BRASSINOSTEROIDS IN THE VEGETABLE KINGDOM

N. V. Kovganko

UDC 547.92

This review gives literature information on the structure of brassinoids, which are natural plant growth regulators, and on their distribution in plants and their structure–biological activity relationships.

Among the large number of natural substances capable of acting on the growth and development of plants that have been detected in recent years [1, 2], a special position is occupied by the brassinosteroids [2-15]. The uniqueness of this group of C_{27}–C_{29} polyhydroxysteroids consists in the fact that they belong to the class of phytohormones and, in plants, exert regulatory functions in combination with other phytohormones (auxins, gibberellins, cytokinins, abscisic acid, and ethylene [16-18]). The discovery of brassinosteroids in plants has forced specialist in the field of physiology to undertake a substantial revision of their ideas on the phytohormone system and the principles of its functioning.

The name of the group of polyhydroxysteroids under discussion was obtained from its first representative [brassinolide (1)] isolated in 1979 from the pollen of Brassica napus [19]. Initially, a complex mixture of compounds called brassins was obtained from this source [20-22]. This mixture possessed the capacity for strongly accelerating the growth of plants. At the same time, its action differed from that of the phytohormones then known [23, 24]. Thus, the brassins caused not only growth but also division of the plant cells. As the result of many years' investigations, the active principle, brassinolide, was isolated from the brassins. The structure of brassinolide has been reliably established by x-ray structural analysis [19, 25].

It follows from Table 1, which gives the structural formulas of the natural brassinosteroids, that brassinolide is a steroidal polyhydroxylactone. Characteristic for the brassinolide molecule is the presence of a C_{28} steroid skeleton with a transformed ring B, which is seven-membered as the result of the insertion of an oxygen atom between C-6 and C-7. It may be concluded from Table 1 that the presence of a 22R,23R-diol grouping in the steroid side chain is characteristic for all brassinosteroids. At the same time, in ring A of a brassinosteroid there may be a 2α,3α-, 2β,3α-, or 2β,3β-diol grouping or an isolated 3α- or 3β-hydroxy group. Depending on the structure of ring B, the brassinosteroids can be divided into three structural types: the first comprises compounds containing a lactone grouping in ring B, while the second (the most numerous) consists of 6-ketosteroids. The brassinosteroids of the third type, having a six-membered ring B without oxygen-containing functional groups is fairly small. The structures of concrete representatives of the brassinosteroids will be considered according to this classification.

An isomer of brassinolide is 24-epibrassinolide (2). This phytohormone was first synthesized by several groups, and only in 1988 was it identifed in the pollen of the vetch Vicia faba by chromato-mass spectrometry. The proof of the identity of the natural and synthetic specimens was based on a comparison of the retention times of their bismethylboronates. While in the brassinolide molecule the C-24 center has the S configuration, the R configuration is characteristic for 24-epibrassinolide.

Using chromato-mass spectrometry, similarly, 28-norbrassinolide (3) and 28-homobrassinolide (4) were detected in higher plants [27, 28]. They were shown to be identical with the corresponding synthetic specimens by a comparison of the chromatographic characteristics of their bismethylboronates.

The brassinosteroid dolicholide (5), also belonging to the lactone group, was isolated in 1982 from the seeds of hyacinth dolichos Dolichos lablab (also called Egyptian or hyacinth

Institute of Bioorganic Chemistry, Academy of Sciences of the Belorussian SSR, Minsk.
Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 159-173, March-April, 1991. Original article submitted December 11, 1990.

0009-3130/91/2702-0135$12.50

beans and lab-lab) [29]. The structure of this compound as the 24(28)-dehydro derivative of brassinolide was established by the combined use of physicochemical methods, with a comparison of the PMR spectra of dolicholide and brassinolide being of decisive importance. There were only slight differences in the spectra, due to the absence of the 24-methyl group and the presence of a 24(28)-methylene group in the dolicholide molecule. A comparison of the chemical shifts of the other signals showed the presence in the dolicholide molecule of 2α,3α- and 22R,23R-diol groupings and a lactone group in ring B.

On continuing the study of the brassinosteroids in D. lablab, another polyhydroxylactone, homodolicholide (6), was isolated [30]. As compared with dolicholide, homodolicholide contains an additional methyl group, attached at C-28. Its presence follows from the PMR spectrum, which contains a signal in the form of a doublet of doublets at 1.72 ppm. In addition, the spectrum includes the signals of protons at C-2, C-3, C-5, C-7, C-22, and C-23 the positions of which coincide with the positions of the analogous signals in the PMR spectra of brassinolide and dolicholide, which shows the presence in the structure of (6) of 2α,3α- and 22R,23R-diol groupings, and also of a lactone ring B.

A representative of the brassinosteroids with a 6-keto group is castasterone (7), which was first isolated from insect galls of the chestnut Castanea crenata [31]. Isomers of castasterone are 24-epicastasterone (8) [32] and 3,24-diepicastasterone (9) [26], the presence of which was first established in the green marine alga Hydrodictyon reticulatum and unripe seeds of the bean Phaseolus vulgaris, respectively.

Brassinone (10) has the structure of 28-norcastasterone. This 6-keto-brassinosteroid was first identified in unripe seeds of the Chinese cabbage Brassica campestris var. pekinensis, the leaves of green tea Thea sinensis, and insect galls of the chestnut Castanea crenata [27]. Another 6-ketone has also been detected in Chineses cabbage and green tea – (24S)-24-ethylbrassinone (11), which is 28-homocastasterone [27]. The identification of the brassinosteroids (10) and (11) by Abe et al. [27] was achieved by a comparison of the retention times of the bismethylboronates of natural and synthetic specimens on gas chromatographic analysis.

The brassinosteroids dolichosterone (12) and homodloichosterone (15) are also 6-ketones. These compounds were first isolated from unripe seeds of hyacinth dolichos Dolichos lablab [33]. The coincidence of the parameters of the PMR spectrum of dolichosterone with those of the spectrum of dolicholide, except for the presence in the second case of signals at 3.11 and 4.09 ppm of protons at C-5 and C-7, was of fundamental importance. The positions of these signals are characteristic for the spectra of brassinosteroids with lactone rings B, and therefore their absence in this region of the spectrum of dolichosterone permitted it to be assigned the structure (22R,23R)-2α,3α,22,23-tetrahydroxy-5α-ergost-24(28)-en-6-one [33]. In its turn, the PMR spectrum of homodolichosterone is very similar to that of dolichosterone, except for the presence of the signals of an ethylene group in the side chain. This fact makes it possible to establish that homodolichosterone is (22R,23R)-2α,3α,22,23-tetrahydroxy-5α-stigmast-24(28)en-6-one.

The 6-ketosteroids include 25-methyhldolichosterone (13) [34] and 25-methyl-2,3-dihydrodolichosterone (4) [35], detected in the seeds of the bean Phaseolus vulgaris. These brassinosteroids are characterized by a not completely usual C-29 steroid skeleton with an additional methyl group at C-25. An investigation of the composition of the sterols in the seeds of P. vulgaris [41] showed that one of the components of the 24-methylsteroids has the same carbon skeleton as brassinosteroids (13) and (14) and is probably a bioprecursor of them. Attention is also attracted by the presence in the 25-methyl-2,3-diepidolichosterone molecule of a 2β, 3β-diol grouping, which is more characteristic of phytoecdysteroids [42, 43].

The brassinosteroids considered so far have contained a 2,3-diol grouping as one of their main structural fragments. However, at the present time two brassinosteroids having only one hydroxy group in ring A, at C-3, are known – typhasterol (16) and teasterol (17). Typhasterol was first detected in the pollen of the cattail Typha latifolia in 1983 [36, 44]. From 25 kg of pollen it was possible to extract only 1.7 mg of the brassinosteroid (16). A comparison of the PMR spectra of typhasterol and castasterone, showing a coincidence of the chemical shifts of the signals of the methyl groups, permitted the identity of their side chains to be established. The hydroxy groups in the PMR spectrum of typhasterol were represented by signals at 3.56 ppm (H-22 and H-23) and 3.72 ppm (H-3). The CD spectrum of typhasterol had a negative Cotton effect with its maximum at 294 nm, which indicated the presence of a 6-keto group and of a trans-A/B linkage. Since the H-3 signal in the PMR spectrum had a half-width of 8 Hz, the 3-hydroxy group geminal to it is axial and therefore has the α configuration. In-

dependently of Schneider et al. [36], although somewhat later, brassinosteroid (16) was isolated under the name 2-deoxycastasterone from the pollen of Pinus thunbergii [37].

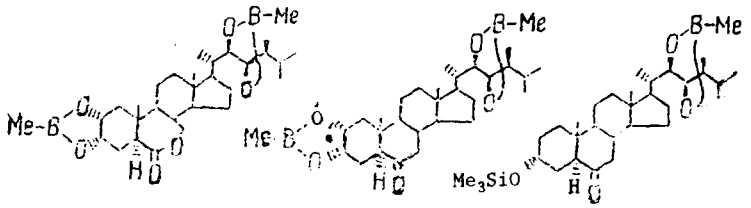
The brassinosteroid teasterone (17) acquired its name by virtue of the fact that it was first detected in the leaves of green tea, Thea sinensis [38]. The structure (22R,23R,24S)-3β,22,23-trihydroxy-5α-ergostan-6-one was established for teasterone by a TLC and GLC comparison of natural and synthesized specimens (as their 3-trimethylsilyl-22,23-methylboronates).

The third structural type of brassinosteroids unites compounds having neither lactone or ketone groups in ring B. These substances include 6-deoxocastasterone (18) and 6-deoxodolichosterone (19), which were first detected in unripe seeds of the bean Phaseolus vulgaris [39]. The structure (22R,23R,24S)-2α,3α,22,23-tetrahydroxy-5α-ergostane was assigned to 6-deoxocastasterone after an analysis of the mass spectrum of its bismethylboronate. The correctness of this structure was confirmed by a comparison of a natural specimen with the substance obtained by the Huang-Minlon reduction of the 6-keto group in the castasterone molecule. Analogously, 6-deoxodolichosterone (19) has the structure (22R,23R)-2α,3α,22,23-tetrahydroxy-5α-ergost-24(28)-ene, which has been confirmed by a comparison with the synthetic substance.

Later [40], the brassinosteroids (18) and (19) were more correctly called 6-deoxodihydrocastasterone and 6-deoxodihydrodolichosterone, as we have reflected in Table 1. However, since the original names have come into wide use in the scientific literature, we have retained them. Yokota et al. [40] have also reported the identification of another new brassinosteroid, 6-deoxodihydrohomodolichosterone (20), which, judging from the mass spectrum of its bismethylboronate, has the structure (22R,23R)-2α,3α,22,23-tetrahydroxy-5α-stigmast-24(28)E-ene. A substance with this structure has been synthesized, and a comparison of it with the natural brassinosteroid showed their complete identity.

Before discussing the distribution of the brassinosteroids in plants we must dwell on methods for their analysis. Since these phytohormones are contained in biological materials in extremely low concentrations, their direct isolation by traditional phytochemical methods is possible only in individual cases. Special methods of instrumental analysis are therefore used to determine brassinosteroids in plants: in the first place, chromato-mass spectroscopy [28, 45-47].

Because of the large number of hydroxy groups, brassinosteroid molecules are distinguished by thermal lability and low volatility. In view of this, volatile derivatives are used for gas-chromatographic analysis. The most convenient products for gas-chromatic analysis are those obtained by heating the brassinosteroids with methylboronic acid [28, 45]. In this reaction, brassinosteroids with hydroxy groups in the 2α,3α,22, and 23 positions form the 2,3, 22,23-bismethylboronates (21) and (22), in which all the hydroxy groups have been substituted. Trihydroxybrassinosteroids, which include, for example, typhasterol, form monomethylboronates under these conditions and therefore additional protection of the 3-hydroxy group by the formation of the trimethylsilyl ether (23) is necessary [47].



(21) (22) (23)

In the combination of gas chromatography with mass spectrometry the brassinosteroid derivatives issuing from the column are recorded from their characteristic peaks in the mass spectrum. Since in the commonest, electron-impact, mass spectra the peaks of the molecular ions of the brassinosteroids are absent or are weak, chemical-ionization mass spectra are used. In these spectra intense $(M + H)^+$ peaks are observed which are usually used for recording in the mass fragmentography regime [28]. This method permits the analysis of nanogram amounts of the brassinosteroids.

TABLE 1. Natural Brassinosteroids

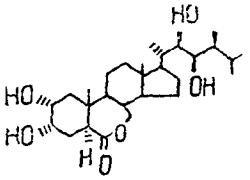
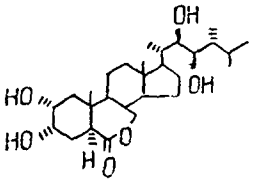
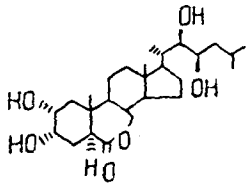
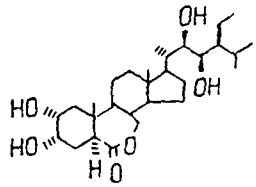
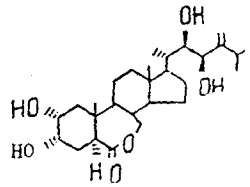
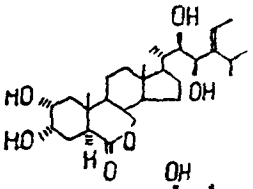
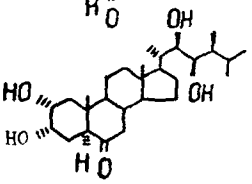
Name, molecular formula	Structural formula	T mp, °C	Literature
1. Brassinolide $C_{28}H_{48}O_6$			19, 25
2. 24-Epibrassinolide $C_{28}H_{48}O_6$			26
3. 28-Norbrassinolide $C_{27}H_{46}O_6$			27
4. 28-Homobrassinolide $C_{29}H_{50}O_6$			28
5. Dolicholide $C_{28}H_{46}O_6$		234—238 (aq. MeCN)	29
6. Homodolicholide $C_{29}H_{48}O_6$		227—228 (aq. MeCN)	30
7. Castasterone $C_{28}H_{48}O_5$		259—261 (aq. MeCN)	31

TABLE 1 (Continued)

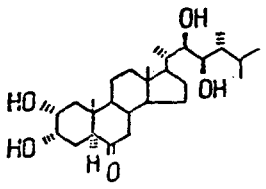
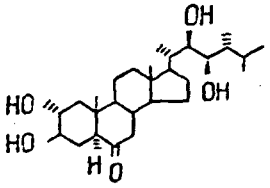
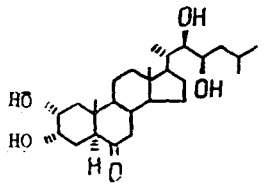
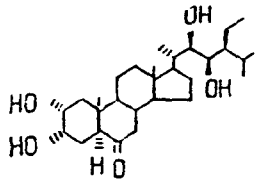
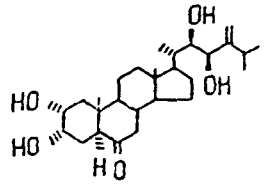
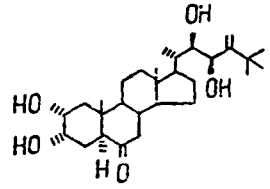
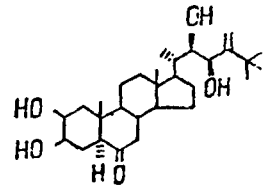
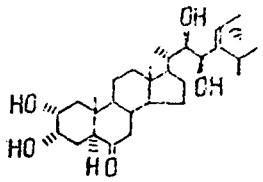
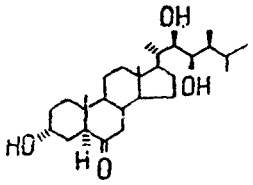
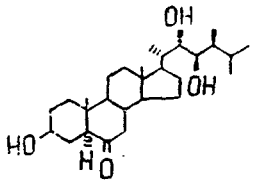
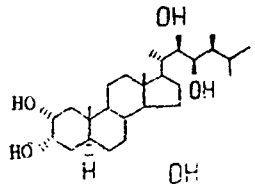
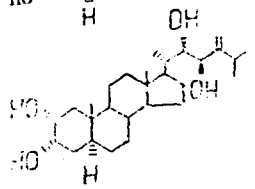
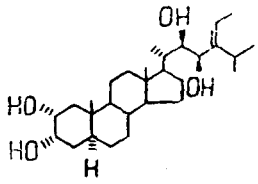
Name, molecular formula	Structural formula	T.mp, °C	Literature
8. 24-Epicastasterone $C_{28}H_{48}O_6$			32
9. 3, 24-Diepicastersterone $C_{28}H_{48}O_6$			26
10. Brassinone $C_{27}H_{46}O_6$			27
11. (24S)-24-Ethylbrassinone $C_{29}H_{50}O_6$			27
12. Dolichosterone $C_{28}H_{46}O_6$		233—237 (aq.MeCN)	33
13. 25-Methyldolichosterone $C_{29}H_{48}O_6$			34
14. 25-Methyl-2,3-diepidolichosterone $C_{29}H_{48}O_6$			35

TABLE 1 (Continued)

Name, molecular formula	Structural formula	T.mp, °C	Literature
15. Homodolichosterone $C_{29}H_{48}O_5$		204—208 (aq.MeCN)	33
16. Typhasterol (2-deoxycastasterone) $C_{28}H_{48}O_4$		227—230 ($CHCl_3$—MeOH) 231—233 (aq.MeCN)	36 37
17. Teasterone $C_{28}H_{48}O_4$			38
18. 6-Deoxocastasterone (6-deoxodihydrocastasterone) $C_{28}H_{50}O_4$			39
19. 6-Deoxodolichosterone (6-deoxodihydrodolichosterone) $C_{28}H_{48}O_4$			39
20. 6-Deoxodihydrohomodolichosterone $C_{29}H_{50}O_4$			40

It has also been proposed to determine brassinosteroids in the form of bisnaphthylboronates by high-performance liquid chromatography [48]. In this method the intense UV absorption of naphthalene rings at 280 nm is used for detection.

The bulk of the information on the distribution of brassinosteroids in plants has been obtained with the aid of chromato-mass spectrometry in the mass fragmentography regime (Table 2). On analyzing this table it is possible to draw the conclusion that brassinosteroids are widely represented in higher plants. They have been detected in the main families of angiosperms, such as the Leguminosae, Cruciferae, Gramineae, Betulaceae, Apocynaceae, etc. There are also brassinosteroids in gymnosperms, representatives of which are the Pinaceae. The detection of (24S)-24-ethylbrassinone and 24-epicastasterone in the green alga Hydrodictyon racemosum [32] may serve as an indication of the presence of brassinosteroids in lower plants, as well.

The largest amount of brassinosteroids is produced in plant pollen. In addition, they have been detected in unripe seeds, shoots, leaves, and insect galls. Also of interest is the recent [49] detection of the presence of brassinolide and castasterone in the cells of galls of Madagascar periwinkle cultivated in vitro.

The most characteristic property of the brassinosteroids is their capacity, in very low concentrations, for exerting an influence on the growth and development of plants. In the very first study on the isolation of brassinolide in the pure form [19] it was established that this compound in doses of >10 ng/plant caused both elongation and division of the cells. Investigations were undertaken on the plant-growth regulating activity of brassinolide in the auxin [60] and the gibberellin and cytokinin [61] bioassays. It was established that the biological action of brassinolide was not similar to those of these phytohormones. It exhibited activity in a number of the tests but in others was only feebly active or not at all. A synergistic action of brassinolide with indolyacetic acid was observed in some biotests.

The quantitative evaluation of the plant-growth regulating activity of the brassinosteroids required the development of special biotests. These include tests on the first and second internodes of the kidney beam proposed by American workers [62-64].

A test using rice, Oryza sativa, shoots is considered highly specific for brassinosteroids [65-69]. In this biotest, the size of the angle between the leaf blades and the stem of rice is used for the quantitative evaluation of the biological activities of brassinosteroids; it depends both on the structures and on the concentrations of phytohormones present in solution. Brassinolide causes an appreciable inclination of rice laminae, in comparison with a control, in a concentration as low as 0.0005 μg/ml. A representative [70] of the auxins (indolylacetic acid) exhibits activity in this biotest only at a concentration of 50 μg/ml.

Radish, Rhapanus sativus, shoots are also used for measuring the plant-growth regulating activity of the brassinosteroids [71]. Under the action of brassinolide a lengthening of the hypocotyles, of the cotyledon petioles, and of the plant as a whole is observed. Brassinolide possesses an appreciable activity even when its solution is diluted to 0.0001 ppm. Similar in use is a biotest on tomato, Lycopersicon esculentum, shoots [71], in which the magnitude of the biological activity is estimated from the lengthening of hypocotyles when shoots are immersed in solutions of the compounds under investigation.

We may also mention a test developed recently [72] using the unrolling of wheat leaves as a specific response to brassinosteroids.

The results of the study of the plant-growth stimulating activity of the brassinosteroids, both natural and synthetic, permit a number of conclusions to be drawn on the structure–function relationships in this series. In Table 3 we have gathered information on the relative activities of brassinosteroids in various biotests. It can be seen from this that, although the biotests do not correlate with one another, brassinolide is the most active phytohormone in all of them. The brassinolide molecule apparently has the optimum set of functional groups for effective binding with the receptors to form hormone-receptor complexes. Only 28-homobrassinolide in the test on the inclination of rice leaf laminae and 28-norbrassinolide and (22S,23R,24R)-brassinolide in the test on radishes are not inferior in activity to brassinolide. It may be concluded that in the brassinosteroid series ketones are, in general, less active than lactones. The 6-deoxobrassinosteroids are still less active [40]. Numerous investigations using synthetic compounds and the rice lamina inclination test [73-76] have shown that the replacement of the oxygen atom in the lactone ring by a nitrogen or sulfur atom, the substitution of the 2α,3α-diol grouping, a shortening of the side chain, and the elimination of the 22,23-diol grouping all lead to a decrease in activity.

It must, however, be mentioned that laboratory biotests cannot give a complete idea of the plant-growth regulating activity of the brassinosteroids and their analogs. This requires more profound investigations under hothouse and field conditions. In this connection we must mention the fact that a synthetic steroid having the structure of (22R,23R,24S)-2α,3α-isopropylidenedioxy-22,23-epoxy-7-oxa-B-homo-5α-stigmastan-6-one exhibited no plant-growth stimulating activity in the biotest for the deviation of rice leaf blades [77], but, nevertheless, under field conditions, when radish seeds were treated with a solution of this compound in a concentration of 0.001 μg/ml a greater increase in yield was obtained than in the case of brassinolide. This and other facts known at the present time open up considerable prospects for finding compounds suitable for practical use in agriculture among brassinosteroid analogs.

It has been shown [78] that, under hothouse and field conditions, some brassinosteroids, such as 24-epibrassinolide, in a concentration of 0.001-0.1 mg/liter considerably accelerate the vegetative growth of barley, kidney-bean, and lettuce shoots and thereby increase their crop yield. A high biological activity of 24-epibrassinolide for the bean has also been confirmed in [79]. However, here it is reported that this brassinosteroid in a concentration of 0.1-1 ppm has no appreciable action on wheat, maize, and cranberry shoots. It was also estab-

TABLE 2. Plants Producing Brassinosteroids

Family	Species	Organ	Brassinosteroid	Amount, ng	Literature
1	2	3	4	5	6
Apocynaceae	*Catharanthus roseus*	Cultivated gall cells	Brassinolide		49
			Castasterone		49
Betylaceae	Alnus glutinosa	Pollen	Brassinolide		50
			Castasterone		50
Brassicaceae	Brassica napus	Pollen	Brassinolide	1×10^5	19
Brassicaceae	Brassica campestris var pekinensis	Unripe seeds	Brassinolide	9,4	28,46,51
			28-Norbrassinolide	1,3	28
			28-Homobrassinolide	Traces	27,28
			Castasterone	1,06	28,46,51
			Brassinone	0,78	27,28
			(24S)-24-Ethylbrassinone	0,13	27,28
Chlorophyceae	Hydrodictyon reticulatum	Whole plant	(24S)-24-Ethylbrassinone	4000	32
			24-Epicastasterone	300	32
Convalovulaceae	Pharbitis purpurea	Unripe seeds	Castasterone	1100	52
			Brassinone	200	52
Fagaceae	Castanea crenata	Galls	Brassinolide	1,0	28,46,53,54
			Castasterone	11,0	28,31,46,53,54
			Brassinone	11,0	27,28,54
			6-Deoxocastasterone		54
Hamamelidaceae	Dystylium racemosum	Leaves	Brassinolide	23	28
			28-Norbrassinolide	156	28
			Castasterone	133	28
			Brassinone	16	28
		Galls	Castasterone	2500	28
			Brassinone	5000	28
Gramineae	Oryza sativa	Epigeal part	Castasterone	13,6	28,55
			Dolichosterone	8,4	28,55
Leguminosae	Dolichos lablab	Unripe seeds	Dolicholide	4700	29,56,57
			Dolichosterone	1500	33,56,57
			Homodolicholide	500	30,56,57
			Homodolichosterone	600	33,56,57
			Brassinolide		30,56
			Castasterone		30,56
			6-Deoxocastasterone		56
			6-Deoxodolichosterone		56

TABLE 2 (continued)

1	2	3	4	5	6
Leguminosae	Phaseolus vulgaris	Unripe seeds	Dolicholide		39,40
			Castasterone		39,40
			6-Deoxocastasterone		39,40
			6-Deoxodolichosterone		39,40
			25-Methyldolichosterone	880	34
			Brassinolide		40
			6-Deoxodihydrohomodolichosterone		40
			25-Methyl-2,3-diepidolichosterone		35
Leguminosae	Vicia faba	Pollen	Brassinolide	$1,9\times10^{5}$	26,48
			24-Epibrassinolide	5000	26,48
			Castasterone		26,48
			Brassinone		26,48
Pinaceae	Picea sitchensis	Shoots	Castasterone	5000	58
			Typhasterol	7000	58
Pinaceae	Pinus thubergii	Shoots	Typhasterol	$8,9\times10^{4}$	37
Theaceae	Thea sinensis	Leaves	Brassinolide	4,6	28,46,59
			Castasterone	110,0	28,46,59
			Brassinone	2,0	27,28
			(24S)-24-Ethylbrassinone	0,46	27,28
			Typhasterol	15,4	38
			Teasterone	61,6	38
Typhaceae	Typha latifolia	Pollen	Typhasterol	$6,8\times10^{4}$	36

TABLE 3. Relative Plant-Growth-Stimulating Activities of Brassinosteroids

Brassinosteroid	Activity in the assay*		
	Oryza sativa [70]	Rhapanus sativus [71]	Lycopersicon esculentum [71]
Lactones:			
Brassinolide	100	100	100
24-Epibrassinolide	10	10	10
(22S,23S,24R)-Brassinolide	5—10	100	3
28-Homobrassinolide	100	10	1
(22S,23S)-28-Homobrassinolide	50	3	1
(24R)-28-Homobrassinolide	10	1	1
(22S,23S,24R)-28-Homobrassinolide	5	0,5	1
28-Norbrassinolide	5	100	10
(22S,23S)-28-Norbrassinolide	1	10	1
(22R,23S)-28-Norbrassinolide	0,1	1	0,1
(22S,23R)-28-Norbrassinolide	0,05	1	0,5
Ketones			
Castasterone	50		
(24S)-24-Ethylbrassinone	50	1	0,3
(22S,23S,24S)-24-Ethylbrassinone	0,5	0,01	0
(24R)-24-Ethylbrassinone	1	0,1	0
(22S,23S,24R)-24-Ethylbrassinone	0,5	0,03	0
Brassinone	5	3	1
(22S,23S)-Brassinone	0,1	0,3	0,3

*In percentages of the activity of brassinolide.

lished that under the action of brassinosteroid (2) the growth of wheat and maize roots is inhibited.

It is reported in a patent [80] that 28-homobrassinolide in a concentration of 100 ppm increases the growth of the epigeal part of tomato shoots by 60% in three days.

An ability to increase the crop yield of maize, wheat, rice, soybeans, and other agricultural plants has also been found for brassinolide [81]. Thus, spraying the plants with a 0.01 ppm solution of brassinolide increases the total mass of grain in ears of wheat.

A new method of growing potatoes has been proposed one of the features of which consists in the spraying of the leaves and stems of the growing plants with a solution of brassinolide [82]. With three sprayings at weekly intervals of a potato plant with solution so brassinolide in a concentration of 10^{-2}-10^{-4} ppm, starting three days after flowering, the weight of tubers increased about one-and-a-half times in comparison with a control.

One of the characteristic features of the biological action of the brassinosteroids and their analogs is their capacity for increasing the resistance of plants to unfavorable environmental conditions. Thus, it has been established [83] that 24-ethylbrassinolide and (22S, 23S,24R)-brassinolide, and also their tetraacetates, decrease the crop losses caused by the salinization of the soil or by the use of herbicides. When rice seeds were treated with a solution of 24-epibrassinolide in a concentration of 10^{-2}-10^{-7} ppm two days before sowing the retardations of growth caused by the herbicides benzothiocarb and butachlor was prevented. The growth of rice at a low temperature is laso stimulated by treatment with brassinolide before budding [84]. Thus, from the combination of their effects the brassinosteroids are antistress agents — a promising group of plant growth regulators [85]. Their use is extremely important for our country, where agriculture is frequently carried out under extreme weather conditions.

Since the brassinosteroids are close in structure to the ecdysteroids, it appears important to study their activity as insect-molting hormones. With this aim, Adam et al. [87] investigated the influence of brassinosteroids on the evagination of the imaginal disks of Phormia terranovae pupae. It was found that, while ecdysterone caused 85 to 100% evagination of the disks in concentrations of 10^{-6} and 10^{-5} M, respectively, the brassinosteroids possessed a considerably lower actiity. Of the compounds studied, only homodolicholide and castasterone exhibited a slight activity in a concentration of 10^{-4} M, and in a concentration of 10^{-5} M this was lost completely.

More interesting proved to be the antiecdysteroid action of the brassinosteroids, which was evaluated from the suppression of the activity of ecdysterone added in a concentration 10^{-7} M. The greatest inhibiting action on the evagination of imaginal disks was shown by castaster-

one and (22S,23S)-28-homobrassinolide, which, at a concentration of 5×10^{-5} M completely suppressed the activity of ecdysterone. In view of the fact that castasterone acts as an ecdysteroid in this test, it may be assumed that the cause of the inhibition is the competitive binding of the btrassinosteroids with the ecdysteroid receptors [86]. It has also been established recently that (22S,23S)-28-homobrassinolide added to its food kills larvae of the cockroach Perplaneta americana as a result of the inhibition of the molting rocess [87].

Thus, an antiecdysteroid action is charcteristic for the brassinosteroids. From an analysis of numerous facts we have drawn the conclusion that various groups of steroids, such as cardiac glycosides, steroid saponins and alkaloids, withasteroids, and phytoecdysteroids, are produced in plants to prevent their being eaten by herbivorous animals, especially insects. At the same time, the brassinosteroids, which are present in plants in extremely low concentrations, are typical regulatory agents. Consequently, at the present day it seems unlikely that the brassinosteroids are involved in the chemical-ecological interrelationship of plants and insects.

LITERATURE CITED

1. N. B. Mandava, in: Plant Growth Substances, N. B. Mandava (ed.), ACS Symposium Series No. 111, American Chemical Society, Washington, D. C. (1979), pp. 135-213.
2. D. Gross, Z. Chem., 20, No. 11, 397-406 (1980).
3. T. H. Maugh, II, Science, 212, No. 4490, 33-34 (1981).
4. X. Li, Zhiwu Shenglixue Tongxun, No. 1, 5-9 (1983).
5. F. Chen, Chem. Canada, No. 9, 13-16 (1983).
6. Q. Guo, Huaxe Tongbao, No. 11, 30-32, 44 (1983).
7. L. Kohout, Chem. Listy, 78, No. 11, 1129-1156 (1984).
8. K. Wada, Kagaku to Kogyo, 37, No. 6, 386-388 (1984).
9. T. Yokota, Shokubutsu no kagaku Chosetsu, 19, No. 2, 102-109 (1984).
10. H. Singh and T. R Bhardwaj, Indian J. Chem., B25, No. 10, 989-998 (1986).
11. Y. Kamuro and S. Takatsuto, Shokoubutsu no Kagaku Chosetsu, 23, No. 2, 142-150 (1988).
12. G. Adam and V. Marquardt, Phytochmistry, k25, No. 8, 1787-1800 (1986).
13. B. Luo, Zhiwu Shenglixue Tongxun, No. 1, 11-14 (1986).
14. K. Z. Gamburg, Usp. Sovrem. Biol., 102, No. 2(5), 314-320 (1986).
15. T. Yokota, Nippon Nogei Kagaku Kaishu, 61, No. 3, 385-388 (1987).
16. O. N. Kulaeva, Cytokinins, Their Structure and Function [in Russian], Nauka (1973).
17. G. S. Muromtsev and V. N. Agnistokova, The Gibberellins [in Russian], Nauka (1984).
18. K. Doeffling, Plant Hormones. A Systemic Approach [Russian translation from the German], Mir, Moscow (1985).
19. M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. Mandava, J. F. Worley, J. D. Warthen, Jr., G. L. Steffens, J. L. Flippen-Anderson, and J. C. Cook, Jr., Nature (London), 281, 216-217 (1979).
20. J. W. Mitchell, N. Mandava, J. F. Worley, J. R. Plimmer, and M. V. Smith, Nature (London), 225, 1065 (1970).
21. N. Mandava, M. Kozempel, J. W. Worley, D. Matthees, J. D. Warthen, Jr., M. Jacobson, G. L. Steffens, H. Kenny, and M. D. Grove, Ind. Eng. Chem. Prod. Res. Dev., 17, No. 4, 351-354 (1978).
22. G. L. Steffens, J. G. Buta, L. E. Gregory, N. B. Manadava, W. J Meudt, and J. F. Worley, in: Advances in Pesticide Science; 4th International Congress of Pesticide Chemistry, Zurich, July 14-28 (1978). H. Geissbühler (ed.), Pergamon Press, Oxford. Part 2 (1979), pp. 343-346.
23. J. F. Worley and J. W. Mitchell, J. Am. Hortic. Soc., 96, No. 3, 270-273 (1971).
24. J. W. Mitchell and L. E. Gregory, Nature, New Biol., 239, No. 95, 253-254 (1972).
25. J. Karle, Lipids, 15, No. 9, 793-797 (1980).
26. N. Ikekawa, F. Nishiyama, and Y. Fujimoto, Chem. Pharm. Bull., 36, No. 1, 405-407 (1988).
27. H. Abe, T. Morishita, M. Uchiyama, S. Takatsuto, N. Ikekawa, M. Ikeda, T. Sassa, T. Kitsuwa, and S. Marumo, Experientia, 39, No. 4, 351-353 (1983).
28. N. Ikekawa, S. Takatsuto, T. Kitsuwa, H. Saito, T. Morishita, and H. Abe, J. Chromatogr., 290, 289-301 (1984).
29. T. Yokota, J. Baba, and N. Takahashi, Tetrahedron Lett., 23, No. 47, 4965-4966 (1982).
30. T. Yokota, J. Baba, and N. Takahashi, Agr. Biol. Chem., 47, No. 6, 1409-1411 (1983).
31. T. Yokota, M. Arima, and N. Takahashi, Tetrahedron Lett., 23, No. 12, 1275-1278 (1982).
32. T. Yokota, S. K. Kim, Y. Fukui, N. Takahashi, Y. Takeuchi, and T. Takematsu, Phytochemistry, 26, No. 2, 503-506 (1987).

33. T. Baba, T. Yokota, and N. Takahashi, Agr. Biol. Chem., 47, No. 3, 659-661 (1983).
34. S. K. Kim, T. Yokota, and N. Takahashi, Agr. Biol. Chem., 51, No. 8, 2303-2305 (1987).
35. K. Mori and T. Takeuchi, Ann. Chem., No. 8, 815-818 (1988).
36. J. A. Schneider, K. Toshihara, K. Nankanishi, and N. Kato, Tetrahedron Lett., 24, No. 36, 3859-3860 (1983).
37. T. Yokota, M. Arima, N. Takahashi, S. Takatsuto, N. Ikekawa, and T. Takematsu, Agr. Biol. Chem., 47, No. 10, 2419-2420 (1983).
38. H. Abe, T. Morishita, M. Uchiyama, S. Takatsuto, and N. Ikekawa, Agr. Biol. Chem., 48, No. 8, 2717-2712 (1984).
39. T. Yokota, M. Morita, and N. Takahashi, Agr. Biol. Chem., 47, No. 9, 2149-2151 (1983).
40. T. Yokota, S. Koba, S. K. Kim, S. Takatsuto, N. Ikekawa, M. Sakakibara, K. Okaba, K. Mori, and N. Takahashi, Agr. Biol. Chem., 51, No. 6, 1625-1631 (1987).
41. S. K. Kim, T. Akihisa, T. Tamura, T. Matsumoto, T. Yokota, and N. Takahashi, Phytochemistry, 27, No. 2, 629-631 (1988).
42. N. K. Abubakirov, Khim. Prir. Soedin., No. 6, 685-702 (1981).
43. A. A. Akhre and N. V. Kovganko, Ecdysteroids: Chemical and Biological Activity [in Russian], Nauka Tekh., Minsk (1989).
44. K. Yoshihara and N. Kato, Japanese Patent No. 6011498 (1985).
45. S. Takatsuto, B. Ying, M. Morisaki, and N. Ikekawa, J. Chromatogr., 239, 233-241 (1982).
46. N. Ikekawa, S. Takatsuto, S. Marumo, H. Abe, T. Morishita, M. Uchiyama, M. Ikeda, T. Sasa, and T. Kitsuwa, Proc. Jpn. Acad. Ser. B, 59, No. 1, 9-12 (1983).
47. S. Takatsuto, and N. Ikekawa, Chem. Pharm. Bull., 34, No. 8, 3435-3439 (1986).
48. K. Gamoh, T. Kitsuwa, S. Takatsuto, Y. Fujimoto, and N. Ikekawa, Anal. Sci., 4, No. 5, 533-535 (1988).
49. K. H. Park, H. Saimoto, S. Nagakawa, A. Sakurai, T. Yokota, N. Takahashi, and K. Shono, Agr. Biol. Chem., 53, No. 3, 805-811 (1989).
50. R. D. Plattner, S. L. Taylor, and M. D. Grove, J. Nat. Prod., 49, No. 3, 540-545 (1986).
51. H. Abe, T. Morishita, M. Uchiyama, S. Marumo, K. Munskata, S. Takatsuto, and N. Ikekawa, Agr. Biol. Chem., 46, No. 10, 2609-2611 (1982).
52. Y. Suzuki, I. Yamaguchi, and N. Takahashi, Agr. Biol. Chem., 49, No. 1, 49-54 (1985).
53. N. Ikekawa, S. Takatsuto, T. Sassa, N. Ikekawa, and M. Nikina, Agr. Biol. Chem., 47, No. 3, 655-657 (1983).
54. M. Arima, T. Yokota, and N. Takahashi, Phytochemistry, 23, No. 8, 1587-1592 (1984).
55. H. Abe, K. Nakamura, T. Morishita, M. Uchiyama, S. Takatsuto, and N. Ikekawa, Agr. Biol. Chem., 48, No. 4,1103-1104 (1984).
56. T. Yokota, J. Baba, S. Koba, and N. Takahashi, Agr. Biol. Chem., 48, No. 10, 2529-2534 (1984).
57. K. Okada, Kagaku to Kogyo, 36, No. 7, 504 (1983).
58. T. Yokota, M. Arima, N. Takahashi, and A. Crozier, Phytochemistry, 24, No. 6, 1133-1135 (1985).
59. T. Morishita, H. Abe, M. Uchiyama, S. Marumo, S. Takatsuto, and N. Ikekawa, Phytochemistry, 22, No. 4, 1051-1053 (L983).
60. J. H. Yopp, N. B. Mandava, and J. M. Sasse, Physiol. Plant, 53, No. 4, 445-452 (1981).
61. N. B. Mandava, J. M. Sasse, and J. H. Yopp, Physiol. Plant, 53, No. 4, 453-461 (1981).
62. M. J. Thompson, N. B. Mandava, W. J. Meudt, W. R. Lusby, and D. W. Spalding, Steroids, 38, No. 5, 567-580 (1981).
63. M. J. Thompson, W. J. Meudt, N. B. Mandava, S. R. Dutky, W. R. Lusby, and W. R. Spaulding, Steroids, 39, No. 1, 89-105 (1982).
64. M. J. Thompson, N. Mandava, J. F. Worley, W. J. Meudt, S. R. Dutky, and W. E. Robbins, US Patent Application No. 182210 (1981): Chem. Abstr., 95, 127425 (1981).
65. S. Marumo, H. Hattori, H. Abe, Y. Nonoyama, and K. Munskata, Agr. Biol. Chem., 32, 528-529 (1968).
66. K. Wada, S. Marumo, N. Ikekawa, and K. Mori, Plant Cell. Physiol., 22, No. 2, 323-325 (1981).
67. K. Wada, S. Marumo, K. Mori, S. Takatsuto, M. Morisaki, and N. Ikekawa, Agr. Biol. Chem., 47, No. 5, 1139-1141 (1983).
68. K. Wada, S. Marumo, H. Abe, T. Morishita, K. Nakamura, M. Uchiyama, and K. Mori, Agr. Biol. Chem., 48, No. 3, 719-726 (1984).
69. N. Ikekawa, in: Natural Products Chemistry, 1984. Collection of Invited Section Colloquium Lectures at the 14th IUPAC International Symposium on the Chemistry of Natural Products, Poznan, July 9-14, 1984, R. I. Zalewski and J. J. Scolik (eds.), Elsevier, Amsterdam (1985), pp. 61-73.

70. S. Takatsuto, N. Yawaza, N. Ikekawa, T. Morishita, and H. Abe, Phytochemistry, 22, No. 6, 1393-1397 (1983).
71. S. Takatsuto, N. Yazawa, N. Ikekawa, T. Takematsu, Y. Takeuchi, and M. Koguchi, Phytochemistry, 22, No. 11, 2437-2441 (1983).
72. K. Wada, H. Kondo, and S. Marumo, Agr. Biol. Chem., 49, No. 7, 2249-2251 (1985).
73. K. Wada and S Marumo, Agr. Biol. Chem., 45, No. 11, 2579-2585 (1981).
74. S. Takatsuto, N. Ikekawa, T. Morishita, and H. Abe, Chem. Pharm. Bull., 35, No. 1, 211-216 (1987).
75. M. Kondo and K. Mori, Agr. Biol. Chem., 47, No. 1, 97-102 (1983).
76. K. Okada and K. Mori, Agr. Biol. Chem., 47, No. 1, 89-95 (1983).
77. T Kakiuchi, Y. Kamuro, S. Takatsuto, and K. Kobayashi, Agr. Biol. Chem., 52, No. 9, 2381-2382 (1988).
78. W. Meudt, M. J. Thompson, N. Mandava, and J. F. Worley, Jr., Canadian Patent No. 1173659 (1984); Chem. Abstr., 102, 19625 (1985).
79. R. M. Devlin, I. I. Zbiec, and S. J. Karczmarszyk, Proc. Plant Growth Reg. Soc. Am., 9, 29-34 (1982).
80. Sumitomo Chemical Co., Ltd. Meiji Seika Kaisha Ltd. Japanese Patent 82118503 (1982); Chem. Abstr. 92, 177029 (1982).
81. A. Kumira, R. Ishii, B. S. Luo, M. Adachi, K. Hamada, and K. Fujita, FRG Patent No. 3533633 (1986); Chem. Abstr., 104, 181755 (1986).
82. T. Gemma, International Patent Application No. 8804890 (1988); Chem. Abstr. 110, 187813 (1989).
83. T. Takematsu, Japanese Patent No. 6366104 (L988); Chem. Abstr., 109, 224716 (1989).
84. Y. Hirai, S. Fujii, and K. Honjo, Japanese Patent No. 63135303 (1988); Chem. Abstr. 109, 224719 (1989).
85. Yu. A. Baskakov. Zh. Vses. Khim. Obshchestva, 33, No. 6, 631-640 (1988).
86. C. Hetru, J. P. Roussel, K. Mori, and Y. Nakatani, C. R. Acad. Sci. Paris, Ser. 2, 302, No. 7, 417-420 (1986).
87. G. Adam, K. Richter, and H. M. Vorbrodt, GDR Patent No. 252751 (1987); Chem. Abstr., 109, 50273 (1988).
88. N. V Kovganko and A A. Akhrem, Steroids: Ecological Functions [in Russian] Nauka Tekh., Minsk (1990).

A COMPARATIVE INVESTIGATION OF THE FATTY ACID COMPOSITIONS OF THE SEEDS OF A NUMBER OF LINES OF A GENETIC COLLECTION OF *Gossypium hirsutum*

S. G. Yunusova, S. D. Gusakova,
A. I. Glushenkova, U. K. Nadzhimov,
Sh. Turabekov, and S A. Musaev

UDC 665.335.9

A comparative analysis has been made of the amounts of lipids and their fatty-acid compositions in the seeds of the lines of agenetic collection of cotton plants of the species *Gossypium hirsutum* and their hybrids and the variety Tashkent-1. The results obtained on the fatty-acid compositions of some hybrids make it possible to recommend the use of indiviudal lines of cotton plants as donors for improving the food-value indices of cottonseed oil.

In various branches of industry a demand exists for vegetable oils with a definite index of oxidation resistance, which depends on the level of polyunsaturated acids in the oil and their distribution in the triglyceride molecules. Thus, a high concentration of the monounsaturated oleic (18:1) acid is preferred in oils subjected to heat treatment (refractory oils),

Institute of Chemsitry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. V. I. Lenin Tashkent State University. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 173-176, March-April, 1991. Original article submitted April 28, 1990.