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INDOLYL-3-ACETOXY DERIVATIVES OF BRASSINOSTEROIDS: SYNTHESIS AND GROWTH-REGULATING ACTIVITY

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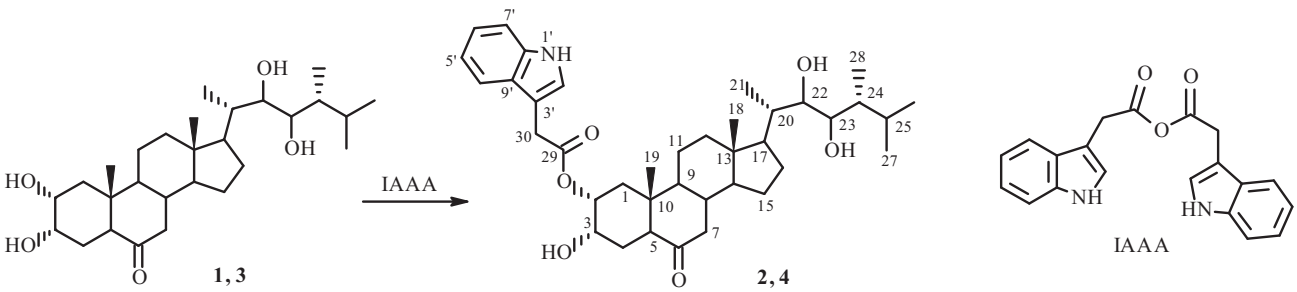
Esters of indolyl-3-acetic acid and brassinosteroids of the 24-epibrassinolide series were synthesized via the reaction of indolyl-3-acetic acid anhydride, which was prepared in situ *from the acid in the presence of dicyclohexylcarbodiimide, and the appropriate brassinosteroid. Tests of the growth-stimulating activity of the synthesized compounds on a wheat-sprout model revealed a synergic effect of the two different phytohormone components included in the ester molecule.*

Keywords: brassinosteroids, indolyl-3-acetic acid, conjugate synthesis.

Brassinosteroid plant hormones (BS) [1–4] are currently known to exhibit growth regulating and adaptogenic activity and to influence the balance of classical phytohormones (auxins, cytokinins, gibberellins, abscisic acid, and ethylene) [5–7]. Synergism in the action of BS and other phytohormones, in particular auxins, was also shown [8, 9]. Thus, ethylene production increased in the presence of indolyl-3-acetic acid (IAA) through the action of BS on etiolated mung bean hypocotyl segments [10].

Keeping in mind the increasing interest in mechanistic studies of cross-hormonal interactions of BS and auxins, it seemed interesting to prepare BS derivatives containing an additional IAA component. We reported recently the synthesis of several IAA esters based on 28-homobrassinosteroids [11]. Based on the results and also on the growth-stimulating and adaptogenic activity of Epin, a derivative of 24-epibrassinolide (EB) that was developed by us and incorporated into agricultural practice [12, 13], we synthesized conjugates of IAA and BS of the EB series.

We synthesized 24-epibrassinosteroids and IAA via the reaction of the appropriate BS and IAA anhydride (IAAA), which was prepared *in situ* by treatment of IAA with dicyclohexylcarbodiimide (DCC) in anhydrous dioxane. Thus, the principal product of the reaction of 24-epicastasterone (**1**) and IAAA was the ester at the 2-hydroxy group (**2**). Increasing the amount of used anhydride and the reaction time had practically no effect on the composition and yield of the reaction products (Scheme 1).



1, 3 **2, 4**

1, 2: 22*R*,23*R*
3, 4: 22*S*,23*S*

Scheme 1

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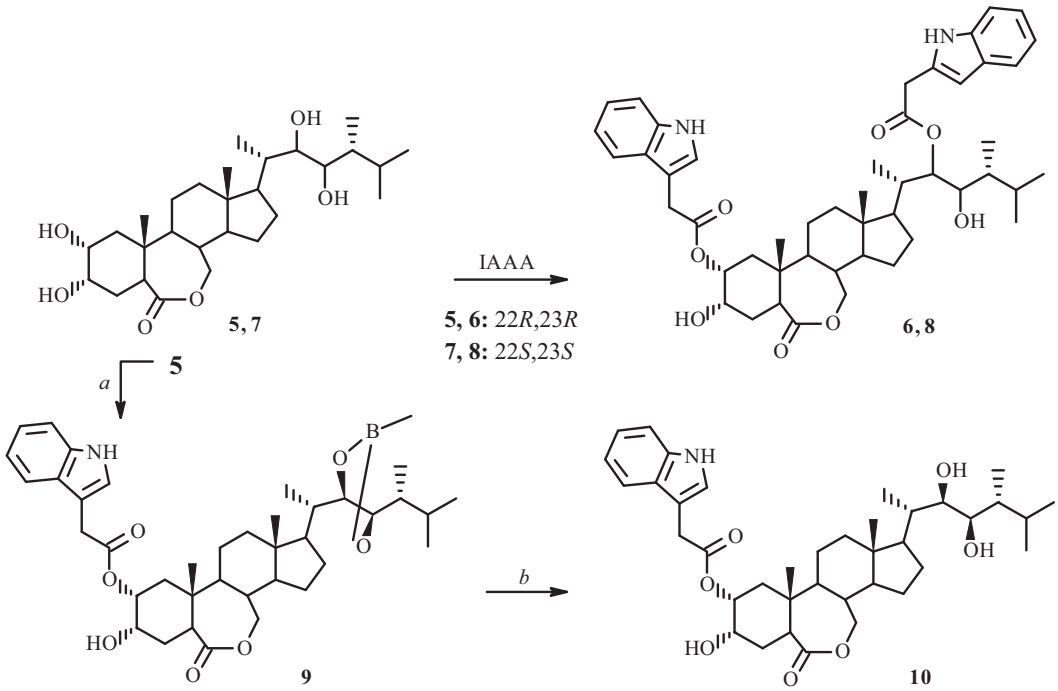
Formation of 2-(3-indolyl)acetoxy derivative **2** was confirmed by the presence in the UV spectrum of absorption bands characteristic of IAA (λ 220 and 280 nm) and a strong-field shift (δ 4.91 ppm) of the resonance for the C-2 proton in the PMR spectrum as a result of formation of the ester bond and the presence of resonances for five indole protons [δ 7.12, 7.19 (2H), 7.34, 7.60 ppm]. The mass spectrum of **2** exhibited a strong peak for the molecular ion and peaks corresponding to loss of water and IAA.

Acylation of the 22*S*,23*S*-isomer of 24-epicastasterone (**3**) was studied in order to determine the stereochemical effect of centers C-22 and C-23 on the course of the reaction. The principal product from its reaction with IAAA was also monoester **4**. Use of a large excess of IAAA and longer reaction times did not give products with greater acylation of **3** in amounts sufficient for their isolation and identification.

In contrast with 24-epicastasterone, the principal product from the reaction of EB **5**, which had a lactone group in ring B, with IAAA was diester **6**. The monoester analogous to **2** practically could not be detected (Scheme 2).



a. 1. $CH_3B(OH)_2$, 2. IAAA; *b*. H_2O_2, K_2CO_3

Scheme 2

The PMR spectrum of **6** contained resonances for the C-2 and C-22 protons (δ 4.86 and 5.12 ppm) that were shifted to stronger field than those in EB **5** and doubled resonances of the indole protons. The IR spectrum of **6** showed broadening of the hydroxyl stretching vibrations. The fragmentation in the mass spectrum corresponded with the proposed structure.

The 22*S*,23*S*-isomer **7** behaved analogously to EB in the studied reaction and gave the product of esterification of the C-2 and C-22 hydroxyls, i.e., 2,22-di(3′-indolylacetoxy) derivative **8**.

We developed a synthetic scheme including preparation of EB with protected hydroxyls in the side chain because of the difficulty in preparing the monoester of EB by direct methods. The reaction of EB **5** with methylboronic acid in Py produced the 22,23-methylboronate, which was treated without isolation from the reaction mixture with IAAA to give monoester **9**.

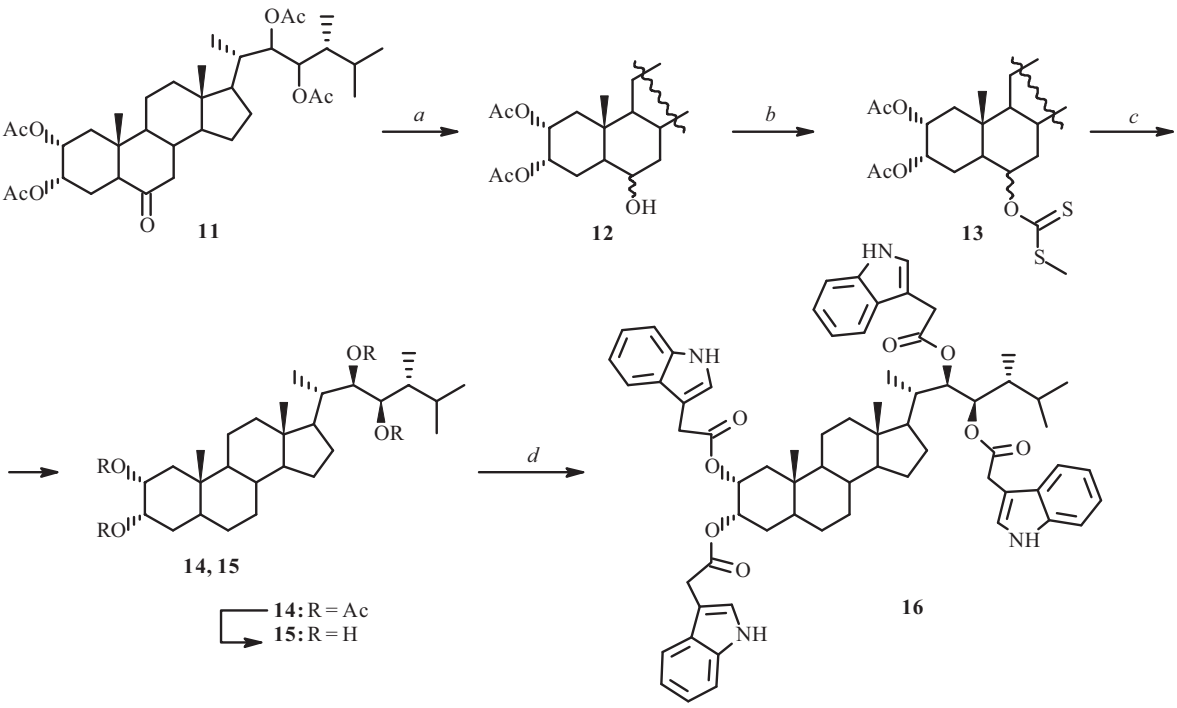
Removal of the methylboronate protection in **9** through the action of H_2O_2 in the presence of K_2CO_3 gave 2-(3′-indolylacetoxy) derivative **10** (Scheme 2).

Having noted that formation of BS conjugates with IAA depended on the structure of steroid ring B, we studied the acylation of 6-deoxoepicastasterone, especially because greater than 10 BS that have now been isolated from natural sources are 6-deoxo derivatives. 6-Deoxo-24-epicastasterone **15** was synthesized from 24-epicastasterone tetraacetate **11** through xanthogenates using the method described recently by us [14] in order to preclude possible isomerization at steroidal C-5. The application of this scheme with certain modifications included reduction of the 6-ketone of **11** by $NaBH_4$ to form a mixture of isomers at the C-6 alcohol (**12**) with isomer ratio 6α:6β ~ 16:1 (according to integration in the PMR spectrum); transformation of them into xanthogenates **13**; deoxygenation of **13**, and removal of the protecting groups (Scheme 3). These transformations enabled the target 6-deoxo-24-epicastasterone **15** to be obtained in ~45% overall yield.

TABLE 1. Influence of Indolyl-3-acetic Acid Esters of Brassinosteroids on Stem Length of Wheat Sprouts

Compound*	Stem length, % of control		Compound	Stem length, % of control	
	5 d	11 d		5 d	11 d
Control (H_2O)	100 ± 1.7	100 ± 1.9	**4**	100.4 ± 1.6	100.7 ± 1.6
24-Epibrassinolide	102.5 ± 1.7	106.9 ± 1.9	**6**	113.1 ± 1.9	108.6 ± 1.9
Indolyl-3-acetic acid	100.4 ± 1.8	100.5 ± 1.7	**8**	101.2 ± 1.7	101.8 ± 1.7
24-Epibrassinolide + indolyl-3-acetic acid	102.7 ± 1.8	106.7 ± 1.7	**10**	114.4 ± 1.9	110.2 ± 1.8
2	106.8 ± 1.6	107.5 ± 1.7			

*Concentration 10^{-7} M.



a. $NaBH_4$; *b*. 1. CS_2, DBU, 2. MeI; *c*. $BuSn_3H$, AIBN, *d*. IAAA

Scheme 3

It turned out that the reaction of **15** with IAAA under the conditions described above formed primarily tetrasubstituted derivative **16**, thereby indicating that the tetraol was exhaustively acylated, which was practically unachievable in the reactions with 24-epicastasterone and EB.

The biological activity of the prepared compounds was studied under laboratory conditions on Karavai variety wheat plants using the literature method [15]. Table 1 presents the results.

Table 1 shows that conjugates of IAA and natural 22*R*,23*R*-brassinosteroids stimulated the growth of wheat-sprout stems in biological tests whereas their 22*S*,23*S*-isomers did not show such an effect. The growth-stimulating effect of each of the hormones taken separately or in a mixture was characteristically inferior to the activity of the ester conjugate in which both phytohormone components were chemically bound.

The obtained derivatives also increased wheat-sprout biomass growth by 7, 13, and 14% in experiments with **2**, **6**, and **10**, respectively.

Thus, a series of IAA esters of 24-epibrassinosteroids were synthesized. BS of the 6-oxo series reacted with IAAA to give primarily monoacylation products; brassinosteroid lactones, diesters; 6-deoxo analogs, tetraesters. Preliminary screening of several prepared compounds in laboratory tests on wheat sprouts showed noticeable growth-stimulating activity that turned out to be greater than in controls and in tests using EB and its mixture with IAA [16].

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD, or Py-d_5 with TMS internal standard on a Bruker A-500 instrument (operating frequency 500 and 125 MHz). UV spectra were taken in MeOH on a Specord UV-Vis instrument. IR spectra were obtained (in film) on a UR-20 instrument. Mass spectra were measured using an Accela HPLC and LCQ-Fleet mass detector (three-dimensional ion trap) with electrospray ionization (ESI). The course of reactions was monitored by TLC on Merck plates (Kieselgel 60 F_{254}). Reaction mixtures were separated chromatographically over a column of silica gel 40/60 (Kieselgel 60, Merck). Melting points were determined on a Kofler block. Starting BS and their 22*S*,23*S*-isomers were synthesized by previously developed laboratory methods [2].

(22*R*,23*R*,24*R*)-3α,22,23-Trihydroxy-2α-(3′-indolylacetoxy)-24-methyl-5α-cholestan-6-one (2). A solution of IAA (100 mg, 0.62 mmol) in anhydrous dioxane (2 mL) was treated with DCC (125 mg) and stirred for 30 min at room temperature. The resulting precipitate of dicyclohexylcarbourea was filtered off. The filtrate was added to a solution of **1** (50 mg, 0.11 mmol) and DMAP (5 mg) in anhydrous dioxane (2 mL) and stirred for 5 h at 40°C. The dioxane was evaporated. The solid was chromatographed over a column of silica gel (eluent petroleum ether:EtOAc, 1:1). Yield of **2**, 50 mg (79%), mp 110–115°C (EtOAc). UV spectrum (λ_{max}, nm) (ε): 220 (30,500), 280 (11,500). IR spectrum (film, ν, cm^{-1}): 3400, 1710, 1590, 1260.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.64 (3H, s, 18-Me), 0.76 (3H, s, 19-Me), 0.83 (3H, d, J = 7, 26-Me), 0.86 (3H, d, J = 7, 27-Me), 0.90 (3H, d, J = 7, 28-Me), 0.96 (3H, d, J = 7, 21-Me), 3.39 (1H, m, H-23), 3.68 (1H, m, H-22), 3.80 (2H, d, J = 7, -$COCH_2$-), 3.99 (1H, m, H-3), 4.90 (1H, m, H-2), 7.12 (1H, H-5′), 7.19 (2H, m, H-6′, 2′), 7.34 (1H, d, J = 8, H-7′), 7.60 (1H, d, J = 8, H-4′), 8.50 (1H, c, NH).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 10.81 (q, C-28), 11.76 (q, C-18), 12.39 (q, C-21), 13.36 (q, C-19), 17.25 (q, C-26), 21.13 (t, C-11), 22.10 (q, C-27), 23.77 (t, C-15), 26.00 (t, C-16), 26.93 (d, C-25), 27.65 (t, C-4), 31.71 (t, C-30), 36.32 (t, C-12), 37.55 (d, C-8), 39.23 (t, C-1), 40.10 (d, C-20), 41.37 (d, C-24), 42.51 (s, C-13), 42.68 (s, C-10), 46.47 (t, C-7), 50.57 (d, C-5), 52.50 (d, C-9), 53.42 (d, C-14), 56.36 (d, C-17), 66.38 (d, C-2), 72.21 (d, C-3), 72.63 (d, C-22), 76.23 (d, C-23), 108.04 (s, C-3′), 111.48 (d, C-7′), 118.39 (d, C-4′), 119.76 (d, C-6′), 122.27 (d, C-5′), 123.12 (d, C-2′), 126.95 (s, C-9′), 136.16 (s, C-8′), 171.12 (s, C-29), 211.95 (s, C-6).

Mass spectrum, *m/z*: 663 $[M + H + MeCN]^+$, 622 $[M + H]^+$, 621 $[M]^+$, 604 $[M + H - H_2O]^+$, 429 $[M + H - IAA]^+$, 411 $[M + H - IAA - H_2O]^+$.

(22*S*,23*S*,24*R*)-3α,22,23-Trihydroxy-2α-(3′-indolylacetoxy)-24-methyl-5α-cholestan-6-one (4). The method described for **2** afforded from **3** (200 mg, 0.431 mmol) ester **4** (110 mg, 41%), mp 100–103°C (EtOAc). UV spectrum (λ_{max}, nm) (ε): 220 (31,500), 280 (10,000). IR spectrum (film, ν, cm^{-1}): 3410, 1710, 1595, 1250.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.67 (3H, s, 18-Me), 0.77 (3H, s, 19-Me), 0.88 (3H, d, J = 7, 26-Me), 0.90 (3H, d, J = 7, 27-Me), 0.97 (3H, d, J = 7, 28-Me), 1.00 (3H, d, J = 7, 21-Me), 3.59 (1H, s, H-23), 3.72 (1H, m, H-22), 3.80 (2H, m, -$COCH_2$-), 3.98 (1H, m, H-3), 4.91 (1H, m, H-2), 7.17 (2H, m, H-5′, 6′), 7.22 (1H, m, H-2′), 7.37 (1H, m, H-7′), 7.62 (1H, m, H-4′), 8.20 (1H, m, NH).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 9.91 (q, C-28), 11.90 (q, C-18), 13.40 (q, C-21), 14.04 (q, C-19), 18.84 (q, C-26), 21.17 (t, C-11), 21.47 (q, C-27), 24.12 (t, C-15), 25.91 (t, C-16), 27.82 (t, C-4), 29.78 (d, C-25), 31.78 (t, C-30), 36.36 (t, C-12), 37.53 (d, C-8), 39.28 (t, C-1), 41.85 (d, C-20), 42.52 (s, C-13), 43.39 (s, C-10), 43.79 (d, C-24), 46.55 (t, C-7), 50.59 (d, C-5), 52.49 (d, C-9), 53.53 (d, C-14), 56.23 (d, C-17), 66.46 (d, C-2), 70.34 (d, C-3), 72.21 (d, C-22), 73.25 (d, C-23), 108.32 (s, C-3′), 111.47 (d, C-7′), 118.44 (d, C-4′), 119.93 (d, C-6′), 122.44 (d, C-5′), 122.99 (d, C-2′), 129.13 (s, C-8′), 136.15 (s, C-9′), 170.93 (s, C-29), 211.57 (s, C-6).

Mass spectrum, *m/z*: 622 $[M + H]^+$, 604 $[M + H - H_2O]^+$, 586 $[M + H - 2H_2O]^+$, 447 $[M + H - IAA - H_2O]^+$, 429 $[M + H - IAA - 2H_2O]^+$, 411 $[M + H - IAA - 3H_2O]^+$.

(22*R*,23*R*,24*R*)-3α,23-Dihydroxy-2α,22-di-(3′-indolylacetoxy)-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (6). The method described for **2** afforded from EB **5** (50 mg, 0.11 mmol) indolylacetoxy derivative **6** (61 mg, 73%), mp 120–123°C (EtOAc). UV spectrum (λ_{max}, nm) (ε): 220 (38,100), 280 (12,000). IR spectrum (film, ν, cm^{-1}): 3400, 1710, 1735, 1620, 760.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.64 (3H, s, 18-Me), 0.68 (3H, s, 19-Me), 0.83 (3H, d, J = 7, 26-Me), 0.85 (3H, d, J = 7, 27-Me), 0.90 (3H, d, J = 7, 28-Me), 0.99 (3H, d, J = 7, 21-Me), 3.09 (1H, m, H-5), 3.42 (1H, m, H-23), 3.70 (1H, m, H-3), 3.86 (4H, m, -$COCH_2$-), 4.01 (2H, m, H-7), 4.86 (1H, m, H-2), 5.12 (1H, m, H-22), 7.13–7.21 (6H, m, H-5′, 6′, 2′), 7.38 (2H, m, H-7′), 7.64 (2H, d, J = 8, H-4′), 8.51 (2H, s, NH).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 10.58 (q, C-28), 11.43 (q, C-18), 13.34 (q, C-21), 13.63 (q, C-19), 16.66 (q, C-26), 21.06 (t, C-11), 21.96 (q, C-27), 23.64 (t, C-15), 26.00 (t, C-16), 26.44 (d, C-25), 27.85 (t, C-4), 31.79 (t, C-30), 31.99 (t, C-30′), 36.44 (t, C-12), 37.40 (d, C-8), 38.77 (t, C-1), 39.59 (d, C-20), 40.87 (d, C-24), 42.42 (s, C-10), 42.47 (s, C-13), 50.62 (d, C-5), 52.43 (d, C-14), 53.34 (d, C-9), 55.70 (d, C-17), 66.49 (d, C-2), 72.27 (d + t, C-3, 7), 76.40 (d, C-22), 76.65 (d, C-23), 108.29 (s, C-3′), 108.88 (s, C-3′), 111.32 (d, C-7′), 111.49 (d, C-7′), 118.45 (d, C-4′), 118.85 (d, C-4′), 119.90 (2d, C-6′), 122.43 (2d, C-5′), 123.03 (d, C-2′), 123.10 (d, C-2′), 127.00 (s, C-9′), 127.17 (s, C-9′), 136.18 (2s, C-8′), 170.95 (s, C-29), 172.20 (s, C-29′), 176.63 (s, C-6).

Mass spectrum, *m/z*: 829 $[M + H + MeCN]^+$, 795 $[M + H]^+$, 777 $[M + H - H_2O]^+$, 620 $[M + H - IAA]^+$, 445 $[M + H - 2IAA]^+$.

(22*S*,23*S*,24*R*)-3α,23-Dihydroxy-2α,22-di-(3′-indolylacetoxy)-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (8). The method described for **2** afforded from 22*S*,23*S*-24-epibrassinolide **7** (100 mg, 0.208 mmol) diester **8** (85 mg, 51%), mp 130–133°C (EtOAc). UV spectrum (λ_{max}, nm) (ε): 220 (37,500), 280 (11,000).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.56 (3H, s, 18-Me), 0.66 (3H, d, J = 7, 26-Me), 0.75 (6H, m, 19-Me and 27-Me), 0.78 (3H, d, J = 7, 28-Me), 0.86 (3H, d, J = 7, 21-Me), 3.07 (1H, m, H-5), 3.61 (2H, m, -CH_2-), 3.72 (2H, m, -CH_2-), 3.81 (1H, m, H-23), 3.85 (1H, m, H-3), 4.04 (2H, m, H-7), 4.76 (1H, m, H-2), 5.25 (1H, m, H-22), 7.07–7.23 (6H, m, H-5′, 6′, 2′), 7.29–7.37 (2H, m, H-7′), 7.48–7.61 (2H, m, H-4′), 8.20–8.30 (2H, m, NH).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 9.48 (q, C-28), 11.25 (q, C-18), 12.14 (q, C-21), 15.11 (q, C-19), 15.70 (q, C-26), 20.47 (q, C-27), 21.86 (t, C-11), 24.38 (t, C-15), 27.38 (t, C-16), 27.98 (d, C-25), 28.78 (t, C-4), 31.39 (2t, C-30, 30′), 37.14 (t, C-12), 37.75 (s, C-10), 38.39 (t, C-8), 38.69 (d, C-20), 39.00 (t, C-1), 40.17 (d, C-5), 41.27 (d, C-24), 42.25 (s, C-13), 50.48 (d, C-14), 53.36 (d, C-17), 56.76 (d, C-9), 68.33 (d, C-2), 69.46 (d, C-3), 70.03 (t, C-7), 84.32 (d, C-22), 85.91 (d, C-23), 108.09 (s, C-3′), 108.80 (s, C-3′), 111.28 (d, C-7′), 111.30 (d, C-7′), 118.86 (d, C-4′), 118.99 (d, C-4′), 119.56 (d, C-6′), 119.64 (d, C-6′), 122.20 (d, C-5′), 122.31 (d, C-5′), 123.27 (d, C-2′), 123.35 (d, C-2′), 127.24 (s, C-9′), 127.36 (s, C-9′), 136.08 (2s, C-8′), 170.83 (s, C-29), 171.34 (s, C-29′), 175.49 (s, C-6).

Mass spectrum, *m/z*: 795 $[M + H]^+$, 777 $[M + H - H_2O]^+$, 602 $[M - IAA - H_2O]^+$, 427 $[M + H - 2IAA - H_2O]^+$.

22,23-Methylborate(22*R*,23*R*,24*R*)-3α-hydroxy-2α-(3′-indolylacetoxy)-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (9). A solution of EB **5** (100 mg, 0.21 mmol) and methylboronic acid (11 mg, 0.23 mmol) in Py (3 mL) was held at room temperature for 6 h and evaporated in vacuo. The residue was dissolved in $CHCl_3$ and passed over a layer of silica gel. The filtrate was evaporated. The residue was dissolved in anhydrous dioxane (2 mL), added to a solution of IAAA obtained from IAA (120 mg, 0.74 mmol) and DMAP (10 mg) in dioxane, and stirred for 24 h at 40°C. The dioxane was evaporated. The residue was chromatographed over a column of silica gel (eluent petroleum ether:EtOAc, 1:1) to afford **9** (104 mg, 76%), mp 115–118°C (EtOAc).

PMR spectrum (CD_3OD, δ, ppm, J/Hz): 0.20 (3H, s, B-Me), 0.67 (3H, s, 18-Me), 0.70 (3H, d, J = 7, 21-Me), 0.83 (3H, d, J = 7, 26-Me), 0.86 (6H, m, 19-Me, 27-Me), 0.91 (3H, d, J = 7, 28-Me), 3.23 (1H, m, H-5), 3.63 (1H, m, H-23), 3.78 (1H, m, H-22), 3.81 (1H, m, H-3), 4.07 (2H, m, H-7), 4.76 (1H, m, H-2), 6.99 (1H, m, J = 8, H-5′), 7.08 (1H, m, J = 8, H-6′), 7.17 (1H, s, H-2′), 7.33 (1H, d, J = 8, H-7′), 7.55 (1H, d, J = 8, H-4′), 7.87 (1H, s, NH).

^{13}C NMR spectrum (CD_3OD, δ, ppm): 8.33 (q, C-B), 10.50 (q, C-28), 10.76 (q, C-18), 14.23 (q, C-21), 15.70 (q, C-19), 20.14 (q, C-26), 24.79 (q, C-27), 27.21 (t + d, C-11, 25), 27.57 (t, C-15), 28.31 (t, C-4), 31.05 (t, C-30), 33.49 (t, C-16), 38.30 (s, C-10), 38.72 (d, C-8), 39.01 (t, C-12), 41.08 (d, C-20), 41.15 (t + d, C-1, 5), 42.14 (s, C-13), 44.51 (d, C-24), 50.37 (d, C-14), 52.53 (d, C-17), 56.67 (d, C-9), 68.48 (d, C-2), 69.72 (d, C-3), 70.20 (t, C-7), 82.03 (d, C-23), 82.58 (d, C-22), 107.37 (s, C-3′), 111.15 (d, C-7′), 118.50 (d, C-4′), 118.75 (d, C-6′), 122.01 (d, C-2′), 127.44 (s, C-8′), 136.71 (s, C-9′), 171.67 (s, C-29), 176.74 (s, C-6).

(22*R*,23*R*,24*R*)-3α,22,23-Trihydroxy-2α-(3′-indolylacetoxy)-24-methyl-B-homo-7-oxa-5α-cholestan-6-one (10). Methylboronate **9** (30 mg, 0.045 mmol) was dissolved in a mixture of H_2O_2 (20 μL) and K_2CO_3 solution (1 mL, 1%) in MeOH, stirred for 2 h, and evaporated. The residue was chromatographed over a column of silica gel with elution by EtOAc. Yield of **10**, 16 mg (56%), mp 120–125°C (EtOAc). UV spectrum (λ_{max}, nm) (ε): 220 (30,700), 280 (9,500). IR spectrum (film, ν, cm^{-1}): 3400, 1720, 1710, 1260, 745.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.66 (3H, s, 18-Me), 0.84 (3H, d, J = 7, 26-Me), 0.86 (3H, d, J = 7, 27-Me), 0.89 (3H, d, J = 7, 28-Me), 0.91 (3H, s, 19-Me), 0.95 (3H, d, J = 7, 21-Me), 3.08 (1H, m, H-5), 3.41 (1H, m, H-23), 3.68–3.72 (2H, m, H-22, H-3), 3.79 (2H, m, -CH_2-), 4.01 (2H, m, H-7), 4.83 (1H, m, H-2), 7.10–7.16 (2H, m, H-5′, 6′), 7.20 (1H, d, J = 8, H-2′), 7.36 (1H, d, J = 8, H-7′), 7.61 (1H, d, J = 7.9, H-4′), 8.37 (1H, s, NH).

^{13}C NMR spectrum (CD_3OD, δ, ppm): 10.86 (q, C-28), 11.58 (q, C-18), 12.34 (q, C-21), 15.26 (q, C-19), 17.31 (q, C-27), 22.13 (q, C-26), 22.67 (t, C-11), 24.66 (t, C-15), 26.99 (d, C-25), 29.33 (t, C-16), 29.66 (t, C-4), 30.69 (t, C-30), 37.61 (s, C-10), 39.08 (d, C-8), 39.46 (t, C-12), 40.89 (d, C-20), 41.37 (t, C-1), 42.37 (s, C-13), 51.09 (d, C-5), 52.48 (d, C-24), 52.51 (d, C-14), 57.94 (d, C-17), 63.58 (d, C-9), 66.14 (d, C-2), 70.37 (d + t, C-3, 7), 72.02 (d, C-22), 76.35 (d, C-23), 108.20 (s, C-3′), 111.51 (d, C-7′), 118.46 (d, C-4′), 119.82 (d, C-6′), 122.36 (d, C-5′), 123.12 (d, C-2′), 127.01 (s, C-9′), 136.19 (s, C-8′), 171.09 (s, C-29), 176.03 (s, C-6).

Mass spectrum, *m/z*: 637 $[M]^+$, 620 $[M + H - H_2O]^+$, 445 $[M + H - IAA]^+$, 427 $[M + H - IAA - H_2O]^+$.

(22*R*,23*R*,24*R*)-2α,3α,22,23-Tetraacetoxy-6ξ-hydroxy-24-methyl-5α-cholestane (12). A solution of 24-epicastasterone tetraacetate **11** (160 mg, 0.24 mmol) in MeOH (5 mL) was stirred, treated in portions with $NaBH_4$ (17 mg, 0.44 mmol), stirred for 3 h at room temperature, treated with CH_3COOH (20 μL) to decompose the excess of reductant, diluted 5× with H_2O, and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, and evaporated. The compound was passed over a column of silica gel (eluent petroleum ether:EtOAc, 5:1) to afford 6-hydroxy derivative **12** (148 mg, 86%) as an oil. IR spectrum (film, ν, cm^{-1}): 3450, 1725 br, 1260 br.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 0.81 (3H, d, J = 7, 28-Me), 0.85 (3H, d, J = 7, 27-Me), 0.92 (3H, d, J = 7, 26-Me), 0.98 (3H, d, J = 7, 21-Me), 1.07 (3H, s, 19-Me), 1.97 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), 3.79 (0.94H, d, J = 2, H_α-6), 3.97 (0.06H, m, H_β-6), 4.96 (1H, m, H-23), 5.06 (1H, dd, J_1 = 7.3, J_2 = 4.9, H-22), 5.23 (1H, t, J = 7.7, H-2), 5.37 (1H, d, J = 2.4, H-3).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 10.77 (q, C-28), 11.73 (q, C-18), 13.39 (q, C-21), 16.04 (q, C-19), 17.16 (q, C-27), 20.70 (t, C-11), 20.87 (q, CO<u>Me</u>), 20.92 (q, CO<u>Me</u>), 21.09 (q, CO<u>Me</u>), 21.22 (q, CO<u>Me</u>), 22.45 (q, C-26), 24.08 (t, C-15), 26.82 (d, C-25), 28.03 (t, C-16), 29.73 (d, C-5), 29.78 (t, C-4), 36.77 (s, C-10), 37.74 (d, C-8), 38.69 (d, C-20), 39.60 (t, C-12), 39.65 (2t, C-7, C-1), 41.98 (d, C-24), 42.51 (s, C-13), 53.06 (d, C-9), 53.89 (d, C-14), 55.85 (d, C-17), 69.35 (d, C-2), 69.78 (d, C-3), 70.72 (d, C-6), 74.76 (d, C-22), 77.56 (d, C-23), 170.36 (2s, 2<u>C</u>OMe), 170.60 (2s, 2<u>C</u>OMe).

***O*-[(22*R*,23*R*,24*R*)-2α,3α,22,23-Tetraacetoxy-24-methyl-5α-cholestan-6ξ-yl]-*S*-methyl Carbonodithionate (13).** A solution of **12** (143 mg, 0.21 mmol) in DMF (3 mL) was treated with CS_2 (200 μL, 3.3 mmol) and DBU (188 μL, 1.26 mmol), held at room temperature for 48 h, treated with MeI (225 μL, 3.61 mmol), stirred at room temperature for 30 min, treated with HCl solution (1 mL, 1 N) to decompose the excess of reagents, diluted with H_2O, and extracted with $CHCl_3$. The combined organic extracts were evaporated. The residue was separated over a column of silica gel (eluent petroleum ether:EtOAc, 7:1) to afford **13** (110 mg, 68%) as an oil.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.69 (3H, s, 18-Me), 0.81 (3H, d, J = 7.0, 28-Me), 0.85 (3H, d, J = 7.0, 27-Me), 0.91 (3H, d, J = 7.0, 26-Me), 0.98 (3H, d, J = 7.0, 21-Me), 1.10 (3H, s, 19-Me), 1.97 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), 2.04 (H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 2.55 (3H, s, S-Me), 4.94–5.01 (1H, m, H-23), 5.06 (1H, dd, J_1 = 7.20, J_2 = 4.9, H-22), 5.19 (0.06H, m, H_β-6), 5.23 (1H, d, J = 7.2, H-2), 5.33 (1H, d, J = 2.2, H-3), 5.79 (0.94H, d, J = 2.0, H_α-6).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 10.77 (q, C-28), 11.79 (q, C-18), 13.40 (q, C-21), 15.78 (q, C-19), 17.13 (q, C-27), 19.06 (q, S<u>Me</u>), 20.66 (t, C-11), 20.85 (q, CO<u>Me</u>), 20.91 (q, CO<u>Me</u>), 21.03 (q, CO<u>Me</u>), 21.15 (q, CO<u>Me</u>), 22.42 (q, C-26), 23.99 (t, C-15), 26.82 (d, C-25), 27.98 (t, C-16), 29.36 (t, C-4), 30.60 (d, C-5), 35.13 (t, C-7), 36.89 (s, C-10), 37.75 (d, C-8), 38.73 (d, C-20), 39.42 (t, C-12), 39.56 (t, C-1), 42.06 (s, C-13), 42.52 (d, C-24), 53.01 (d, C-9), 53.52 (d, C-14), 55.76 (d, C-17), 68.81 (d, C-2), 69.41 (d, C-3), 74.69 (d, C-22), 77.53 (d, C-23), 82.62 (d, C-6), 170.11 (s, <u>C</u>-OMe), 170.26 (s, <u>C</u>-OMe), 170.55 (2s, 2<u>C</u>-OMe), 216.12 (s, <u>C</u>=S).

Mass spectrum (*m/z*, I_{rel}, %): 665 $[M - AcOH]^+$ (22), 617 $[M - HOCS_2Me]^+$ (60), 557 $[M - HOCS_2Me - AcOH]^+$ (20), 497 $[M - HOCS_2Me - 2AcOH]^+$ (100), 437 $[M - HOCS_2Me - 3AcOH]^+$ (13), 377 $[M - HOCS_2M - 4AcOH]^+$ (18).

(22*R*,23*R*,24*R*)-2α,3α,22,23-Tetraacetoxy-24-methyl-5α-cholestane (14). A solution of xanthogenate **13** (104 mg, 0.13 mmol) in anhydrous C_6H_6 (4 mL) under Ar was treated with 2,2′-azobisisobutyronitrile (AIBN, 1 mg), heated to 100°C, treated under Ar with Bu_3SnH (130 μL, 0.49 mmol), and stirred for 1 h at 100°C. The mixture was evaporated to dryness and passed over a column of silica gel (eluent petroleum ether:EtOAc, 7:1) to afford **14** (87 mg, 97%) as an oil. IR spectrum (film, ν, cm^{-1}): 1725 br, 1250 br.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.65 (3H, s, 18-Me), 0.81 (3H, d, J = 7.0, 28-Me), 0.85 (6H, d, J = 6.2, 26-Me and 27-Me), 0.92 (3H, s, 19-Me), 0.99 (3H, d, J = 6.7, 21-Me), 1.97 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), 2.04 (1H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 4.92–4.98 (1H, m, H-23), 5.06 (1H, dd, J_1 = 7.3, J_2 = 4.9, H-22), 5.25 (2H, m, H-2 and H-3).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 10.79 (q, C-28), 11.73 (q, C-18), 12.48 (q, C-19), 13.38 (q, C-21), 17.18 (q, C-27), 20.87 (q, CO<u>Me</u>), 20.93 (q + t, CO<u>Me</u> + C-11), 21.12 (q, CO<u>Me</u>), 21.23 (q, CO<u>Me</u>), 22.46 (q, C-26), 24.05 (t, C-15), 26.83 (d, C-25), 27.32 (t, C-6), 28.07 (t, C-16), 31.61 (t, C-7), 32.31 (t, C-4), 34.75 (d, C-5), 36.94 (s, C-10), 37.74 (d, C-8),

38.05 (t, C-12), 38.71 (d, C-20), 39.32 (d, C-24), 39.77 (t, C-1), 42.46 (s, C-13), 53.07 (d, C-9), 54.04 (d, C-14), 56.19 (d, C-17), 69.32 (d, C-2), 70.03 (d, C-3), 74.76 (d, C-22), 77.57 (d, C-23), 170.40 (s, C-OMe), 170.45 (s, C-OMe), 170.56 (2s, 2C-OMe).

(22*R*,23*R*,24*R*)-2α,3α,22,23-Tetrahydroxy-24-methyl-5α-cholestane (15). A solution of NaOH (5%) in MeOH (5 mL) was treated with 6-deoxo-24-epicastasterone **14** (73 mg, 0.11 mmol), refluxed for 30 min, treated with H_2O (20 mL), and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4. The solvent was evaporated. The residue was separated over a column of silica gel (eluent $CHCl_3$:MeOH, 20:1) to afford **15** (45 mg, 80%), mp 214–216°C (EtOAc:hexane).

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.77 (3H, s, 18-Me), 0.87 (3H, s, 19-Me), 0.98 (3H, d, J = 6.9, 28-Me), 1.04 (3H, d, J = 6.8, 26-Me), 1.08 (3H, d, J = 6.8, 27-Me), 1.32 (3H, d, J = 6.6, 21-Me), 3.73 (1H, m, H-23), 4.09 (2H, m, H-2 and H-22), 4.38 (1H, m, H-3).

^{13}C NMR spectrum (C_5D_5N, δ, ppm): 11.27 (q, C-28), 12.06 (q, C-18), 12.65 (q, C-21), 13.15 (q, C-19), 17.41 (q, C-27), 21.18 (t, C-11), 22.36 (q, C-26), 24.36 (t, C-15), 27.13 (d, C-25), 28.16 (t, C-16), 28.35 (t, C-6), 32.25 (t, C-7), 35.00 (d, C-5), 35.49 (t, C-4), 37.00 (s, C-10), 38.58 (d, C-8), 40.28 (t, C-12), 41.24 (d, C-20), 41.80 (t, C-1), 42.04 (d, C-24), 42.58 (s, C-13), 53.39 (d, C-9), 54.55 (d, C-14), 56.56 (d, C-17), 68.97 (d, C-2), 69.69 (d, C-3), 72.31 (d, C-22), 76.18 (d, C-23).

Mass spectrum (*m/z*, I_{rel}, %): 415 $[M - 2H_2O]^+$ (100), 397 $[M - 3H_2O]^+$ (63).

(22*R*,23*R*,24*R*)-2α,3α,22,23-Tetra-(3′-indolylacetoxy)-24-methyl-5α-cholestane (16). The method described for **2** afforded from 6-deoxoepicastasterone **15** (40 mg, 0.089 mmol) tetraindolylacetoxy derivative **16** (66 mg, 69%) as an oil. UV spectrum (λ_{max}, nm) (ε): 220 (113,400), 280 (27,000). IR spectrum (film, ν, cm^{-1}): 3420, 1720, 1255.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.49 (3H, s, 18-Me), 0.74 (9H, m, 19-Me, 26-Me, 27-Me), 0.81 (3H, d, J = 6.8, 21-Me), 0.81 (3H, d, J = 6.9, 28-Me), 3.49 (2H, s, -CH_2-), 3.56 (2H, s, -CH_2-), 3.62 (2H, d, J = 5.8, -CH_2-), 3.72 (2H, s, -CH_2), 4.95 (1H, ddd, J_1 = 7.4, J_2 = 4.1, J_3 = 2.9, H-23), 5.16 (1H, dd, J_1 = 7.9, J_2 = 4.3, H-22), 5.26 (1H, br.d, J = 7.9, H-2), 5.3 (1H, m, H-3), 6.90 (1H, d, J = 2.2, H-5′), 6.94 (1H, d, J = 2.2, H-5′), 6.99 (1H, d, J = 2.2, H-5′), 7.04 (1H, d, J = 2.2, H-5′), 7.16 (8H, m, H-6′, 2′), 7.24–7.31 (4H, m, H-7′), 7.54 (1H, d, J = 7.9, H-4′), 7.57–7.67 (3H, m, H-4′), 8.03 (2H, s, NH), 8.06 (1H, c, NH), 8.10 (1H, c, NH).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 10.95 (q, C-28), 11.63 (q, C-18), 12.46 (q, C-19), 13.19 (q, C-21), 17.28 (q, C-27), 20.89 (t, C-11), 22.57 (q, C-26), 24.01 (t, C-15), 26.90 (d, C-25), 27.33 (t, C-6), 28.14 (t, C-16), 31.39 (2t, C-30), 31.50 (2t, C-30′), 31.79 (t, C-7), 32.32 (t, C-4), 34.64 (d, C-5), 36.86 (s, C-10), 37.90 (t, C-12), 38.13 (d, C-8), 38.91 (d, C-20), 39.24 (d, C-24), 39.34 (d, C-1), 42.21 (s, C-13), 52.54 (d, C-9), 53.63 (d, C-14), 55.72 (d, C-17), 70.04 (d, C-2), 70.79 (d, C-3), 75.11 (d, C-22), 77.72 (d, C-23), 108.30 (s, C-3′), 108.32 (s, C-3′), 108.74 (s, C-3′), 108.85 (s, C-3′), 111.28 (d, C-7′), 111.36 (3d, 3C-7′), 119.03 (2d, 2C-4′), 119.19 (2d, 2C-4′), 119.63 (d, C-6′), 119.74 (d, C-6′), 119.75 (d, C-6′), 119.81 (d, C-6′), 122.15 (d, C-5′), 122.22 (d, C-5′), 122.31 (2d, C-5′), 123.16 (d, C-2′), 123.36 (d, C-2′), 123.47 (d, C-2′), 123.54 (d, C-2′), 127.41 (4s, C-9′), 136.18 (3s, C-8′), 136.24 (s, C-8′), 171.64 (3s, C-29), 171.88 (s, C-29′).

Mass spectrum (*m/z*, I_{rel}, %): 1079 $[M + H]^+$ (100), 904 $[M - IAA]^+$ (50), 747 (16), 729 (15), 663 (27).

Study of Growth-stimulating Activity of the Synthesized Compounds. The biological activity of the synthesized compounds was tested under laboratory conditions on Karavai variety wheat plants by the published method [15]. Wheat grain was wetted in solutions of the IAA and BS derivatives at concentration 1×10^{-7} M for 1 d, placed in a row on filter paper (three repetitions, 20 each), and turned into a roll. The rolls were placed in pure water and grown first for 3 d in the dark and then in light. The length of the wheat-sprout stems and the sprout mass were measured on the fifth and eleventh day. Table 1 compares the average values with statistical significance at the 0.05 level.

REFERENCES

1. M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. Mandava, J. F. Worley, J. D. Warthen, G. L. Steffens, J. L. Flippen-Anderson, and J. C. Cook, *Nature*, **281**, 216 (1979).
2. V. A. Khripach, F. A. Lakhvich, and V. N. Zhabinskii, *Brassinosteroids* [in Russian], Navuka i Tekhnika, Minsk, 1993, 287 pp.
3. V. A. Khripach, V. N. Zhabinskii, and A. de Groot, *Brassinosteroids: A New Class of Plant Hormones*, Academic Press, San Diego, 1999, 456 pp.
4. A. Sakurai, T. Yokota, and S. Clouse, *Brassinosteroids: Steroidal Plant Hormones*, Springer, Berlin, 1999, 253 pp.

5. J.-S. Eun, S. Karaishi, and N. Sakurai, *Plant Cell. Physiol.*, **30**, 807 (1989).
6. F. Vandenbussche, P. Callebert, P. Zadnikova, E. Benkova, and D. V. D. Straeten, *Am. J. Bot.*, **100**, 215 (2013).
7. A. M. Aval′baev, M. V. Bezrukova, and F. M. Shakirova, *Dokl. Ross. Akad. Nauk*, **391**, 413 (2003).
8. J. H. Yopp, G. C. Colclasure, and N. Mandava, *Physiol. Plant.*, **46**, 247 (1979).
9. F. Bao, J. Shen, S. R. Brady, G. K. Muday, T. Asami, and Z. Yang, *Plant Physiol.*, **134**, 1624 (2004).
10. R. N. Arteca, D.-S. Tai, C. Shlagnhaufer, and N. B. Mandava, *Physiol. Plant.*, **59**, 539 (1983).
11. R. P. Litvinovskaya, M. E. Raiman, V. I. Anikeev, and V. A. Khripach, *Vestn. BRFFI*, 50 (2009).
12. *Catalog of Pesticides Allowed for Use in the Republic of Belarus* [in Russian], Minsk, 2000, p. 189.
13. *List of Pesticides and Agrochemicals Allowed for Use in the Russian Federation* [in Russian], Moscow, 2002, p. 287.
14. V. A. Khripach, V. N. Zhabinskii, O. V. Gulyakevich, and Yu. V. Ermolovich, *Khim. Prir. Soedin.*, 539 (2012).
15. O. A. Rozhanskaya, N. V. Yudina, O. I. Lomovskii, and V. G. Korolev, *Khim. Rastit. Syr′ya*, No. 3, 29 (2003).
16. RB Pat. 14,986 (2009); *Of. Byul.*, No. 3 (2011).