Synthesis of Brassinosteroids and Relationship of Structure to Plant Growth-Promoting Effects

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ABSTRACT

A number of brassinosteroids with and without hydroxyl groups or an alkyl stubstituent in their side chain were synthesized. The alkyl substituent at C-24 highly influenced the oxidation of the C-22 double bond with osmium tetroxide and, hence the ratio of the 22β,23β- and 22α,23α-glycolic isomers obtained. Two different bean bioassays used to compare the plant growth-promoting capabilities of these compounds and of brassinolide and its three side chain 22,23-cis-glycolic isomers showed that brassinolide was the most active. The next most active brassinosteroids were generally those with 22α-OH, 23α-OH orientation and a β-methyl or α-ethyl substituent at C-24. Similarly, of the synthetic precursor tetrahydroxy ketones of the brassinosteroids, those with 22α-OH, 23α-OH orientation (like brassinolide) and an alkyl group at C-24 were also the most active in both bioassays. The results indicate stringent structural features are required for a steroid to induce brassin activity. The structural requirements are: a trans A/B ring system (5α-hydrogen), a 6-ketone or a 7-oxa-6-ketone system in ring B, cis α-oriented hydroxyl groups at C-2 and C-3, cis hydroxy groups at C-22 and C-23 as well as a methyl or ethyl substituent at C-24.

INTRODUCTION

The structure and stereochemistry of brassinolide (I, 2α,3α,22α,-23α-tetrahydroxy-24α-methyl-B-homo-7-oxa-5α-cholestan-6-one), a novel plant growth-promoting steroidal lactone isolated from the pollen [1-4] of rape (Brassica napus L.), were determined by X-ray crystallography [5]. Subsequent to the identification of brassinolide, two side chain 22,23-cis-glycolic C-24 methyl isomers of I were synthesized [6]. More recently, brassinolide was synthesized via C_{22} unsaturated steroids [7,8] and via 22-dehydrocampesterol [9]. Our synthesis of brassinolide and its 22β,23β-isomer [9] via 22-dehydrocampesterol

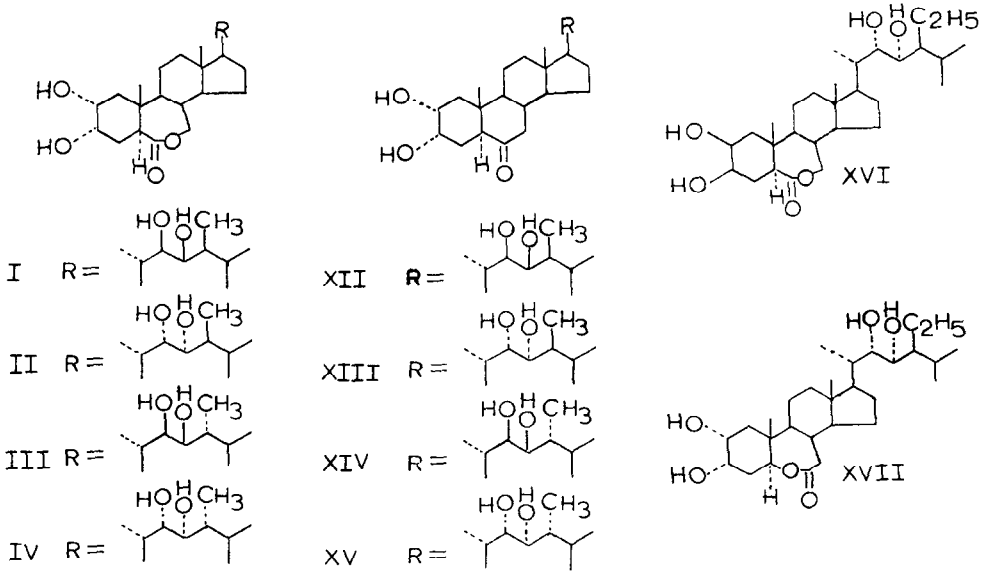


Figure 1

completed the series and made available I and all of its 22,23-cis-glycolic isomers for physiological studies. In the bean second internode bioassay [10], these brassinosteroids (I-IV, Fig. 1) showed varying degree of brassin activity; that is, they induced the unique biological response of cell division and cell elongation that results in elongation, curvature, swelling, and, finally, splitting of the treated bean internodes at hormonal levels (10^{-6} - 10^{-9}M).
In the bean first internode bioassay, an auxin-induced growth system [11], the isomers were also highly active. The synthetic precursor tetrahydroxy ketones of I and III were also active in both bioassays [9,6]. Both ketones, like brassinolide, have 22α,23α-oriented hydroxyl groups. Their corresponding isomers with 22β,23β-oriented hydroxyl groups were inactive.

To better define the relationship of brassinosteroid structure to plant growth-promoting activity, we synthesized an additional number of

brassinosteroids and tetrahydroxy ketones and then evaluated the plant growth-promoting effects of these compounds and those of brassinosteroids I-IV in two different bioassay systems. We now report our findings.

EXPERIMENTAL

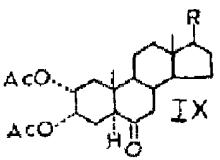
Instrumentation: Melting points were determined on the Kofler block [12] and are uncorrected. Rotations were determined at 25° of approximately 1% solutions of compounds in chloroform. Infrared spectra were obtained with a Perkin-Elmer Model 221 prism grating spectrophotometer. EI-MS were recorded on an LKB Model 9000 GC-MS (LKB-Produkter AB, Stockholm) equipped with a Varian Spectro System 100 MS data system. The samples were introduced directly into the ion chamber and the ionization energy was 70 ev. CI-MS were obtained with a Finnigan 4000 mass spectrometer. Methane chemical ionization was used to produce ions, and samples were introduced by way of the solid probe. The source temperature was 160°. Gas liquid chromatography (GLC) analyses were made on a Barber Colman Model 10 Chromatograph equipped with an argon-ionization detector and argon was used as carrier gas. The liquid phase used was 0.75% SE-30 and 1% OV-17.

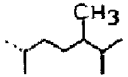
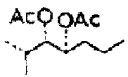
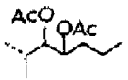
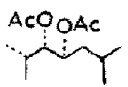
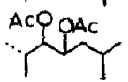
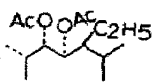
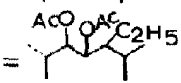
Materials: Silicic acid (Unisil 100 to 200 mesh) was obtained from Clarkson Chemical Co. (Williamsport, PA). Woelms neutral alumina was obtained from ICN Nutritional Biochemicals (Cleveland, OH), and the required amount of water was added to make activity Grade II or III alumina. Analab Anasil H scored plates (North Haven, CT) were used for TLC analyses. Analab Anasil H plates dipped into 4% silver nitrate solution prepared by a published method [13] were used for thin layer argentation chromatography.

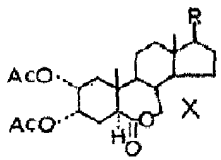
Bean Second Internode Bioassay: This test system [10] was used to evaluate the growth-promoting activity of the brassinosteroids. Plants were grown in growth rooms (temperature 25-27°, light 7.5 Klux for 12 hr), and groups of three 6-day-old bean (Phaseolus vulgaris L., Pinto var.) seedlings with second internodes 2 mm long were treated with the different concentrations of the test compounds in 250 µg of lanolin. The control plants were treated with lanolin alone. Measurements were taken after 4 days and those of the treated and control plants were compared and scored as previously reported [9].

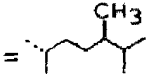
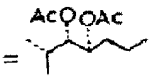
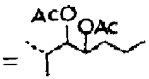
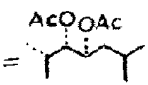
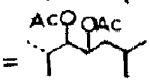
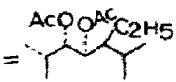
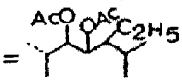
Bean First Internode Curvature Bioassay: The growing of etiolated bean (P. vulgaris L. cv. Bush Burpee Stringless) seedlings and collecting of the excised first internodes (4.0-5.0 cm long) from the 6-day-old bean seedlings were conducted according to a published procedure [11]. Ten nanograms of test compounds, solubilized in ethanol, was applied to the base of excised bean internode sections 1 hr prior to the application of 0.1 nM of auxin (indole-3-acetic acid, IAA). The control plant was treated with auxin alone. The growth of the sections was measured, as previously reported [9,11], 1 and 3 hr after the application of auxin.

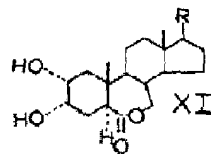
V
VI
VII
VIII
C°
M.P.
[α]25D
a) R =
b) R =
c) R =
d) R =
e) R =
f) R =
g) R =
h) R =
160-162 -35°
165-167 -28°
148-149 -16°
165-167 -30°
150-151 -27°
162-164 -16°
102-104 -2°
114-115 ±0°
121-122 +21°
109-110 +35°
88-90 +34°
206-207 +7°
230-231 +13°
208-210 -1°
227-228 -14°
231-233 +28°
221-222 -14°
213-214 +4°
243-244 +13°

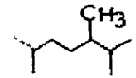
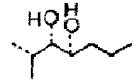
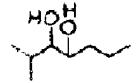
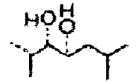
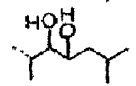
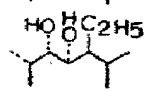
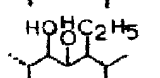


	C° M.P.	$[\alpha]^{25}D$
a) R = 	156-157	+2°
b) R = 	164-166	+5°
c) R = 	-	-
d) R = 	177-178	-9°
e) R = 	163-165	-14°
f) R = 	172-173	-17°
g) R = 	150-151	-10°
h) R = 	144-145	+16°



	C° M.P.	$[\alpha]^{25}D$
a) R = 	187-188	+51°
b) R = 	193-194	+44°
c) R = 	185-186	+32°
d) R = 	179-180	+19°
e) R = 	109-110 resolidified; melted 165-166	-
f) R = 	205-206	+8°
g) R = 	193-194	+19°
h) R = 	143-145	+41°



	C° M.P.	$[\alpha]^{25}D$
a) R = 	134-136	+53°
b) R = 	203-204	+51°
c) R = 	-	-
d) R = 	212-213	+11°
e) R = 	282-284	-
f) R = 	238-239	+24°
g) R = 	197-198	+35°
h) R = 	271-272	+33°

Synthetic scheme for brassinosteroids

Sterols Used in the Synthesis. Cholesterol (m.p. 149-150°) was obtained from Fisher Scientific Co. and used without any further purification. Campesterol (>96% purity, m.p. 160-161°, $[\alpha]^{25}D$ -33°) was obtained by fractional crystallization of soybean sterols from which the stigmasterol had been removed. Stigmasterol (m.p. 170-171°) was a gift of the Upjohn Co. (Kalamazoo, MI). 22(Z)-Dehydrocholesterol (m.p. 137-139°, $[\alpha]^{25}D$ -65°) and 22(Z)-dehydro-27-norcholesterol (m.p. 119-121°, $[\alpha]^{25}D$ -50°) were made via a Corey-modified Wittig reaction of 3,5-cyclodinorcholan-22-al methyl ether with 3-methylbutyltriphenylphosphonium bromide and with butyltriphenylphosphonium bromide, respectively, as previously reported [14].

Synthesis of Brassinosteroids. The methods of synthesizing the brassinosteroids and of purifying of intermediates were essentially the same as previously described for our synthesis of brassinolide and its 22,23-cis glycolic C-24 methyl isomers [9,6]. The sequence of reactions and the physical properties of the brassinosteroids and intermediates are shown above. The TLC, IR, and mass spectral analysis of the intermediates and final products were fully compatible with the structures indicated. The mass spectral data of the previously unreported brassinosteroids are presented. EI-MS were obtained on brassinosteroids without hydroxyl group in their side chain. Since it was difficult by EI-MS to obtain molecular ions for the compounds with hydroxyl groups in the side chain, CI-MS were obtained.

Mass Spectral Data of Previously Unreported Brassinosteroids.

2α,3α-Dihydroxy-B-homo-7-oxa-5α-cholestan-6-one(XIa). EI-MS $C_{27}H_{46}O_4$ (m/z, rel. intensity), 434(M^+, 5), 416(M - H_2O, 31), 389(29), 334(10), 261(22), 250(13), 155(13), 149(42), 137(16), 135(31), 121(21), 109(36), 107(37), 93(42), 43(100).

2α,3α-Dihydroxy-24α-methyl-B-homo-7-oxa-5α-cholestan-6-one(XIb). EI-MS $C_{28}H_{48}O_4$ (m/z, rel. intensity), 448(M^+, 4), 430(M - H_2O, 19), 403(16), 348(4), 250(8), 155(13), 149(27), 137(16), 135(24), 122(22), 121(18), 109(34), 107(31), 95(48), 93(42), 43(100).

2α,3α,22β,23β-Tetrahydroxy-27-nor-B-homo-7-oxa-5α-cholestan-6-one(XIc). CI-MS $C_{26}H_{44}O_6$ (m/z, rel. intensity), 453(M + 1, 99), 435(M + 1 - H_2O, 77), 417(M + 1 - $2H_2O$, 100), 399(M + 1 - $3H_2O$, 36), 381(M - C_4H_7O, 11), 379(11), 361(12), 331(10), 319(14), 315(12), 257(4), 177(5), 149(9), 111(9).

2α,3α,22α,23α-Tetrahydroxy-27-nor-B-homo-7-oxa-5α-cholestan-6-one(XId). CI-MS $C_{26}H_{44}O_6$ (m/z, rel. intensity), 453(M + 1, 100), 435(M + 1 - H_2O, 74), 417(M + 1 - $2H_2O$, 71), 399(M + 1 - $3H_2O$, 38), 381(M - C_4H_7O, 14), 379(15), 361(17), 331(17), 319(17), 315(18), 177(7), 149(12), 111(8).

2α,3α,22β,23β-Tetrahydroxy-B-homo-7-oxa-5α-cholestan-6-one(XIe). CI-MS $C_{27}H_{46}O_6$ (m/z, rel. intensity), 467(M + 1, 100), 449(M + 1 - H_2O, 20), 431(M + 1 - $2H_2O$, 12), 413(M + 1 - $3H_2O$, 8), 379(M - $C_5H_{11}O$, 13), 331(7), 315(7), 205(6), 177(5), 149(10), 111(10).

2α,3α,22α,23α-Tetrahydroxy-B-homo-7-oxa-5α-cholestan-6-one(XIf). CI-MS $C_{27}H_{46}O_6$ (m/z, rel. intensity), 467(M + 1, 100), 449(M + 1 - H_2O, 80), 431(M + 1 - $2H_2O$, 74), 413(M + 1 - $3H_2O$, 27), 379(M - $C_5H_{11}O$, 13), 331(14), 319(15), 315(21), 205(19), 177(18), 149(31), 123(23), 111(40).

2α,3α,22β,23β-Tetrahydroxy-24α-ethyl-B-homo-7-oxa-5α-cholestan-6-one(XIg). CI-MS $C_{29}H_{50}O_6$ (m/z, rel. intensity), 495(M + 1, 90), 477(M + 1 - H_2O, 63), 461(25), 459(M + 1, 90), 477(M + 1 - H_2O, 63), 461(25), 459(M + 1 - $2H_2O$, 21), 379(M - $C_7H_{15}O$, 65), 363(11), 361(18), 351(41), 349(21), 333(12), 279(13), 155(20), 145(57), 127(10), 115(100).

2α,3α,22α,23α-Tetrahydroxy-24α-ethyl-B-homo-7-oxa-5α-cholestan-6-one(XIh). CI-MS $C_{29}H_{50}O_6$ (m/z, rel. intensity), 495(M + 1, 25), 477(M + 1 - H_2O, 63), 461(11), 459(M + 1 - $2H_2O$, 10), 379(M - $C_7H_{15}O$, 34), 363(9), 361(16), 351(21), 349(13), 333(11), 279(16), 257(15), 145(27), 127(8), 115(100).

2α,3α,22β,23β-Tetrahydroxy-24α-ethyl-B-homo-6-oxa-5α-cholestan-7-one (XVII). CI-MS $C_{29}H_{50}O_6$ (m/z, rel. intensity) 495(M + 1, <1), 477(M + 1 - H_2O, 19), 459(M + 1 - $2H_2O$, 100), 343(24), 315(16), 257(8), 205(11), 177(11), 145(26), 139(18), 137(19), 127(91), 115(96).

2β,3β,22β,23β-Tetrahydroxy-24α-ethyl-B-homo-7-oxa-5α-cholestan-6-one(XVI). CI-MS $C_{29}H_{50}O_6$ (m/z, rel. intensity) 495(M + 1, 100), 477(M + 1 - H_2O, 62), 461(17), 459(M + 1 - $2H_2O$, 41), 441(23), 379(M - $C_7H_{15}O$, 26), 350(12), 331(13), 321(22), 319(30), 303(16), 285(16), 155(18), 139(17), 127(51), 115(10).

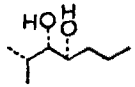
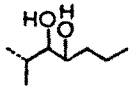
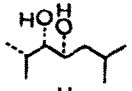
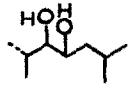
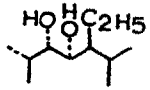
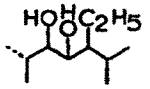
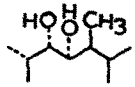
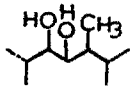
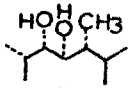
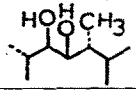
RESULTS AND DISCUSSION

Chemistry

Two isomeric end products were obtained, via the reaction pathway shown, from each of the $\Delta^{5,22}$-sterols used as starting material. The overall yields of the purified pairs ranged from 12 to 20%, the pair comprising XIg and XIh being obtained in the highest yield. Brassinosteroid XIa was obtained in a 30% yield from cholesterol. According to the reaction pathway shown above, intermediates Va,Vb,VIa, and VIb were not obtained, since we had on hand compounds VIIa and VIIb obtained via a different sequence of reactions, which had been used to synthesize ecdysteroids with molting hormone activity [15].

Oxidation of compounds VIIc - VIIe with 2 molar equivalents of

Table 1. TLC analyses of brassinosteroids (BR) and their corresponding synthetic precursor tetrahydroxy ketones and percentages of tetrahydroxy ketone side chain isomers formed in the synthesis.

Side chain	BR	R_f value*	Tetrahydroxy ketone	R_f value*	% Isomer
	XIc	0.41	VIIIc	0.52	30
	XId	0.38	VIIId	0.47	70
	XIe	0.38	VIIIe	0.52	30
	XIf	0.40	VIIIf	0.48	70
	XIg	0.57	VIIIg	0.63	87
	XIh	0.38	VIIIh	0.45	13
	II	0.55	XIII	0.62	82
	I	0.35	XII	0.42	18
	IV	0.52	XV	0.60	50
	III	0.40	XIV	0.47	50

*TLC plate developed 3 times (in chamber with wick) in chloroform/ethanol (9:1)

osmium tetroxide followed by reductive cleavage of the osmate esters resulted in their nearly quantitative conversion to a mixture of 22β,23β- and 22α,23α-isomers [16] of tetrahydroxy ketones with the expected 2α,3α-cis-diol orientation [17]. The ratio of side chain

isomers varied. The steroids without an alkyl substituent at C-24 yielded mainly the 22α,23α-isomer, whereas the steroid VIIe, which has a 24α-ethyl group, gave only about 13% of the 22α,23α-isomer and 87% of the 22β,23β-isomer (Table 1). The conversion of VIIe primarily to the 22β,23β-isomer agrees with the results we obtained when we used osmium tetroxide to oxidize a homologue of VIIe in the synthesis of brassinolide[9]. The homologue, which contained a 24α-methyl rather than a 24α-ethyl gave better than 80% of the 22β,23β-isomer (XIII) and less than 20% of the 22α,23α-isomer (XII, Table 1). Thus, our results suggest that the reported synthesis of homobrassinolide (a 24α-ethyl brassinosteroid) isomers [18], consisted of about 90% of the 22β,23β-isomer. The two 22,23-cis glycolic isomers of various brassinolide-related tetrahydroxy ketones we synthesized in this and our earlier studies are shown in Table 1 along with their ratios of formation and other data. The R_f values shown indicate the difficulty of separating the isomers that lack an alkyl substituent at C-24. The pair of isomers with a 24α-ethyl or 24α-methyl were readily separated by column chromatography and the pair of isomers with a 24α-ethyl were more easily separated than the pair of isomers with a 24β-methyl substituent.

A Baeyer-Villiger oxidation of the tetraacetates IXa-IXh with trifluoroperacetic acid in dichloromethane (a very rapid oxidation) or with m-chloroperbenzoic acid gave the tetraacetates Xa-Xh in 85% yield. Also produced in 12 to 15% yield were their corresponding more polar (by TLC) tetraacetoxy 6-oxa-ketones, which were easily separated from Xa-Xh by column chromatography. The oxidations were conducted with the tetraacetates since the tetrahydroxy 7-oxa-ketones and their

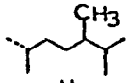
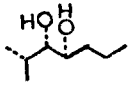
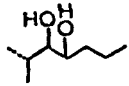
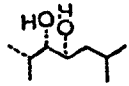
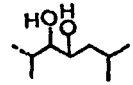
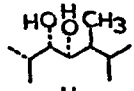
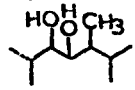
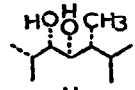
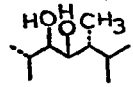
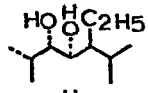
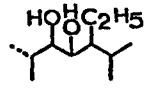
corresponding tetrahydroxy 6-oxa-ketones were not separable by TLC with various solvent systems [for example chloroform-ethanol (7:1 or 9:1)]. The tetraacetoxy 6-oxa-ketone is the major product when the Baeyer-Villiger oxidation is conducted in dichloromethane containing 1% sulfuric acid and 10% glacial acetic acid by volume. Compound XVII was prepared by this method (Fig. 1).

Biology

In the bean second internode bioassay brassinolide may cause one or more of the following changes: elongation, swelling, curvature and splitting of the treated internodes [5]; thus, the morphological change(s) induced by a brassinosteroid depends upon its structure and dosage. We used different dosages to evaluate the efficacies of brassinolide and the synthetic brassinosteroids in this test system (Table 2). The evaluation was based on two scoring methods: 1) percentage elongation over control, and 2) a growth response coded from 0 to 5 (Table 2 - see numbers in parenthesis; also see Fig. 2 in Reference 9).

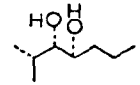
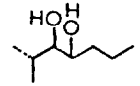
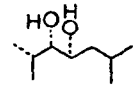
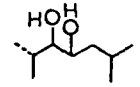
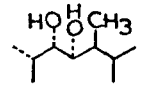
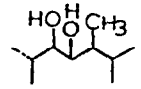
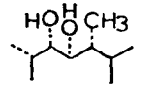
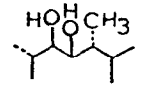
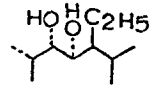
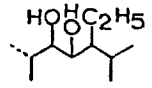
Using the response code, we concluded that compounds without hydroxyl groups in their side chain (XIa and XIb) are relatively inactive (Table 2). Except for XIc, those brassinosteroids with cis hydroxyl groups in their side chain but without an alkyl substituent at C-24 elicited a weak response. The brassinosteroids (II-IV, XIg and XIh) with cis hydroxyl groups at C-22 and C-23 and a substituent at C-24 were highly active, though less active than brassinolide (I). The isomeric pair, XIg and XIh, with a 24α-ethyl substituent were the least active of the group. Again, the isomer XIh with the substituents in the side chain oriented similar to those of brassinolide was the more

Table 2. Effects of brassinosteroids (BR) and their concentrations on the elongation and growth response of internodes in the bean second internode bioassay.

BR	Side chain	% Elongation over controls and growth response *				
		50µg	10µg	1µg	0.1µg	0.01µg
XIa		65(1)	58(1)			
XIb		136(1)	137(1)			
XIc		193(3)	188(2)			
XId		0	9(0)			
XIe		105(1)	104(1)			
XIf		51(2)	94(1)			
II			257(5)	129(4)	116(2)	80(1)
I			136(5)	136(5)	156(5)	183(4)
IV		460(5)	163(5)	136(5)	152(3)	140(2)
III		366(5)	237(5)	178(5)	228(4)	224(3)
XIg		283(4)	274(4)	26(1)	22(1)	7(0)
XIh		467(5)	411(4)	317(4)	278(3)	200(2)

*Growth response of the treated internodes was rated arbitrarily on a 0 to 5 scale (see numbers in parenthesis): 0. no elongation; 1. elongation only; 2. elongation with slight curvature and swelling; 3. elongation with good curvature and swelling; 4. elongation with excellent curvature and swelling; and 5. elongation with split internodes.

Table 3. Effects of tetrahydroxy ketones (THK) and their concentrations on the elongation and growth response of internodes in the bean second internode bioassay.

THK	Side chain	% Elongation over controls and growth response *			
		50µg	10µg	1µg	0.1µg
VIIIc		81(3)	72(2)		
VIIId		0	46(1)		
VIIIe		75(1)	30(1)		
VIIIf		59(1)	0		
XIII			0	71(1)	21(0)
XII			152(4)	120(4)	105(3)
XV		6(0)	0	21(0)	0
XIV		426(4)	295(3)	336(3)	352(2)
VIIIg		32(1)	0		
VIIIh		321(3)	171(1)		

*See footnote in Table 2.

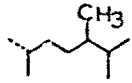
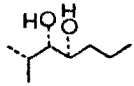
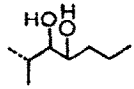
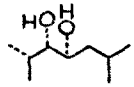
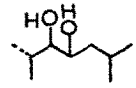
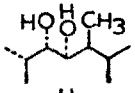
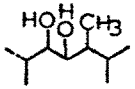
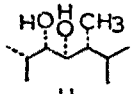
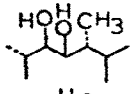
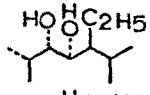
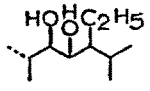
active of the pair. Surprisingly, of the compounds with a C-24 methyl, the least active was II, with 22β-OH, 23β-OH, and 24α-Me (Me configuration identical with that of brassinolide). The brassinosteroids III and IV were about one-tenth as active as brassinolide. The results also suggest that compound III was slightly more active

than compound II.

The synthetic precursor tetrahydroxy ketones of the brassinosteroids showed somewhat a dissimilar pattern of biological activity from that of their corresponding brassinosteroids in the bean second internode bioassay and were generally less active (Table 3). Except for VIIIc, those tetrahydroxy ketones without a methyl or ethyl substituent at C-24 generally caused no appreciable plant growth-promoting activity. Of the isomeric pair with a 24α-ethyl, only VIIIh, with 22α-OH, 23α-OH orientation, was active. Similar results were obtained for the 24α- and 24β-methyl tetrahydroxy ketones; only XII and XIV (Fig. 1), the isomers with 22α-OH, 23α-OH orientation, were active. In fact, ketone XII (side chain structure of brassinolide) was not only more active than ketone XIV, but also more active than II, the 22β,23β-isomer of brassinolide, and as active as compounds III and IV in this assay system.

The brassinosteroids were further evaluated in a bean first internode bioassay for their effectiveness in stimulating auxin-induced growth [11]. The brassinosteroids and tetrahydroxy ketones were tested at a level of 10 ng which is both low enough for brassinolide to induce a not too high response, and high enough for less active compounds to induce a measurable response. Brassinolide was the most active compound (Table 4), and the patterns of activity shown by the brassinosteroids and tetrahydroxy ketones were similar to those they showed in the bean second internode assay. That is, the compounds with 22α-OH, 23α-OH orientation and with an alkyl substituent at C-24 were generally more active than the corresponding 22β,23β-isomers. Of the paired compounds VIIIc-VIIId and XIc-XId, however, the isomers with the

Table 4. Effect of brassinosteroids (BR) and tetrahyroxy ketones on the elongation of internodes in the bean first internode bioassay

Side chain	BR	% Growth* over auxin-treated control	Tetrahydroxy ketone	% Growth* over auxin-treated control
	XIa	0	VIIIa	
	XIb	0	VIIIb	
	XIc	211	VIIIc	86
	XId	33	VIIId	35
	XIe	7	VIIIe	32
	XIf	25	VIIIf	17
	II	249	XIII	35
	I	547	XII	273
	IV	189	XV	171
	III	198	XIV	216
	XIg	141	VIIIg	9
	XIh	180	VIIIh	75

*After 3 hours.

22β-OH,23β-OH orientation were the more active, for reasons we cannot yet explain.

The acetates of the most active brassinosteroids including the tetraacetate of brassinolide, caused only marginal elongation in both bioassays. Compounds XVI with the brassinolide lactone system but with 2β-OH, 3β-OH, and compound XVII with a 6-oxa-ketone system, and the corresponding 6-oxa-ketones of compounds III and IV (Fig. 1) were also not biologically active. This information and the data in Tables 2-4 are evidence that specific structural features are necessary for a steroid to induce brassin activity in the bean bioassays. We concluded that the structural requirements are: a trans A/B ring system (5α-hydrogen), a 6-ketone or a 7-oxa-6-ketone system in ring B, cis α-oriented hydroxyl groups at C-2 and C-3, cis hydroxy groups at C-22 and C-23 as well as a methyl or ethyl substituent at C-24. Preliminary results of greenhouse and field plot evaluation of the effects of these unique hormonal growth substances on plant growth and crop production parallel results obtained in the bean internode bioassays, and brassinosteroids III and IV are presently the best available material for field evaluation of these growth substances on plant growth and crop yield.

Our structure-activity relationship study showed that the structural requirements of brassinosteroids for promoting plant growth are quite stringent. Even though certain tetrahydroxy ketones were active, mainly those ketones containing most of the structural features and configuration of brassinolide were active, and they differed from brassinolide only in the alkyl substituent or orientation of the methyl group at C-24. This would suggest that the active tetrahydroxy ketones

fit the brassinolide receptor site(s) and/or are converted rapidly to the corresponding brassinosteroids. Whether we can design and synthesize a more active brassinosteroid than brassinolide may, perhaps, depend on our gaining a better understanding and knowledge of the physiological role of these unique growth promoting substances in plants.

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