Phytochemistry, Vol. 25, No. 8, pp. 1787–1799, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

REVIEW ARTICLE NUMBER 19
BRASSINOSTEROIDS

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(*Received* 20 *September* 1985)

Key Word Index—Brassinolide; brassinosteroids; structure; occurrence; biosynthesis; synthesis; bioactivity; structure–activity relationships; mode of action.

Abstract—Brassinosteroids are a new class of plant growth regulators. The developments in this field since the discovery of brassinolide in 1979 are discussed including the synthesis of brassinosteroids and the structure, isolation and occurrence of the natural compounds. Their bioactivity, structure–activity relationships and possible physiological role are also reviewed.

INTRODUCTION

Seeking new natural constituents with plant growth regulating activity Mitchell *et al.* [1, 2] found that 'brassins' or the 'brassin complex' extracted from rape pollen (*Brassica napus* L.) produced a powerful growth accelerating effect when applied to young pinto bean plants. This phenomenon involved an increase in cell elongation and cell division [3]. The active principle possessing this bioactivity was isolated from 40 kg rape pollen to yield 4 mg of a crystalline compound named brassinolide. Its structure was elucidated as (22*R*,23*R*, 24*S*)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (**1**) by spectroscopic data including X-ray analysis [4]. Thus, brassinolide represents the first naturally occurring steroid with an unprecedented seven-membered lactone B-ring and two vicinal diol functions at ring A and the side chain. Because of its scarcity, interesting novel structural features and dramatic ability to accelerate plant growth, much effort has been devoted in recent years to the search for further natural brassinosteroids, to the synthesis of brassinolide and its analogues and to the study of their biological activity and physiological function. Promising results in the application of brassinosteroids to produce increased crop yields have also been reported [5–13]. Thompson *et al.* [14] described the discovery of brassinolide (**1**) as 'perhaps the most important discovery of the plant physiologists and biochemists since the discovery of gibberellic acid'. The present review includes the structure, occurrence, synthesis and bioactivity of this new class of steroidal plant growth regulators which may be regarded possibly as a sixth group of phytohormones.

STRUCTURE AND OCCURRENCE

Since the discovery of brassinolide (**1**) as many as 13 further native brassinosteroids **2**–**14** (Scheme 1) have been found in species of different plant families. Structurally they exhibit either the 7-membered 7-oxalactone B-ring but differing in the C-24 substitution of the side chain moiety (**1**–**5**) or they are the corresponding 6-oxo steroids with a normal 6-membered B-ring (**6**–**12**). 6-Deoxocastasterone (**13**) and 6-deoxodolichosterone (**14**) lack the B-ring oxygen function. All these brassinosteroids are 2α,3α,22*R*,23*R*-tetrols with the exception of 2-deoxycastasterone (typhasterol, **11**) and teasterone (**12**) which show only one 3α- and 3β-hydroxy group in ring A, respectively.

The isolation of brassinolide (**1**) from rape pollen was achieved by successive silica gel chromatography of the *i*-propanol extract followed by C_{18}Hi-flosil chromatography and HPLC. To guide the separation the bean second-internode bioassay was used [4]. Most of the later published isolation procedures of brassinosteroids include methanol extraction, column chromatography, gel filtration, partition processes and finally HPLC combined with a bioassay (lit. see Table 1). In a few cases preparative TLC was involved [35] or used instead of HPLC for the final separation [18].

The microanalysis of brassinolide (**1**) and other brassinosteroids using GLC and GC/MS has been investigated and the corresponding bismethaneboranates were found to be more suitable derivatives then the trimethylsilyl ethers [17, 36]. By means of GC/CIMS and a computerized selected ion monitoring program picogram amounts of brassinosteroids could be detected in plants [19, 36, 37] and this method has been widely applied for screening and detection of new brassinosteroids [16, 18, 20–22, 25, 28–30, 35]. Very recently the production of monoclonal antibodies to the synthetic 24-epibrassinolide in CAF_1 mice has been described and an enzyme-linked immunosorbent assay (ELISA) used for the examination of brassinolide (**1**) distribution in *Brassica napus* tissues [38].

The occurrences of native brassinosteroids are shown in Table 1. Among the investigated plant sources are such agriculturally important species as rape, rice, tea, Chinese cabbage and beans as well as the Gymnosperms *Pinus*

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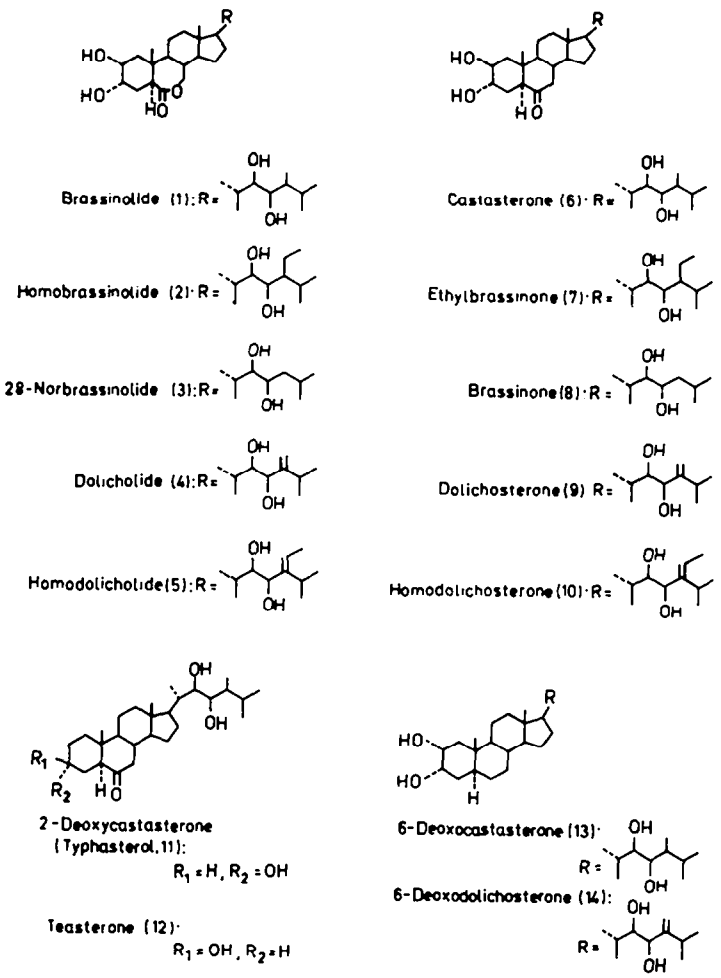


Scheme 1. Structure of natural brassinosteroids.

thubergii and *Picea sitchensis* indicating a wide distribution in the plant kingdom. The detected amounts vary from 0.1 ng/kg (ethylbrassinone, 7, from fruit of *Brassica campestris* var. *pekinensis* [16]) up to 100 μg/kg (brassinolide, 1, from pollen of *Brassica napus* [4]). The chemotaxonomical aspects of brassinosteroid distribution have not been considered. However, the results obtained so far show that, as in the case of gibberellin phytohormones, a spectrum of different compounds are found but the major component can vary in different plant species. Thus, brassinolide (1) represents the main constituent in *Brassica napus* [4] whereas castasterone (6) is the predominant compound in *Thea sinensis* [16] or *Pharbitis purpurea* [29] while dolicholide (4) predominates in *Dolichos lablab* [22].

Interestingly, in insect galls of *Distylium racemosum* the amounts of castasterone (6) and brassinone (8) were found to be 20–300 times higher than in the leaves of the same plant and the lactone type brassinosteroids were present only in leaves but not in insect galls [19]. On the other hand in comparative investigations of shoot, leaf, flower-bud and insect gall of the chestnut tree all tissues were found to contain castasterone (6) and 6-deoxocastasterone (13) with a higher content of the latter compound. Additionally, a small amount of brassinolide (1) was detected in the gall. The larvae from the galls contained only a trace of bioactivity showing that activity of the galls is exclusively due to the plant tissue [20, 26].

No results concerning the biosynthesis of brassinosteroids have been published to date and experiments using radioactive compounds are necessary. The biosynthesis should presumably commence from the corresponding sterols, e.g. brassicasterol or 24-methylenecholesterol for the C_{28}-brassinosteroids. For the biosynthesis of the 2α,3α-dihydroxy moiety from a 3β-hydroxy precursor different pathways via ketol or epoxide intermediates have been discussed [39]. The 6-oxo type brassinosteroids are assumed to be biosynthetic precursors of the corresponding 7-oxolactone compounds, especially castasterone (6) for brassinolide (1) [18, 23, 40] and brassinone (8) for 28-nor-brassinolide (3) [16]. The 6-deoxo brassinosteroids 13 and 14 have been considered [25] as putative precursors for their respective 6-oxo analogues 6 and 9, respectively, and the 3β-hydroxy compound teasterone (12) was assumed to be the biosynthetic intermediate to typhasterol (11) and further to castasterone (6) and brassinolide (1) on the basis of their common occurrence in *Thea sinensis* [33]. On the other hand from the absence of 7-oxalactone type brassinosteroids, e.g. in *Pharbitis purpurea* [29], as well as from the

Table 1. Occurrence of brassinosteroids

Compound	Plant	Part used	Yield/kg	References
Brassinolide (1)	*Brassica campestris* var. *pekinensis*	seeds, sheaths	9.4 ng	15–17
	Brassica napus	pollens	100 μg	4
	Castanea crenata	galls	1 μg	18–20
	Distylium racemosum	leaves	23 ng	19
	Dolichos lablab	seeds		21, 22
	Thea sinensis	leaves	5 ng	17, 23
Homobrassinolide (2)	*Brassica campestris* var. *pekinensis*	seeds, sheaths	traces	16, 19
28-Norbrassinolide (3)	*Brassica campestris* var. *pekinensis*	seeds, sheaths	1 μg	16, 17, 19
	Distylium racemosum	leaves	156 ng	19
Dolicholide (4)	*Dolichos lablab*	seeds	5 μg	22, 24
	Phaseolus vulgaris cv. Kentucky Wonder	seeds		25
Homodolicholide (5)	*Dolichos lablab*	seeds	0.35 μg	21, 22
Castasterone (6)	*Brassica campestris* var. *pekinensis*	seeds, sheaths	1.6 ng	15, 17
	Castanea crenata	galls	4–12 μg	16, 18, 20, 26, 27
		shoots, leaves, flower buds	2–6 μg	20
	Distylium racemosum	leaves	133 ng	19
		galls	2500 μg	19
	Dolichos lablab	seeds		21, 22
	Oryza sativa cv. Arborio J1	shoots	13.6 ng	17, 28
	Pharbitis purpurea	seeds	1.1 μg	29
	Phaseolus vulgaris cv. Kentucky Wonder	seeds		25
	Picea sitchensis	shoots	5 μg	30
	Pinus thubergil			31
	Thea sinensis	leaves	7.2 μg	16
Ethylbrassinone (7)	*Brassica campestris* var. *pekinensis*		0.1 ng	16, 17
	Thea sinensis	leaves	0.5 ng	16, 23
Brassinone (8)	*Brassica campestris* var. *pekinensis*		0.8 ng	16
	Castanea crenata	galls	10 ng	16
	Distylium racemosum	leaves	16 ng	19
		galls	5000 ng	19
	Pharbitis purpurea	seeds	0.2 μg	29
	Thea sinensis		2 ng	16
Dolichosterone (9)	*Dolichos lablab*	seeds	1.5 μg	22, 32
	Oryza sativa cv. Arborio J1	shoots	8.4 ng	19, 28
Homodolichosterone (10)	*Dolichos lablab*	seeds	0.6 μg	22, 32
2-Deoxycastasterone (Typhasterole, 11)	*Picea sitchensis*	shoots	7 μg	30
	Pinus thubergii	pollens	90 μg	31
	Thea sinensis	leaves	0.01 μg	33
	Typha latifolia	pollens	70 μg	34
Teasterone (12)	*Thea sinensis*	leaves	0.05 μg	33
6-Deoxocastasterone (13)	*Castanea crenata*	shoots, leaves, flower buds	15–30 μg	20
		galls	9–26 μg	
	Dolichos lablab	seeds		22
	Phaseolus vulgaris cv. Kentucky Wonder	seeds		25
6-Deoxodolichosterone (14)	*Dolichos lablab*	seed		22
	Phaseolus vulgaris cv. Kentucky Wonder	seeds		25

time course of rice lamina inclination induced by castasterone (6) and brassinolide (1), it was suggested [40] that the 6-oxo type members have their own physiological function in plants without prior transformation to the corresponding lactones.

SYNTHESIS OF BRASSINOSTEROIDS

Soon after the structure elucidation of brassinolide (1) its first syntheses were published [41, 42] and since that time brassinosteroid synthesis has remained a field of

tremendous activity. For the chemical synthesis of brassinosteroids starting from a suitable steroidal precursor the typical A/B ring functionalization and the construction of the dihydroxylated side chain moiety including the asymmetric centres at C-22, C-23 and C-24 are necessary. The latter problem has been solved by two different strategies. Either a C-22 aldehyde was used for the possible stereoselective side chain construction or the desired 22,23-diol function was introduced into a precursor with an intact sterol side chain. In the following account these fundamental pathways are discussed and their application is described in the synthesis of specific brassinosteroids (Table 2).

A/B-Functionalization

In nearly all syntheses a Δ^5-3β-hydroxy starting steroid (**15**) is transformed into a Δ^2-6-oxo compound (**22**) representing a suitable intermediate for the synthesis of both 6-oxo and 7-oxalactone type brassinosteroids. This transformation has been achieved via cyclosteroid formation or hydroboration as key reactions (Scheme 2).

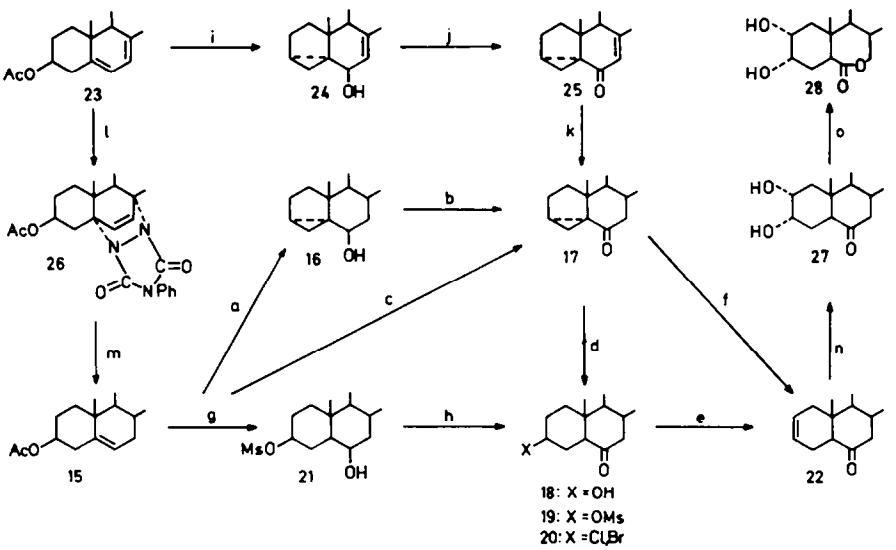
In the first case a mesylate or tosylate of **15** was solvolysed to the corresponding 3,5-cyclosteroid **16** which gave upon oxidation, e.g. with the CrO_3–pyridine complex or PCC, a 3,5-cyclo-6-oxo-compound **17** [14, 26, 43–47]. Upon reaction with DMSO–NaOAc the mesylate of **15** could be transformed also directly to **17** in 32% yield [48]. The conversion of **17** to **22** was realized via reaction to the hydroxyketone **18** [14, 43] followed by treatment of a corresponding mesylate **19** or tosylate with Li_2CO_3 [42] or LiBr [14, 39, 41, 43, 49]. A modified sequence to **22** leads via hydrogen halogenide catalysed rearrangement of **17** to **20** and subsequent elimination [47, 50, 51]. Compound **22** was also directly obtained from **17** upon reaction with *p*-TsOH–sulpholane [44–46].

The second pathway to **22** involves hydroboration of a **15**-mesylate followed by oxidation of the obtained 6β-hydroxy compound **21** with Jones reagent [42] or PCC [41, 49] to **19** and subsequent elimination as described above. With an overall yield of 63% this sequence represents the most superior way to the key intermediate **22**. Also, ergosteryl acetate (**23**) has been used as starting material for **22** via **17**. Thus, compound **23** was transformed to the corresponding 3,5-cyclosteroid **24** and further oxidized to the Δ^7-6-oxo derivative **25**. Subsequent reduction with Li–liquid NH_3 gave **17** [43]. On the other hand reduction of the ergosterol 1,4-cyclo adduct **26** led to the corresponding 3β-hydroxy-Δ^5-compound **15** as well as its Δ^7-isomer [50].

Reaction of Δ^2-6-ketones **22** with OsO_4 [42] or OsO_4–NMMNO [41] results in stereospecific hydroxylation to the 2α,3α-dihydroxy-6-oxo compounds **27** thus yielding the typical A/B structural feature of the native brassinosteroids (**6–10**). Subsequent Bayer–Villiger oxidation of **27** or preferably oxidation of the corresponding 2,3-diacetate and saponification, gives up to 90% of the desired 7-oxalactones **28** with the final A/B-structural

Table 2. Synthesis of brassinosteroids and selected analogues

Starting compound	Final product	Steps	Yield	References
(20*S*)-6β-Methoxy-3α,5-cyclo-5α-pregnane-20-carboxyaldehyde	1	12		42
(20*S*)-3β-Hydroxy-pregn-5-en-20-carboxyaldehyde-		23	4.5 %	41
3-tetrahydropyranylether	1	23	6.6 %	52
Brassicasterol/22-dehydrocampesterol	1	11	1 %	14
Stigmasterol	1	19	1.3 %	45, 46
		16	3 %	53, 60
(20*R*)-3β,20-Dihydroxy-pregn-5-en-3-tetrahydro-				
pyranylether-20-tosylate	1	16	4.3 %	66
(20*S*)-6β-Methoxy-3α,5-cyclo-5α-pregnane-20-	1	6	4.7 %	74
carboxyaldehyde	1		5.9 %	74
(20*S*)-6β-Methoxy-3α,5-cyclo-5α-pregnane-20-	(22*R*, 23*R*, 24*S*)-6β-			
carboxyaldehyde	Methoxy-24-methyl-			
	3α,5-cyclo-5α-			
	cholestan-22,23-diol	7	19.5 %	65
Stigmasterol	2	11	6.4 %	67
		16	3.4 %	68
22-Dehydrocampesterol	3	11	6 %	49
		17	8 %	62
Stigmasterol	4	13	1.6 %	61
		17	9.5 %	59, 62
Stigmasterol	5	13	1 %	69, 76
(20*S*)-6β-Acetoxy-3α,5-cyclo-5α-pregnane-				
20-carboxyaldehyde	6	9	8 %	47
Brassicasterol	(22*R*, 23*R*)- and			
	(22*S*, 23*S*)-24-			
	Epibrassinolide	5	20 %	48
Stigmasterol	(22*S*, 23*S*)-Homo-			
	brassinolide	9	10 %	45
(22*R*)-3β,22-Dihydroxy-24-nor-chol-5-en-23-	26,27-Bisnorbras-			
aldehyde-3,22-bismethoxymethyl ether	sinolide	13	16 %	63



a) $KHCO_3/Me_2CO/H_2O$, b) Jones reagent, c) DMSO/NaOAc, d) AcOH/H_2SO_4/K_2CO_3, e) HCl/AcOH, HBr/AcOH, f) Li_2CO_3, LiBr, p-TsOH/sulfolane, g) BH_3·THF, h) Jones reagent, PCC, i) $KHCO_3/Me_2CO/H_2O$, j) CrO_3/Py, k) Li/NH_3, l) 4-Phenyl-1,2,4-triazolin-3,5-dione, m) Li/$EtNH_2$, n) OsO_4, OsO_4/NMMO, o) CF_3CO_3H, MCPBA

Scheme 2. Pathways for A/B-functionalization in the synthesis of brassinosteroid.

features of the lactone-type brassinosteroids **1–5** [14, 41, 42, 45, 52, 53]. Thus, in the syntheses of 7-oxa-lactones the corresponding 6-oxo type members often occur as intermediates. Huang–Minlon reduction of the 6-oxo precursors (**27**) with the appropriate side chain led smoothly to 6-deoxocastasterone (**13**) and 6-deoxo-dolichosterone (**14**), respectively [53].

For structure–activity relationships a multitude of brassinosteroid analogues of the 6-oxo and lactone type have been prepared in which the substitution pattern of ring A is modified. Among them are compounds with unsubstituted ring A [39, 54], 2α,3α-epoxy, 2β-methoxy, 3α-hydroxy, 2β,3α-dihydroxy [39], 3α-hydroxy, 3β-hydroxy, 3-oxo,3α,4α-dihydroxy and Δ^3 unsaturated [54] functions. Also included is the synthesis of the native precursor typhasterol (**11**) [52]. Efforts to synthesize lactam and thiolactam analogues via Beckmann rearrangement of corresponding 6-oximes and subsequent thiation have led only to the isomeric 6-aza-7-oxo lactams and the corresponding thiolactams [27, 55].

Side chain construction

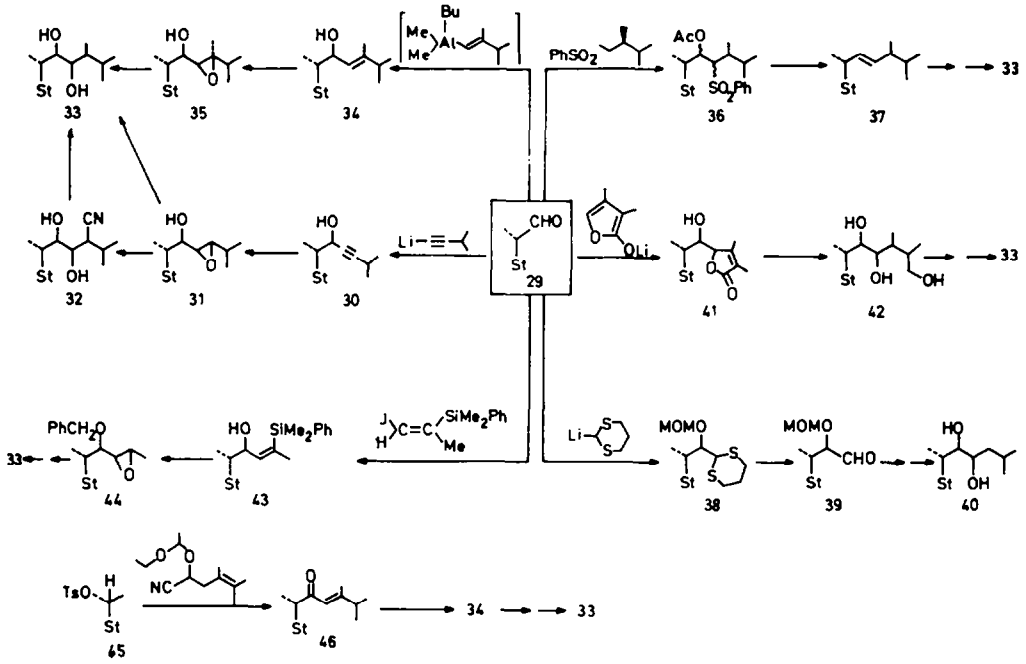
The starting material for most of the brassinolide syntheses is a 22-aldehyde of the type **29** (Scheme 3) which is readily available from dinorcholenic acid [56] or stigmasterol [46, 57–59]. Ishiguro *et al.* [41, 52] used a 22-aldehyde (**29**) with a THP-ether protected 3β-hydroxy group which was reacted with 3-methylbut-1-ynyl-Li to give a 1:1 ratio of epimeric 22-alcohols. Reduction of the separated (22*R*)-compound (**30**) with Lindlar catalyst to a *cis*-allylic alcohol followed by Sharpless epoxidation with *t*-BuOOH–VO(acac)$_2$ led stereospecifically to the (22*R*,23*R*)-epoxide (**31**). Attempts to introduce the 24-methyl group, e.g. with Me_3Al, Me_2CuLi or MeMgBr/CuI, were unsuccessful. Therefore, the nitrile alcohol **32** was prepared and the cyano-function transformed in a further sequence to the (24*S*)-methyl group of the brassinolide side chain (**33**). Later it was found [53, 60] that a direct methylation of **31** can be realized with Me_3Al–*n*-BuLi which enabled a significant shortening of the pathway and a higher yield of brassinolide (**1**).

The brassinolide synthesis described by Fung and Siddall [42] involves stereoselective alkylation of a 3,5-cyclo-6-methoxyaldehyde (**29**) with $Li^+[Me_2BuAlCH{=}CMeCHMe_2]^-$ to yield the major (22*R*)-alcohol **34**. Epoxidation to **35** followed by anti-Markovnikov reduction with $LiBH_4$–BH_3·THF gave after inversion at C-24 the preferred (22*R*,23*R*,24*S*)-brassinolide side chain **33**.

More recently similar principles of side chain construction were applied to the synthesis of other brassinosteroids using various alkylation reagents such as $Me_2CHC{\equiv}CMgBr$ [47], $Br_2C{=}CHCHMe_2$/LiBu [60], $Li^+[Me_2BuAlCH{=}CMeCHMe_2]^-$ [53, 61] and $LiC{\equiv}CCH_2Me_2$ [52].

In a further brassinolide synthesis published by the Mori group [45, 46] (2*R*)-(+)-(2,3-dimethyl)-pentylphenylsulphone was used as a preformed chiral building block, available in 10 steps from (*R*)-(+)-citronellic acid. Carbanion addition to **29** and acetylation led to the acetoxysulphon **36** which was reduced with Na-amalgam to the (24*S*)-Δ^{22}-olefin **37**. Its further transformation to **33** followed the epoxidation pathway as described in the next section. More recently another reaction sequence to the (24*S*)-Δ^{22}-olefin side chain (**37**) starting from **29** was developed by Anastasia *et al.* [47].

A quite variable method for the brassinosteroid side chain construction has been developed by Takatsuto and Ikekawa [59, 62, 63] using the chelation-controlled Grignard reaction for a stereoselective introduction of the desired (22*R*,23*R*)-diol function. Thus, from **29** with 1,3-dithiane lithium the (22*R*)-dithian was obtained and its MOM-ether **38** was treated with HgO–BF_3 to yield the 23-aldehyde **39**. Chelation-controlled Grignard reaction with *iso*-BuMgBr and removal of the MOM group gave



Scheme 3. Construction of the brassinosteroid side chain.

the 28-norbrassinolide side chain **40** [62]. Similarly Grignard addition of CH_2=C(MgBr)$CHMe_2$ permitted synthesis of dolicholide (**4**) [59, 62] and dolichosterone (**9**) [59]. With *iso*-Pr-MgBr the brassinosteroid analogues of the 26,27-bisnor series are available [63].

The synthesis of the brassinolide side chain developed by Donaubauer *et al.* [64] involves the aldol reaction of **29** with the Li-salt of 2,3-dimethylbutenolide as a key reaction leading to the major (22*R*,23*R*)-isomeric hydroxybutenolide **41**. Hydrogenation followed by reductive lactone opening with $LiAlH_4$ gave **42**. An elimination sequence for the additional 26-hydroxyl function led to the desired compound **33**. A modified reaction sequence to **33** using 3-*iso*-propylbut-2-enolide as side chain precursor was also described by the same authors [64].

Another stereoselective route to the brassinolide side chain involves reaction of **29** with the lithium salt of 2-(Me_2PhSi)-1-iodo-1-propene to give the major (22*S*)-allylic alcohol **43**. Silyl-group assisted Sharpless epoxidation and elimination of the Me_2PhSi function with *n*-Bu_4NF led to an epoxy alcohol which was transformed to the benzylether **44**. The completion of the side chain moiety was achieved upon alkylation of **44** with (*iso*-Pr)$_2$Cu(CN)Li_2 followed by Li–NH_3 deprotection to give **33** [65].

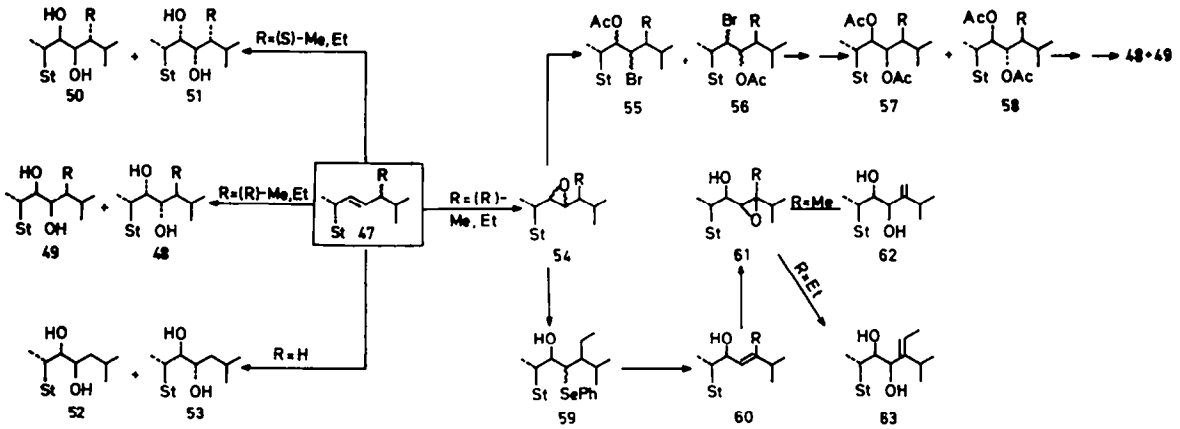
Very recently a further pathway to **33** starting from a (20*R*)-tosyloxy steroid **45** prepared from pregnenolone has been described [66]. Thus, the construction of the brassinolide side chain was carried out by alkylation of **45** with a protected C_8-cyanhydrin building block followed by acid and base treatment to give the Δ^{23}-22-oxo intermediate **46**. Reduction of **46** with Dibal-H led stereoselectively to the (22*R*)-allylic alcohol **34** which was further transformed to **33** as described above.

Modification of an intact side chain

As a starting material with a preformed side chain the $\Delta^{5,22}$-unsaturated compounds 22-dehydrocampesterol-brassicasterol [14], brassicasterol [48], stigmasterol [39, 44, 45, 53, 55, 60, 67–71], ergosterol [43, 50, 72, 73], 22-dehydrocholesterol [49] and porifasterol [74] have been used for brassinosteroid synthesis. In all these cases the introduction of the necessary vicinal 22,23-diol function was effected either upon direct OsO_4-hydroxylation or epoxidation followed by epoxide fission (Scheme 4). When applied to the synthesis of native brassinosteroids by both routes the main problem consists in the achievement of sufficient stereoselectivity with regard to the desired (22*R*,23*R*)-configuration.

The stereochemistry of the hydroxylation of (22*E*)-Δ^{22}-precursors **47** with OsO_4 or OsO_4–NMMNO was found to be influenced strongly by the configuration of the 24-alkyl group. Unfortunately (24*S*)-alkyl substitution directed the hydroxylation preferentially to the (22*S*,23*S*)-configuration **48** and the desired (22*R*,23*R*)-epimer **49** was either not isolated [27, 44, 45] or only obtained in low yield [14, 39, 47, 68, 71, 75]. Thus, starting from the 24-epimeric mixture of 22-dehydrocampesterol and brassicasterol this reaction was used for the synthesis of brassinolide (**1**) with a quite low overall yield [14]. Otherwise, the direct OsO_4-hydroxylation of (24*S*)-Δ^{22}-precursors was applied as a suitable method for the preparation of (22*S*,23*S*)-epimeric brassinosteroid analogues such as (22*S*,23*S*)-homobrassinolide from stigmasterol [45].

From (24*R*)-alkylated Δ^{22}-precursors (**47**) OsO_4-hydroxylation gave both epimeric (22*R*,23*R*)- and (22*S*,23*S*)-diols **50** and **51**, respectively, in either an equal 1:1 ratio [43, 50, 72, 75], a 3:5 ratio [48] or a 1:2 ratio [74]. In this manner brassinosteroid analogues of the



Scheme 4. Modification of a preformed side chain in the synthesis of brassinosteroid.

(24*R*)-series, including 24-epibrassinolide, were available in a five-step sequence starting from brassicasterol [48].

Hydroxylation of (22*E*)-22-dehydrocholesterol with OsO_4–NMMNO led to both epimeric diols **52** and **53** in 7:3 [75] and 2:5 [49] ratios, respectively, from which the (22*R*,23*R*)-epimer **52** was further transformed to 28-norbrassinolide (**3**) [49]. However, when this hydroxylation was applied to a Δ^{22}-precursor with a Δ^{2}-6-oxo-function the reaction proceeded stereoselectively to a (22*S*,23*S*)-diol (**53**) [55]. OsO_4-hydroxylation of (22*Z*)-22-dehydrocholesterol under more drastic conditions yielded the corresponding (22*R*,23*S*)- and (22*S*,23*R*)-diols in a 7:1 ratio [49].

As a more stereoselective route to the (22*R*,23*R*)-diol side chain in the (24*S*)-alkylated series, the intermediate **47** was reacted with MCPBA to give a mixture of stereoisomeric epoxides (**54**). *Trans*-opening of the epoxide ring with HBr and acetylation to regio- and stereoisomeric bromoacetates **55** and **56** followed by SN_2-substitution of bromine by the acetoxy function led to both separable epimeric diacetates **57** and **58** and further to the diols **48** and **49**. When applied to the synthesis of brassinolide (**1**) [45, 46] and homobrassinolide (**2**) [62, 67] thus gave a 3:2 [45, 67] and 10:9 ratio [62] of the corresponding (22*R*,23*R*)- and (22*S*,23*S*)-diol intermediates **49** and **48**, respectively.

In a modified procedure, ring opening of the stereoisomeric epoxides (**54**) with Ph_2Se_2–$NaBH_4$ yielded a regioisomeric mixture containing **59** which was transformed with 30% H_2O_2 to the allylic alcohol **60**. Corresponding intermediates of **60** (R = Me or Et) are also available from 22-aldehydes as described in the foregoing section [53, 61] or from ergosterol [73]. Epoxidation with MCPBA to **61** and aluminium isopropoxide catalysed rearrangement afforded, depending upon the C-24 substituent, the methylenediol or ethylidenediol side chain **62** and **63**, respectively. This reaction procedure has been used for the synthesis of dolicholide (**4**) [53, 61] and homodolicholide (**5**) [53, 69, 76] as well as the corresponding 6-oxo-type members dolichosterone (**9**) [53] and homodolichosterone (**10**) [53, 70, 76].

BIOACTIVITY

Brassinosteroids are potent plant growth promoters in a number of different test systems and act in many cases in a synergistic manner with auxin. In the bean second-internode bioassay brassinolide (**1**) produced nearly 200% increase in elongation of the internode. The increased cell division response was manifested by curvature, swelling and a dramatic splitting of the second internode [4]. Also, a high biological activity in the elongation test with wheat coleoptile has been observed [41]. In comparative studies in selected auxin bioassays compound **1** elicited responses similar to IAA in bioassays based upon bean hypocotyl hook opening, elongation of maize mesocotyl, pea epicotyl and azuki bean epicotyl sections, and fresh weight increases in Jerusalem artichoke and pea epicotyl sections. However, only about 50% of the IAA responses were elicited in the maize mesocotyl, bean hypocotyl hook and Jerusalem artichoke bioassays. A powerful synergism between compound **1** and IAA was observed in the azuki bean, pea epicotyl and bean hypocotyl hook bioassay [77]. With compound **1** and IAA a synergistic effect has been observed on growth of sheath pulvini of grasses [78].

Compared to GA_3 brassinolide (**1**) was found to be highly active in elongation bioassays employing the dwarf pea epicotyl and etiolated bean hypocotyl. Like GA_3 compound **1** also inhibited betacyanin accumulation in *Amaranthus* seedlings and prevented adventitious root initiation in hypocotyls of mung bean, dwarf bean and cucumber. Unlike the case with IAA, compound **1** does not interact synergistically with GA_3 [79]. The growth rate of mung bean hypocotyls was used for evaluating the effect of **1** on cell elongation whereas growth stimulation was observed at 10^{-10} M and above. GA_3 showed an additive relationship at 10^{-9}–10^{-8} M of **1** in this test system which suggested that the two growth promoters may independently act at the cellular level [80]. Recent studies on the place of 24-epibrassinolide in the sequential response to plant hormones in elongating tissue from peas and wheat showed that the sensitivity to the brassinosteroid occurs after that to GA_3 and begins before sensitivity to IAA [81].

With the rice lamina inclination test a specific and highly sensitive bioassay for brassinosteroids has been developed in which compounds **1** and **2** stimulated the lamina inclination of excised leaf lamina at concentrations of 0.0005 and 0.005 μg/ml, respectively. IAA showed a similar, but only weak effect, at 50 μg/ml [82]. Subsequently the rice cultivars Arborio J-1 and

Nihonbare were found to be the most suitable plants for this microquantitative brassinosteroid bioassay. Addition of cytokinin reduced the promoting effect of brassinolide suggesting that this test can be used also for detection of antibrassinosteroids [40]. A similar leaf lamina bending in intact dwarf rice seedlings was observed for the synthetic brassinolide analogue with (22*S*,23*S*,24*R*)-configuration [83]. For the bean first internode bioassay used for brassinosteroid detection [14, 75] a very sensitive modification has been developed allowing the detection of 1 p mole of 24-epibrassinolide [84]. For bioassay of brassinosteroids with intact plants radish (*Raphanus sativus*) and tomato (*Lycopersicon aesculentum*) have been introduced as suitable objects whereas promoted elongations of cotyledon petioles and hypocotyls of young plants at brassinolide concentrations as low as 0.01 ppm were observed. Auxins and gibberellins are also active but only at the 10 ppm level [85].

For structure–activity relationships most of the native brassinosteroids and many of the synthetic analogues have been tested in different bioassays [14, 39, 43, 74, 75, 82, 85]. Table 3 summarizes the relative activities of side chain modified brassinosteroids in four different test systems related to the most active member, brassinolide (**1**). Going from the 7-oxalactone to the 6-oxo type (e.g. **1** → **6** or **2** → **7**) the responses were much weaker. Thus, castasterone (**6**) gave about 50% of the brassinolide response in the rice lamina inclination and bean first internode bioassays. The relatively high activities of some synthetic analogues with a modified side chain such as 24-epibrassinolide, (22*S*,23*S*,24*R*)-brassinolide [74, 75, 85] and 27,28-bisnorbrassinolide

Table 3. Relative bioactivity of natural brassinosteroids and side chain modified analogues

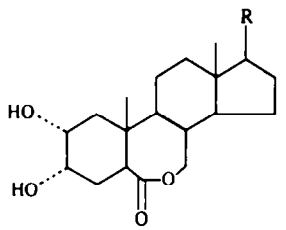
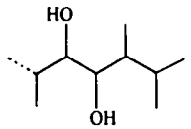
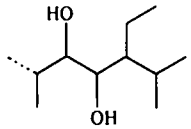
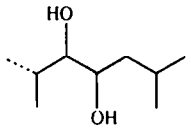
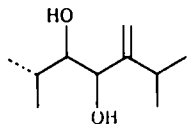
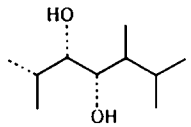
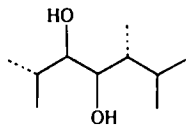
7-Oxalactone type		Raphanus*	Tomato*	Rice lamina*	Bean 1st internode†	References
						
R = 	(1)	100	100	100	547	74, 75, 85
	(2)	10	1	100	180	74, 75, 85
	(3)	100	10	5	25	74, 75, 85
	(4)			10		85
					249	75
			10	10	198	74, 75, 85

Table 3. (*Continued*)

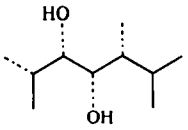
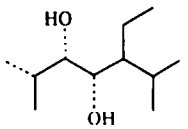
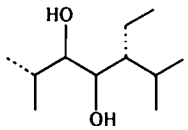
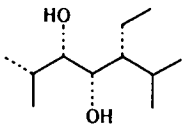
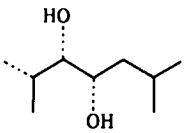
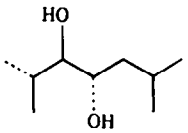
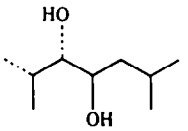
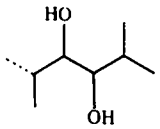
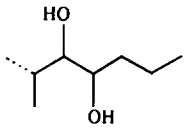
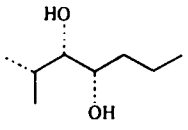
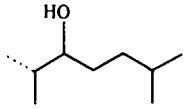
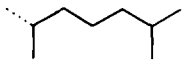
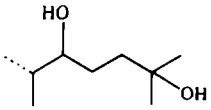
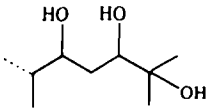
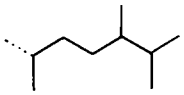
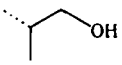
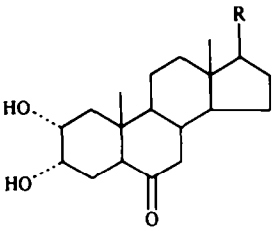
7-Oxalactone type	Raphanus*	Tomato*	Rice lamina*	Bean 1st internode†	References
	100	3	5–10	189	74, 75, 85
	3	1	50	141	74, 75, 85
	1	1	10		74, 85
	0.5	1	5		74, 85
	10	1	1	7	74, 75, 85
	1	0.1	0.1		74, 85
	1	0.5	0.05		74, 85
	100		100		63
				33	75
				210	75
	1	0.3	2		85, 86

Table 3. (*Continued*)

7-Oxalactone type	Raphanus*	Tomato*	Rice lamina*	Bean 1st internode†	References
			1–2		85, 86
	0	0			85
	0	0			85
				0	75
			2		86
			0.001		86

6-Ketotype



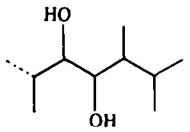
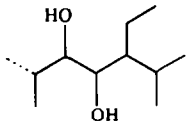
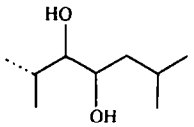
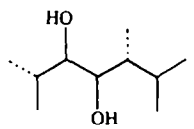
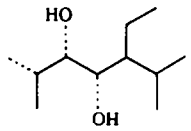
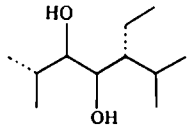
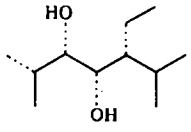
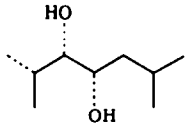
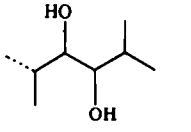
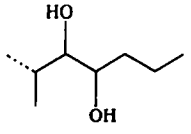
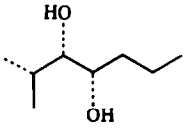
R =						
	(6)			50	273	74, 75
	(7)	1	0.3	50	75	74, 75, 85
	(8)	3	1	5	17	74, 75, 85
					216	75

Table 3. (*Continued*)

6-Ketotype	Raphanus*	Tomato*	Rice lamina*	Bean 1st internode†	References
	0.01		0.5	9	74, 75, 85
	0.1		1		74, 85
	0.03		0.5		74, 85
	0.3	0.3	0.1	32	74, 75, 85
	10		50		63
				35	75
				86	75

*Activity expressed as a percentage of the activity of brassionolide (1).
† %Growth over auxin-treated control.

[63] seems remarkable. For the 2α,3α-vicinal diol and the 22,23-vicinal diol function of 1 two binding sites on a receptor has been assumed [85]. However, systematic modifications at ring A of homobrassinolide showed that analogues with a 3α,4α-diol or 3α-ol function instead of a 2α,3α-diol still gave 50 and 10% responses, respectively, of 2 in the rice lamina test [54]. Also, the native 3α-substituted member 2-deoxycastasterone (11), as well as the 3β-hydroxylated teasterone (12), exhibit 10% brassinolide activity in the rice lamina inclination bioassay [39]. Substitution of the 6-oxo-7-oxalactone function by the isomeric 6-oxa-7-oxolactone led to a dramatic loss of activity [54, 75] similar to that found for 6-aza-7-oxo lactams and thiolactam analogues [27, 55].

The knowledge about the structure–activity relationships of brassinosteroids has been further developed in several publications [14, 54, 63, 75, 85]. The most recently postulated structural requirements for a high brassinosteroid activity are the following: (22*R*,23*R*)-vicinal diol moiety, (24*S*)-methyl or ethyl group, 7-oxalactone or 6-oxo functionality in the B-ring, 3α-hydroxy group, 2α,3α-vicinal diol or 3α,4α-vicinal diol and A/B-*trans*-fused ring junction [54]. However, until now A/B-*cis*-fused brassinosteroids have not been prepared and in some patents synthetic brassinosteroids with high plant growth promoting activity having a 17-alkyl-, 17-alkenyl- [9] and 22-ether side chain [10] were claimed.

Efforts to explore the physiological function and mode of action of the brassinosteroids are focussed on two main directions. It has been shown that 24-epibrassinolide induced growth stimulation in Azuki bean epicotyl segments was associated with an increase of acid secretion and by an early hyperpolarization of the transmembrane electrical potential as found for IAA [87]. In root

segments of maize 24-epibrassinolide induced a significant stimulation of root growth, also associated with an increase of acid secretion accompanied by hyperpolarization of the transmembrane electric potential. Contrariwise, IAA inhibited acid secretion and depolarized the membrane potential in maize root segments, suggesting an action of two different hormone types on the proton pump via two different mechanisms [88].

On the other hand it has been found that the (22*S*,23*S*,24*R*)-analogue of compound **1** stimulates several fold the auxin-induced ethylene production by etiolated mung bean hypocotyl segments in a synergistic manner with active auxins [89]. Also cytokinin interacts with this brassinosteroid in stimulating ethylene production [90] and Ca^{2+} acts synergistically [9]. The particular step of the brassinosteroid-induced ethylene production process influenced in mung bean hypocotyls was found to be between SAM and ACC. The results led to the conclusion that this effect occurs through increased IAA activity resulting in increased ACC synthase activity [91]. Recently, studies on the relationship of steroidal structure to ethylene production by etiolated mung bean segments, using compound **1** and a series of analogues confirmed stringent structural requirements for this effect and a brassinolide-like activity. Thus, changing the configuration from (22*R*,23*R*) to (22*S*,23*S*) or from (24*S*)- to (24*R*)-methyl did not very much reduce the efficiency to stimulate ethylene production alone or in combination with IAA. However, removal of the side chain hydroxyls caused inactivity similar to that found for removing the 7-oxa function on ring B. Thus, concordant structural requirements for activity in growth-based bioassays of brassinosteroids and brassinosteroids plus IAA enhancement of ethylene biosynthesis have been confirmed. These findings provided more support for the hypothesis that brassinosteroids may require endogenous auxin for activity [92]. Further physiological effects of brassinosteroids are summarized in ref. [89].

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