ISSN 1070-4280, Russian Journal of Organic Chemistry, 2006, Vol. 42, No. 9, pp. 1325–1332. © Pleiades Publishing, Inc., 2006.
Original Russian Text © R.P. Litvinovskaya, M.A. Aver'kova, A.V. Baranovskii, V.A. Khripach, 2006, published in Zhurnal Organicheskoi Khimii, 2006, Vol. 42, No. 9, pp. 1344–1350.

Synthesis of 6-Deoxo-24-epiteasterone and Its Analogs

R. P. Litvinovskaya, M. A. Aver'kova, A. V. Baranovskii, and V. A. Khripach

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus,
ul. Akademika Kuprevicha 5/2, Minsk, 220141 Belarus
e-mail: litvin@iboch.bas-net.by

Received July 12, 2005

Abstract—Selective reduction of the C^8=C^{14} double bond in 3-hydroxyergosta-8(14),22-dien-15-one, followed by *cis*-hydroxylation of the double bond in the side chain and reduction of the 15-oxo group gave new 3β-hydroxy-6-deoxobrassinosteroids, their 22*S*,23*S* isomers, and the corresponding esters. The side chain in the products is identical to that in such known natural brassinosteroids as 24-epibrassinolide and 24-epicastasterone.

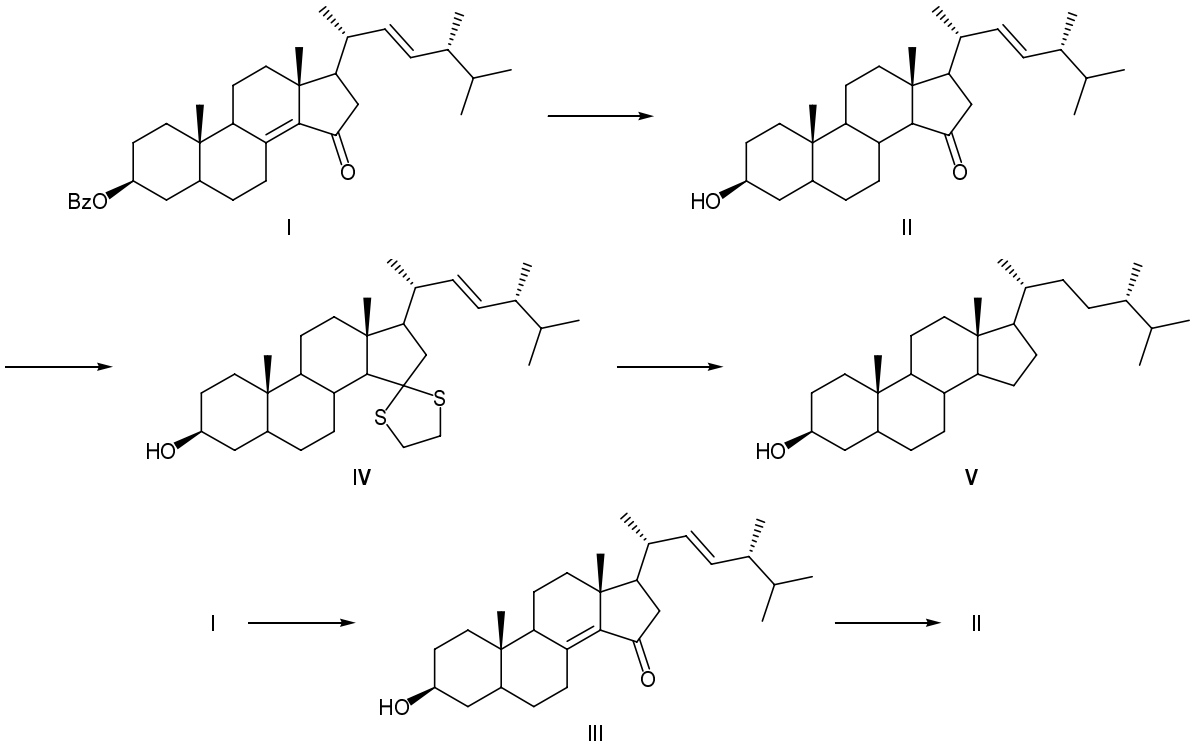
DOI: 10.1134/S1070428006090120

Among brassinosteroids isolated so far from natural sources, more then ten are 6-deoxo derivatives [1–5]. Even early studies on the structure–activity relationships in the series of brassinosteroids showed that the presence in a steroid molecule of 2α,3α,22*R*,23*R*-tetrahydroxy- and B-homo-7-oxa-6-oxo moieties is crucial for their biological activity. However, some 6-deoxo analogs [6], 2-deoxy derivatives [7], and those possessing a 3β-hydroxy group [8] were found to exhibit an appreciable activity. Two more things must be noted. First, extensive studies on isolation and structure determination of brassinosteroids lead to the discovery of new representatives of this class of compounds [9, 10], and second, various derivatives are necessary to elucidate mechanisms of their action and paths of biosynthesis and metabolism, as well as to establish the structure of new brassinosteroids. According to published data on the biosynthesis of brassinosteroids [11], campestanol is converted into castasterone through either so-called early C^6-oxidation or late C^6-oxidation (early C^{22}-oxidation). Study on the mechanism of the latter process required development of synthetic routes to 6-deoxocastasterone, 3-dehydrocastasterone, 6-deoxothyphasterol, 6-deoxoteasterone, and their labeled analogs [12].

Taking into account the above stated, we focused on the development of methods for the synthesis of new 6-deoxo analogs of brassinosteroids, specifically 3β-hydroxy-6-deoxo derivatives with a side chain typical of 24-epibrassinolide and 24-epicastasterone. By analogy with brassinolide and castasterone, such compounds may be precursors of 24β-methylbrassinosteroids in vivo. Up to now, the known procedures for the synthesis of 6-deoxobrassinosteroids are based on the hydrogenation of the C^5=C^6 double bond in Δ^5-derivatives in the presence of various catalysts [13] and on the reduction of the oxo group in 6-oxobrassinosteroids according to Wolff–Kishner [14] or desulfurization of the corresponding ethylenedisulfanyl derivatives [15].

In the present work we used as starting compound 3β-benzoyloxyergosta-8(14),22-dien-15-one (**I**) which was synthesized previously in four steps from ergosterol (for the synthesis of zymosterol) [16]. The synthetic sequence included selective reduction of the C^8=C^{14} double bond, *cis*-hydroxylation of the side-chain C^{22}=C^{23} bond, and removal of the carbonyl group. The most effective procedure for the reduction of conjugated double bond turned out to be treatment with metallic lithium in a mixture of liquid ammonia with tetrahydrofuran. The reaction was carried out at –30 to –40°C (30 min), and compound **II** was formed as the major product; however, the yield was poor (23%). When the reaction was performed with 3β-hydroxy derivative **III** prepared by hydrolysis of benzoate **I**, we obtained ketone **II** in 68% yield in 15 min (Scheme 1). Compound **II** showed in the IR spectrum an absorption band at 1730 cm^{-1}, which is typical of stretching vibrations of unconjugated carbonyl group. The ^{13}C NMR spectrum of **II** contained downfield signals at δ_C 133.3 and 134.25 ppm from the C^{23} and C^{22} atoms and a signal at δ_C 216.19 ppm from the carbonyl carbon atom.

Scheme 1.

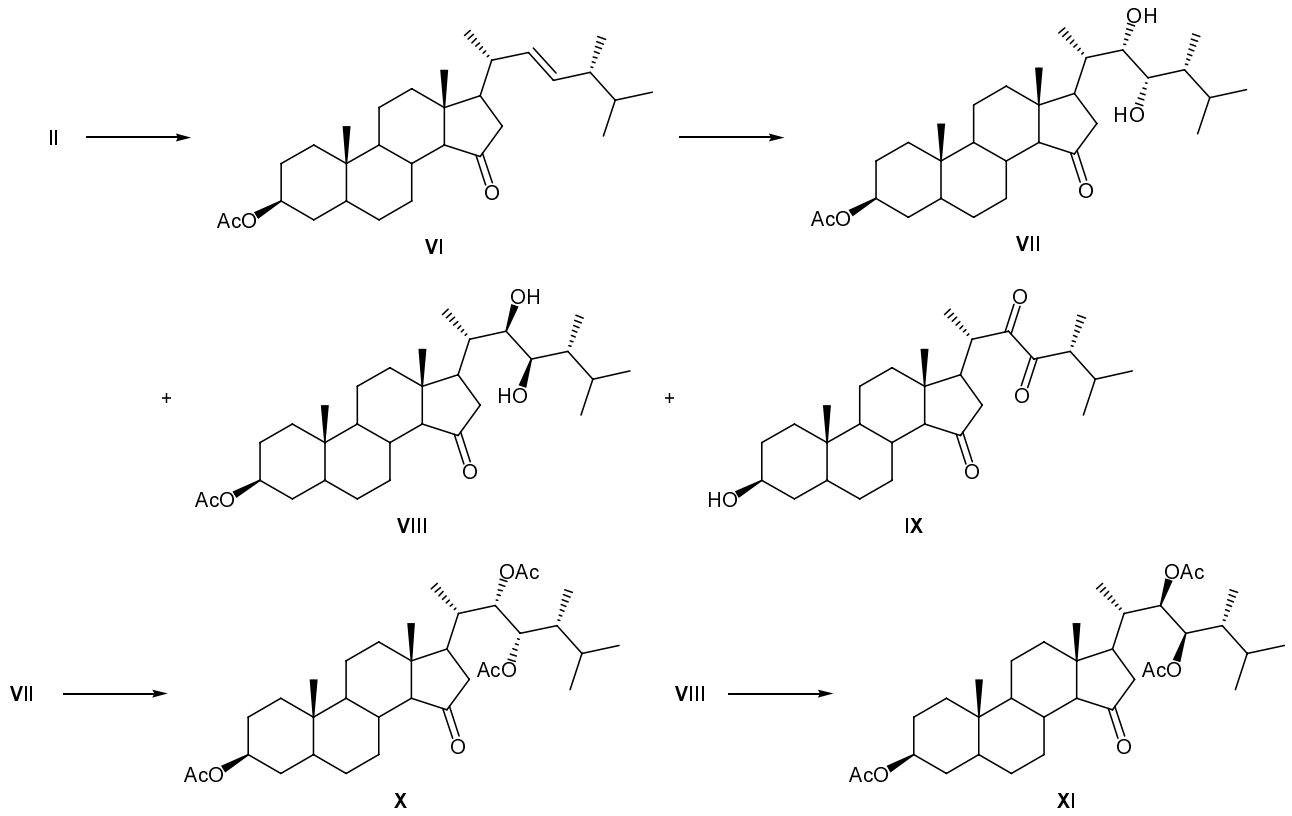


We failed to remove the carbonyl group from compound **II**. It remained unchanged under the Kishner–Wolff conditions [14] (heating in boiling diethylene glycol for 3 days), presumably due to strong shielding of the C^{15}=O carbonyl group, as was reported previously for 11-oxo steroids [17]. Therefore, we tried another procedure, desulfurization of ethylene dithioacetals [15]; for this purpose compound **II** was converted in a small yield (28%) into ethylene dithioacetal **IV**. Desulfurization of the latter over Raney nickel was accompanied by hydrogenation of the side-chain double bond (C^{22}=C^{23}) to give compound **V**. These results prompted us to change the scheme of synthesis so that appropriate initial modification of the side chain at the double bond excluded its further undesirable transformations.

We found that *cis*-hydroxylation of the C^{22}=C^{23} bond with osmium tetraoxide occurs in a specific fashion due to the presence of an oxo group in the 15-position. Direct hydroxylation of 3β-acetoxy derivative **VI** (prepared by acetylation of alcohol **II**) with an equimolar amount of OsO_4 in pyridine (24 h) gave a mixture of isomeric (22*S*,23*S*)- and (22*R*,23*R*)-diols **VII** and **VIII** at a ratio of ~2:1 (according to the NMR data) in an overall yield of 98% (Scheme 2). It was difficult to separate isomers **VII** and **VIII** by column chromatography on silica gel. Therefore, mixture **VII/VIII** was subjected to acetylation with acetic anhydride in pyridine in the presence of 4-dimethylaminopyridine; isomeric 3,22,23-triacetoxy derivatives **X** and **XI** thus obtained were readily separated by chromatography. The structure of compounds **X** and **XI** was confirmed by IR and NMR spectroscopy. In the IR spectra of both isomers we observed strong bands due to stretching vibrations of the ester groups (1745 and 1240 cm^{-1}). The ^1H NMR spectrum of the 22*R*,23*R* isomer contained two one-proton multiplets centered at δ 5.08 and 5.13 ppm, while the 22*S*,23*S* isomer displayed the corresponding signals at δ 4.98 and 5.19 ppm. The steric configuration of **X** and **XI** was assigned on the basis of published data [18, 19] and our results obtained while building up side chains of 24-epicastasterone and 24-epibrassinolide [20].

The hydroxylation of compound **VI** in the presence of chiral catalysts followed a different pattern. For instance, the reaction of **VI** with commercial AD-$mix_β$ [21] was very slow. After two weeks, 26% of (22*R*,23*R*)-diol **VIII** and 14% of 22,23-dioxo derivative **IX** were formed, the conversion of **VI** being 70%. No (22*S*,23*S*)-epimeric diol was detected among the products. Prolonged reaction favored formation of the more profound oxidation product, diketone **IX** (Scheme 2). The most effective procedure for the synthesis of the target diol with (22*R*,23*R*) configuration

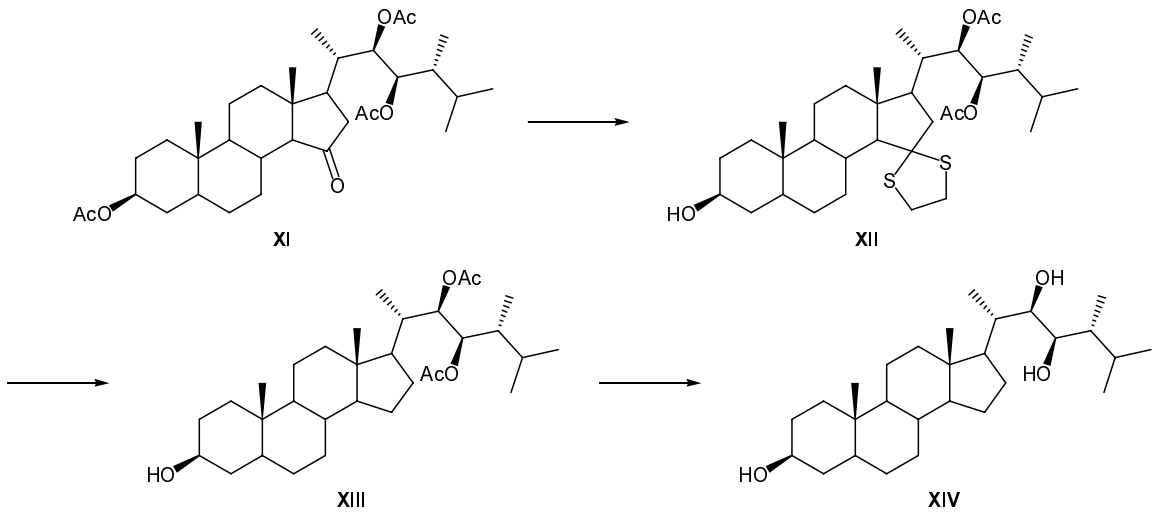
Scheme 2.



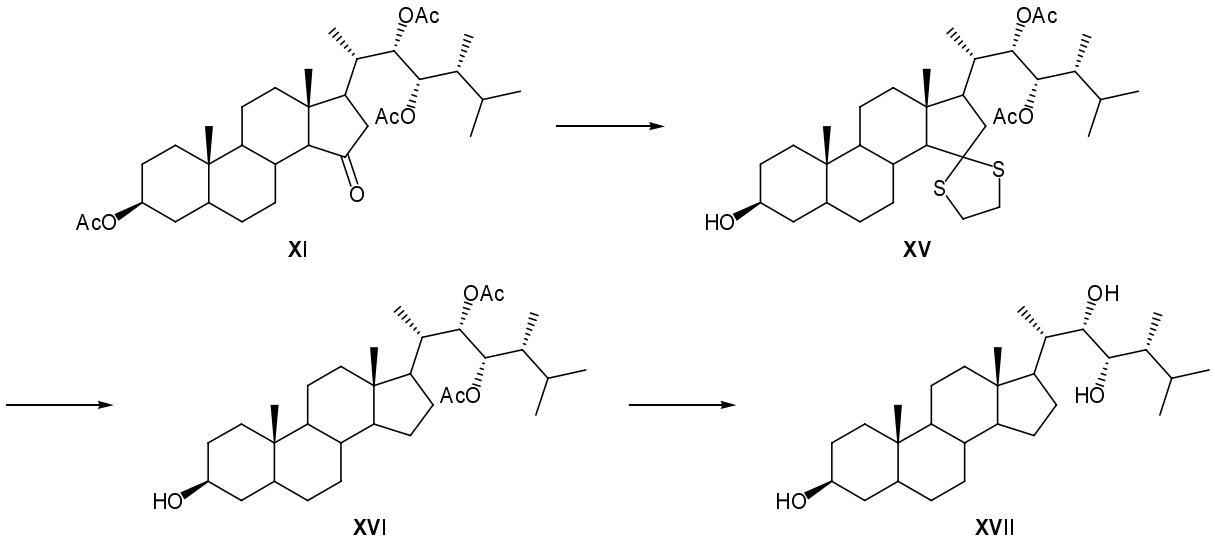
of the side chain, typical of brassinosteroids, was hydroxylation in aqueous *tert*-butyl alcohol with potassium osmate dihydrate in the presence of 10,11-dihydroquinidine *p*-chlorobenzoate, $K_3[Fe(CN)_6]$, and K_2CO_3 [22]. To avoid formation of by-products, the reaction mixture was treated after 3 days. We thus isolated 45% of (22*R*,23*R*)-diol **VIII**, the conversion of initial olefin **VI** being 59%.

The 15-oxo group was removed through the corresponding dithioacetals as described above for compound **II**. However, the application of this procedure to 22,23-diol **VIII** was unsuccessful. A complex mixture of products was formed even in the stage of synthesis of ethylene dithioacetal by treatment of **VIII** with ethane-1,2-dithiol in the presence of boron trifluoride–ether complex. A probable reason is the reaction at the

Scheme 3.



Scheme 4.



vicinal hydroxy groups; therefore, diols **VII** and **VIII** were converted into the corresponding 3,22,23-triacetates **X** and **XI**. The reaction of triacetoxy derivative **XI** with ethane-1,2-thiol in the presence of boron trifluoride–ether complex in a boiling methanol–chloroform mixture gave dithioacetal **XII** and was accompanied by unexpected selective deacetylation of the hydroxy group on C^3. Desulfurization of **XII** was effected by heating in boiling methanol over Raney nickel; the subsequent removal of protecting groups from 15-deoxo derivative **XIII** by the action of potassium hydroxide in methanol–methylene chloride gave 6-deoxo-24-epiteasterone (**XIV**) (Scheme 3). Following a similar scheme, from 3,22,23-triacetoxy-15-oxo derivative **X** we obtained (22*S*,23*S*) diastereoisomer **XVII** (Scheme 4).

Thus, starting from 3β-hydroxyergosta-8(14),22-dien-15-one, we have synthesized new 6-deoxobrassinosteroids: 6-deoxo-24-epiteasterone, its 22,23-epimer, and the corresponding 15-oxo derivatives. The side chain in the products is analogous to that present in such known natural brassinosteroids as 24-epibrassinolide and 24-epicastasterone. The prepared compounds may be useful both for studying biosynthesis of brassinosteroids and detecting them in natural sources and as potential biologically active substances.

EXPERIMENTAL

The IR spectra were recorded on a UR-20 spectrometer from samples prepared as films or KBr pellets. The ^1H and ^{13}C NMR spectra were obtained on Bruker A-200 (200 MHz) and Bruker Avance-500 instruments (500 MHz for ^1H and 125 MHz for ^{13}C) from solutions in $CDCl_3$ using TMS as internal reference. The progress of reactions was monitored by TLC on Kieselgel 60 F_{254} plates (Merck). Silica gel Kieselgel 60 (40–60 μm, Merck) was used for column chromatography. The melting points were determined on a Kofler melting point apparatus.

(22*E*)-3β-Hydroxyergost-22-en-15-one (II). Metallic lithium, 0.087 g (12.9 mmol), was added to 25 ml of liquid ammonia on cooling with liquid nitrogen–acetone, and the mixture was stirred until lithium dissolved completely. A solution of 0.303 g (0.586 mmol) of (22*E*)-15-oxoergosta-8(14),22-dien-3β-yl benzoate (**I**) [16] was added in one portion to the solution, the mixture was stirred for 10 min, and 1.358 ml (12.9 mmol) of bromobenzene was added. The mixture was then poured into a cold mixture of 50 ml of a saturated solution of ammonium chloride and 50 ml of ethyl acetate, the organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The extracts were combined with the organic phase, washed with two portions of water, and dried over $MgSO_4$, the solvent was removed, and the residue was separated by column chromatography on silica gel using cyclohexane–ethyl acetate (10:1) as eluent to isolate 0.058 g (23%) of 15-oxo steroid **II**, mp 183–184°C (from MeOH). IR spectrum (film), ν, cm^{-1}: 3450, 1730. ^1H NMR spectrum, δ, ppm: 0.75 s (3H, $C^{18}H_3$), 0.81 s (3H, $C^{19}H_3$), 0.81 d (6H, $C^{26}H_3$, $C^{27}H_3$), 0.89 d (3H, $C^{28}H_3$, J = 7 Hz), 1.07 d (3H, $C^{21}H_3$, J = 7 Hz), 2.29 d.d (1H, 16α-H, J_1 = 9, J_2 = 19 Hz), 2.65 m (1H, 7β-H, $J_{w/2}$ = 15 Hz), 3.58 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 5.13 m (2H, 22-H), 5.23 m (2H,

23-H). ^{13}C NMR spectrum, δ_C, ppm: 12.20 q (C^{19}), 13.21 q (C^{18}), 17.65 q (C^{28}), 19.62 q (C^{27}), 19.94 q (C^{26}), 20.76 d (C^{11}), 21.34 q (C^{21}), 28.23 t (C^{6}), 30.64 d (C^{7}), 31.40 d (C^{2}), 31.87 d (C^{28}), 32.96 d (C^{25}), 35.58 t (C^{10}), 36.95 t (C^{1}), 38.05 t (C^{4}), 39.73 d (C^{20}), 39.76 t (C^{12}), 42.22 s (C^{13}), 42.23 t (C^{16}), 42.85 d (C^{24}), 44.80 s (C^{5}), 51.26 d (C^{17}), 53.97 d (C^{9}), 65.97 d (C^{14}), 71.19 d (C^{3}), 133.30 d (C^{22}), 134.25 d (C^{23}), 216.19 s (C^{15}).

Following a similar procedure, from 0.364 g (0.882 mmol) of compound **III** we obtained 0.294 g (68.4%) of ketone **II**.

(22*E*)-3β-Hydroxyergosta-8(14),22-dien-15-one (III). A solution of 0.039 g (0.695 mmol) of potassium hydroxide in 0.5 ml of methanol containing 10% of water was added to a solution of 0.239 g (0.463 mmol) of 3β-benzoate **I** in 1 ml of benzene. The mixture was heated for 36 h under reflux, diluted with ethyl acetate, washed with a saturated solution of ammonium chloride, and dried over $MgSO_4$. The solvent was removed, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (10:1) as eluent to isolate 0.115 g (60%) of 3-hydroxy derivative **III**, mp 152–154°C (from MeOH). IR spectrum (film), ν, cm^{-1}: 3340, 1715, 1630, 1540, 1460. ^{1}H NMR spectrum, δ, ppm: 0.72 s (3H, $C^{18}H_3$), 0.82 d and 0.84 d (6H, $C^{26}H_3$, $C^{27}H_3$, J = 7 Hz), 0.92 d (3H, $C^{28}H_3$, J = 7 Hz), 1.00 s (3H, $C^{19}H_3$), 1.08 d (3H, $C^{21}H_3$, J = 7 Hz), 3.64 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 4.14 m (1H, 7β-H, $J_{w/2}$ = 15 Hz), 5.22 m (2H, 22-H, 23-H).

(22*E*)-15,15-Ethylenedisulfanylergost-22-en-3β-ol (IV). Ethane-1,2-dithiol, 0.029 ml (0.343 mmol), and boron trifluoride–ether complex, 0.012 ml (0.137 mmol), were added in one portion to a solution of 0.095 g (0.229 mmol) of 15-oxo steroid **II** in a mixture of 5 ml of methanol and 2 ml of chloroform. The mixture was stirred for 3 h at room temperature, an additional portion of ethane-1,2-dithiol, 0.029 ml (0.343 mmol), was added, and the mixture was stirred for 1 h and left overnight. The mixture was then heated for 12 h under reflux, 0.029 ml (0.343 mmol) of ethane-1,2-dithiol was added, the mixture was heated again for 12 h under reflux, 0.040 ml (0.498 mmol) of pyridine was added, and the mixture was stirred for 15 min, poured into a solution of sodium hydrogen carbonate, and extracted with chloroform. The extract was dried over $MgSO_4$ and evaporated, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (7:1) as eluent to isolate 0.064 g (67%) of initial ketone **II** and 0.032 g (28%) of dithioacetal **IV** as an oily substance. ^{1}H NMR spectrum, δ, ppm: 0.76 s (3H, $C^{18}H_3$), 0.77–0.94 m (12H, $C^{19}H_3$, $C^{26}H_3$, $C^{27}H_3$, $C^{28}H_3$), 1.02 d (3H, $C^{21}H_3$, J = 7 Hz), 3.05–3.37 m (4H, SCH_2), 3.60 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 5.12 m (2H, 22-H, 23-H).

Ergostan-3β-ol (V). Compound **IV**, 0.018 g (0.0366 mmol), was dissolved in 6.5 ml of methanol, a catalytic amount of an aqueous suspension of Raney nickel was added, and the mixture was heated under reflux in a stream of argon, additional portions of Raney nickel being added every hour. After 7 days, the catalyst was filtered off through a layer of silica gel, and the filtrate was evaporated to obtain 0.011 g (74%) of compound **V** as an oily material. IR spectrum (film), ν, cm^{-1}: 3340. ^{1}H NMR spectrum, δ, ppm: 0.64 s (3H, $C^{18}H_3$), 0.74–0.94 m (15H, $C^{19}H_3$, $C^{21}H_3$, $C^{26}H_3$, $C^{27}H_3$, $C^{28}H_3$), 3.60 m (1H, 3-H, $J_{w/2}$ = 19 Hz).

(22*E*)-15-Oxoergost-22-en-3β-yl acetate (VI). Hydroxy steroid **II**, 0.136 g (0.328 mmol), was dissolved in 5 ml of anhydrous methylene chloride, and 0.046 ml (0.492 mmol) of acetic anhydride, 0.138 ml (0.985 mmol) of triethylamine, and 0.004 g (0.0328 mmol) of 4-dimethylaminopyridine were added to the solution. The mixture was stirred for 4 h, 0.2 ml of methanol was added, and the mixture was stirred for 30 min, washed with water, and extracted with ethyl acetate. The extract was dried over $MgSO_4$, the solvent was removed, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (9:1) as eluent to isolate 0.150 g (99%) of compound **VI**, mp 173–175°C (from MeOH). IR spectrum (film), ν, cm^{-1}: 1740, 1260. ^{1}H NMR spectrum, δ, ppm: 0.68 s (3H, $C^{18}H_3$), 0.76–0.8 m (9H, $C^{19}H_3$, $C^{27}H_3$, $C^{28}H_3$), 0.84 d (3H, $C^{26}H_3$, J = 7 Hz), 1.01 d (3H, $C^{21}H_3$, J = 7 Hz), 1.96 s (3H, OAc), 2.24 d.d (1H, 16-H, J_1 = 9, J_2 = 17 Hz), 2.59 m (1H, 7β-H, $J_{w/2}$ = 15 Hz), 4.61 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 5.12 m (2H, 22-H, 23-H).

Hydroxylation of the C^{22}=C^{23} bond. *a.* Osmium tetraoxide, 0.047 g (0.188 mmol), was added to a solution of 0.086 g (0.188 mmol) of compound **VI** in 5 ml of pyridine. The mixture was stirred for 24 h at room temperature, 3 ml of a 10% solution of Na_2SO_3 and 0.1 ml of concentrated sulfuric acid were added, and the mixture was stirred for 30 min on heating on a water bath (40–50°C). The mixture was cooled, diluted with water, and extracted with chloroform, the extract was dried over $MgSO_4$, the solvent was removed, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (2:1) as eluent to isolate 0.090 g (98%) of

a mixture of diols **VII** and **VIII** as an oily material. IR spectrum (film), ν, cm^{-1}: 3500, 1740, 1720 sh, 1260. ^1H NMR spectrum, δ, ppm: 0.76–0.79 m (12H, $C^{18}H_3$, $C^{19}H_3$, $C^{27}H_3$, $C^{28}H_3$), 0.86 d (3H, $C^{26}H_3$, J = 7 Hz), 1.08 d (3H, $C^{21}H_3$, J = 7 Hz), 2.04 s (3H, OAc) 2.36 m (1H, 16-H), 2.66 m (1H, 7β-H, $J_{w/2}$ = 14 Hz), 3.42 t (1H, 22*R*-H, J = 6 Hz), 3.5 m (1H, 22*S*-H, $J_{w/2}$ = 7 Hz), 3.58 m (1H, 23*R*-H, $J_{w/2}$ = 8 Hz), 3.7 m (1H, 23*S*-H, $J_{w/2}$ = 11 Hz), 4.68 m (1H, 3-H, $J_{w/2}$ = 20 Hz).

b. Compound **VI**, 0.307 g (0.685 mmol), was dissolved in a mixture of *tert*-butyl alcohol and water, 1.644 g of ADmix-β and 0.123 g of methanesulfonamide were added, the mixture was stirred for 2 weeks, a saturated aolution of Na_2SO_3 was added, and the mixture was stirred for 1 h, diluted with water, and extracted with ethyl acetate. The extract was dried over $MgSO_4$, the solvent was removed, and the residue was separated by column chromatography on silica gel using cyclohexane–ethyl acetate (5:1) as eluent to isolate (in the order of elution) 0.091 g (30%) of initial olefin **VI**, 0.047 g (14%) of triketone (**IX**), and 0.087 g (26%) of diol **VIII**.

(22*R*,23*R*)-22,23-Dihydroxy-15-oxoergostan-3β-yl acetate (VIII). mp 204–206°C (from hexane). IR spectrum (film), ν, cm^{-1}: 3450, 1740, 1260. ^1H NMR spectrum, δ, ppm: 0.75 s (3H, $C^{18}H_3$), 0.82 s (3H, $C^{19}H_3$), 0.83 d (3H, $C^{28}H_3$, J = 7 Hz), 0.86 d (3H, $C^{27}H_3$, J = 7 Hz), 0.91 d (3H, $C^{26}H_3$, J = 7 Hz), 1.03 d (3H, $C^{21}H_3$, J = 6 Hz), 2.02 s (3H, OAc) 2.58 d.d (1H, 16α-H, J_1 = 9, J_2 = 17 Hz), 2.66 d.d (1H, 7β-H, J_1 = 3.5, J_2 = 13.1 Hz), 3.42 m (1H, 23-H, $J_{w/2}$ = 7 Hz), 3.58 t (1H, 22-H, J = 6 Hz), 4.68 m (1H, 3-H, $J_{w/2}$ = 20 Hz). ^{13}C NMR spectrum, δ_C, ppm: 10.92 q (C^{28}), 12.25 q (C^{19}), 12.97 q (C^{18}), 13.13 q (C^{21}), 17.33 q (C^{27}), 21.60 q ($COCH_3$), 22.21 q (C^{26}), 20.90 t (C^{11}), 27.20 d (C^{25}), 27.54 t (C^2), 28.25 t (C^6), 30.67 t (C^7), 32.02 d (C^8), 33.98 t (C^4), 35.71 s (C^{10}), 36.82 t (C^1), 40.27 d (C^{20}), 39.99 t (C^{12}), 41.58 d (C^{24}), 41.79 t (C^{16}), 42.36 s (C^{13}), 44.71 d (C^5), 48.21 d (C^{17}), 53.84 d (C^9), 65.89 d (C^{14}), 72.55 d (C^{22}), 73.67 d (C^3), 76.32 d (C^{23}), 170.85 s ($COCH_3$), 215.69 s (C^{15}).

15,22,23-Trioxoergostan-3β-yl acetate (IX). mp 129–131°C (from hexane). IR spectrum (film), ν, cm^{-1}: 1740, 1720 sh, 1255. ^1H NMR spectrum, δ, ppm: 0.80 s (3H, $C^{18}H_3$), 0.84 d (3H, $C^{28}H_3$, J = 7 Hz), 0.85 s (3H, $C^{19}H_3$), 0.93 d and 1.00 d (6H, $C^{26}H_3$, $C^{27}H_3$, J = 7 Hz), 1.14 d (3H, $C^{21}H_3$, J = 7 Hz), 2.02 s (3H, CH_3CO), 3.26 m and 3.20 m (2H, 20-H, 24-H), 4.68 m (1H, 3-H).

c. Compound **VI**, 0.099 g (0.217 mmol), was dissolved in a mixture of 5 ml of *tert*-butyl alcohol and 5 ml of distilled water, and 0.0016 g (0.00434 mmol) of $K_2OsO_4 \cdot 2H_2O$, 0.004 g (0.009 mmol) of 10,11-dihydroquinidine *p*-chlorobenzoate, 0.214 g (0.651 mmol) of $K_3[Fe(CN)_6]$, 0.090 g (0.651 mmol) of K_2CO_3, and 0.062 g (0.651 mmol) of methansulfonamide were added. The mixture was stirred for 72 h, a saturated solution of Na_2SO_3 was added, and the mixture was stirred for 1 h, diluted with water, and extracted with ethyl acetate. The extract was dried over $MgSO_4$, the solvent was removed, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (5:1) as eluent to isolate (in the order of elution) 0.041 g (41%) of initial olefin **VI** and 0.048 g (45%) of (22*R*,23*R*)-diol **VIII**.

(22*R*,23*R*)-15-Oxoergostane-3β,22,23-triyl triacetate (XI). 22,23-Dihydroxy steroid **VIII**, 0.055 g (0.112 mmol), was dissolved in 1.5 ml of anhydrous pyridine, 0.105 ml (1.12 mmol) of acetic anhydride, and a catalytic amount of 4-dimethylaminopyridine were added, and the mixture was stirred for 24 h at room temperature. Methanol, 0.5 ml, was added, and the mixture was stirred for 30 min, washed with a solution of $NaHCO_3$, and extracted with ethylacetate. The extract was dried over $MgSO_4$, the solvent was removed, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (9:1) as eluent. Yield 0.060 g (93%), mp 180–182°C (from MeOH). IR spectrum (film), ν, cm^{-1}: 1745, 1540, 1240. ^1H NMR spectrum, δ, ppm: 0.73 s (3H, $C^{18}H_3$), 0.81 d (3H, $C^{28}H_3$, J = 6 Hz), 0.82 s (3H, $C^{19}H_3$), 0.84 d (3H, $C^{27}H_3$, J = 7 Hz), 0.91 d (3H, $C^{26}H_3$, J = 7 Hz), 1.05 d (3H, $C^{21}H_3$, J = 6 Hz), 2.02 s (3H, OAc), 2.03 s (3H, OAc), 2.07 s (3H, OAc), 2.72 m (1H, 16α-H), 2.64 m (1H, 7β-H), 4.67 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 5.08 m (2H, 23-H, $J_{w/2}$ = 7 Hz), 5.13 m (2H, 22-H, $J_{w/2}$ = 7 Hz). ^{13}C NMR spectrum, δ_C, ppm: 10.85 q (C^{28}), 12.25 q (C^{19}), 12.76 q (C^{18}), 13.99 q (C^{21}), 17.19 q (C^{27}), 20.87 t (C^{11}), 20.96 q ($CO\mathbf{C}H_3$), 21.06 q ($CO\mathbf{C}H_3$), 21.59 q ($CO\mathbf{C}H_3$), 22.55 q (C^{26}), 27.10 d (C^{25}), 27.54 t (C^2), 28.20 t (C^6), 30.60 t (C^7), 31.99 d (C^8), 33.96 t (C^4), 35.68 s (C^{10}), 36.85 t (C^1), 37.83 d (C^{20}), 39.97 t (C^{12}), 38.92 d (C^{24}), 41.97 t (C^{16}), 42.20 s (C^{13}), 44.74 d (C^5), 48.73 d (C^{17}), 53.84 d (C^9), 65.89 d (C^{14}), 74.46 d (C^{22}), 73.63 d (C^3), 77.25 d (C^{23}), 170.66 s ($\mathbf{C}OCH_3$), 170.79 s ($\mathbf{C}OCH_3$), 170.84 s ($\mathbf{C}OCH_3$), 214.82 s (C^{15}).

Following a similar procedure, from 0.057 g (0.116 mmol) of a mixture of (22*S*,23*S*)-diol **VII** and (22*R*,23*R*)-diol **VIII** we obtained 0.016 g (23%) of (3β,22*S*,23*S*)-triacetate **X** and 0.008 g (11.5%) of (3β,22*R*,23*R*)-triacetate **XI**.

(22*S*,23*S*)-15-Oxoergostane-3β,22,23-triyl triacetate (X). mp 127–128°C (from MeOH). IR spectrum (film), ν, cm^{-1}: 1745, 1540, 1240. ^1H NMR spectrum, δ, ppm: 0.72 s (3H, $C^{18}H_3$), 0.76 d (3H, $C^{28}H_3$, J = 7 Hz), 0.81 s (3H, $C^{19}H_3$), 0.82 d (3H, $C^{27}H_3$, J = 7 Hz), 0.90 d (3H, $C^{26}H_3$, J = 7 Hz), 1.01 d (3H, $C^{21}H_3$, J = 7 Hz), 2.02 s (3H, OAc), 2.08 s (3H, OAc), 2.10 s (3H, OAc), 2.42 d.d (1H, 16α-H, J_1 = 7, J_2 = 17 Hz), 2.65 m (1H, 7β-H, $J_{w/2}$ = 15 Hz), 4.68 sept (1H, 3-H, J = 5.2 Hz), 4.98 d.d (1H, 22-H, J_1 = 2.9, J_2 = 4.2 Hz), 5.19 d.d (1H, 23-H, J_1 = 2.9, J_2 = 7.4 Hz). ^{13}C NMR spectrum, δ_C, ppm: 9.86 q (C^{28}), 12.22 q (C^{19}), 12.88 q (C^{18}), 14.99 q (C^{21}), 17.15 q (C^{27}), 20.83 t (C^{11}), 21.20 q ($CO\mathbf{C}H_3$), 21.52 q ($CO\mathbf{C}H_3$), 21.59 q ($CO\mathbf{C}H_3$), 21.78 q (C^{26}), 28.67 d (C^{25}), 27.53 t (C^2), 28.22 t (C^6), 30.55 t (C^7), 32.01 d (C^8), 33.97 t (C^4), 35.67 s (C^{10}), 36.83 t (C^1), 38.63 d (C^{20}), 39.77 t (C^{12}), 40.36 d (C^{24}), 41.41 t (C^{16}), 42.75 s (C^{13}), 44.73 d (C^5), 47.47 d (C^{17}), 53.74 d (C^9), 65.26 d (C^{14}), 73.85 d (C^{22}), 73.64 d (C^3), 72.85 d (C^{23}), 170.47 s ($\mathbf{C}OCH_3$), 170.83 s ($\mathbf{C}OCH_3$), 170.88 s ($\mathbf{C}OCH_3$), 214.67 s (C^{15}).

(22*R*,23*R*)-15,15-Ethylenedisulfanyl-3β-hydroxyergostane-22,23-diyl diacetate (XII). Following the procedure described above for the synthesis of compound **IV**, from 0.052 g (0.090 mmol) of 15-oxo steroid **XI** we obtained 0.050 g (93%) of dithioacetal **XII** as an oily substance. IR spectrum (film), ν, cm^{-1}: 3420, 1740, 1250. ^1H NMR spectrum, δ, ppm: 0.72 d (3H, $C^{28}H_3$, J = 7 Hz), 0.77 s (3H, $C^{18}H_3$), 0.78 d and 0.81 d (6H, $C^{26}H_3$, $C^{27}H_3$, J = 6 Hz), 0.82 s (3H, $C^{19}H_3$), 0.94 d (3H, $C^{21}H_3$, J = 6 Hz), 1.97 s (3H, OAc), 1.98 s (3H, OAc), 2.96 d.d (1H, 16-H, J_1 = 9, J_2 = 19 Hz), 3.10–3.37 m (4H, SCH_2), 3.53 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 5.00 m (2H, 22-H, 23-H).

(22*R*,23*R*)-3β-Hydroxyergostane-22,23-diyl diacetate (XIII). Following the procedure described above for the synthesis of compound **V**, from 0.042 g (0.0197 mmol) of dithioacetal **XII** we obtained 0.010 g (97%) of compound **XIII** as an oily substance. ^1H NMR spectrum, δ, ppm: 0.78 s (3H, $C^{18}H_3$), 0.88–0.92 m (9H, $C^{19}H_3$, $C^{27}H_3$, $C^{28}H_3$), 0.94 d (3H, $C^{26}H_3$, J = 7 Hz), 0.98 d (3H, $C^{21}H_3$, J = 7 Hz), 2.02 s (3H, OAc), 2.06 s (3H, OAc), 3.58 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 5.06 d.d (1H, 23-H, J_1 = 5, J_2 = 7 Hz), 5.24 m (1H, 22-H, $J_{w/2}$ = 7 Hz).

(22*R*,23*R*)-Ergostane-3β,22,23-triol (6-deoxo-24-epiteasterone) (XIV). Potassium hydroxide, 0.019 g (0.35 mmol), was added to a solution of 0.045 g (0.0872 mmol) of 22,23-diacetate **XIII** in a mixture of 0.7 ml of methanol and 0.5 ml of methylene chloride. The mixture was stirred for 72 h, 3 ml of ethyl acetate was added, and the mixture was stirred for 1 h, diluted with ethyl acetate, washed with a saturated solution of NH_4Cl, and dried over $MgSO_4$. The solvent was removed, and the residue was subjected to column chromatography on silica gel using cyclohexane–ethyl acetate (2:1) as eluent to isolate 0.011 g (30%) of triol **XIV**, mp 243–245°C (from hexane). IR spectrum (KBr): ν 3450 cm^{-1}. ^1H NMR spectrum (pyridine), δ, ppm: 0.74 s (3H, $C^{18}H_3$), 0.79 s (3H, $C^{19}H_3$), 0.94 d (3H, $C^{26}H_3$, J = 7 Hz), 1.00 d (3H, $C^{28}H_3$, J = 7 Hz), 1.03 d (3H, $C^{27}H_3$, J = 7 Hz), 1.30 d (3H, $C^{21}H_3$, J = 6.4 Hz), 3.70 t (1H, 23-H, J = 5.1 Hz), 3.83 sept (1H, 3-H, J = 4.8 Hz), 4.04 d (1H, 22-H, J = 3.2 Hz). ^{13}C NMR spectrum (pyridine), δ_C, ppm: 11.46 q (C^{28}), 12.23 q (C^{18}), 12.56 q (C^{19}), 13.35 q (C^{21}), 17.60 q (C^{27}), 21.62 t (C^{11}), 22.54 q (C^{26}), 24.57 t (C^{15}), 27.31 d (C^{25}), 28.55 t (C^{16}), 29.21 t (C^6), 32.41 t (C^7), 32.56 t (C^2), 35.82 d (C^8), 35.80 s (C^{10}), 37.52 t (C^1), 39.38 t (C^4), 40.55 t (C^{12}), 41.41 d (C^{20}), 42.21 d (C^{24}), 42.76 s (C^{13}), 45.20 d (C^5), 53.36 d (C^{17}), 54.63 d (C^9), 56.82 d (C^{14}), 70.65 d (C^3), 72.53 d (C^{22}), 76.36 d (C^{23}).

(22*S*,23*S*)-15,15-Ethylenedisulfanyl-3β-hydroxyergostane-22,23-diyl diacetate (XV). Following the procedure described above for the synthesis of compound **IV**, from 0.020 g (0.034 mmol) of 15-oxo steroid **X** we obtained 0.012 g (58%) of dithioacetal **XV** as an oily substance. ^1H NMR spectrum, δ, ppm: 0.82 s (3H, $C^{18}H_3$); 0.84–0.94 m (9H, $C^{19}H_3$, $C^{27}H_3$, $C^{28}H_3$); 0.94 d (3H, $C^{26}H_3$, J = 7 Hz); 0.98 d (3H, $C^{21}H_3$, J = 7 Hz); 2.06 s (3H, OAc); 2.08 s (3H, OAc); 2.78 d.d (1H, 16-H, J_1 = 7, J_2 = 15 Hz); 3.08 m, 3.24 m, and 3.42 m (4H, SCH_2), 3.6 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 4.86 t (1H, 22-H, J = 4 Hz), 5.22 d.d (1H, 23-H, J_1 = 3, J_2 = 6 Hz).

(22*S*,23*S*)-3β-Hydroxyergostane-22,23-diyl diacetate (XVI). Following the procedure described above for the synthesis of compound **V**, from 0.012 g (0.0197 mmol) of compound **XV** we obtained 0.010 g (97%) of diacetate **XVI** as an oily substance. ^1H NMR spectrum (pyridine), δ, ppm: 0.62 s (3H, $C^{18}H_3$), 0.78–0.84 m (15H, $C^{19}H_3$, $C^{21}H_3$, $C^{26}H_3$, $C^{27}H_3$, $C^{28}H_3$), 2.04 s (3H, OAc), 2.06 s (3H, OAc), 3.6 m (1H, 3-H, $J_{w/2}$ = 19 Hz), 5.12 m (1H, 22-H), 5.24 m (1H, 23-H). ^{13}C NMR spectrum (pyridine), δ_C, ppm: 10.69 q (C^{28}), 12.21 q (C^{18}), 12.52 q (C^{19}), 14.82 q (C^{21}), 19.31 q (C^{27}), 21.56 t (C^{11}), 21.88 q (C^{26}), 28.62 t (C^{16}), 29.18 t (C^6), 30.48 d (C^{25}), 32.38 t (C^7), 32.54 t (C^2), 35.74 d (C^8), 35.79 s (C^{10}), 37.53 t (C^1), 39.35 t (C^4), 40.7 d, 41.5 d, 42.2 s, 43.4 t, 46.1 t, 50.0 d, 52.3 d, 56.9 d, 60.4 d, 70.5 d, 122.5 d, 140.8 s, 212.8 s.

(22*S*,23*S*)-Ergostane-3β,22,23-triol (XVII). Following the procedure described above for the synthesis

of compound **XIV**, from 0.010 g (0.019 mmol) of 22,23-diacetate **XVI** we obtained 0.005 g (61%) of triol **XVII**, mp 149–151°C (from hexane). ^{1}H NMR spectrum, δ, ppm: 0.67 s (3H, $C^{18}H_3$), 0.79 s (3H, $C^{19}H_3$), 0.98 d (3H, $C^{27}H_3$, J = 6.7 Hz), 1.05 d (3H, $C^{26}H_3$, J = 6.7 Hz), 1.19 d (3H, $C^{28}H_3$, J = 6.7 Hz), 1.34 d (3H, $C^{21}H_3$, J = 6.7 Hz), 3.84 sept (1H, 3-H, J = 5 Hz), 3.94 t (1H, 22-H, J = 3.05 Hz), 4.08 d.d (1H, 23-H, J_1 = 2.72, J_2 = 4.33 Hz). ^{13}C NMR spectrum (pyridine), δ_C, ppm: 10.69 q (C^{28}), 12.21 q (C^{18}), 12.52 q (C^{19}), 14.82 q (C^{21}), 19.31 q (C^{27}), 21.56 t (C^{11}), 21.88 q (C^{26}), 24.79 t (C^{15}), 28.62 t (C^{16}), 29.18 t (C^6), 30.48 d (C^{25}), 32.38 t (C^7), 32.54 t (C^2), 35.74 d (C^8), 35.79 s (C^{10}), 37.53 t (C^1), 39.35 t (C^4), 40.35 t (C^{12}), 42.92 d (C^{20}), 43.38 s (C^{13}), 44.97 d (C^{24}), 45.22 d (C^5), 53.27 d (C^{17}), 54.56 d (C^9), 56.42 d (C^{14}), 69.95 d (C^{23}), 70.62 d (C^3), 73.20 d (C^{22}).

REFERENCES

1. Yokota, T., Morita, M., and Takahashi, N., *Agric. Biol. Chem*., 1983, vol. 47, p. 2149.
2. Yokota, T., Koba, S., Kim, S.K., and Takatsuto, S., *Agric. Biol. Chem*., 1987, vol. 51, p. 1625.
3. Kim, S.K., *Brassinosteroids: Chemistry, Bioactivity, and Applications*, Cutler, H.G., Yokota, T., and Adam, G., Eds., Washington, DC: Am. Chem. Soc., 1991, ACS Symp. Series, vol. 474, p. 26.
4. Spengler, B., Schmidt, J., Voigt, B., and Adam, G., *Phytochemistry*, 1995, vol. 40, p. 907.
5. Griffits, P.G., Sasse, J.M., Yokota, T., and Cameron, D.W., *Biosci. Biotech. Biochem*., 1995, vol. 59, p. 956.
6. Kishi, T., Wada, K., Marumo, S., and Mori, K., *Agric. Biol. Chem*., 1986, vol. 50, p. 1821.
7. Takatsuto, S. and Ikekawa, N., *J. Chem. Soc. Perkin Trans. 1*, 1984, p. 439.
8. Wada, K. and Marumo, S., *Agric. Biol. Chem*., 1981, vol. 45, p. 2579.
9. Khripach, V.A., Zhabinskii, V.N., and de Groot, A.E., *Brassinosteroids: A New Class of Plant Hormones*, San Diego: Academic, 1999.
10. *Brassinosteroids: Steroidal Plant Hormones*, Sakurai, A., Yokota, T., and Clouse, S.D., Eds., Tokyo: Springer, 1999.
11. Fujioka, S. and Yokota, T., *Annu. Rev. Plant Biol*., 2003, vol. 54, p. 137.
12. Choi, Y.H., Fujioka, S., and Harada, A., *Phytochemistry*, 1996, vol. 43, p. 593.
13. Watanabe, B., Yamamoto, S., Sasaki, K., Nakagawa, Y., and Miyagawa, H., *Tetrahedron Lett*., 2004, vol. 45, p. 2767.
14. Watanabe, T., Noguchi, T., Yokota, T., Shibata, K., Koshino, H., Seto, H., Kim, S., and Takatsuto, S., *Phytochemistry*, 2001, vol. 58, p. 343.
15. Mori, K., Sakakibara, M., and Okada, K., *Tetrahedron*, 1984, vol. 40, p. 1767.
16. Baranovskii, A.V., Litvinovskaya, R.P., and Khripach, V.A., *Bioorg. Khim*., 2002, vol. 28, p. 277.
17. Huang-Minlon, *J. Am. Chem. Soc*., 1949, vol. 71, p. 3301.
18. Thompson, M.J., Mandava, N., Flippen-Anderson, J.L., and Worley, J.F., *J. Org. Chem*., 1979, vol. 44, p. 5002.
19. Anastasia, M., Ciuffreda, P., and Fiecchi, A., *J. Chem. Soc., Perkin Trans. 1*, 1983, p. 379.
20. Khripach, V.A., Zhabinskii, V.N., Zhernosek, E.V., Lakhvich, F.A., Ol'khovik, V.K., and Ivanova, G.V., *Vestsi Akad. Navuk BSSR, Ser. Khim. Navuk*, 1991, no. 2, p. 271.
21. Hellrung, B., Voigt, B., Schmidt, J., and Adam, G., *Steroids*, 1997, vol. 62, p. 415.
22. Takatsuto, S., Watanabe, T., Fujioka, S., and Sakurai, A., *J. Chem. Res., Synop*., 1997, p. 134.