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Alkamides: a critical reconsideration of a multifunctional class of unsaturated fatty acid amides

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Abstract Alkamides are natural products formed by connecting straight-chain, mostly unsaturated, aliphatic acids with various amines by an amide linkage. More than 300 derivatives are known from eight plant families consisting of various combinations of 200 acids with 23 amines. Apart from a few saturated derivatives alkamides with unsaturated acid parts are grouped into compounds with purely olefinic patterns and those with olefinic and acetylenic linkages. Derived from C_{18} oleic acid the acid parts are modified either by chain elongations to C_{28} or by oxidative shortenings to C_4 acid residues. Substrate and regiospecific desaturases and acetylenases are responsible for their characteristic patterns of unsaturation. Amine parts are derived from various amino acids by decarboxylation. Beside the widespread isobutylamines alkamides with six- and five-membered ring amines and those with phenylalanine derived amines are characteristic for the Asteraceae and Piperaceae while benzylamines are restricted to the Brassicaceae. Within the Asteraceae 2-methylbutylamine distinguishes the tribe Heliantheae from Anthemideae characterized by ring amines. Alkamides with elongated olefinic acid parts are mainly found in Piperaceae and Brassicaceae while acetylenic acid parts are typical for Asteraceae. A wide variety of biological activities ranges from the characteristic pungent/tingling property and high insecticidal toxicity to significant antifungal, antibacterial, antiprotozoal, molluscicidal, cercaricidal, and acaricidal activity. They also act as plant growth-promoting substances. Position and stereochemistry of the double bonds are essential for the different qualities of the pungent taste. Medically alkamides possess anti-inflammatory and analgesic properties and are responsible for immunomodulatory and cannabinomimetic effects.

Keywords Structural diversity · Biogenetic trends · Chemotaxonomy · Biological activities · Structure–activity relationships

Introduction

Historically, the interest in alkamides comes from the unique form of tingling pungency of some derivatives that is distinct from that produced by capsaicin from chili peppers, *Capsicum annuum* L., and piperine from black pepper, *Piper nigrum* L., and is usually accompanied by local anesthesia and salivation. Thus, plants producing these compounds, mainly species of the genera *Acmella* (*Spilanthes*), *Anacyclus*, *Echinacea*

Dedicated to my teacher and friend the late Professor Robert Hegnauer, University of Leiden, The Netherlands.

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and *Heliopsis* of the Asteraceae, and *Zanthoxylum* of the Rutaceae family were used medicinally since ancient times, particularly in cases of toothache. The active material was first obtained in the crude state from the flower heads of *Acmella oleracea* (L.) R. K. Jansen (published as *Spilanthes oleracea* Jacquin) by Gerber (1903), who designated it spilanthol (**92a**). The pungent principle from the roots of *Anacyclus pyrethrum* (L.) Link, used medicinally under the names pellitory root or Pyrethri radix, was extracted by Gulland and Hopton (1930) and named pellitorine (**101a**). Moreover, insecticidal activities were reported for all these plant species which were attributed to the accumulation of alkamides (for ref. Greger 1984, 1988; Boonen et al. 2012a; Rios 2012). In Asia the fruits of various *Zanthoxylum* species, known as "hua jiao" in China or "sansho" in Japan, are widely used as spice because of the distinctive tingling taste caused by alkamides (Yang 2008; Menozzi-Smarrito et al. 2009). This particular sensation also contributes to the use of *A. oleracea* as vegetable, known as "pará cress" or "jambú" in Brazil. Later, the interest in alkamides was greatly inspired by their anti-inflammatory and immuno-modulatory effects investigated in *Echinacea* species (Gertsch et al. 2004, 2006; Woelkart and Bauer 2007; Ardjomand-Woelkart and Bauer 2014). In addition, various promising pharmacological activities of the lipophilic fraction of the methanolic extract of *Lepidium meyeni* Walp. of the Brassicaceae family, known as Peruvian ginseng or "maca", may at least partly be attributed to the presence of species-specific alkamides and related olefinic fatty acids (Zhao et al. 2005; Wang et al. 2007).

As reviewed previously alkamides are regarded as a distinct class of natural products formed by connecting straight-chain, mostly unsaturated, aliphatic acids with various amines by an amide linkage (Greger 1984, 1988). As derivatives of the straight-chain fatty acid synthesis the acid parts differ biosynthetically from structurally similar but branched carbon chains from amides of the genera *Capsicum* of the Solanaceae (Curry et al. 1999; Aza-González et al. 2011; Kehie et al. 2015) or *Ipomoea* and *Merremia* of the Convolvulaceae family (Tofern et al. 1999). In Piperaceae the alkamides are only known from the genus *Piper*, where they are frequently accompanied by so-called piperamides, another class of amides, differing by an aromatic ring in the acid moiety (Parmar et al. 1997; Do Nascimento et al. 2012; Dawid et al. 2012). From the various amides of the Rutaceae fatty acid derived alkamides were mostly found in the genus *Zanthoxylum*, but were also reported for *Tetradium daniellii* (Reisch et al. 1985) and *Pilocarpus trachylophus* (Andrade-Neto et al. 1996). The other amides of this family are characterized by different acid parts derived from cinnamic acid (Mester 1983; Kubo et al. 1984; Riemer et al. 1997), cysteine, anthranilic or isovalerianic acid (Hofer et al. 1995; Greger et al. 1996). The biogenetic origin of the different amine parts of the alkamides was not clarified fully to date, but they are most likely derived from amino acids by decarboxylation and, sometimes, by additional transformation processes (Greger 1984, 1988; Minto and Blacklock 2008; Cortez-Espinosa et al. 2011). Other straight-chain fatty acid amides, such as the physiologically active *N*-acylethanolamines (Kim et al. 2010) or derivatives with *N*-unsubstituted amino groups (Sittie et al. 1998; Dembitsky et al. 2000) as well as those with more complex amine rests, e.g. in the malyngamides (Ainslie et al. 1985) and conioidines (Chan et al. 1993), are derived from different biosynthetic routes and, thus, are not included in the present survey (Fig. 1).

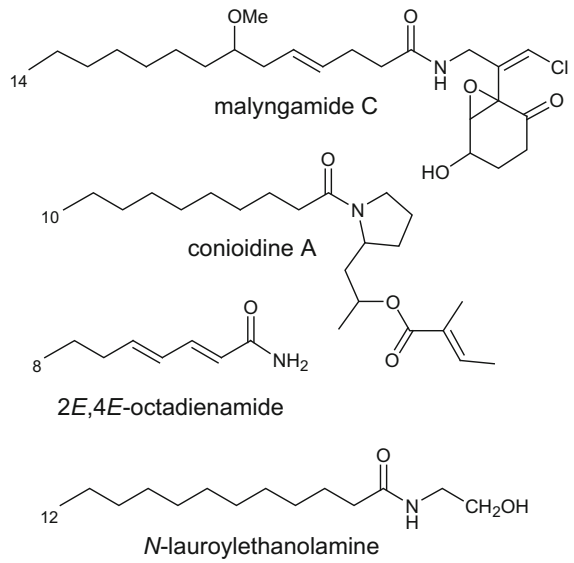


Fig. 1 Straight-chain fatty acid amides derived from different biosynthetic routes not regarded as alkamides

Following these biosynthetic considerations structures of more than 300 alkamides have been reported consisting of various combinations of 200 acid parts (Tables 1, 2, 3; Figs. 4, 6, 7, 8) with 23 amine parts (**a**–

w) (Fig. 2). Apart from a few derivatives with fully saturated acid parts, mainly isolated from *Piper* species and *L. meyeni*, alkamides with unsaturated acids fall into two groups: derivatives with olefinic patterns (Tables 1, 2) and those with olefinic and acetylenic linkages (Table 3). So far 180 alkamides with purely olefinic patterns were isolated from eight plant families, namely the Asteraceae, Piperaceae, Rutaceae, Brassicaceae, Euphorbiaceae, Aristolochiaceae, Menispermaceae, and Poaceae. The majority of the 135 acetylenic alkamides is known from the Asteraceae, where they are accumulated solely in the two tribes Anthemideae and Heliantheae (Greger 1984, 1988; Christensen and Lam 1991; Christensen 1992). Apart from the plant-derived alkamides the C_{17} acetylenic alkamide callyspongamide A (**128c**) was isolated from the Red Sea sponge *Callyspongia fistularis* (Youssef et al. 2003) and an acetylenic C_9 amide linked with the amino acid valine from the fungus *Poria sinuosa* (Cambie et al. 1963).

The wide range of biological activities of alkamides and their distribution has recently been summarized in three reviews, where, however, various classes of amides have been combined which are derived from different biosynthetic pathways (Boonen et al. 2012a; Rios 2012; Rios and Olivo 2014). Although these overviews provide a plethora of interesting data, they do not offer a biologically orientated evaluation of the structural diversity of the straight-chain fatty acid derived alkamides and its chemotaxonomic significance. A simple schematic of the many chemical structures of these amides would greatly help to grasp the biogenetic background of the various differentiation processes. However, their long carbon chains combined with different patterns of double and triple bonds complicate a structural overview of a greater number of derivatives. Here, simple formula depictions are presented to comprehend many derivatives and their possible structural relationships at a glance. Particular attention was drawn to the different carbon chain lengths of the acid residues as well as the characteristic clustering of olefinic and acetylenic linkages, whereas the different amine parts are indicated by small letters only (Fig. 2; Tables 1, 2, 3). This comprehensive overview of more than 300 different structures should serve as a broad-based summary to establish specific biogenetic trends and as a guide for exploiting these compounds for further pharmaceutical development, pest control, or uses in taste and food chemistry.

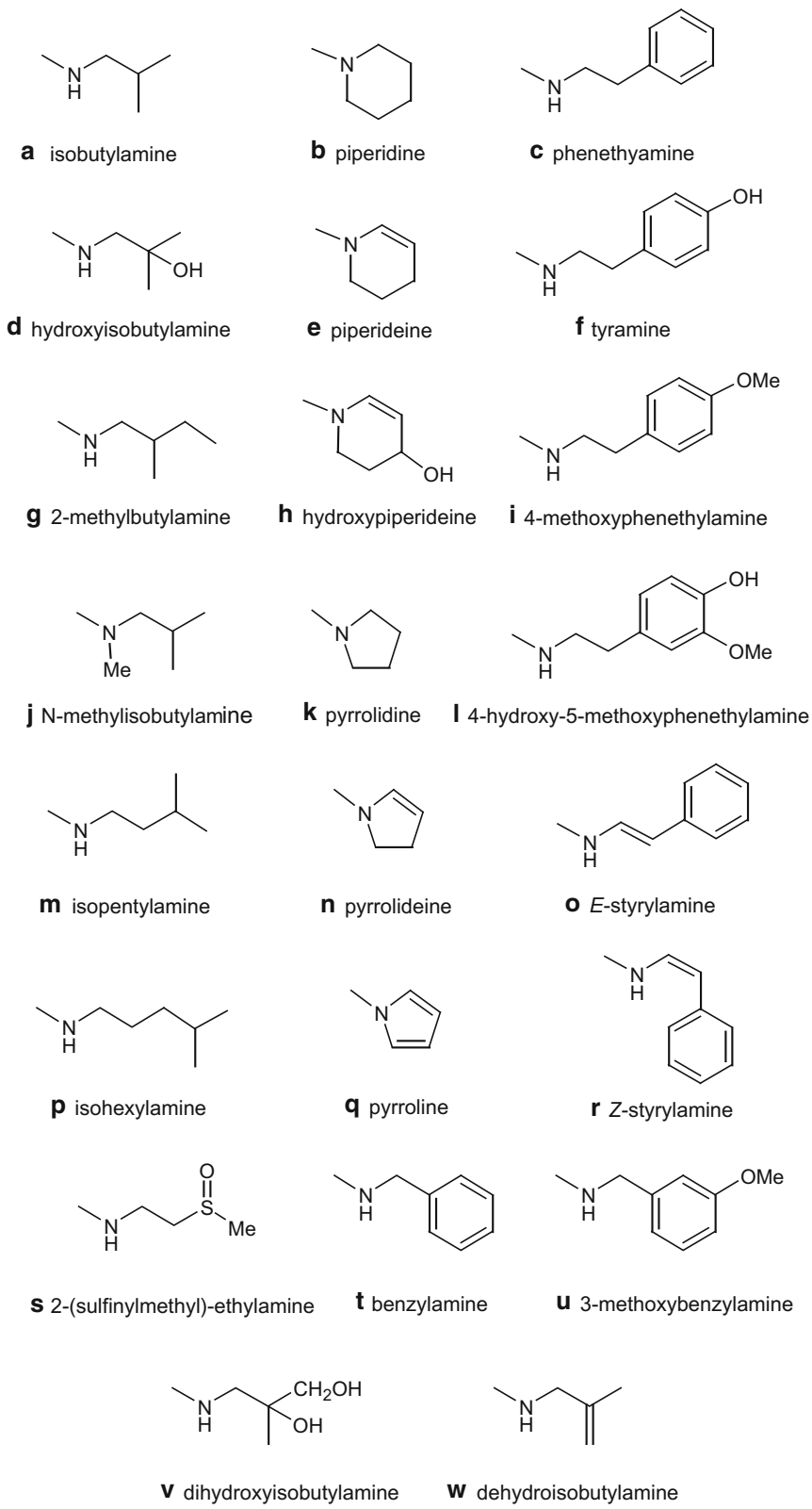
Structural relationships

Amine parts

The amine parts of the alkamides are most likely derived from various amino acids by decarboxylation (Bohlmann and Zdero 1973; Greger 1984, 1988; Cortez-Espinosa et al. 2011). On the basis of the present overview isobutylamine (**a**) is shown to be the most widespread amine part, followed by piperidine (**b**) and pyrrolidine (**k**), and, less frequently, phenethylamine (**c**), hydroxyisobutylamine (**d**), 2-methylbutylamine (**g**), isopentylamine (**m**), and benzylamine (**t**). The remaining amines shown in Fig. 2 are known only in a few, sometimes even only in a single amide: i.e. **37p**, **58w**, **101h**, **101i**, and **101s** (Tables 1, 2, 3). Biosynthetically, the amino acid valine was regarded as precursor of isobutylamine (**a**), dihydroxyisobutylamine (**v**), and dehydroisobutylamine (**w**). A possible biosynthetic intermediate was found in the Basidiomycete fungus *Poria sinuosa* Fr., where a nona 2*E*-en-4,6,8-triynoic acid is linked with valine without decarboxylation (Cambie et al. 1963). The formation of piperideine (**e**) was suggested to be derived from lysine (Bohlmann and Zdero 1973) and a similar way may be expected for the other six-membered ring amines piperidine (**b**) and hydroxypiperideine (**h**). Correspondingly, ornithine can be regarded as precursor of the five-membered ring of pyrrolidine (**k**), pyrrolideine (**n**), and pyrroline (**q**). However, decarboxylation of a proline derivative also leads to pyrrolidines (Strunz 2000). Isopentylamine (**m**) and 2-methylbutylamine (**g**) are most likely derived from leucine and isoleucine, and the aromatic amines, at least those with a phenylpropanoid unit (**c**, **f**, **i**, **l**, **o**, **r**), can be interpreted as derivatives of phenylalanine or tyrosine, respectively (Cortez-Espinosa et al. 2011). The biosynthetic origin of the rare isohexylamine (**p**), found in *P. nigrum* (Siddiqui et al. 2004), remains unclear, whereas the only sulfur-containing 2-(sulfinylmethyl)-ethylamine (**s**), detected in *P. boehmeriifolium* (Tang et al. 2011), suggests to be derived from cysteine.

Regarding the wide distribution of isobutylamine (**a**) as part of the alkamides of seven from eight different plant families its absence in *L. meyeni* of the Brassicaeae deserves special attention. The divergent position of *Lepidium* alkamides, known as macamides, is also indicated by the specific formation of

Fig. 2 Various amine parts of alkamides



benzylamines (**t**, **u**) (Wang et al. 2007). The alkamides isolated from the Aristolochiaceae, Menispermaceae, and Poaceae are exclusively characterized by an isobutylamine residue (**a**). With the exception of a tyramine residue (**f**) in three amides of *Zanthoxylum gilletii* (Wansi et al. 2009), isobutylamine (**a**) is also predominating in the Rutaceae. However, the fruits of *Zanthoxylum* species deviate by an additional hydroxylation leading to an accumulation of hydroxyisobutylamine (**d**) and sometimes even to dihydroxyisobutylamine (**v**). The greatest diversity of different amine parts was shown in the Asteraceae and Piperaceae, generated primarily by the additional presence of six- (**b**, **e**, **h**) or five-membered ring amines (**k**, **n**, **q**), respectively. In addition, both families were shown to contain aromatic amine parts with phenylpropanoid structures (**c**, **f**, **i**, **l**, **o**, **r**). Within the Asteraceae the presence of 2-methylbutylamine (**g**) distinguishes the tribe Heliantheae from the Anthemideae. The latter, by contrast, is characterized by the frequent formation of piperidine (**b**, **e**, **h**) and pyrrolidine (**k**, **n**, **q**) derived amines, which were not found so far in the Heliantheae (Greger 1984, 1988; Christensen and Lam 1991; Christensen 1992). Especially some species of the genus *Achillea* were shown to be a rich source of amides containing these ring amines, from which the formation of pyrrolideine (**n**) even appears to be restricted to the genus (Bohlmann and Zdero 1973; Greger et al. 1982, 1983, 1987b; Hofer et al. 1986; Greger and Hofer 1989) (Fig. 9).

Acid parts

The acid parts are grouped together in Tables 1, 2, 3 in order of different carbon chain lengths. Most of them are regarded as derivatives of C_{18} oleic acid formed by various oxidative degradations leading to chain shortenings at the carboxyl site as well as at the terminal methyl end (Bohlmann and Dallwitz 1974) (Tables 2, 3). By contrast, fatty acid elongations up to C_{26}[1] lead to the alkamides listed in Table 1. Apart from different chain lengths the acid parts are characterized by different patterns of double and triple bonds formed by successive desaturase and acetylenase activities, respectively, frequently accompanied by isomerization (Minto and Blacklock 2008). Only a few amides are known with fully saturated acid parts (Tables 1, 2, 3). Generally, double bonds are frequently formed in conjugation with the carboxyl group mostly showing *E*-configuration. Further patterns of unsaturation along the carbon chain are often separated by a dimethylene bridge. Whereas most of the 117 saturated and olefinic acid parts, listed in Tables 1 and 2, are terminated with a methyl end, some deviate by an oxidation of the terminal carbon atom: i.e. the C_{10} derivatives **93**, **94**, **97**, **98**, the C_6 derivatives **115**, **116**, and the C_4 derivative **117**. Insertion of oxygen at other positions results in the formation of keto-acids, frequently found in the C_{18} series of saturated and olefinic alkamides, and various hydroxylations mainly in the olefinic C_{12} and C_{10} series. Oxygenation also leads to cyclisation products (**83**, **100**, **189**) and endoperoxide rings (**80**, **81**) (Devkota et al. 2013) (Table 2), as well as the unique pipercycliamide shown in Fig. 4 (Wei et al. 2004).

Most of the acetylenic alkamides listed in Table 3 are derived from oleic acid by chain shortening from C_{18} to C_9 (Greger 1984, 1988). In this group successive oxidative degradations at the methyl end frequently lead to a terminal acetylenic hydrogen, a widespread feature of acetylenic alkamides, which can easily be detected in IR-analysis by strong signals at 3.306–3.308 cm^{-1} in CCl_4. A graphical comparison of IR-spectra from various alkamides was presented previously (Greger 1985). Although the thiophenes **174** and **184** do not show acetylenic linkages, they are included in Table 3 due to biosynthetic considerations. As indicated by feeding experiments with ^{14}C labelled compounds thiophene **174**, named otanthic acid, is formed by incorporation of H_2S into the C_{11} diyne **167** (Bohlmann et al. 1974). An acetylenic C_{13} precursor was suggested for the formation of thiophene **184** (Bohlmann et al. 1973).

Biosynthesis of the acid parts

The acid parts of the alkamides are regarded as products of fatty acid synthases, which append in a head-to-tail fashion malonyl units to a growing acyl chain (Minto and Blacklock 2008). Although limited studies exist on the biosynthesis of alkamides, the double and triple bonds of the acyl chains are most likely formed by similar enzymatic activities known from naturally occurring olefinic and acetylenic fatty

[1] In addition, a saturated C_{28} tyramide (**f**) has been isolated from *Zanthoxylum gilletii* (Wansi et al. 2009).

acids. In this case substrate and regiospecific desaturases and acetylenases, respectively, were shown to be responsible for the characteristic patterns of unsaturation (Cahoon et al. 1997, 2001; Shanklin and Cahoon 1998; Uttaro 2006; Minto and Blacklock 2008). On the basis of feeding experiments with *Echinacea purpurea* Bohlmann and Dallwitz (1974) already showed the biosynthetic connection between C_{18} oleic acid and acetylenic alkamides of the C_{14}, C_{12}, and C_{11} series: starting with the C_{18} acetylenic crepenynate pathway two β-oxidations at the carboxyl site lead to anacyclin (**141a**), which is further shortened to **160a** and **167a** by successive oxidative degradations at the methyl end. The co-existence of various crepenynic acid derived C_{18} amides in *Achillea lycaonica* and *A. chamaemelifolia* suggested possible biosynthetic connections (Greger et al. 1982, 1987a), indicating successive desaturase (**118b**) and acetylenase activity (**119b**), as well as the elimination of the terminal propyl group by oxidative degradations (**136b**) (Fig. 3). The insertion of the Δ^2-double bond in **120b**, **122b** and **136b** can be interpreted as a result of oxidation and dehydration. The formation of the conjugated 8,10-diene-12-yne system in **122b**, interpreted as allylic oxidation and rearrangement by Bohlmann et al. (1973), is possibly catalyzed by a specific conjugase activity (Cahoon et al. 1999) (Fig. 3). This characteristic group of unsaturation was also reported for C_{18} isobutylamides from *Heliopsis buphthalmoides* and *H. helianthoides* (**124a**–**127a**) (Bohlmann et al. 1983; Jakupovic et al. 1986), and a series of C_{16} amides with different amine parts (**132k**, **133a**,**k**,**n**,**q**; **134a**) from *Achillea ageratifolia* (Greger et al. 1983, 1987b). Besides acetylenic C_{18} piperidides, *A. lycaonica* was shown to accumulate also olefinic and saturated C_{18} piperidides (**30b**, **31b**,**k**; **32b**) and pyrrolidides (**20b**,**k**) as major compounds. Biosynthetically, they were interpreted either as more primitive

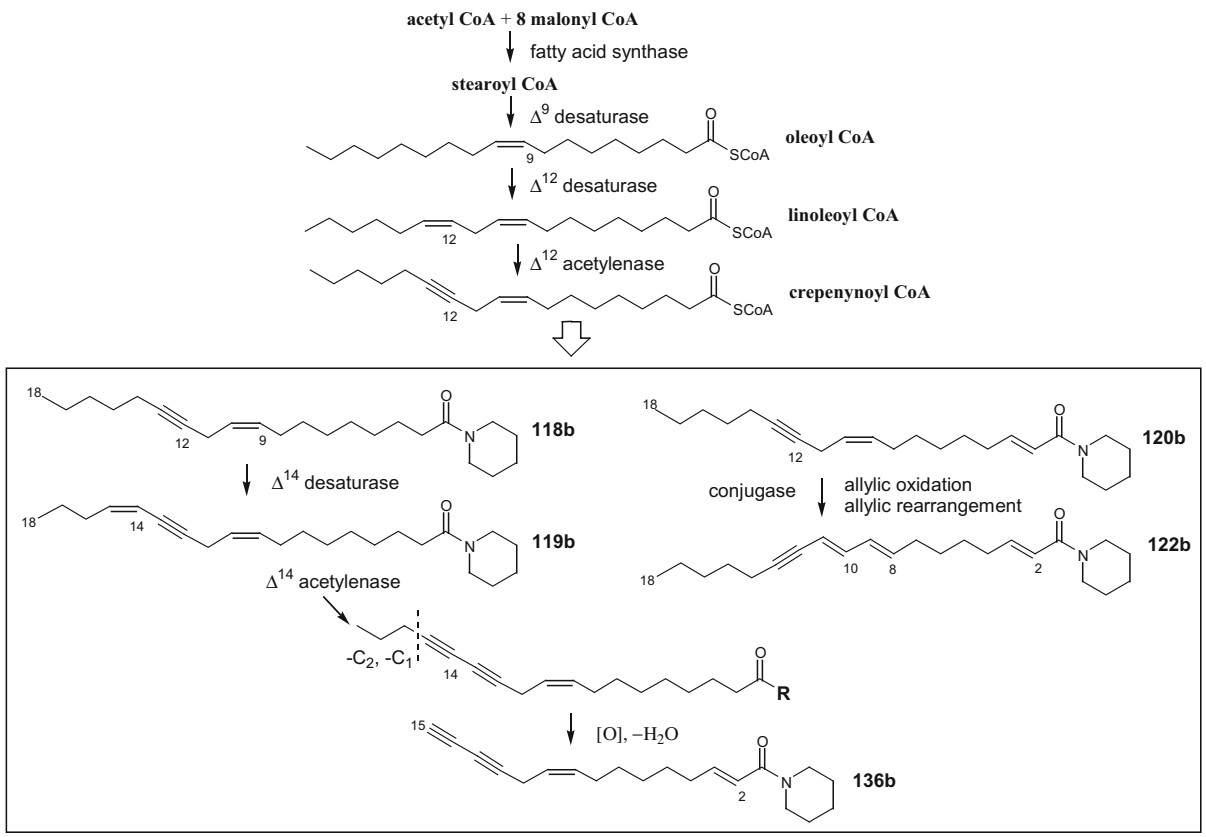


Fig. 3 Proposed biosynthetic connections of five piperidides co-occurring in *A. lycaonica*

or, with regard to the keto-acids **20b**,**k** and **31b**,**k** as hydrogenation products of formerly more unsaturated derivatives (Greger et al. 1982, 1987a). With respect to the co-existence of a series of amides with the same pattern of unsaturation in *Acmella ciliata*, Martin and Becker (1985) suggested a common biosynthetic sequence for C_{12}, C_{10}, and C_8 olefinic amides derived from linolenic acid. On the basis of the present overview some general conclusions can be drawn as to the major biosynthetic trends of alkamide formation and their chemotaxonomic significance.

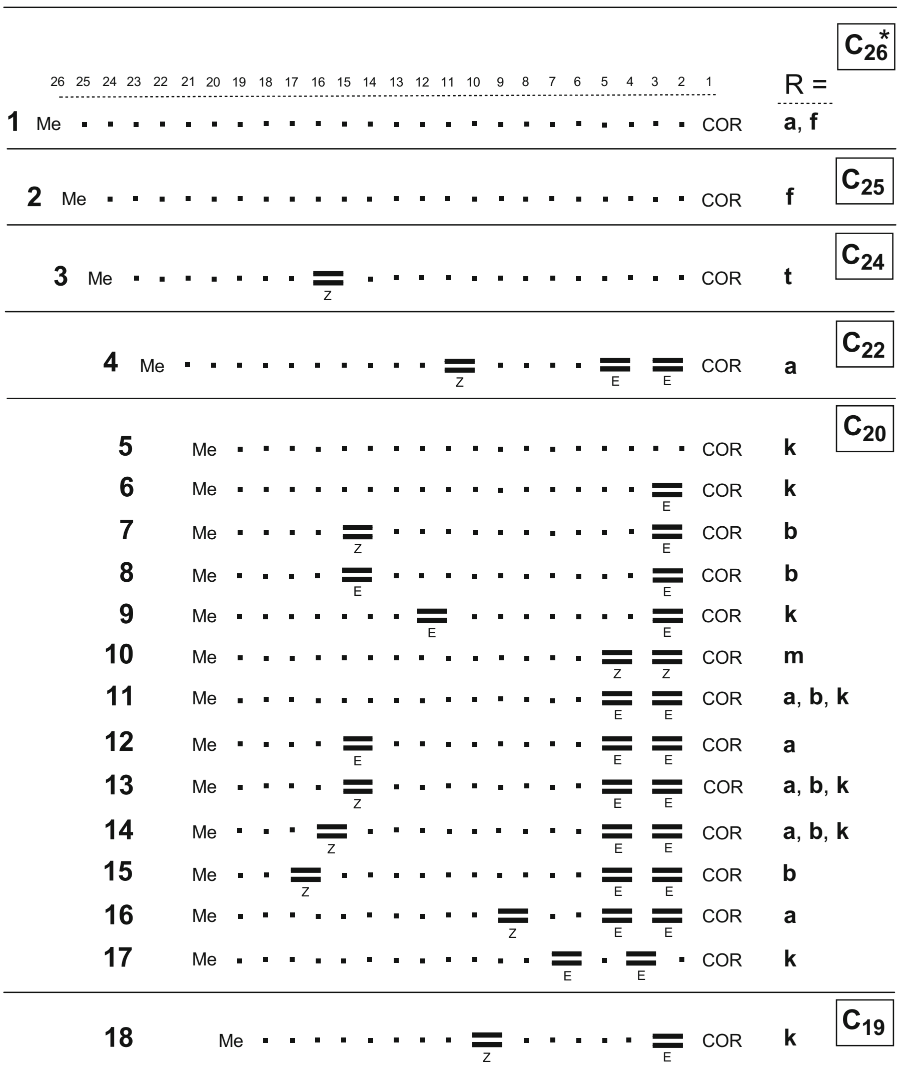
Major biosynthetic trends

Olefinic alkamides

Elongated saturated and olefinic C_{19}–C_{28} alkamides

The acyl parts of the very long-chain amides ranging from C_{19} to C_{28} (Table 1) can be regarded as products of specific desaturase/elongase activities (Leonard et al. 2004; Uttaro 2006). Apart from the C_{28} and C_{26} tyramides (**1f**) from *Z. gilletii* (Wansi et al. 2009), the

Table 1 Alkamides with elongated saturated and olefinic C_{26}–C_{19} acid chains



Amine parts indicated by small letters are listed in Fig. 2

* In addition, a saturated C_{28} tyramide (**f**) has been isolated from *Zanthoxylum gilletii* (Wansi et al. 009)

C_{25} tyramide **2f** from *Senecio erechtitoides* (Ndom et al. 2010), the C_{24} benzylamide **3t** from *L. meyeni* (Zhao et al. 2005), and the C_{20} isobutylamide **11a** from *Mallotus lianus* (Jiang et al. 2009), the remaining elongated alkamides were isolated from *Piper* species. In this case they are mostly characterized by olefinic C_{20} acyl chains frequently accompanied by derivatives of the C_{18}, C_{16}, and C_{10} series. Compounds with longer acyl chains show a restricted distribution each known so far from single species only: the C_{26} longamide (**1a**) from *P. longum* (Koul et al. 1988), the C_{22} filfiline (**4a**) from *P. retrofractum* (=*P. officinarum*) (Gupta et al. 1976), and the C_{19} brachystine (**18k**) from *P. brachystachyum* (Koul et al. 1988). The patterns of unsaturation in the elongated acid parts are mostly characterized by one or two *E*-configurated double bonds conjugated with the carboxyl group together with a further single double bond located at different positions along the acyl chain. As shown in Table 1 no acetylenic linkages are formed in this group.

Saturated and olefinic C_{18} alkamides

The C_{18} alkamides are separated into two groups comprising saturated and olefinic derivatives in the first (Table 2), and acetylenic derivatives in the second (Table 3). Many derivatives of the first group were isolated from *Piper* species (for ref.: Parmar et al. 1997; Do Nascimento et al. 2012) (Table 4). Similar patterns of unsaturation suggest close biosynthetic connections with the elongated amides mentioned above, but also with those of the C_{16} series. Octadeca-2*E*,4*E*,12*Z*-trienoic acid derived amides were isolated from *P. retrofractum* (**37a**) (Kikuzaki et al. 1993), *P. nigrum* (**37a**,**k**,**p**) (Siddiqui et al. 2004), and *P. longum* (**37**?) (Sun et al. 2007), but were also detected in *M. lianus* of the Euphorbiaceae (**37a**,**b**) (Jiang et al. 2009). By contrast, C_{18} alkamides of *L. meyeni* clearly deviate by different patterns of unsaturation in the acid parts, lacking Δ^2-, Δ^4-double bonds (**19**, **21**, **24**–**29**), as well as by specific combinations with the benzylamines **t** and **u**. Otherwise, olefinic acid parts containing only these two Δ^2-, Δ^4-double bonds are widespread occurring with various chain lengths in different plant families and genera (Table 2). However, further patterns of unsaturation along the acyl chains, mostly separated by a dimethylene interruption, represent characteristic biosynthetic trends of restricted distribution.

Saturated and olefinic C_{16} alkamides

Apart from the two saturated hexadecanoic acid amides **41t** and **41u** from *L. meyeni* (Zhao et al. 2005), most of the C_{16} amides listed in Table 2 were found in *Piper amalago* (**41k**, **42k**, **43k**) and *P. retrofractum* (**46a**, **47a**, **48a**). It is interesting to note that all twelve alkamides detected in the root extract of the former were shown to be pyrrolidides (Achenbach et al. 1986), whereas in the fruit extract of the latter isobutylamides prevail (Kubo et al. 2013). The hexadeca-2*E*,7*Z*-dienoic acid pyrrolide (**44q**) and hexadeca-2*E*,9*Z*,12*Z*,14*E*-tetraenoic acid isobutylamide (**45a**) show an erratic distribution known so far only from the roots of *A. ageratifolia* (Greger et al. 1987b) and flower heads of *E. purpurea* (Christensen et al. 2009), respectively.

Olefinic C_{14} alkamides

Olefinic C_{14} alkamides with Δ^2-, Δ^4-double bonds (**51a**,**b**,**d**,**k**,**f**) are widespread in different plant families. In contrast, higher desaturated derivatives were mainly found in *Zanthoxylum* species of the Rutaceae family. The co-existence of the highly unsaturated tetradecapentaenoic acid isobutylamides γ- (**58a**) and δ-sanshool (**59a**) with a series of less desaturated derivatives, ranging from dien- (**51a**, **52a**) to trien- (**53a**, **54a**), and tetraenoic acid isobutylamides (**55a**,**d**, **56a**,**d**) in *Z. bungeanum* (Xiong et al. 1997) and *Z. integrifolium* (Chen et al. 1999), suggests a common biosynthetic sequence. Alkamides with the acid residues **53** and **56** were also isolated from the propably closely related Asteraceae genera *Leucocyclus* (**53a**, **56a**) (Greger et al. 1981), *Achillea* (**53a**, **56ak**) (Greger et al. 1984, 1987b), and *Otanthus* (**53a**,**b**, **56a**,**b**) (Christodoulopoulou et al. 2005). However, the conjugated triene system in **58** and **59**, separated from the Δ^2-, Δ^4-double bonds by a dimethylene bridge, can be regarded as a characteristic chemical feature of *Zanthoxylum*. Another common chemical character of the genus is the hydroxylation of the isobutylamine part (**d**) in the fruits (Yasuda et al. 1982; Mizutani et al. 1988; Kashiwada et al. 1997) (Table 2). In *Sanvitalia ocymoides* (Asteraceae–Heliantheae) the co-existence of the unique tetradeca-2*E*,4*E*,8*Z*,10*Z*,12*E*-pentaenoic acid isobutylamide (**60a**) with a corresponding olefinic C_{14} methylester, showing the same configurations of the

five conjugated double bonds, points to a common biosynthetic origin (Domínguez et al. 1987).

Olefinic C_{12} alkamides

The accumulation of olefinic C_{12} alkamides (Table 2) represents another major biosynthetic trend of *Zanthoxylum* (Menozzi-Smarrito et al. 2009), and particularly of *Echinacea* species of the Asteraceae tribe Heliantheae (Woelkart and Bauer 2007). Moreover, they were also shown to accumulated in the genera *Asiasarum* (Yasuda et al. 1981a) and *Asarum* (Zhang et al. 2005) of the Aristolochiaceae. Like the C_{14} amides, the C_{12} derivatives of *Zanthoxylum* are characterized by a conjugated triene group near the methyl end, but deviate by possessing only one Δ^2-double bond (**72**–**74**). Their formation can be explained by an elimination of a C_2-unit from corresponding C_{14} amides by β-oxidation. This relationship is also indicated by the common vernacular names α- (**72a**) and β-sanshool (**74a**) of the C_{12} series, and γ- (**58a**) and δ-sanshool (=γ-isosanshool) (**59a**) of the C_{14} series (Fig. 7). In view of their high instability (Crombie and Tayler 1957; Yasuda et al. 1981b) derivatives with oxygen functions, isolated from the fruits of *Z. piperitum* (**75d**–**78d**) (Hatano et al. 2004), *Z. bungeanum* (**77d**, **79d**) (Huang et al. 2012), and *Z. armatum* (**75d**, **80d**, **81d**) (Devkota et al. 2013), can be regarded as products of successive oxidation and hydration processes. However, it remains unknown, whether these processes take place in the plant or during extraction procedures.

The accumulation of dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (**66a**), with only two conjugated double bonds near the methyl end, is typical for the related genera *Echinacea*, *Salmea*, and *Acmella* (*Spilanthes*), but is also known from *Asiasarum* and *Asarum*. The co-occurrence of the isomeric pair **65a/66a** with dodeca-2*E*,4*E*-dien- (**62a**) and 2*E*,4*E*,8*Z*-trienoic acid derived amides (**63a**) in *E. purpurea* (Bauer et al. 1988), *E. angustifolia* (Hinz et al. 2007), and *E. atrorubens* (Dietz and Bauer 2001), suggests a common biosynthetic sequence. From the other C_{12} isomers (**67a**–**69a**) dodeca-2*E*,4*E*,8*E*,10*Z*-tetraenoic acid isobutylamide (**67a**) was isolated from the first two *Echinacea* species (Matovic et al. 2011) and *Acmella mauritiana* (Jondiko 1986), but was also detected in *Leucanthemum hosmariense* of the tribe Anthemideae (Bohlmann et al. 1980a). The dodeca-2*E*,4*Z*,8*Z*,10*E*-tetraenoic acid isobutylamide (**68a**) was reported for *A. ciliata* (Martin and Becker 1985), *A. radicans* (Rios-Chavez et al. 2003), and for *Heliopsis longipes*, *H. procumbens*, and *H.* ex aff. *novogaliciana* (García-Chávez et al. 2004). Together with the 2*E*,4*Z*,8*Z*,10*Z*-isomer (**69a**) it was also isolated from *Asarum forbesii* (Zhang et al. 2005). A significant chemical character of *Echinacea* and *Acmella* amides is the frequent combination with 2-methylbutylamine (**g**). Due to the unusual terminal vinyl end a different biosynthetic pathway was suggested for **64a** (Table 2). It was isolated from the aerial parts of the Australian *Brachycome ciliocarpa* (Asteraceae–Astereae) (Zdero et al. 1988), and, with uncertain configuration, from the toothache grass *Ctenium aromaticum* (Poaceae) (Gamboa-Leon and Chilton 2000).

Olefinic C_{10} alkamides

The well-known C_{10} amide pellitorine (**101a**) (Crombie 1955b) is the most widespread olefinic alkamide reported for Asteraceae, Piperaceae, Rutaceae, Aristolochiaceae, Menispermaceae, and Poaceae. In the first two families the deca-2*E*,4*E*-dienoic acid (**101**) was shown to be combined with twelve different amines (Table 2). In the roots of *Cissampelos glaberrima* of the Menispermaceae the predominating **101a** was accompanied by octa-2*E*,4*E*-dienoic isobutylamide (**112a**) and traces of the more saturated 2*E*-decenoic (**89a**) and decanoic acid isobutylamide (**86a**) (Loureiro-Rosario et al. 1996). Pellitorine (**101a**) was also isolated from *Ctenium aromaticum* of the Poaceae, where it is accompanied by deca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide (**102a**), as yet only known from *Achillea* species (Gamboa-Leon and Chilton 2000). In view of the wide distribution of pellitorine (**101a**) its restricted occurrence within the Asteraceae is noteworthy. Here, it was reported so far only for the tribe Anthemideae, while in the Heliantheae olefinic C_{10} amides are mainly represented by the well-known deca-2*E*,6*Z*,8*E*-trienoic acid isobutylamide spilanthol (=affinin) (**92a**) (Crombie et al. 1963; Yasuda et al. 1980). With respect to the co-existence of **92a** with the C_{12} amide **66a** in *Acmella* species, showing a similar pattern of unsaturation (Martin and Becker 1985; Keipert and Melzig 2009; Bae et al. 2010; Sharma et al. 2011), the formation of spilanthol (**92a**) may be explained by an elimination of C_2 from **66a** by β-

oxidation. Derivatives with oxygen functions in *A. ciliata* (**88a**, **97a**, **98a**) (Martin and Becker 1985; Keipert 2009), *H. longipes* (López-Martínez et al. 2011), and *A. oleracea* (Simas et al. 2013) (**95a**, **96a**) can be regarded as oxidation products of spilanthol (**92a**). The detection of a bornylester of **92** in *H. longipes* and *H. novogaliciana* is of biogenetic interest (García-Chávez et al. 2004). Another group of oxidized C_{10} amides (**99a**,**b**, **100a**, **103b**) and the unique cyclisation product pipercycliamide was reported for the roots of *P. nigrum* together with pellitorine (**101a**) and related derivatives (**101b**,**k**, **89b**) (Wei et al. 2004). In this series the diol **99b** was separated into the two *erythro* and *threo* configurated isomers (Fig. 4). The *erythro* isomer of **99a**, named sylvamide, was isolated from the seeds of *P. sylvaticum* Roxb. (Banerji and Pal 1983).

The accumulation of various olefinic C_{10} amides represents a major biogenetic trend of the genus *Achillea* of the tribe Anthemideae. Apart from the many combinations of the widespread deca-2*E*,4*E*-dienoic acid residue (**101**) with different amine parts (**a**,**b**,**c**,**e**,**f**,**h**,**i**,**m**), deca-2*E*,4*E*,8*Z*-trienoic acid derived amides (**102a**,**b**,**e**) were shown to be typical for some *Achillea* species (Bohlmann et al. 1974; Greger 1988). However, of special chemotaxonomic significance is the formation of C_{10} amides with three and even four conjugated double bonds in the acid moiety (**105e**–**108e**) (Fig. 9). Beside a single report on the unique sencolaminic acid derived amides (**104c**,**m**) from *Senecio colaminus* (Asteraceae–Senecioneae) (Bohlmann and Zdero 1979), these highly conjugated system is typical for the taxonomically complex *Achillea millefolium* group: besides the predominating deca-2*E*,4*E*,6*Z*-trienoic acid piperideide **105e** (Bohlmann and Zdero 1973), the underground parts of different species were shown to be characterized by various accumulation trends towards the two isomeric decatetraenoic acid piperideides **107e** and **108e** (Greger and Hofer 1989; Greger and Werner 1990) (Fig. 9).

Olefinic C_8–C_4 alkamides

As products of extensive oxidative degradations the short-chain olefinic alkamides of the C_8 to C_4 series represent a small group in the Asteraceae, Piperaceae, Rutaceae, and Menispermaceae. The oxidation of the terminal carbon atom in **115c** of *Salmea scandens* (Bohlmann et al. 1985), **116a** of *A. ciliata* (Keipert 2009), **116d** (timuramide D) of *Zanthoxylum armatum* (Devkota et al. 2013), and the shortest alkamide **117a** of *Piper hancei* (Narui et al. 1995) suggests chain shortening from the methyl end. However, it should be

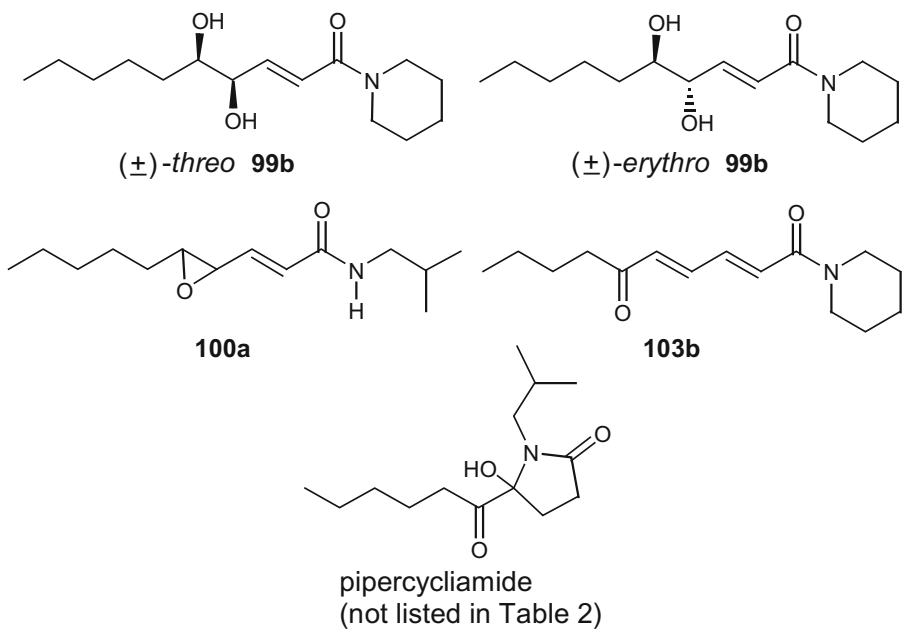


Fig. 4 Various oxidized C_{10} amides from roots of *P. nigrum* (Wei et al. 2004)

Table 2 Alkamides with saturated and olefinic C_{18}–C_4 acid chains

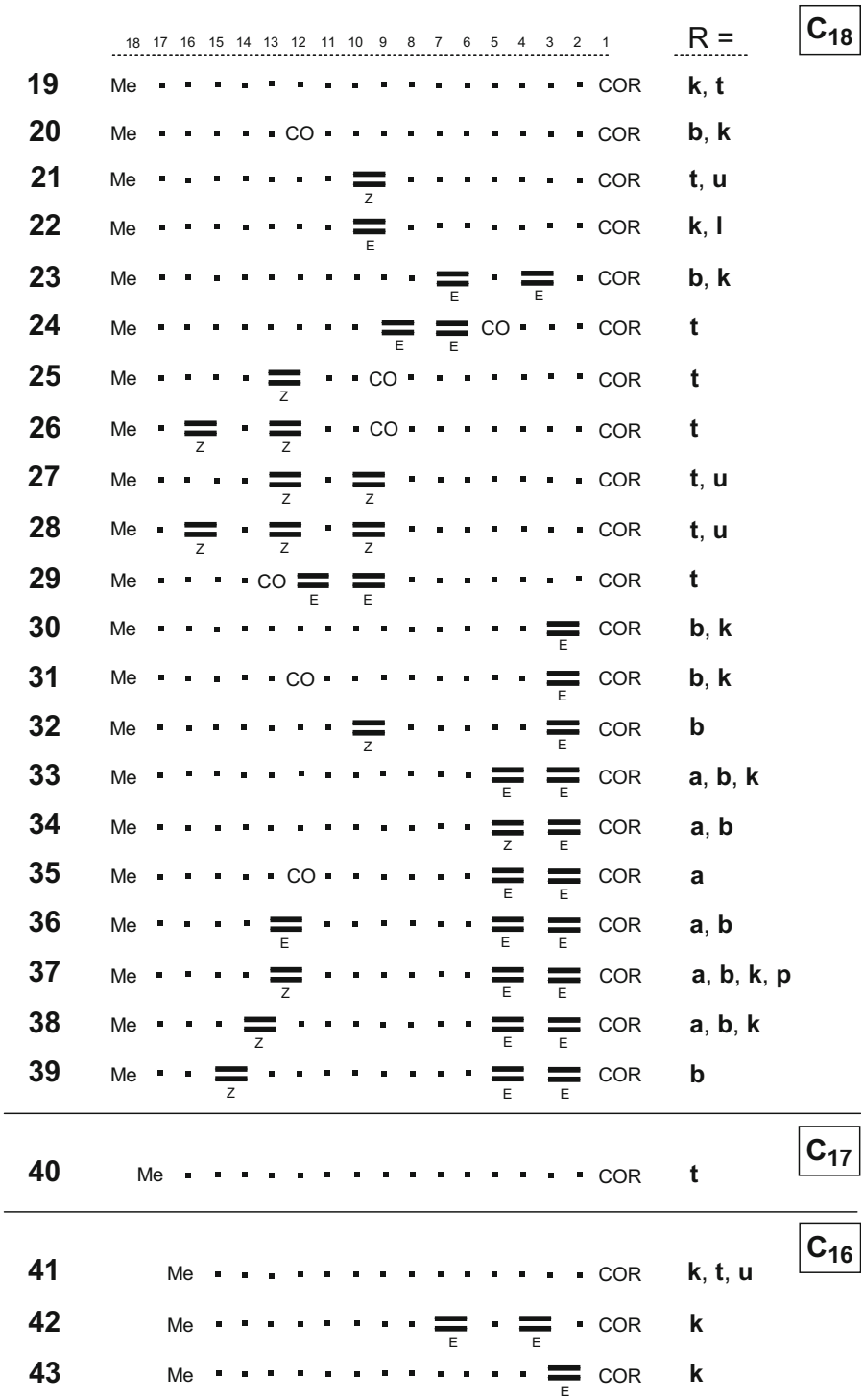


Table 2 continued

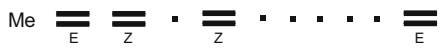
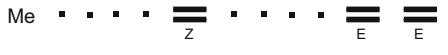
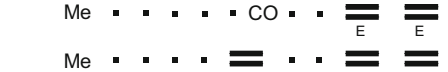
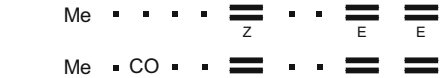
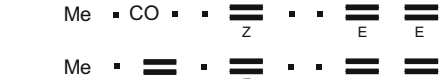
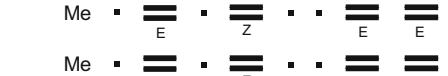
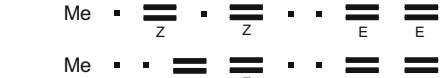
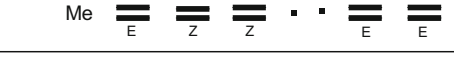
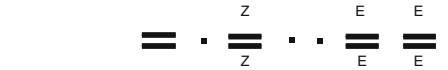
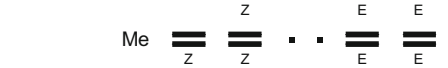
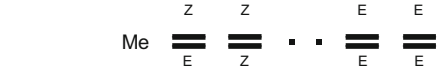
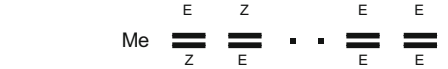
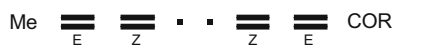
44		**q**
45		**a**
46		**a**
47		**a**, **b**
48		**a**
C15		
49		**k**, **t**, **u**
C14		
50		**b**
51		**a**, **b**, **d**, **f**, **k**
52		**a**
53		**a**, **b**, **d**
54		**a**
55		**a**, **d**
56		**a**, **b**, **d**, **k**
57		**a**
58		**a**, **d**, **w**
59		**a**, **d**
60		**a**
C13		
61		**a**
C12		
62		**a**, **f**, **g**, **k**
63		**a**, **g**
64		**a**
65		**a**, **g**
66		**a**, **g**
67		**a**

Table 2 continued

68		**a**, **g**
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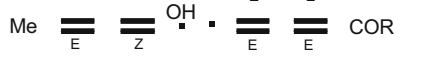
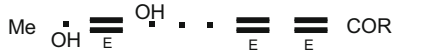
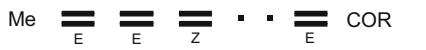
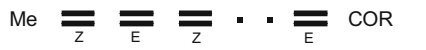
70		**a**
71		**a**
72		**a**, **d**, **v**
73		**d**
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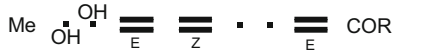
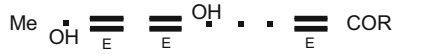
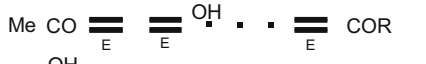
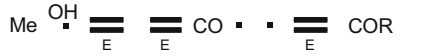
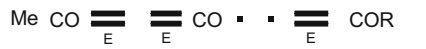
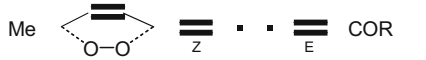
75		**d**
76		**d**
77		**d**
78		**d**
79		**d**
80		**d**
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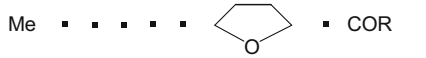
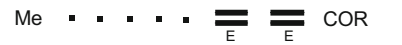
82		**k**
83		**q**
84		**a**
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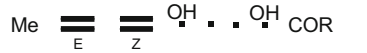
86		**a**, **f**
87		**a**, **d**
88		**a**
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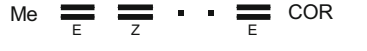
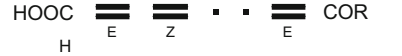
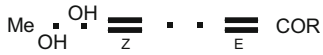
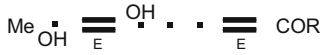
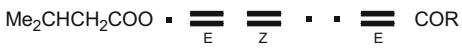
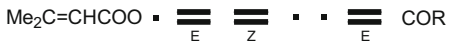
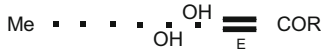
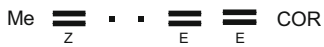
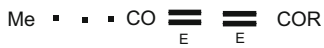
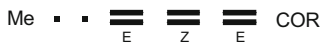
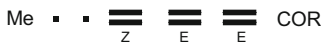
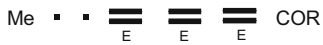
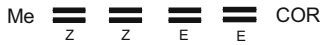
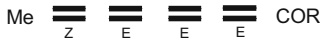
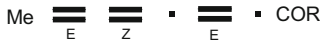
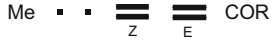
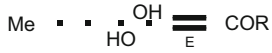
90		**a**
91		**a**
92		**a**, **c**, **g**
93		**d**
94		**d**

C11

C10

Table 2 continued

95			**a**
96			**a**
97			**a**
98			**a**
99			**a**, **b**
100			**a**
101			**a**, **b**, **c**, **e**, **f**, **h**, **i**, **k**, **l**, **m**, **q**, **s**
102			**a**, **b**, **e**
103			**b**
104			**c**, **m**
105			**e**
106			**e**
107			**e**
108			**e**
109			**c**
		C_8	
110			**a**, **c**
111			**a**, **c**
112			**a**
113			**a**
		C_7	
114			**a**
		C_6	
115			**c**
116			**a**, **d**
		C_4	
117			**a**

Amine parts indicated by small letters are listed in Fig. 2

pointed out that a large amount of fumaric acid was found together with the fumarylisobutylamide **117a** in *A. ciliata* (Keipert 2009), possibly indicating a different biogenetic origin of this acid moiety.

Acetylenic alkamides

Acetylenic C_{18}–C_{15} alkamides

Acetylenic C_{18} alkamides are only known so far from the two genera *Heliopsis* (Fig. 6) (Bohlmann et al. 1983; Ramírez-Noya et al. 2011) and *Achillea* (Greger et al. 1987a). As shown in Fig. 3 the acid parts can be derived from crepenynic acid demonstrating basic steps of desaturase and acetylenase activities as well as the formation of conjugated diene-yne sytems (**122–127**). The insertion of Δ^2-, Δ^4-double bonds (**120–127**) appears to be the result of independent biosynthetic activity. A similar pattern of unsaturation was reported for a series of acetylenic C_{16} alkamides from *A. ageratifolia* (**129–134**) (Greger et al. 1983, 1987b) and *A. tomentosa* (**134**) (Greger et al. 1981), suggesting chain shortening by β-oxidation at the carboxyl end. By contrast, shortening at the methyl end possibly leads to the C_{16} amides **135a** in *Echinacea angustifolia* (Bauer et al. 1989), *E. purpurea* (Binns et al. 2002) and in the four derivatives detected by GC/MS analysis in *Heliopsis suffruticosa* (Fig. 6) (Ramírez-Noya et al. 2011). The elimination of the terminal propyl group results in the formation of acetylenic C_{15} alkamides (**136a**,**b**,**d**) found in *Achillea* as well as in *Echinacea* species (Fig. 3). The unusual pattern of unsaturation in the C_{17} callysponga-mide A (**128c**), isolated from the sponge *C. fistularis* (Youssef et al. 2003), clearly deviates, indicating a different biosynthetic origin (Table 3).

Acetylenic C_{14}–C_{11} alkamides

Two different accumulation trends either towards acetylenic C_{14} or C_{12} alkamides represent important chemotaxonomic criteria differentiating between the tribes Anthemideae and Heliantheae. Derived from C_{18} crepenynic acid, the C_{14} amides are formed by two β-oxidations at the carboxyl site, whereas the C_{12} amides are additionally shortened at the methyl end. In the C_{13} acyl chains **150–154** the patterns of unsaturation suggest a cleavage of a terminal propyl group from corresponding C_{16} precursors. They were exclusively reported for the Heliantheae genera *Acmella* and *Echinacea* (Nakatami and Nagashima 1992) (Table 4). Apart from two isomeric tetradeca-2,4,10-triene-8-ynoic acid isobutylamides (**138a**, **139a**) from *H. buphthalmoides* (Bohlmann et al. 1983; Jakupovic et al. 1986) and tetradeca-2-en-10,12-diynoic acid isobutylamide (**145a**), isolated in small amounts from *E. angustifolia* (Woelkart et al. 2005), the accumulation of acetylenic C_{14} alkamides listed in Table 3 was detected solely in the tribe Anthemideae. They were mainly reported for *Achillea* species (Table 4), where they are characterized by highly desaturated acid moieties, sometimes even leading to fully conjugated systems (**147–149**). Whereas most derivatives have two triple bonds, the piperidide **144b**, isolated from *Achillea spinulifolia*, possesses three acetylenic linkages, the highest number of triple bonds found in the alkamides (Greger et al. 1982). By contrast, acetylenic C_{12} acid moieties (**155–165**) were mostly isolated from *Echinacea* species, where they are usually linked to isobutylamine (**a**), and frequently also to 2-methylbutylamine (**g**) (Table 4).

With the exception of **173a** from *Artemisia dracunculus* (Saadali et al. 2001) and the thiophenes **174a** from *Anacyclus clavatus* (=*A. tomentosus*) and **174a**,**b**,**e** from *Otanthus maritimus* (Bohlmann et al. 1974), the acetylenic C_{11} alkamides, listed in Table 3, are uniformly characterized by a terminal acetylenic hydrogen. Biosynthetically, they can be regarded as a result of oxidative chain shortening at the methyl end. The thiophenes (**174a**,**b**,**e**) are known to be formed by an incorporation of H_2S into the C_{11}-diynoic acid **167** (Bohlmann et al. 1974). Most of the C_{11} amides were isolated from *Echinacea* and *Acmella* species and are characterized by a 8,10-diyne group (**166–172**). Only derivatives of the undeca-2*E*,4*E*-dien-8,10-diynoic acid (**167**) are more widely distributed: they were also isolated from the Anthemideae genera *Achillea*, *Anacyclus*, *Otanthus*, *Argyranthemum*, and *Chamaemelum*. In this case the acyl rest **167** was shown to be linked to seven different amine parts (**a**,**b**,**c**,**e**,**g**, **j**,**m**) (Table 4). An unusual pattern of unsaturation was reported for decumbine (**175a**), an undeca-4*E*,6*E*-dien-10-ynoic acid isobutylamide, which is only known so far from *Acmella decumbens* (Casado et al. 2009). The unique C_{11} amide **173a**, isolated from *A. dracunculus*, deviates by a terminal methyl group. It is possibly derived from a C_{13} precursor by an elimination of C_2 at the methyl end (Bohlmann et al. 1973).

Acetylenic C_{10} and C_9 alkamides

With respect to their various patterns of unsaturation acetylenic C_{10} amides can be derived from two major biosynthetic pathways (Table 3). The formation of highly unsaturated acid parts, leading even to fully conjugated systems (**179**–**182**), represents a characteristic trend of the Anthemideae genera *Achillea*, *Anacyclus*, and *Cladanthus* (Table 4). They are biosynthesized by direct oxidative cleavage of a C_8-group from an octadec-9*Z*-en-12,14,16-triynoic acid precursor (Jente and Richter 1976). By contrast, the C_{10} acyl parts **177** and **178** from the Heliantheae genera *Acmella* and *Salmea* are most likely formed by an elimination of C_2 from corresponding C_{12} derivatives by β-oxidation. This hypothesis is supported by the predominant formation of similar acetylenic C_{12} amides in the related genus *Echinacea*. The biosynthetic origin of the C_{10} tyramide acmelline (**183f**), isolated from *A. decumbens* (Casado et al. 2009), remains unclear. Like the C_{11} amide decumbine (**175a**), mentioned above, the patterns of unsaturation clearly deviate from those known from other *Acmella* species (Table 3). According to Bohlmann et al. (1973) the biosynthesis of thiophene **184a**, isolated from *Argyranthemum* (=*Chrysanthemum*) *frutescens* (Bohlmann and Zdero 1967), can be explained by a terminal cleavage of a C_2-unit from a trideca-2,4-dien-7,9-diynoic acid precursor, followed by incorporation of H_2S and elimination of the terminal methyl group. The acetylenic C_9 derivatives were isolated only from the two closely related genera *Acmella* and *Salmea* (Table 3). They are characterized by a terminal acetylenic hydrogen (**185**–**189**), apparently derived from corresponding C_{12} (**160**–**162**) or C_{10} alkamides (**177**, **178**), respectively, by an oxidative chain shortening at the methyl end. Another dominant biogenetic trend in both genera is the combination with aromatic amine parts (**c**,**o**,**r**) (Table 4).

Biological activities

Pungent and tingling properties

Pungent and tingling alkamides were mainly reported for species of *Acmella* (*Spilanthes*), *Heliopsis*, and *Anacyclus* of the Asteraceae, and *Zanthoxylum* of the Rutaceae family. Similar taste profiles caused by alkamides are also known from *Echinacea*, *Argyranthemum*, *Salmea*, *Aaronsohnia*, *Piper*, and the grass *C. aromaticum*. The most prominent representatives are the four olefinic isobutylamides spilanthol (=affinin) (**92a**), pellitorine (**101a**), α-sanshool (=neoherculin) (**72a**), and γ-sanshool (**58a**). Due to their high instability isolation of pure compounds and structure elucidation turned out to be difficult (Fig. 5).

Spilanthol (=affinin)

Spilanthol (**92a**) was first obtained in the crude state from the flower heads of pará cress, *A. oleracea*, by Gerber (1903), who already supposed the existence of a fatty acid amide. Asano and Kanematsu (1927) initially concluded an unsaturated C_{10} isobutylamide with an allenic structure, and later (1932), a deca-4,6-dienoic acid isobutylamide. The correct structure of spilanthol, isolated from the roots of *H. longipes*, was suggested to be either a deca-2,6,8- or 2,5,7-trienoic acid isobutylamide (Acree et al. 1945). Since this plant was originally wrongly identified as *Erigeron affinis*, the compound was named affinin. Later, spilanthol was confirmed to be identical with affinin (Jacobson 1957a), and its stereochemistry finally assigned as 2*E*,6*Z*,8*E* (**92a**) by Crombie et al. (1963).

Pellitorine

The pungent constituent of pellitory root, *A. pyrethrum*, a plant native to North Africa, was first examined by Buchheim (1876), who named it "pyrethrin" and classified it erroneously as an amide related to piperine. Later, Dunstan and Garnett (1895) renamed the active material as pellitorine without any progress in elucidating the structure. Gulland and Hopton (1930) regarded it as a decadienoic isobutylamide with indefinite position of the double bonds. They retained the name pellitorine in order to avoid confusion with the insecticidal pyrethrins of Pyrethrum flowers (*Tanacetum cinerariifolium*). In order to determine the positions of the double bonds Jacobson (1949) investigated 6 kg of ground dry pellitory roots and concluded that pellitorine is an *N*-isobutyl-2,6-decadienamide. However, a re-examination by Crombie (1955b) has shown, that pellitorine contained at least three compounds with deca-2*E*,4*E*-dienoic acid isobutylamide (**101a**) as major component.

Table 3 Alkamides with acetylenic C_{18}–C_9 acid chains

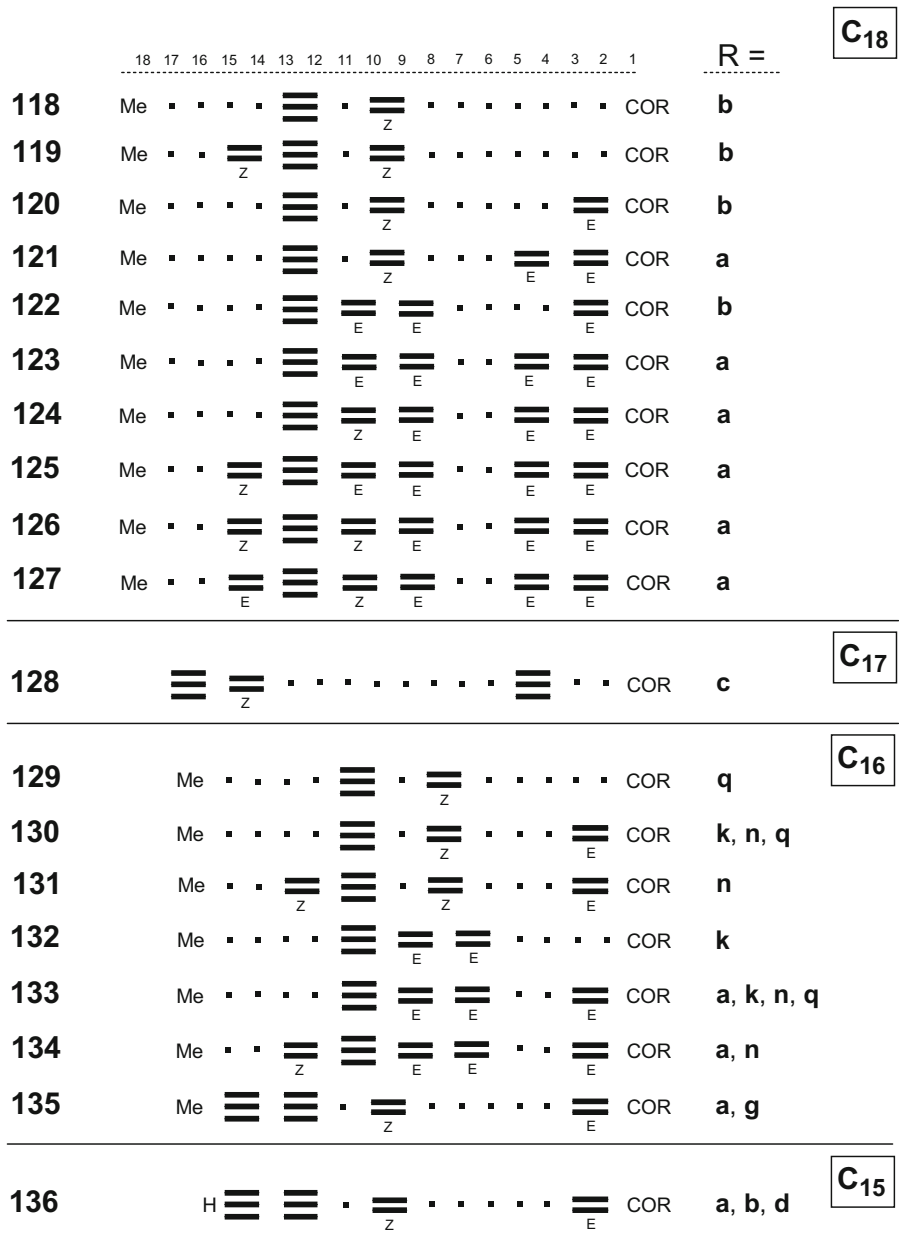


Table 3 continued

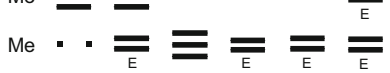
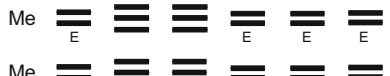
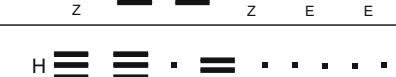
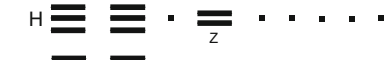
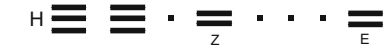
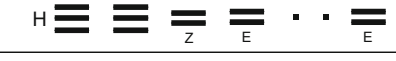
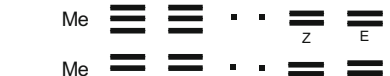
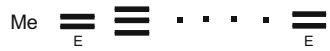
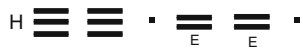
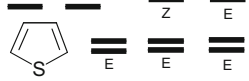
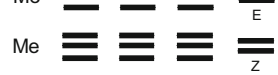
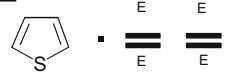
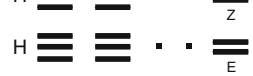
137		**k**	C_{14}
138		**a**	
139		**a**, **b**, **k**	
140		**a**	
141		**a**, **b**, **f**, **j**, **k**, **m**	
142		**a**, **b**, **k**	
143		**a**, **b**, **m**	
144		**b**	
145		**a**	
146		**b**	
147		**b**	
148		**a**, **m**	
149		**a**, **m**	
150		**a**	C_{13}
151		**a**, **g**	
152		**a**	
153		**a**	
154		**a**	
155		**g**	C_{12}
156		**a**	
157		**a**, **g**	
158		**a**	
159		**a**	
160		**a**, **g**	
161		**a**, **g**	
162		**a**, **g**	
163		**a**, **g**	

Table 3 continued

164			**a**
165			**a**
166		**C11**	**a**, **c**, **g**
167			**a**, **b**, **c**, **e**, **g**, **j**, **m**
168			**a**, **c**, **g**
169			**a**, **g**
170			**a**, **g**
171			**a**
172			**c**
173			**a**
174			**a**, **b**, **e**
175			**a**
176		**C10**	**a**
177			**c**
178			**a**, **c**, **o**, **r**
179			**j**
180			**a**, **c**, **j**, **m**
181			**a**, **c**, **m**
182			**a**
183			**f**
184			**a**
185		**C9**	**c**
186			**c**
187			**a**, **c**, **o**, **r**
188			**c**
189			**c**, **o**, **r**

Amine parts inidicated by small letters are listed in Fig. 2

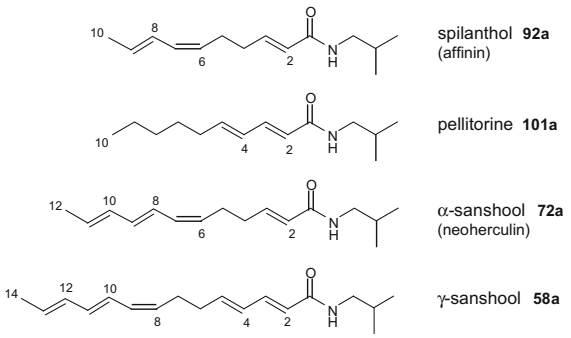


Fig. 5 Four prominent representatives of pungent and tingling alkamides

α-Sanshool (=neoherculin)

The pungent principle of the fruits of Japanese pepper *Zanthoxylum piperitum* ("Asakura Sansho") was first investigated by Murayama and Shinozaki (1931) and named sanshool. After hydrogenation they already supposed the presence of a C_{12} lauric acid amide. In a subsequent study Asano and Kanematsu (1931) regarded sanshool as a dodecadienoic acid isobutylamide, which was later shown to be a mixture of two homologous substances (Aihara 1950). One of which, named sanshoöl I, had a strong pungent taste and was very unstable in pure state when exposed to air. Aihara (1950) concluded a dodecatrienoic acid isobutylamide with the double bonds located at 2,4,8-position. The bark of *Z. clava-herculis*, commonly known as southern prickly ash, Herculesclub, or toothache tree in the southern United States, also gives a persistent burning and paralyzing effect on the lips and tongue. The active substance was first isolated by Jacobson (1948) and named herculin. He concluded the presence of an *N*-isobutylamide of a C_{12} acid containing two double bonds at 2- and 8-position. Crombie (1954) disproved the structure by syntheses and isolated a highly unstable but pure compound, named neoherculin. It was shown to be a dodeca-2,6,8,10-tetraenoic isobutylamide, whose stereochemistry was finally assigned as 2*E*,6*Z*,8*E*,10*E* (**72a**) (Crombie 1955a). In a synthetical investigation Crombie and Tayler (1957) showed that sanshoöl I has not the gross structure assigned to it by Aihara (1950). They isolated a very unstable amide from *Z. piperitum*, named α-sanshoöl (later referred to as α-sanshool). Its structure was confirmed to be the same as that of neoherculin (Crombie and Tayler 1957).

γ-Sanshool and related isobutylamides

From the fruits of *Z. ailanthoides* ("Karasu Shansho") two pungent compounds were isolated, named γ-sanshoöl and hydroxy γ-sanshoöl (later referred to as γ-sanshools). Their structures were determined as tetradeca-2*E*,4*E*,8*Z*,10*E*,12*E*-pentaenoic acid isobutylamide (**58a**) and the corresponding hydroxyisobutylamide (**58d**), respectively. The pungent taste of **58a** was shown to be stronger than that of **58d** (Yasuda et al. 1981b). In addition, two pungent C_{14} tetraenoic acid hydroxyisobutylamides were reported for the fruits of the Chinese *Z. bungeanum* ("hua jiao") (Mizutani et al. 1988), which were later designated as bungeanool (**56d**) and isobungeanool (**55d**) (Xiong et al. 1997). The corresponding pungent isobutylamide, named hazaleamide (**56a**), was isolated from the bark of the Indonesian *Z. rhetsa* ("hazalea") (Shibuya et al. 1992).

Dodeca-2,4,8,10-tetraenoic acid isobutylamides ("echinacein")

The numbing and pungent principle of the roots of the American coneflower, *E. angustifolia*, was first isolated by Jacobson (1954), who named it echinacein. He supposed an isobutylamide of a highly unsaturated C_{12} straight-chain acid which might be identical with neoherculin (=α-sanshool). Twelve years later a mixture of isomeric dodeca-2,4,8,10-tetraenoic acid isobutylamides was reported for the roots of *E. angustifolia* and *E. purpurea*, which was supposed to represent Jacobsońs echinacein (Bohlmann and Grenz 1966). One of the isomers was isolated from *Acmella alba* and its stereochemistry was assigned as 2*E*,4*E*,8*Z*,10*E* (**66a**) (Bohlmann et al. 1980b). Its co-occurrence with the isomeric 2*E*,4*E*,8*Z*,10*Z*-tetraene (**65a**) was then reported for *Asiasarum heterotropoides* (Yasuda et al. 1981a) and later for *E. purpurea* (Bauer et al. 1988). This pair of isomers was suggested to be responsible for the numbing effect of the root extract of *S. scandens* (Herz and Kulanthaivel 1985).

Pungent C_{18} isobutylamides (scabrin, heliopsin)

Special interest deserves the discovery of the pungent principle in the roots of *Heliopsis helianthoides* var. *scabra*, which was thought to consist of two highly unsaturated C_{18} isobutylamides. One of which was

described as a pale yellow, viscous oil which could not be distilled without decomposition, even under high vacuum. It was named scabrin, for which analysis and molecular weight determination indicated the formula $C_{22}H_{35}NO$ (Jacobson 1951). Similar characteristics were found in the second compound with the molecular formula $C_{22}H_{33}NO$, designated as heliopsin (Jacobson 1957b). Both compounds exhibited an intense burning and paralytic effect which was produced only after an induction period of approximately 10 min with scabrin, and 20 min with heliopsin. The correct structures of both amides were not elucidated so far. Regarding the investigations of *H. helianthoides* var. *scabra* and *H. buphthalmoides* by Bohlmann et al. (1983) and Jakupovic et al. (1986), it is tempting to assume that the four acetylenic C_{18} isobutylamides **124a**–**127a** (Fig. 6) isolated from the aerial parts and roots, respectively, are closely related or even identical with scabrin and heliopsin. Further experiments will have to show whether the delayed perception of the pungent taste can be explained by an enzymatic transformation or even a cleavage of these long-chained C_{18} alkamides. In this connection it would be interesting to confirm the structures of the acetylenic C_{18} and C_{16} alkamides of *H. suffruticosa* deduced from GC/MS analysis and test them for their pungent properties (Fig. 6) (Ramírez-Noya et al. 2011).

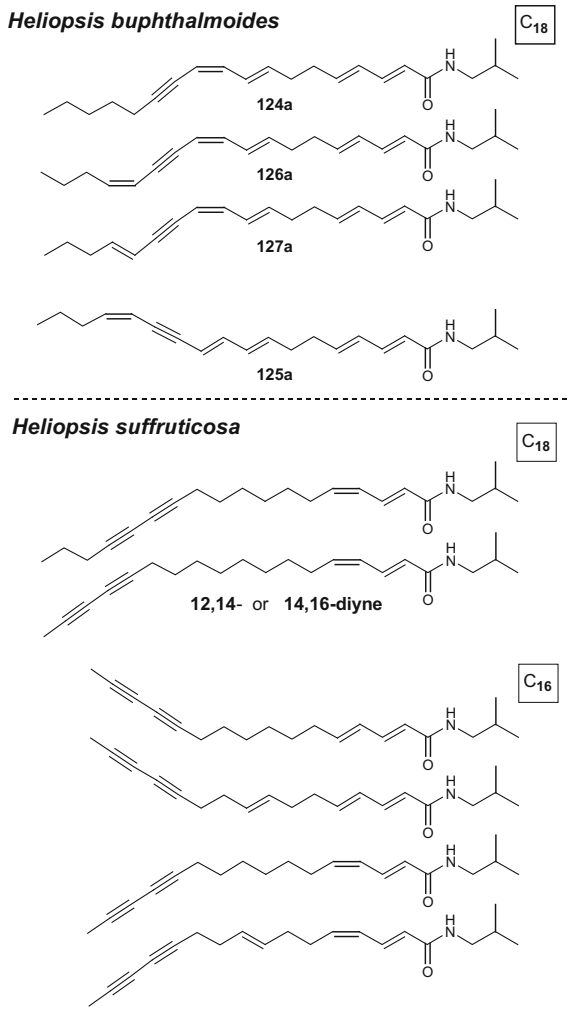


Fig. 6 Acetylenic C_{18} and C_{16} alkamides from *Heliopsis* species. Structures detected in *H. suffruticosa* have been deduced from GC/MS analysis (Ramírez-Noya et al. 2011) and are not listed in Tables 3 and 4

Structure–activity relationships

Active alkamides were shown to excite different populations of sensory neurons than does capsaicin or other similar pungent spices (Bryant and Mezine 1999; Bautista et al. 2008). In addition to pungency they are known to create a characteristic tingling sensation. Human judgments of 25–50 μg of α-hydroxysanshool (**72d**) applied directly to the tongue indicated that this sensation was more similar to a mild electric shock or a weakly carbonated solution. At higher concentrations (>100 μg), the sensation was painful. While ε-hydroxysanshool (**73d**), having one more *Z*-double bond, was as active as **72d** at the same concentration, the all-*E* isomer β-hydroxysanshool (**74d**) was inactive even at 100 μg (Bryant and Mezine 1999). The fact that *Z*/*E* isomerism influences the perception of pungency was also reported for the fruits of *Zanthoxylum bungeanum* containing a series of related hydroxyisobutylamides. While those having a *Z*-double bond (**55d**, **56d**, **58d**, **72d**) exhibited a strong pungency, the corresponding all-*E* isomers (**59d**, **74d**) were tasteless (Mizutani et al. 1988). Shibuya et al. (1992) prepared four derivatives related to hazaleamide (**56a**) and found that those with a fully saturated acid part or having only the 2*E*-double bond swap the pungent with a bitter sensation. In order to provide more information about structure–activity relationships of the pungent taste for the sanshool-related compounds (Fig. 7), a variety of derivatives was synthesized by Galopin et al. (2004). These results confirmed that a *Z*-double bond in the acyl chain is a

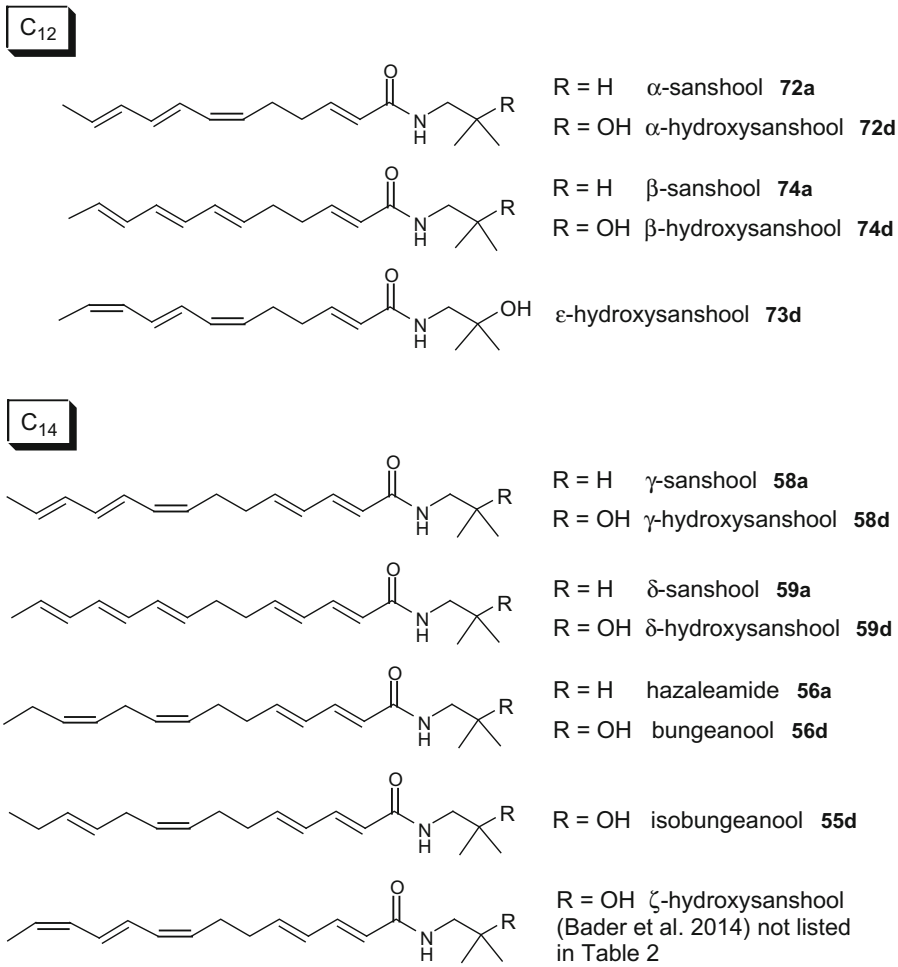


Fig. 7 *Z/E* Isomers of the sanshool group

key element for the sensory properties, but it is not the only one. Chain length and a certain pattern of unsaturation, sharing a common motif, were also shown to be required for activity. Detection threshold and taste characteristics of six sanshools were examined by sensory evaluation. In these studies pungent qualities of each derivative were shown to be different: e.g. the stimulus perceived as burning and tingling was predominantly detected with α-sanshool (**72a**) which lasted for the longest time. γ-Sanshool (**58a**) was perceived as burning and fresh, and α-hydroxysanshool (**72d**) as tingling and numbing (Sugai et al. 2005a, b). In a more recent psychophysical half-tongue experiments using filter paper rectangles as vehicle it was confirmed that alkamides of the sanshool-group possessing at least one *Z*-configurated double bond (**55d**, **56d**, **58d**, **72d**, **73d**, ζ-hydroxysanshool) (Fig. 7) elicited the well-known tingling and paresthetic orosensation above threshold levels of 3.5–8.3 nmol/cm^2. In contrast, the all-*E* configurated derivatives **59d** and **74d** induced a numbing and anesthetic sensation above thresholds of 3.9 and 7.1 nmol/cm^2, respectively (Bader et al. 2014).

To elucidate the cellular and molecular basis of the action of **72d**, a series of specific receptors was identified and cloned. Thus, **72d** binds to and inhibits the two-pore potassium channels KCNK3 (TASK-1), KCNK9 (TASK-3), and KCNK18 (TRESK), a class of pH- and general anestethics-sensitive ion channel (Bautista et al. 2008). *Ex vivo* skin-nerve preparation was used to examine the pattern and intensity with which the sensory terminals of cutaneous neurons respond to **72d**. It was found that it excited virtually all

D-hair afferents, a distinct subset of ultrasensitive light-touch receptors in the skin, and targeted novel populations of Aβ and C fiber nerve afferents. Thus, **72d** was shown to provide a novel pharmacological tool for discriminating functional subtypes of cutaneous mechanoreceptors (Lennertz et al. 2010).

Various trigeminal effects for artificial and naturally occurring alkamides related to spilanthol (**92a**) were reported (Ley et al. 2006a). From 26 derivatives it was shown that **92a**, having one *Z*-double bond, was the most active tingling and saliva-inducing compound. Pellitorine (**101a**), lacking a *Z*-double bond, exhibited mainly the same sialogogic activity but without the strong tingling effect. Interestingly, the structurally corresponding 2-ketol ester acmellonate, possessing the two conjugated *Z*/*E* double bonds near the methyl end as **92a**, showed similar numbing and tingling effects (Ley et al. 2006b). In order to elucidate the hypothesis that saliva induction may be correlated with a reduction of astringency sensation the potential masking effect of ten structurally related olefinic alkamides were evaluated. Among the selected compounds, the saliva-inducing **101a** significantly lowered the intensity of the astringency of epigallocatechin-3-gallate (Obst et al. 2013).

In a taste dilution analysis Dawid et al. (2012) tested the active alkamides of black pepper, *P. nigrum*, to what extent they contribute to the pungent impression of the well-known aromatic amide piperine. While eight aromatic piperine analogues exhibited a clear pungent sensory profile, twelve long-chain C_{20}- and C_{18}-2*E*,4*E*-dienoic acid derived alkamides, possessing an additional isolated *Z*-double bond, induced a pungent impression as well as a long-lasting tingling sensation. In accordance with the sanshools (Fig. 7) the *Z*-double bond was shown again to be a key element for the tingling effect: when compared to the tingling activity of octadeca-2*E*,4*E*,12*Z*- (**37a**) and 2*E*,4*E*,13*Z*-trienoic acid isobutylamide (**38a**), lacking of the *Z*-double bond in octadeca-2*E*,4*E*-dienoic acid isobutylamide (**33a**) induced a complete loss of that sensation. Moreover, amides with an isobutyl (**a**) or pyrrolidine part (**k**) were less pungent and tingling than the corresponding derivatives with a piperidine part (**b**). For example, eicosa-2*E*,4*E*,14*Z*- (**13b**) and 2*E*,4*E*,15*Z*-trienoic acid piperidide (**14b**) showed lowest threshold concentrations for pungency when compared to the corresponding pyrrolidides (**13k**, **14k**) and isobutylamides (**13a**, **14a**) (Dawid et al. 2012).

Insecticidal activities

LaForge et al. (1942) reported on the presence of an insecticidal principle in the bark of *Zanthoxylum clava-herculis* which was later isolated and identified as α-sanshool (=neoherculin) (**72a**) (Crombie 1955a). It was proved to have approximately the same order of paralyzing action and toxicity to house flies (*Musca domestica*) as the pyrethrins (Jacobson 1948). Similar insecticidal activities are known from the roots of *H. longipes* traded in Mexico under the Nahuatl names of "chilcuague" and "chilmecatl" (Molina-Torres et al. 1996). Here, the major insecticidal compound was identified as spilanthol (**92a**) (Crombie et al. 1963), originally published as "affinin" (Acree et al. 1945). Toxicity to house flies was also observed in petroleum ether extracts of three further American *Heliopsis* species (Gersdorff and Mitlin 1950). Especially the roots of *H. helianthoides* var. *scabra* were shown to be very toxic. In contrast to *H. longipes* two highly unsaturated C_{18} isobutylamides were isolated and named scabrin and heliopsin, from which the former proved to be appreciably more toxic than pyrethrins to house flies. Their structures were published only tentatively as C_{18}-pentaenoic and -hexaenoic acid isobutylamides, respectively (Jacobson 1951, 1957b). As mentioned above they are probably closely related to or even identical with the acetylenic C_{18} isobutylamides **124a**–**127a** isolated from that species by Bohlmann et al. (1983) and Jakupovic et al. (1986).

The pronounced insecticidal activity of spilanthol (**92a**) was also detected in bioassays against the American cockroach, *Periplaneta americana*. The acute toxicity became apparent when compared to three conventional insecticides. It was found to be 1.3, 2.6, and 3.8 times more toxic than carbaryl, bioresmethrin, and lindan, respectively. Electrophysiological studies suggested a neurotoxic action indicating immediate hyperexcitation followed by complete inhibition of the cercal nerve activity (Kadir et al. 1989). In addition to **92a** the structurally similar undeca-2*E*,7*Z*,9*E*-trienoic acid isobutylamide (**85a**) and the acetylenic undeca-2*E*-en-8,10-diynoic acid isobutylamide (**169a**), isolated from *Acmella paniculata* (=*Spilanthes acmella*), were shown to be very active against larvae of the mosquito *Aedes aegypti* (=*Stegomyia aegypti*) and neonates of the moth *Helicoverpa zea*, a major agricultural pest. While the two olefinic alkamides **92a** and **85a** showed 50 %

mortality at 6.25 μg/ml, the co-occurring acetylenic derivative **169a** showed only 30 % mortality at the same concentration. However, in antifeedant tests **169a** showed significantly higher weight reduction of the larvae of *H. zea* compared to **92a** and **85a** (Ramsewak et al. 1999). In search of ecofriendly and easily biodegradable naturally-occurring insecticides against *Tuta absoluta* (Lepidoptera), one of the key pests of tomato crops, hexane and ethanol extracts from 23 mostly South American plant species out of 16 different families were tested (Moreno et al. 2012). The hexane extract from aerial parts of *A. oleracea* exhibited by far the highest activity. In the bioactive fractions **92a** was identified as major component together with the two acetylenic C_{11} alkamides **169a** and **169g**. In accordance with previous findings (Kadir et al. 1989) **92a** was shown to be the most active compound being approximately 5 times more toxic than the commercial permethrin and a good deal more potent than the seed extract from Neem-tree, *Azadirachta indica*. The two acetylenic derivatives **169a** and **169g** exhibited activities similar to that of permethrin (Moreno et al. 2012). Bioassay-guided chromatographic fractionation of the leaf extract of *A. oleracea* led to a mixture of active alkamides consisting of nona-2*Z*-en-6,8-diynoic (**187c**) and deca-2*Z*-en-6,8-diynoic acid phenethylamide (**178c**). This mixture was shown to be active against *A. aegypti* larvae at $LC_{50} = 7.6$ ppm (Simas et al. 2013).

Pellitorine (**101a**) was reported to be a major contributor to the antilarval properties of the methanolic extract of *A. millefolium* showing 100 % mortality at 5 ppm against 24-h -old *Aedes triseriatus* larvae. However, it could not be ascertained if **101a** is the sole active compound. To gain information regarding the minimum molecular structural requirement for anitlarval activity three analog isobutylamides of 2*E*-decenoic (**89a**), decanoic (**86a**), and 2*E*,4*E*-hexadienoic (sorbic) acid were prepared. While the first two derivatives showed 96 and 59 % mortality, respectively, at 20 ppm, the sorbamide was inactive at the same concentration (LaLonde et al. 1980). In an artificial diet feeding assay **101a** and the closely related C_8 isobutylamide **112a** together with three cinnamic acid derived isobutylamides from the bark of *Z. gilletii* (=*Fagara macrophylla*) were tested against four species of lepidopteran larvae of agricultural importance. Concerning growth-inhibitory activity **101a**, the second most abundant amide, was shown to be the most active compound, especially against *Pectinophora gossypiella* ($ED_{50} = 15$ ppm). It also caused death ($LD = 25$ ppm) to *P. gossypiella* larvae, but not to those of *Heliothis virescens*, *H. zea*, and *Spodoptera frugiperda*. The closely related 2*E*,4*E*-octadienoic acid isobutylamide (**112a**) also caused mortality to *P. gossypiella* only ($LD_{90} = 100$ ppm). **101a** also proved to be the most toxic amide against the house mosquito *Culex pipiens* with a LD_{100} value at 5 ppm (Kubo et al. 1984).

The two isomeric C_{12}-tetraenoic isobutylamides **65a** and **66a**, isolated from *E. purpurea* roots, were reported to exhibit mosquito larvicidal activity against *A. aegypti*. They proved to be most effective with 87.5 % mortality within 15 min at a concentration of 100 μg/ml. A significant activity was still shown at 10 μg/ml, with 63 % mortality in 1 h. Slightly less active was the co-occurring acetylenic undeca-2*E*,4*Z*-dien-8,10-diynoic acid isobutylamide (**168a**) with 71 and 100 % mortality by 2 and 9 h, respectively, while the related C_{11} and C_{12} isobutylamides **166a** and **161a** showed activity at the end of 9 h, with 78 and 50 % mortality, respectively. Interestingly, the corresponding 2-methylbutylamides **168g** and **161g** demonstrated the lowest effects (Clifford et al. 2002). Mosquitocidal activity was also reported for the isomeric C_{12}-tetraenoic isobutylamide **67a**, isolated from the aerial parts of *A. mauritiana* (Jondiko 1986). While most of the insecticidal alkamides mentioned above are characterized by olefinic C_{10}-, C_{12}- or acetylenic C_{11}-acyl chains, the mosquitocidal activity of *Piper nigrum* fruits was ascribed to the C_{18} isohexylamide pipnoohine (**37p**) (Siddiqui et al. 2004). Insecticidal activity with longer acyl chains was also shown in Chrysanthemum varieties, *Dendranthema morifolium*, where concentration of the acetylenic C_{14} isobutylamide **140a** was positively correlated with the degree of resistance against the western flower thrips, *Frankliniella occidentalis*, a major insect pest in the greenhouse industry (Tsao et al. 2003). Six olefinic C_{14} alkamides, isolated from *Otanthus maritimus*, were shown to be toxic against the ant *Crematogaster scutellaris* and the termite *Reticulitermes balkanensis*. Among them tetradeca-2*E*,4*E*-dienoic acid piperidide (**51b**) exhibited the highest activity, whereas the corresponding isobutylamide (**51a**) showed the lowest response (Christodoulopoulou et al. 2005). Bioassays of fifty mostly synthetic 2,4-dienamides against house flies (*M. domestica*) and mustard beetles (*Phaedon*

cochleariae) showed that insecticidal activity was influenced by structural differences at the non-amide end of the acyl chain. These results presented clear indications concerning the necessity for a functional group containing unsaturation at the non-amide end and the strong dependence of the relationships detected on test species. Thus, pellitorine (**101a**) and the whole series of methyl-terminated amides displayed a much lower activity against *M. domestica* and *P. cochleariae* than phenyl- or vinyl-terminated derivatives (Elliott et al. 1987). Stimulated by the insecticidal activities of 2*E*,4*E*-dienamides derived from isobutylamine, piperidine, and pyrrolidine an efficient synthesis of corresponding derivatives was stereoselectively achieved by Abarbri et al. (1998).

Antibacterial and antifungal activities

Spilanthol (**92a**) was also shown to exhibit pronounced antibacterial and antifungal properties. Growth of *Escherichia coli* and *Saccharomyces cerevisiae* was inhibited at concentrations as low as 25 μg/ml. However, higher concentrations were necessary to inhibit growth of *Pseudomonas solanacearum* and *Bacillus subtilis* (Molina-Torres et al. 1999). In order to evaluate the importance of unsaturated bonds in the in vitro bacteriostatic and fungistatic properties the two less desaturated 2*E*-decenoic (**89a**) and decanoic acid isobutylamide (**86a**) were prepared from spilanthol (**92a**) by catalytic hydrogenation and tested against a number of different fungi and bacteria. While **92a** was very active against *Sclerotium rolfsii*, *S. cepivorum*, *Phytophthora infestans*, *S. cerevisiae*, and *Rhizoctonia* groups AG3 and A-5, displaying a growth inhibition around 100 %, the more saturated derivatives **89a** and **86a** showed no fungitoxic activity. **92a** was also shown to have a definite negative effect on the growth of the bacteria *E. coli* and *B. subtilis*, but *Erwinia carotovora* was not sensitive even at the highest dose. By contrast, **89a** was more potent against *E. coli* and *E. carotovora*, than **92a**, and only the saturated **86a** displayed a significant activity on the growth of *B. subtilis*. These data suggested a different mechanism of action of alkamides against fungi and bacteria, indicating that the 2*E*-unsaturation is insufficient for the fungitoxic action. It requires further unsaturation in conjunction with the unsaturation in either positions 6*Z*, 8*E* or both (Molina-Torres et al. 2004). UV light-mediated antifungal activity was reported for extracts of some *Echinacea* species. In this case the combination of high levels of the C_{12} tetraene amides **65a**/**66a** together with various polyacetylenes exhibited a very effective phototoxic action against a variety of clinically isolated human-pathogenic fungi (Merali et al. 2003). In a more recent study the hypothesis was tested, that alkamides from *Echinacea* exert antifungal activity by disrupting the fungal cell wall/membrane complex (Cruz et al. 2014). The results showed that *S. cerevisia*e cells exposed to sub-inhibitory concentrations of each of seven synthetic alkamides found in *Echinacea* extracts exhibited increased frequencies of cell wall damage and death that were comparable to the positive control caspofungin, a lipopeptide antifungal drug of the new class of echinocandins. Among the alkamides tested, the acetylenic C_{11} derivatives **166a** and **169a** showed the greatest antifungal and cell wall disruption activities, as opposed to the five remaining less active C_{12} derivatives, suggesting that the length of the acyl chain has an effect on the biological activity. The presence of a diynoic moiety in **166a** and **169a** enhanced cell wall disruption activity while an opposite trend was observed in the membrane disruption assay. In the latter case the dienoic group in the olefinic C_{12} derivatives **62a**, **63a**, **65a**, and **66a** was shown to be more effective. Based on these findings the authors proposed that alkamides found in *Echinacea* act synergistically to disrupt the fungal cell wall/membrane complex (Cruz et al. 2014).

Antiprotozoal activities

Acmella species are used as traditional herbal medicines in Africa and India to treat malaria. Spilanthol (**92a**) and the acetylenic C_{11} alkamide **169a**, isolated from *A. paniculata* (=*Spilanthes acmella*), were tested against the chloroquine-resistent strain K1 of *Plasmodium falciparum*, originating from Thailand, and the mildly chloroquine-resistent strain PFB, originating from Brazil. For the Brazilian strain the IC_{50} (half maximal inhibiting concentration) for **92a** and **169a** were 16.5 and 41.4 μg/ml, respectively, while for the Thai strain the effect was significantly greater, with 5.8 and 16.3 μg/ml, respectively. Moreover, a comparison of fresh plant water extract with an ethanol extract, containing ten times the concentration of **92a**, was performed in vivo on *Plasmodium yoellii yoellii-*

infected mice. Surprisingly, the water extract exhibited a higher activity with 53 % reduction in parasitemia than the ethanol extract with 36 % reduction. This suggested that in addition to **92a**, there may be water soluble constituents that are also active against *Plasmodium*, or the treatment could have induced immunological activity (Spelman et al. 2011). In the course of an ongoing screening of plants of the family Asteraceae for antiprotozoal activity, a CH_2Cl_2-extract from the flowering aerial parts of *Achillea ptarmica* was found to be active in vitro against *Trypanosoma brucei rhodesiense* with $IC_{50} = 0.67$ - μg/ml and *P. falciparum* with $IC_{50} = 6.6$ μg/ml. From the bioactive fractions six alkamides were isolated from which pellitorine (**101a**) and 8,9-*Z*-dehydropellitorine (**102a**) were shown to be the major components accompanied by the acetylenic C_{11} derivatives **167a**,**b**,**c** and C_{14} anacyclin (**141a**). Pellitorine (**101a**) exhibited the highest activity against *P. falciparum* with an IC_{50} value at 3.26 μg/ml. The antiplasmodial activity was thus about twofold higher than that of the crude extract. Although 8,9-*Z*-dehydropellitorine (**102a**) was shown to be the most active compound against *T. brucei* with an $IC_{50} = 2.0$ μg/ml, it corresponded only to a threefold lower activity in comparison with the crude extract. Since the promising activity of the crude extract could not be attributed to any of the isolated alkamides on its own it was hence conceivable that either some minor, not as yet isolated, constituents were responsible for the high activity, or a synergistic effect was at work (Althaus et al. 2014).

Miscellaneous properties

Spilanthol (**92a**) was reported to be also a potential agent to control schistosomiasis. It was tested against the freshwater snail *Physa occidentalis* and the cercariae released by the mollusc. Above 50 mg/l in H_2O snails were shown to be inactive after 60 min and dead within 18 h. At 150 mg/l, the solubility limit for **92a**, cercarial emergence ceased and the snails showed immobility after 30 min. The cercariae ceased to move after 5 s and convulsed after 1 min (Johns et al. 1982). Acaricidal activity was detected in the hexane extract of the aerial parts of *A. oleracea* which most likely can be attributed to the presence of the major constituent **92a**. It was highly effective against larvae of the cattle tick *Rhipicephalus microplus* with an $LC_{50} = 0.8$ mg/ml, and against engorged females, where it reduced oviposition and hatchability of eggs with an $LC_{50} = 79.7$ mg/ml (Castro et al. 2014). **92a** was also shown to act as plant growth-promoting substance. Together with the two more saturated alkamides dec-2*E*-enoic (**89a**) and decanoic acid isobutylamide (**86a**) it was found to alter the architecture of the root system and regulate cell division and differentiation processes in *Arabidopsis thaliana* (Ramírez-Chávez et al. 2004; López-Bucio et al. 2006; Morquecho-Contreras et al. 2010).

Medical properties

Anti-inflammatory and analgesic activities

The roots of *H. longipes* are claimed to be effective for the alleviation of toothache pain and are used extensively in the rural areas of Mexico. Spilanthol (**92a**) was identified as the active principle and its analgesic activity was first evaluated by Ogura et al. (1982). The topical anti-inflammatory effects of **92a** and its saturated analog **86a** were evaluated in the mouse ear edema test using arachidonic acid (AA) and phorbol myristate acetate (PMA) as irritating agents. **92a** was shown to inhibit the AA-induced edema in a dose-dependent manner with an ED_{50} value at 1.2 mg/ear, while **86a** displayed the same effect with an ED_{50} at 0.9 mg/ear. The acute PMA-induced inflammation was inhibited with ED_{50} values at 1.3 mg/ear with **92a** and 1.1 mg/ear with **86a** (Hernández et al. 2009). Various pharmacological experiments were carried out to study the mechanism of action of the antinociceptive and analgesic effects of *H. longipes* root extract and specifically of its main alkamide **92a** (Rios et al. 2007; Cilia-López et al. 2010; Cariño-Cortés et al. 2010; Déciga-Campos et al. 2010, 2012).

Spilanthol (**92a**) was reported to exhibit a dose-dependent inhibition of 5-lipoxygenase with an IC_{50} value at 50 μmol, and was assumed to be the anti-inflammatory principle of the extract of *A. oleracea*, topically used in anti-rheumatic therapy. In contrast, it did not show an inhibiting effect on prostaglandin synthase. A similar inhibitory effect on 5-lipoxygenase was found for an alkamide fraction of the root extract of *E. purpurea*, consisting of ten different polyunsaturated isobutylamides. This activity contributed to the antiphlogistic activity of the drug

formerly attributed to the water extract and the polysaccharides (Wagner et al. 1989). Since *Echinacea* and *Achillea* species were used in traditional medicine in North America and Europe for anti-inflammatory purposes, various alkamides from both genera were tested for in vitro inhibition of 5-lipoxygenase and cyclooxygenase (Müller-Jakic et al. 1994). The isomeric C_{12}-tetraenoic isobutylamides **65a/66a** were shown to be the major constituents of the root extract of *E. angustifolia* and a 1:1 mixture inhibited both, cyclooxygenase 54.7 % and 5-lipoxygenase 62.2 % at 50 µmol. However, in comparison to the n-hexan extract with 62.4 and 81.8 % inhibition, respectively, the mixture **65a/66a** was shown to be less potent suggesting the existence of other active compounds or synergistic effects. In order to determine the main active principle, and to perform structure–activity relationship studies eight alkamides from *E. angustifolia* and ten from *Achillea* species together with one each from *Anacyclus pyrethrum* and *Aaronsohnia factorovskyi* (=*Matricaria pubescens*) were tested. In summary, all compounds appeared to be more or less potent inhibitors of cyclooxygenase, but only few inhibited 5-lipoxygenase. For the latter the mixture **65a/66a** from *E. angustifolia* showed the highest activity followed by the C_{14} acetylenic piperidide **144b** from *Achillea spinulifolia* and the C_{16} acetylenic pyrrolide **130q** from *A. ageratifolia*. For cyclooxygenase the two closely related C_{16} and C_{15} acetylenic isobutylamides **135a** and **136a** from *E. angustifolia*, the thiophene **184a** from *A. factorovskyi*, and the C_{14} pyrrolidide **139k** from *Achillea nana* exhibited higher inhibition than the dominating isomers **65a/66a** (Müller-Jakic et al. 1994). In the study of Clifford et al. (2002) the latter did not show activity at 100 µg/ml for either cyclooxygenase (COX)-1 or -2. Here, the two isomers undeca-2*E*,4*Z*-dien-8,10-diynoic (**168a**) and undeca-2*Z*,4*E*-dien-8,10-diynoic isobutylamide (**166a**) exhibited 36 and 60 % inhibition, respectively, of COX-1 activity. **166a** exhibited the highest inhibition to both COX-1 and -2 enzymes. The two 2-methylbutylamides **161g** and **168g**, differing by one methyl group in the amine part, demonstrated higher activity against the two enzymes when compared to the corresponding isobutylamides (**161a**, **168a**). The two latter, by contrast, differing among each other by one terminal methyl group in the acid chain, exhibited equal inhibitory activity (Clifford et al. 2002).

Since inhibition of COX-2 was proven as an effective strategy to suppress pain and inflammation, the impact of several alkamides, isolated from the roots of *E. angustifolia*, on both activity and expression of this enzyme was investigated. A 48-h treatment of H4 human neuroglioma cells with the CO_2 extract led to a significant suppression of prostaglandin E_2 (PGE_2) formation, the major product of the COX-2 pathway. From eight different alkamides the three acetylenic derivatives undeca-2*Z*-ene-8,10-diynoic isobutylamide (**170a**), dodeca-2*E*-en-8,10-diynoic isobutylamide (**163a**), and dodeca-2*E*,4*Z*-dien-8,10-diynoic 2-methylbutylamide (**161g**) were shown to contribute to this response and interfere with COX-2 activity (Hinz et al. 2007). Moreover, inhibition of PGE_2 formation in lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophage cells was assessed with an enzyme immunoassay following treatments with *Echinacea* extracts or synthesized alkamides. All of the 13 alkamides screened significantly inhibited the production of PGE_2 at 50 µmol. The C_{12}-tetraene **66a** and the acetylenic C_{12} derivatives **162a** and **163a** were shown to reduce the PGE_2 levels at 25 µmol. Only **163a** significantly inhibited PGE_2 production at 10 µmolar. Because the innate concentrations of individual alkamides found in crude extracts did not reach concentrations shown to have significant PGE_2 inhibition, it was assumed that they might have contributed toward the anti-inflammatory activity in a synergistic or additive manner (LaLone et al. 2007). Similar results were obtained in a previous study where *Echinacea* alkamides showed anti-inflammatory activity as measured by inhibition of nitric oxide (NO) production in LPS-stimulated RAW264.7 cells. As a pro-inflammatory mediator NO was significantly reduced by a mixture of several alkamides ranging from 1.6 to 30 µg/ml (Chen et al. 2005). On the basis of a mitogen-induced murine skin inflammation study a comparative metabolomics approach coupled with cell- and gene-based assays was used to evaluate the anti-inflammatory activity of three *Echinacea* species. The order of efficacy was *E. angustifolia* > *E. purpurea* > *E. pallida* (Hou et al. 2010). In order to characterize the anti-inflammatory activity of the specific alkamides the authors compared an alkamide-enriched fraction with the predominant C_{12} isomers **65a/66a**. Immunoblotting analysis of COX-2 protein expression in LPS-stimulated macrophages showed better suppression for the alkamide fraction of

the *E. purpurea* root extract than the isolated isomers **65a/66a**. In accordance with previous findings (Müller-Jakic et al. 1994; LaLone et al. 2007) it was suggested, that the other alkamide(s) present in the mixture, apart from **65a/66a**, also contributed to the inhibition of COX-2 activity. The anti-inflammatory activity of **65a/66a** in mice was shown to be directly associated with the protective effect in LPS/D-GalN-induced acute hepatitis and liver injury (Hou et al. 2011).

Immunomodulation and cannabinomimetic effects

In the prevention and treatment of common cold *Echinacea* plant preparations are widely used in North America and Europe. The standardized tincture Echinaforce™ was analyzed and found that it induced de novo synthesis of tumor necrosis factor α (TNF-α) mRNA in primary human monocytes/macrophages, but not TNF-α protein. Moreover, LPS-stimulated TNF-α protein was potently inhibited in the early phase but prolonged in the late phase. Among the main constituents of the tincture the olefinic C_{12} isobutylamides **65a/66a**, **62a**, and **63a** were shown to be responsible for this effect which was ascribed to their interactions with the cannabinoid type 2 receptor (CB_2) on monocytes (Gertsch et al. 2004). These findings were independently confirmed by Woelkart et al. (2005). Due to the structural similarity of alkamides with the endogenous cannabinoid ligand anandamide (*N*-arachidonoyl ethanolamide) (Fig. 8) they investigated the receptor binding of twelve *Echinacea* alkamides to rodent cannabinoid receptors CB_1 and CB_2. Concerning selectivity the acetylenic pentadeca-2*E*,9*Z*-dien-12,14-diynoic acid isobutylamide (**136a**) showed the highest affinity for CB_1 with an inhibitory constant (*Ki*) of 2.0 µmol, followed by dodeca-2*E*-en-8,10-diynoic acid 2-methylbutylamide (**163g**) with a *Ki* of 4.1 µmol, while tetradeca-2*E*-en-10,12-diynoic acid isobutylamide (**145a**) with a *Ki* of 1.9 µmol was shown to be the most selective and most affine ligand for CB_2. A further evidence of CB-receptor-binding of alkamides was demonstrated by Raduner et al. (2006). At concentrations below 100 nmol, the two olefinic C_{12}-isobutylamides **65a** and **62a** potently displaced the radioligand from membrane recombinantly overexpressing CB_2 receptors with *Ki* values of 57 ± 14 and 60 ± 13 nmol, respectively. Immunomodulatory effects of **65a** and **62a** were also investigated by

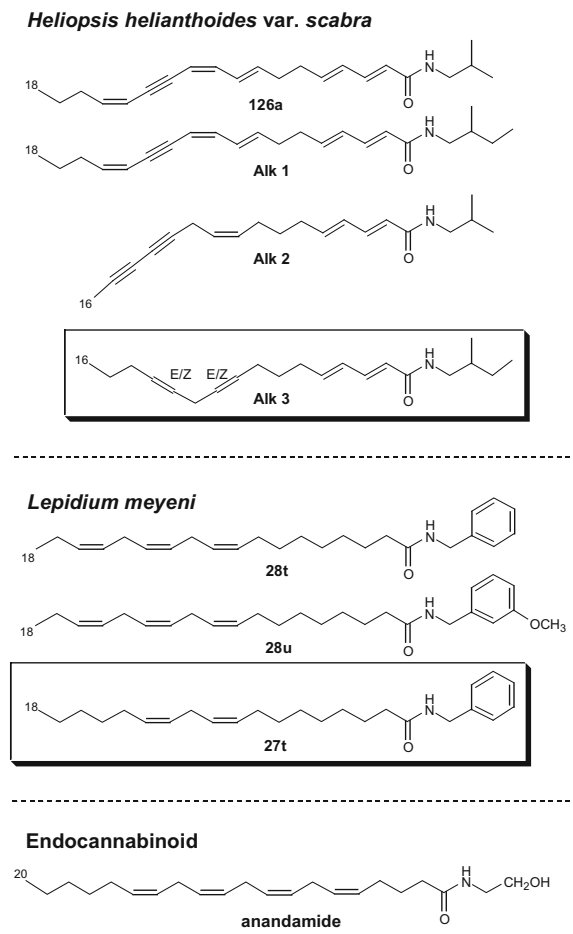


Fig. 8 C_{18} and C_{16} alkamides isolated from *H. helianthoides* var. *scabra* and *L. meyeni* compared with the C_{20} endocannabinoid anandamide. Compounds in the boxes showed submicromolecular binding affinities for the CB_1 recepetor (Hajdu et al. 2014). The newly described alkamides **Alk 1–3** are not listed in Tables 2, 3 and 4

Sasagawa et al. (2006) who reported on their inhibitory effect to interleukin-2 formation on human Jurkat T cells. In the review of Gertsch et al. (2006) *Echinacea* alkamides were described as a new class of cannabinomimetics which specifically engage and activate the CB_2 receptors and are likely to provide novel lead structures for the development of CB_2-directed drugs. The affinity of alkamides to cannabinoid receptors was shown to depend on their solubility and thus it is important to understand their physicochemical behavior in an aqueous environment. Raduner et al. (2007) discovered that **62a** and **65a** form micelles in aqueous medium which could not be observed for the acetylenic C_{11} derivative **169a** (which

has no affinity to cannabinoid receptors) or for structurally related endogenous cannabinoids, such as anandamide (Fig. 8). Depending on concentration microscopy images showed that **62a** formed globular and rod-like supermicelles which did not bind to CB_2. While anandamide did not aggregate and thus freely interacted with CB_2, **62a** exhibited differential receptor affinity competing with self-aggregation as a function of concentration. Woelkart and Bauer (2007) summarized the results of pharmacological experiments with *Echinacea* extracts and demonstrated the significant anti-inflammatory and immunomodulatory properties of individual alkamides. Gertsch (2008) reported on the increasing evidence that fatty acid derived alkamides can modulate the action of endogenous lipid signals. In a more recent study the functional interaction of alkamides from *Heliopsis helianthoides* var. *scabra* and *Lepidium meyeni* ("maca") with the endocannabinoid system was investigated. The newly described hexadeca-2,4,9,12-tetraenoic acid 2-methylbutylamide (**Alk 3** in Fig. 8) from *H. helianthoides* var. *scabra* and **27t** from *L. meyeni* showed submicromolar and selective binding affinities for CB_1 with *Ki* values of 0.31 and 0.48 μmol, respectively. Due to the structural similarities of the 9,12 double-bond system of the lineleoyl group in **27t** with anandamide the results provided additional strong evidence of the endocannabinoid substrate mimicking of some alkamides (Fig. 8) (Hajdu et al. 2014).

On the basis of a newly developed plate-based CB_1/CB_2 receptor functional assay the extracts of several *Zanthoxylum* species were screened for active compounds. The objective was to identify novel antagonists of CB_1, which simultaneously display agonist activity against CB_2. Compounds matching this criterion could be potential candidates for the treatment of type-1 diabetes. The extract from *Z. bungeanum* was deemed active, leading to the identification of eight alkamides of the sanshool group (Fig. 7). One of them, δ-sanshool (**59a**) (erroneously published as γ-sanshool), was obtained as a promising lead compound (Dossou et al. 2013).

Pharmacokinetics and bioavailability

The bioavailability of the isomeric C_{12} tetraenes **65a/66a** in human blood after oral administration was reported by Dietz et al. (2001), and their permeability through Caco-2 monolayers described by Jager et al. (2002). The isomers were found to be nearly completely transported from the apical to the basolateral side of the monolayers in 6 h by passive diffusion and that no significant metabolism occurred. The apparent permeability of twelve *Echinacea* alkamides was investigated by Matthias et al. (2004). They found that most of the alkamides readily cross the Caco-2 monolayer with many more than 50 % transported after 90 min and that increasing unsaturation gave rise to increases in the apparent permeability: e.g. the 2,4-dienes **161g** and **168a** appeared to be more readily transported than the equivalent 2-enes **163g** and **169a**. Methylation at the terminal diyne group, e.g. in **161a**, caused a reduction of permeability compared to the corresponding structure of **168a** with a terminal acetylenic hydrogen. Woelkart et al. (2009) showed that the tetraenes **65a/66a** were available in plasma and rat tissues with a rapid passage across the blood–brain barrier. For these compounds an LC–MS/MS assay was developed and validated for quantification in human plasma. The results showed that they can be accurately and precisely quantified and are chemically stable under relevant conditions (Goey et al. 2012). Furthermore, the interaction of eight *Echinacea* alkamides with P-glycoprotein, a major constituent of the blood–brain barrier, showed that four derivatives inhibited P-glycoprotein in primary endothelial cells freshly isolated from porcine brain blood vessels (Mahringer et al. 2013). The transdermal permeation behavior of spilanthol (**92a**) from *Acmella paniculata* (=*Spilanthes acmella*) extracts was investigated by Boonen et al. (2010a, b), who reported on the influence of ethanol (65 %) and propylene glycol (10 %) as solvents. Similar permeation properties were recently also described for the *Anacyclus pyrethrum* extract and the main alkamide pellitorine (**101a**) (Veryser et al. 2014).

Miscellaneous properties

As part of the crude drug Dai-kenchu-to, a Chinese prescription frequently used in recent Japan to treat paralytic ileus after laparotomy and severe constipation, the fruits of *Zanthoxylum piperitum* are known to relax the gastric body as well as contract the ileum and distal colon. Using the gastrointestinal tract isolated from guinea pig it was shown that the alkamides γ- (**58a**), β-sanshool (**74a**), and especially β-

hydroxysanshool (**74d**) isolated from *Z. piperitum* potently induced ileal contraction and acted directly on smooth muscle of the gastric body (Hashimoto et al. 2001).

The nuclear factor peroxisome proliferator-activated receptor (PPAR) γ is predominantly found in adipose tissue and is known to regulate adipocyte differentiation as well as glucose homeostasis. A hexane extract of the flowers of *Echinacea purpurea* was found to significantly activate PPARγ without stimulating adipocyte differentiation. Bioassay-guided fractionations yielded the C_{12} alkamides **62a**, **65a**, **66a**, **157a**, and the newly described hexadeca-2*E*,9*Z*,12*Z*,14*E*-tetraenoic acid isobutylamide (**45a**) together with three fatty acids that all activated PPARγ. The C_{16} alkamide **45a** exhibited a significant activation of more than tenfold at 30 µmol without a concurrent stimulation of adipocyte differentiation, but still retained the insulin-sensitizing effects. This makes it a potential beneficial PPARγ partial agonist (Christensen et al. 2009).

The subterranean parts (hypocotyl tubers) of *Lepidium meyeni* ("maca") are used as foodstuff in the central Andes of Peru and are also known for its aphrodisiac properties (Zhao et al. 2005). The effect of a lipid extract on sexual behavior in mice and rats was investigated by Zheng et al. (2000). They proposed that alkamides ("macamides") and the structurally corresponding 5-oxo-6*E*,8*E*-octadecadienoic acid ("macaene") (**24**) are involved in improving sexual performance. However, in spite of the many studies already carried out on this drug, the effective substances and the mechanism of action were not fully elucidated so far (Wang et al. 2007). The roots of *Anacyclus pyrethrum*, commonly referred to as "Akarkara" in Ayurvedic system of Indian medicine are also considered aphrodisiac and sexual stimulant (Sharma et al. 2009; Annalakshmi et al. 2012). Administration of alkamide-rich extracts of *A. pyrethrum* showed improvement in sexual behavior of male rats. With respect to the similar properties observed in *L. meyeni* the authors suspected the presence of pellitorine (**101a**) as possible contributor to these effects and hypothesized that alkamides may mimic the action of testosterone or stimulate secretion of testosterone that improves sexual function (Sharma et al. 2010, 2013). Similar effects on sexual behavior in male rats were detected in the ethanolic flower extract of *Acmella paniculata*. In this case spilanthol (**92a**) and other alkamides of the extract were supposed as causative agents (Sharma et al. 2011).

The lipophilic extract from the fruit husks of *Z. bungeanum* (Zanthalene®), mainly consisting of α- (**72d**) and β-hydroxysanshool (**74d**), was validated as an anti-itching cosmetic ingredient and was shown to potently inhibit synaptic transmission. Thus, its capacity to relax subcutaneous muscles and to act as topical lifting agent for wrinkles was investigated. The results of this study fully qualified the extract as a functional cosmetic ingredient for the temporary improvement of skin wrinkles (Artaria et al. 2011).

Chemotaxonomic significance

Due to the absence of Δ^2-, Δ^4-double bonds in the unsaturated acid parts, and the combination with benzylamines (**t**, **u**), the "macamides" from *Lepidium meyeni* of the Brassicaceae can be clearly distinguished from other plant-derived alkamides. The accumulation of alkamides with elongated saturated and olefinic acid moieties (Table 1) represents a typical biogenetic trend of the Piperaceae and Brassicaceae, while the formation of acetylenic derivatives (Table 3) is a characteristic chemical feature of the Asteraceae solely known from the two tribes Anthemideae and Heliantheae. Compounds with six- (**b**, **e**, **h**) or five-membered ring amines (**k**, **n**, **q**) and those with phenylalanine derived residues (**c**, **f**, **i**, **l**, **o**, **r**) typify the two families Asteraceae and Piperaceae. Within the Asteraceae derivatives with a 2-methylbutylamine residue (**g**) distinguishes the tribe Heliantheae from Anthemideae which is characterized by alkamides with dominating ring amines. Specific desaturase activity in the acid part leads to the conjugated triene system of the sanshool group characteristic for *Zanthoxylum* species comprising C_{14}-pentaene and C_{12}-tetraene derivatives (Fig. 7; Table 2). By contrast, the isomeric C_{12}-tetraenes **65a**/**66a**, containing a conjugated diene group near the methyl end, represent a typical chemical character of the related Heliantheae genera *Echinacea*, *Acmella*, and *Salmea*.

Broad-based phytochemical comparisons within the tribe Anhemideae exhibited a predominant alkamide formation in many *Achillea* species, while most of the other genera were characterized by a variety of polyacetylenes (Greger 1977, 1988; Christensen 1992). Alkamide formation was also reported for the genera *Anacyclus*, *Leucocyclus* and *Otanthus* (Table 4) whose close relationships to *Achillea* were already suggested previously (Greger 1978; Greger et al. 1981). Meanwhile multidisciplinary studies presented a new circumscription of *Achillea* including the former unispecific genera *Leucocyclus* (as *Achillea formosa* (Boiss.) Sch. Bip.) and *Otanthus* (as *Achillea maritima* (L.) Ehrend. and Y.-P. Guo). Extended DNA

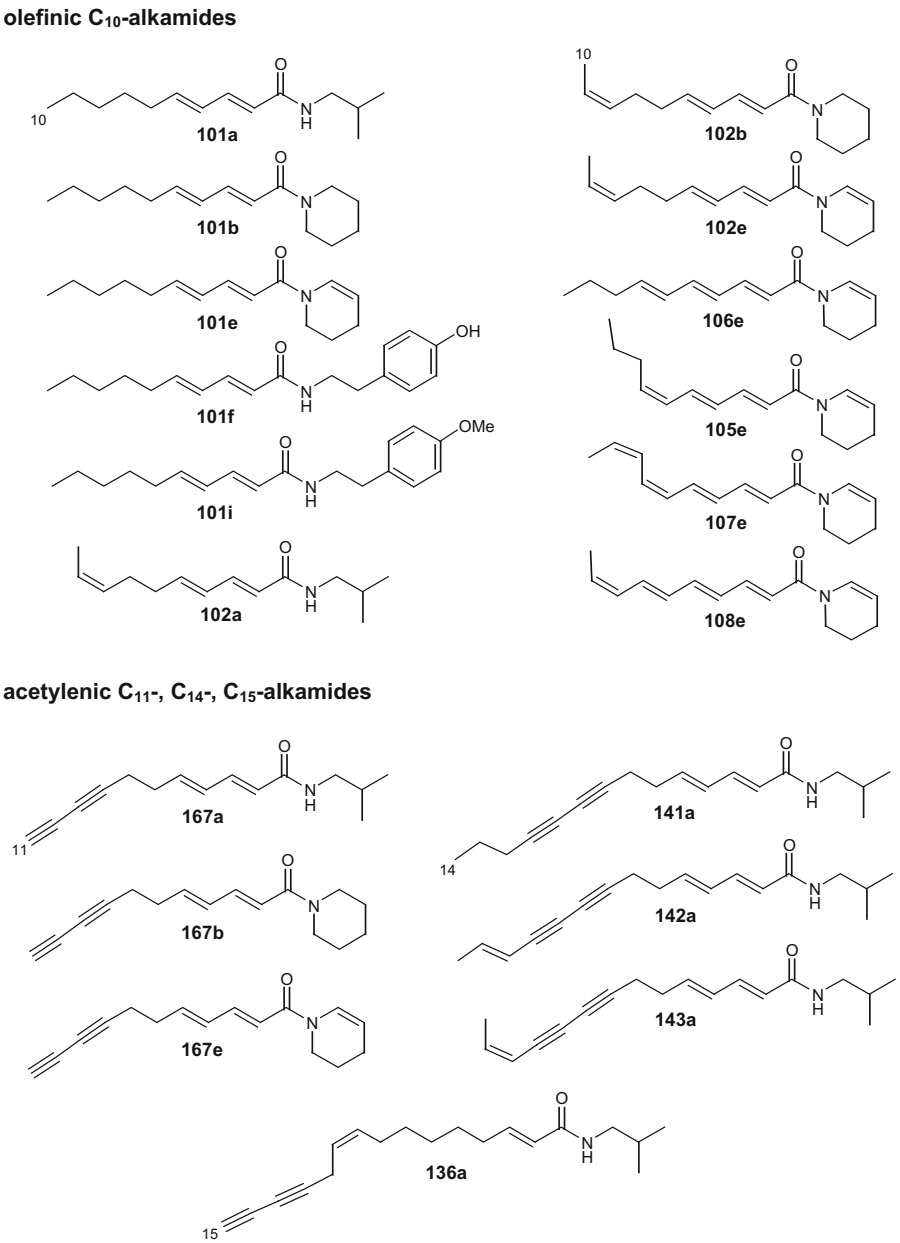


Fig. 9 Structural diversity of olefinic and acetylenic alkamides isolated from the underground parts of *A. millefolium*

Table 4 Distribution of alkamides in plant species

PLANT SPECIES	ALKAMIDES
Asteraceae–Anthemideae	
Aaronsohnia factorovskyi Warb. and Eig [syn.: *Matricaria pubescens* (Desf.) *Sch.Bip.*]	**101a**, **184a** (Greger and Hofer 1984)
Achillea ageratifolia (Sibth. and Smith) Boiss.	**44q**, **56k**, **83q**, **129q**, **130knq**, **131n**, **132k**, **133aknq**, **134a**, **137k** (Greger et al. 1983, 1987b)
A. biebersteinii Afan.	**143b** (Greger et al. 1981)
A. chamaemelifolia Pourr.	**35a**, **120b**, **121a**, **122b**, **123a**, **124a** (Greger et al. 1987a)
A. crithmifolia Waldst. and Kit.	**101e**, **105e** (Greger et al. 1981)
A. falcata L.	**101abeh**, **102abe**, **166abc** (Greger et al. 1983; Hofer et al. 1986)
A. fragrantissima (Forssk.) Sch. Bip.	**101a**, **139b** (Greger unpubl.)
A. grandifolia Friv.	**142b**, **147b** (Greger et al. 1982)
A. ligustica All.	**146b** (Greger et al. 1984)
A. lycaonica Boiss. and Heldr.	**20bk**, **30bk**, **31bk**, **32b**, **118b**, **119b**, **120b**, **122b**, **136b** (Greger et al. 1982, 1987a)
A. macrophylla L.	**101a**, **102a**, **167a** (Greger et al. 1984)
A. millefolium L. agg.	**101abefi**, **102ae**, **105e**, **106e**, **107e**, **108e**, **136a**, **141ab**, **142ab**, **143a**, **160a**, **167abe** (Bohlmann and Zdero 1973; Bohlmann et al. 1974; Greger and Hofer 1989)
A. nana L.	**51k**, **53a**, **56a**, **139ak**, **141ak**, **142ak** (Greger et al. 1984)
A. ptarmica L.	**101abe**, **102abe**, **141ab**, **143ab**, **167abcem**, **181a** (Kuropka et al. 1986; Kuropka and Glombitza 1987)
A. spinulifolia Fenzl ex Boiss.	**101a**, **102a**, **139b**, **144b**, **181a** (Greger et al. 1982)
A. tomentosa L.	**134an**, **139a** (Greger et al. 1981, 1982)
A. wilhelmsii C. Koch	**101cm**, **141m**, **143m**, **148am**, **149am**, **180am**, **181acm** (Greger and Hofer 1987)
Anacyclus pyrethrum (L.) Link	**51af**, **62af**, **101afj**, **141afj**, **142a**, **167acj**, **179j**, **180j**, **182a** (Burden and Crombie 1969; Jente et al. 1972; Boonen et al. 2012a, b)
A. clavatus (Desf.) Pers.	**101a**, **167a**, **174a** (Bohlmann et al. 1974)
Argyranthemum foeniculaceum (Willd. Webb ex Sch. Bip. (syn.: *Chrysanthemum anethifolium* Brouss.)	**184a** (Doskotch and Beal 1970)
A. frutescens (L.) Sch. Bip. (syn.: *Chrysanthemum frutescens* L.)	**51a**, **167a**, **184a** (Winterfeldt 1963; Bohlmann and Zdero 1967)
A. gracile Sch. Bip.	**167a**, **184a** (Bohlmann and Zdero 1967)
Artemisia dracunculus L.	**101ab**, **173a** (Saadali et al. 2001)
Chamaemelum fuscatum (Brot.) Vasc.	**101a**, **142ab**, **143a** (Bohlmann and Zdero 1970; Greger 1988)
C. nobile (L.) All.	**101a**, **142ab** (Greger 1988)
C. scariosum (Ball) Benedi	**101a**, **142ab**, **167a** (Greger 1988)
Cladanthus arabicus (L.) All.	**101a**, **181a** (Bohlmann et al. 1974)
Dendranthema morifolium (Ramat.) Tzvelev. (syn.: *Chrysanthemum morifolium* Ramat.)	**140a**, **142a**, **143a** (Tsao et al. 2003)
D. zawadskii var. *latilobum* (Maxim.) Kitam.	**143ab** (Rahman et al. 2007)
Leucanthemum hosmariense (Ball) Font Quer.	**67a** (Bohlmann et al. 1980a)
Leucocyclus formosus Boiss. ssp. *formosus*	**51a**, **53a**, **56a**, **61a**, **62a**, **84a**, **101a**, **139a** (Greger et al. 1981)
Otanthus maritimus (L.) Hoffm. and Link	**51ab**, **53ab**, **56ab**, **102a**, **167abe**, **174abe** (Bohlmann et al. 1974; Christodoulopoulou et al. 2005)
Asteraceae–Astereae	
Brachycome ciliocarpa W. Fitzg.	**64a** (Zdero et al. 1988)

Table 4 continued

PLANT SPECIES	ALKAMIDES
Asteraceae–Heliantheae	
Acmella alba (L′ Hér.) R. K. Jansen	**66a**, **166a**, **178cor**, **187cor**, **189r** (Bohlmann et al. 1980b)
A. calva (DC. in Wight) R. K. Jansen (syn.: *Spilanthes callimorpha* A. H. Moore)	**66a**, **71a**, **151a**, **153a**, **167c**, **168c**, **187c** (Li et al. 2007)
A. ciliata (H. B. K.) Cass.	**66ag**, **68a**, **70a**, **85a**, **87a**, **88a**, **92acg**, **97a**, **98a**, **109c**, **110c**, **111a, 116a**, **117a**, **152a**, **163a**, **166acg**, **167acg**, **168g**, **169a**, **172c**, **186c**, **187ac**, **188c**, **189c** (Martin and Becker 1984, 1985; Keipert 2009; Keipert and Melzig 2009)
A. decumbens (Sm.) R. K. Jansen	**175a**, **183f**, **187c** (Casado et al. 2009)
A. mauritiana A. Rich. ex Pers.	**67a** (Jondiko 1986)
A. oleracea (L.) R. K. Jansen (syn.: *Spilanthes oleracea* L.)	**92ag**, **95a**, **96a**, **150a**, **151a**, **169ag**, **178ac**, **185c**, **187ac** (Greger et al. 1985; Nakatami and Nagashima 1992; Simas et al. 2013)
A. oppositifolia (Lam.) R. K. Jansen (syn.: *Spilanthes americana* (L.f.) Hieron.	**66a**, **92ag**, **176a** (Ospina de Nigrinis et al. 1986; Molina-Torres et al. 1996)
A. paniculata (Wall.ex DC.) R. K. Jansen (syn.: *Spilanthes acmella* (L.) J. A. Murr.)	**65a**, **66a**, **85a**, **91a**, **92ag**, **151a**, **167a**, **168a**, **169ag**, **185c**, **187a**, **189c** (Ramsewak et al. 1999; Bae et al. 2010; Boonen et al. 2010a, b; Sharma et al. 2011)
A. radicans (Jacq.) R. K. Jansen	**68a**, **89a**, **92acg**, **110c**, **111c**, **167a**, **187c**, **188c** (Rios-Chavez et al. 2003)
Echinacea angustifolia DC.	**62a**, **63a**, **65a**, **66a**, **67a**, **135a**, **136a**, **145a**, **157a**, **159a**, **161ag**, **162a**, **163ag**, **166a**, **168ag**, **169ag**, **170ag** (Bohlmann and Grenz 1966; Bauer et al. 1989; Woelkart et al. 2005; Chen et al. 2005; Matovic et al. 2011; Mahringer et al. 2013)
E. atrorubens (Nutt.) Nutt.	**62ag**, **63ag**, **65ag**, **66ag**, **136a**, **151a**, **157ag**, **161ag**, **163a**, **164a**, **165a**, **167a**, **168a**, **169a** (Dietz and Bauer 2001; Dietz 2002)
E. pallida (Nutt.) Nutt.	**136a**, **162a**, **166a** (Thomsen et al. 2012)
E. purpurea (L.) Moench	**45a**, **62a**, **63a**, **65ag**, **66ag**, **67a**, **135a**, **136ad**, **151ag**, **154a**, **155g**, **156a**, **157a**, **158a**, **160g**, **161ag**, **162ag**, **166a**, **168ag**, **170a** (Bohlmann and Hoffmann 1983; Bauer et al. 1988; Binns et al. 2002; Matovic et al. 2011; Hohmann et al. 2011; Thomsen et al. 2012)
Heliopsis annua Hemsl.	**92a** (García-Chávez et al. 2004)
H. buphthalmoides (Jacq.) Dunal	**124a**, **125a**, **126a**, **127a**, **138a**, **139a** (Bohlmann et al. 1983; Jakupovic et al. 1986)
H. helianthoides var. *scabra* (Dunal) Fernald	**124a**, **126a** (Bohlmann et al. 1983)
H. longipes (A. Gray) S. F. Blake	**68a**, **89a**, **90a**, **92ag**, **95a**, **96a**, **166a**, **167a**, **169a**, **171a** (García-Chávez et al. 2004; López-Martínez et al. 2011)
H. novogaliciana B. L. Turner	**92a** (García-Chávez et al. 2004)
H. ex aff. *novogaliciana*	**68ag**, **87a**, **92ag**, **167a**, **169a** (García-Chávez et al. 2004)
H. procumbens Hemsl.	**68a**, **92a** (García-Chávez et al. 2004)
Salmea scandens (L.) DC.	**65a**, **66a**, **115c**, **177c**, **178c**, **187c**, **189c** (Herz and Kulanthaivel 1985; Bohlmann et al. 1985)
Sanvitalia ocymoides DC.	**57a**, **60a** (Domínguez et al. 1987)
Wedelia parviceps S. F. Blake	**92a** (Johns et al. 1982)
Asteraceae–Senecioneae	
Senecio colaminus Cuatrec.	**104cm** (Bohlmann and Zdero 1979)
S. erechtidoides Baker	**2f** (Ndom et al. 2010)
Aristolochiaceae	
Asarum forbesii Maxim.	**68a**, **69a** (Zhang et al. 2005)
Asiasarum heterotropoides (Schmidt) F. Maek.	**65a**, **66a**, **101a** (Yasuda et al. 1981a)

Table 4 continued

PLANT SPECIES	ALKAMIDES
Brassicaceae	
Lepidium meyeni Walp.	**3t**, **19t**, **21tu**, **24t**, **25t**, **26t**, **27tu**, **28tu**, **29t**, **40t**, **41tu**, **49t** (Muhammad et al. 2002; Zhao et al. 2005; Wang et al. 2007)
Euphorbiaceae	
Mallotus lianus Croiz.	**11a**, **37ab** (Jiang et al. 2009)
Menispermaceae	
Cissampelos glaberrima A.St.-Hil.	**86a**, **89a**, **101a**, **112a** (Loureiro-Rosario et al. 1996)
Piperaceae	
Piper amalago L.	**5k**, **6k**[a], **9k**[a], **17k**[a], **19k**, **22k**[a], **23k**[a], **30k**[a], **33k**[a], **41k**, **42k**[a] (Achenbach et al. 1986)
P. boehmeriifolium (Miq.) Wall.	**62ak**, **82k**, **101akls** (Tang et al. 2011)
P. brachystachyon Vahl.	**18k** (Koul et al. 1988)
P. guineense Schumach. and Thonn.	**33a**, **101a** (Tackie et al. 1975)
P. hancei Maxim.	**101a**, **117a** (Narui et al. 1995)
P. longum L.	**1a**, **11a**, **13b**[b], **16a**, **33a**, **36ab**, **37**[b], **39b**, **46a**, **47ab**, **50b**, **51a**, **53b**, **84a**, **101ab** (Dhar and Atal 1967; Koul et al. 1988; Sun et al. 2007; Li et al. 2013)
P. nigrum L.	**8b**, **13abk**, **14abk**, **16a**, **33a**, **34a**, **37abkp**, **38abk**, **41k**, **51a**, **62ak**, **89b**, **99ab**, **100a**, **101abk**, **103b**, **112a**, pipercycliamide (Wei et al. 2004; Siddiqui et al. 2004; Ee et al. 2010; Dawid et al. 2012)
P. novae-hollandiae Miq.	**101a**, **112a** (Loder et al. 1969)
P. peepuloides Roxb.	**33a**, **101a** (Dhar and Atal 1967)
P. retrofractum Vahl (syn.: *P. officinarum* (Miq.) C.DC; *P. chaba* Hunter)	**4a**, **7b**, **11b**, **13ab**, **15b**, **16a**, **33ab**, **37ab**, **39b**, **46a**, **47a**, **48a**, **101a** (Gupta et al. 1976; Ahn et al. 1992; Kikuzaki et al. 1993; Morikawa et al. 2004; Kubo et al. 2013)
P. sarmentosum Roxb.	**101ak** (Likhitwitayawuid et al. 1987)
P. sylvaticum Roxb.	**99a**, **101a** (Banerji and Pal 1982)
P. trichostachyon (Miq.) C.DC.	**11k** (Singh et al. 1971)
P. tuberculatum Jacq.	**101ab** (Leitão da-Cunha and de Oliveira Chaves 2001)
Poaceae	
Ctenium aromaticum (Walter) Alph.Wood	**64a**, **101a**, **102a** (Gamboa-Leon and Chilton 2000)
Rutaceae	
Pilocarpus trachylophus Holmes	**101a** (Andrade-Neto et al. 1996)
Tetradium daniellii (Benn.) T.G.Hartley (syn.: *Euodia hupehensis* Dode)	**46a**, **101a** (Reisch et al. 1985)
Zanthoxylum ailanthoides Siebold and Zucc.	**58ad**, **114a** (Yasuda et al. 1981b; Chen et al. 2009)
Z. armatum DC.	**72d**, **74d**, **75d**, **76d**, **77d**, **79d**, **81d**, **93d**, **116d** (Devkota et al. 2013)
Z. bungeanum Maxim.	**51d**, **53d**, **55d**, **56d**, **58adw**, **59d**, **72d**, **74d**, **77d** (Mizutani et al. 1988; Xiong et al. 1997; Huang et al. 2012)
Z. clava-herculis L.	**72a** (Crombie 1955a)
Z. gilletii (De Willd.) P.G.Waterman (syn.: *Fagara macrophylla* Engl.)	**1f**, **58a**, **62a**, **86f**, **101a**, **112a**, octacosantyramide (Kubo et al. 1984; Adesina and Reisch 1988; Wansi et al. 2009)
Z. integrifolium (Merr.) Merr.	**51d**, **52a**, **54a**, **55ad**, **56ad**, **58ad** (Chen et al. 1999)
Z. liebmannianum (Engl.) P.Wilson	**72a** (Navarrete and Hong 1996)
Z. piperitum DC.	**58a**, **59ad**, **72adv**, **73d**, **74adv**, **75d**, **76d**, **77d**, **78d** (Crombie and Tayler 1957; Hatano et al. 2004; Jang et al. 2008)
Z. rhetsa DC.	**56a** (Shibuya et al. 1992)

Table 4 continued

PLANT SPECIES	ALKAMIDES
Z. schinifolium Siebold and Zucc.	**72d** (Iseli et al. 2007)
Z. zanthoxyloides (Lam.) Zepern and Timler	**101a**, **110a** (Bowden and Ross 1963; Chaaib et al. 2003)

[a] With unknown configuration of the double bonds (Achenbach et al. 1986)

[b] With unsolved structure of a pentylamine moiety (Sun et al. 2007)

sequences also suggested *Anacyclus* to be the sister genus of *Achillea* (Ehrendorfer and Guo 2005, 2006). The European species/cytotypes of the taxonomically complex *A. millefolium* group can be distinguished from the Asian and North-American representatives by the predominance of the C_{10}-2*E*,4*E*,6*Z*-trienoic acid piperideide (**105e**) (Greger and Hofer 1989; Greger and Werner 1990) (Fig. 9). With regard to the enormous capacity for vegetative reproduction and gregarious clonal growth due to the abundant formation of rootsuckers in many *Achillea* species, e.g. in *A. wilhelmsii*, the accumulation of alkamides in these parts deserves also ecological attention (Greger and Hofer 1987). Taking into account that unsaturated alkamides were considered to be unstable when heated and to be non-volatile with water vapour it is noteworthy that the three piperideides **101e**, **105e**, **106e** together with **101a** and **101b** were detected after distillation in a GC/MS analyses of the root oil of *A. distans* ssp. *distans*, a member to the *A. millefolium* group (Lazarević et al. 2010).

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