Phthalides: Distribution in Nature, Chemical Reactivity, Synthesis, and Biological Activity

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Contents

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1 Introduction

Phthalides are a relatively small group of natural compounds found in several higher and lower plant and fungal genera. Classifiable by structure, the monomeric and dimeric phthalides are known principally as the bioactive constituents in several plants used in traditional medicine in Asia, Europe, and North America. Phthalides are also isolated from several species of fungi.

Although the ancient historical record is fragmentary, there is evidence of the exchange of medicinal herbs between Asia and Europe along the Silk Road trading

routes established by Alexander the Great (356–326 BC), and several old Chinese texts mention medicinal plants that contain phthalides that were included in these routes. In northern Mexico and the southern United States, the medicinal use of the phthalide-containing rootstock of *Ligusticum porteri* has been recorded since the eighteenth century. Relatively few reviews have addressed the phthalides [1–3]. This contribution aims to provide a broad treatment of the topic, with an overview of phthalide chemical structures, natural sources, research methodologies, selected chemical syntheses and reactions, and the main reported bioactivities of phthalides.

1.1 Traditional Uses of Plants that Contain Phthalides

Compiled in ca. 200 AD from ancient oral traditions (ca. 2800 BC), the "Shen Nong Bencaojing" is one of the oldest Chinese texts on agriculture and plants used traditionally to include a description of the use of "Danggui" (*Angelica sinensis* (Oliv.) Diels roots, family Umbelliferae, a plant that contains phthalides) "for enriching the blood" [4]. This plant is included in the Pharmacopoeia of the People's Republic of China, together with other two phthalide-containing plants, *Ligusticum sinense* Oliv. ("Rhizoma Ligustici", "Chinese Lovage", "Gaoben", used to relieve pain) and *Ligusticum chuanxiong* S.H. Qiu, Y.Q. Zeng, K.Y. Pan, Y.C. Tang & J.M. Xu ("Rhizoma Chuanxiong" or "Szechwan Lovage Rhizome", used to promote the flow of blood) [5]. The traditional uses, as well as the chemical constituents and bioactivities of the latter species have been reviewed [6, 7], including bioactivities with other plants [8].

A tea prepared with the rootstock of the North American phthalide-containing species, *Ligusticum porteri* J.M. Coult. & Rose, is commonly used to alleviate stomachache and colic [9], ulcers and diarrhea as well as to treat diabetes and circulatory problems [10, 11]. Infusions of this plant also play a role in the ritual-curing ceremonies in northern Mexico and the southern United States, for which this medicinal plant is highly regarded, mainly by the native Raramuri ethnic group [12]. Some illustrations of this plant material in different stages are shown in Fig. 1.

Not confined in the human sphere of activity, Kodiak bears have been reported to chew the roots of this plant, and to rub the root-saliva mixture into their fur [13].

1.2 Early Chemical Studies (1897–1977) of Phthalides in the Family Umbelliferae

Several of the first reports on the chemistry of phthalides appeared at the end of the nineteenth century, where they were identified as the odor constituents of celery (*Apium graveolens* L.) by the Italian researchers Ciamician and Silber in 1897. The provision of essential oil from celery seed (by the Schimmel Company, Leipzig, Germany) allowed Ciamician and Silber (working in Bologna, Italy) to isolate what

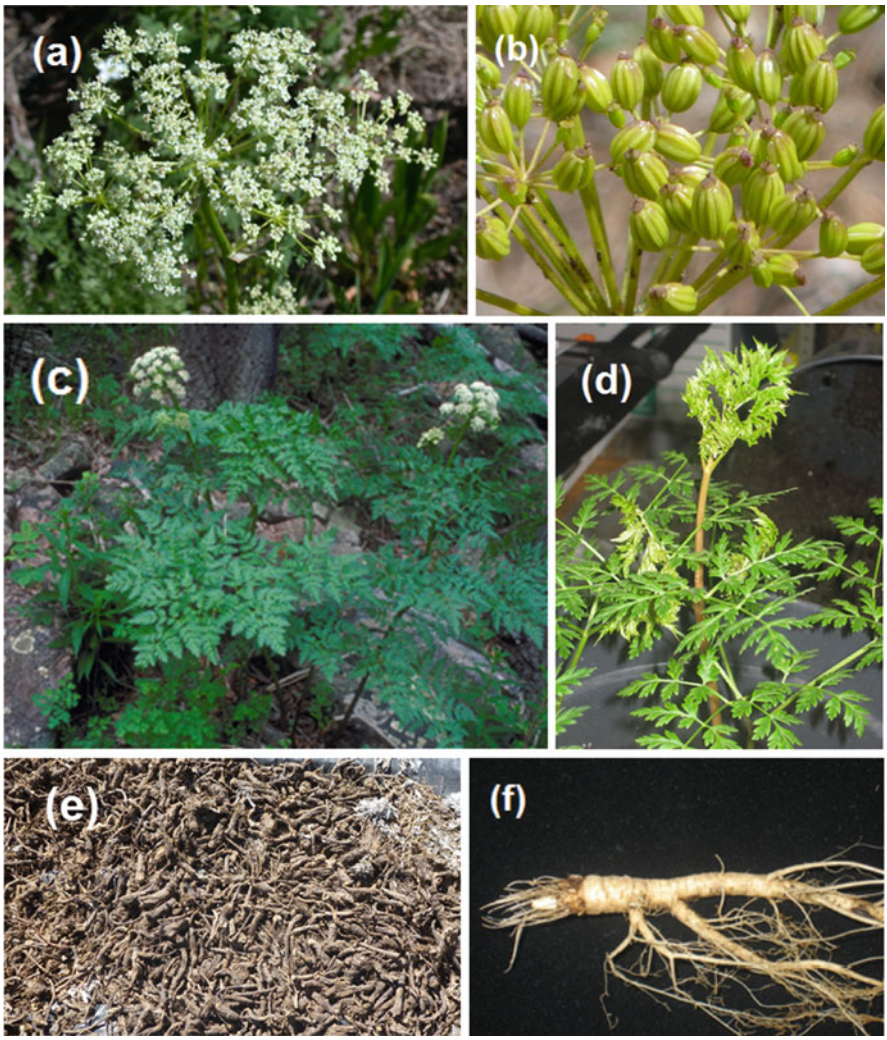


Fig. 1 *Ligusticum porteri* J. M. Coult. & Rose (Umbelliferae). (**a**) Flowers of *L. porteri*, photo: M. E. Harte, Bugwood.org; (**b**) Immature flowers of *L. porteri*, photo: R. Bye and E. Linares, Instituto de Biología, Universidad Nacional Autónoma de México; (**c**) Wild plant, *L. porteri* (Colorado, USA), photo: D. Powell, USDA Forest Service, Bugwood.org; (**d**) Cultivated plant, *L. porteri* (Mexico City), photo: G. Delgado; (**e**) Rootstocks of mature plants of *L. porteri*, photo: R. Bye and E. Linares, Instituto de Biología, Universidad Nacional Autónoma de México; (**f**) Young rootstock of cultivated *L. porteri*, photo: G. Delgado

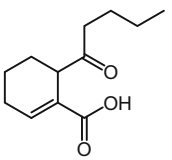
they called sedanolide and sedanonic anhydride [14]; "sedane" is the Italian translation of celery. These substances could be structurally characterized as a result of their transformation to sedanonic acid [15], for which structure **1** was proposed and later, following the analyses of derivatives [16] and intermediates [16, 17], proven to be correct. The published account on these experiments [18] was, according to Barton, "one of the early classics of natural products chemistry"

[19]. A decade later, in 1910, Swenholt obtained the volatile fraction from celery seeds provided by the John A. Salzer Seed Company (La Crosse, Wisconsin, USA). This fraction was saponified and from the organic residue sedanonic acid (**1**) was characterized [20]. Fourteen years later, Berlingozzi established that the odorant properties of celery correlated with the nature of the side chain of phthalides [21–25], a finding subsequently confirmed by other authors [26, 27].

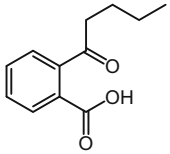
In 1921, Murayama, when leading a chemical investigation of the highly regarded Japanese drug "Sen-Kyu", isolated "cnidium lactone", a compound that had been named previously by Sakai in 1916. This compound was also obtained from the roots of *Cnidium officinale* Makino, which has a long history in traditional Asian medicine, and is named "Hsiung-Ch'uang" in mainland China [28]. Following re-isolation from a second population of the same species [26], it was concluded that "cnidium lactone" was similar in structure to sedanolide, isolated by Ciamician and Silber. However, the instability and the practical difficulties of isolating phthalides hampered any further characterization or identification of these substances.

In 1934, Noguchi reisolated "cnidium lactone" and noting its close structural relationship with sedanolide, proposed that stereoisomeric characteristics may underlie any structural differences [27].

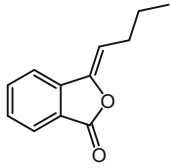
From the saponified extract of the fruits of another species in the Umbelliferae, *Ligusticum acutilobum* Siebold & Zucc., Kariyone and Kotani [29], isolated an acid, which could be transformed to a lactone; and these two compounds were later characterized as valerophenone *o*-benzoic acid (**2**) and (*Z*)-butylidenephthalide (**3**) [30, 31]. Although the structures of sedanolide and "cnidium lactone" remained unclear [32], a study of the essential oil of lovage by Naves [33], resulted in the characterization of (*Z*)-butylidenephthalide (**3**), butylphthalide (**4**) and what Ciamician and Silber had named sedanonic anhydride (sedanonic acid lactone), which was found to be (*Z*)-6,7-dihydro-ligustilide (**5**). Compound **2** was obtained as a saponification product of the essential oil of the crude drug named "Toki" (*Angelica acutiloba* (Siebold & Zucc.) Kitag.) [34].



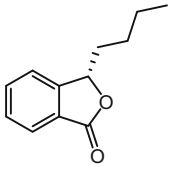
1 (sedanonic acid)



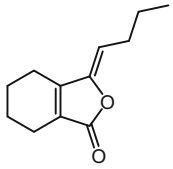
2 (valerophenone *o*-benzoic acid)



3 ((*Z*)-butylidenephthalide)



4 ((*S*)-3-butylphthalide)

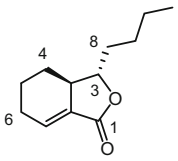


5 ((*Z*)-6,7-dihydroligustilide, sedanonic anhydride)

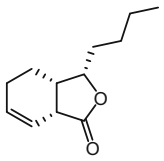
Barton and de Vries [19] then determined the chemical formulas of sedanolide **6** (by $NaBH_4$-mediated reduction of sedanonic acid (**1**)), and cnidium lactone (**7**, cnidilide), although without assigning the configurations of the chiral carbons.

(*Z*)-Ligustilide (**8**) was characterized from the roots of *Ligusticum acutilobum* (common name in Japanese: "Hokkai-Toki") and from *Cnidium officinale* by Mitsuhashi and Nagai [35]. The 6,7-dihydro derivative **5** was found to generate sedanonic acid (**1**) following its saponification. The structures of neocnidilide (**6**) and cnidilide (**7**), determined following extraction from the roots of *C. officinale*, indicated that sedanolide (represented by formula **6**, leaving aside the configurational assignments), actually comprised a mixture of neocnidilide (**6**) and butylphthalide (**4**) [36]. The configurations at C-3, C-3a, and C-7a for cnidilide (cnidium lactone) and at C-3 of isocnidilide (*trans*-sedanolide) were determined as shown in formulas **7** and **9**, respectively, by using chiroptical methods [37, 38]. The synthesis of butyltetra- and hexahydrophthalides was used to establish the identity of neocnidilide and *trans*-sedanenolide with the configurational assignments showed in formula **6**, and also confirmed structures **7** and **9** for cnidilide and isocnidilide, respectively [39].

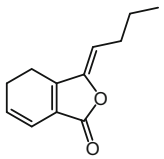
At the same time, phthalides **3**, **4**, and **8** were characterized from *Meum athamanticum* Jacq. [40] and some experimental improvements for the separation and characterization of phthalides were reported [41].



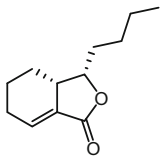
6 (neocninilide, (*trans)*-sedanolide)



7 (cnidilide, cnidium lactone)

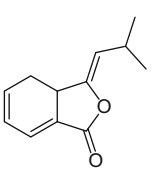


8 ((*Z*)-ligustilide)

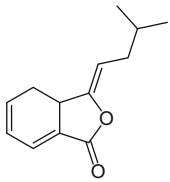


9 (isocnidilide, (*cis)*-sedanolide)

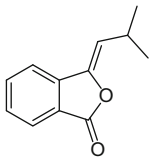
Phthalides **10**–**13** were isolated from celery, indicating that they are responsible for its characteristic odor [42], since these substances were structurally similar to those reported by Berlingozzi and associates more than three decades earlier [21–25]. Butylphthalide (**4**), sedanolide (**6**), 3-*n*-butylhexahydrophthalide (**14**) [43], as well as senkyunolide A (formerly named sedanenolide) (**15**) were also isolated from celery [44].



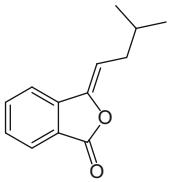
10 ((*Z*)-isobutylidene-3a,4-dihydrophthalide)



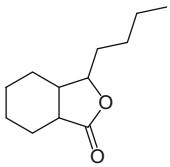
11 ((*Z*)-isovalidene-3a,4-dihydrophthalide)



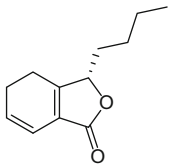
12 ((*Z*)-isobutylidene-phthalide)



13 ((*Z*)-isovalidene-phthalide)



14 (3-butyl-hexahydrophthalide)



15 (sedanenolide, senkyunolide A)

3-Butylphthalide (**4**) and cnidilide (**7**) were characterized from the essential oil of the Chinese medicinal plant "Gaoben" (*Ligusticum sinense*) [45]. Reinvestigation of *Cnidium officinale* subsequently allowed the characterization of compounds **3**–**5**, **8** and **15** (permitting the (3*S*)-configuration for **15** to be defined), and a mass spectrometric fragmentation pattern for these compounds was proposed [46].

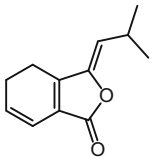
A 1979 review of the phthalides in the family Umbelliferae included their chemotaxonomic aspects, biosynthesis, and stereochemical assignments [47]. It is interesting to note that, at that time, no dimeric phthalides had yet been isolated.

2 Distribution of Phthalides in Nature

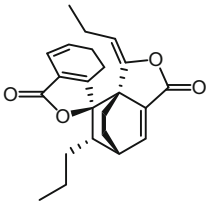
2.1 Phthalides in the Umbelliferae (syn. Apiaceae)

A number of studies on phthalides from Umbelliferae family members have been conducted to verify the presence of phthalides, with investigations on the volatile odor constituents of celery (*Apium graveolens*). These studies permitted the characterization of compounds **3**, **4**, **8**, and **10** [48], and **3**, **6**, **8**, and **16** [49]. Additionally, the monomeric phthalides **3**, **4**, **6**, **7**, **8**, and **15** were identified from *Cnidium officinale* [50].

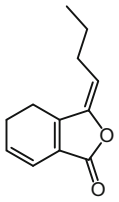
The first dimeric phthalide reported in the literature was angeolide (**17**), which was isolated from *Angelica glauca* Edgew. (a species distributed in the Western Himalayas). The chemical structure of angeolide was confirmed to be a Diels–Alder adduct of (*E*)-ligustilide (**18**), which acts as diene and dienophile. Both (*E*)-**17** and (*Z*)-ligustilide (**8**) were isolated and characterized from this plant species [51]. The direct nomenclature used to name the ligustilide dimers incorporates: (a) the numbers of the connected atoms (describing the adduct derived from the reaction diene + dienophile); (b) the stereochemical descriptors *endo*- and *exo*-, and (c) the name of the monomers. Therefore, angeolide (**17**) could be named as *endo*-[3.3′a,8.6′]-(*E*,*E*′)-diligustilide.



16 ((*Z*)-isobutylidene-4,5-dihydrophthalide)

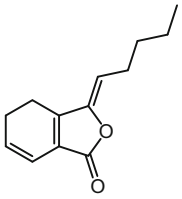


17 (angeolide)

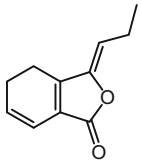


18 ((*E*)-ligustilide)

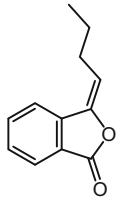
The monomeric phthalides **4**, **6**, **8**, and **18** were identified from the roots of *Cenolophium denudatum* (Fisch. ex Hornem.) Tutin and *Coriandrum sativum* L. (coriander) [52]; compounds **3**, **8**, **15**, **18**, and **19–21** were found as constituents of the essential oil from the roots of *Levisticum officinale* W.D.J. Koch [53], and from the roots of *Silaum silaus* (L.) Schinz & Thell. and *Anethum sowa* Roxb. ex Fleming, **5**, **6**, **8**, and **15** were characterized [54]. Neocnidilide (**6**), (*Z*)-ligustilide (**8**), and senkyunolide A (**15**) were present in *Anethum graveolens* L. (dill); phthalide **8** was characterized from *Todaroa montana* Webb ex Christ [55] and compounds **3**, **4**, **8**, and **15** were identified from the roots of *Opopanax chironium* Koch.



19 ((*Z*)-validene-4,5-dihydrophthalide)

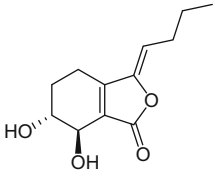


20 ((*Z*)-propylidene-phthalide)

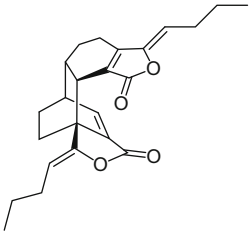


21 ((*E*)-butylidene-phthalide)

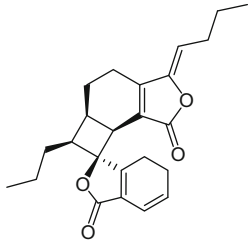
From *Ligusticum wallichii* Franch. were isolated a *trans*-diol named (*Z*)-ligustidiol (**22**) [56] (later renamed as senkyunolide I, see below), and the Diels–Alder adduct of (*Z*)-ligustilide, termed (*Z*,*Z*′)-diligustilide (**23**) [57] (later renamed by Höfle as levistolide A, see below). This last compound could be named *endo*-[3a.7′,6.6′]-(*Z*,*Z*′)-diligustilide, following the nomenclature that indicates the connections between the monomers. The [π2s + π2s] dimer, [6.8′,7.3′]-(*Z*,*Z*′)-diligustilide **24**, named riligustilide, was characterized from *Ligusticum acutilobum* [58].



22 (6,7-*trans*-ligustidiol, senkyunolide I)



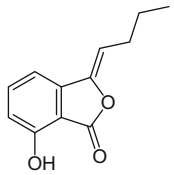
23 ((*Z*,*Z*′)-diligustilide, levistolide A)



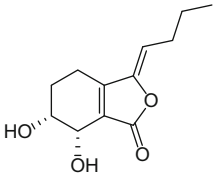
24 (riligustilide)

Compounds **25** ((*Z*)-3-butylidene-7-hydroxy-phthalide, later renamed senkyunolide B), **26** (*cis*-6,7-dihydroxy-ligustilide, later renamed senkyunolide H), and **22** (senkyunolide I) were isolated from *Ligusticum wallichii*, together with a dimer named wallichilide **27** [59]. It is interesting to note that methyl ester **27** could be an artifact derived from the ring opening of diligustilide (levistolide A, **23**) and esterification, given its isolation from a hot water extract, followed by HPLC purification using MeOH–H_2O–HOAc.

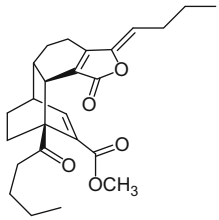
A series of hydroxyphthalide derivatives were isolated from the rhizomes of *Cnidium officinale* by Mitsuhashi and associates; these were senkyunolides A (**15**), B (initially **25**, but later corrected to **37**, see below), C (**28**), D (**29**), E (**30**), F (**31**), G (**32**), H (**26**), I (**22**), and J (**33**). With the exception of senkyunolide J, all were optically inactive [60].



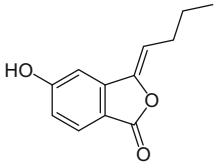
25 (3-butylidene-7-hydroxyphthalide)



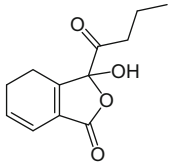
26 (6,7-*cis*-ligustidiol, senkyunolide H)



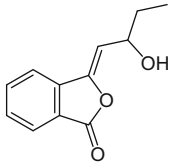
27 (wallichilide)



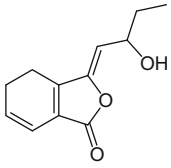
28 (senkyunolide C)



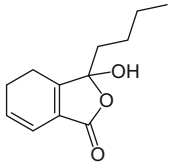
29 (senkyunolide D)



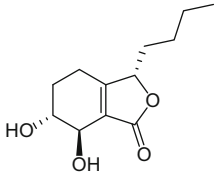
30 (senkyunolide E)



31 (senkyunolide F)



32 (senkyunolide G)

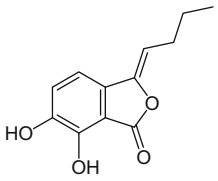


33 (senkyunolide J)

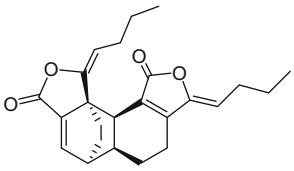
(*Z*)-Ligustilide (**8**) was found in the roots of *Capnophyllum peregrinum* Lange, while compounds **7**, **8**, and **15**, were identified from the roots of *Peucedanum ostruthium* (L.) W.D.J. Koch [61]. (*Z*)-5-Hydroxy-butylidene-phthalide ((**28**) senkyunolide C) and the dihydroxyphthalide **34**, were characterized from the rhizomes of *Ligusticum wallichii* [62].

(*Z*)- and (*E*)-Butylidenephthalides ((**3**) and (**21**)), butylphthalide (**4**), (*Z*)-ligustilide (**8**), senkyunolide A (**15**), angeolide (**17**), (*Z*,*Z*′)-diligustilide ((**23**), renamed by Höfle as levistolide A), and levistolide B (**35**), were all identified from the underground parts of *Levisticum officinale* ("Radix Levici") [63]. This last compound could be also named *endo*-[3a.7′,6.6′]-(*E*,*Z*′)-diligustilide.

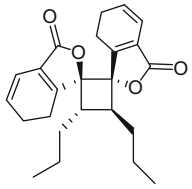
The [π2s + π2s]-cyclodimer derived from ligustilide, termed angelicolide (**36**), was found as an additional constituent from *Angelica glauca*, and its structure was confirmed by X-ray analysis as a derivative of (*E*)-ligustilide (**18**) [64].



34 ((*Z*)-4,5-dihydroxy-3-butylidenephthalide)



35 (levistolide B)

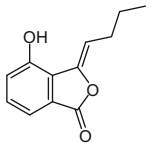


36 (angelicolide, [8,8'.3,3']-(*E*,*E*')-diligustilide)

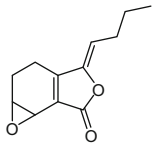
Three phthalide derivatives, **15**, **22**, and **23**, were isolated from the rhizomes of *Meum athamanticum* [65]. Additional compounds including senkyunolides I (**22**), H (**26**), C (**28**), E (**30**), and F (**31**) were also identified. The structure of senkyunolide B was corrected from 7-hydroxy- (**25**) to 4-hydroxy-butylidenephthalide (**37**) by comparison of spectroscopic properties, which was possible by their occurrence in this natural source [66].

From the roots of *Apium graveolens* were identified phthalides **4**, **6**, **7**, **8**, and **15**, and from *A. graveolens* var. *rapaceum* (Mill.) DC., compounds **3**, **4**, **6**, **8**, **15**, and **18** were characterized. *Petroselinum crispum* (Mill.) Fuss. var. *tuberosum* (Bernh. ex Richb.) Soó (parsley) was used to isolate **8** and **15**, while from *Bifora testiculata* (L.) Roth, compounds **6**, **8**, and **15** were found [67]. A study of Angelicae Radix ("Chinese Tang-kuei") allowed the characterization of (*Z*)-butylidenephthalide (**3**), butylphthalide (**4**), and (*Z*)-ligustilide (**8**) [68].

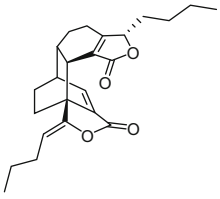
Diligustilide (levistolide A (**23**)), riligustilide (**24**), (*Z*)-6,7-epoxy-ligustilide (**38**), and an additional dimer, 3,8-dihydro-[6.6′,7.3a′]-(*Z*′)-diligustilide (**39**), were all identified from the rhizomes of *Ligusticum wallichii* [69]. The structure of this last dimer was corrected to structure **40** [70], which was then reisolated from *Ligusticum chuanxiong* and later renamed senkyunolide P [71].



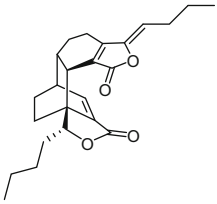
37 (4-hydroxy-butylidenephthalide)



38 ((*Z*)-6,7-epoxy-ligustilide)

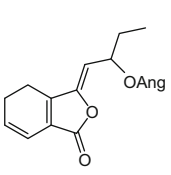


39 ((*Z*')-3,8-dihydro-6.6',7.3'a-diligustilide)

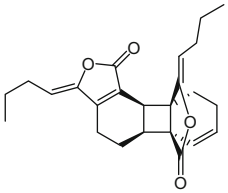


40 (senkyunolide P)

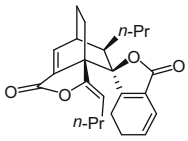
The known senkyunolides I (**22**), H (**26**), E (**30**), F (**31**), as well as levistolide A (**23**), were found as constituents of *Angelica acutiloba*, along with 11-angeloyl-senkyunolide F (**41**), tokinolide A (**42**), and tokinolide B (**43**) [72]. A series of known monomeric phthalides, together with senkyunolides K (**44**), L (**45**), and M (**46**), was characterized from *Ligusticum wallichii* [73]. (*Z*)-Ligustilide (**8**), (*Z*,*Z*′)-diligustilide ((**23**) levistolide A) and riligustilide (**24**) were found as constituents of *Ligusticum porteri* [70], and senkyunolides O (**47**) and P (**40**) were identified from *Ligusticum chuanxiong* [71]. The ^{1}H and ^{13}C NMR spectroscopic data of some monomeric phthalides have been reported in the literature [74].



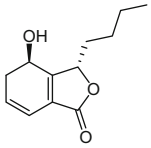
41 (angeloyl-senkyunolide F)



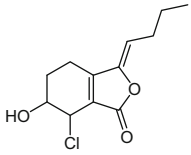
42 (tokinolide A)



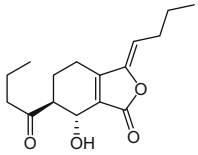
43 (tokinolide B)



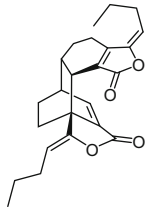
44 (senkyunolide K)



45 (senkyunolide L)



46 (senkyunolide M)

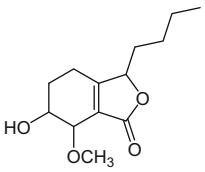


47 (senkyunolide O)

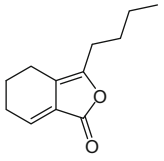
(*Z*)-Ligustilide (**8**) has been proposed as a benchmarking constituent for preparations of *Ligusticum officinale* [75], and its relative abundance in the essential oil of this species has been studied [76]. Both (*Z*)-butylidenephthalide (**3**), and (*Z*)-ligustilide (**8**) have been found in *Pituranthos tortuosus* (Desf.) Benth. & Hook. f. ex Asch. & Schweinf. [77], and these compounds together with (*E*)-ligustilide and monoterpenes were found as constituents of the rootstock of *Ligusticum porteri* [78].

The volatile aroma constituents of celery and related species have been the subject of several investigations [79, 80], and despite a wide variation in the chemical constituents reported [81, 82], these studies confirmed early observations that monomeric phthalides were responsible for the characteristic aroma of celery. Volatile components isolated from celery plants grown with different fertilizers have also been analyzed [83]. Compound NG-072 (**48**), purported as being useful for the treatment of Alzheimer's disease, was characterized from celery, although without assigning the configuration of the chiral centers [84]. Phthalides **3**, **4**, **6**, **9**, **15**, and **21**, as well as the unstable compound **49**, were characterized from parsley (*Petroselinum crispum*) [85]. The Diels–Alder adduct **50**, derived from (*Z*)-ligustilide (**8**) (diene) and (*E*)-ligustilide (**18**) (dienophile), were isolated from *Angelica sinensis* and named E-232 [86]. An additional series of phthalides was isolated from *Ligusticum chuanxiong*, including (*E*)-senkyunolide E (**51**),

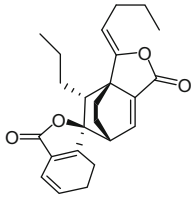
senkyunolide N (**52**), and senkyunolide J (**33**) [44]. The absolute configurations of these last two compounds were established as depicted in their structural formulas [87]. From this source were isolated senkyunolide Q (**53**) and methyl 2-(1-oxo-pentyl)-benzoate (**54**) [88], which is the methyl ester of compound **2** characterized by Noguchi in earlier investigations of *Ligusticum* species [30, 31].



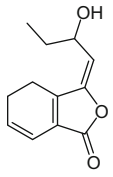
48 (NG-072)



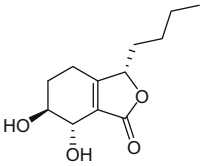
49 (3-butyl-5,6-dihydro-4*H*-isobenzofuran-1-one)



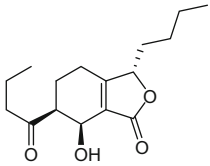
50 (E-232)



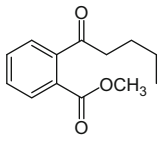
51((*E*)-senkyunolide E)



52 (senkyunolide N)



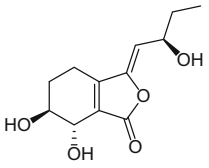
53 (senkyunolide Q)



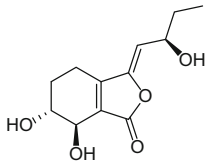
54 (methyl 2-(1-oxo-pentyl)-benzoate)

The preparation of derivatives of the monomeric and dimeric phthalides has been limited to structural studies. The reactivity of (*Z*)-ligustilide (**8**) acting as a biological electrophile, has been explored by Beck and Stermitz [89], and their interesting results obtained are described in Sect. 5.1.4.

(*Z*)-Ligustilide (**8**) was characterized from *Ligusticum mutellina* (L.) Crantz [90] and *Angelica sinensis* [91] and the monomeric phthalides **3**, **8**, **21**, and **22** were found in *Angelica glauca* roots [92]. Both senkyunolide R (**55**) and senkyunolide S (**56**) were characterized as constituents of *Ligusticum chuanxiong* [93].



55 (senkyunolide R)

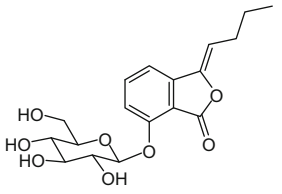


56 (senkyunolide S)

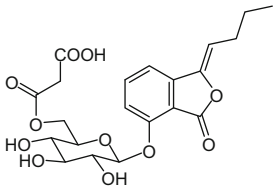
The separation of 3-butylphthalide enantiomers ((*S*)-enantiomer: structure **4**) and their odor thresholds have now been established [94]. Enantioselective analyses of the flavor-imparting compounds (3-butylphthalide derivatives) in celery, celeriac, and fennel have also been investigated [95] with seasonal variations in the composition of volatile components (including phthalides) from different parts of the lovage plant reported [96]; compound **8** was found in the essential oils of

Lomatium torreyi J.M. Coult. & Rose [97], *Meum athamanticum* [98] and *Trachyspermum roxburghianum* H. Wolff [99]. (*Z*)-Ligustilide (**8**) was also found as a constituent of non-polar extracts of the roots from *Ligusticum porteri*, *L. filicinum*, and *L. tenuifolium* [100].

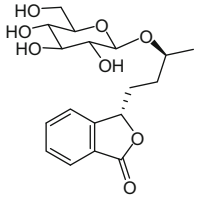
From elicitor-treated parsley cell suspension cultures were isolated four phthalides, namely, 3-butylidene-7-hydroxy-phthalide (**25**), and 3-butylidene-5-hydroxy-phthalide (senkyunolide C (**28**)) and its 7-*O*-*β*-D-glucopyranoside (**57**) and 7-*O*-(6′-malonyl)-*β*-D-glucopyranoside (**58**) derivatives [101]. An analysis of the water-soluble fraction of the methanol extract of celery seed afforded three more phthalide glycosides, named celephthalide A (**59**), celephthalide B (**60**) (with an unresolved configuration at C-3), and celephthalide C (**61**) [102]. As noted in Beck and Chou's review on phthalides [2], the structure of celephthalide C (**61**) was found to be similar to that of neocnidilide (**6**).



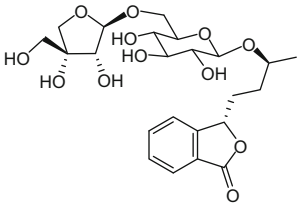
57 (7-hydroxy-butylidenephthalide 7-*O*-*β*-D-glucopyranoside)



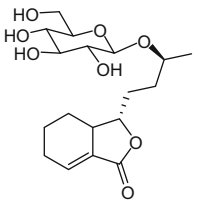
58 (7-hydroxy-butylidenephthalide 7-*O*-(6'malonyl)-*β*-D-glucopyranoside)



59 (celephthalide A)



60 (celephthalide B)

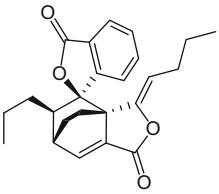


61 (celephthalide C)

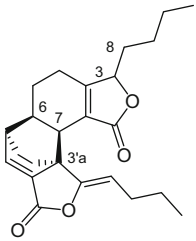
The accumulation of some secondary metabolites of *Ligusticum chuanxiong* (including phthalides) has been correlated with the developmental stages of the plant [103], with (*Z*)-butylidenephthalide (**3**) and (*Z*)-ligustilide (**8**) found as volatiles of *Angelica tenuissima* Nakai [104] and *Meum athamanticum* [105].

Several dimeric phthalides were isolated from *Ligusticum chuanxiong*, and characterized as levistolide A (**23**), riligustilide (**24**), tokinolide B (**43**), 4,5-dehydrotokinolide B (**62**), and 3,8-dihydrolevistolide A (**63**) [106]. This last compound had been previously prepared by catalytic reduction of [6.6′,7,3a′]-(*Z*,*Z*′)-diligustilide A (syn: levistolide A (**23**)) and its structure was firmly established [70]; therefore, the compound isolated from *L. chuanxiong* requires structural revision. A series of phthalides, including butylphthalide (**4**), cnidilide (**7**), (*Z*)-ligustilide (**8**), senkyunolide I (**22**), levistolide A (**23**), riligustilide (**24**), (*Z*)-7-hydroxy-butylidenephthalide (**25**), senkyunolide H (**26**), tokinolide B (**43**), the triol **64** [107], (*S*)-4-hydroxy-butylphthalide (**65**) [108] and the dimeric

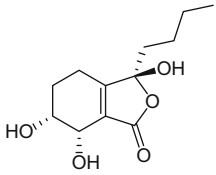
phthalides chuanxiognolides A (**66**) and B (**67**), were also reported as constituents of *L. chuanxiong* [103].



62 (4,5-dehydro-tokinolide B)



63 (3,8-dihydro-levistolide A)



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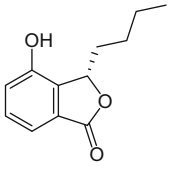

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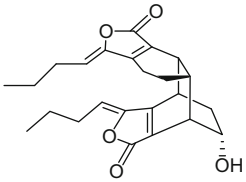
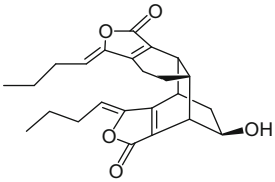
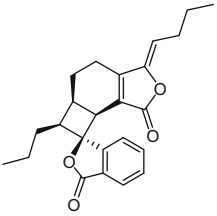


66 (chuangxiognolide A)

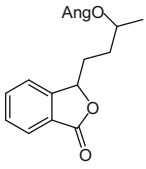


67 (chuangxiognolide B)

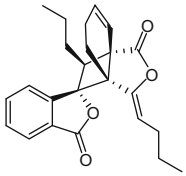
The dimeric phthalides riligustilide (**24**) and gelispirolide (**68**) were isolated from *Angelica sinensis* [109], with three new phthalides (**69**–**71**) purified from the same plant [110]. (*Z*)-Butylidenephthalide (**3**), (*Z*)-ligustilide (**8**), levistolide A (**23**), riligustilide (**24**), and compounds **72** and **73** were also isolated from a population of *A. sinensis* [111]. From an aqueous extract of *Ligusticum chuanxiong* was isolated a lactone derivative (**74**) considered as a phthalide analog [112].



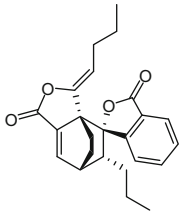
68 (gelispirolide)



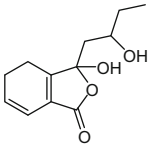
69 (10-angeloyloxi-butylphthalide)



70 (sinaspirolide)



71 (ansaspirolide)



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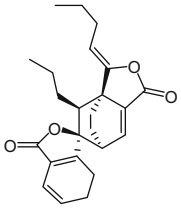
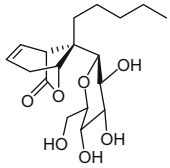


73 ((3Z,3a*R*,6*S*,3'*R*,8'*S*)-3a.8',6.3'-diligustilide)

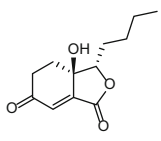


74 (ligusticoside)

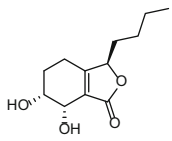
Ligusticum chuanxiong is recognized widely as an effective medicinal plant. Of more than 200 compounds that have been isolated from this species, the phthalides are considered to be the characteristic metabolites. Recent reviews compiling the chemical profile of *L. chuanxiong* [113] and its pharmacological properties [114] have been published.

Four not previously reported phthalides (**75**–**78**), together with compounds **4**, **7**, **8**, **22**, **23**, **27**, and **43**, have also been isolated from *Ligusticum chuanxiong* [115].

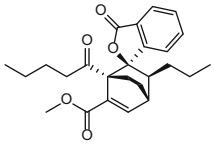
Sedanonic acid (**1**) and phthalides **6**, **22**, **26**, **52**, and **79**–**85**, were isolated from *Ligusticum sinense* Oliv. cv. *chaxiong*, with some compounds displaying activity against neuronal injury [116]. From the roots of the same species were isolated (*Z*)-ligustilide (**8**), and the dimeric phthalides chaxiongnolide A (**86**) and chaxiongnolide B (**87**) [117]. This last-mentioned compound had been previously characterized as a semisynthetic substance that was obtained by the differentiated cyclization of the ketoacid derived from tokinolide B (**43**) [118]. 7-Acetyl-senkyunolide H (**88**) was isolated from the roots of *Angelica sinensis* [119], and (*Z*)-ligustilide (**8**) has been found in good yields in different plant parts of *Kelussia odoratissima* Mozaff [120].



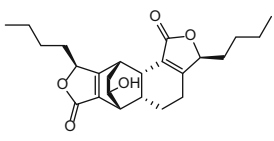
75 (chuanxiongnolide R1)



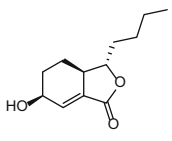
76 (chuanxiongnolide R2)



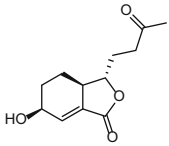
77 (chuanxiongdiolide R1)



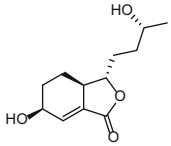
78 (chuanxiongdiolide R2)



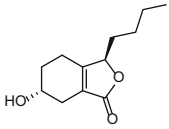
79 ((3*S*,3a*R*,6*S*)-6-hydroxysedanolide)



80 (6-hydroxy-sedanolide-10-one)



81 (10-hydroxysedanolide)



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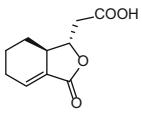
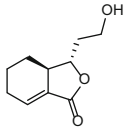


83 ((3*S*,3a*R*)-3-(2-carbonyl)-ethyl-3a,4,5,6-tetrahydrophthalide)



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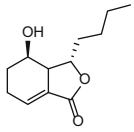
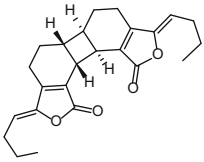
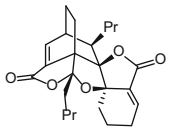


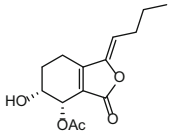
85 ((3*S*,3a*R*,4*R*)-4-hydroxy-sedanolide)



86 (chaxiongnolide A)



87 (chaxiongnolide B, cyclization product of the ketoacid derived from tokinolide B)



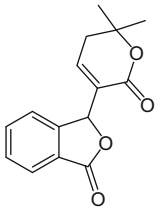
88 (7-acetyl-senkyunolide H)

2.2 Phthalides in Other Plant Families

This section refers to the presence of phthalides that have been found in several plant families, other than the Umbelliferae (Apiaceae).

2.2.1 Bignoniaceae

From a methanol extract of the wood of *Catalpa ovata* G. Don, used traditionally as a diuretic in Japan, was isolated catalpalactone (**89**) [121]. Inouye and co-workers confirmed its structure, by preparing several derivatives. Compound **89** was obtained from the same plant several years later [122, 123].



89 (catalpalactone)

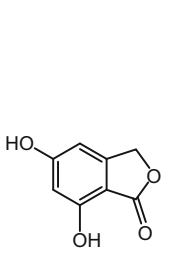
2.2.2 Cactaceae

Compounds from the leaves, flowers and fruits of *Opuntia leindheimeiri* var. *linguiformis* (Griffiths) L.D. Benson, the leaves and flowers of *O. macrorhiza* Engelm., and the leaves of *O. microdasys* (Lehm.) Pfeiff. were extracted by steam distillation, and (*E*)-butylidenephthalide (**21**) was identified by GC-MS [124].

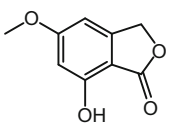
2.2.3 Compositae (syn. Asteraceae)

Several species of the genus *Helichrysum* have yielded phthalides. 5,7-Dihydroxyphthalide (**90**) and 5-methoxy-7-hydroxy-phthalide (**91**) were isolated from *H. italicum* (Roth) G. Don [125, 126]. Both phthalides and arenophthalide A (**92**) were contained in the organic extracts of *H. arenarium* (L.) Moench [127, 128]. On the other hand, *H. platypterum* DC. yielded platyphterophthalide (**93**) [129]. Venditti and co-workers carried out a chemical

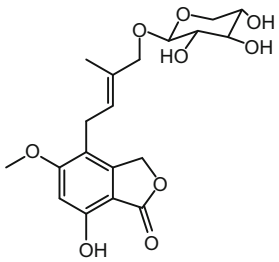
analysis of a chromatographic fraction of *H. microphyllum* (Willd.) Benth. & Hook. f. ex Kirk of medium polarity, and characterized phthalides **94** and **95** [130].



90 (5,7-dihydroxyphthalide)



91 (7-hydroxy-5-methoxyphthalide)



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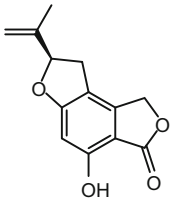
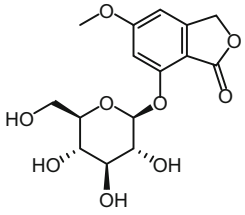


93 (platyphterophthalide)



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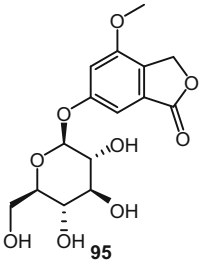
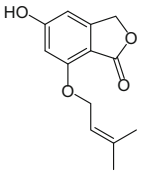
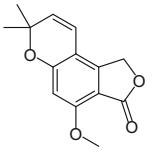


95

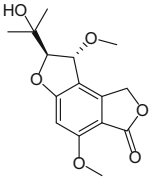
Talapatra and co-workers [131] analyzed the petroleum ether, chloroform, and alcoholic extracts of *Anaphalis contorta* (D. Don) Hook. f., and from these were isolated 5,7-dihydroxyphthalide (**90**), 5-methoxy-7-hydroxyphthalide (**91**), and 5-hydroxy-7-*O*-(3′-methyl-but-2′-enyl)phthalide (anaphatol, **96**). Phthalidochromene (**97**), araneophthalide (**98**) and aranochromanophthalide (**99**) were later obtained from the aerial parts of *Anaphalis araneosa* DC. [129].



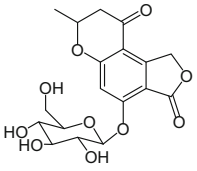
96 (anaphatol)



97 (phthalidochromene)



98 (araneophthalide)

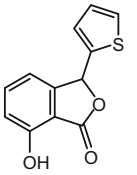


99 (arachromanophthalide)

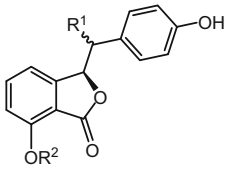
A 3-substituted phthalide with thiophene, which was called chrycolide (**100**), was isolated from an extract of *Chrysanthemum coronarium* L. [132].

Stuppner's research group analyzed *Scorzonera tomentosa* L., a plant that has been used traditionally for the treatment of infertility and as an analgesic, anthelmintic, and antirheumatic in Turkey. From the methanol extract were isolated three phthalides as racemic mixtures, namely, (±)-scorzophthalide (**101**), (±)-hydramacrophyllol A (**102**), and (±)-hydromacrophyllol B (**103**) [133].

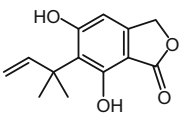
From the aerial parts of *Gnaphalium adnatum* DC. (Wall.) ex Thwaites [134] were isolated compounds **90** and **104**–**108**.



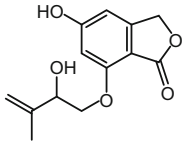
100 (chrycolide)



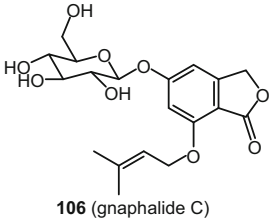
(±)-**101** (scorzophthalide) $R^1 = \alpha$-H, R^2 = Me_2
(±)-**102** (hydramacrophyllol A) $R^1 = \alpha$-OH, R^2 = H
(±)-**103** (hydramacrophyllol B) $R^1 = \beta$-OH, R^2 = H



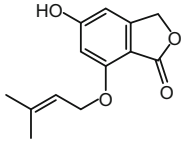
104 (gnaphalide A)



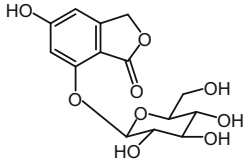
105 (gnaphalide B)



106 (gnaphalide C)



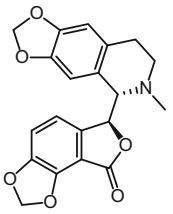
107



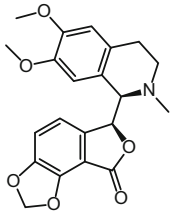
108

2.2.4 Fumaraceae

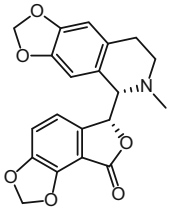
In a search for spirobenzyl-isoquinolines from *Fumaria parviflora* Lam., four phthalideisoquinolines were found, namely, (+)-adlumidine (**109**), (–)-corlumine (**110**), (+)-bicuculline (**111**), and (+)-α-hydrastine (**112**) [135].



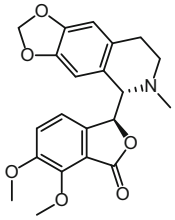
109 ((+)-adlumidine)



110 ((–)-corlumine)



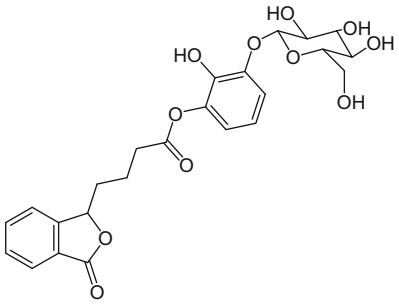
111 ((+)-bicuculline)



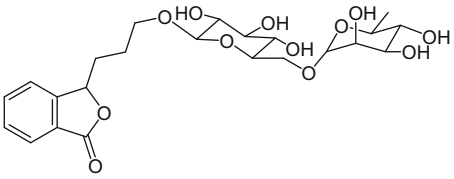
112 ((+)-α-hydrastine)

2.2.5 Gentianaceae

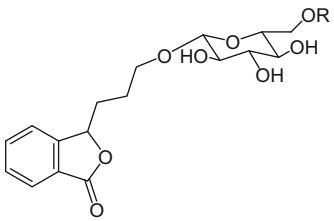
From the leaves of *Gentiana pedicellata* (Wall. ex D. Don) Griseb., pedicelloside (**113**) [136] and pedirutinoside (**114**) were isolated by Chulia and co-workers [137]. Garcia and associates analyzed the aerial parts of *Gentiana pyrenaica* L. and obtained 3-(3-*O*-*β*-D-glucosylpropyl)phthalide, which was named pediglucoside (**115**), and 3-[3-(6-vanilloyloxy-*O*-*β*-D-glucosyl)propyl]phthalide, or 6′-vanilloylpediglucoside (**116**) [138].



113 (pedicelloside)



114 (pedirutinoside)



115 (pediglucoside) R = H
116 (6'-vanilloylpediglucoside) R = vanilloyl

2.2.6 Lamiaceae

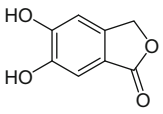
Scutellaria baicalensis Georgi has been used in Chinese traditional medicine for the treatment of diarrhea and inflammatory diseases. Its phytochemical investigation has yielded butylidenephthalide (**3**), (*S*)-butylphthalide (**4**), neocnidilide (**6**), cnidilide (**7**), (*Z*)-ligustilide (**8**), and senkyunolide A (**15**) [139].

2.2.7 Leguminosae (syn. Fabaceae)

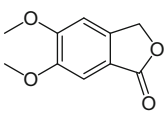
Malan and Roux performed the isolation of 5,6-dihydroxyphthalide (**117**), identified as meconine (**118**) after methylation with diazomethane, in the chemical analysis of *Peltogyne pubescens* Benth. and *Peltogyne venosa* (Vahl) Benth. [140]. 4,6-Dimethoxyphthalide (**119**) was isolated from a methanolic extract of *Albizzia julibrissin* Durazz. [141].

2.2.8 Loganiaceae

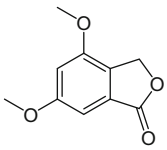
Preparations from the stem bark of *Anthocleista djalonensis* A. Chev. have been used traditionally for curing fever, as a purgative, and for stomachache, and from the organic extract of this species, 4-carbomethoxy-5,7-dimethoxy-6-methylphthalide (**120**) (djalonensin) was obtained [142].



117



118



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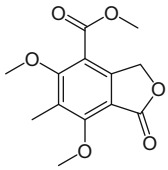


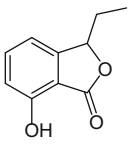
120 (djalonensin)

2.2.9 Oleaceae

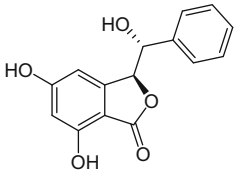
From the essential oil of the stem bark of *Forsythia japonica* Makino, Kameoka and co-workers isolated and characterized 3-ethyl-7-hydroxyphthalide (**121**) [143].

2.2.10 Onocleaceae

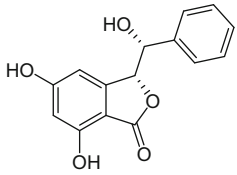
(±)-Matteucen C (**122**) and (±)-matteucen D (**123**) were isolated as racemic products, along with some isocoumarins, from the rhizomes of *Matteuccia orientalis* (Hook.) Trevis. [144].



121



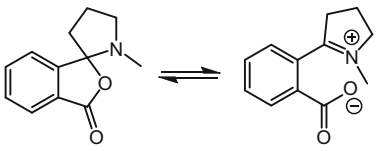
122 ((±)-matteucen C)



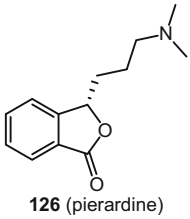
123 ((±)-matteucen D)

2.2.11 Orchidaceae

Shihunine (**124**) is a secondary metabolite of *Dendrobium lohohense* Tang & F.T. Wang. It was found as a racemic mixture, as deduced by the lack of optical properties [145, 146]. Pierardine (**126**) was isolated from the methanol extract of *Dendrobium pierardii* Roxb. ex Hook. as an optically active compound [147]. Later, it was synthesized and its absolute configuration (*S*) was assigned by comparison of its physical characteristics with those previously reported for (3*S*)-butylphthalide (**4**) [148]. Shihunine (**124**) was also reported as a metabolite of *D. pierardii*, as well as betaine (**125**), which exists in polar solvents.



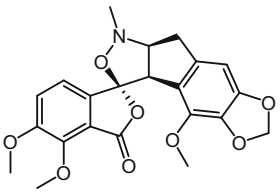
124 (shihunine) **125** (betaine)



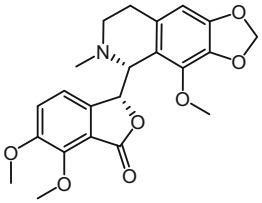
126 (pierardine)

2.2.12 Papaveraceae

Setigerumine I (**127**) was isolated from *Papaver setigerum* DC., which also yielded the well known α-noscapine (**128**). The relative configuration of the new phthalide was determined through NMR spectroscopic experiments, and it was isolated as a racemic mixture [149].



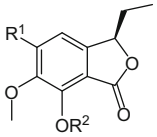
127 (setigerumine I)



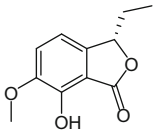
128 (α-noscapine)

2.2.13 Pittosporaceae

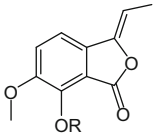
From the Chinese and Taiwanese *Pittosporum illicioides* Makino var. *illicioides*, were isolated six hitherto unknown phthalides, **129**–**134**. According to the method described, enantiomers **129** and **132** eluted differentially by column chromatography over silica gel [150]; since this is not possible, a compound configurational error in this report seems probable [150]. The absolute configurations of the compounds were determined by comparison of their specific rotations with known 3-alkylphthalides [151].



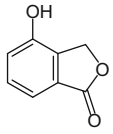
129 $R^1 = R^2 = H$
130 $R^1 = OMe$, $R^2 = H$
131 $R^1 = OMe$, $R^2 = Me$



132



133 R = H
134 R = Me



135 (4-hydroxyphthalide)

2.2.14 Poaceae (syn. Gramineae)

4-Hydroxyphthalide (**135**) was isolated from an acetone extract of crushed oat grain (both *Avena fatua* L. and *Avena sativa* L.). Considering that 4-oxygen-substituted phthalides are seldom found in Nature, the author suggested that it cannot be ruled out that 4-oxy-phthalides have another biosynthetic origin than that through the more common 3-alkyl and 3,5- and/or 7-oxygen substituted phthalides [152].

2.2.15 Polygonaceae

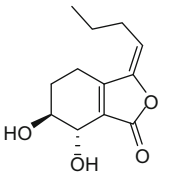
From the methanol extract of the root tubers of *Polygonum multiflorum* Thunb., a medicinal plant used traditionally for the treatment of hyperlipidemia, were obtained *trans*- and *cis*-(*E*)-3-butylidene-4,5,6,7-tetrahydro-6,7-dihydroxy-3*H*-isobenzofuranone **136** and **137**. The absolute configurations of these compounds were not determined [153].

2.2.16 Saxifragaceae

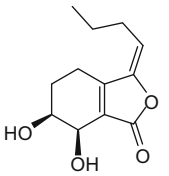
Thunberginol F (**138**) is a phthalide isolated from the methanol extract of "Hydrangeae Dulcis Folium", i.e. the fermented and dried leaves of *Hydrangea macrophylla* (Thunb.) Ser. var. *thunbergii* Makino. The double bond configuration was established by NOE experiments of its trimethyl derivative [154, 155]. From the ethyl acetate-soluble part of the same extract, were found hydramacrophyllols A (**102**) and B (**103**), the former with low optical purity and the last as a racemic mixture, suggesting that **103** is an artifact. The absolute configuration of **102** was not determined [155–157].

2.2.17 Typhaceae

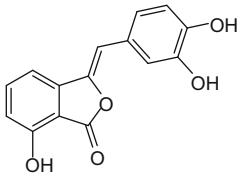
The phytochemical investigation of the rhizomes of *Typha capensis* (Rohrb.) N.E. Br. yielded typhaphthalide (**139**) and radulanolide (**140**) [158].



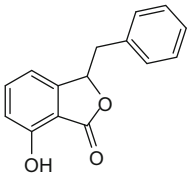
136



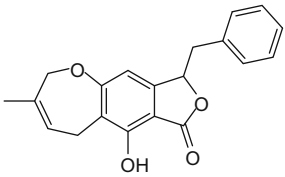
137



138 (thunberginol F)



139 (typhaphthalide)

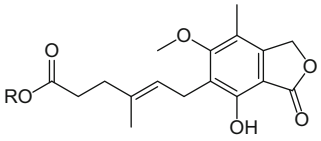


140 (radulanolide)

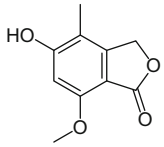
2.3 Phthalides in Fungi

In 1913, Alsberg and Black reported the isolation of an acid of molecular formula $C_{17}H_{20}O_6$, which they called mycophenolic acid (MPA (**141**)), from *Penicillium stonoliferum* Thom [159]. Its structure was not correctly determined until the late 1940s and early 1950s as **141** [160–162]. This phthalide was also found in cultures of fifteen strains of *Penicillium brevicompactum* Dierckx and *Penicillium biourgeianum* K.M. Zalessky [163], as well as *Penicillium brunneostoloniferum* S. Abe [164], *Penicillium echinulatum* Raper & Thom ex Fassat. [165], *Penicillium roqueforti* Thom [166], *Penicillum verrucosum* Dierckx [167], and *Phomopsis longicolla* Hobbs [168]. San Martin and co-workers reported that *P. brevicompactum* produces not only mycophenolic acid, but also its methyl ester **142** [169]. From *Penicillium crustosum* Thom was also isolated 5-hydroxy-7-methoxy-4-methylphthalide (**143**) [170].

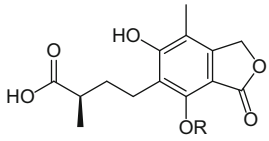
Structurally similar compounds to **141** have been isolated from different sources. Euparvic acid (**144**) and the phthalides **145**–**147** were isolated from *Eupenicillium parvum* Raper et Fennell [171], and compound **147** and penicacids A–C (**148**–**150**) were found to be metabolites from *Penicillium* sp. SOF07 [172]. Phthalides **151** [173], **152**, and **153** [174] were obtained from *Penicillium brevicompactum*, but their configurations were not established. In all these cases, MPA (**141**) was isolated along with the aforementioned compounds.



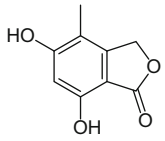
141 (mycophenolic acid) R = H
142 R = Me



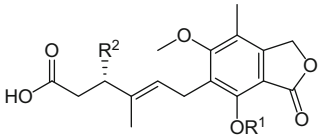
143 (5-hydroxy-7-methoxy-4-methylphthalide)



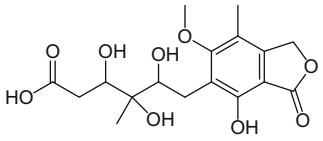
144 (euparvic acid) R = H
145 R = Me



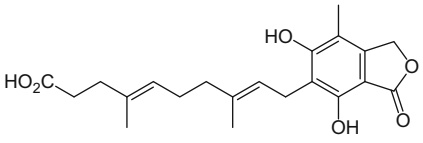
146



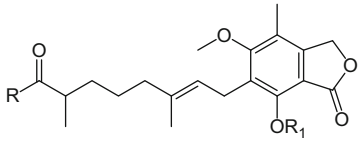
147 R^1 = H; R^2 = OH
148 (penicacid A) R^1 = CH_3; R^2 = OH
149 (penicacid B) R^1 = H; R^2 = OGlc



150 (penicacid C)



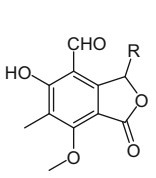
151



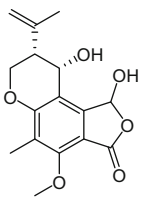
152 R = OH
153 R = Me

Birkingshaw and co-workers [175] isolated cyclopaldic acid (**154**) from cultures of two strains of *Penicillium cyclopium* Westling. This compound has also been found to be a secondary metabolite of *Aspergillus duricaulis* Raper et Fennell [176], *Seiridium cupressi* (Guba) Boessew. [177], *Penicillium commune* Thom, and *Penicillium mononematosum* (Frisvad, Filt. & Wicklow) Frisvad [178]. Compound **154** was found in some *Penicillium* spp. along with the related metabolite deoxycyclopaldic acid (**155**) [179], which was also isolated from *Microsphaeropsis arundinis* PSU-G18 [180]. *Aspergillus duricaulis* also yielded chromanols **156**–**159** as additional terpenoidal phthalides [181].

Two sesquiterpene-cyclopaldic acid hybrid derivatives were found to be metabolites from *Pestalotiopsis* sp., an endophytic fungus isolated from the leaves of the mangrove *Rhizophora mucronata* Lam. These phthalides were named pestaliotiopens A (**160**) and B (**161**), and their configurations were determined through spectroscopic methods and theoretical calculations. The sesquiterpene moiety is derived from altiloxin B, which preserves its absolute configuration in the hybrid compounds. The authors suggested that the formation of each individual scaffold (mycophenolic acid and altiloxin B) occurs previously and then both moieties join to form these compounds [182].



154 (cyclopaldic acid) R = OH
155 (deoxycyclopaldic acid) R = H



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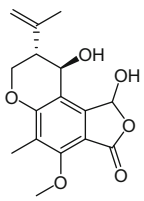

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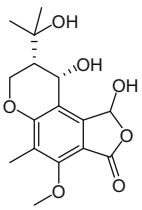

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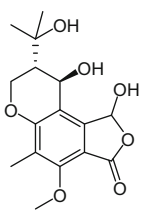

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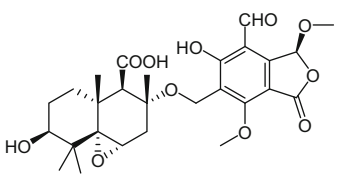
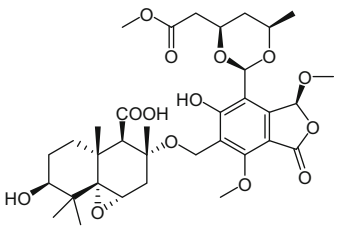


160 (pestalotiopen A)

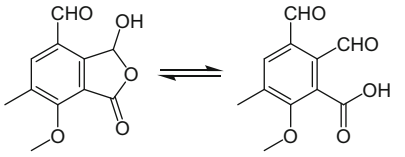


161 (pestalotiopen B)

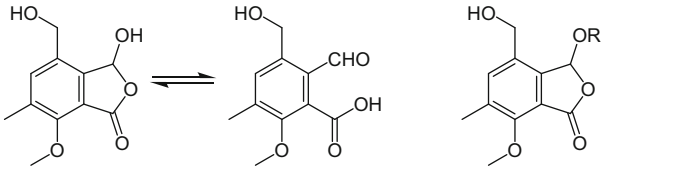
McGowan and coworkers isolated gladiolic acid (**162**) from a culture of *Penicillium gladioli* L. McCullogh & Thom. This compound was found to display antibacterial and fungistatic activities [183]. Grove established its structure, suggesting that should there be a tautomeric equilibrium between the

hydroxylactone **162** and the aldehydic acid **163**, as occurs with mycophenolic acid (**141**) [184, 185].

Other studies have shown that gladiolic acid (**162**) and dihydrogladiolic acid (**164**) (which also exists in an equilibrium with aldehydic acid **165**) are constituents of the culture of *Penicillium gladioli* [186–188]. A modification of the experimental procedure originally employed for the isolation, allowed the characterization of compound **166**, which was considered an artifact [189]. From the endophytic fungal strain *Phomopsis* sp. A123 was isolated dihydrogladiolic acid (**164**) as an optically active compound, along with its 3-ethoxy derivative, **167**, named phomotone [190].



162 (gladiolic acid) **163**



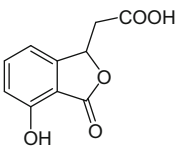
164 (dihydrogladiolic acid) **165**

166 R = Me
167 R = Et (phomotone)

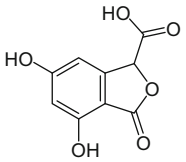
Alternaria kikuchiana S. Tanaka is a well-known parasite, which causes black spot disease in Japanese pears. Chemical investigation of the culture filtrates of the broth yielded *iso*-ochracinic acid (**168**) [191], and this compound has also been characterized from a fungicolous hyphomycete resembling *Cladosporium* [192].

Herbaric acid (**169**), an analog of *iso*-ochracinic acid, is produced by *Cladosporium herbarium* (Pers.) Link, a fungus associated with the Indonesian sponge *Callyspongia aerizusa*. It is interesting to note that other strains of this fungus, isolated from *Aplysina aerophoba*, collected in the Mediterranean Sea, did not produce this phthalide [193]. A closely related phthalide to herbaric acid is acetophthalidin (**170**), which was isolated from the fungal strain BM923 [194].

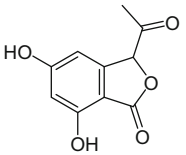
Phthalide **171** and its β-D-glucopyranoside **172** were isolated from a mycophilic *Hansfordia* species, along with other natural products [195].



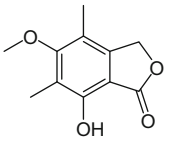
168 (*iso*-ochracinic acid)



169 (herbaric acid)



170 (acetophthalidin)



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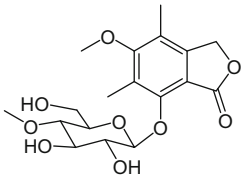
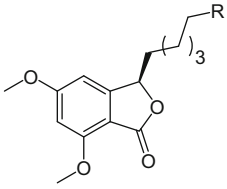
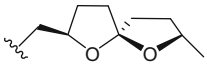


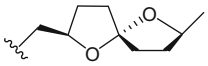
172

Several anti-*Helicobacter pylori* phthalides (**173**–**179**) were isolated from the basidiomycete *Phanerochaete velutina* CL6387, but these phthalides did not display antibacterial activities against other microorganisms against which they were evaluated. The stereochemical assignments of some of these compounds were not completed [196].

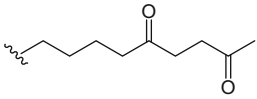




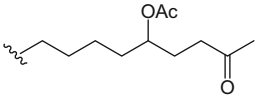
173 (CJ-12,954)



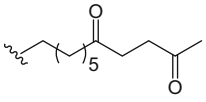
174 (CJ-13,014)



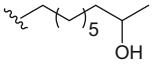
175 (CJ-13,015)



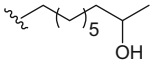
176 (CJ-13,102)



177 (CJ-13,103)



178 (CJ-13,104)

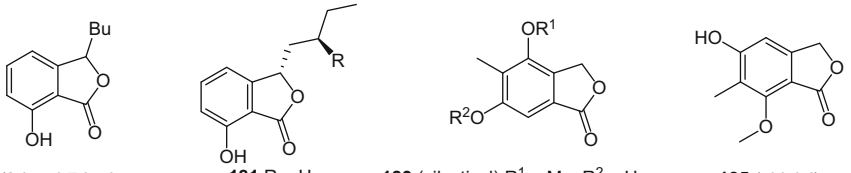


179 (CJ-13,108)

From the culture broth of *Penicillium vulpinum* (Cooke & Massee) Seifert & Samson were isolated several natural products including 3-butyl-7-hydroxyphthalide (**180**), which did not display cytotoxic activity [197].

The phthalide **181**, as well as its derivative **182**, were isolated by Sobolevskaya and co-workers from the mycelial fungus *Penicillium claviforme* Bainter, as found on the surface of the seagrass, *Zostera marina* L. They determined the absolute configuration of **181** by comparison of its specific rotation with previously reported data [198]. The absolute configuration at the carbinolic carbon of **182** was determined through the modified Mosher method as (*R*) (the corrected drawing is depicted in the present contribution since in the original paper the (*S*)-enantiomer appeared).

Chemical analysis of the culture filtrate of *Aspergillus silvaticus* Fennell and Raper IFO8173 yielded silvaticol (**183**), *O*-methylsilvaticol (**184**), and nidulol (**185**) [199].



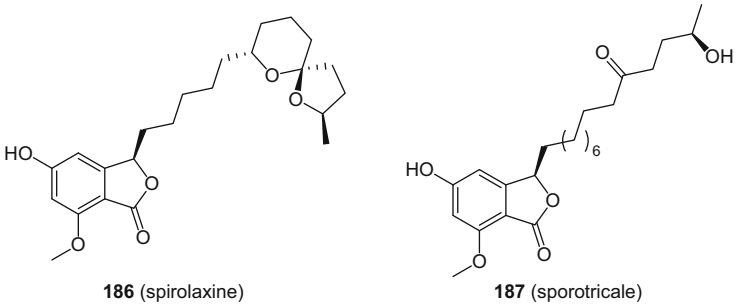
180 (3-butyl-7-hydroxy-phthalide)

181 R = H
182 R = OH

183 (silvaticol) R^1 = Me; R^2 = H
184 (*O*-methylsilvaticol) $R^1 = R^2$ = Me

185 (nidulol)

From *Sporotrichum laxum* CBS 578.63 were isolated two long-chain phthalides named spirolaxine (**186**) and sporotricale (**187**) [200].

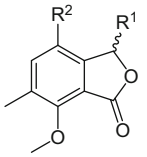


186 (spirolaxine)

187 (sporotricale)

The fungus *Phomopsis convolvulus* Ormeno-Nuñez, Reedeler, & A.K. Waston is a pathogen of the perennial plant *Convolvulus arvensis* L. (Convolvulaceae), and has been studied for the potential biological control of this plant. A chemical investigation of this fungus afforded the phthalides convolvulanic acid A (**188**), convolvulanic acid B (**189**), and convolvulol (**190**) [201].

Compounds **189–191** and xylariphthalide A (**192**) were also isolated from the fungus *Everniastrum cirhatum* (Fr.) Hale ex Sipman (Xylariaceae) [202]. The authors reported that compound **192** displayed a low specific rotation value, presumably due to tautomerism of the hemiacetal group.



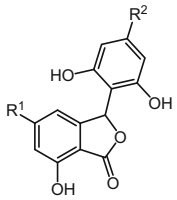
	R^1	R^2
188 (convolvulanic acid A)	OH	COOH
189 (convolvulanic acid B)	H	COOH
190 (convolvulol)	H	CH_2OH
191	H	Me
192 (xylariphthalide A)	OH	CH_2OAc

Isopestacin (**193**) is a 3-phenylsubstituted phthalide found as a racemic mixture in a culture of *Pestalotiopsis microspore* (Speg.) But. & Peres, an endophyte from *Terminalia morobensis* Coode [203]. A similar phthalide is cryphonectric acid (**194**), an optically active abundant metabolite of *Cryphonectria parasitica* (Murrill) M.E. Barr [204].

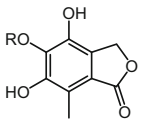
An antioxidant phthalide, 4,5,6-trihydroxy-7-methylphthalide, named epicoccone (**195**), was isolated from the fungus *Epicoccum* sp. [205]. Phthalides **195** and **196** were purified and characterized from a culture of the fungus *Cephalosporium* sp. AL031 [206].

From the antibacterial active culture broth of *Cytospora* sp. and *Diaporthe* sp. collected in Costa Rica, several octaketides were obtained, including the bioactive phthalide cytosporone E (**197**) [207].

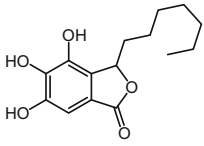
During a screening protocol to discover compounds that bind to the cancer target Akt1, it was found that the fungal culture of *Oidiodendron* sp. displayed activity. From this sample, a new phthalide was isolated and characterized as 3-methyl-4,5,6-trihydroxy-phthalide (**198**) [208].



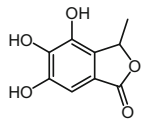
193 (isopestacin) R^1 = Me, R^2 = H
194 (cryphonectric acid) R^1 = OH, R^2 = COOH



195 (epicoccone) R = H
196 R = Me



197 (cytosporone E)



198 (3-methyl-4,5,6-trihydroxyphthalide)

The fungus *Alternaria porri* (Ellis) Cif. is a pathogen of onion, from a culture broth of which 5-(3′,3′-dimethylallyloxy)-7-methoxy-6-methylphthalide (**199**) was characterized [209], along with **200** [210]. Phthalide **199** was also isolated from a liquid culture of endophytic *Pestalotiopsis photiniae* (Thüm) Y.X. Chen, obtained from the plant *Podocarpus macrophyllus* D. Don [211, 212].

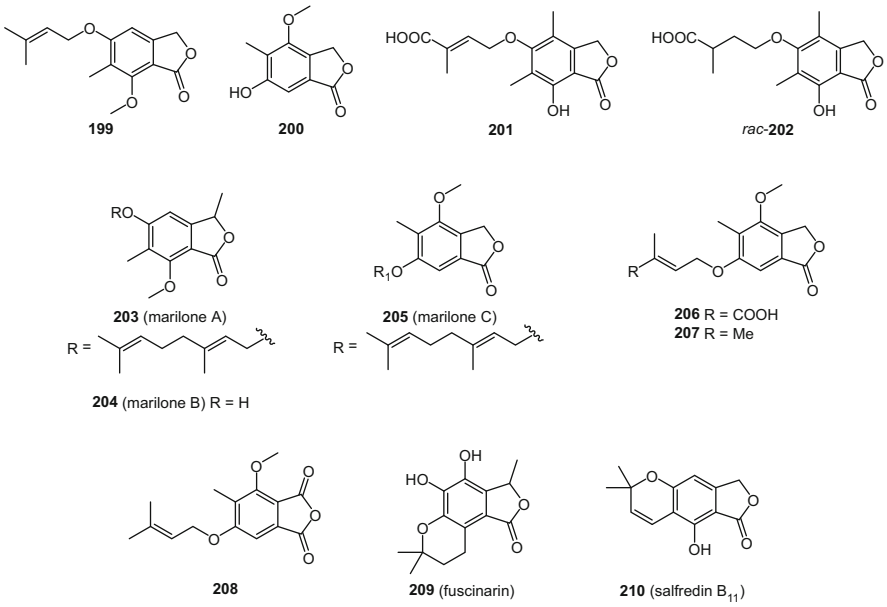
The O-prenylated phthalides **201** and **202** were isolated from an unidentified fungus named "Sterile Dark". Both of these displayed modest antifungal activity against

Cladosporium herbarium, but only phthalide **201** was active against *Gaeumannomyces gramini* var. *tritici* J. Walker, which causes the "take-all" disease in plants [213].

Silvaticol (**185**) and marilones A–C (**203**–**205**) were obtained from the culture medium of the fungus *Stachylidium* sp., which was isolated from the sponge *Callispongia* sp. Compound **203** displayed antiplasmodial activity, and **205** showed antagonistic activity towards the 5-HT_{2B} serotonin receptor [214].

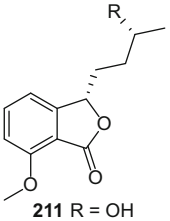
Compounds **199**, **200**, and **206**–**208** were characterized from *Pestalotiopsis photiniae* as antifungal constituents against *Fusarium graminearum*, *Botrytis cinerea* and *Phytophtora nicotianae*, which are considered plant pathogens [211]. Yoganathan and co-workers [215] isolated fuscinarin (**209**) from the soil fungus *Oidiodendron griseum* Robak.

Salfredin B_{11} (**210**) is a prenylated phthalide isolated from *Crucibilum* sp. (strain RF-3817), which displayed aldose reductase inhibitory activity [216].

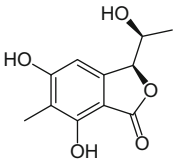


From a marine fungus of the order Pleosporales were isolated (3*S*,3′*R*)-3-(3′-hydroxybutyl)-7-methoxy-phthalide (**211**) and the deoxy derivative **212**. This last compound displayed weak cytotoxic activity against selected cancer cell lines [217]. The absolute configuration of **211** was determined through the Mosher ester method, and the absolute configuration of **212** was determined by comparison of the specific rotations of both these compounds.

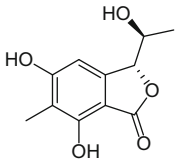
The organic extract of the fermentation culture of the endophytic fungus *Pestalotiopsis foedan* exhibited activity against *Candida albicans*, *Geotrichum candidum*, and *Aspergillus fumigatus*. From this extract were isolated pestaphthalides A (**213**) and B (**214**), and compounds **215**–**217**. Phthalides **213** and **214** exhibited modest activity toward the above-mentioned fungi [218].



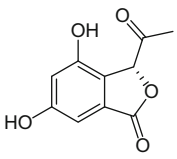
211 R = OH
212 R = H



213 (pestaphthalide A)



214 (pestaphthalide B)



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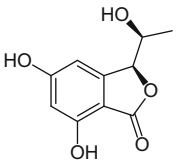

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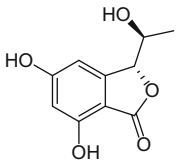
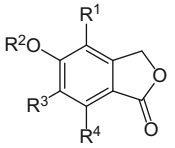


217

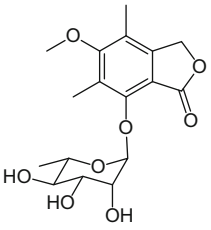
From the edible and cultivable mushroom *Sparassis crispa* (Japanese common name: "Hanabiratake"), were purified the phthalides **218**–**223**, in addition to other constituents [219]. Compounds **218**–**220** were named hanabiratakelides A–C, respectively [219]. Phthalides **221**–**223** were previously found from other sources [131, 220]. These compounds displayed discernible antioxidant, antiinflammatory, and cytotoxic activities.

The fungus *Pestalotiopsis heterocornis* (Guba) Y.X. Chen was isolated from the stems of *Bruguiera gymnorhiza* (L.) Lam. (Rhizophoraceae), and phthalides **171**, **224**, and **227** were isolated a fermentation broth [221].

Several radical scavenging and cytotoxic isocoumarins along with the antioxidant phthalide **226** were isolated from the endophytic fungus *Colletotrichum* sp. [222].



	R^1	R^2	R^3	R^4
218 (hanabiratakelide A)	OH	H	OMe	H
219 (hanabiratakelide B)	H	H	OMe	OH
220 (hanabiratakelide C)	OH	H	OH	OMe
221	H	Me	H	OMe
222	H	Me	OH	OMe
223	OH	Me	H	OMe



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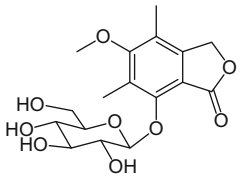

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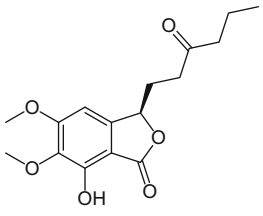
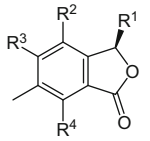
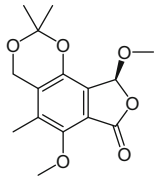


226 (colletotrialide)

Microsphaeropsis arundinis PSU-G18 is a source of a wide range of phthalides. From its broth and mycelial ethyl acetate extract were characterized deoxycyclopaldic acid (**155**), microsphaerophthalides A–G (**227**–**233**), and another four highly substituted phthalides **234**–**237**. Microsphaerophthalides C–G (**229**–**233**) belong to the less common 3-oxygenated phthalides. The absolute configurations of these compounds were determined by comparison of their specific rotations [180].



	R^1	R^2	R^3	R^4
227 (microsphaerophthalide A)	H	CH_2OEt	OH	OMe
228 (microsphaerophthalide B)	H	CHO	OMe	OH
229 (microsphaerophthalide C)	OEt	CHO	OMe	OH
230 (microsphaerophthalide D)	OEt	CH_2OMe	OH	OMe
231 (microsphaerophthalide E)	OEt	CH_2OEt	OH	OMe
232 (microsphaerophthalide F)	OMe	OMe	OH	OMe
234	H	Me	OH	OH
235	H	CH_2OMe	OH	OMe
236	H	Me	OH	OMe
237	Me	Me	Me	OMe



233 (microsphaerophthalide G)

A crude extract obtained from the culture broth of the fungus *Acremonium* sp., an endophyte from the mangrove plant *Rhizophora apiculata* Blume (Rhizophoraceae), displayed antibiotic activity towards *Candida albicans* and *Cryptococcus neoformans*. Several isocoumarin derivatives and a phthalide named acremonide (**238**) were obtained from this endophytic fungus, and these compounds displayed activity toward both microorganisms [223].

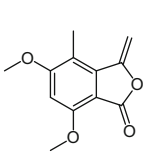
The fungus *Bipolaris* sp. was isolated from the seagrass *Halophila ovalis* (R. Br.) Hook. f., and from this fungus were purified and characterized several chromanones, anthraquinones, and phenolic compounds, including the phthalide bipolaride (**239**) [224].

The absolute configuration of sporotricale (**187**) was determined using the Mosher ester method, and 6-hydroxysporotricale (**240**) was characterized from *Sporotrichum laxum* (syn: *Phanerochaete pruinosum*) CBS 578.63 [225]. This fungus was recently reinvestigated and the anti-*Helicobacter pylori* phthalides spirolaxine (**186**) and sporotricale (**187**) were reisolated [226].

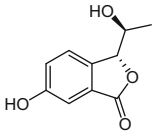
Pseudaboydins A (**241**) and B (**242**) were obtained from the fungus *Pseudallescheria boydii* associated with the starfish *Acanthaster planci*. The

configuration of both phthalides was established using their CD spectra, using previously developed empirical rules [227]. A *Penicillium* sp. (strain ZH58) was found to produce phthalide **243** [228]. Phthalide **244** was isolated from the fermentation broth of the fungus *Pezicula* sp., occurring in the twigs of *Forsythia viridissima* Lindl. (Oleaceae) [229].

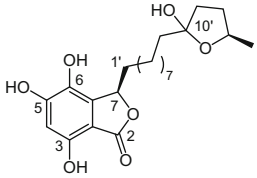
Paecilocin A (**245**) was isolated from *Paecilomyces variotii*, a fungus obtained from the jellyfish *Nepolinema nomurai*. The absolute configuration of paecilocin A (**245**) was assigned by comparison of its specific rotation with that of (3*S*)-butylphthalide (**4**) [230]. 5,7-Dihydroxy-4-methylphthalide (**148**) was characterized from a culture filtrate of *Aspergillus flavus* [231]. Xylaral (**246**) was isolated from *Xylaria polymorphus* (Pers.) Grev., the well-known "dead man's fingers" fungus [232].



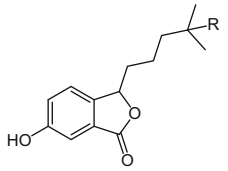
238 (acremonide)



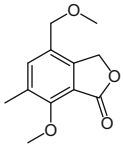
239 (bipolaride)



240 (6-hydroxysporotricale)



241 (pseudaboydin A) R = OH
242 (pseudaboydin B) R = H



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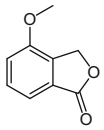

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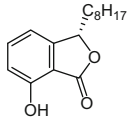
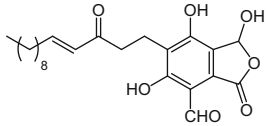


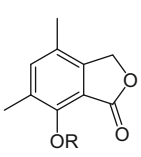
245 (paecilocin A)



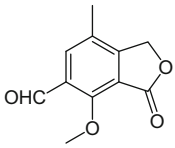
246 (xylaral)

7-Hydroxy-4,6-dimethylphthalide (**247**) was isolated from *Penicillium megasporum* NHL2977 [233]. It was also found in a culture of *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. [234]. Compounds **248**, **249**, and **250** were characterized from *Phomopsis* sp. A123 [235]. Phthalide **250** has been previously isolated from the marine fungus *Diaporthe* sp. [236].

Excelsione, also named phomopsidone (**251**), was almost simultaneously isolated from an unidentified fungus growing in the inner stem of the tree *Knightia excelsa* R. Br. [237], and from *Phomopsis* strain E02091 [238]. Phomopsidone A (**252**), a phthalide that includes an oxetane ring in its structure, was found also in this last-named fungus [235].



247 R = H
248 R = Me



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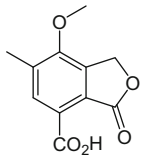

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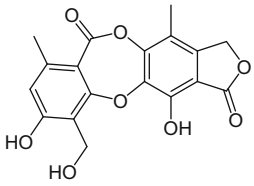
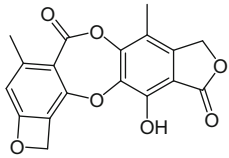


251 (excelsione, phomopsidone)



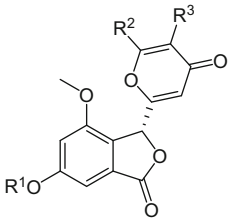
252 (phomopsidone A)

As a result of an investigation of *Penicillium vermiculatum* Dang., a cytotoxic compound was isolated and named vermistatin [239–241]. Its structure was later elucidated as **253**, and its absolute configuration was assigned by analysis of the CD spectrum [241]. Compound **253** has also been found in *Penicillium verruculosum* [242], and *Talaromyces flavus* FKI-0076 and IFM52668 [243, 244]. This compound was named fijiensin when it was isolated from *Mycosphaerella fijiensis* Morelet in 1990 [245].

From *Mycosphaerella fijiensis*, **253** was isolated with its dihydro- (**254**), acetoxydihydro- (**255**), and hydroxydihydro- (**256**) derivatives, as well as penisimplicissin (**257**). The absolute configurations of **254** and **255** were determined through the Mosher ester method [246]. Compounds **253** and **257** were also isolated from a culture of *Talaromyces thailandiasis* T. Douthop, L. Manoch, A. Kijjoa, M. Pinto, L. Gales, A. Damas, A.M.S. Silva, G. Eaton & W. Herz, together with **256** [247], and from *Penicillium rubrum* Stoll together with **254** [248].

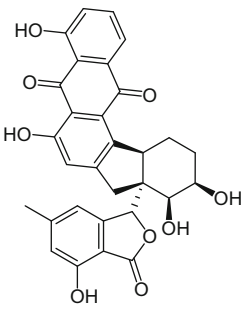
The absolute configuration of **253** was confirmed through X-ray analysis, when it was isolated from the fungus *Guignardia* sp. no. 4382, along with two new derivatives, **258** and **259**, for which the absolute configurations were in turn assigned by comparison of their CD spectra with that of **253**. Compounds **253** and **258** were characterized from the fungus *Eurotium rubrum* [249].

The fungus *Penicillium* sp. HN29-2B1 was found to be a source of several derivatives. From its mycelium and culture medium were characterized **253**, **258**–**259**, 6-demethylvermistatin (**260**), 6-demethylpenicimplissinin (**261**), 5′-hydroxypenisimplicissin (**262**), and 2″-*epi*-hydroxydihydrovermistatin (**263**). The absolute configurations of **260** and **261** were determined by analysis of their CD data, while that of **263** was assigned as (3*R*,2″*S*) by means of single-crystal X-ray diffraction [250]. Phthalide **260** has been previously isolated from *Guignardia* sp. no. 4832 [251]. Compounds **257**, **258**, **260**, and neosarphenol A (**264**) were isolated from an ethanol extract of the culture of *Neosartorya glabra* CGMCC32286 by Liu and co-workers [252].

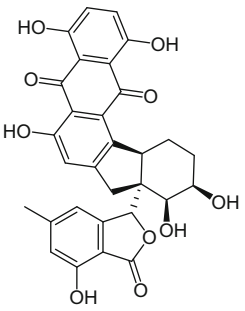


	R^1	R^2	R^3
253 (vermistatin)	Me		H
254 (dihydrovermistatin)	Me		H
255 (acetoxydihydrovermistatin)	Me		H
256 (hydroxydihydrovermistatin)	Me		H
257 (penisimplicissin)	Me	Me	H
258 (methoxyvermistatin)	Me		OMe
259 (hydroxyvermistatin)	Me		OH
260 (6-demethylvermistatin)	H		OH
261 (6-demethylpenisimplicissin)	H	Me	H
262 (5'-hydroxypenisimplicissin)	Me	Me	OH
263 (2"-epihydroxydihydrovermistatin)	Me		H
264 (neosarphenol)	H		OMe

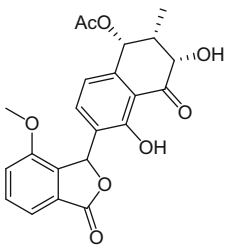
Two anthraquinone phthalides, namely, rubellins C and D (**265** and **266**, respectively), were found in extracts from a strain of *Mycosphaerella rubella* (Niessl & J. Schröt.) Magnus [253]. Rubiginone H (**267**) was isolated from the methanol extract of the mycelium of *Streptomyces* sp. (strain Go N1/5) [254].



265 (rubellin C)



266 (rubellin D)

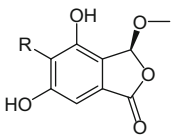


267 (rubiginone H)

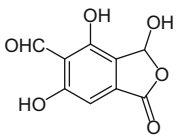
An extract from the culture broth of *Penicillium rubrum* Stoll yielded rubralides A–C (**268**–**270**) [255]. The absolute configurations of **268** and **270** were established by comparison of their CD spectra with that of vermistatin (**253**), while the absolute configuration of **269** was not determined. Compound

269 and talaromycolides A–C (**271**–**273**) were isolated from *Talaromyces pinophilus* AF-02 [256].

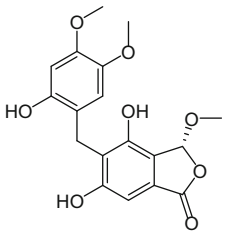
From a methanol extract of the culture of *Penicillium* sp. IFB-E022, an endophytic fungal strain residing in the stems of *Quercus variabilis* Blume (Fagaceae), were isolated penicidones A (**274**) and B (**275**) by Tan and co-workers [257]. The absolute configuration at C-8 for both compounds was established as (8*R*) by comparison of the specific rotation with those of vermistatin (**253**), dihydrovermistatin (**254**), and penisimplicissin (**257**).



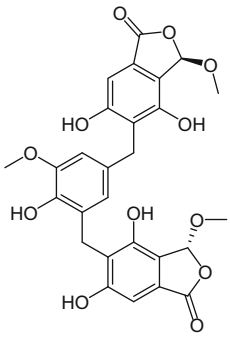
268 (rubralide A) R = Me
269 (rubralide C) R = CHO



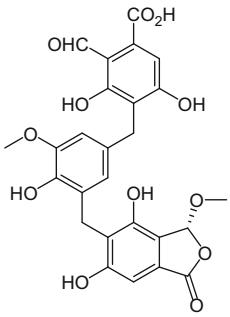
270 (rubralide B)



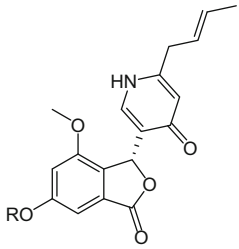
271 (talaromycolide A)



272 (talaromycolide B)

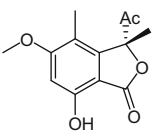


273 (talaromycolide C)



274 (penicidone A) R = Me
275 (penicidone B) R = H

Phthalides bearing two substituents at C-3 are not found frequently as natural products. One example is compound **276**, which was isolated from an ethyl acetate extract of the culture broth of *Halloroselinia oceanica* BCC 5149 [258]. This phthalide was also found in broth cultures of *Leptosphaeria* sp. KTC 727 [259] and *Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White [260]. Hashimoto and coworkers characterized compounds **276** and **277** from *Leptosphaeria* sp. KTC 727 [259]. Another example of this class of phthalides is compound **278**, isolated from an extract of the culture of *Emericella unguis* Malloch & Cain [261]. Corollosporine (**279**) is a compound from *Corollospora maritima* Werderm., which was characterized as a racemic mixture. It displayed antibacterial activity against *S. aureus* and other bacteria [262].



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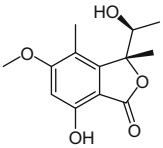

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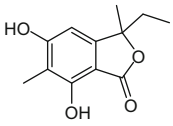
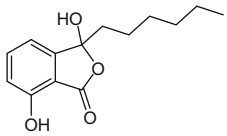


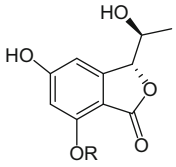
278 (3-ethyl-5,7-dihydroxy-3,6-dimethylphthalide)



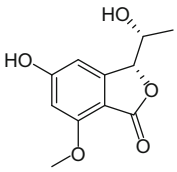
279 (corollosporine)

2.4 Phthalides in Lichens

Takenaka and co-workers isolated 3,5-dihydroxyphthalic acid and the phthalides **280–282** from the polyspore-derived mycobionts of *Graphis proserpens* Vain. [263].



280 R = Me
281 R = H



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2.5 Phthalides in Liverworts

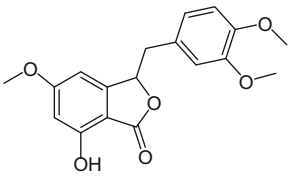
Asakawa and co-workers reported that radulanolide (**140**) was isolated from an organic extract from *Radula complanata* (L.) Dumont, a liverwort which causes allergic contact dermatitis [264]. The methanol extract of *Balantiopsis rosea* Berggr. yielded balantiolide (**283**), for which the structure was established by analysis of its spectroscopic data and by the preparation of its acetyl derivative (**284**) [265].

Asakawa's group [266] obtained 3-(4′-methoxy-benzyl)-5,6-dimethoxy-phthalide (**285**) from the ether extract of the liverwort *Frullania falciloba* Taylor ex Lehm. This structure was similar to 3-substituted phthalides previously isolated from *Radula complanata* [264] and *Balantiopsis rosea* [265]. The same group reported the phthalide **286** [267]).

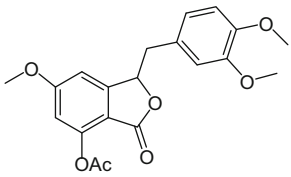
Kraut and co-workers [268] analyzed the constituents of the liverwort *Frullania muscicola* Steph., and from a crude extract was purified the previously isolated balantiolide (**283**) [265] as well as 3-(3′,4′-dimethoxybenzyl)-5,7-dimethoxyphthalide (**287**) and 3-(4′-hydroxy-3′-methoxybenzyl)-5,7-dimethoxyphthalide (**288**). From an organic extract of *Plagiochila killarniensis* Pears., Rycroft and co-workers characterized killarniesolide (**289**). Acetylation of compound **289** afforded **290**, establishing the substitution of the benzylic ring [269].

Chemical investigation of *Plagiochila buchtiniana* Steph. provided 3-(4′-methoxybenzyl)-7-hydroxyphthalide (**291**), whereas work-up of *P. diversifolia* Lindenb. & Gottsche yielded 3-(4′-methoxybenzyl)-7-methoxyphthalide (**292**), 3-(3′,4′-dimethoxybenzyl)-7-methoxyphthalide (**293**), and 3-(3′,4′,5′-trimethoxybenzyl)-7-methoxyphthalide (**294**) [270].

Chemical analysis of the organic extracts of *Frullania falciloba* afforded 3-(4′-methoxybenzyl)-5,7-dimethoxyphthalide (**295**) [271], for which the structure was drawn in an erroneous manner in reference [266].



283 (balantiolide)



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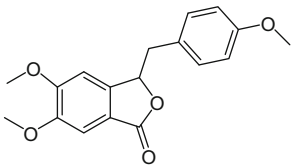

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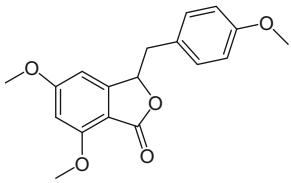

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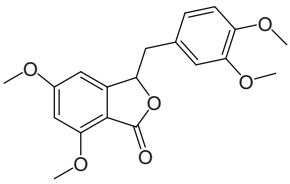

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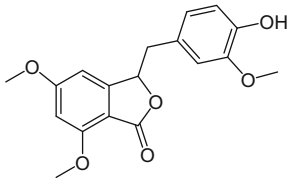

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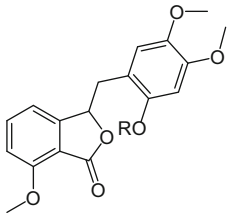
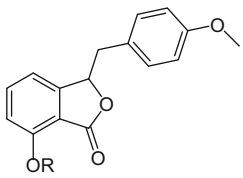
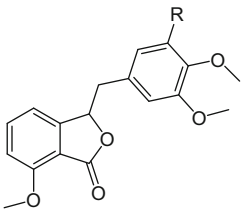


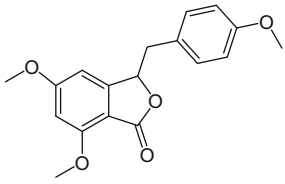
289 R = H (killarniesolide)
290 R = Ac



291 R = H
292 R = Me



293 R = H
294 R = OMe



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3 Analytical Aspects

This section summarizes some methods employed for the extraction, isolation, chemical characterization, dereplication, and to achieve quality control of phthalides.

3.1 Extraction, Isolation, and Chemical Characterization

Historically, the extraction techniques for obtaining phthalides have focused on the use of non-polar solvents such as petroleum ether [127, 140, 272], hexane [36, 78, 142], and pentane [50]. Steam distillation has been employed for the extraction of several phthalides, such as sedanenolide ((**15**) senkyunolide A), (*Z*)- (**8**) and (*E*)-lingustilide (**18**), (*Z*)- (**3**) and (*E*)-butylidenephthalide (**21**), and butylphthalide (**4**) [42, 44, 273–275]. For obtaining polar compounds such as the diols, senkyunolide I (**22**) and senkyunolide H (**26**), in older work the plant rhizomes were defatted with non-polar solvents and then extracted with more polar solvents such as chloroform [65], or with water, followed by partition with an organic solvent [59], or extracted with acetone and methanol [60, 101, 276]. Several conventional procedures such as decoction [277, 278], percolation [279], sonication [279, 280], and reflux [281] have been used. Other techniques employed include supercritical fluid extraction (SFE) [274, 282–284], solid-phase microextraction (SPME) [285], microwave-assisted extraction [113, 286], and the use of biomembranes [287]. Pressurized liquid extraction (PLE) is an option that allows the quantification of phthalides [288–290]. A recently developed high-pressure ultrasonic-assisted extracted technology method has been applied for the purification of this type of phytochemicals [291, 292].

Regarding phthalide isolation, in earlier work, crude organic extracts were subjected to basic aqueous partitioning to remove acid and phenolic compounds [42, 293]. The organic layer obtained was then subjected to distillation for obtaining several fractions, yielding phthalides [19, 293]. A frequently used method for the isolation of phthalides is column chromatography (CC) over adsorbents or solid supports such as silica gel [103, 116], alumina [51], polyamide (CC6) [69], Sephadex LH-20 [66], and reversed-phase (C_{18}) silica gel [153]. Other reported methods are preparative thin-layer chromatography (PTLC) [294], vacuum-liquid chromatography (VLC) [294, 295], medium-pressure liquid chromatography (MPLC) [294], high-vacuum distillation [80, 106], centrifugal circular thin-layer chromatography (CCTLC) [69], high-speed countercurrent chromatography (HSCCC) [106, 296–298], and droplet-countercurrent chromatography (DCCC) [110]. Normal- [299], reversed-phase [110, 295], and high-performance liquid chromatography (HPLC) are common methods used for the isolation of phthalides.

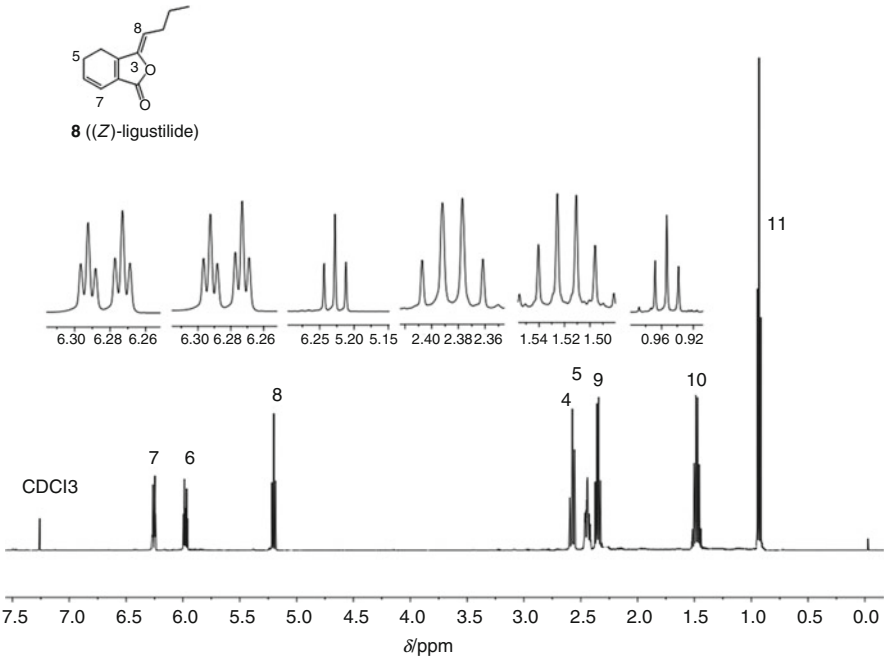


Fig. 2 ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of (*Z*)-ligustilide (**8**)

The chemical characterization of phthalides has involved the determination of melting points [293], boiling points [36, 42], and chemical transformations such as saponification [293], hydrolysis [293], hydrogenation [35], ozonolysis [36], and oxidation [36], among others. Later on, these procedures were complemented with methods including infrared spectrometry [41, 44], ultraviolet spectroscopy [42, 44, 293], refractive indices [19, 293], optical rotations [293], gas chromatography (GC) [42], mass spectrometry (MS) [42], and NMR spectroscopy [36, 41]. Later, GC coupled to selective mass detectors and high resolution mass spectrometry (GC-MS) [44, 48] were included. The use of NMR spectroscopy [41, 51] and X-ray diffraction analysis has increased [51, 103], and a combination of both has been applied [93, 103, 300].

Figures 2, 3, and 4 show the ^{1}H NMR spectra for compounds **8**, **23**, and **43**, which are natural constituents of *Ligusticum porteri* [70].

3.2 Dereplication and Quality Control (HPLC, MS, NMR)

Dereplication is a process that facilitates the determination of the composition of a mixture of substances or of an extract [301]. It is focused on the rapid analysis of

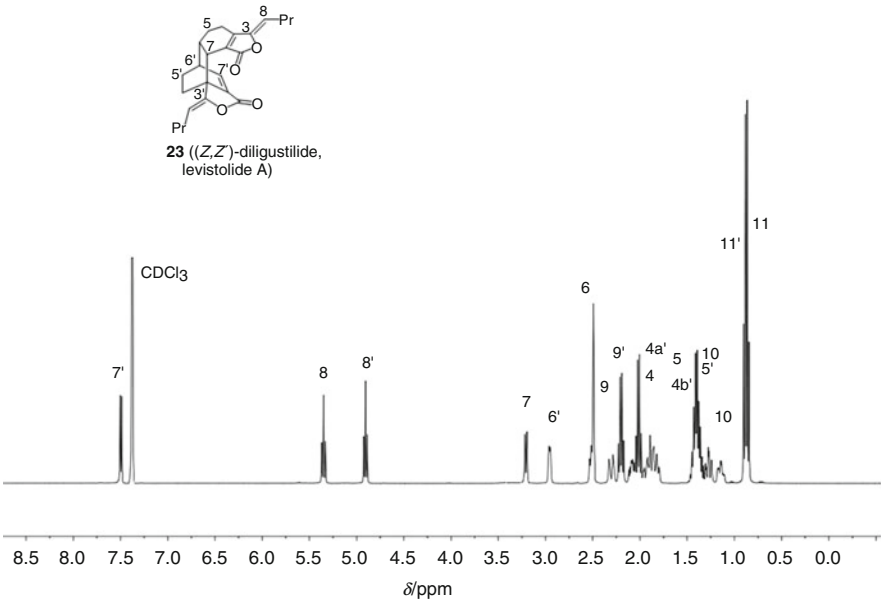


Fig. 3 ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of diligustilide (**23**)

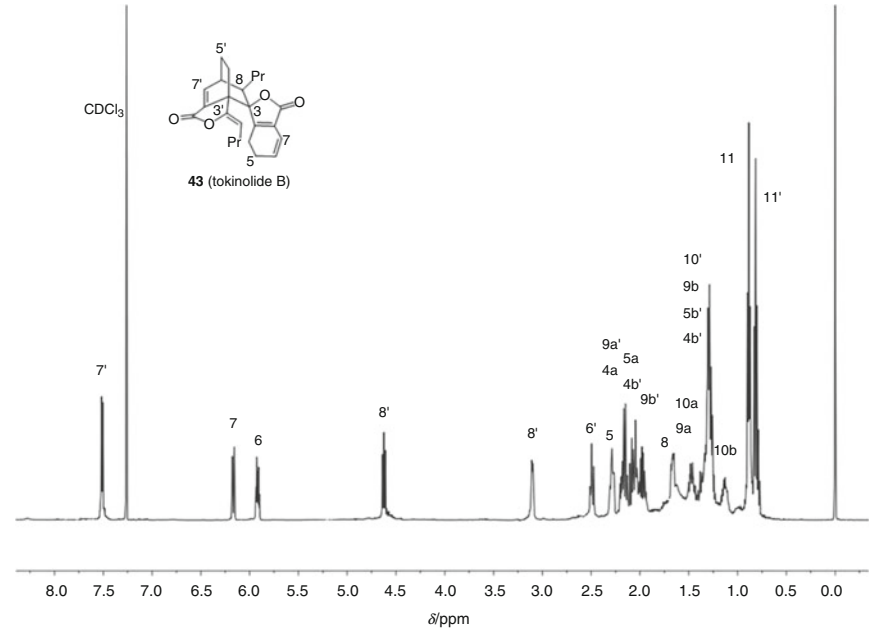


Fig. 4 ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of tokinolide B (**43**)

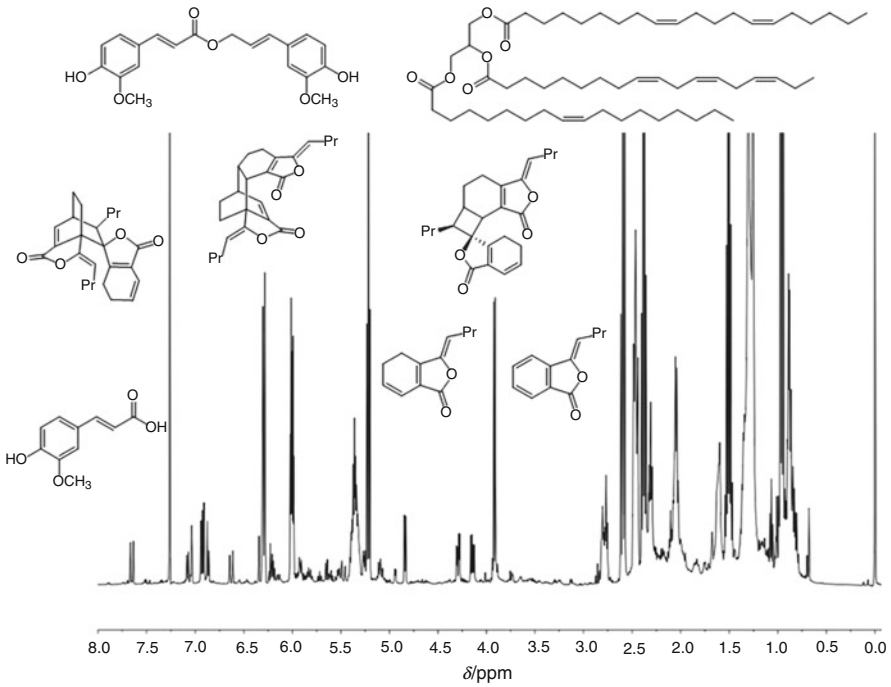


Fig. 5 Analysis of the components of *Ligusticum porteri* acetone extract by ^{1}H NMR spectroscopy (500 MHz, $CDCl_3$) [300]

known components present in crude plant material or medicinal herbal products without the isolation of compounds, and is based on the use of TLC, HPLC, and HPLC-coupled spectroscopic techniques, for instance, LC-MS and LCMS/MS [302, 303], and GC-MS [304]. Access to 1D ^{1}H NMR data at the initial steps of dereplication of crude extracts can accelerate substantially the whole process, e.g. the identification of the constituents in a crude acetone extract from rhizomes of *Ligusticum porteri* [300] (Fig. 5).

Quality control aims to ensure the consistency, efficacy, and safety of preparations from plants used in traditional medicine. A chemical fingerprint indicates the presence of multiple chemical markers within a sample. It has been used for determining the presence of phthalides in several Asian medicinal plants and herbal remedies [277, 305]. Among the phthalides present, (*Z*)-ligustilide (**8**) typically has been selected as a marker compound to perform the quality control of the roots of *Angelica sinensis* or *Ligusticum chuanxiong*, and HPLC and GC-MS are the main analytical methods for its quantification [281, 288, 305–308].

The identification and quantification of two major phthalides from *Ligusticum porteri* were established using a HPLC-diode array (DAD) method for quality

control purposes [309]. The secondary metabolite profiles of plants may be affected by many factors, including seasonal changes, harvesting time, cultivation sites, post-harvesting processing, adulterants or substitutes of raw materials, and procedures of extraction and preparation [60, 310, 311]. A practical tool for determining the variation of the constituents of plants (in the form of crude fresh extracts) is NMR spectroscopy. A qualitative chemical analytical procedure of an acetone extract of the rhizomes of *Ligusticum porteri* using ^{1}H NMR spectroscopy has been reported to establish the presence of the individual components (Fig. 5). This analysis verified that the dimeric phthalides diligustilide (**23**), riligustilide (**24**), and tokinolide B (**43**) occur as natural products in fresh *L. porteri* rhizomes. A protocol involving NMR spectroscopy has been developed for quantifying some of the constituents from this natural source [300].

Qin and co-workers reported the use of NMR spectroscopy to analyze *Ligusticum chuanxiong* rhizomes of several commercial types, collected from different regions in mainland China. The ^{1}H NMR spectra and HPLC profiles allowed comparison of the characteristics of the major constituents [311].

3.3 DOSY Experiments of Extracts of Ligusticum porteri

NMR spectroscopy is a powerful analytical technique for the examination of mixtures of organic compounds, which includes a specific procedure called Pulsed Gradient Spin Echo (PGSE) NMR, or the so-called Diffusion Ordered SpectroscopY (DOSY). This experimental technique is a tool for analyzing complex mixtures based on different translation diffusion coefficients, D, which depend on the molecular weight, size and shape of each compound. DOSY spectra show the diffusion coefficients on the vertical axis and the ^{1}H NMR chemical shifts on the horizontal axis [312, 313].

DOSY analysis [300] allowed the determination of the presence of (*Z*)-butylidenephthalide (**3**), (*Z*)-ligustilide (**8**), tokinolide B (**43**), diligustilide (**23**), ferulic acid (**296**), and coniferyl ferulate (**297**) in an acetone extract of the dried rhizomes of *Ligusticum porteri*. The NMR spectrum revealed four main diffusion rate levels: A, B, C, and D (Figs. 6 and 7). Looking at the δ 7.00–4.3 ppm region, the signals that appeared with a diffusion coefficient of 1.75×10^{-10} m^2/s (highlighted as level A), corresponding to a mixture of ferulic acid (**296**) and coniferyl ferulate (**297**). At levels B and C (diffusion coefficient range $2.20–2.45 \times 10^{-10}$ m^2/s), the most representative signals were found for diligustilide (**23**) (H-7′ at δ 7.50, H-8 at δ 5.35 and H-8′ at δ 4.90 ppm) and tokinolide B (**43**) (H-7′ at δ 7.64 and H-8′ at δ 4.45 ppm). This analysis confirmed the occurrence of dimeric phthalides. The monomer (*Z*)-ligustilide (**8**) displayed a diffusion coefficient of 3.65×10^{-10} m^2/s (level D). DOSY NMR is a useful tool for detection of adulterants in plant extracts,

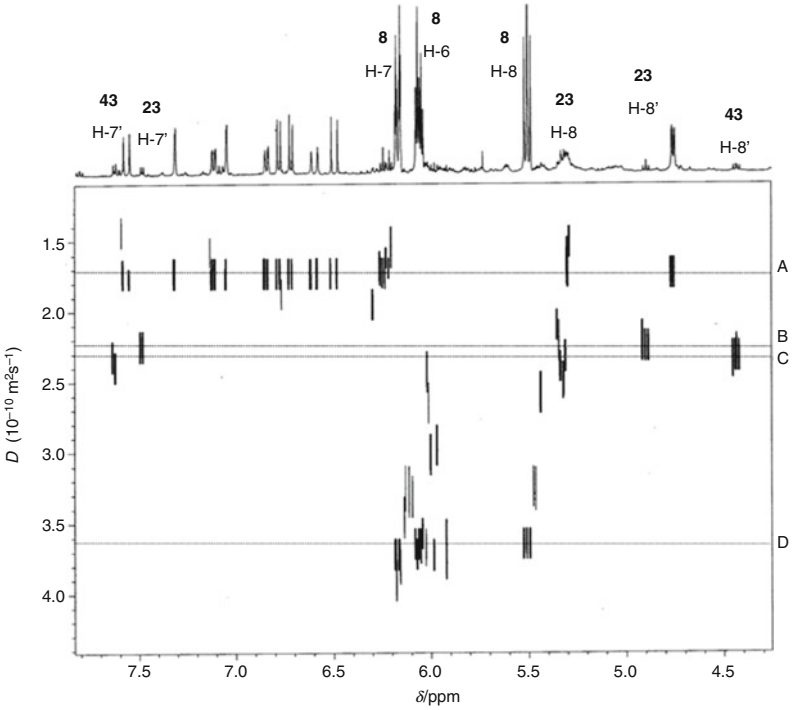


Fig. 6 DOSY spectrum of the acetone extract of *Ligustium porteri*. The ^{1}H NMR spectrum of the acetone extract is shown at the top. The assignments of some signals for (*Z*)-ligustilide (**8**), diligustilide (**23**), and tokinolide B (**43**) are displayed

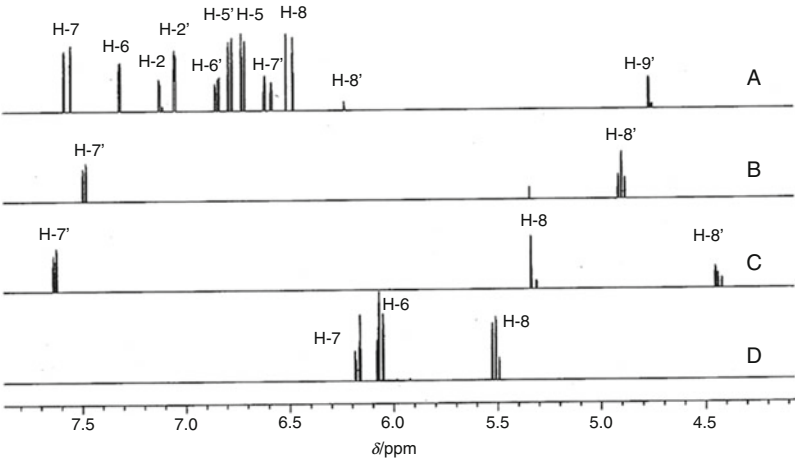


Fig. 7 DOSY slice spectrum with different diffusion coefficients: level A, mixture of ferulic acid (**296**) and coniferyl ferulate (**297**); levels B and C, diligustilide (**23**) and tokinolide B (**43**), respectively, and level D, (*Z*)-ligustilide (**8**)

or for fast and complete analysis of the phytochemical content of extracts and herbal medicines.

4 Biosynthesis of Phthalides

The study of the biosynthesis of phthalides began with the structural determination of mycophenolic acid (**141**), which is constituted by a phthalide fragment (derived from the polyketide pathway) and a terpene fragment (derived from the isoprenoid pathway). Birch and co-workers reported labeling studies with [1-^{14}C], identifying the polyketide and terpenoid pathways [314]. Afterwards, the presence of methoxy and methyl groups in the benzene ring of mycophenolic acid was demonstrated by the same group of investigators, using feeding experiments incorporating [$^{14}CH_3$]-methionine in cultures of *Penicillium brevicompactum* [315].

In 1966, the biosynthesis of phthalides was investigated also by Mitsuhashi and Nomura [272]. They studied the biogenetic origin of butylphthalides by conducting feeding experiments to explain the formation of ligustilide (**8**) in *Levisticum officinale*, and determined that the alkylphthalides have polyketide precursors.

In further work of this type, Canonica and co-workers [316] demonstrated by labeling experiments that the methyl group at C-4 in mycophenolic acid is incorporated at the tetraketide step, and that the formation of the benzene ring was carried out followed by subsequent transformations, yielding 5,7-dihydroxy-4-methylphthalide. Bedford et al. [317] studied the nature of the polyketide intermediates in the biosynthetic pathway from basic units, as acetate and mevalonate. Their study was performed with comparative incorporation experiments using [1′-^{14}C]-orsellinic acid and [1′-^{14}C]-4,6-dihydroxy-2,3-dimethylbenzoic acid, showing that the latter compound is a precursor of mycophenolic acid (**141**). A detailed review including the biosynthesis of mycophenolic acid (**141**) was published by Bentley [318].

The production of MPA (**141**) and analogs has been proposed using metabolic engineering as shown in Chart 1. Regueira et al. carried out experiments on the discovery of the involved enzymes (polyketide synthases, starter unit acyl carrier protein transacylase, β-ketoacylsynthase, acyltransferase, and methyltransferase, as well as the product template and acyl carrier protein responsible for the backbone synthesis of **141**) by means of the production of mpaC (which assembled the phthalide fragment of **141**) in a "gene cluster" in *Penicillium brevicompactum* [319].

Recently, Su and co-workers reported that phthalides could be biosynthesized through the acetate-malonate pathway. (*Z*)-Ligustilide (**8**), sedanolide (**6**), and some other derivatives are the result of reductions, oxidations, decarboxylation, cyclization, and dehydration [116].

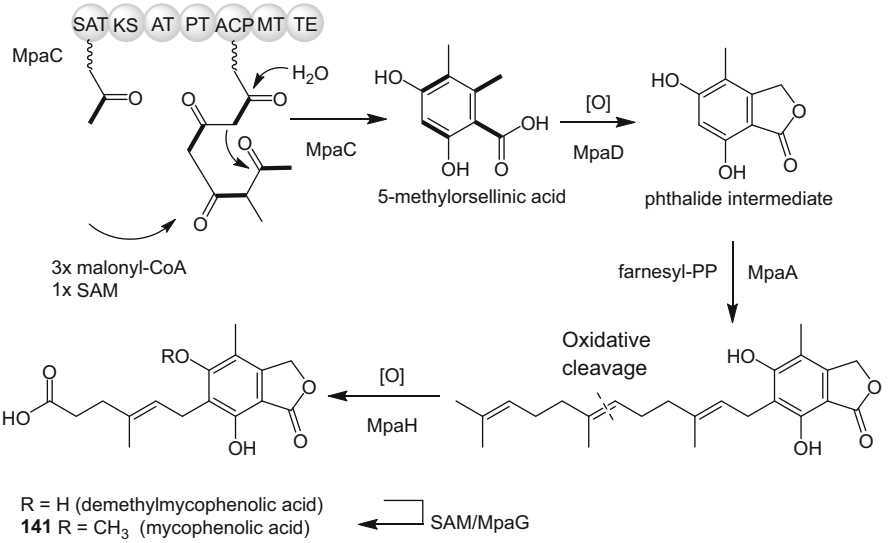


Chart 1 Biosynthesis route for mycophenolic acid ((**141**) MPA) (adapted from [319])

5 Reactions of Phthalides

Phthalides have been studied widely by some investigators, in attempts to understand the reactivity of this class of natural products, as well as aiming to establish structure-activity relationships (SAR) of biologically active natural phthalides, or determining their structures.

5.1 Derivatives of Monomeric Phthalides

5.1.1 Diels–Alder Adducts from (*Z*)-Ligustilide

One remarkable feature of the apparently simple structure of (*Z*)-ligustilide (**8**) is the conjugated cyclohexadiene moiety, which makes it able to undergo Diels–Alder reactions, both as diene and dienophile. Several natural dimeric phthalides, such as diligustilide (**23**) and tokinolide B (**43**), are Diels–Alder adducts of (*Z*)-ligustilide (**8**), and have been partially synthesized from this compound [320, 321] (see Sect. 6.2.1).

Some semisynthetic derivatives have been prepared from (*Z*)-ligustilide (**8**) and several dienophiles through Diels–Alder reactions. Thus, in the early 1960s, Mitsuhashi and co-workers [35] carried out the reaction of this phthalide with maleic anhydride, obtaining both *endo*-**298** and *exo*-**298** isomers. A 3:1 ratio for the products was reported more recently (see Fig. 8) [322]. The reaction with ethyl acrylate afforded *exo*- and *endo*-**299**, with this last compound being the major product. Theoretical calculations agreed with the experimental results, since the transition state involved in the formation of the major isomer was lower in energy.

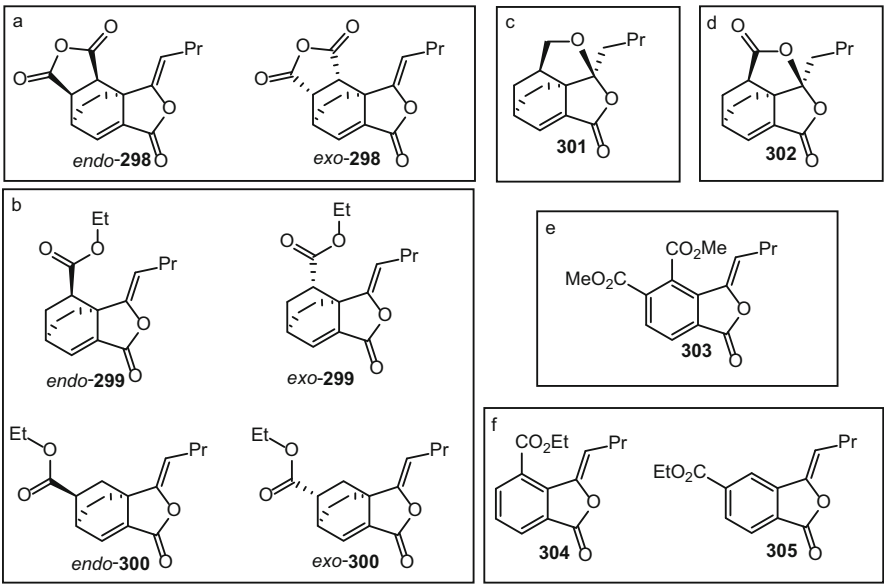
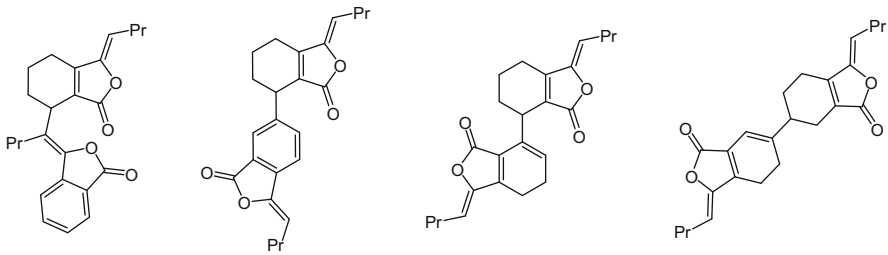


Fig. 8 Diels–Alder adducts of (*Z*)-ligustilide (**8**) with: (**a**) maleic anhydride; (**b**) ethyl acrylate, (**c**) acrylic acid, and (**d**) allyl alcohol. Alder–Rickert reaction products of (*Z*)-ligustilide (**8**) with (**e**) DMAD and (**f**) ethyl propiolate

When (*Z*)-ligustilide (**8**) was reacted with allyl alcohol in the presence of *p*-TsOH, or with acrylic acid **301** and **302** were obtained. The regio- and stereoselectivity of both reactions is noteworthy, since only one product was observed in each case. In the same study, Alder–Rickert reactions of (*Z*)-ligustilide (**8**) with ethyl propiolate or dimethyl acetylenedicarboxylate (DMAD) were carried out, yielding butylidenephthalide-type derivatives **303**–**305** [322].

5.1.2 Preparation of Linear Dimers from (*Z*)-Ligustilide

In an attempt to explore the [π4s + π2s] cycloadditions of (*Z*)-ligustilide (**8**) catalyzed by Lewis acids, the formation of the linear dimers **306**–**309** was reported, rather than of Diels–Alder adducts [323].



306 **307** **308** **309**

The authors suggested that complexation of Lewis acids with carbonyl oxygen or olefinic carbons, promoted cationic mechanisms. Thus, as depicted in Chart 2, it was proposed that the formation of the major product proceeded by a nucleophilic attack from C-6–C-7 double bond electrons towards C-8, in a 1,6-addition, facilitated by the complexation of Lewis acid with oxygen. Subsequent isomerizations through proton transfer reactions led to a cyclohexadiene that was dehydrogenated to yield the observed product **306** [323] (Chart 2).

Similarly, the presence of Lewis acid promoted 1,2 addition of one olefin moiety of (*Z*)-ligustilide (**8**) to the C-6–C-7 double bond of another (*Z*)-ligustilide (**8**) molecule through other carbocations (Chart 3). It is interesting to note that the second major product corresponds to the formation of an allyl cation at C-7, which is more stable than that formed when the cation is formed at C-6 [323].

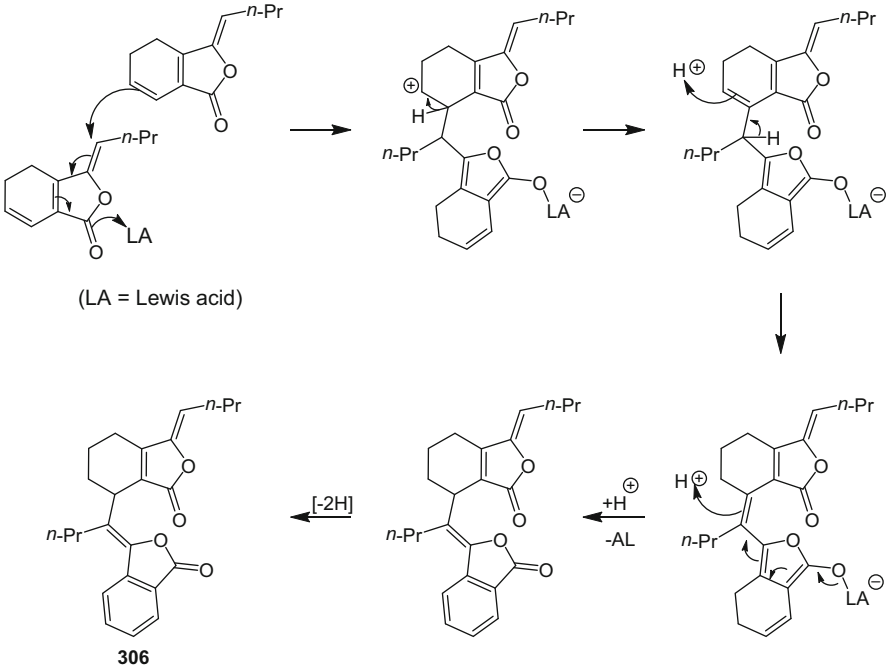


Chart 2 Formation of the major linear dimer **306**

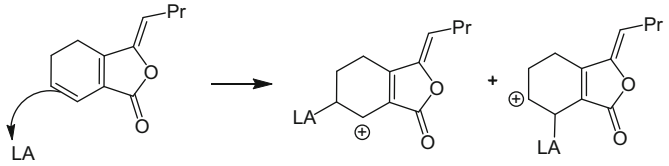
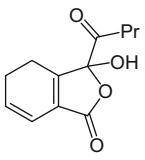


Chart 3 Other carbocations in linear dimer formation

Then, nucleophilic attack of one molecule of **8** to one of the cationic intermediates produces the carbon–carbon bonds necessary to yield dimers **307–309**, for which the formation takes place after acid–base equilibration steps, and dehydrogenation (in the case of **307**) [323].

5.1.3 Instability of (*Z*)-Ligustilide

Pauli and co-workers evaluated the purity and relative stability of isolates of (*Z*)-ligustilide (**8**) through quantitative NMR spectroscopy and GC-MS, and found that this compound decomposed rapidly when stored in $CDCl_3$ solution, or without solvent, even at −30°C. It was observed that the degradation process was slower when (*Z*)-ligustilide (**8**) was stored in hexane, methanol, DMSO, or in a mixture of hexane, ethyl acetate, methanol, and water (9:1:9:1). The degradation pathway was characterized by combining NMR and GC-MS techniques, leading to the determination of an epoxide, 4,5-dihydro-3-hydroxy-8-oxo-butylphthalide (**310**), butyraldehyde, and phthalic anhydride as degradation products [324].



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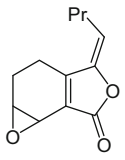

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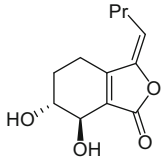

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Lin and co-workers detected that (*Z*)-ligustilide (**8**) spontaneously produced minor amounts of the dimeric phthalides diligustilide (levistolide A, **23**), riligustilide (**24**), and a mixture of *cis*- and *trans*-ligustidiol (**22** and **26**), suggesting that these phthalides could be artifacts [310]. However, various attempts to transform (*Z*)-ligustilide (**8**) into its Diels–Alder adducts on a preparative scale, did not proceed in good yields [35, 320, 321]. In addition, dimeric phthalides have been found in freshly prepared extracts of *L. porteri* [300], confirming their existence as natural products.

Hu and co-workers established that decomposition of (*Z*)-ligustilide (**8**) is influenced by temperature, light, and oxygen, and that the addition of vitamin C delays its transformation [325].

Additional evidence of the facile transformation of (*Z*)-ligustilide (**8**) were provided by Lau and co-workers. They analyzed the chemical composition of crude extracts of *Angelica sinensis* roots and *Ligusticum chuangxiong* rhizomes by gas chromatography-triple quadrupole mass spectrometry, and comparison of the extracts of the same plants before and after treatment with wine. (*S*)-Butylphthalide (**4**), (*Z*)-butylidenephthalide (**3**), senkyunolide A (**15**),

(*Z*)-ligustilide (**8**), and ferulic acid (**296**) were used as chemical markers. It was concluded that there were variations of the relative content of these compounds after wine treatment, indicating that the stability of phthalides depends on the presence of other compounds [326].

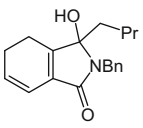
More recently, it was observed that (*Z*)-ligustilide (**8**), when exposed to sunlight at room temperature, was transformed into (*Z*)-6,7-epoxyligustilide (**38**), senkyunolide I (**22**), senkyunolide H (**26**), **311**, and **312**, as racemic mixtures, confirming the main degradation products of (*Z*)-ligustilide (**8**) [327].

5.1.4 Functional Group Transformations

Many reactions of phthalides have been carried out to determine the reactivity of this group of compounds, to establish structure–activity relationships, or as a tool for their structure elucidation.

Mitsuhashi and Kobayashi reported the epoxidation of (*Z*)-ligustilide (**8**) followed by hydrolysis, yielding senkyunolides H (**26**) and G (**22**), while senkyunolide A (**15**) gave senkyunolide J (**33**) [328]. When the hydrolysis of epoxyligustilide was conducted with hydrochloric acid, senkyunolide L (**45**), a chlorohydrin, was formed [73]. The same group also obtained reduced derivatives of ligustilide (**8**) [35], and, in an attempt to prepare the Diels–Alder adducts (tokinolide B (**43**) or diligustilide (**23**)), they subjected (*Z*)-ligustilide (**8**) to pyrolysis. The dimers were not observed, but instead small amounts of a dialdehyde, a product of oxidation of the C-6–C-7 double bond, was observed [72].

Beck and Stermitz submitted (*Z*)-ligustilide (**8**) to nitrogen and sulfur nucleophiles, obtaining a 1,2-addition product from the former nucleophile (**313**). It was found that the sulfur nucleophile gave a 1,6-addition to the α,β,γ,δ–unsaturated carbonyl fragment (**314**), and another addition–elimination product (**315**), and a disubstitution product (**316**). The results were in agreement with hard and soft acid and base theory [89].



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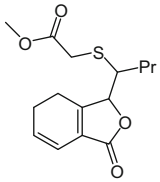

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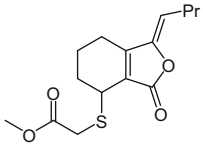

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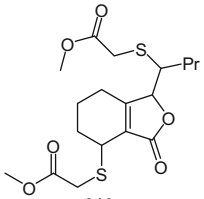

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Cyclopaldic acid (**154**) exhibited insect-biting deterrent and larvicidal activities. Thus, in order to establish a structure–activity relationship (SAR) profile, Cimmino and co-workers [329] synthesized isocyclopaldic acid (**317**) and prepared other

cyclopaldic acid derivatives: this compound was mono- and tetraacetylated to afford **318** and **319**. The aldehyde reacted with 2,4-dinitrophenylhydrazine to give the corresponding hydrazone (**320**). Treatment of cyclopaldic acid with dansyl hydrazine yielded products **321** and **322**. The natural phthalide was also treated with 5-azidopentanoic acid and *N*,*N*′-dicyclohexylcarbodiimide, giving **323**. Finally, when the natural phthalide was treated with $NaBH_4$, the products **324** and **325** were obtained (see Chart 4) [329].

Wu and co-workers [330] prepared derivatives of mycophenolic acid (**141**). Its protected derivative was subjected to aminolysis, yielding the amidophenol **326**. The phenolic group was then transformed to thioacetate **327**, azide **328** and mesylate **329**. Furthermore, the mesyl derivative was used for the preparation of three new heterocyclic compounds, the corresponding 2,3-dihydroisoindolone (**330**), 2,3-dihydro-*N*-methylisoindolone (**331**), and benzothiophenone (**332**).

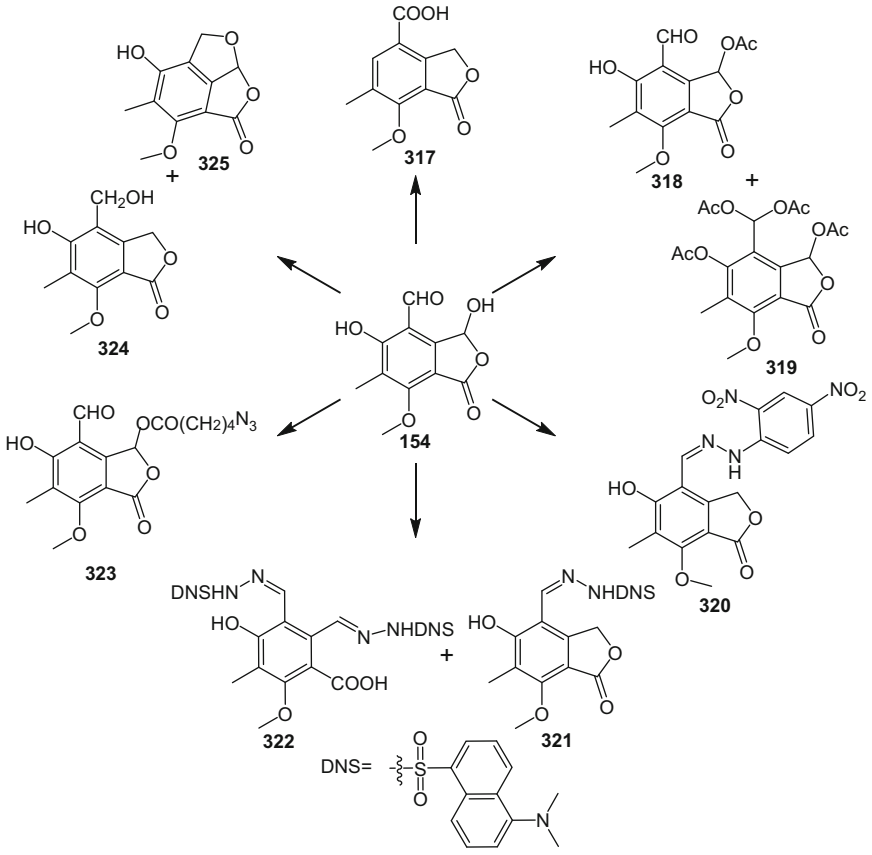
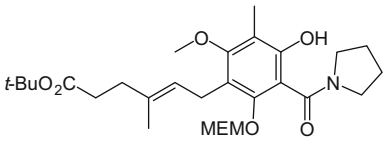


Chart 4 Cyclopaldic acid derivatives



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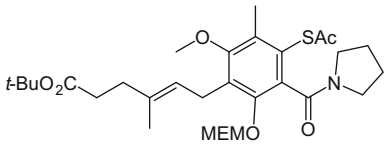

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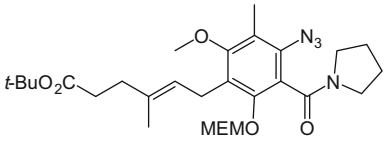

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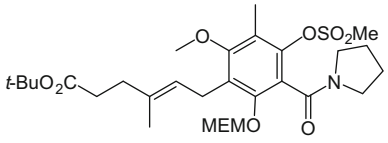

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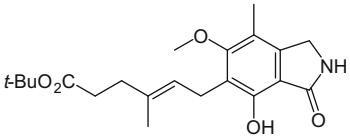

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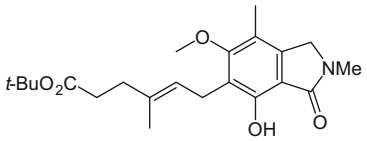

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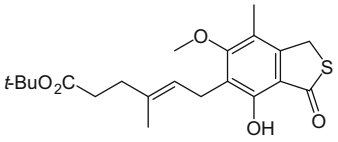

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5.2 *Derivatives of Dimeric Phthalides*

The natural dimeric phthalides are obtained basically as [π4s + π2s] and [π2s + π2s] cycloadducts from two units of monomeric phthalides such as (*Z*)-ligustilide (**8**) and from (*Z*)-butylidenephthalide (**3**). They display interesting reactivities due to their topological characteristics and the presence of several reactive sites.

One of the first reports concerning the reactivity of dimers led to the correction of a structure obtained from *Ligusticum wallichii* by means of the catalytic hydrogenation of diligustilide (**23**), which yielded a mixture of 3,8,7′,7a′-tetrahydrodiligustilide (**333**) and (*Z*′)-3,8-dihydro-[6.6′,7.3a′]-diligustilide (**39**). This last compound had been previously reported as a natural compound, but spectroscopic data analysis permitted a structural correction to **40** (Chart 5) [70].

5.2.1 Intramolecular Condensations of Dimeric Phthalides

Alkaline treatment of diligustilide (**23**) under different conditions yielded the intramolecular condensation products **339**, **340** and **343**. The mechanism was

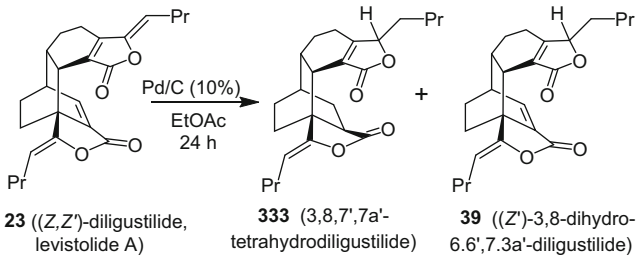


Chart 5 Hydrogenation of diligustilide (**23**)

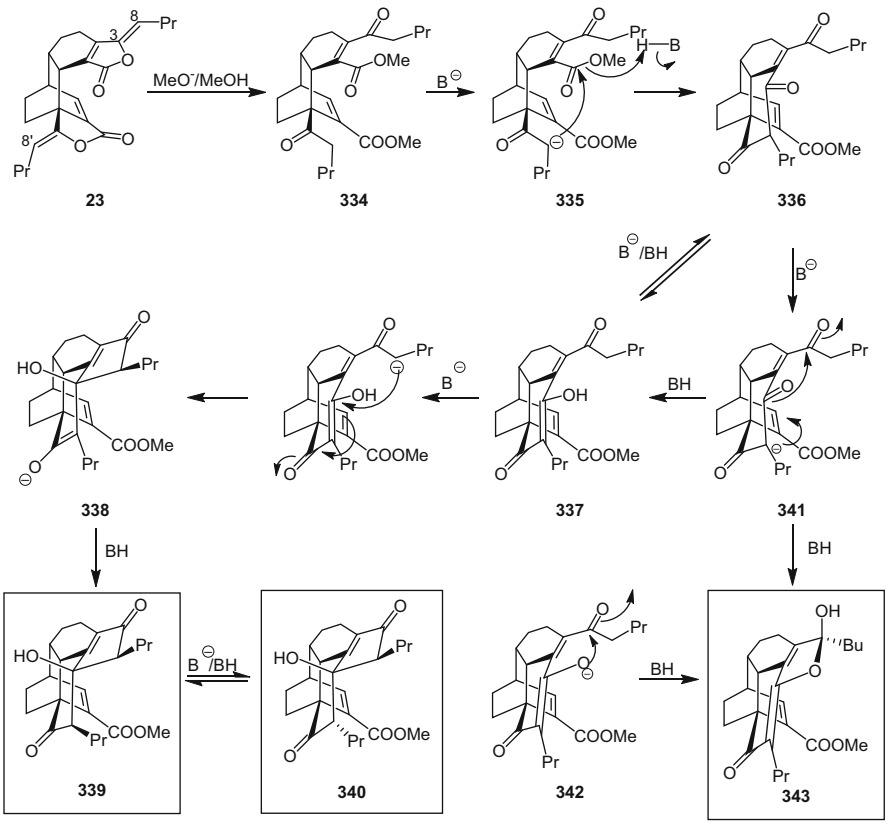


Chart 6 Base-catalyzed intramolecular condensation of diligustilide (**23**)

proposed as follows: the diketo diester **334** (obtained from the methanolysis of diligustilide (**23**)) underwent intramolecular reaction through deprotonation of the methylene at C-8′ (intermediate **335**), and subsequent addition to the carbonyl group-generated intermediates **336** and **337**. The carbanion of this last compound reacted intramolecularly to yield intermediate **338**, which equilibrated yielding **339** and **340** (Chart 6). O-Alkylation of tautomers **341** and **342** afforded **343** [331].

Treatment of diligustilide (**23**) with Na_2CO_3 in $Me_2CO/MeOH/H_2O$ afforded **340**, **339**, **343**, **344** (demethylwallichilide), and **345** (Chart 7).

Attempts to find better conditions to obtain products **339** and **343** and the hydrolysis products **344** and **345** from diligustilide (**23**) were made [332].

Treatment of tokinolide B (**43**) under basic conditions (NaOH in THF) yielded cyclotokinolide B (**346**) derived from an intramolecular condensation procedure. Its formation began with a chemoselective nucleophilic attack of the hydroxide ion to the carbonyl group at C-1, to produce an enolate (intermediate **A**), followed by Michael addition of the carbanion to the enone, by means of 5-*exo*-trigonal cyclization, yielding intermediate **B**, which produced cyclotokinolide B (**346**) (Chart 8). The results showed that intramolecular cyclizations are a general feature for these dimeric phthalides [333].

Treatment of tokinolide B (**43**) with base in acetone under reflux afforded ketoacid **347** by chemoselective lactone ring opening.

The reaction of ketoacid **347** with the chiral amines ((–)-(*S*)-α-methylbenzylamine and (+)-(*R*)-α-methylbenzylamine) under pressure afforded product **87**, tokinolide B (**43**), and the starting material (Chart 9).

The ketoacid of tokinolide B (**347**) displayed chemoselectivity under basic conditions. Strong alkaline conditions afforded **346** via C-alkylation, while mild alkaline conditions produced compound **87** (via O-alkylation) [118] (Chart 10).

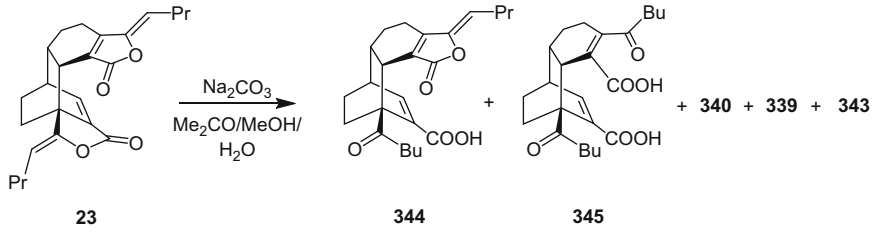


Chart 7 Products derived from basic hydrolysis of diligustilide (**23**)

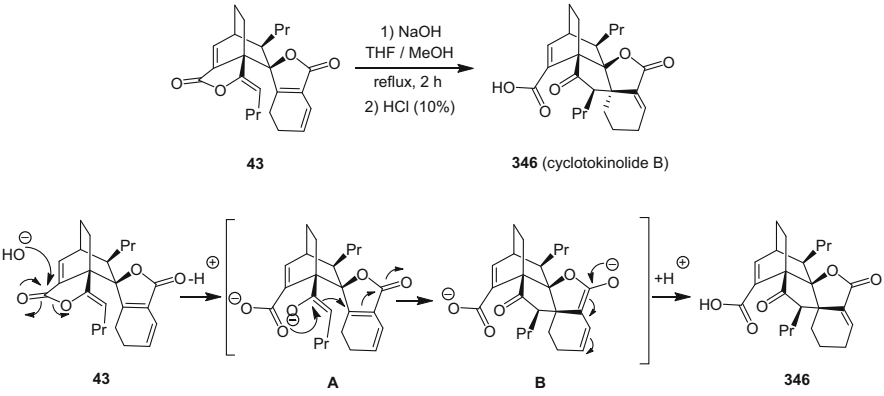


Chart 8 Formation of cyclotokinolide B (**346**)

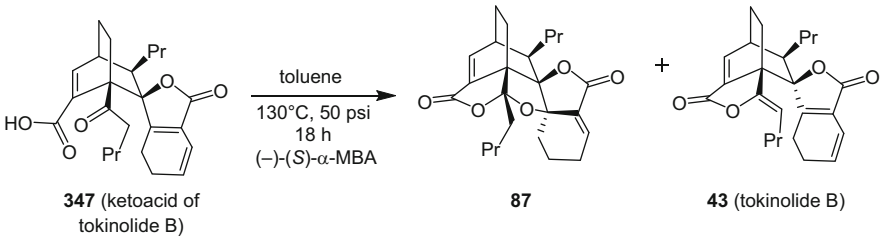


Chart 9 Derivatives obtained from the ketoacid of tokinolide B (**347**)

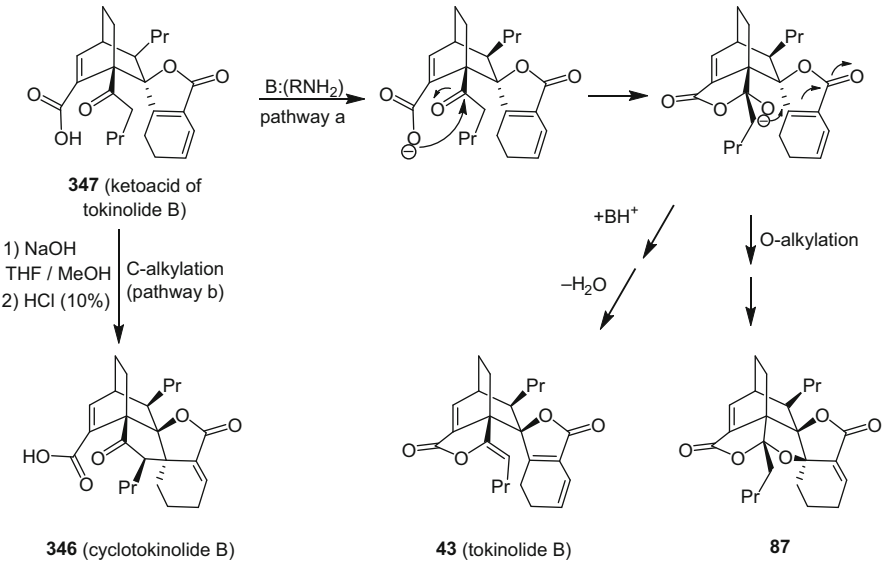


Chart 10 Proposed mechanism for the formation of **87** and **347**

This last compound was later characterized as a natural product from *Ligusticum sinense* cv. *chaxiong* and named chaxiongnolide B (**87**) [117] (see Chart 10).

Comparison of calculated energies for compounds **87**, **346**, and **347** indicated that **87** had a lower energy, followed by **346**, and this outcome may be correlated with the number of rings and conformational constraints of the structures (Fig. 9) [118].

The results on derivatives of intramolecular condensation provided evidence of the particular chemical reactivity of the natural dimeric phthalides.

5.2.2 Synthesis and Stereochemical Assignments of Enantiopure Derivatives

Taking in consideration that natural dimeric phthalides are found as racemic mixtures [70], enantiomeric derivatives of tokinolide B (**43**) and diligustilide

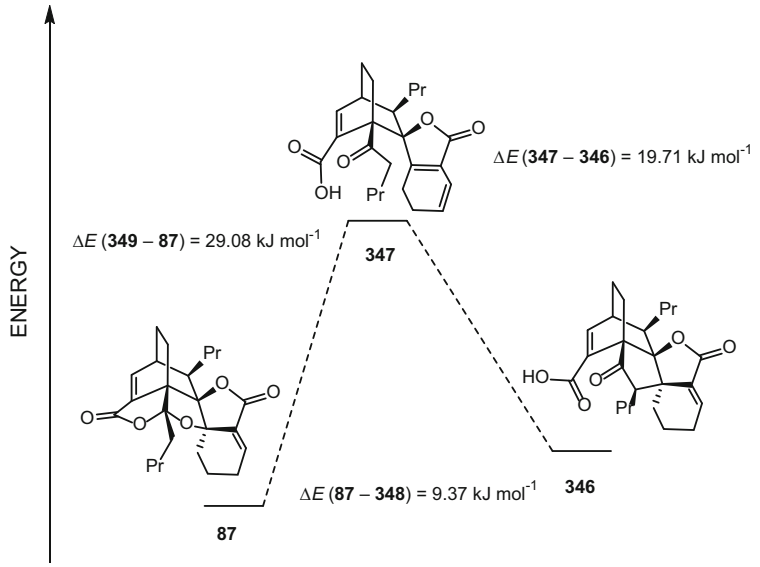


Fig. 9 Representation of total energies of **87**, **346**, and **347**. (Molecular computations were done at the B3LYP/6-311G level of theory)

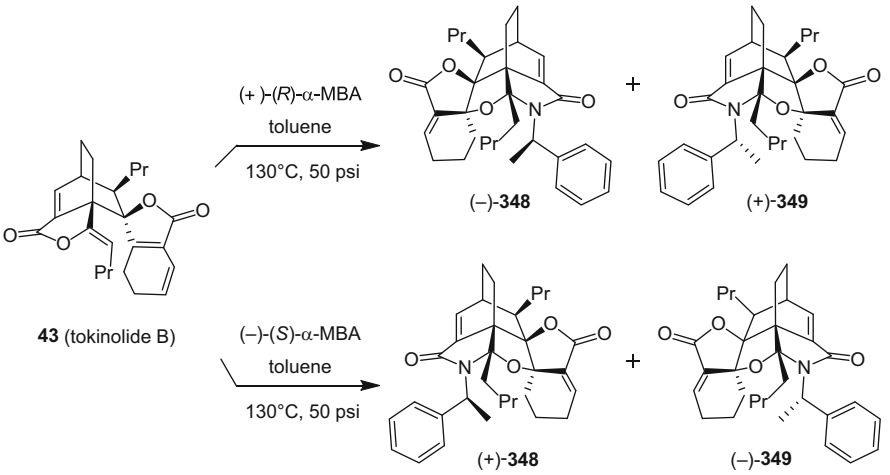


Chart 11 Diastereomeric mixtures of enantiomerically pure derivatives of tokinolide B (**43**)

(**23**) were prepared and evaluated as cytotoxic agents. Treatment of **43** with (+)-(*R*)-α-methylbenzylamine ((*R*)-MBA) and (–)-(*S*)-α-methylbenzylamine ((*S*)-MBA) afforded pairs of diastereomeric products, namely, (−)-**348** + (+)-**349** and (+)-**348** + (−)-**349** (Chart 11) [334].

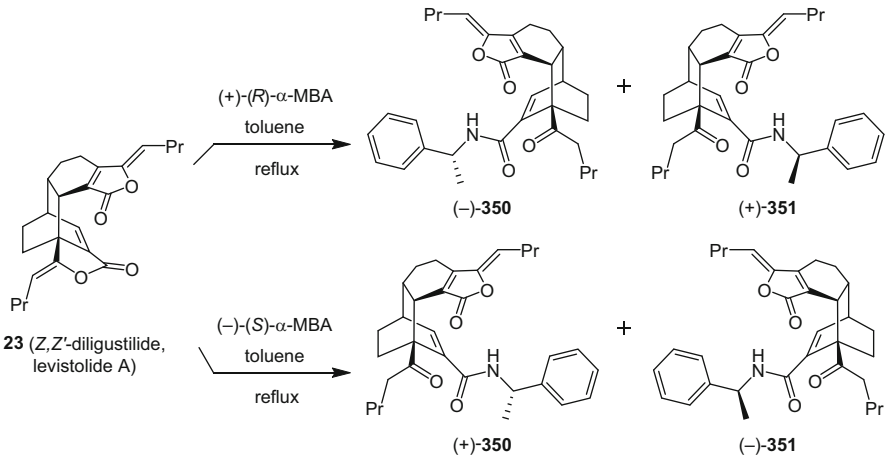


Chart 12 Diastereomeric mixtures of enantiomerically pure derivatives obtained from diligustilide (**23**)

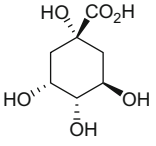
The absolute configurations of (−)-**348**, (+)-**349**, (+)-**348**, and (−)-**349** were determined by analyzing their ECD curves, using the exciton chirality method and defining the direction of the transition dipole moments of the chromophores.

In a complementary manner, the enantiopure derivatives (−)-**350** + (+)-**351**, and (+)-**350** + (−)-**351**, were obtained, in turn, by treatment of diligustilide (**23**) with (*R*)- and (*S*)-α-MBA (Chart 12) [335].

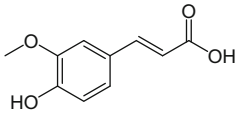
The absolute configurations of the amides were determined by the interpretation of the electronic circular dichroism curves (ECD), as previously described for the derivatives of tokinolide B (**43**) [334, 335].

5.3 *Biotransformations*

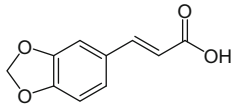
Mycophenolic acid (**141**) and **143** were isolated from a culture of *Penicillium crustosum*, when mixtures of either ferulic (**296**) and quinic acids (**352**) or 3-methoxy-4-hydroxycinnamic acid (**353**) and 3,4-methylenedioxycinnamic (**354**) acids were added to the medium [170].



352 (quinic acid)



353 (3-methoxy-4-hydroxy cinnamic acid)



354 (3,4-methylenedioxycinnamic acid)

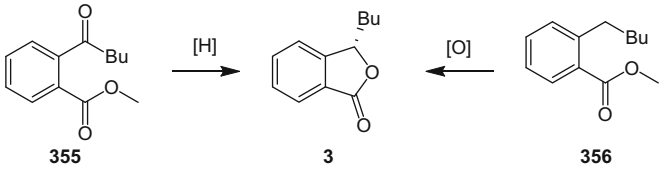
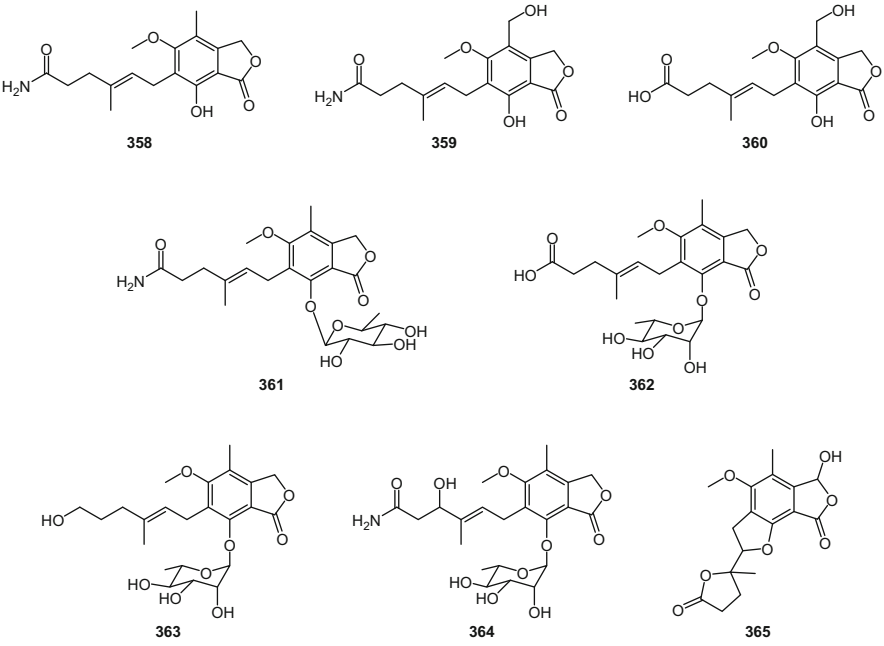


Chart 13 Microbial preparation of (*S*)-butylphthalide (**3**)

(*S*)-Butylidenephthalide (**3**) was prepared in 99% enantiomeric excess through microbial reduction of methyl 2-butyrylbenzoate (**355**) or microbial oxidation of methyl 2-pentylbenzoate (**356**) [336] (Chart 13).

Other derivatizations have been carried out for the resolution of racemic mixtures of phthalides. For example, the enzymatic resolution of racemic 3-butylidenephthalide (**3**) was achieved with Novozyme 435, which catalyzed the reaction between (*S*)-butylidenephthalide (**3**) and acetic anhydride to afford 2-((1*S*)-acetoxypentyl)-benzoic acid (**357**) in 98% *ee*, with up to 50.9% of unreacted 3-butylidenephthalide (**3**) remaining in 95.7% *ee* of the (*R*)-enantiomer [337–340] (Chart 14).

Several derivatives (**358**–**365**) of mycophenolic acid (**141**) were obtained by treatment with *Streptomyces* sp. [341, 342].



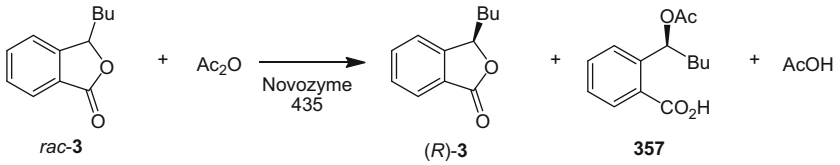
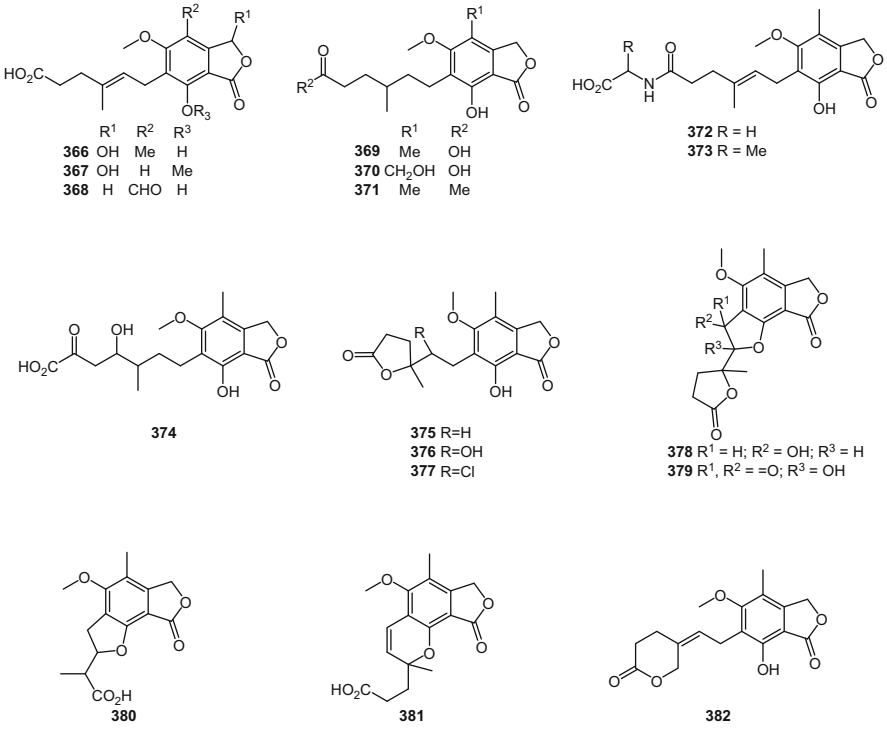


Chart 14 Enzymatic resolution of *rac*-**3**

Other modifications of **141** have been carried out by subjecting this phthalide to microbial transformation by 21 different species of bacteria, fungi and algae, furnishing phthalides **360** and **366–382**. The most common and abundant transformation products were the hydroxylactone **367**, resulting from oxidation at C-3, and **360**, by benzylic oxidation of the methyl group. Compound **372** was also obtained in relatively good yield. It is noteworthy that several *Penicillium* spp. were able transform mycophenolic acid (**141**) [343].



When *Polyporus brumalis* (Pers.) Fr. was supplemented with the phthalideisoquinoline derivative, (–)-β-hydrastine (**383**), this compound was hydroxylated with retention of configuration, yielding **384**, probably due to the action of a cytochrome-P450-dependent monooxygenase [344] (Chart 15).

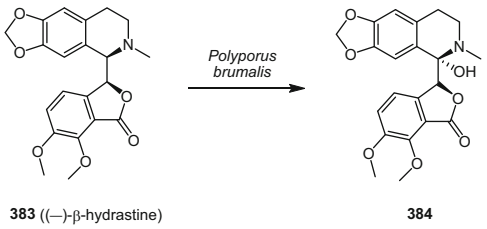


Chart 15 Hydroxylation of (–)-β-hydrastine (**383**)

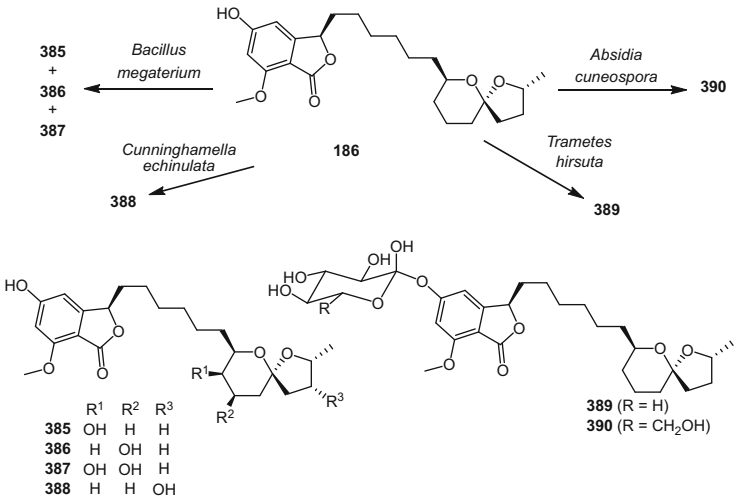


Chart 16 Microbial transformation of spirolaxine (**186**)

Spirolaxine (**186**) has been biotransformed by several microorganisms. *Bacillus megaterium* yielded phthalides **385–387** and *Cunninghamella echinulata* yielded **388** [345]. *Trametes hirsuta* transformed **186** into **389**, while *Absidia cuneospora* produced **390** [346] (Chart 16).

6 Synthesis of Phthalides

In view of the relevant biological properties of phthalides and, in particular, their chemical reactivity, many investigations have been devoted to the synthesis of these compounds. Research on this topic has resulted in a number of specific and interesting methodological procedures. In this section, selected approaches concerned with this topic are described. As a prior consideration, it is important to mention that Mal and co-workers [3] recently published a review covering part of this topic; nonetheless, in the present chapter the specific syntheses of naturally occurring phthalides are featured.

6.1 Synthesis of Monomeric Phthalides

The most direct approach for the synthesis of natural phthalides is to start from other natural phthalides. For example, Cimmino and co-workers prepared isocyclopaldic acid through a Canizzaro reaction, by treatment of cyclopaldic acid with base, reducing C-3 and oxidizing the formaldehyde at C-5 [329]. Other examples of this approach are the semisynthesis of senkyunolides H-J (**26**, **22**, and **33**) and L (**45**) [73, 328] (see above).

Salfredin B_{11} (**210**) was synthesized by Babu and Mali [347] from **90** and 3-chloro-3-methylbutyne, and subsequent thermal cyclization with dimethylphenylamine (Chart 17).

The terpenoid phthalide **151** was proved to be involved in the biosynthesis of mycophenolic acid (**141**), and was prepared by semi- and total synthesis [173] (Chart 18). Mycophenolic acid (**141**) was reduced to the corresponding aldehyde

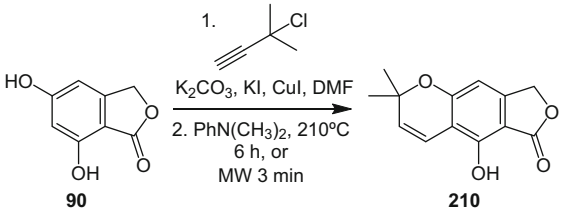


Chart 17 Synthesis of salfredin B_{11} (**210**)

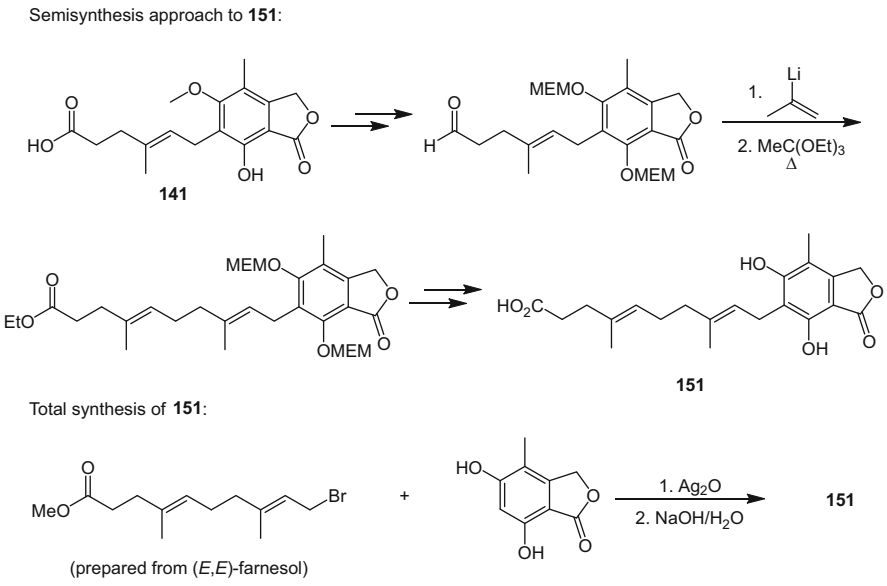


Chart 18 Semisynthesis and total synthesis of phthalide **151**

and coupled with propenyl lithium. The resulting compound yielded the natural product **151** after a Claisen-type rearrangement and hydrolysis. On the other hand, the total synthesis consisted basically of transforming (*E*,*E*)-farnesol into 10-bromo-4,8-dimethyl-deca-4,8-dienoic acid, and conducting the alkylation of 5,7-dihydroxy-3-methylphthalide with the former compound, in the presence of Ag_2O.

A number of more complex total syntheses of natural phthalides have been developed and some selected examples are described below.

6.1.1 Formation of the Cyclohexane Ring: The Alder–Rickert Reaction

The Diels–Alder reaction between cyclohexadienes and acetylenes, followed by retrocycloaddition, yields substituted benzenes and ethylene. This transformation is called the Alder–Rickert reaction and has been employed widely for the synthesis of phthalides substituted at C-4, C-5, C-6, and/or C-7 [348].

One of the first reports using the Alder–Rickert reaction was Birch and Wright's total synthesis of mycophenolic acid (**141**) [315], devoted to the formation of the benzene ring needed for the phthalide moiety, as depicted in Chart 19. The synthesis started from resorcinyl dimethyl ether, which was subjected twice to sequential Vilsmeier–Haack formylation/Wolff–Kishner reduction steps, followed by Birch's reduction and isomerization of the product. The resulting cyclohexadiene was subjected to an Alder–Rickert reaction with dimethyl acetylene dicarboxylate (DMAD), yielding a substituted dimethyl phthalic ester. It was then demethylated and converted into the corresponding phthalic anhydride, which was in turn

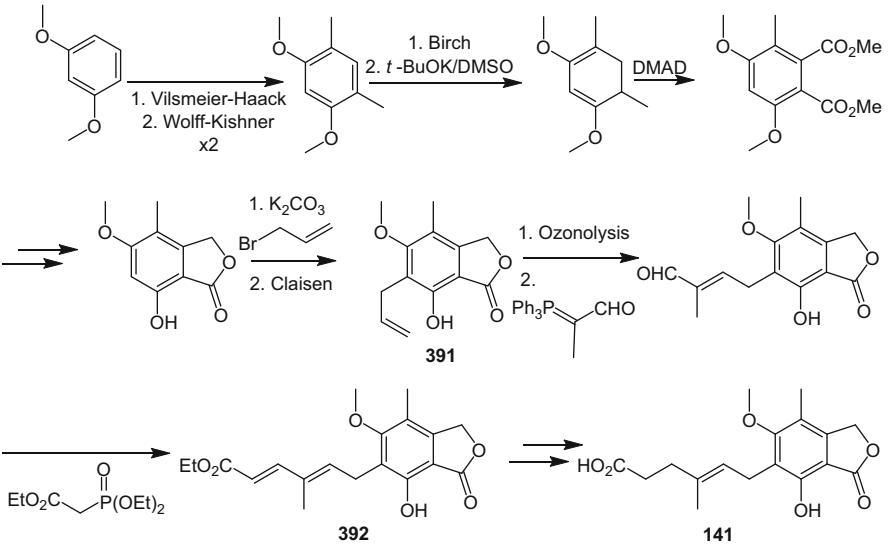


Chart 19 Birch's synthesis of mycophenolic acid (**141**)

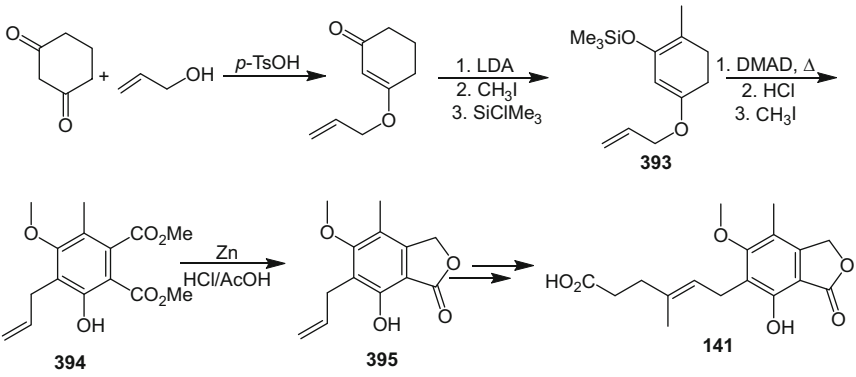


Chart 20 Patterson's synthesis of mycophenolic acid (**141**)

selectively reduced with Zn/HCl. Alkylation of the hydroxy group followed by Claisen rearrangement furnished **391**. This last compound was subjected to ozonolysis, then to a Wittig reaction, and next to the Horner–Wadsworth–Emmons reaction, yielding the ethyl ester of dehydromycophenolic acid **392**. Finally, this compound was hydrolyzed and reduced with diimide to yield MPA (**141**) (see Chart 19).

Patterson also reported a synthesis of **141** involving the Alder–Rickert reaction between trimethylsilyloxy enol **393** and DMAD, as shown in Chart 20 [349]. The product was then isomerized through a Claisen rearrangement. The resulting dimethyl *o*-dicarboxylbenzoate **394** was reduced with Zn to yield phthalide **395**, which was subjected to ozonolysis. This aldehyde was reacted with 2-propenyl magnesium bromide, and the thermolysis of the resulting alcohol with triethyl orthoacetate in the presence of propionic acid yielded MPA (**141**). More recently, Barrett and co-workers [350] reported an additional total synthesis of MPA (**141**) in 12 steps, which included a biomimetic cyclization–aromatization step starting from a polyketide-like compound.

The fungal phthalides 5-(3′,3′-dimethylallyloxy)-7-methoxy-6-methylphthalide (**199**), 6-(3′,3′-dimethylallyloxy)-4-methoxy-5-methylphthalide (**207**), and silvaticol (**183**) were prepared by Hariprakasha and co-workers [351] using the Alder–Rickert reaction between diene **396** and DMAD to furnish a polysubstituted benzene ring that was then *O*-prenylated and hydrolyzed to furnish **397** (see Chart 21).

Acid-catalyzed dehydration of diacid **397** yielded the corresponding phthalic anhydride, which was reduced with $NaBH_4$ and hydrolyzed with K_2CO_3, yielding silvaticol (**183**) (Chart 22).

A mixture of prenylated phthalides **199** and **207** was obtained, accomplishing the cyclization of phthalic acid **397** with DCC, and then reducing with $NaBH_4$. An alternative approach to these phthalides is to reduce the dimethyl phthalic ester **398** with DIBAL, followed by oxidative cyclization of the diol with PCC. It is interesting to note that the use of each procedure produces a switch in regioselectivity. Thus, the former methodology forms phthalides **199** and **207** in a 1:4 ratio; on the other hand, the ratio using the second methodology was 3:1 (see Chart 23) [351].

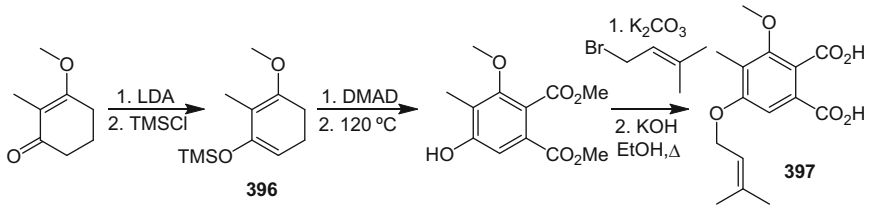


Chart 21 Synthesis of silvaticol (**183**) (Part 1)

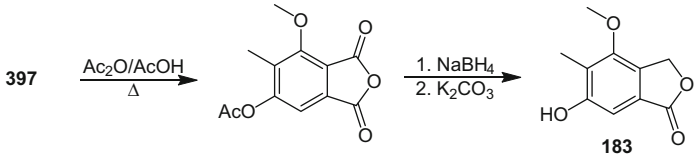


Chart 22 Synthesis of silvaticol (**183**) (Part 2)

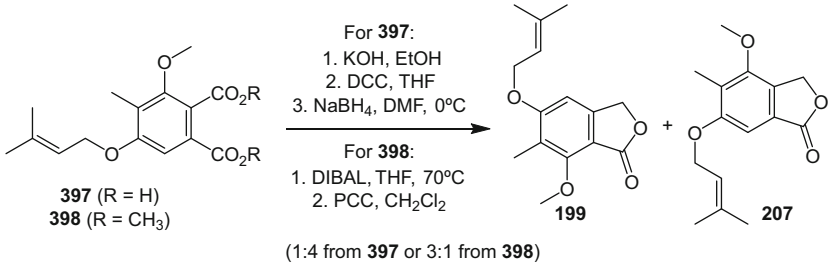


Chart 23 Synthesis of phthalides **199** and **207**

Kuwahara and co-workers [352] prepared both enantiomers of the fungal phthalide **202** starting from the protected dienol **399**, which underwent an Alder–Rickert reaction and then deprotection to yield 4-hydroxy-6-methoxy-3,5-dimethyl-1,2-benzene-dicarboxylate (**400**). Alkaline hydrolysis and reduction with Zn in aqueous HCl furnished phthalide **401**. After O-alkylation with the appropriate bromoester, deprotection, and oxidation, both enantiomers of **202** were obtained. The preparation of both enantiomers allowed identification of (*S*)-**202** as the natural product (see Chart 24).

6.1.2 Preformed Cyclohexane Ring and Formation of the Lactone Ring

The syntheses of less substituted phthalides, and mainly 3-substituted phthalides, have been investigated widely. In these cases, the use of accessible preformed benzene rings is a common feature, and there are several procedures for obtaining the lactone ring. A procedure for the preparation of 3-(2,6-dihydroxyphenyl)

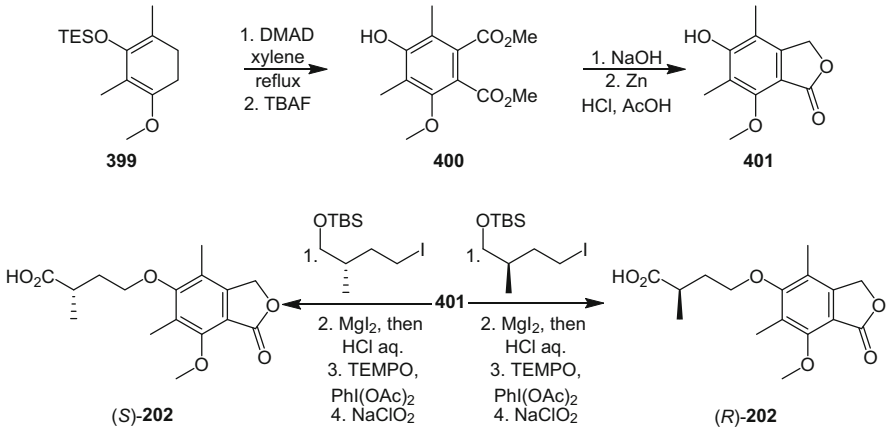


Chart 24 Total synthesis of **202**

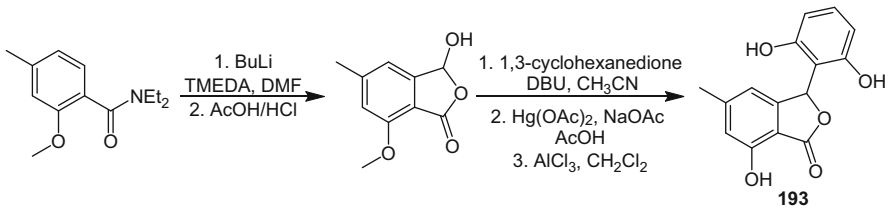


Chart 25 Preparation of isopestacin (**193**)

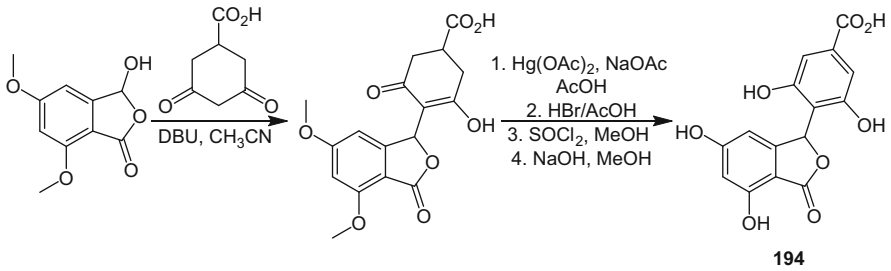


Chart 26 Preparation of cryphonectric acid (**194**)

phthalides was developed by Mal and co-workers. It is based on the reaction of phthalaldehydic acids with enamines of 1,3-cyclohexanediones and subsequent aromatization, and it was used for the preparation of isopestacin (**193**) and cryphonectric acid (**194**). This latter compound was esterified and hydrolyzed for its characterization. Attempts to prepare these natural products in an enantioselective manner were futile (see Charts 25 and 26) [353].

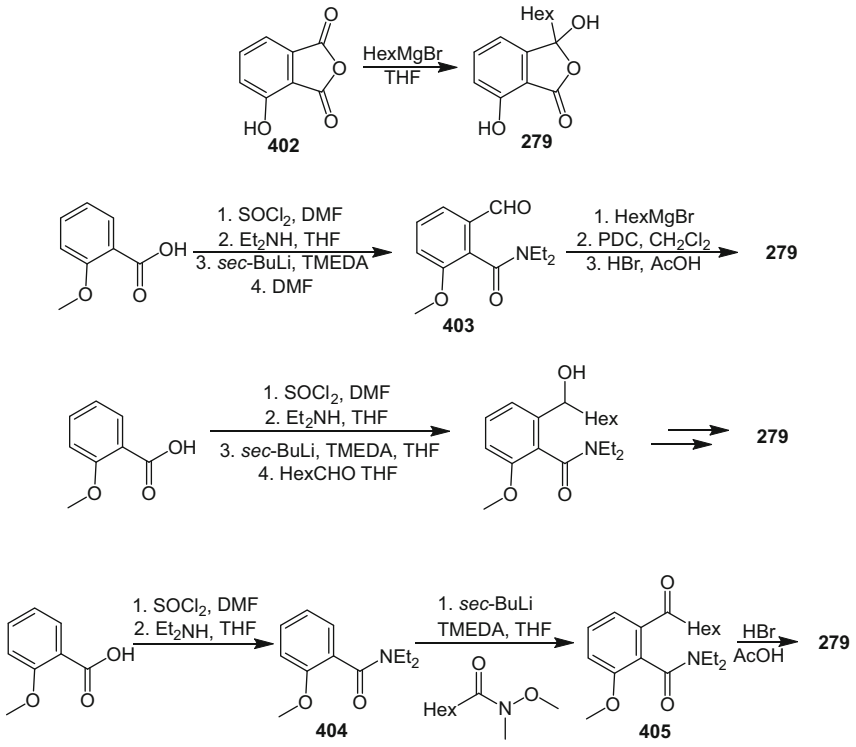


Chart 27 Synthesis methodologies for corollosporine (**281**)

Ohzeki and Mori carried out four approaches to obtain corollosporine (**279**), which are shown in Chart 27. The first of these consisted of a one-step reaction of 3-hydroxyphthalic anhydride (**402**) with hexylmagnesium bromide, which was the most direct route (36% yield), although it lacked effectiveness because of difficulties in purification. The second method involved the preparation of *N,N*-diethylacetamide of *o*-methoxybenzoic acid, followed by the *ortho*-metalation of Snieckus conducted with *sec*-butyllithium in the presence of tetramethylethylenediamine (TMEDA), and then *N,N*-dimethylformamide (DMF), furnishing **403**. This product was first converted into a secondary alcohol through a Grignard reaction with hexylmagnesium bromide, then oxidized to the ketone, and finally hydrolyzed and demethylated with hydrobromic acid to yield **279**. Another synthetic route avoided the Grignard reaction of **403** by treatment of an *ortho*-metallated anion with heptanal and following the above-described steps (oxidation, hydrolysis, and demethylation), afforded the desired compound. The last strategy consisted of a reaction of *N,N*-diethylacetamide **404** with the appropriate Weinreb amide and hydrolysis and demethylation of the furnished ketone **405** to yield **279** [354].

In the procedure described by Ranade and co-workers, ethyl 3,5-dimethoxybenzoate (**406**) was reduced, acetylated, and formylated (by means of the Vilsmeier–Haack reaction) to produce **407**. An oxidative cyclization of this last compound led

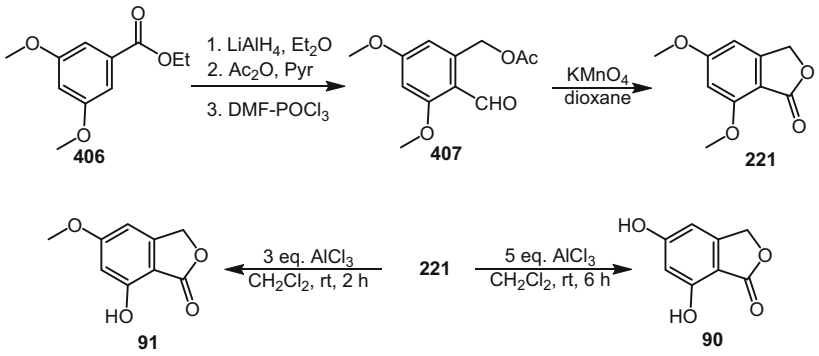


Chart 28 Synthesis of **90**, **91** and **221**

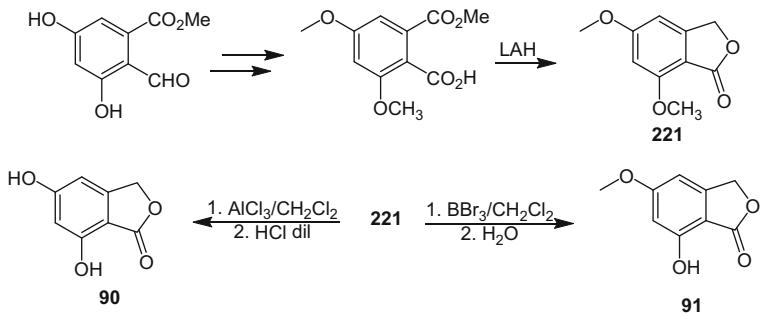


Chart 29 Alternative synthesis of **90**, **91** and **221**

to naturally occurring 5,7-dimethoxyphthalide (**221**), which was mono- or bidemethylated with $AlCl_3$ to yield the corresponding natural phthalides **90** and **91** (see Chart 28) [355].

Similarly, Talapatra and Talapatra synthesized these three natural phthalides starting from methyl 2-formyl-3,5-hydroxybenzoate, with protection and selective reduction with LAH, yielding one of the natural phthalides (**221**). Compounds **90** and **91** were obtained through partial or total demethylation, almost in the same conditions reported previously (see Chart 29) [356].

The synthesis of 3-substituted phthalides, among them senkyunolides B (**37**) and C (**28**), was achieved starting from appropriate 3-hydroxybenzoates (**408** or **409**) that were converted into nonaflates, to effect cross coupling of the resulting products with alkynes through a palladium-catalyzed Negishi type reaction. Hydrolysis of the resulting 2-alkynylbenzoates (**410** or **411**) and selective 5-*exo-dig* cyclization catalyzed by silver powder gave these phthalides in good yield. It is worth mentioning that when $AgNO_3$ was used as the catalyst instead of Ag powder, the resulting products were the analogous isocoumarins. In addition, senkyunolide E (**30**) was synthesized by saponification of methyl 2-(3-hydroxypentynyl)benzoate [357]. This procedure is shown in Chart 30.

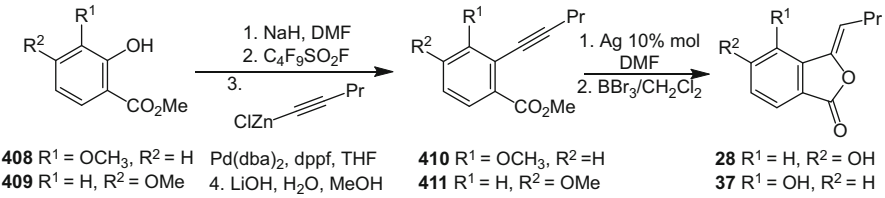


Chart 30 Synthesis of 3-alkenylphthalides from alkynyl benzoates

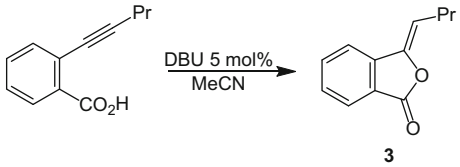


Chart 31 Synthesis of (*Z*)-butylidenephthalide (**3**)

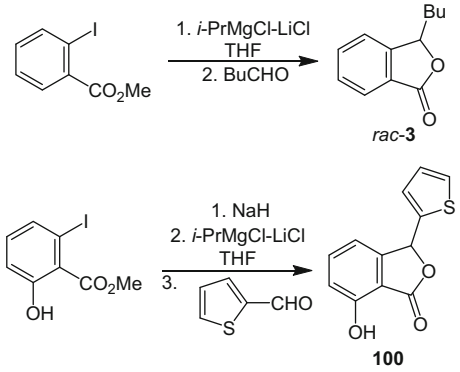


Chart 32 Synthesis of 3-substituted phthalides through Barbier reactions

In a similar manner, Kanazawa and Terada synthesized (*Z*)-butylidenephthalide (**3**) from *o*-pentynylbenzoic acid by means of a nucleophilic intramolecular addition, catalyzed by DBU (see Chart 31) [358].

Kuethe and Maloney employed a method essentially based on halogen–metal exchange of methyl *o*-iodoesters via a Barbier-type reaction with *i*-PrMgCl-LiCl, followed by quenching with carbonyl compounds, yielding racemic mixtures of the natural phthalides 3-butylidenephthalide (*rac*-**3**) and chrycolide (**100**) [359] (Chart 32).

Mondal and Argade reported regioselective procedures starting from 5,7-dihydroxyphthalide (**90**) and an α,β-unsaturated aldehyde, through which it proved possible to obtain selectively two kinds of skeletons, representing an adequate synthetic procedure for salfredin B_{11} (**210**) and phthalidochromene (**97**). When

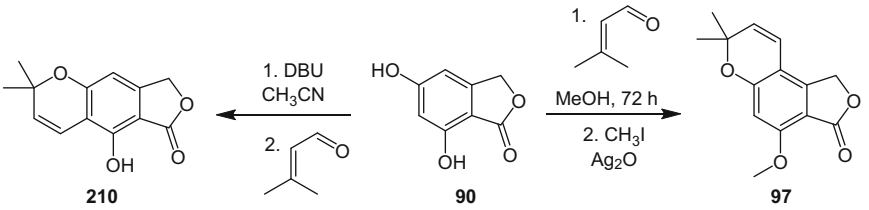


Chart 33 Synthesis of tricyclic terpenoid phthalides

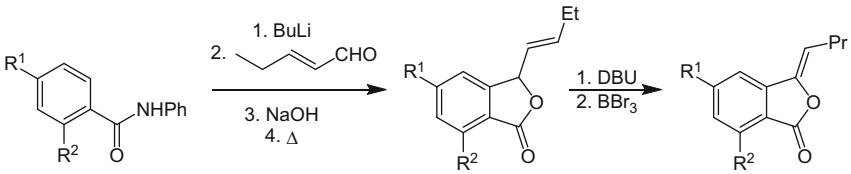


Chart 34 Wakamatsu's synthesis approach to 3-alkenylhydroxyphthalides

the starting phthalide was treated with DBU or other diaza-bases, a dianion was formed, and the more reactive C-6 anion underwent a nucleophilic attack (1,2-addition) at the carbonyl carbon from 3-methyl-3-butenal. The subsequent attack from oxygen at the remaining vinylic system, followed by dehydration, furnished the linear structure of salfredin B_{11}-like products. However, if 5,7-dihydroxyphthalide (**90**) was refluxed in methanol, with subsequent additions of the aldehyde, the "angular" tricycle was obtained exclusively, after methylation in the presence of Ag_2O, leading to the natural product **97** (see Chart 33) [360].

Wakamatsu and co-workers developed a synthetic pathway consisting of the lithiation of 2-methoxy-*N*-phenylbenzamide **412** (prepared from the corresponding carboxylic acid) with butyllithium in the presence of TMEDA, followed by nucleophilic attack on (*trans*)-2-pentenal, hydrolysis, and thermal cyclization, affording phthalide **414**, which was further isomerized and demethylated to yield the natural compound (*Z*)-3-butylidene-7-hydroxyphthalide (**25**). Similarly, (*Z*)-3-butylidene-5-hydroxyphthalide (**28**) was obtained starting from benzamide **413** and phthalide **415** as the intermediate (see Chart 34) [361].

Li's group synthesized (*Z*)-ligustilide (**8**) starting from *o*-formylbenzoic acid, which was converted into a 1:1 (*E*)/(*Z*) mixture of 2-butylidenebenzoic acid through a Wittig reaction, then oxidized with H_2O_2. The resulting *threo*/*erythro* mixture of 8-hydroxy-3-butylphthalide was reduced under Birch conditions, and the hydroxyphthalide obtained was dehydrated, affording **8** (see Chart 35) [362].

Beck and Stermitz reported an improved methodology for synthesizing phthalide **8** in three steps, starting from phthalide, which was treated with lithium diisopropyl amide (LDA) and then butyraldehyde, followed by Birch reduction and dehydration with MsCl (see Chart 36) [89].

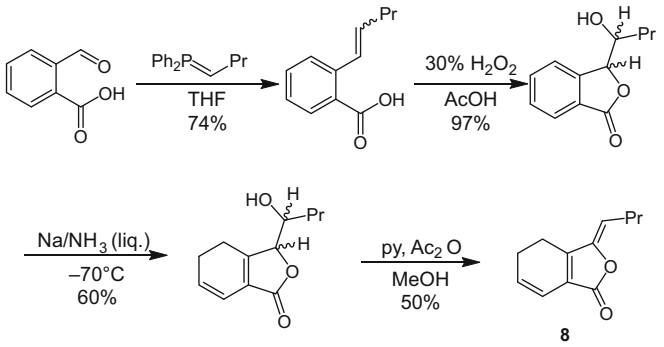


Chart 35 Li's synthesis of (*Z*)-ligustilide (**8**)

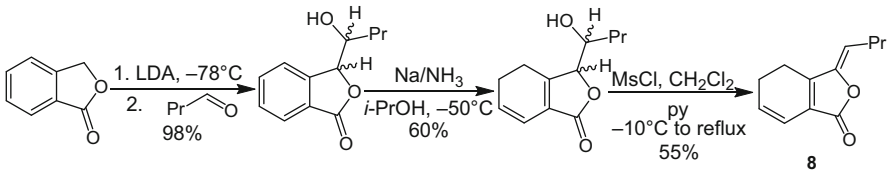


Chart 36 Beck's synthesis of (*Z*)-ligustilide (**8**)

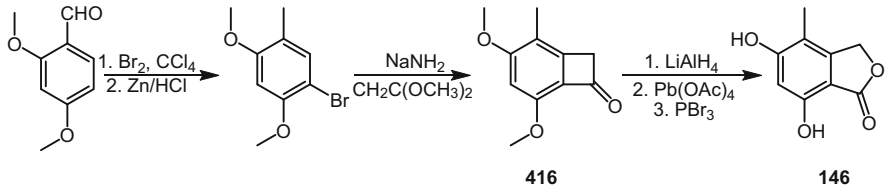


Chart 37 Synthesis of phthalide **146**

Kobayashi and co-workers developed a procedure in which 5,7-dihydroxy-4-methylisobenzofuran-1-(3*H*)-one (**146**) was synthesized from benzocyclobutenone **416** (prepared from 2,4-dimethoxybenzaldehyde, which was brominated and then reduced through a Clemmensen reaction to afford 1-bromo-2,4-dimethoxy-5-methylbenzene, and then treated with 1,1-dimethoxyethylene under Birch conditions. This compound (**416**) was transformed into the natural phthalide through reduction ($LiAlH_4$) and subsequent oxidation (with $Pb(OAc)_4$) [363]. Phthalide **146** can be used for the synthesis of MPA (**141**), so it constitutes a formal synthesis of the last-mentioned compound (see Chart 37).

The synthesis of naturally occurring 7-hydroxy-4,6-dimethylphthalide (**247**) was achieved by Takei and co-workers by means of the silylation of butenolide **417** with trialkylsilyl chloride, furnishing a furan-type diene **418**. The key step in this synthetic procedure was the Diels–Alder reaction between the former compound and maleic anhydride, for which the product, under hydrolysis, yielded the substituted phthalic anhydride **419**. This product was selectively reduced with

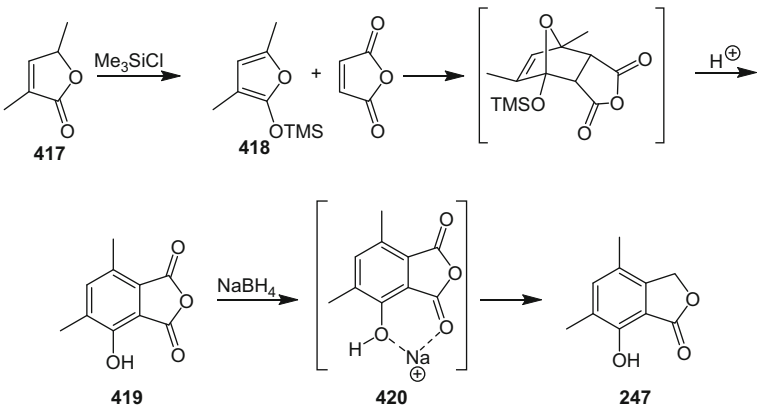


Chart 38 Synthesis of compound **247**

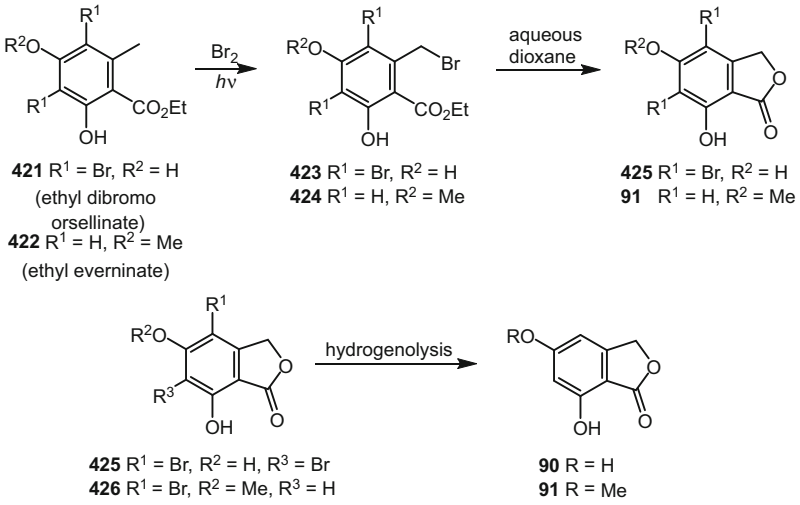


Chart 39 Synthesis of **90** and **91**

$NaBH_4$ (as a result of chelation of sodium cation with the hydroxy and carbonyl groups; intermediate **420**), yielding **247** (see Chart 38) [364].

Allison and Newbold accomplished the synthesis of naturally occurring 5,7-dihydroxyphthalide (**90**) and 7-hydroxy-5-methoxyphthalide (**91**) by benzylic bromination of ethyl dibromo orsellinate (**421**) or ethyl everninate (**422**) (producing **423** and **424**, respectively), followed by treatment with aqueous dioxane, which furnished **91**. To obtain **90**, a further step of hydrogenolysis of **425** was necessary. An alternative method to obtain **91**, also using the hydrolysis/cyclization process as an essential feature, took advantage of the by-product of the bromination reaction, i.e. the dibromo derivative of ethyl everninate, which, after hydrolysis, furnished phthalide **426**. Through a hydrogenolysis of the resulting product, the bromine atom attached to C-4 was replaced by hydrogen, yielding **91** [365] (Chart 39).

Canonica and co-workers carried out the preparation of the phthalide framework necessary for the synthesis of MPA (**141**), via Michael addition of sodium diethylmalonate to 3-methyl-3-penten-2-one and subsequent Dieckmann condensation. The resulting product was aromatized and then methylated; the corresponding acid was obtained under hydrolysis, and its chloride was prepared and reacted with ammonia, producing an amide, which was N-chlorinated. The product was photolytically converted and demethylated to produce **146**, which after alkylation and other functional group transformations, yielded **141** (see Chart 40) [366].

Mali and Patil reported a synthesis procedure in which a Wittig reaction between **427** and *n*-butylidenetriphenylphosphorane provided the corresponding vinyl benzoic acid, which was iodinated and cyclized with I_2/KI aqueous solution. After treatment with NaOAc in EtOH, HI was eliminated. Finally, the oxygen at C-7 was demethylated with $AlCl_3$ in CH_2Cl_2 to yield (*Z*)-3-butylidene-7-hydroxyphthalide (**25**), a natural compound isolated from *Ligusticum wallichii* (see Chart 41) [367].

Thibonnet's group synthesized natural phthalides **432** and **433**, using as a key step a Sonogashira coupling-oxacyclization, between *o*-iodobenzoic acid **428** and acetylenes **429** or **430**. It is noteworthy that due to the presence of methoxy substituents on benzene, the only observed products are phthalides **431** and **432**, from a 5-*endo-dig* oxacyclization, and not the coumarin, which would be formed through a 6-*exo-dig* attack (see Chart 42) [368].

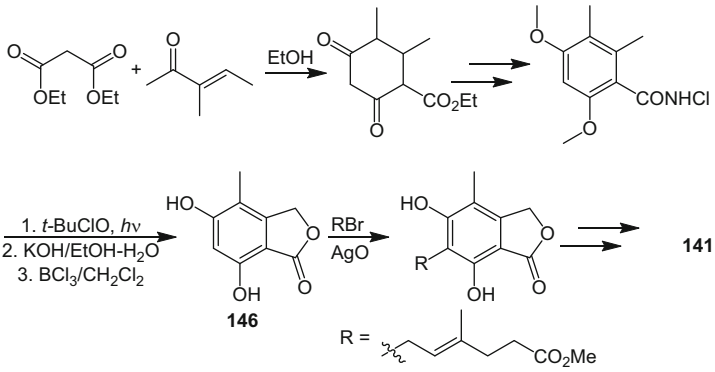


Chart 40 Canonica's synthesis of compound **141**

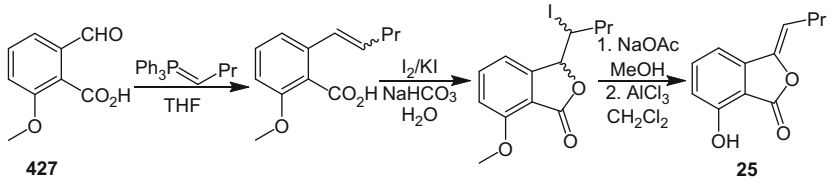


Chart 41 Synthesis of phthalide **25**

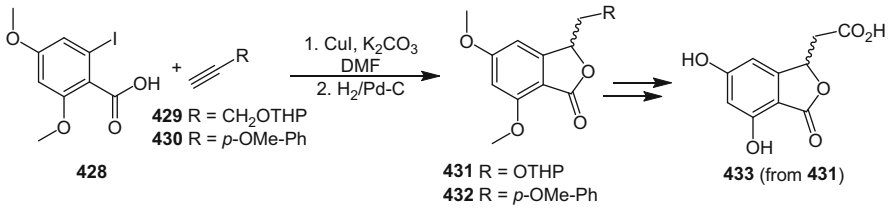


Chart 42 Synthesis of compounds **432** and **433**

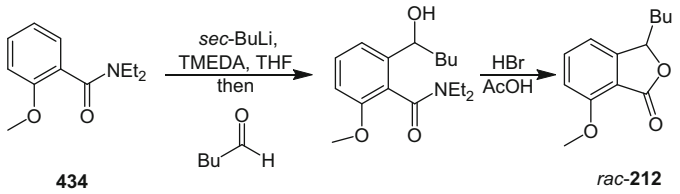


Chart 43 Synthesis of *rac*-**212**

Ohzeki and Mori used a simple two-step procedure consisting of *ortho*-lithiation of *N*,*N*-diethyl-*o*-methoxybenzamide (**434**) followed by nucleophilic attack on pentanal and lactonization to obtain a racemic mixture of 3-butyl-7-hydroxyphthalide (*rac*-**212**) (see Chart 43) [369].

6.1.3 Lactone Ring Formed Prior to Benzene Ring

Maldonado and co-workers reported an original synthesis of demethyl nidulol (**435**), a natural phthalide from *Aspergillus nidulans* (Eidam) G. Winter and *A. duricaulis*, where the formation of the lactone preceded the formation of the benzene ring. The procedure consisted of the preparation of compounds **436** and **437**, which were able to undergo an intramolecular Michael addition from the anion of the diactivated methylene moiety to the α,β-unsaturated propargyl or iodovinyl carbonyl fragments to afford the lactone ring. A subsequent Dieckmann condensation led to **435** (see Chart 44) [370].

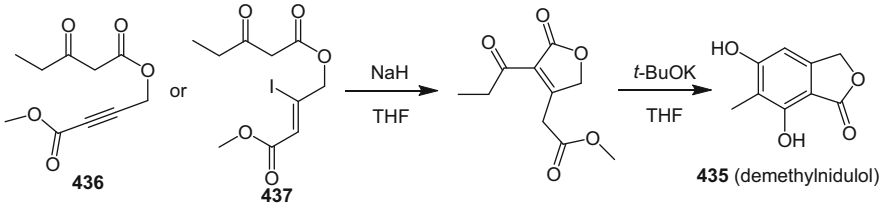


Chart 44 Preparation of demethyl nidulol (**435**)

6.1.4 Stereoselective Syntheses of Phthalides

Butylphthalide (**4**), (+)-matteucen C ((+)-**122**), (−)-matteucen C ((−)-**122**), and demethyl pestaphthalide (**216**), were synthesized by Santhosh and co-workers, as shown in Chart 45, via oxidative cyclization of the corresponding *o*-cyanostyrenes (**437**, **438**, and **439**), achieved with chiral oxo osmium complexes AD-mix (α- or β-). The mechanism of cyclization was investigated through indirect experiments, and was suggested to consist of oxidation of the carbon–carbon double bond with two of the oxygen atoms bonded to osmium, followed by nucleophilic attacks of the benzylic oxygen to nitrile carbon and of nitrile nitrogen to osmium. Subsequent hydrolysis yielded the desired phthalide. It is worth mentioning that the synthesis of both (+)- and (−)-matteucen C (**122**) confirmed the *syn* relationship of the substituents at C-3 and C-8 (See Chart 45) [371].

Koert and co-workers prepared (+)-pestaphthalide A (**213**) and (−)-pestaphthalide B (**214**), as depicted in Chart 46, by a stereodivergent synthesis from 2,6-dimethoxytoluene, which was selectively *meta* borylated. The resulting arylboronate was submitted to Suzuki-Miyaura coupling with (*Z*)-1-bromopropene, delivering a (*Z*)-alkene, which was epoxidized asymmetrically under Katsuki–Jacobsen conditions, and subsequently hydrolyzed either with aqueous perchloric acid in the presence of manganese III catalyst, or with aqueous 10-camphorsulphonic acid, leading to 4:1 and 1:3 mixtures of *cis*/*trans* diols,

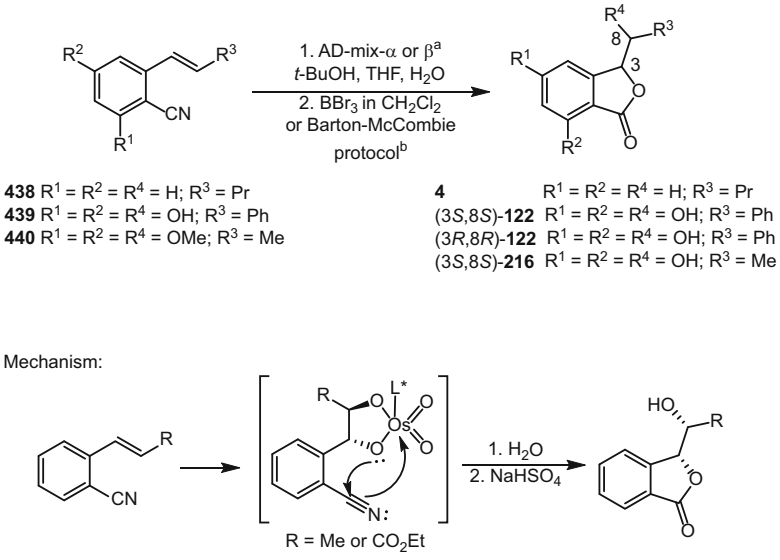


Chart 45 Enantioselective synthesis of matteucen C (**122**), demethylpestephthalide (**216**) and butylphthalide (**4**), and the underlying stereoselective determining step. (**a**) AD-mix-β was used for (−)-matteucen C (**122**); (**b**) Barton–McCombie protocol was used for synthesizing butylphthalide (**4**)

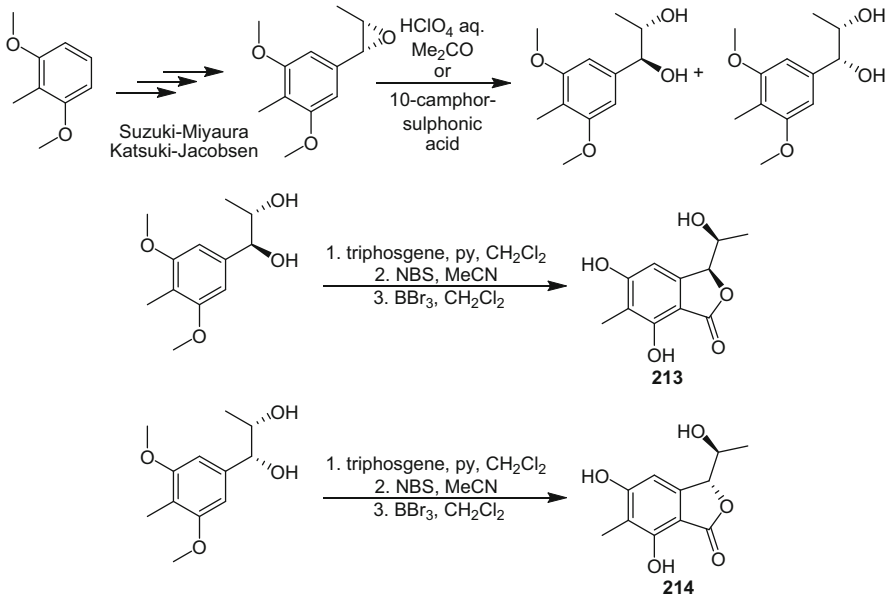


Chart 46 Syntheses of pestaphthalides A (**213**) and B (**214**)

respectively. The former was converted into cyclic carbonates with triphosgene. The convenient carbonate was subjected to bromination (with NBS), and then bromine–lithium exchange yielded an intermediate that rearranged to the corresponding phthalide, when heated to 20°C (see Chart 46) [372].

Watanabe and co-workers prepared a mixture of enantiomerically pure (−)-(*S*)-sedanenolide (senkyunolide A, **15**) and (−)-(3*S*)-butylphthalide (**4**) and a mixture of their enantiomers. This was achieved by esterification of 2,4-pentadienoic acid with the appropriate enantiomer of 1-heptyn-3-ol. The resulting ester was cyclized through a Diels–Alder reaction to give the corresponding mixture of (–)-(*S*)-sedanenolide (**15**) and (−)-(3*S*)-butylphthalide (**4**) or their enantiomers (see Chart 47) [373].

In another approach, summarized in Chart 48, (*R*)-butylphthalide ((*R*)-**4**) and other phthalides were prepared enantioselectively via Grignard-type reactions with *o*-oxazynyl-substituted benzaldehydes as electrophiles, yielding the appropriate alcohol in a diastereoselective manner, according to the Felkin–Ahn model. Hydrolysis of the oxazine moiety led to the corresponding ethylacetal, which, after oxidation with MCPBA and $BF_3{\cdot}OEt_2$, afforded phthalide (*R*)-**4** [374].

A reverse Wacker oxidation, aided by the presence of lactone oxygen, was used by Brimble and co-workers to prepare (−)-herbaric acid ((−)-**169**), in the following manner. An enantiomerically pure benzylic alcohol (accessible by enzymatic resolution), was reacted with carbonyl diimidazole (CDI) and diethylamine, yielding a carbamate, which was lactonized by bromine–lithium exchange. The desired product ((−)-**169**) was obtained from the 5,7-dimethoxy-3-vinyl-phthalide, by

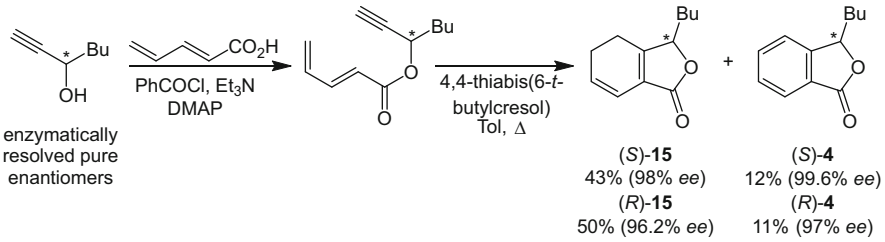


Chart 47 Syntheses of sedanenolide (**15**) and butylphthalide (**4**)

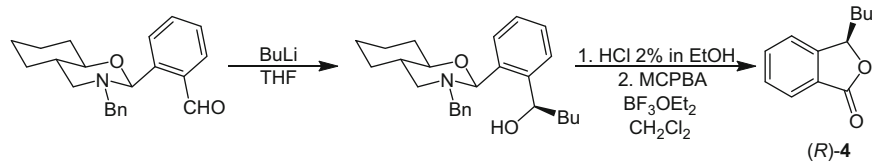


Chart 48 Enantioselective synthesis of (*R*)-butylphthalide ((*R*)-**4**)

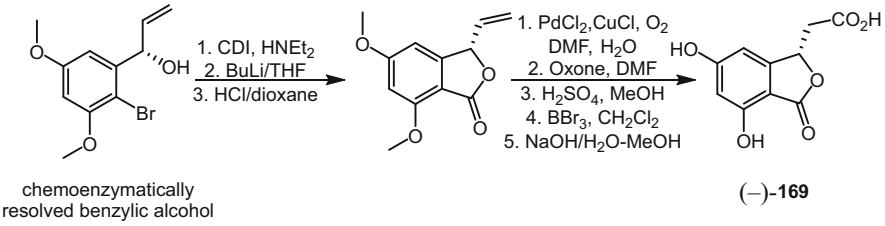


Chart 49 Synthesis of (−)-herbaric acid ((−)**169**)

reverse Wacker oxidation ($PdCl_2$, CuCl, O_2, DMF), oxidation of the resulting aldehyde (oxone, DMF), esterification, demethylation, and hydrolysis. This procedure is depicted in Chart 49 [375].

The syntheses of both enantiomers of acetophthalidin (*S*)-(**170**) and (*R*)-(**170**) were accomplished by Kitahara and co-workers, through stereoselective Sharpless dihydroxylation of 5-(1-propenyl)-bisbenzyl-resorcinol with either AD-mix-α or AD-mix-β, yielding (*S*,*S*)- and (*R*,*R*)- hydroxyphthalides, respectively. Oxidation of the alcohol to the ketone with Dess–Martin periodinane, followed by hydrogenolysis, yielded the enantioenriched (*S*)-(**170**) and (*R*)-(**170**) (see Chart 50) [376].

In order to confirm the configuration of (−)-3-butyl-4-hydroxyphthalide (**65**), Mitsuhashi's research group developed an asymmetric synthesis for this compound, as shown in Chart 51. Thus, the chiral aminal of *m*-methoxybenzaldehyde (**441**) was *ortho*-alkylated stereoselectively with *n*-pentanal, and, after acidulation, a diastereomeric mixture of lactols was obtained. This mixture was oxidized to the

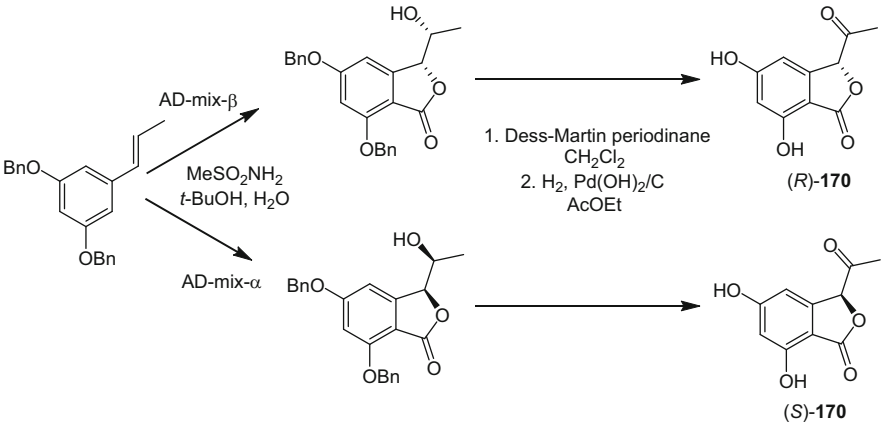


Chart 50 Synthesis of both enantiomers of acetophthalidin ((*S*)-**170**) and (*R*)-(**170**))

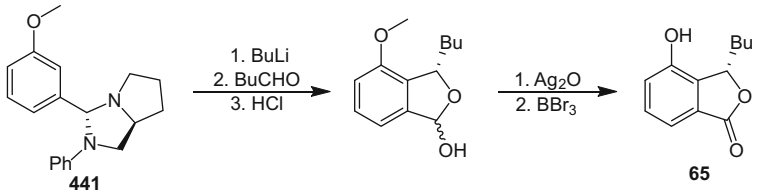


Chart 51 Synthesis of (−)-3-butyl-4-hydroxyphthalide (**65**)

corresponding lactone and the methyl ether was deprotected with BBr_3, affording phthalide **65** [108].

Another approach for the syntheses of chiral 3-substituted alkylphthalides with high enantiomeric excesses, was the use of *o*-phthalaldehyde. This, after reaction with an appropriate enantiomerically pure *N*-alkylvalinol, yielded oxazolidinyl benzaldehyde **442**, which was reacted with alkylmetallic reagents. The resulting product was further transformed to give enantioenriched 3-substituted natural phthalides (**4**, **443**, **444**). The stereoselective alkylation step was strongly influenced by the solvent, achieving enantiomeric excesses up to 90% of the (*R*)-enantiomer in a mixture of THF and dioxane, and 33% of the (*S*)-enantiomer in diethyl ether (see Chart 52) [151].

Both enantiomers of 3-butyl-7-hydroxyphthalide (**212**) were synthesized by Ohzeki and Mori, starting from methyl 2,6-dihydroxybenzoate, which was alkylated through a Suzuki–Miyaura coupling. The resulting olefin was dihydroxylated with either AD-mix-α or AD-mix-β to obtain enantiomerically pure diols. Further transformations gave both (*R*)- and (*S*)-enantiomers of the phthalide. The well-known stereochemistry of the Sharpless' epoxidation was used to confirm the configuration of the natural product as (*S*) (see Chart 53) [369].

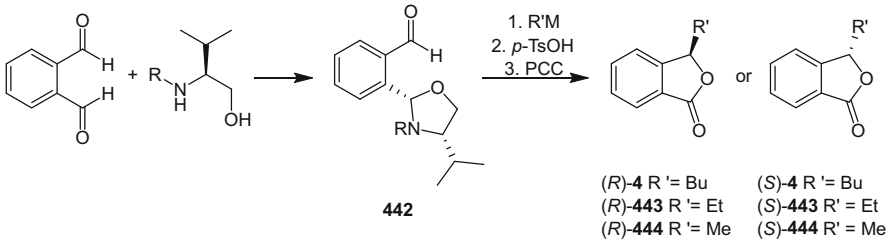


Chart 52 Syntheses of enantiomerically pure 3-alkylphthalides

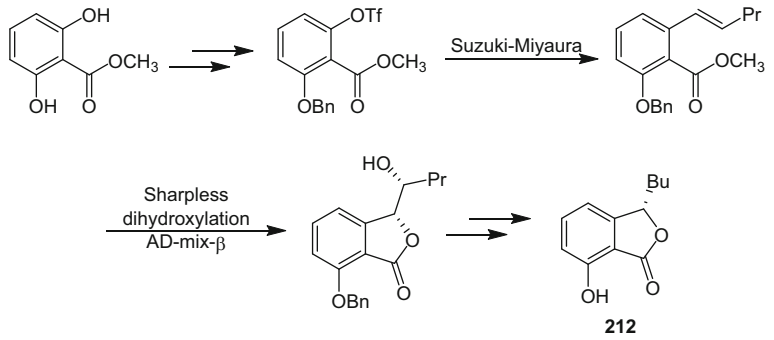


Chart 53 Synthesis of (*S*)-**212**. The enantiomer ((*R*)-**212**) was obtained using AD-mix α

6.2 Synthesis of Dimeric Phthalides

The synthesis of dimeric phthalides has been studied, mainly using (*Z*)-ligustilide (**8**) as starting material. It is interesting to note that dimeric phthalides have been isolated as racemic mixtures from members of the Apiaceae (Umbelliferae) plant family, and have displayed several biological activities (see Sect. 7). Diligustilide (levistolide A, **23**) and tokinolide B (**43**) have been derivatized to enantiomerically pure compounds, as described in Sect. 5.2.2.

6.2.1 [π4s + π2s] Cycloadditions

Wakamatsu and co-workers [320] described the preparation of diligustilide (levistolide A, **23**) and tokinolide B (**43**) from (*Z*)-ligustilide (**8**), by a Diels–Alder process. It was observed that tokinolide B (**43**) was transformed partially to levistolide A (**23**) under the reaction conditions (Chart 54). Calculations of HOMO and LUMO of (*Z*)-ligustilide (**8**) were also carried out to explain the regioselectivity of the dimers formed. A similar thermal reaction in a sealed tube of (*Z*)-ligustilide (**8**) allowed its conversion to diligustilide (**23**), confirming the regio- and stereoselectivity of the reaction [321] (see Chart 54).

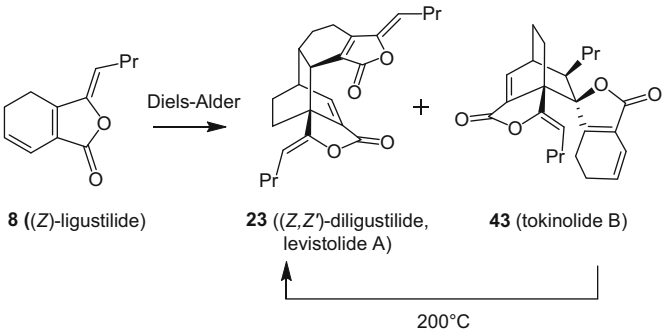
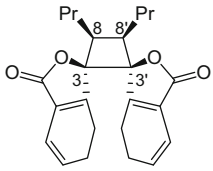


Chart 54 Diels–Alder reaction of (*Z*)-ligustilide (**8**)

6.2.2 [π2s + π2s] Cycloadditions

Although the majority of natural dimeric phthalides are formed by [π4s + π2s] reactions, several dimeric phthalides such as riligustilide (**24**), tokinolide A (**42**), and *endo*-(*Z*,*Z*′)-[3.3′,8.8′]-diligustilide (**445**) are biosynthesized through [π2s + π2s] cycloadditions [72, 304, 377, 378].



445 (*endo*-(*Z*,*Z'*)-[3.3',8.8']-diligustilide)

The situ-, regio- and stereochemical possibilities of the three olefins of (*Z*)-ligustilide (**8**) have been considered in the formation of [π2s + π2s] photocyclodimers, but there are no direct guidelines available to predict the structure of the products. (*Z*)-Ligustilide (**8**) was exposed to photochemical conditions, affording the natural product riligustilide (**24**), *endo*-(*Z*,*Z*′)-[3.8′,8.3′]-diligustilide (**446**), *endo*-(*Z*,*Z*′)-[3a.7a′,7a.3a′]-diligustilide (**447**) and *exo*-(*Z*,*Z*′)-[3a.7a′,7a.3a′]-diligustilide (**448**) (Chart 55). It was found that in the triplet state the carbon atoms of the side chain of **8** were quasi-coplanar with the lactone ring, bringing down the steric hindrance for the transition states, and also that the regioselectivity was determined by orbital coefficients and energies. Frontier molecular orbitals and Mülliken charge calculations agreed with the experimental yields obtained for the reaction products [378].

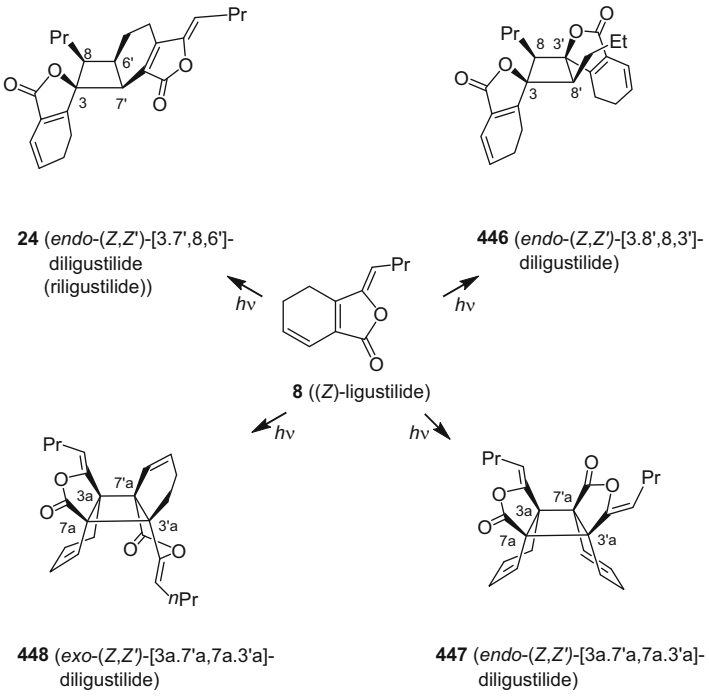


Chart 55 Photocyclodimerization of (*Z*)-ligustilide (**8**)

7 Biological Activity

The evaluation of biological activity has been a prominent aspect of phthalide research. While a number of biological activities have been attributed to natural product extracts containing phthalides, these data have been complicated by the presence of more than one active constituent (i.e. the biologically active constituents are not exclusively phthalides). With this in mind, the present contribution reviews mainly the bioactivities of natural and semisynthetic phthalides as pure compounds.

Several reviews on related topics have been published [1, 114, 379–382], with some of them focusing exclusively on one compound, such as mycophenolic acid (**141**) [318] or noscapine (**128**) [383–386]. The current review is neither intended to be a comprehensive treatment of the biological activity of natural phthalides as whole, nor on the individual compounds mentioned. Instead, an integrated overview is presented of the most relevant biological activities of this type of compounds is presented here.

7.1 Antioxidant Effects

Several human diseases are associated with oxidative damage. The overproduction of reactive oxygen species (ROS) damages the cell [387], and eukaryotic cells have developed defensive enzymatic systems. (*Z*)-Butylidenephthalide (**3**), (*Z*)-ligustilide (**8**), senkyunolide I (**22**), sinaspirolide (**70**), and ansaspirolide (**71**), were screened for their antioxidant activity at 100 μ*M*. All these compounds showed activity in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Also, ansaspirolide (**71**) was the most active in inducing the activity of NAD(P)H-quinone oxidoreductase 1 (NQO1), but was also cytotoxic for the host hepatoma cells (Hepa1c1c7). (*Z*)-Butylidenephthalide (**3**) and (*Z*)-ligustilide (**8**) also successfully induced NQO1. The transcription of several antioxidant enzymes is regulated by antioxidant response elements (ARE) in promoter units. (*Z*)-Ligustilide (**8**) induced ARE reporter activity in a dose-dependent manner (5–20 μ*M*) [388]. Senkyunolides I (**22**) and H (**26**) both induced heme oxygenase-1 (HO-1), with senkyunolide H showing the most potent effect, and the induction was related to the activation of Nrf2 (nuclear factor E2-related factor-2)/ARE pathway. Both compounds were inhibitors of ROS formation and lipid peroxidation in human liver hepatocellular carcinoma cells (HepG2) [389]. Colletotrialide (**226**) demonstrated a low antioxidant activity, scavenging DPPH with an IC_{50} value of >324 μ*M*. It also inhibited weakly superoxide anion radical formation (by xanthine/xanthine oxidase) ($IC_{50} > 648$ μ*M*); superoxide anion radical generation (in differentiated human promyelocytic leukemia cells (HL-60)) ($IC_{50} > 130$ μ*M*); xanthine oxidase ($IC_{50} > 648$ μ*M*), and aromatase ($IC_{50} > 16.2$ μ*M*) [222].

The antioxidant properties of (*Z*)-ligustilide (**8**) and *cis*-(*Z*,*Z*′)-3a.7a′,7a.3a′-diligustilide (**447**) have been assessed using human umbilical vein endothelial cells (HUVECs), evaluating the oxidative damage caused by hydrogen peroxide (H_2O_2). Treatment with **447** protected HUVECs ($IC_{50} = 15.14$ μ*M*), with (*Z*)-ligustilide (**8**) displaying an IC_{50} of 0.55 μ*M*. Lactate dehydrogenase (LDH) leakage provoked by H_2O_2 was also reduced by **447** at concentrations of 25, 50, and 100 μ*M*. The application of **447** also increased superoxide dismutase (SOD) activity and decreased malondialdehyde (MDA) levels, confirming its antioxidant properties [387].

Epicoccone (**195**) prevented lipid peroxidation (62%) when used at 37 μg/cm^3 [205], while isopestacin (**193**) was found to scavenge hydroxyl radicals at a concentration of 0.22 m*M* and the superoxide radicals at 0.185 m*M* [203].

7.2 Analgesic Effects

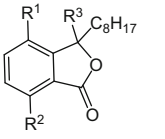
(*Z*)-Ligustilide (**8**) has analgesic effects, since administration to mice at doses of 2.5, 5, and 10 mg/kg (p.o.), caused a dose-dependent reduction in the both the

writhing response induced by acetic acid and formalin-induced licking time [390]. Compound **8** has also been evaluated at the higher dosages of 20, 60, and 100 mg/kg, with the same results: a delayed licking time and reduced writhing response both occurred [391]. In a similar study, (*Z*)-butylidenephthalide (**3**), (*Z*)-ligustilide (**8**), and diligustilide (**23**), suppressed the irritation induced by acetic acid, with diligustilide (**23**) showing the greatest effect. (*Z*)-Butylidenephthalide (**3**) and (*Z*)-ligustilide (**8**) also demonstrated an antinociceptive effect when using a hot-plate assay [392].

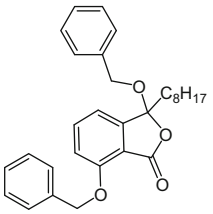
7.3 Antihyperglycemic Effects

Type 2 diabetes mellitus is a chronic condition associated with abnormal levels of blood glucose. Both (*Z*)-butylidenephthalide (**3**) and (*Z*)-ligustilide (**8**) decreased the postprandial blood glucose peak in mice treated with streptozotocin [393]. (*Z*)-Butylidenephthalide (**3**) also inhibited the activity of yeast α-glucosidase in vitro in a concentration-dependent manner ($IC_{50} = 2.35$ m*M*). Docking analysis (using the (*E*)-isomer (**21**)) showed that this compound binds close to the catalytic site [393].

In screens for competitive binding to PPAR-γ, paecilocin A (**245**) used at 100 μ*M* demonstrated comparable activities to rosiglitazone, a PPAR-γ agonist used for the treatment of type 2 diabetes mellitus. Compounds **449** and **450** also showed comparable binding properties to rosiglitazone, whereas **451**–**454**, which contain benzyl or methyl groups, were less effective. The introduction of additional substituents failed to enhance activity, as shown for compounds **452**–**454** and **457**–**460**. Phthalides **455** and **456** showed no enhanced activity, with compound **455** slightly more active than **456** [394].



	R^1	R^1	R^3
449	OH	H	H
450	H	OH	H
451	H	OMe	H
455	H	H	H
459	OH	H	OH
460	H	OH	OH



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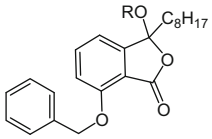
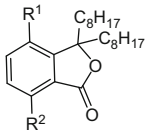


453 R = H
454 R = Me



	R^1	R^2
456	H	H
457	OH	H
458	H	OH

7.4 Antithrombotic and Antiplatelet Effects

Thrombosis is the main cause of the thromboembolic complications of ischemic disorders. One pharmacological strategy has been to re-establish the blood flow to the ischemic site by dissolving the clot. Another strategy is to prevent clot formation. To this end, the search for new antithrombotic agents has continued [395]. (*Z*)-Ligustilide (**8**), administered for three days at doses of 10 or 40 mg/kg (p.o.), demonstrated both antithrombotic and antiplatelet activities [395]. (*Z*)-Butylidenephthalide (**3**) showed antiplatelet activity, and inhibited the aggregation of washed rabbit-derived platelets, induced by collagen, arachidonic acid, platelet activating factor, and adenosine diphosphate (ADP). (*Z*)-Butylidenephthalide (**3**) also inhibited the release of adenosine triphosphate (ATP) from these platelets [396]. (*R*)-Butylphthalide (*ent*-**4**) and (*S*)-butylphthalide (**4**) inhibited also platelet aggregation [397], with blood viscosity reduced by *rac*-butylphthalide (*rac*-**4**), cnidilide (**7**), senkyunolide A (**15**), senkyunolide P (**40**), and tokinolide B (**43**) [398].

7.5 Neurological Effects

7.5.1 Stroke

Stroke is a leading cause of disability in adults and is the third most prevalent cause of mortality in the world [399, 400]. Treatment options are currently limited. Intraperitoneal administration of (*Z*)-ligustilide (**8**) to mice undergoing transient forebrain cerebral ischemia/reperfusion (I/R), at dosages of 5 and 20 mg/kg, reduced infarct volume in a dose-dependent manner. The administration of **8** also decreased MDA content, restored the activities of glutathione peroxidase (GSH-PX) and SOD in ischemic brain tissues, and regulated pro- and antiapoptotic effector proteins [401]. Oral administration of (*Z*)-ligustilide (**8**) at doses of 20 or 80 mg/kg to rats with middle cerebral artery occlusion (MCAO) also showed, after 24 h of obstruction, a marked reduction in infarct volume and brain edema. (*Z*)-Ligustilide also ameliorated neurobehavioral impairment and improved survival rate [400]. In terms of the immune response, microglia are activated during ischemia. Both (*Z*)-ligustilide (**8**) and senkyunolide A (**15**) inhibited neuroinflammation, blocked the production of TNF-α and nitrites in murine microglial cells (BV-2), and reduced TNF-α production from peripheral blood monocyte-derived macrophages (PBMac) [399].

Oral administration of (*Z*)-ligustilide (**8**) at doses of 20, 40 or 80 mg/kg, 3 and 0.5 h before the MCAO procedure, reduced the neurological deficit score, and the infarct volume in a dose-dependent manner. The expression of erythropoietin (EPO, an endogenous protective factor) was also enhanced and the level of the stress-induced protein RTP801 (an endogenous detrimental factor) was reduced.

The cytoprotection conferred by EPO could be mediated by the phosphorylation of ERK promoted by **8**. (*Z*)-Ligustilide (**8**) also increased cell viability and decreased the leakage of LDH, although concentrations above of 5 μ*M* were cytotoxic to neurons maintained in cell culture. Transfection of human neuroblastoma cells (SH-SY5Y) with the pcDNA3.1-RTP801 plasmid DNA increased LDH leakage and RTP801 expression, both of which were inhibited by (*Z*)-ligustilide (**8**) [402]. Compound **8** protected PC12 cells from apoptosis induced by oxygen-glucose deprivation (OGD), induced tolerance to oxidative stress, induced HO-1 expression, and promoted translocation of Nrf2 [403] to the nucleus (an inducible transcription factor that regulates multiple cellular antioxidant systems during stroke) [404]. (*Z*)-Ligustilide (**8**) also regulated the homeostasis of glutathione (GSH) [403].

Zhu et al. evaluated the effect of (*Z*)-ligustilide (**8**) on the Nrf2/HO-1 pathway, and it was found that this compound provoked a significant decrease of infarct volume, improved neurological function, and attenuated neuronal loss (at 16 and 32 mg/kg, i.v.) in transient MCAO-induced damage [404]. The results concerning the Nrf2 and HO-1 proteins were similar to those obtained by Rong et al. [403]. (*Z*)-Ligustilide (**8**) activates the Nrf2 pathway, with its protective action possibly mediated by the Nrf2/HO-1 pathway [404]. Noscapine (**128**) improved clinical prognosis in ischemic stroke patients [405].

Subarachnoid hemorrhage (SAH) is a stroke subtype that can lead to cerebral vasospasm. The treatment of rats with (*Z*)-ligustilide (**8**) at a dose of 20 mg/kg improved neurobehavioral scores, reduced edema, improved the permeability of the blood brain barrier, and with vasospasms diminished. (*Z*)-Ligustilide (**8**) may ameliorate tissue damage caused by SAH by mechanisms that involve apoptosis [406].

Permanent bilateral ligation of the common carotid artery is an experimental model for cerebral hypoperfusion (used for the study of dementia), which impairs memory and learning. Administration of (*Z*)-ligustilide (**8**) prevented the structural and functional abnormalities in the brain of rats subjected to this procedure. (*Z*)-Ligustilide also protected the hippocampus from damage, ameliorated cognitive deficits, decreased MDA and acetylcholinesterase (AChE) levels, and increased the activity of the SOD and choline acetyltransferase (ChAT) [407].

7.5.2 Alzheimer's Disease and Cognitive Impairment

Alzheimer's disease is a progressive neurodegenerative disease characterized by damage to the regions of the brain that regulate cognitive function in the elderly [408]. The cytotoxicity induced by the amyloid β-peptide (Aβ) in Alzheimer's disease, together the effects of (*Z*)-ligustilide (**8**) and 11-angeloylsenkyunolide F (**41**), have been evaluated. Cell viabilities following exposure to $A\beta_{1-40}$ were 61.6 and 69.4%, respectively, at 10 and 50 μg/cm^3 for (*Z*)-ligustilide (**8**). The same concentrations of 11-angeloylsenkyunolide F (**41**) produced viabilities of 59.5 and

67.1%. The toxicities of these compounds were also analyzed, with 50 μg/cm^3 (*Z*)-ligustilide (**8**) shown to be toxic (53.0% of cell viability) [408].

A second group of investigators reported cytotoxicity data using $A\beta_{25–35}$ and oral administration of **8** (40 mg/kg) for 15 days (from the 6th to 20th day). (*Z*)-Ligustilide (**8**) prevented cognitive dysfunction and attenuated the morphologic changes and neuronal loss induced by injection of $A\beta_{25–35}$. The injection of $A\beta_{25–35}$ increased the expression of Aβ, amyloid precursor protein, and Tau protein, with (*Z*)-ligustilide (**8**) preventing all of these effects [409].

Some studies have suggested that the widespread loss of ACh-containing neurons, and the reduction in activity of ChAT, are early biological signs of Alzheimer's disease. (*Z*)-Ligustilide (**8**) (at 10 or 40 mg/kg daily for 26 days) was tested on an model of Alzheimer's disease using scopolamine, which increases AChE activity and decreases ChAT activity. Phthalide **8** improved spatial long-term memory, prevented spatial short-term memory deficits, inhibited AChE activity ($IC_{50} = 6.48$ mg/kg), and increased ChAT activity ($ED_{50} = 7.66$ mg/kg) [410]. (*Z*)-Ligustilide (**8**) has also demonstrated a cytoprotective effect, and protected against the cognitive impairment and neurotoxicity induced by D-galactose (at a dose of 80 mg/kg/8 weeks) in aged mice brains, by improving spatial learning and memory. MDA levels and the expression of cleaved caspase-3 were both diminished on the administration of **8**. The decline in activity of Na^+–K^+-ATPase provoked by D-galactose was also prevented by (*Z*)-ligustilide (**8**), with a diminution of astrocytic activation [411].

Phthalide NG-072 (**48**) has been reported to be effective in the potential treatment of Alzheimer's disease, by enhancing axon growth [84].

7.5.3 Parkinson's Disease

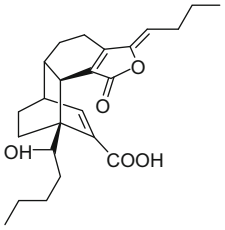
Parkinson's disease is a neurodegenerative pathology characterized by a progressive loss of dopaminergic nigrostriatal neurons. Current therapies for Parkinson's disease depend mainly on dopamine replacement using levodopa (L-dopa) and antioxidants; however, there is certain evidence of the toxicity of dopamine and its metabolites. Dopamine at concentrations ranging from 200 to 800 μ*M* affected the viability of PC12 cells in a concentration-dependent manner. (*Z*)-Ligustilide (**8**) used at 50 μ*M* decreased cell viability by 9.6%, but the combination of **8** (50 μ*M*) and dopamine (500 μ*M*) was even more cytotoxic to PC12 rat dopaminergic cells, reducing viability by almost 90%. The same treatment as for PC12 on the SH-SY5Y (human neuroblastoma), HepG2 (human hepatoma), MCF-7 (human breast adenocarcinoma), HeLa (human epithelial carcinoma), and PC3 (human prostate cancer) cell lines, revealed that only the dopaminergic cell lines were adversely affected. Cells treated with dopamine or (*Z*)-ligustilide (**8**) died via apoptosis and necrosis, with a mixture of these compounds increasing levels of cell death to 56.8%, and decreasing GSH levels to 28.8% [412].

7.5.4 GABAergic and Sedative Effects

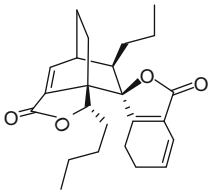
The $GABA_A$ receptor is a target for drugs that modulate sedative, anxiolytic, anticonvulsant, muscle relaxant, and amnesic activities. Binding to the $GABA_A$ receptor using [^{3}H] flunitrazepam and diazepam as positive controls, demonstrated that both gelispirolide (**68**) and riligustilide (**24**) inhibited [^{3}H]diazepam binding to $GABA_A$ receptors (the IC_{50} values were 29 and 24 μ*M*) [109].

(*Z*)-Ligustilide (**8**) at 5–20 mg/kg, and (*Z*)-butylidenephthalide (**3**) at 10–30 mg/kg (i.p.) reversed pentobarbital-induced sleep shortened by social isolation stress. Both phthalides (at 20 mg/kg) attenuated the effects of methoxamine and yohimbine, which decreased the time of the pentobarbital sleep period. (*Z*)-Ligustilide (**8**) potentiated the effects of diazepam in pentobarbital-induced sleep in mice, suggesting noradrenergic suppression. Both phthalides also attenuated the effect of a benzodiazepine receptor inverse agonist. Taken together, the $GABA_A$ receptor may be implicated in the activity of these compounds [413].

The hypnotic time induced by sodium pentobarbital in mice increased significantly by pretreatment with 50 mg/kg of (*Z*)-ligustilide (**8**), diligustilide (**23**), tokinolide B (**43**), senkyunolide F (**31**) and several semi-synthetic products, including the diketo diacid of diligustilide (**345**), demethylwallichilide (**344**), *rel*-(3′*S*)-3′,8′-dihydrodemethylwallichilide (**461**), and *rel*-(3′*R*)-3′,8′-dihydrotokinolide B (**462**). The increases in hypnosis, expressed as percentages, were 46.3, 24.6, 70.8, 34.6, 66.0, 52.3, 36.2, and 100%, respectively. Compounds **43** and **462** demonstrated the highest activities [276]. In the same model, compounds **15** and **4** displayed similar effects [414]. Phthalideisoquinolines, (+)-bicuculline (**111**) [415, 416], (+)-hydrastine (**112**), and corlumine (**110**) were found to be antagonists of the $GABA_A$ receptor, with the most potent antagonist proving to be **112**, with **111** more potent than **110**; all three phthalides are convulsive agents [417].



461 (*rel*-(3′*S*)-3′,8′-dihydro demethylwallichilide)



462 (*rel*-(3′*R*)-3′,8′-dihydrotokinolide B)

7.5.5 Anticonvulsive Effects

Epilepsy is a disorder characterized by abnormal neuronal electrical activity [418] with periodic and unpredictable seizures [419]. *rac*-Butylphthalide (*rac*-**4**) and senkyunolide A (**15**) were shown to be anticonvulsive agents against metrazole, electroshock-, and audio-induced seizures [420]. Yang et al. confirmed the

anticonvulsive effects of both phthalides [421]. *rac*-Butylphthalide protected against chronic epilepsy induced by coriaria lactone [422], and at 700 mg/kg prevented abnormalities in the hippocampus [423]. Both enantiomers of butylphthalide (**4**) protected from the seizures induced by electro-shock [424].

7.6 Progestogenic Effects

The hormone progesterone is necessary for menstrual and reproductive health. During menopause, hormone replacement therapy is an effective treatment against hormonal disorders. Some phthalides have shown progesterone-like activity. For example, 3,8-dihydrodiligustilide (**63**) ($EC_{50} = 91$ nM) was shown to be a potent and specific activator of the progesterone receptor, with riligustilide (**24**) ($EC_{50} = 81$ μM) displaying weaker activity. Levistolide A ((**23**) (*Z*,*Z*′)-diligustilide) was inactive, which demonstrates the importance of minor structural variations in this type of molecule for biological activity [106].

7.7 Cytotoxic Effects

The current lack of specificity for multiple antitumor therapies has led to a search for novel, more targeted agents [219]. 3-Methyl-4,5,6-trihydroxyphthalide (**198**) is an agent that has been tested for activity against the serine/threonine-protein kinase Akt1, which regulates metabolism, proliferation, and cell survival, and showed an IC_{50} value of 19.7 μM. The IC_{50} for the functional inhibition of Bad phosphorylation by Akt1 was 30.4 μM [208]. Cytotoxicities for **198** against several cancer cell lines are listed in Table 1.

In the treatment of liver fibrosis, suppression of the growth of liver stellate cells (HSC) with the induction of apoptosis has been suggested to be a plausible therapeutic approach. (*Z*,*Z*′)-[6.8′,7.3′]-Diligustilide (**24**) and levistolide A (**23**)

Table 1 IC_{50} values for 3-methyl-4,5,6-trihydroxyphthalide (**198**) against several human cancer cell lines [208]

Cell line	IC_{50}/μM
T cell lymphoblast (Jurkat)	20
Myeloma cells derived from peripheral blood lymphocytes (RPMI-8226)	67
Central nervous system cancer (SNB-75)	60
Melanoma (SK-MEL-28)	37
Ovarian cancer (OVCAR-5)	74
Breast cancer (BT-549)	24
Lymphoma (U937)	60

((*Z*,*Z*′)-diligustilide) were found to reduce the cell proliferation stimulated by platelet-derived growth factor (PDGF-BB) in immortalized liver stellate cells (HSC-T6) and in immortalized human stellate cells (LI-90), with **23** showing a higher potency than **24**. Both compounds induced apoptosis in HSC stimulated by PDGF-BB, without significant toxicity to primary hepatocytes, when used at 5–40 μ*M* for **24**, and 1–20 μ*M* for **23** [425].

Noscapine (**128**) has been in Phase I/II clinical trials for non-Hodgkin's lymphoma or chronic lymphocytic leukemia refractory to chemotherapy [426]. In addition, noscapine also displayed activity against HT-29, colon carcinoma (SW480), and the human colon adenocarcinoma (LoVo) cell lines, with selectivity against the latter cell line ($IC_{50} = 75$ μ*M*) [427]. There have been several studies of the bioactivity of noscapine (**128**), which concluded that its mechanism of action is related to microtubule assembly [428–430].

Topoisomerases are enzymes that are involved in the progression of the cell cycle and their inhibition can be used as targets for cancer chemotherapy. Senkyunolides N (**52**) and J (**33**), and sedanolide (**6**) exhibited inhibitory effects against topoisomerases I and II, with **6** completely inhibiting both enzymes at 100 μg/cm^3 [431]. The cytotoxic and antiproliferative effects of senkyunolide A (**15**), (*Z*)-ligustilide (**8**), and (*Z*)-butylidenephthalide (**3**) were evaluated using the human colon cancer cell line (HT-29) and the normal human colon fibroblast cell line (CCD-18Co). The phthalides decreased cell viability for tumor-derived cell lines (IC_{50} values ranging from 8.6 to 51.2 μ*M*), without any significant effect on the viability of normal cells. Of these agents, senkyunolide A (**15**) was the most selective [432].

(*Z*)-Butylidenephthalide (**3**) prevented cell cycle entry in glioblastoma multiforme brain tumor cells, when used at 75 μg/cm^3. This compound also induced apoptosis and prolonged the survival of mice after malignant brain tumor cell implantation [433].

(*S*)-3-Butyl-7-methoxyphthalide (**212**) is a natural product that has been previously synthesized; its IC_{50} values against several cell lines are shown in Table 2 [217].

Compound **199** displayed activity against HeLa and KB cells (IC_{50} 36.0 and 14.0 μg/cm^3) [210, 212]. Porriolide (**200**) also displayed activity against KB cells

Table 2 IC_{50} values for (*S*)-3-butyl-7-methoxyphthalide (**212**) against several cancer cell lines

Cell line	IC_{50}/μg cm^{-3}
Human lung carcinoma (A549)	44.0
Human epidermoid carcinoma of the mouth (KB)	32.0
HeLa	31.0
Human mammary adenocarcinoma (T47 D)	30.0
Murine leukemia cell line (P388)	25.8

($IC_{50} = 59.0$ μg/cm^3) [209]. Phthalide **199** induced apoptosis, with the authors suggesting that proliferation was inhibited by a G1 phase arrest in HeLa cells [212].

Hanabiratakelides A ((**218**) HA), B ((**219**) HB), and C ((**220**) HC) were found to display cytotoxic activities against colon cancer cells (Caco-2 and colon-26 cells). The respective IC_{50} values for HA (**218**) and HC (**220**) were 342 and 535 μ*M* in Caco-2 cells. In turn, the IC_{50} values for colon-26 cells were 96 μ*M* for HA (**218**), 18 μ*M* for HB (**219**), and 49 μ*M* for HC (**220**). These compounds also showed superoxide dismutase (SOD)-like activity, with IC_{50} values of 15.7, 49.0, and 3.2 μ*M* for HA (**218**), HB (**219**), and HC (**220**), respectively [219].

Marilones A (**203**), and C (**205**) also showed weak cytotoxic activities against three cell lines: NCI-H460 (lung), MCF7 (breast), and SF268 (central nervous system). Cytotoxicities were comparable for **203** ($LC_{50} > 100$ μ*M*) and **205** ($LC_{50} = 99.6$ μ*M*) against NCI-H460 and MCF7 [214].

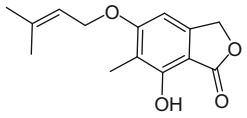
Colletotrialide (**226**) has been tested against several cell lines with IC_{50} values of 162.1 μ*M* for HuCCA-1, HepG2, and the A549 cell lines, and slightly less, at 147.8 μ*M*, for the acute lymphoblastic leukemia line (MOLT-3) [222].

Several additional natural and semi-synthetic phthalides have been assayed for their bioactivities against three cancer cell lines. The enantiomers (–)-**348**, (+)-**349**, (+)-**348**, (–)-**349**, (–)-**350**, (+)-**350**, (+)-**351**, (–)-**351**, were more active (see Table 3), with helenalin used as a positive control [118, 334, 335].

Table 3 IC_{50} values of several natural and semi-synthetic phthalides against three human cancer cell lines

	IC_{50}/μ*M*		
	Cell line		
Compound	Leukemia (K562)	Colon (HCT-15)	Lung (SK-LU-1)
Dilustilide (**23**)	26.6	10.5	7.1
Rilugustilide (**24**)	46.1	44.8	13.2
Tokinolide B (**43**)	26.6	10.5	7.1
Chaxiongnolide B (**87**)	30.6	23.1	37.4
Demethylwallichilide (**344**)	47.2	>100	>100
Diketo diacid of dilustilide (**345**)	19.9	71.6	42.6
Cyclotokinolide (**346**)	21.9	28.4	22.9
Ketoacid of tokinolide B (**347**)	>100	>100	>100
(–)-**348**	5.7	5.4	4.1
(+)-**348**	13.9	7.5	4.9
(–)-**349**	5.2	5.2	4.3
(+)-**349**	21.7	8.5	5.9
(–)-**350**	13.8	36.7	27.0
(+)-**350**	4.4	12.2	7.3
(–)-**351**	17.1	9.6	7.1
(+)-**351**	10.4	32.5	26.9

5-(3′,3′-Dimethylallyloxy)-7-hydroxy-6-methylphthalide (**463**) exhibited moderate activity against the myeloid liver carcinoma (SMMC-7721) and MCF-7 cell lines, with IC_{50} values of 1.8 and 29.0 μ*M* [434].



463

Multi-drug resistance (MDR) is an obstacle for many current cancer therapies. One of the mechanisms involved in MDR is the elimination of compounds by conjugating them by phase II enzymes, including glutathione *S*-transferase (GST). 11-Angeloylsenkyunolide F (**41**) and tokinolide B (**43**) inhibited GST enzyme (IC_{50} 16.8 and 7.3 μ*M*, respectively), in a reversible and noncompetitive process, docking analysis showed that both compounds interacted with the active site. Compounds **41** and **43** showed low cytotoxicity against the A549 and MDA-MB-231 cell lines, with both reversing MDR in these cell lines [435].

7.8 *Inhibition of the Abnormal Proliferation of Vascular Smooth Muscle Cells*

Another biological activity that has been investigated for natural occurring phthalide derivatives is the proliferation of vascular smooth muscle cells. Abnormal proliferation is seen in atherosclerosis and in atherosclerotic plaques [436, 437]. Some phthalides have been reported to inhibit this proliferation in a concentration-dependent manner. Senkyunolide H (**26**) was the most active ($IC_{50} = 0.1$ μg/cm^3) of the following compounds: (*Z*)-butylidenephthalide (**3**) ($IC_{50} = 3.25$ μg/cm^3), (*Z*)-ligustilide (**8**) ($IC_{50} = 1.68$ μg/cm^3), senkyunolide A (**15**) ($IC_{50} = 1.52$ μg/cm^3), and neocnidilide (**6**) ($IC_{50} = 6.22$ μg/cm^3). 3-Butylphthalide (**4**), cnidilide (**7**), and senkyunolide I (**22**) also demonstrated weak effects [436].

Kobayashi et al. also investigated the effect of various phthalides on the competence and progression of the cell cycle proliferation. The most active phthalide was senkyunolide L (**45**), followed by senkyunolide H (**26**), senkyunolide J (**33**), senkyunolide I (**22**), (*Z*)-ligustilide (**8**), senkyunolide A (**15**), and (*Z*)-butylidenephthalide (**3**) [437].

The effect of phthalide **8** on the abnormal proliferation of vascular smooth muscle cells was related to its inhibition of ROS production [438]. (*Z*)-Butylidenephthalide (**3**) and (*Z*)-ligustilide (**8**) both inhibited the proliferation of vascular smooth muscle cells stimulated with basic fibroblast growth factor [439]. Compound **8** also displayed positive effects in a rat model of atherosclerosis [440].

7.9 Insecticidal Effects

The larvicidal activities of (*Z*)-butylidenephthalide (**3**) and (*Z*)-ligustilide (**8**) were evaluated against *Drosophila melanogaster*. Compound **3** was found to be more active than **8** ($LC_{50} = 0.94$ μmol/cm^3 and $LC_{50} = 2.54$ μmol/cm^3), although both were less effective than rotenone. Acute adulticidal activity, resulting in 100% mortality, was seen when compound **3** was used at a dose of 5.0 μg/adult ($LD_{50} = 0.84$ μg/adult), and was more potent than the value obtained for rotenone [441].

(*Z*)-Ligustilide (**8**) deterred the biting of both *Aedes aegypti* and *Anopheles stephensi* at 25 nmol/cm^2 more effectively than *N*,*N*-diethyl-3-methylbenzamide, which is considered to be one of the most effective mosquito repellents [442]. Sedanolide (**6**) showed 100% of mortality at 50 μg/cm^3 against *A. aegypti* [294].

Bemisia tabaci is one of the most important insect pests and participates in the transmission of numerous plant-pathogenic viruses. The most pathogenic biotypes of *B. tabaci* are the B- and Q-biotypes. The residual contact toxicities of (*Z*)-ligustilide (**8**) ($LC_{50} = 268.4$ ppm), and (*Z*)-butylidenephthalide (**3**) ($LC_{50} = 254.2$ ppm) were comparable to cypermethrin, but lower than other insecticides; **3** was more toxic than (*S*)-butylphthalide (**4**) ($LC_{50} = 338.9$ ppm) against the B-biotype females. The toxicity of these compounds against the Q-biotype females was also tested. (*Z*)-Ligustilide (**8**) and (*Z*)-butylidenephthalide (**3**) showed more pronounced toxicity against the B-biotype females than the Q-biotype. (*Z*)-Butylidenephthalide (**3**) also demonstrated acaricidal activity against two dust mite species, *Dermatophagoides farina* and *D. pteronyssinus* [443].

7.10 Bactericidal, Antifungal, Antiviral, Immunosuppressant, and Antiparasitic Effects

Mycophenolic acid (**141**) is an antibiotic agent with activity against a broad range of microorganisms including *Cryptococcus neoformans*, *Candida albicans*, *C. stellatoidean*, *C. tropicalis*, *C. parakrusei*, and *Trichophyton* species, and showed moderate inhibition of *Staphylococcus aureus* [444], which has developed some resistance to this antibiotic [445]. Mycophenolic acid (**141**; MPA) was effective in suppressing psoriasis [446], and its morpholine ester was useful in reducing episodes of allograft rejection [447]. The pharmacokinetics and pharmacodynamics of MPA analogs have been reviewed recently [448, 449].

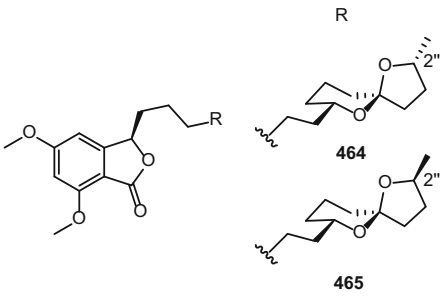
The increasing prevalence of multidrug resistant organisms has led to the search for new, more effective, and nontoxic agents. (*Z*)-Ligustilide (**8**) showed a moderate potentiation of norfloxacin activity against a norfloxacin-resistant strain of *S. aureus*. It also reduced the minimum inhibitory concentration of norfloxacin ($MIC_{\text{norfloxacin}} = 16$ μg/cm^3) at 50 μg/cm^3 [450].

Sedanolide (**6**) displayed 100% mortality against *Panagrellus redivivus* at 25 μg/cm^3, and also against *Caenorhabditis elegans* at 50 μg/cm^3. Senkyunolides N (**52**) and J (**33**) also showed nematicidal effects against *P. redivivus* at 100 μg/cm^3 [294].

(*Z*)-Ligustilide (**8**) and the semi-synthetic product [7-(methyl thioglycolyl)-(6,7-dihydro)]-(*Z*)-ligustilide (**315**) showed weak activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Sacharomyces cerevisiae*, and *Klebsiella pneumoniae*. Phthalide **8** also displayed weak antiviral activity. (*Z*)-Ligustilide has a number of electrophilic sites and can accept nucleophiles, which might explain some of the mechanisms related to these bioactivities [89].

Cytosporone E (**197**) showed activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans* [207], while corollosporine (**279**) was active against *S. aureus* [262]. The antibacterial activity of some synthetic analogs was determined. Data for *MIC* values and the minimum bactericidal concentrations (*MBC*) after 24 h against the *Helicobacter pylori* strain 11637 are listed in Table 4.

Epimers **173** and **178** containing a 5,5-spiroacetal functionality were found to be potent anti-*Helicobacter pylori* derivatives. The (2″*R*) diastereomer **464** showed less potent bioactivity than the (2″*S*) isomer **465** [451].



Spirolaxine (**186**) and sporotricale (**187**) showed specific activity against *Helicobacter pylori*. Awaad et al. also found that (–)-β-hydrastine (**383**), inhibited the growth of *H. pylori* (*MIC* 100 μg/cm^3) [452]. Marilone A (**203**) exhibited

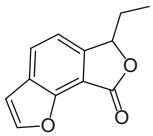
Table 4 Efficacy of some phthalides against *Helicobacter pylori*

Compound	*MIC*/μg cm^{-3}	*MBC*/μg cm^{-3}
CJ-13,102 (**176**)	1.25	2.5
CJ-13,104 (**178**)	12.5	50
CJ-13,108 (**179**)	10	10–20
CJ-13,015 (**175**)	2.5	5
CJ-12,954 (**173**)	0.02	0.02
CJ-13,014 (**174**)	0.02	0.02
Spirolaxine (**186**)	0.2	>2
464	0.5	1
465	0.125	0.125

antiplasmodial activity (against *Plasmodium berghei*) in a dose-dependent manner ($IC_{50} = 12.1$ μ*M*) [214].

Microsphaerophthalide B (**228**) and microsphaerophthalide F (**232**) both displayed activity against *Microsporum gypseum* SH-MU-4 and *Cryptococcus neoformans*, respectively, both with a *MIC* value of 64 μg/cm^3. Compound **232** showed weak activity against *M. gypseum* (*MIC* = 200 μg/cm^3) [180].

(*Z*)-Butylidenephthalide (**3**), (*S*)-butylphthalide (**4**), (*Z*)-ligustilide (**8**), and (*E*)-butylidenephthalide (**21**), were evaluated against *Mycobacterium tuberculosis* H_{37}Rv, and *M. bovis* BCG. All had comparable IC_{50} values, ranging from 200 to 250 mg/dm^3 [453]. (±)-Concentricolide (**466**) inhibited the cytopathic effect ($EC_{50} = 0.31$ μg/cm^3) induced by HIV-1 in C8166 cells [454].



466 ((±)-concentricolide)

7.11 Herbicidal and Antifungal Effects on Plant Pathogens

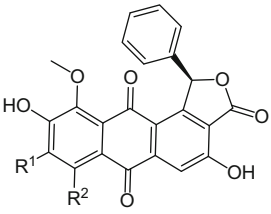
The search for agents with phytotoxic and antifungal activities is relevant to the control of weeds in agricultural crops. Convolvulanic acid B (**189**) was found to be a potent phytotoxic substance, inhibiting growth (100%) and chlorosis of *Lemna paucicostata* plants at concentrations of 5.9×10^{-4} and 3.5×10^{-4} *M*, respectively. Convolvulanic acid A (**188**) and convolvulol (**190**) also inhibited the growth of *L. paucicostata*, in turn, by 80% and 50% [201]. Phthalide **201** showed antifungal activity against *Gaeumannomyces graminis* var. *tritici* and *Cladosporium herbarum*. Compound **202** also displayed activity against *Cladosporium herbarum* [352].

Phthalides **199**, **206**, **208**, and porriolide (**200**) were evaluated for their activity against *Fusarium graminearum*, *Botrytis cinerea*, and *Phytophthora nicotianae* (see Table 5). Porriolide (**200**) was found to be the most active phthalide, with *MIC* values comparable to ketoconazole, which was used as a reference compound [211].

Table 5 Activities of phthalides against some fungal plant pathogens

Compound	*MIC*/μg cm^{-3}		
	F. graminearum	*B. cinerea*	*P. nicotianae*
206	6.3	6.3	6.3
208	6.3	12.5	6.3
199	3.1	25	6.3
200	3.1	6.3	6.3

Some phthalides alter the development of plants and fungi. Thus, rubralides A (**268**) and B (**269**) inhibited the root growth of *Lactuca sativa* at 100 mg/dm^3 [255], cryphonectric acid (**194**) influenced the formation of tomato seedlings at 100 μ*M* [204], basidifferquinones A (**467**), B (**468**), and C (**469**) induced fruiting-body formation of *Favolus arcularius* [455, 456], and isopestacin (**193**) had an inhibitory effect against *Pythium ultimum*, a plant pathogenic oomycete [203].



	R^1	R^2
467 (basidifferquinone A)	Me	OH
468 (basidifferquinone B)	H	OH
469 (basidifferquinone C)	H	H

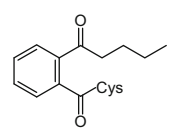
7.12 Bioavailability and Routes of Administration

The majority of previous studies have analyzed the effects of (*Z*)-butylidenephthalide (**3**) and (*Z*)-ligustilide (**8**), with reports of the low bioavailability of these compounds.

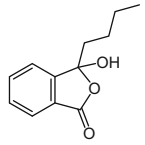
The absorption, distribution, metabolism and excretion of isotopically labeled (*Z*)-butylidenephthalide (**3**) after hot or cold dermal administration have been evaluated. Compound **3** was subsequently detected in the liver, bile, and kidney at 0 h, and in the intestinal contents at 4 h. Radioactivity was maximal at 0 h in the skin and plasma (and then decreased, $t_{1/2}$ 0.5–1 h), and was sustained in the liver, bile, and kidney until 1 h, and thereafter accumulated in the small and large intestines, cecum and its contents, reaching maximal values 1–2 h later. Altogether, 70% of the unaltered or metabolized (*Z*)-butylidenephthalide (**3**) was captured from the urine at 8 h, increasing to 80% within 24 h; only 5% was excreted into the feces within 24 h. The cysteine conjugate **470** was detected in both the urine and feces. It was demonstrated that (*Z*)-butylidenephthalide (**3**) immediately permeated through the skin into the circulatory system [457].

Multiple types of pharmacokinetic studies on (*Z*)-ligustilide (**8**) have been conducted. After intravenous (i.v.) administration, compound **8** (15.6 mg/kg) exhibited extensive distribution through the body (V_d 3.76 L/kg), with rapid elimination from the plasma ($t_{1/2}$ 0.31 h). When (*Z*)-ligustilide (**8**) was administered intraperitoneally (i.p.) at a low dose (26 mg/kg), it was rapidly absorbed (T_{max} 0.05 h) and eliminated ($t_{1/2}$ 0.36 h), with i.p. bioavailability estimated to be 52%, which indicated an extensive hepatic first-pass metabolism. At a higher dose (52 mg/kg), the bioavailability was 98%, suggesting nonlinear and dose-dependent pharmacokinetics. In the case of oral administration, pharmacokinetic parameters could only be obtained at a concentration of 500 mg/kg. Compound **8** was found to be rapidly absorbed (C_{max} 0.66 μg/cm^3), with oral bioavailability established at

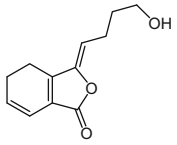
2.6%. Eight metabolites were identified, among them (*Z*)-butylidenephthalide (**3**), senkyunolides I (**22**) and H (**26**), and 3-hydroxybutylphthalide (**471**), as well as 11-hydroxyligustilide (**472**), **473**, and **474**. All metabolites were generated by NADPH-dependent monooxygenases [458]. Ding et al. also evaluated metabolite production after the oral administration of compound **8** (200 mg/kg), and obtained similar results to those obtained by Yan et al. [458], in addition to characterizing the metabolites **28**, **475**, and **476** [459].



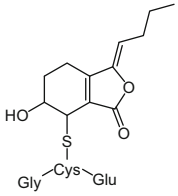
470 (cysteine conjugate)



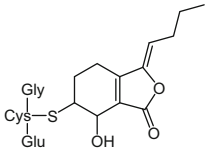
471 (3-hydroxybutylphthalide)



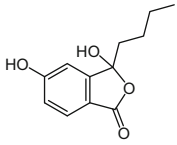
472 (11-hydroxyligustilide)



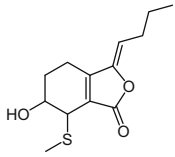
473 (isomer of hydroxyligustilide glutathione conjugate)



474 (isomer of hydroxyligustilide glutathione conjugate)



475 (3,5-dihydroxy-3-butylphthalide)



476 (6,7-dihydro-6-hydroxy-7-sulfomethylligustilide)

Compound **8** has a neuroprotective effect (see above) with a rapid onset of action following direct transport from the nasal cavity to the central nervous system (CNS). Phthalide **8** was administered to each rat nostril at 45 mg/kg, and brain tissues were collected at sequential periods of time (5–240 min) after administration. HPLC analyses of the brain tissue homogenates, together with a pharmacokinetic study, showed that **8** could be detected 5 min after administration. It was concluded that intranasal administration of (*Z*)-ligustilide (**8**) could have a rapid effect and might be more effective in the treatment of acute CNS diseases [460].

The use of nano-emulsions as a strategy to increase bioavailability is under active consideration. For instance, the anti-inflammatory effects (endotoxin-induced uveitis in rats) of orally administered (*Z*)-ligustilide (**8**) versus a nano-emulsion of ligustilide (LIGNE) were evaluated. The emulsion improved absorption given that (*Z*)-ligustilide (**8**) (20 mg/kg) was not detectable in plasma, while LIGNE remained detectable for up to 1.5 h. The nano-emulsion also improved the anti-inflammatory effect of **8** [461].

A complex of (*Z*)-ligustilide (**8**) and hydroxypropyl-β-cyclodextrin (LIG/HP-β-CD) was also prepared and quantified in rat plasma; its absolute bioavailability was found to be higher than that for (*Z*)-ligustilide (**8**) alone [462].

The pharmacokinetics of noscapine (**128**) were evaluated in male and female mice following oral (75, 150, and 300 mg/kg) and i.v. (10 mg/kg) administration. Noscapine (**128**) was easily absorbed (C_{max} 13.37, 24.48 and 49.47 μg/cm^3 in male mice, and 12.18, 22.00, and 44.00 μg/cm^3 in female mice). The AUC_{last} at 75 and 150 mg/kg were similar, but at 300 mg/kg were threefold higher, which suggested a nonlinear or saturable behavior. The $t_{1/2}$ values were similar at 75 and 300 mg/kg, but were lower at 150 mg/kg for both male and female mice. After i.v. administration, **128** was almost undetectable after 3–4 h of infusion; the $t_{1/2}$ values were 0.39 and 1.05 for males and females, respectively. It was shown that noscapine (**128**) was absorbed rapidly, and widely distributed at all doses [426].

8 Concluding Remarks

Studies on the occurrence of phthalides in Nature suggest that they are mainly confined to several higher plant families, fungi, lichens, and liverworts, with some sections of the Apiaceae plant family providing the major natural sources of these compounds. Structural analogues of (*Z*)-ligustilide and their dimers, together with mycophenolic acid analogues, could be considered as chemical markers for plant and fungal phthalides, respectively.

Chemical derivatization studies on monomeric and dimeric phthalides have demonstrated that their distinct chemical reactivities could be explained in terms of specific stereoelectronic characteristics and relative instabilities.

In the future, new analytical techniques will accelerate the structural characterization of additional minor compounds from different natural sources, establishing their interactions with macromolecular receptors and their metabolism as xenobiotic agents.

Synthesis strategies for phthalides have evolved from linear preparations to convergent ones that include efficient enantiodifferentiated reactions using new catalysts. It is foreseeable that progress in the chemistry of phthalides will focus on the exploration of their chemical and biological spaces by means of greener methodologies, including more efficient syntheses and bioassays.

Phthalides have been extensively evaluated in terms of their bioactivity, with a considerable recent literature being available on this topic. For instance, mycophenolic acid analogues are commercially available immunosupressants prescribed for autoimmune diseases, with other applications under study. Many natural phthalides display a variety of biological activities, and, in the case of compounds from the Apiaceae, most agree with the traditional medicinal uses of their natural plant sources. It has been stated that "phthalides are responsible for numerous bioactivities; however their exact mode of action is not yet realized. . ." [2]. One would envisage that future efforts to investigate the biological activities of phthalides, particularly in terms of neurological diseases, might show considerable promise.

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