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Synthesis of (22*R*,23*R*,24*S*)-24-Methyl-5α-cholestane-3β,6α,22,23-tetraol, a Biosynthetic Precursor of Brassinolide

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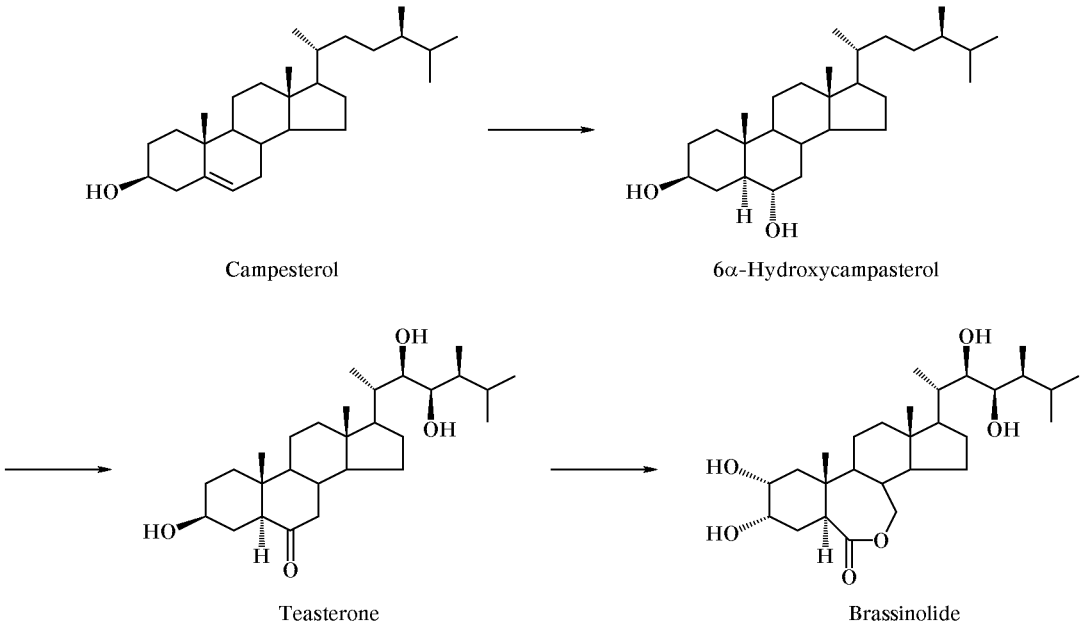
Abstract—A biosynthetic precursor of brassinolide, (22*R*,23*R*,24*S*)-24-methyl-3α-cholestane-3β,6α,22,23-tetraol was synthesized from Δ^{23}-22-keto steroid which was obtained from the corresponding 20-carbonitrile oxide. The side chain was built up by a series of successive transformations: hydride reduction of the initial enone, epoxidation of the allyl-like alcohol, and copper(I) cyanide-catalyzed opening of the 23,24-epoxy ring. The cyclic fragment was completed by opening of the cyclopropane ring with subsequent hydroboration and oxidation of the Δ^5-bond.

The most important advances in the field of brassinosteroids are the results of recent studies of biosynthesis of these compounds [1, 2]. Fujioka and Sakurai showed [3, 4] that there are at least two paths of biosynthetic transformations of campesterol into brassinolide in plants. One of these includes functionalization of the steroid **B** ring in the early stages [5, 6]; here, 6α-hydroxycampesterol is formed as intermediate (Scheme 1). An alternative path of brassinolide biosynthesis involves initial functionalization of the side chain (formation of the 22*R*,23*R*-diol moiety) [7, 8]. Thus the available data suggest that appropriate functional groups can be introduced independently in the course of biosynthesis of brassinolide. Therefore, different, hitherto unknown ways of biosynthesis of this compound are possible. The present work was aimed at synthesizing tetrahydroxy steroid **IX** which possesses already completed brassinolide side chain

Scheme 1.



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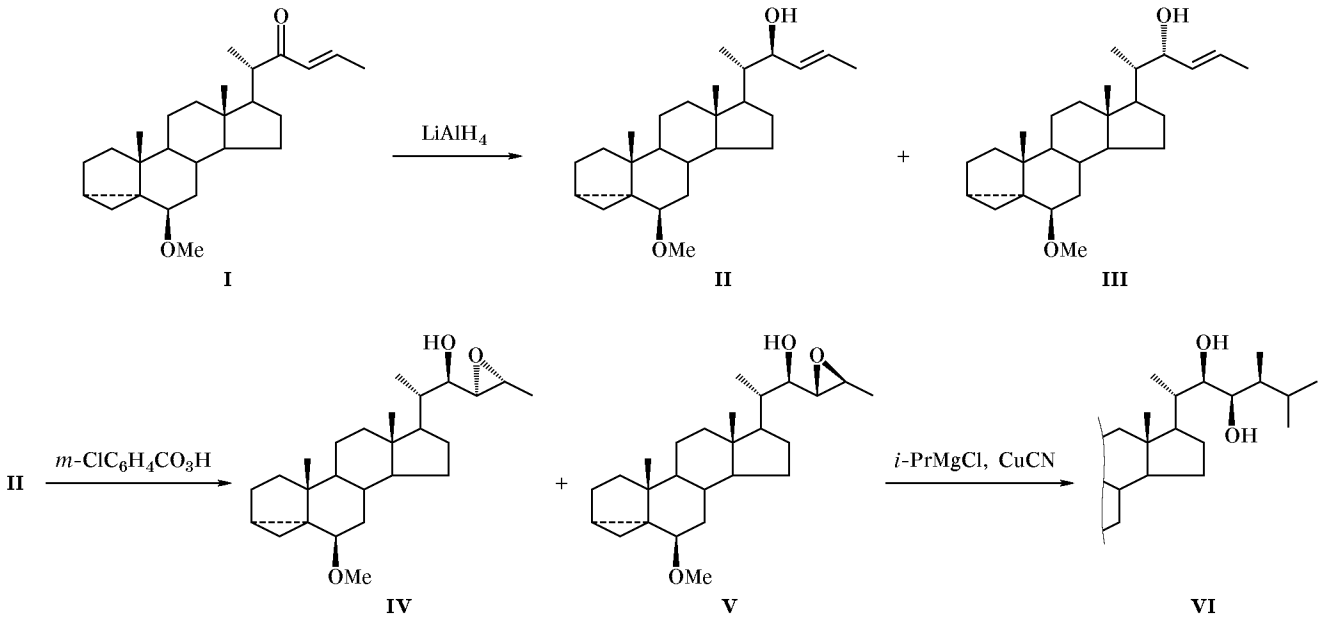
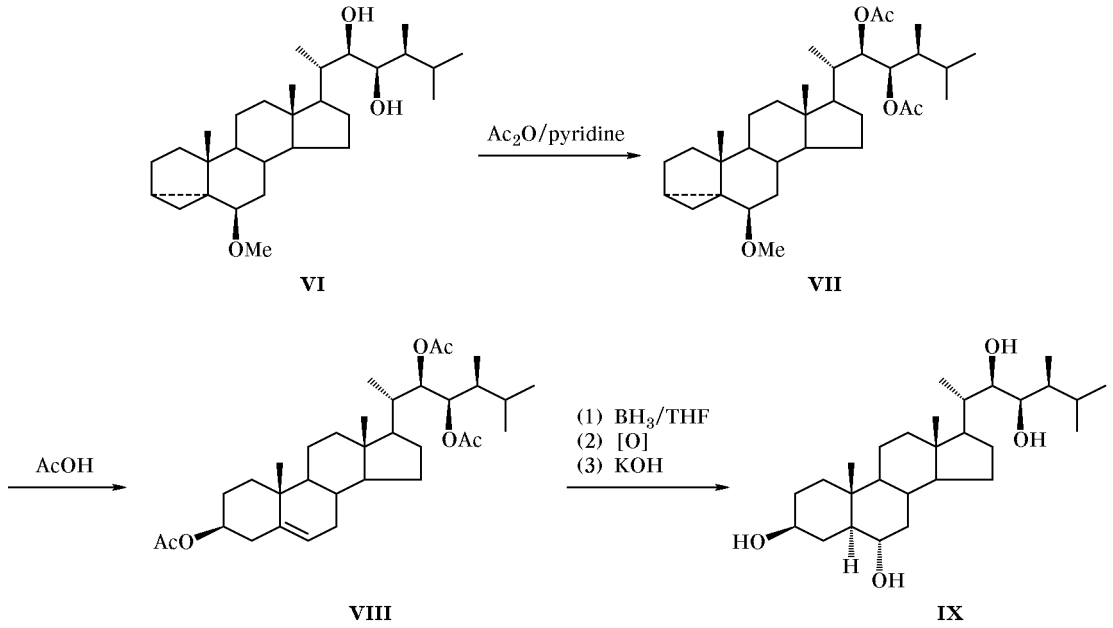
Scheme 2.



and 3β,6α-diol moiety in the polycyclic fragment and is considered [9, 10] to be a biosynthetic precursor of brassinolide. As starting compound we used enone **I** which was synthesized [11] from the corresponding 20-carbonitrile oxide. Related Δ^{23}-22-keto steroids are intermediate products in the synthesis of brassinosteroids [12–15]. The reduction of **I** with lithium aluminum hydride gave an equimolar mixture of epimeric allyl-like alcohols **II** and **III** whose ratio almost did not depend on the reaction temperature. Compounds **II** and **III** were separated by column chromatography on silica gel. Further building of the side chain was accomplished following the procedure developed by Back *et al.* [16–18]. Epoxidation of the double bond in **II** by the action of *m*-chloroperoxybenzoic acid in methylene chloride led to formation of an inseparable mixture of epoxy derivatives **IV** and **V**; the isomer ratio (6 : 4) was determined from

Scheme 3.



the 22-H signal intensities in the ^{1}H NMR spectrum, δ 3.88 and 3.63 ppm, respectively. The spectrum of isomer mixture **IV**/**V** also contained multiplet signals from 23-H at δ 2.76 and 2.65 ppm, doublets of doublets from 24-H at δ 3.14 and 2.91 ppm, and signals from the other fragments of the molecule.

To complete the brassinolide side chain, isomer mixture **IV**/**V** was treated with isopropylmagnesium chloride in the presence of copper(I) cyanide. The products were separated by column chromatography to isolate alcohol **II**, epoxy steroid **IV**, and diol **VI** formed by opening of the oxirane ring (Scheme 2).

Before proceeding with functionalization of the polycyclic fragment, the diol moiety in molecule **VI** was protected via its transformation into the corresponding diacetoxy derivative by treatment with acetic anhydride in pyridine. Subsequent heating of diacetate **VII** in boiling acetic acid was accompanied by formation of 3β-acetoxy group and Δ^5-bond. The ^{1}H NMR spectrum of **VIII** contained a singlet at δ 2.04 ppm from the 3β-acetoxy group, a doublet at δ 5.36 ppm from 6-H, and a multiplet at δ 4.50–4.68 ppm from 3-H. The 6α-hydroxy group was introduced by hydroboration followed by oxidation of the double bond in the **B** ring. Subsequent hydrolysis of the acetoxy groups gave the desired tetrahydroxy steroid **IX** (Scheme 3).

Thus we were the first to synthesize (22*R*,23*R*,24*S*)-24-methyl-5α-cholestane-3β,6α,22,23-tetraol (**IX**) which is a biosynthetic precursor of brassinolide.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Bruker AC-200 spectrometer (200 MHz) in $CDCl_3$ using tetramethylsilane as internal reference. The IR spectra (700–3600 cm^{-1}) were measured on a UR-20 instrument from samples prepared as thin films or KBr pellets. The progress of reactions was monitored by TLC on Kieselgel 60 F_{254} plates (Merck).

Reduction of enone I. Lithium aluminum hydride, 12 mg, was added at room temperature to 103 mg of enone **I** in 15 ml of tetrahydrofuran. When the reaction was complete, ammonium chloride was added, and the mixture was diluted with water and extracted with ethyl acetate. The extract was dried over sodium sulfate and evaporated, and the residue was subjected to column chromatography on silica gel. The column was eluted with cyclohexane–ethyl acetate mixtures (30:1, 20:1, and 10:1). Three fractions were collected. The first fraction contained 47 mg (45%) of (22*S*)-6β-methoxy-3α,5-cyclo-26,27-bisnor-5α-cholest-23-en-22-ol (**II**). IR spectrum, ν, cm^{-1}: 3600–3300, 3050–2800, 1465, 1385, 1330, 1280, 1210, 1190, 1145, 1105, 1025, 1010, 975, 935, 900, 870. ^{1}H NMR spectrum, δ, ppm: 0.69 s (3H, 18-Me), 0.87 d (3H, 21-Me, *J* = 6 Hz), 0.99 s (3H, 19-Me), 1.68 d (3H, 25-Me, *J* = 5 Hz), 2.76 m (1H, 6-H), 3.31 s (3H, OMe), 4.16 d (1H, 22-H, *J* = 4 Hz), 5.50–5.62 m (2H, 23-H, 24-H).

The second fraction contained 38 mg (36%) of (22*R*)-6β-methoxy-3α,5-cyclo-26,27-bisnor-5α-cholest-23-en-22-ol (**III**). ^{1}H NMR spectrum, δ, ppm: 0.74 s (3H, 18-Me), 0.94 d (3H, 21-Me, *J* = 6.5 Hz), 1.02 s (3H, 19-Me), 1.72 d (3H, 25-Me, *J* = 6.5 Hz), 2.77 m (1H, 6-H), 3.33 s (3H, OMe), 4.11 d.d (1H, 22-H), 5.42–5.76 m (2H, 23-H, 24-H).

Epoxidation of alcohol II. *m*-Chloroperoxybenzoic acid, 351 mg, was added at room temperature to 592 mg of hydroxy derivative **II** in 50 ml of CH_2Cl_2. After 30 min, the mixture was treated with a 25% solution of ammonia and extracted with methylene chloride. The extract was dried over sodium sulfate and evaporated, and the residue was applied to a column charged with silica gel. The column was eluted with toluene–ethyl acetate mixtures (20:1 and 10:1) to isolate 572 mg (93%) of a mixture of isomeric epoxy derivatives **IV** and **V**, (22*R*,23*S*,24*R*)- and (22*R*,23*R*,24*S*)-6β-methoxy-23,24-epoxy-3α,5-cyclo-26,27-bisnor-5α-cholestan-22-ols. IR spectrum, ν, cm^{-1}: 3600–3220, 3070, 3050–2800, 1730, 1465, 1390, 1355, 1330, 1300, 1280, 1210, 1190, 1140, 1105, 1025, 1010, 990, 975, 935. ^{1}H NMR spectrum, δ, ppm: 0.75 s (3H, 18-Me), 0.96 d (3H, 21-Me, *J* = 6.5 Hz), 1.02 s (3H, 19-Me), 1.33 d, 1.34 d (3H, 25-Me, *J* 5 = Hz), 2.65 m (1H, 23-H), 2.76 m (2H, 6-H, 23-H), 2.91 d.d and 3.14 d.d (1H, 24-H), 3.32 s (3H, OMe), 3.63 m and 3.88 m (1H, 22-H).

Reaction of isomeric epoxy steroids IV and V with isopropylmagnesium chloride. A suspension of 47 mg of copper(I) cyanide in 3 ml of ether was cooled to –60°C, and 2 ml of a 2 M solution of isopropylmagnesium chloride was added under argon. The mixture was kept for 1 h at –60°C, 430 mg of isomer mixture **IV**/**V** in 6 ml of ether was added, and the mixture was kept for 1 h at –60°C and for 4 h at –5°C. It was then allowed to warm up to room temperature, treated with a 20% solution of ammonium chloride, and extracted with ether. The extract was dried over sodium sulfate and evaporated, and the residue was applied to a column charged with silica gel. The column was eluted with hexane–ethyl

acetate mixtures (30 : 1, 15 : 1, and 5 : 1). Three fractions were collected. The first fraction contained 56 mg (13%) of alcohol **II**. The second fraction contained 239 mg (56%) of (22*R*,23*S*,24*R*)-6β-methoxy-23,24-epoxy-3α,5-cyclo-26,27-bisnor-5α-cholestan-22-ol (**IV**). IR spectrum, ν, cm^{-1}: 3600–3220, 3070, 3050–2800, 1730, 1465, 1390, 1355, 1330, 1300, 1280, 1210, 1190, 1140, 1105, 1025, 1010, 990, 975, 935. ^{1}H NMR spectrum, δ, ppm: 0.75 s (3H, 18-Me), 0.96 d (3H, 21-Me, *J* = 6.5 Hz), 1.02 s (3H, 19-Me), 1.34 d (3H, 25-Me, *J* = 5 Hz), 2.66 m (1H, 23-H), 2.76 m (1H, 6-H), 3.15 d.d (1H, 24-H), 3.32 s (3H, OMe), 3.90 br.s (1H, 22-H).

From the third fraction we isolated 103 mg (22%) of (22*R*,23*R*,24*S*)-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholestane-22,23-diol (**VI**). IR spectrum, ν, cm^{-1}: 3600–3200, 3050–2800, 1735, 1470, 1380, 1325, 1270, 1205, 1190, 1130, 1105, 1020, 1005, 985, 970, 930, 900, 865. ^{1}H NMR spectrum, δ, ppm: 0.74 s (3H, 18-Me), 0.84–1.03 m (15H, 19-Me, 21-Me, 26-Me, 27-Me, 28-Me), 2.78 m (1H, 6-H), 3.33 s (3H, OMe), 3.57 d (1H, 23-H), 3.74 d (1H, 22-H).

(22*R*,23*R*,24*S*)-22,23-Diacetoxy-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholestane (VII). To a mixture of 96 mg of diol **VI** in 3 ml of pyridine we added 1 ml of acetic anhydride and 52 mg of 4-dimethylaminopyridine. The mixture was heated for 24 h at 60°C, diluted with water, and extracted with ether. The extract was dried over sodium sulfate and evaporated, and the residue was applied to a column charged with silica gel. The column was eluted with hexane–ethyl acetate mixtures (20 : 1, 10 : 1, and 5 : 1) to isolate 94 mg (82%) of diacetate **VII**. IR spectrum, ν, cm^{-1}: 3055–2810, 1745, 1470, 1375, 1250, 1105, 1025, 980. ^{1}H NMR spectrum, δ, ppm: 0.75 s (3H, 18-Me), 0.89–1.02 m (15H, 19-Me, 21-Me, 26-Me, 27-Me, 28-Me), 1.99 s and 2.02 s (6H, Ac), 5.16 d (1H, 23-H), 5.34 d (1H, 22-H).

(22*R*,23*R*,24*S*)-3β,22,23-Triacetoxy-24-methylcholest-5-ene (VIII). Diacetate **VII**, 80 mg, was dissolved in 2 ml of acetic acid, and the solution was refluxed for 30 min and evaporated. The residue was subjected to column chromatography on silica gel using hexane–ethyl acetate mixtures (40 : 1, 30 : 1, and 20 : 1) as eluent. Yield 81 mg (96%). IR spectrum, ν, cm^{-1}: 3030–2800, 1750, 1470, 1380, 1255, 1035, 980. ^{1}H NMR spectrum, δ, ppm: 0.70 s (3H, 18-Me); 0.90–1.03 (15H, 19-Me, 21-Me, 26-Me, 27-Me; 28-Me); 1.99 s, 2.01 s, and 2.04 s (9H, Ac); 4.50–4.68 m (1H, 3-H); 5.16 d (1H, 23-H, *J* = 9.5 Hz); 5.33 d (1H, 22-H, *J* = 9.5 Hz); 5.36 d (1H, 6-H, *J* = 5 Hz).

(22*R*,23*R*,24*S*)-24-Methyl-5α-cholestane-3β,6α,-22,23-tetraol (IX). To a solution of 74 mg of triacetate **VIII** we added at room temperature 2.8 ml of a 1 M solution of BH_3 in tetrahydrofuran. The mixture was kept for 12 h at room temperature, and 2 ml of a 2 N solution of NaOH and 3 ml of a 30% solution of hydrogen peroxide were added. After 0.5 h, the mixture was extracted with ethyl acetate, and the extract was dried over sodium sulfate and evaporated. The residue was dissolved in 10 ml of a 5% solution of potassium hydroxide in methanol, and the solution was refluxed for 1 h. It was then neutralized with acetic acid and extracted with ethyl acetate, the extract was dried over sodium sulfate and evaporated, and the residue was applied to a column charged with silica gel. The column was eluted with toluene–ether–methanol (10 : 2 : 5) to isolate 26 mg (43%) of compound **IX**. ^{1}H NMR spectrum, δ, ppm: 0.71 s (3H, 18-Me), 0.83–0.98 m (15H, 18-Me, 19-Me, 21-Me, 26-Me, 27-Me, 28-Me), 3.51 d (1H, 22-H, *J* = 8.5 Hz), 3.68 d (1H, 23-H, *J* = 8.5 Hz).

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