Chapter 1

The Brassinosteroids Family – Structural Diversity of Natural Compounds and Their Precursors

Marco Antonio Teixeira Zullo and Andrzej Bajguz

Abstract The members of the brassinosteroids family, defined as the 3-oxygenated (20β)-5α-cholestane-22α,23α-diols or their derived compounds isolated from plants, bearing additional alkyl or oxy substituents, are presented. Further, brassinosteroids are grouped into C_{27}, C_{28}, and C_{29} depending upon the number of carbons in their skeletons. Their structural variations occur due to the substitution in A and B-rings as well in the side chain. They occur in both free and conjugated forms to sugars, fatty and inorganic acids. Their presence in Algae, Bryophyta, Pteridophyta and Angiosperms indicates a ubiquitous distribution in the plant kingdom. The related brassinosteroids precursors, as well as their occurrence, are also presented. Brassinosteroids are considered as the 6th class of plant hormones which have been established after the discovery of brassinolide and other related compounds.

Keywords Natural brassinosteroids · Brassinosteroids precursors · Brassinosteroids occurrence

1 Introduction

Intrigued with previous reports of growth regulating properties of pollen extracts, Mitchell and Whitehead (1941) examined the growth responses and histological changes that resulted from the application of ethereal extracts of corn pollen on intact bean plants or on the cut surfaces of decapitated stems. They observed that the first internode of the plants where these extracts were applied grew significantly more and faster than the untreated ones or treated with some known auxins, as well as gained more fresh and dry weights than the controls. They demonstrated that

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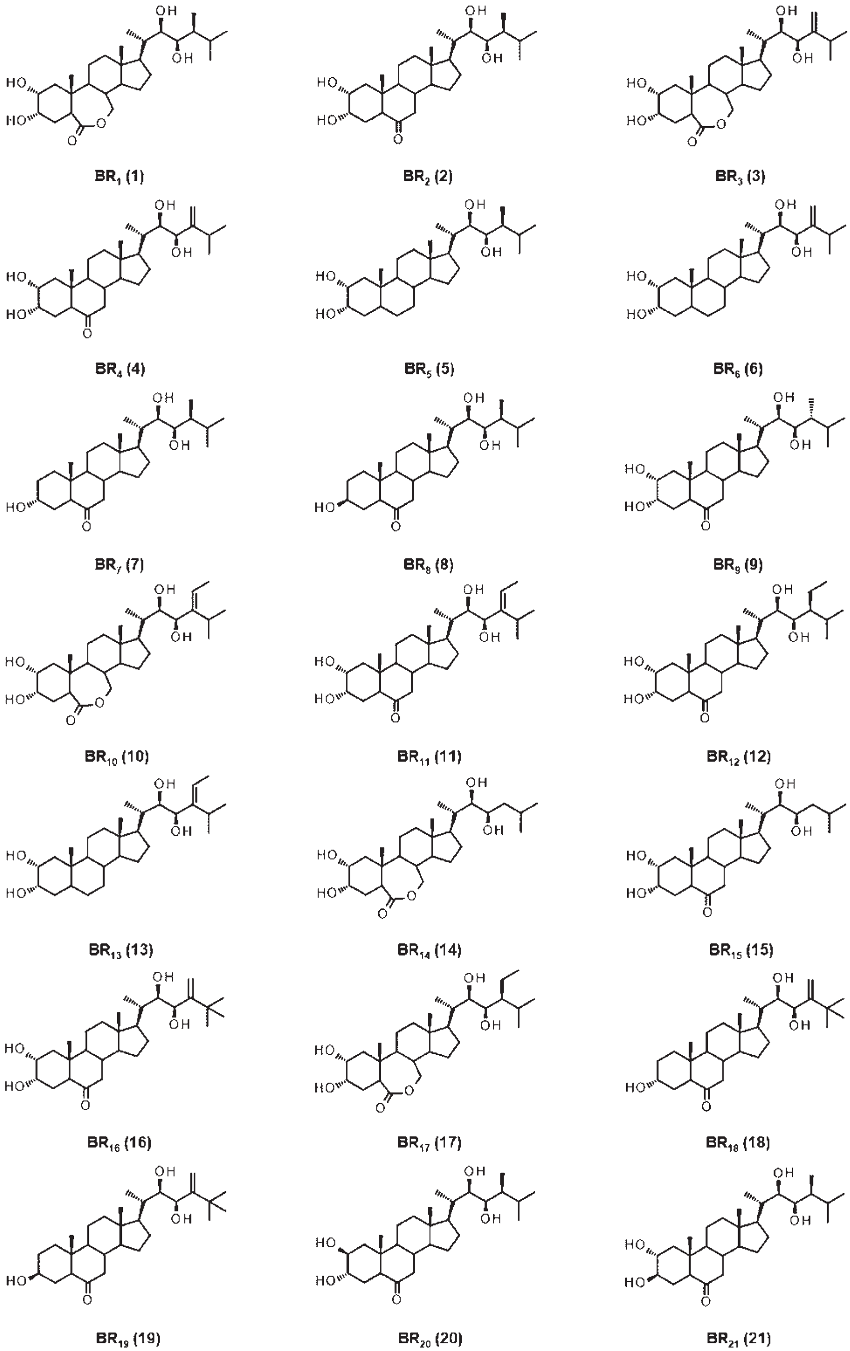
these were light dependent phenomena, and due to cell elongation rather than cell division. When applied to tap roots, these extracts inhibited root elongation and provoked the appearance of small tumors distal to the application point. When these pollen extracts where applied to the cut surfaces of decapitated stems they caused pronounced radial elongation of epidermal, cortical parenchyma, and endothelial cells. Later Mitchell et al., reported that immature bean seeds also contained plant growth-stimulating hormones (Mitchell et al. 1951) and that *Brassica napus* pollen contained new, yet unknown, hormones they called *brassins* (Mitchell et al. 1970), all of them with properties similar to those reported earlier (Mitchell and Whitehead 1941).

About 60 kinds of pollen were then screened for plant growth activity in the bean second internode assay, and "a few samples, notably the pollen from rape plant (*Brassica napus* L.) and alder tree (*Alnus glutinosa* L.), produced an unusual response that combined elongation (the typical gibberellin response) with swelling and curvature" (Mandava 1988). At the same time, some experiments showed that application of brassins to young bean and Siberian elm tree plants promoted overall plant growth (Mitchell and Gregory 1972), what led United States Department of Agriculture (USDA) to initiate an effort aimed to explore the agricultural perspectives of brassins and to isolate their component(s). After processing 500 libers of rape pollen, finally, the USDA team announced the isolation and structure elucidation of the active principle, brassinolide (**1**) (Grove et al. 1979), the first plant hormone of steroidal nature, presenting, unlike animal steroidal hormones, (*i*) a 22α,23α-dihydroxylated campestane side chain, (*ii*) a B-ring lactone, and, (*iii*) a 2α,3α-dihydroxylated ring A. Bean second internodes exhibited elongation, curvature, swelling and even splitting when treated with increasing amounts of brassinolide (**1**) (Grove et al. 1979) (Fig. 1.1), a very distinct effect never observed with any other known plant hormone. Its isolation was followed by its partial synthesis (Fung and Siddall 1980; Ishiguro et al. 1980) and of its analogues (Thompson et al. 1979, 1981, 1982; Mori 1980; Takatsuto et al. 1981; Sakakibara and Mori 1982; Sakakibara et al. 1982; Mori et al. 1982), some later recognized as plant hormones themselves.

The early synthetic work furnished many compounds with similar or weaker brassin activity, what prompted natural products chemists to search for brassinolide related compounds in plant species other than rape. To the first of them, the 6-ketosteroid castasterone (**2**) (Yokota et al. 1982a), the putative biosynthetic precursor of brassinolide (**1**), followed that of dolicholide (**3**) (Yokota et al. 1982b), dolichosterone (**4**) (Baba et al. 1983), both with a 24-methylene-5α-cholestane structure, and 28-homodolichosterone (**11**) (Baba et al. 1983), with a 24(*E*)-ethylidene-5α-cholestane skeleton instead of a 5α-campestane basis as in brassinolide (**1**) and castasterone (**2**), and then a multitude of brassinosteroids (BRs) of different side chain structures and oxygenation patterns were isolated, giving rise to the class of brassinosteroids phytohormones, the components of which will be described ahead.



$\mathbf{BR_1}$ **(1)** $\mathbf{BR_2}$ **(2)** $\mathbf{BR_3}$ **(3)**

$\mathbf{BR_4}$ **(4)** $\mathbf{BR_5}$ **(5)** $\mathbf{BR_6}$ **(6)**

$\mathbf{BR_7}$ **(7)** $\mathbf{BR_8}$ **(8)** $\mathbf{BR_9}$ **(9)**

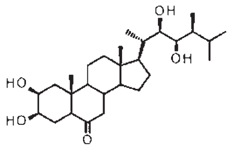
$\mathbf{BR_{10}}$ **(10)** $\mathbf{BR_{11}}$ **(11)** $\mathbf{BR_{12}}$ **(12)**

$\mathbf{BR_{13}}$ **(13)** $\mathbf{BR_{14}}$ **(14)** $\mathbf{BR_{15}}$ **(15)**

$\mathbf{BR_{16}}$ **(16)** $\mathbf{BR_{17}}$ **(17)** $\mathbf{BR_{18}}$ **(18)**

$\mathbf{BR_{19}}$ **(19)** $\mathbf{BR_{20}}$ **(20)** $\mathbf{BR_{21}}$ **(21)**

Fig. 1.1 Natural brassinosteroids



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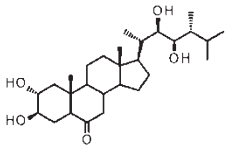

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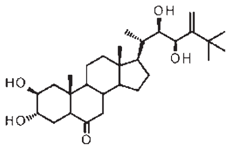

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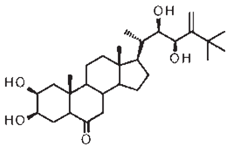

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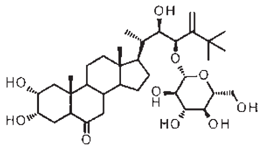

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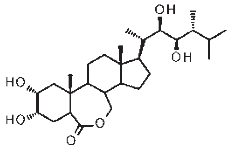

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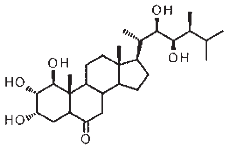

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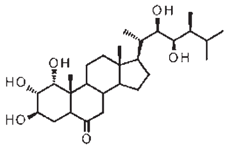

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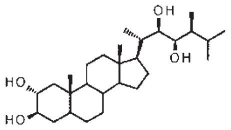

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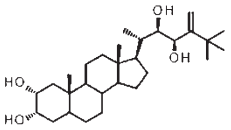

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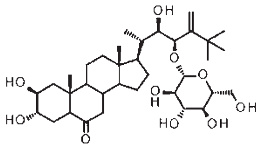

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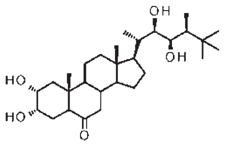

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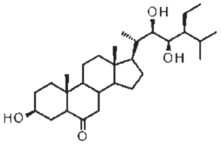

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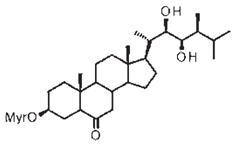

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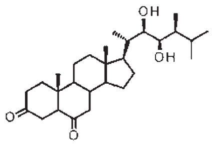

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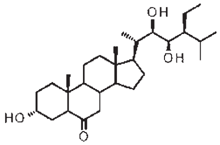

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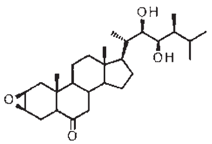

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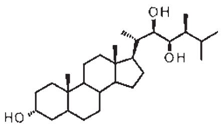

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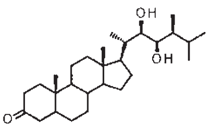

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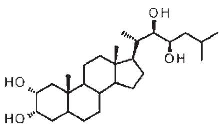

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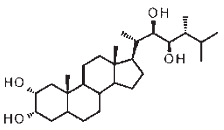

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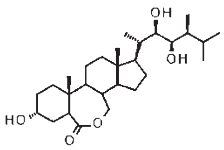
Fig. 1.1 (continued)



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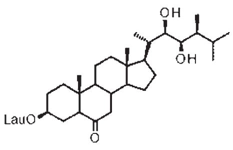

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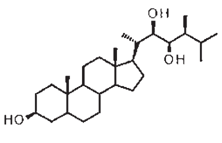

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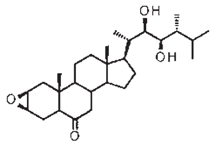

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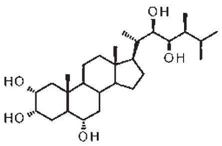

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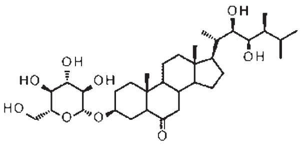

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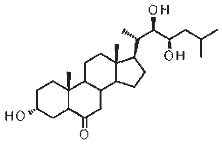

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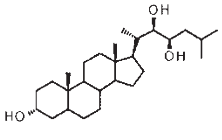

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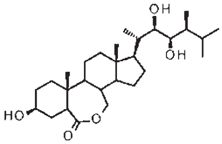

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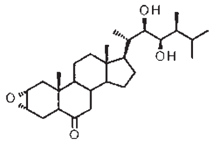

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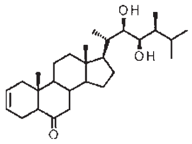

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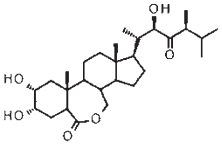

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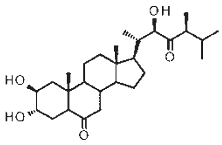

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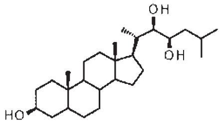

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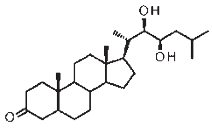

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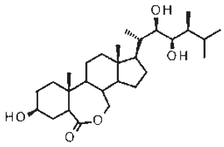

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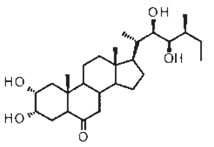

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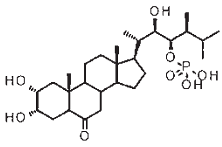

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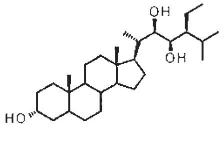

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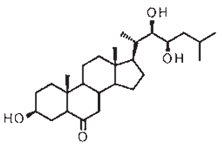


BR$_{62}$ (62)

Fig. 1.1 (continued)

2 Natural Brassinosteroids

About sixty compounds with structures related to that of brassinolide (**1**) were isolated from or detected in plant materials in the last forty years (see Table 1.1 and Fig. 1.1). They were found in 26 species of 6 families of Algae, in 2 species of 2 families of Bryophyte, in 15 species of 8 families of Pteridophyte, in 6 species of 4 families of Gymnospermae, in 74 species of 35 families of Angiospermae (in 18 species of 6 families of Monocotyledoneae and 56 species of Dicotyledoneae), and in some plant derived products. About 15 biosynthetic precursors of brassinosteroids, some presenting brassinosteroid activity themselves, were found in many plant species.

Table 1.1 First report of a natural brassinosteroid

BR_n	Trivial name	References
1	Brassinolide	Grove et al. (1979)
2	Castasterone	Yokota et al. (1982a)
3	Dolicholide	Yokota et al. (1982b)
4	Dolichosterone	Baba et al. (1983)
5	6-Deoxocastasterone	Yokota et al. (1983c)
6	6-Deoxodolichosterone	Yokota et al. (1983c)
7	Typhasterol	Schneider et al. (1983)
8	Teasterone	Abe et al. (1984a)
9	24-Epicastasterone	Yokota et al. (1987b)
10	28-Homodolicholide	Yokota et al. (1983b)
11	28-Homodolichosterone	Baba et al. (1983)
12	28-Homocastasterone	Abe et al. (1983)
13	6-Deoxo-28-homodolichosterone	Yokota et al. (1987c)
14	28-Norbrassinolide	Abe et al. (1983)
15	28-Norcastasterone	Abe et al. (1983)
16	25-Methyldolichosterone	Kim et al. (1987)
17	28-Homobrassinolide	Ikekawa et al. (1984)
18	2-Deoxy-25-methyldolichosterone	Takahashi et al. (1988)
19	3-Epi-2-deoxy-25-methyldolichosterone	Yokota and Takahashi (1988)
20	2-Epicastasterone	Takahashi et al. (1988)
21	3-Epicastasterone	Takahashi et al. (1988)
22	2,3-Diepicastasterone	Takahashi et al. (1988)
23	3,24-Diepicastasterone	Takahashi et al. (1988)
24	2-Epi-25-methyldolichosterone	Takahashi et al. (1988)
25	2,3-Diepi-25-methyldolichosterone	Takahashi et al. (1988)
26	23-*O*-β-*D*-Glucopyranosyl-25-methyldolichosterone	Yokota et al. (1987a)
27	24-Epibrassinolide	Ikekawa et al. (1988)
28	1β-Hydroxycastasterone	Takahashi et al. (1988)

(continued)

Table 1.1 (continued)

BR_n	Trivial name	References
29	1α-Hydroxy-3-epicastasterone	Kim (1991)
30	3-Epi-6-deoxocastasterone	Kim (1991)
31	6-Deoxo-25-methyldolichosterone	Kim (1991)
32	23-*O*-β-*D*-Glucopyranosyl-2-epi-25-methyldolichosterone	Kim (1991)
33	25-Methylcastasterone	Taylor et al. (1993)
34	28-Homoteasterone	Schmidt et al. (1993b)
35	Teasterone-3-myristate	Asakawa et al. (1994)
36	3-Dehydroteasterone	Abe et al. (1994)
37	28-Homotyphasterol	Abe et al. (1995a)
38	Secasterone	Schmidt et al. (1995b)
39	6-Deoxotyphasterol	Griffiths et al. (1995)
40	3-Dehydro-6-deoxoteasterone	Griffiths et al. (1995)
41	6-Deoxo-28-norcastasterone	Spengler et al. (1995)
42	6-Deoxo-24-epicastasterone	Spengler et al. (1995)
43	2-Deoxybrassinolide	Schmidt et al. (1995c)
44	Teasterone-3-laurate	Asakawa et al. (1996)
45	6-Deoxoteasterone	Fujioka et al. (1998b)
46	24-Episecasterone	Friebe et al. (1999)
47	6α-Hydroxycastasterone	Fujioka et al. (2000b)
48	3-*O*-β-*D*-Glucopyranosylteasterone	Soeno et al. (2000b)
49	28-Nortyphasterol	Fujioka et al. (2000a)
50	6-Deoxo-28-nortyphasterol	Yokota et al. (2001)
51	3-Epibrassinolide	Konstantinova et al. (2001)
52	2,3-Diepisecasterone	Antonchick et al. (2003)
53	Secasterol	Antonchick et al. (2003)
54	Cryptolide	Watanabe et al. (2000)
55	23-Dehydro-2-epicastasterone	Hwang et al. (2006)
56	6-Deoxo-28-norteasterone	Bhardwaj et al. (2007)
57	3-Dehydro-6-deoxo-28-norteasterone	Bhardwaj et al. (2007)
58	3-Epi-2-deoxybrassinolide	Katsumata et al. (2008)
59	26-Norcastasterone	Son et al. (2013)
60	Castasterone 23-phosphate	Kim et al. (2015)
61	6-Deoxo-28-homotyphasterol	Xin et al. (2016)
62	28-Norteasterone	Oklestkova et al. (2017)

The finding that the rice lamina inclination assay, developed by Maeda (1965), to test for auxin activity could be used to detect the activity of brassinosteroids at even nanomolar or subnanomolar concentrations (Wada et al. 1981) and the development of a microanalytical method for the quantification of 22α, 23α-dihydroxybrassinosteroids (Takatsuto et al. 1982), allowed a rapid expansion of the number of known brassinosteroids. The first brassinosteroids isolated presented, as common features, (*i*) a 5α-cholestane or a 6,7-seco-5α-cholestane derived skeleton, (*ii*) ring A with one to three oxygen functions (one always at carbon 3), (*iii*) ring B fully saturated or with varying degree of oxidation at carbon 6, (*iv*) all-*trans* ring junctions and (*v*) 22α,23α-dihydroxylation. In this sense, 3-oxygenated (20β)-5α-cholestane-22α,23α-diols of plant origin, bearing additional alkyl or oxy substituents, were considered as natural brassinosteroids (Zullo and Adam 2002). A more restricted definition states that, in the biosynthetic route to a brassinosteroid lactone, "one would consider as brassinosteroids only those compounds originated after the 22α,23α-dihydroxylation (i.e., those between teasterone or 6-deoxoteasterone and brassinolide), and hence as *brassinosteroid precursors* those before dihydroxylation occurs (i.e., those compounds up to cathasterone and 6-deoxocathasterone)" (Zullo et al. 2003; Zullo and Kohout 2004). After then some other brassinosteroids presenting 2,3-epoxy, 23-dehydro, 23-glycosidic, 23-ester functions, or 26-nor side chain or even 2,3-unsaturation were isolated, "allowing to consider as *natural brassinosteroids* the 3-oxygenated (20β)-5α-cholestane-22α,23α-diols or their derived compounds isolated from plants, bearing additional alkyl or oxy substituents" (Zullo 2018).

The unconjugated brassinosteroids so far isolated present 27 (C_{27}), 28 (C_{28}) or 29 (C_{29}) carbons, with 5α-cholestane or 26-nor-5α-campestanes (= 26-nor-24α-methyl-5α-cholestane) structures for the C_{27} series, 5α-campestane (= 24α-methyl-5α-cholestane), 5α-ergostane (= 24β-methyl-5α-cholestane) or 24-methylene-5α-cholestane skeletons for the C_{28} series, and 5α-sitostane (= 24α-ethyl-5α-cholestane), 24(*Z*)-ethylidene-5α-cholestane, 25-methyl-5α-campestane and 24-methylene-25-methyl-5α-cholestane structures for the C_{29} series (Fig. 1.2). Only one of the side chains of isolated brassinosteroids is of a 26-nor sterol, although C_{26}-demethylation of brassinosteroids have been demonstrated in metabolic studies with some species (Joo et al. 2012, 2015; Kim et al. 2000a, b). From the 12 different side chains of natural brassinosteroids, 9 of them present 22α,23α-dihydroxylation, while one presents a 22α-hydroxy-23-oxo group, another one presents conjugation of one glucose unit at the 23α-hydroxyl, and a last different side chain shows phosphorylation at the 23α-hydroxyl. Feeding studies shows that side chain glucosylation can occur at either C-23 (Poppenberger et al. 2005) or C-22 (Soeno et al. 2006), and also at C-25 or C-26 after hydroxylation at these carbons (Hai et al. 1996). Phosphorylation (Kim et al. 2015) and sulfonation (Rouleau et al. 1999) have been demonstrated to occur at the side chain of brassinosteroids, but while the first occurs at C-23, the second occurs at C-22, at least with the actual experimental data available.

It is known that the bioactivity of brassinosteroids is dependent on the structure of the side chain and of the A/B rings (Takatsuto et al. 1983b; Takatsuto et al. 1983a; Brosa et al. 1996; Takatsuto et al. 1987; Mandava 1988; Liu et al. 2017; Zullo and Adam 2002). Regarding to the side chain, as general rules, employing the rice lamina inclination assay on any of its versions (Maeda 1965; Wada et al. 1981; Fujioka et al. 1998a), for the same A/B ring structures, 22α,23α-dihidroxybrassinosteroids of the brassinolide series are so active as of the 28-homobrassinosteroids series (Takatsuto et al. 1983a), and more active than those of 24-epi- or 28-norbrassinosteroids (Takatsuto et al. 1983a; Wada et al. 1983),

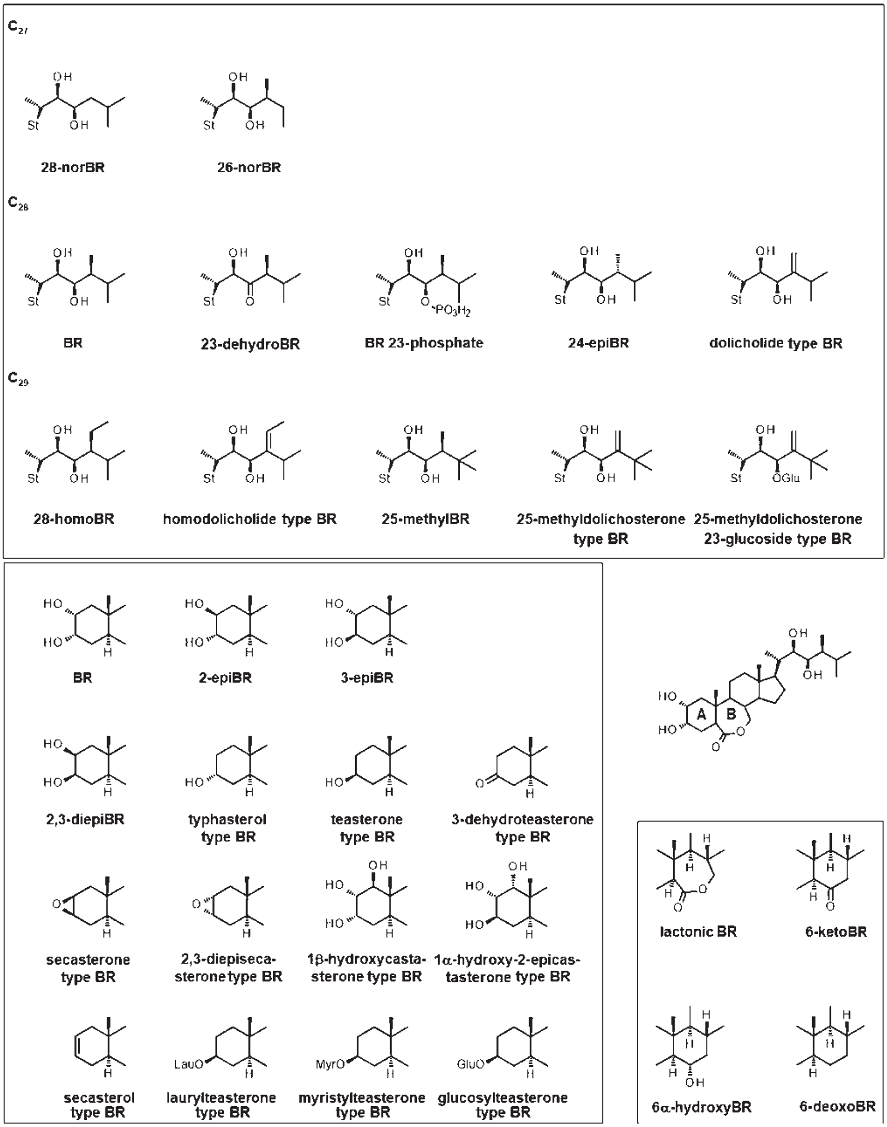


Fig. 1.2 Brassinolide and structural variations of brassinosteroids

which are more active than 26-norbrasssinosteroids (Kim et al. 2000a; Watanabe et al. 2001). 23-Dehydrogenation (Watanabe et al. 2001), or conjugation at one of the side chain hydroxyls (Suzuki et al. 1993b; Kim et al. 2015; Rouleau et al. 1999), diminishes (Yokota et al. 1998; Suzuki et al. 1993b) or abolishes the biological activity (Kim et al. 2015; Rouleau et al. 1999), an effect contrary to that observed with 25-methylation (Mori and Takeuchi 1988). It is to note that the relative biological activity of brassinosteroids vary according to the biological assay performed for their evaluation, not only in relation to the side chain but also to the other active sites of their molecules (Takatsuto et al. 1983b; Watanabe et al. 2001; Zullo and Adam 2002; Liu et al. 2017).

A greater structural variation is observed in ring A, with 15 different structures reported, ranging from $\Delta^{2,3}$-unsaturated to trioxygenated and conjugated brassinosteroids: even so, this variation still does not reflect all the possible substructures at this ring, presumed either by efforts of large scale isolation of brassinosteroids (Kim 1991; Fujioka 1999), or by the study of the metabolism of brassinosteroids (Zullo 2018). The biological activity for brassinosteroids with A/B *trans* ring junctions increases as substitution in ring A changes in the order 3β-hydroxy ≤ 3-oxo ≤ 3α-hydroxy < 2α,3α-dihydroxy, and diminishes as deviates from these patterns (Mandava 1988; Zullo and Adam 2002; Liu et al. 2017; Takatsuto et al. 1987; Fujioka et al. 1995a).

The structural variations in ring B reflect the main steps in the biosynthesis of brassinosteroids (Vriet et al. 2013), being more active as its oxidation state increases (Mandava 1988) sequentially from the 6-deoxo to the 6α-hydroxy to the 6-oxo and to the 7-oxalactone types. Therefore, brassinosteroids can be classified, according to the B ring structure, as: (a) **6-oxo-7-oxalactonic brassinosteroids:** (*i*) 2α,3α-dihydroxylated: brassinolide (**1**), dolicholide (**3**), 28-homodolicholide (**10**), 28-norbrassinolide (**14**), 28-homobrassinolide (**17**), 24-epibrassinolide (**27**), cryptolide (**54**); (*ii*) 2α, 3β-dihydroxylated: 3-epibrassinolide (**51**); (*iii*) 3α-hydroxylated: 2-deoxybrassinolide (7-oxatyphasterol, **43**); (*iv*) 3β-hydroxylated: 3-epi-2-deoxybrassinolide (7-oxateasterone, **58**); (b) **6-oxo (or 6-keto) brassinosteroids:** (*i*) 2α,3α-dihydroxylated: castasterone (**2**), dolichosterone (**4**), 24-epicastasterone (**9**), 28-homodolichosterone (**11**), 28-homocastasterone (**12**), 28-norcastasterone (**15**), 25-methyldolichosterone (**16**), 25-methylcastasterone (**33**), 26-norcastasterone (**59**); (*ii*) 2β,3α-dihydroxylated: 2-epicastasterone (**20**), 2-epi-25-methyldolichosterone (**24**), 23-dehydro-2-epicastasterone (**55**); (*iii*) 2α,3β-dihydroxylated: 3-epicastasterone (**21**), 3,24-diepicastasterone (**23**); (*iv*) 2β,3β-dihydroxylated: 2,3-diepicastasterone (**22**), 2,3-diepi-25-methyldolichosterone (**25**); (*v*) 3α-monohydroxylated: typhasterol (**7**), 2-deoxy-25-methyldolichosterone (**18**), 28-homotyphasterol (**37**), 28-nortyphasterol (**49**); (*vi*) 3β-monohydroxylated: teasterone (**8**), 3-epi-2-deoxy-25-methyldolichosterone (**19**), 28-homoteasterone (**34**), 28-norteasterone (**62**); (*vii*) 1β,2α,3α-trihydroxylated: 1β-hydroxycastasterone (**28**); (*viii*) 1α,2α,3β-dihydroxylated: 1α-hydroxy-3-epicastasterone (**29**); (*ix*) 2α,3α-epoxide: 2,3-diepisecasterone (**52**); (*x*) 2β,3β-epoxide: secasterone (**38**), 24-episecasterone (**46**); (*xi*) Δ^2-olefin: secasterol (**53**); (*xii*) 3β-conjugates: teasterone-3-myristate (**35**), teasterone-3-laurate (**44**), 3-O-β-D-glucopyranosylteasterone (**48**); (*xiii*) 23α-conjugates: 23-*O*-β-*D*-glucopyranosyl-25-methyldolichosterone (**26**), 23-*O*-β-*D*-glucopyranosyl-2-epi-25-methyldolichosterone (**32**), castasterone 23-phosphate (**60**); (*xiv*) 3-dehydro: 3-dehydroteasterone (**36**); (c) **6α-hydroxybrassinosteroids:** 6α-hydroxycastasterone (**47**); (d) **6-deoxobrassinosteroids:** (*i*) 2α,3α-dihydroxylated: 6-deoxocastasterone (**5**), 6-deoxodolichosterone (**6**), 6-deoxo-

28-homodolichosterone (**13**), 6-deoxo-25-methyldolichosterone (**31**), 6-deoxo-28-norcastasterone (**41**), 6-deoxo-24-epicastasterone (**42**); (*ii*) 2α,3β-dihydroxylated: 3-epi-6-deoxocastasterone (**30**); (*iii*) 3α-monohydroxylated: 6-deoxotyphasterol (**39**), 6-deoxo-28-nortyphasterol (**50**), 6-deoxo-28-homotyphasterol (**61**); (*iv*): 3β-monohydroxylated: 6-deoxoteasterone (**45**), 6-deoxo-28-norteasterone (**56**); (*v*) 3-dehydro: 3-dehydro-6-deoxoteasterone (**40**), 3-dehydro-6-deoxo-28-norteasterone (**57**).

3 Brassinosteroids Precursors

A series of papers revealed the main steps of brassinosteroids biosynthesis, from the plant sterols to the brassinosteroid lactones, especially that from campesterol (**CR**) or campestanol (**CN**) to brassinolide (**1**). From these studies it became clear that, if the natural brassinosteroids can be easily recognized from their chemical structures, similar observation does not happen with their precursors (see Fig. 1.3 and Table 1.2). The first experiments established the biosynthesis of brassinolide (**1**) from teasterone (**8**) via, sequentially, 3-dehydroteasterone (**36**), typhasterol (**7**), and castasterone (**2**) (Suzuki et al. 1993a, 1994a, c) (follow by Fig. 1.4). Soon after it was found that campesterol (**CR**) was converted to campestanol (**CN**) and to 6α-hydroxycampestanol (**63**), 6-oxocampestanol (**64**), 22α-hydroxy-6-oxocampestanol (**65**), named cathasterone, and this one to teasterone (**8**) (Fujioka et al. 1995b). The complete biosynthetic sequence of brassinolide starting from campesterol (**CR**) via cathasterone (**65**) is known as the early C-6 oxidation pathway (a route in which C-6 oxidation occurs earlier than 22α,23α-dihydroxylation).

The frequent isolation or detection of 6-deoxobrassinosteroids brought the suspicion that another biosynthetic route to brassinosteroid lactones could exist. Feeding experiments with labeled precursors established the sequence 6-deoxoteasterone (**45**), 3-dehydro-6-deoxoteasterone (**40**), 6-deoxotyphasterol (**39**), 6-deoxocastasterone (**5**), castasterone (**2**), brassinolide (**1**), which was called the late C-6 oxidation pathway (a route in which C-6 oxidation occurs later than 22α, 23α-dihydroxylation) (Choi et al. 1997). It was further demonstrated the conversion of campestanol (**CN**) to 6-deoxoteasterone (**45**) through 6-deoxocathasterone (**66**) (Bishop et al. 1999), and the presence of 3-epi-6-deoxocathasterone (**67**), a putative brassinosteroid precursor, in cultured cells of *Catharantus roseus* (Fujioka et al. 2000b).

A thorough examination of the sterols present in cultured cells of *C. roseus* and in *Arabidopsis* seedlings, conjugated with metabolic studies with deuterated substrates, revealed that the conversion of campesterol (**CR**) to campestanol (**CN**) occurs through campest-4-en-3-one (**4en3one**) and campestan-3-one (**3one**) (Fujioka et al. 2002). Moreover, it revealed the operation of intermediates in the conversion of campesterol (**CR**) to 6-deoxocathasterone (**66**), originating

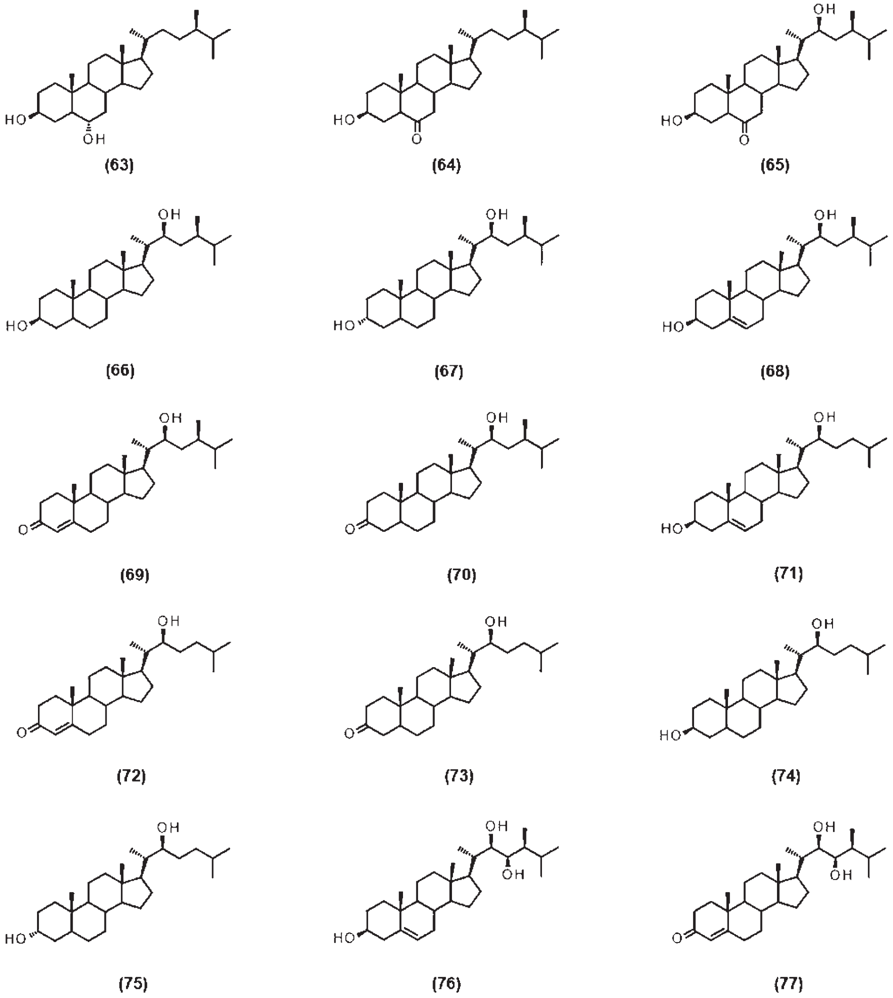


Fig. 1.3 Brassinosteroids precursors

22α-hydroxycampesterol (**68**), 22α-hydroxycampest-4-en-3-one (**69**), and 22α-hydroxy-5α-campestan-3-one (**70**) from, respectively, campesterol (CR), campest-4-en-3-one (**4en3one**) and campestan-3-one (**3one**). In the same extracts were found also the 28-norhomologues 22α-hydroxycholesterol (**71**), 22α-hydroxycholest-4-en-3-one (**72**), 22α-hydroxy-5α-cholestan-3-one (**73**), 6-deoxo-28-norcathasterone (**74**) and 3-epi-6-deoxo-28-norcathasterone (**75**). Later, studying the action of *Arabidopsis* CYP90C1 and CYP90D1, it was found that these enzymes act on 3-epi-6-deoxocathasterone (**67**), 22α-hydroxycampesterol (**68**), 22α-hydroxy-5α-campestan-3-one (**70**), and 22α-hydroxycampest-4-en-3-

Table 1.2 Brassinosteroid precursors

Compound	Trivial name	References
63	6α-Hydroxycampestanol	Fujioka et al. (1995b)
64	6-Oxocampestanol	Fujioka et al. (1995b)
65	Cathasterone	Fujioka et al. (1995b)
66	6-Deoxocathasterone	Bishop et al. (1999)
67	3-Epi-6-deoxocathasterone	Fujioka et al. (2000b)
68	22α-Hydroxycampesterol [(22*S*)-22-Hydroxycampesterol]	Fujioka et al. (2002)
69	22α-Hydroxycampest-4-en-3-one [(22*S*,24*R*)-22-Hydroxyergost-4-en-3-one]	Fujioka et al. (2002)
70	22α-Hydroxycampestan-3-one [(22*S*,24*R*)-22-Hydroxy-5α-ergostan-3-one]	Fujioka et al. (2002)
71	22α-Hydroxycholesterol [(22*S*)-28-Nor-22-hydroxycampesterol]	Fujioka et al. (2002)
72	22α-Hydroxycholest-4-en-3-one [(22*S*)-28-Nor-22-hydroxyergost-4-en-3-one]	Fujioka et al. (2002)
73	22α-Hydroxycholestan-3-one [(22*S*)-28-Nor-22-hydroxy-5α-ergostan-3-one]	Fujioka et al. (2002)
74	6-Deoxo-28-norcathasterone	Fujioka et al. (2002)
75	3-Epi-6-deoxo-28-norcathasterone	Fujioka et al. (2002)
76	22α,23α-Dihydroxycampesterol [(22*R*,23*R*)-22,23-Dihydroxycampesterol]	Ohnishi et al. (2006b)
77	22α,23α-Dihydroxycampest-4-en-3-one [(22*R*,23*R*)-22,23-Dihydroxycampest-4-en-3-one]	Ohnishi et al. (2006b)

one (**69**) to yield, respectively, 6-deoxotyphasterol (**39**), 22α, 23α-dihydroxycampesterol (**76**), 3-dehydro-6-deoxoteasterone (**40**), and 22α, 23α-dihydroxycampest-4-en-3-one (**77**), revealing a new shortcut in the biosynthesis of brassinosteroids. Compounds **63-77**, isolated from plant material, present side chains with no oxygen function or 22α-monohydroxylated or 22α,23α-dihydroxylated and rings A/B typical of common plant sterols (as 3β-hydroxy-Δ^5-sterols or 3β-hydroxy-5α-stanols) or less usual ones [like Δ^4-sten-3-ones (Franke et al. 2004; Georges et al. 2006; Pinto et al. 2002) or 5α-stan-3-ones (Guillen and Manzanos 2001)] or reflecting the steps for the construction of typical A/B rings of brassinosteroids (5α-stan-3β,6α-diols, 5α-stan-3β-ol-6-one, 5α-stan-3α-ol) (Fig. 1.5). None of these fragments, *per se*, can be attributed exclusively to brassinosteroids (Zullo 2018).

It is to note that only brassinosteroids precursors of campestane and cholestane skeletons had been isolated to date, what does not exclude the possibility of similar biosynthetic reactions can occur at the remaining skeletons (ergostane, sitostane, 24-methylenecholestane, 24-ethylydenecholestane, 25-methylcampestane and 24-methylene-25-methylcholestane), for all the possible sequences in the grid (as shown in Fig. 1.4) or through conversions of skeletons while functional-

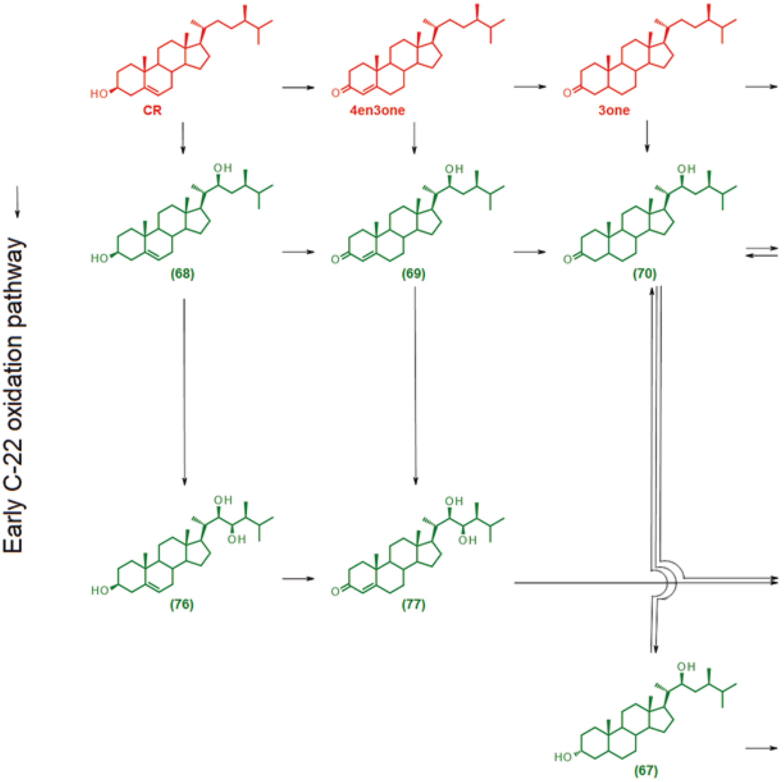


Fig. 1.4 Biosynthesis of brassinolide (1) from campesterol (red: sterols; green: brassinosteroids precursors; blue: brassinosteroids). Adapted from Zullo 2018

izing them towards the synthesis of castasterone-like or brassinolide-like brassinosteroids, what could explain the isolation or detection of brassinosteroids of different skeletons in the same plant materials. The fact that total sterols usually comprise 2–3 × 10^{-3} g/g of plant dry weight (Benveniste 2004) and that brassinosteroids are present usually in 10^{-12}–10^{-9} g/g fresh weight in plant material (Bajguz and Tretyn 2003; Takatsuto 1994), immersed in a matrix of tens of compounds of similar structure (and, hence, of similar polarity and similar chromatographic behavior), turns a very difficult task to determine the brassinosteroids profile of a given plant material, including the compounds of transient existence,

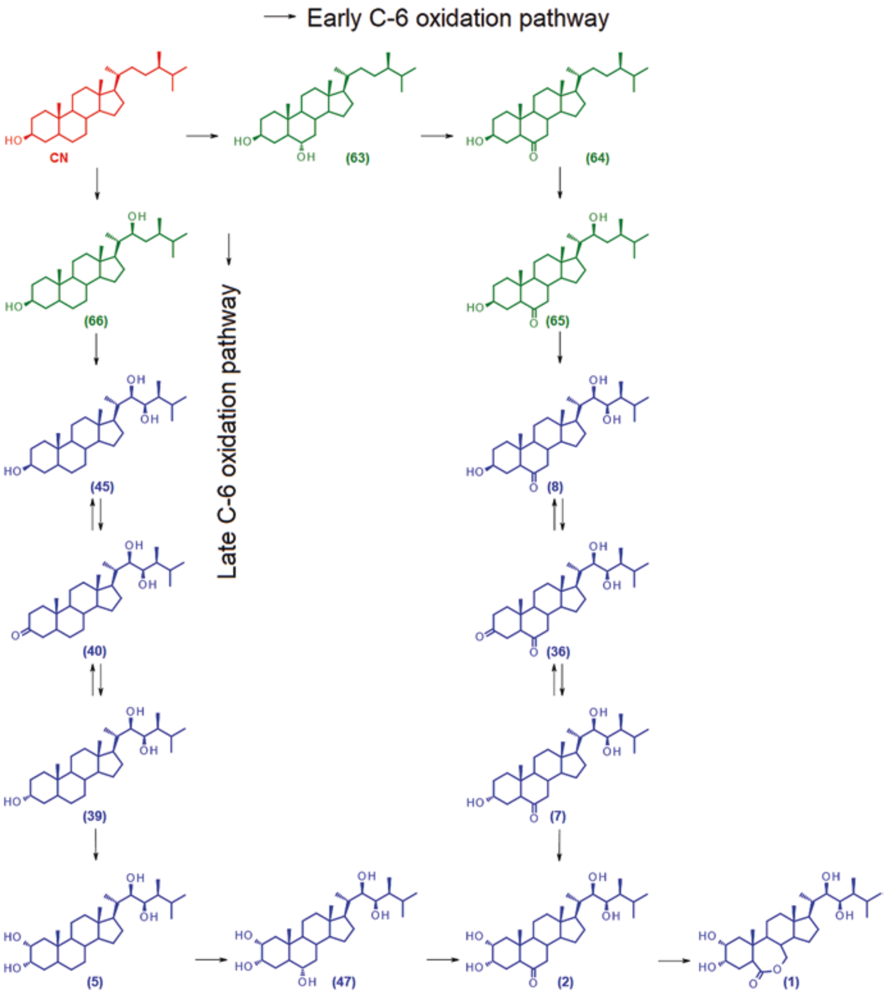


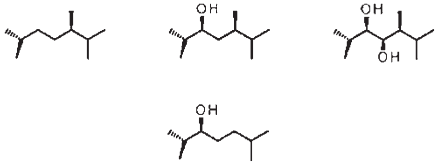
Fig. 1.4 (continued)

like their precursors, can explain why precursors of different skeletons have not been isolated yet.

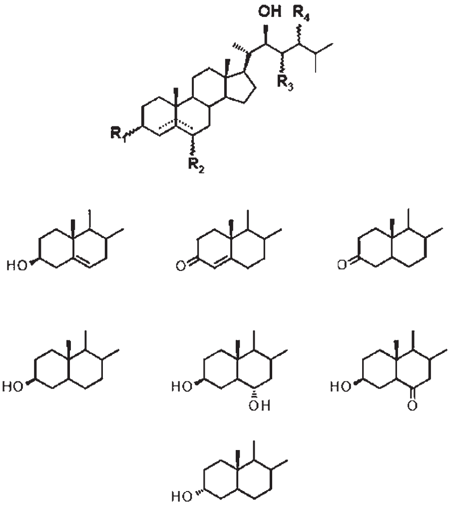
4 Brassinosteroids with Partially Elucidated Structure

A few natural brassinosteroids were isolated in pure state in enough amount to identify them by the usual spectroscopic methods, but usually they are detected by comparison with authentic compounds prepared by synthesis. Sometimes, due to small

Fig. 1.5 Fragments found in brassinosteroids precursors



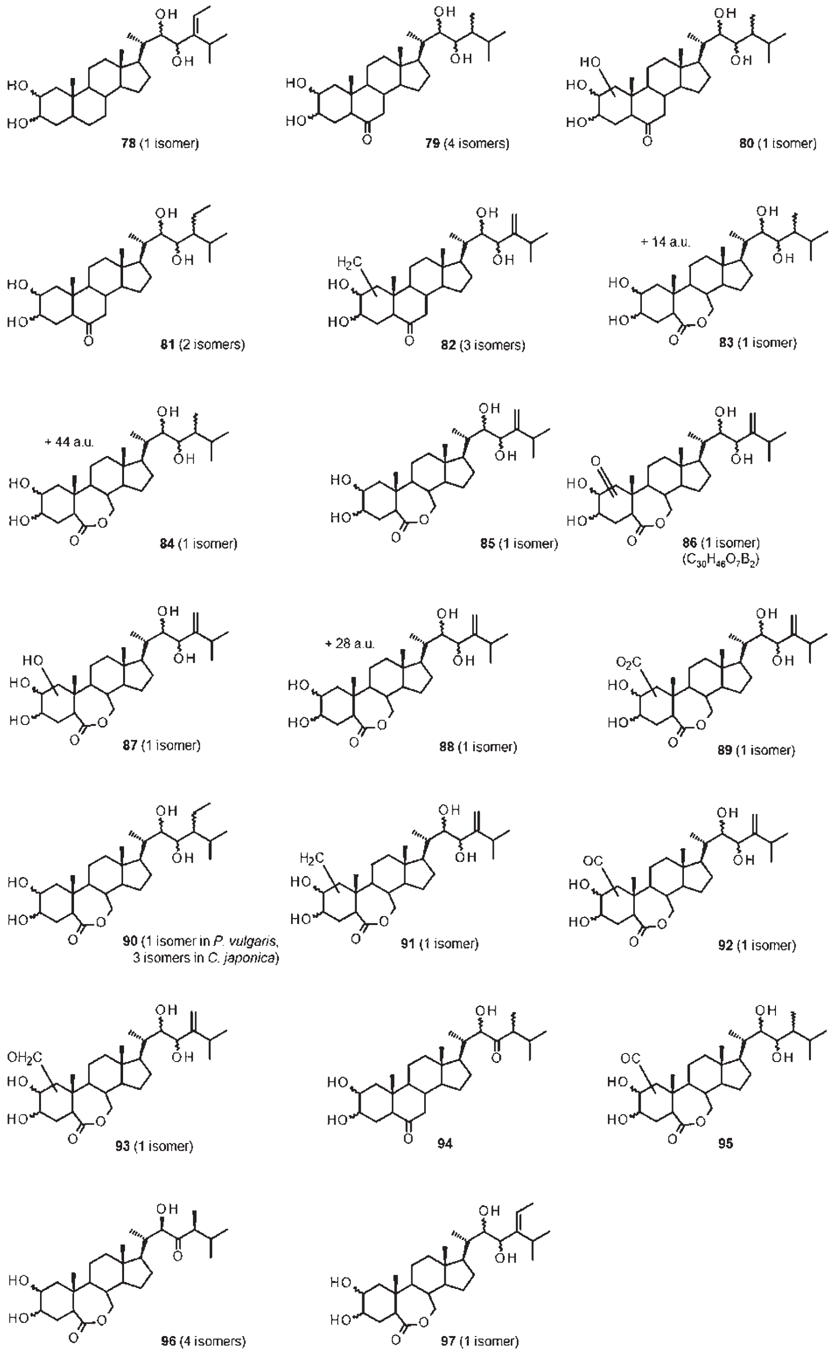
Fragments found in the side chain



Fragments found in rings A and B

amounts of samples, to similar spectroscopic characteristics but different chromatographic behavior, it is not possible to determine the structure of all compounds present in a given brassinosteroids extract. Eventually the complete structure of one of these compounds is correctly elucidated.

One of the richest sources of brassinosteroids, the seeds of kidney beans, presents about 60 compounds of partially known structure (Hwang et al. 2006), for which some of them were described (Yokota et al. 1987c) (see Fig. 1.6). Among them is cited 1 isomer of 6-deoxo-28-homodolichosterone (**78**), 4 isomers of castasterone (**79**), 1 isomer of a hydroxylated castasterone (**80**), 2 isomers of 28-homocastasterone (**81**), 3 isomers of a homologue of dolichosterone (**82**), 1 isomer of a brassinolide derivative with 14 atomic units higher (**83**), 1 isomer of a brassinolide derivative with 44 atomic units higher (**84**), 1 isomer of dolicholide (**85**), 1 isomer of dolicholide with an extra oxygen (**86**), another one with an extra hydroxyl (**87**), a dolicholide derivative 28 atomic units higher (**88**), and another one



78 (1 isomer)
79 (4 isomers)
80 (1 isomer)
81 (2 isomers)
82 (3 isomers)
83 (1 isomer)
84 (1 isomer)
85 (1 isomer)
86 (1 isomer) ($C_{30}H_{46}O_7B_2$)
87 (1 isomer)
88 (1 isomer)
89 (1 isomer)
90 (1 isomer in *P. vulgaris*, 3 isomers in *C. japonica*)
91 (1 isomer)
92 (1 isomer)
93 (1 isomer)
94
95
96 (4 isomers)
97 (1 isomer)

Fig. 1.6 Brassinosteroids with partially elucidated structure with a carboxy group (**89**), an isomer of 28-homobrassinolide (**90**), an homologue of dolicholide (**91**) and its carbonyl derivative (**92**), a carbonyl homologue of dolicholide (**93**) (Yokota et al. 1987c). Two other brassinosteroids were reported in *Phaseolus vulgaris*, ξ-epi-23-dehydrocastasterone (**94**) and an homologue with a carbonyl group (**95**) (Kim 1991). Three isomers of 28-homobrassinolide (**90**), four isomers of 23-dehydrobrassinolide (**96**), and one isomer of 28-homodolicholide (**97**) were reported in pollen and anthers of *Cryptomeria japonica* (Yokota et al. 1998). 25-Methyldolichosterone (**16**) was later identified as one of the isomers of (**82**) (Kim et al. 1987), as well as cryptolide (**54**) as one of the four isomers of 23-dehydrobrassinolide (**96**) (Watanabe et al. 2000).

5 Occurrence of Brassinosteroids

Brassinosteroids have been isolated from different plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, and grain as well as in insect and crown galls. The endogenous level of brassinosteroids varies from plant's organ and the age of the plant. Pollen and immature seeds are found to have the highest concentration of brassinosteroids, however, young growing tissues contain higher levels of brassinosteroids than mature tissues. The presence of some bioactive brassinosteroids viz., castasterone (**2**, BR_2), brassinolide (**1**, BR_1), 6-deoxocastasterone (**5**, BR_5), teasterone (**8**, BR_8), typhasterol (**7**, BR_7) and 3-dehydro-6-deoxoteasterone (**40**, BR_{40}) was confirmed in at least 103, 71, 40, 34, 28 and 28 plant species, respectively. Brassinolide (**1**) and castasterone (**2**) are widely distributed in algae and flowering plants, but only castasterone (**2**) was detected in lower non-flowering plants (liverwort, moss, lycophytes and ferns). Their presence in so many species, from the simplest algae to the more complex phanerogams, as well as the increasing detection in many new species indicates their ubiquitous distribution in the plant kingdom, what is expected from their role as plant hormones.

Table 1.3 lists the occurrence of brassinosteroids in plant species and Table 1.4 the occurrence of the established brassinosteroids precursors. It does not discriminate from which organ they were isolated or detected, or the concentration which they were found, so, primary source of information must be retrieved for proper use of their data.

Brassinosteroids were also found in plant derived products, as 24-epibrassinolide (**27**) in biodiesel cakes of *Brassica carinata* A. Braun or *Brassica napus* L. (Bardi and Rosso 2015); brassinolide (**1**), castasterone (**2**), typhasterol (**7**), teasterone (**8**) and 28-homocastasterone (**12**) in a vermicompost leachate (Aremu et al. 2015); and brassinolide (**1**), castasterone (**2**), 28-norbrassinolide (**14**) and 28-norcastasterone (**15**) in date (*Phoenix dactilifera* L.), medlar (*Eryobotrya japonica* Lindl.), milkvetch (*Astragalus* sp.), rape (*Brassica napus* L.) and robinia (*Robinia pseudo-acacia* L.) honeys, and also 28-homobrassinolide (**17**) in the last four honeys (Wang et al. 2017).

Table 1.3 Occurrence of natural brassinosteroids

Species	Family	Brassinosteroids	References
Acutodesmus acuminatus (Lagerh.) Tsarenko	*Chlorophyceae*	BR_1 BR_2 BR_7 BR_{12}	Stirk et al. (2013, 2018)
Acutodesmus incrassatulus (Bohlin) Tsarenko	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Aegle marmelos Correa	*Rutaceae*	BR_{27}	Sondhi et al. (2008)
Alnus glutinosa Gaertn.	*Betulaceae*	BR_1 BR_2	Plattner et al. (1986)
Amaranthus inamoenus	*Amaranthaceae*	BR_2	Takatsuto et al. (1999)
Apium graveolens L.	*Umbelliferae*	BR_{43}	Schmidt et al. (1995c)
Arabidopsis thaliana (L.) Henyh.	*Brassicaceae*	BR_1 BR_2 BR_4 BR_5 BR_7 BR_8 BR_{15} BR_{27} BR_{36} BR_{39} BR_{40} BR_{45} BR_{49} BR_{51} BR_{58} BR_{59} BR_{60}	Fujioka et al. (1996, 1997, 1998b, 2000a), Schmidt et al. (1997), Noguchi et al. (1999, 2000), Choe et al. (2001, 2002), Konstantinova et al. (2001), Nomura et al. (2001), Bancos et al. (2002, 2006), He et al. (2003), Kim et al. (2005a, 2006a, 2015), Carland et al. (2010), Shimada et al. (2003), Turk et al. (2003, 2005), Nakamura et al. (2005), Poppenberger et al. (2005), Takahashi et al. (2005), Chung et al. (2010), Beste et al. (2011), Schneider et al. (2012), Choi et al. (2013), Zhu et al. (2013), Best et al. (2016), Antonchick et al. (2006), Lee et al. (2006, 2010), Ohnishi et al. (2006b, 2012),

(continued)

Table 1.3 (continued)

Species	Family	Brassinosteroids	References
			Swaczynova et al. (2007), Katsumata et al. (2008), Huo et al. (2012), Villiers et al. (2012), Roh et al. (2012), Polko et al. (2013), Son et al. (2013), Xin et al. (2013), Singh et al. (2014), Lv et al. (2014), Kasote et al. (2016), Youn et al. (2016), Ding et al. (2016), Xu et al. (2016), and Chen et al. (2018)
Areca catechu L.	*Arecaceae*	BR_1	Wang and Lu (2008)
Atractylodes lancea	*Compositae*	BR_1	Ren et al. (2014)
Atryrium yokoscence (Fr. & Sav.) C. Ch.	*Woodsiaceae*	BR_2 BR_5 BR_8 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Attalea vitrivir Zona	*Arecaceae*	BR_1 BR_2	Dias et al. (2017)
Banksia grandis Willd.	*Proteaceae*	BR_1 BR_2	Takatsuto (1994)
Beta vulgaris L.	*Chenopodiaceae*	BR_2 BR_9	Schmidt et al. (1994)
Brassica campestris var. *pekinensis*	*Brassicaceae*	BR_1 BR_2 BR_{12} BR_{14} BR_{15} BR_{17} BR_{27}	Abe et al. (1982, 1983), Ikekawa et al. (1984), Ikekawa and Takatsuto (1984), Pan et al. (2013), and Lv et al. (2014)
Brassica carinata A. Braun	*Brassicaceae*	BR_{27}	Bardi and Rosso (2015)
Brassica juncea L.	*Brassicaceae*	BR_2 BR_7 BR_8 BR_{27}	Kanwar et al. (2012, 2013, 2015)
Brassica napus L.	*Brassicaceae*	BR_1 BR_2 BR_4 BR_5 BR_7 BR_8 BR_{12} (or BR_{33}) BR_{14} BR_{15} BR_{27} (or BR_{51}) BR_{28} BR_{29} BR_{40} BR_{41} BR_{47}	Grove et al. (1979), Swaczynova et al. (2007), Ding et al. (2014a, b), Zhang et al. (2010), Pan et al. (2012), Ding et al. (2013a, b, 2016), Oklestkova et al. (2017), and Yu et al. (2017)
Brassica napus var. *oleifera*	*Brassicaceae*	BR_{27}	Bardi and Rosso (2015)
Butia capitata (Mart.) Becc.	*Arecaceae*	BR_1 BR_2	Dias et al. (2017)
Camellia sinensis (O) Kuntze (= *Thea sinensis* L.)	*Theaceae*	BR_5 BR_7 BR_{10} BR_{27} BR_{36} BR_{39} BR_{41} BR_{50} BR_{56} BR_{57}	Gupta et al. (2004) and Bhardwaj et al. (2007)
Cannabis sativa L.	*Cannabaceae*	BR_2 BR_8	Takatsuto et al. (1996b)

(continued)

Table 1.3 (continued)

Species	Family	Brassinosteroids	References
Castanea crenata Sieb. et Zucc	*Fagaceae*	BR_1 BR_2 BR_7 BR_8 BR_{15}	Park et al. (1994a), Yokota et al. (1982a), Abe et al. (1983), Ikeda et al. (1983), Arima et al. (1984), Ikekawa et al. (1984)
Catharanthus roseus Don.	*Apocynaceae*	BR_1 BR_2 BR_5 BR_7 BR_8 BR_{21} BR_{25} BR_{39} BR_{40} BR_{45} BR_{47}	Park et al. (1989), Yokota et al. (1990a), Choi et al. (1993, 1996, 1997), Suzuki et al. (1993a, 1994a, 1995), Fujioka et al. (1995b, 2000b), and Fujioka and Sakurai (1997)
Centella asiatica (L.) Urban	*Apiaceae*	BR_2	Sondhi et al. (2010)
Chlamydomonas reinhardtii P.A. Dang.	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Chlorella minutissima Fott et Nováková	*Trebouxiophyceae*	BR_1 BR_2 BR_{42}	Stirk et al. (2013, 2014a)
Chlorella pyrenoidosa Chick	*Trebouxiophyceae*	BR_1 BR_2	Stirk et al. (2013)
Chlorella vulgaris Beijerinck	*Trebouxiophyceae*	BR_1 BR_2 BR_5 BR_7 BR_8 BR_{12} BR_{39} BR_{40} BR_{45}	Stirk et al. (2013, 2018), Bajguz (2009), and Bajguz and Piotrowska-Niczyporuk (2013, 2014)
Chlorococcum ellipsoideum Deason et Bold	*Chlorophyceae*	BR_1 BR_2 BR_7 BR_{12}	Stirk et al. (2013, 2018)
Cistus hirsutum Theill.	*Cistaceae*	BR_1 BR_2	Takatsuto (1994)
Citrus sinensis Osbeck	*Rutaceae*	BR_1 BR_2	Motegi et al. (1994)
Citrus unshiu Marcov.	*Rutaceae*	BR_1 BR_2 BR_7 BR_8	Takatsuto (1994)
Coccomyxa sp.	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Coelastrum microporum Nägeli	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Cryptomeria japonica D. Don.	*Taxodiaceae*	BR_3 BR_7 BR_{10} BR_{17} BR_{36} BR_{54}	Watanabe et al. (2000), Takatsuto (1994), and Yokota et al. (1998)
Cucumis sativus L.	*Cucurbitaceae*	BR_1	Hou et al. (2017)
Cucurbita moschata Duchesne	*Cucurbitaceae*	BR_1 BR_2	Jang et al. (2000) and Pachthong et al. (2006)
Cupressus arizonica E. Greene	*Cupressaceae*	BR_1 BR_2 BR_4 BR_5 BR_7 BR_8 BR_{12} BR_{36} BR_{39} BR_{40}	Griffiths et al. (1995)

(continued)

Table 1.3 (continued)

Species	Family	Brassinosteroids	References
Cyrtomium laetevirens (Hiyama) Nakaike	*Dryopteridaceae*	BR_2 BR_{40} BR_{45}	Yokota et al. (2017)
Daucus carota ssp. *sativus* L.	*Apiaceae*	BR_1 BR_2 BR_9 BR_{27}	Schmidt et al. (1998), Swaczynova et al. (2007), and Lv et al. (2014)
Deparia japonica (Thunb.) M. Kato	*Woodsiaceae*	BR_2 BR_5 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Desmodesmus armatus (R. Chodat) E. Hegewald	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Diospyros kaki Thunb.	*Ebenaceae*	BR_2	Takatsuto (1994)
Distylium racemosum Sieb et Zucc.	*Hammamelidaceae*	BR_1 BR_2 BR_7 BR_8 BR_{14} BR_{15} BR_{36}	Ikekawa et al. (1984), Ikekawa and Takatsuto (1984), and Abe et al. (1994)
Dolichos lablab Adans.	*Leguminosae*	BR_1 BR_2 BR_3 BR_4 BR_5 BR_6 BR_{10} BR_{11}	Yokota et al. (1982b, 1983b, 1984) and Baba et al. (1983)
Dryopteris crassirhizoma Nakai (1920)	*Dryopteridaceae*	BR_2 BR_5 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Dryopteris erythrososa (D.C.Eaton) Kuntze	*Dryopteridaceae*	BR_2 BR_5 BR_8 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Echium plantagineum L.	*Boraginaceae*	BR_1 BR_2	Takatsuto (1994)
Ecklonia máxima (Osbeck) Papenfuss	*Phaeophyceae*	BR_1 BR_2	Stirk et al. (2014b)
Elaeis guineenses Jacq. var. *tenera*	*Palmae*	BR_1	Habib et al. (2012)
Equisetum arvense L.	*Equisetaceae*	BR_2 BR_4 BR_5 BR_{14} BR_{15} BR_{39} BR_{40} BR_{45}	Yokota et al. (2017) and Takatsuto et al. (1990a)
Eriobotrya japonica Lindl.	*Rosaceae*	BR_2	Takatsuto (1994)
Erythronium japonicum Decne	*Liliaceae*	BR_7	Yasuta et al. (1995)
Eucalyptus calophylla R. Br.	*Myrtaceae*	BR_2	Takatsuto (1994)
Eucalyptus marginata Sn.	*Myrtaceae*	BR_4	Takatsuto (1994)
Fagopyrum esculentum Moench.	*Polygonaceae*	BR_1 BR_2	Takatsuto et al. (1990b)
Ginkgo biloba L.	*Gingkoaceae*	BR_8	Takatsuto et al. (1996a)
Gyoerffyana humicola Kol et Chodat	*Chlorophyceae*	BR_1 BR_2 BR_7 BR_{12}	Stirk et al. (2013, 2018)

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Table 1.3 (continued)

Species	Family	Brassinosteroids	References
Gypsophila perfoliata L.	*Caryophyllaceae*	BR_{27}	Schmidt et al. (1996)
Helianthus annuus L.	*Asteraceae*	BR_1 BR_2 BR_{15} BR_{27}	Takatsuto et al. (1989) and Pan et al. (2012)
Hordeum vulgare L.	*Poaceae*	BR_2 BR_{12} BR_{27}	Dockter et al. (2014) and Gruszka et al. (2016)
Humulus lupulus L.	*Cannabaceae*	BR_1 BR_2 BR_3 BR_7 BR_9 BR_{11} BR_{14} BR_{17} BR_{27} BR_{62}	Oklestkova et al. (2017) and Chen et al. (2018)
Hydrodictyon reticulatum (L.) Lagerheim	*Hydrodictyaceae*	BR_9 BR_{12}	Yokota et al. (1987b)
Klebsormidium flaccidum (Kütz.) P.C. Silva, K.R. Mattox et W.H. Blackw.	*Charophyceae*	BR_1 BR_2	Stirk et al. (2013)
Lagenaria ciceraria	*Cucurbitaceae*	BR_2	Takatsuto and Makiuchi (2000)
Lilium elegans Thunb.	*Araceae*	BR_1 BR_2 BR_7 BR_8	Suzuki et al. (1994b)
Lilium longiflorum Thunb.	*Araceae*	BR_1 BR_2 BR_7 BR_8 BR_{35} BR_{36} BR_{44} BR_{48}	Asakawa et al. (1994, 1996), Abe (1991), Abe et al. (1994), Soeno et al. (2000a, b)
Lolium perenne L.	*Poaceae*	BR_{33}	Taylor et al. (1993)
Luffa cylindrica (L.) M.J. Roem	*Cucurbitaceae*	BR_1 BR_2	Pachthong et al. (2007)
Lychnis viscaria L.	*Caryophyllaceae*	BR_9 BR_{46}	Friebe et al. (1999)
Lycopersicon esculentum Mill.	*Solanaceae*	BR_1 BR_2 BR_5 BR_7 BR_8 BR_9 BR_{14} BR_{15} BR_{27} BR_{39} BR_{40} BR_{41} BR_{42} BR_{45} BR_{47} BR_{50} BR_{60}	Yokota et al. (1997), Bishop et al. (1999), Koka et al. (2000), Nomura et al. (2001, 2005), Yokota et al. (2001), Van Meulebroek et al. (2012), Wu et al. (2013), and Kim et al. (2015)
Lygodium japonicum (Thunb.) Sw.	*Lygodiaceae*	BR_2 BR_5 BR_8 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Malus prunifolia (Willd.) Borkh.	*Rosaceae*	BR_2 BR_5 BR_7 BR_8 BR_{39} BR_{40} BR_{45}	Pereira-Netto et al. (2009)
Marchantia polymorpha L.	*Marchantiaceae*	BR_2 BR_5 BR_8 BR_{40}	Kim et al. (2002) and Yokota et al. (2017)
Matricaria recutita L.	*Compositae*	BR_{17} BR_{34}	Pradko et al. (2015)
Matteuccia struthiopteris (L.) *Tod.*	*Woodsiaceae*	BR_2 BR_5 BR_{40}	Yokota et al. (2017)

(continued)

Table 1.3 (continued)

Species	Family	Brassinosteroids	References
Monoraphidium contortum (Thur.) Komárková-Legnerová	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Myrmecia bisecta Reisigl	*Trebouxiophyceae*	BR_1 BR_2	Stirk et al. (2013)
Nautococcus mamillatus Korschikov	*Chlorophyceae*	BR_1 BR_2 BR_7 BR_{12}	Stirk et al. (2013, 2108)
Nicotiana tabacum L.	*Solanaceae*	BR_2 BR_5 BR_8 BR_{39} BR_{40} BR_{45}	Ohnishi et al. (2006a)
Onoclea sensibilis L.	*Woodsiaceae*	BR_2 BR_5 BR_{40}	Yokota et al. (2017)
Ornithopus sativus Brot.	*Fabaceae*	BR_2 BR_5 BR_9 BR_{41} BR_{42}	Schmidt et al. (1993a) and Spengler et al. (1995)
Oryza sativa L.	*Poaceae*	BR_1 BR_2 BR_4 BR_5 BR_7 BR_8 BR_9 BR_{12} BR_{15} BR_{17} BR_{27} BR_{34} BR_{36} BR_{37} BR_{39} BR_{40} BR_{42} BR_{45} BR_{61}	Abe et al. (1995a, 1984b), Mori et al. (2002), Wu et al. (2008), Nakamura et al. (2006), Asahina et al. (2014), Tanabe et al. (2005), Sakamoto et al. (2006, 2012); Kim et al. (2008), Ding et al. (2013a, b, 2014b, 2016), Li et al. (2013), Xin et al. (2013, 2016), Wang et al. (2014), Joo et al. (2015), Yu et al. (2016), Qian et al. (2017), Deng et al. (2016), Tamiru et al. (2016), Yokota et al. (2017), Ikekawa and Takatsuto (1984), Abe (1991); Shim et al. (1996), Park et al. (1994b), and Chen et al. (2018)
Osmunda japonica Thunb.	*Osmundaceae*	BR_2 BR_5 BR_7 BR_8 BR_{36} BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Perilla frutescens Britton.	*Labiatae*	BR_2 BR_{10}	Park et al. (1994b)
Petunia hybrida line W138	*Solanaceae*	BR_1 BR_2 BR_5 BR_7 BR_8 BR_{36} BR_{39} BR_{40} BR_{45}	Verhoef et al. (2013)
Phalaris canariensis L.	*Poaceae*	BR_2 BR_8	Shimada et al. (1996)
Pharbitis nil (L.) Choisy	*Convolvulaceae*	BR_2 BR_5 BR_7 BR_8 BR_{39} BR_{45}	Suzuki et al. (2003)
Pharbitis purpurea Voigt	*Convolvulaceae*	BR_2 BR_{15}	Suzuki et al. (1985)

(continued)

Table 1.3 (continued)

Species	Family	Brassinosteroids	References
Phaseolus vulgaris L.	*Fabaceae*	BR_1 BR_2 BR_3 BR_4 BR_5 BR_6 BR_7 BR_8 BR_{11} BR_{12} BR_{13} BR_{14} BR_{16} BR_{18} BR_{19} BR_{20} BR_{21} BR_{22} BR_{23} BR_{24} BR_{25} BR_{26} BR_{27} BR_{28} BR_{29} BR_{30} BR_{31} BR_{32} BR_{37} BR_{55} BR_{62}	Yokota et al. (1983c), Kim et al. (1987), Yokota and Takahashi (1988); Park et al. (2009a, b), Yokota et al. (1987a, c, 1990b); Kim (1991); Kim et al. (2000c, 2006b); Hwang et al. (2006, 2007), Swaczynova et al. (2007), Lee et al. (2011), and Oklestkova et al. (2017)
Phoenix dactylifera L.	*Arecaceae*	BR_9	Zaki et al. (1993)
Physcomitrella patens (Hedw.) Bruch & Schimp.	*Funariaceae*	BR_2 BR_5 BR_8 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Picea sitchensis (Bong.) Carr.	*Pinaceae*	BR_2 BR_7	Yokota et al. (1985)
Pinus silvestris Lour.	*Pinaceae*	BR_1 BR_2	Kim et al. (1990)
Pinus thunbergii Parl.	*Pinaceae*	BR_2 BR_7	Yokota et al. (1983a)
Pisum sativum L.	*Fabaceae*	BR_1 BR_2 BR_5 BR_7 BR_{36} BR_{39} BR_{40} $BR_{43}BR_{45}$	Yokota et al. (1996) and Nomura et al. (1997, 2001, 2004, 2007)
Poloidion didymos Pascher	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Protococcus viridis C. Agardh	*Chlorophyceae*	BR_1 BR_2 BR_7 BR_{12}	Stirk et al. (2013, 2018)
Protosiphon botryoides G.A. Klebs	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Psophocarpus tetragonolobus DC	*Fabaceae*	BR_1 BR_2 BR_5 BR_{12}	Yokota et al. (1991) and Takatsuto (1994)
Pteridium aquilinum (L.) Kuhn in Kersten (1879)	*Dennstaedtiaceae*	BR_2 BR_5 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Pyrus communis L.	*Rosaceae*	BR_1 BR_2	Oikawa et al. (2015)
Raphanus sativus L.	*Brassicaceae*	BR_1 BR_2 BR_8 BR_{34}	Schmidt et al. (1991, 1993b)
Raphidocelis subcapitata (Korshikov) G. Nygaard, J. Komárek, Kristiansen et Skulberg	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)

(continued)

Table 1.3 (continued)

Species	Family	Brassinosteroids	References
Rheum rhabarbarum L.	*Polygonaceae*	BR_1 BR_2 BR_9	Schmidt et al. (1995a)
Robinia pseudo-acacia L.	*Fabaceae*	BR_2 BR_5 BR_7	Abe et al. (1995b)
Scotiellopsis terrestris (Reisigl) Pun_coch. et Kalina	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Secale cereale L.	*Poaceae*	BR_2 BR_5 BR_7 BR_8 BR_{12} BR_{15} BR_{20} BR_{21} BR_{38} BR_{52} BR_{53}	Schmidt et al. (1995b), Antonchick et al. (2003, 2005), and Pociecha et al. (2016)
Selaginella moellendorffii Hieronymus	*Sellaginellaceae*	BR_2 BR_5 BR_{40}	Yokota et al. (2017)
Selaginella uncinata (Desv. ex Poir.) Spring	*Sellaginellaceae*	BR_2 BR_5 BR_8 BR_{39}	Yokota et al. (2017)
Solidago altissima L.	*Asteraceae*	BR_1	Takatsuto (1994)
Spongiochloris excentrica Starr	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Sporobolus stapfianum Gand.	*Poaceae*	BR_1 BR_2 BR_3	Sasse et al. (1998)
Stichococcus bacillaris Nägeli	*Trebouxiophyceae*	BR_1 BR_2	Stirk et al. (2013)
Stigeoclonium nanum (Dillwyn) Kütz.	*Chlorophyceae*	BR_1 BR_2	Stirk et al. (2013)
Thea sinensis L. (= *Camellia sinensis* (O) Kuntze)	*Theaceae*	BR_1 BR_2 BR_7 BR_8 BR_{12} BR_{15}	Abe et al. (1983, 1984a), Morishita et al. (1983), and Ikekawa and Takatsuto (1984)
Thelypteris decursive-pinnata (H.C. Hall) Ching, 1936	*Thelypteridaceae*	BR_2 BR_5 BR_{39} BR_{40} BR_{45}	Yokota et al. (2017)
Thelypteris palustris Schott	*Thelypteridaceae*	BR_5 BR_8 BR_{40} BR_{45}	Yokota et al. (2017)
Triticum aestivum L.	*Poaceae*	BR_1 BR_2 BR_5 BR_7 BR_8 BR_{12} BR_{17} BR_{27} BR_{36}	Yokota et al. (1994) and Janeczko and Swaczynova (2010)
Tulipa gesneriana L.	*Liliaceae*	BR_7	Takatsuto (1994)
Typha latifolia Mey.	*Typhaceae*	BR_7	Schneider et al. (1983) and Yoshihara and Katou (1985)

(continued)

Table 1.3 (continued)

Species	Family	Brassinosteroids	References
Ulothrix sp.	*Ulvophyceae*	BR_1 BR_2	Stirk et al. (2013)
Vicia faba L.	*Fabaceae*	BR_1 BR_2 BR_5 BR_7 BR_{15} BR_{27} BR_{39} BR_{45}	Ikekawa et al. (1988), Park et al. (1987), Fukuta et al. (2004), and Pan et al. (2013)
Vitis vinífera L.	*Vitaceae*	BR_1 BR_2 BR_5	Xu et al. (2015)
Zea mays L.	*Poaceae*	BR_1 BR_2 BR_3 BR_5 BR_7 BR_8 BR_9 BR_{11} BR_{14} BR_{15} BR_{27} BR_{36} BR_{39} BR_{40} BR_{45} BR_{62}	Suzuki et al. (1986); Sekimoto et al. (1997), Kim et al. (2005b, 2006c), Hartwig et al. (2011), Pan et al. (2013), Yokota et al. (2017), and Oklestkova et al. (2017)
Zinnia elegans Jacq.	*Asteraceae*	BR_2 BR_5 BR_7 BR_{39} BR_{45}	Yamamoto et al. (2001, 2007)

Table 1.4 Occurrence of brassinosteroids precursors

Species	Family	Brassinosteroid precursors	References
Arabidopsis thaliana (L.) Henyh.	*Brassicaceae*	**63 64 65 66 67 68 69 70 71 72 73 74**	Fujioka et al. (2002), Lee et al. (2006), Ohnishi et al. (2006b), Shahnejat-Bushehri et al. (2016), Roh et al. (2012), and Zhu et al. (2013)
Attalea vitrivir Zona	*Arecaceae*	**64**	Dias et al. (2017)
Atryrium yokoscence (Fr. & Sav.) C. Ch.	*Woodsiaceae*	**65 66 67 69**	Yokota et al. (2017)
Butia capitata (Mart.) Becc.	*Arecaceae*	**64**	Dias et al. (2017)
Camellia sinensis (O.) Kuntze	*Theaceae*	**73**	Bhardwaj et al. (2007)
Catharanthus roseus Don.	*Apocynaceae*	**64 65 66 67 68 69 70 71 72 73 74**	Fujioka et al. (1995b, 2000b, 2002)
Chlorella minutissima Fott et Nováková	*Trebouxiophyceae*	**64**	Stirk et al. (2014a)
Cyrtomium laetevirens (Hiyama) Nakaike	*Dryopteridaceae*	**65 66 67**	Yokota et al. (2017)
Dryopteris crassirhizoma Nakai (1920)	*Dryopteridaceae*	**67 66**	Yokota et al. (2017)
Dryopteris erythrosoa (D.C.Eaton) Kuntze	*Dryopteridaceae*	**65 66 67**	Yokota et al. (2017)

(continued)

Table 1.4 (continued)

Species	Family	Brassinosteroid precursors	References
Deparia japonica (Thunb.) M. Kato	*Woodsiaceae*	**65 66 67**	Yokota et al. (2017)
Equisetum arvense L.	*Equisetaceae*	**65 66 67 69**	Yokota et al. (2017)
Lycopersicon esculentum	*Solanaceae*	**73**	Yokota et al. (2001)
Lygodium japonicum (Thunb.) Sw.	*Lygodiaceae*	**65 66 67**	Yokota et al. (2017)
Malus prunifolia (Willd.) Borkh.	*Rosaceae*	**63 65**	Pereira-Netto et al. (2009)
Marchantia polymorpha L.	*Marchantiaceae*	**65 66 67**	Yokota et al. (2017)
Osmunda japonica Thunb.	*Osmundaceae*	**65 66 67 69**	Yokota et al. (2017)
Onoclea sensibilis L.	*Woodsiaceae*	**66**	Yokota et al. (2017)
Oryza sativa L.	*Poaceae*	**65 66 67 68 69 73**	Yokota et al. (2017), Wu et al. (2008), and Tamiru et al. (2016)
Petunia hybrida	*Solanaceae*	**65 66 67 69**	Verhoef et al. (2013)
Pteridium aquilinum (L.) Kuhn in Kersten (1879)	*Dennstaedtiaceae*	**65 66 69**	Yokota et al. (2017)
Physcomitrella patens (Hedw.) Bruch & Schimp	*Funariaceae*	**65 66 67**	Yokota et al. (2017)
Selaginella moellendorffii Hieronymus	*Sellaginellaceae*	**66**	Yokota et al. (2017)
Selaginella uncinata (Desv. ex Poir.) Spring	*Sellaginellaceae*	**65 66 67**	Yokota et al. (2017)
Thelypteris decursive-pinnata (H.C. Hall) Ching, 1936	*Thelypteridaceae*	**65 66 69**	Yokota et al. (2017)
Thelypteris palustris Schott	*Thelypteridaceae*	**65 66 67**	Yokota et al. (2017)
Zea mays L.	*Poaceae*	**65 66 67 69**	Yokota et al. (2017)

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