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A New Synthesis of Zymosterol

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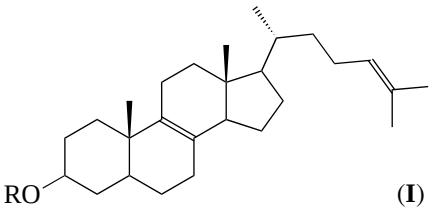
Abstract—A modified scheme for the synthesis of zymosterol, one of the biosynthetically important yeast sterols, starting from 3-benzoyloxyergosta-8(14),22-dien-15-one has been suggested.

Key words: *steroids, synthesis of steroid side chains, zymosterol*

INTRODUCTION

Zymosterol (**I**) is an important intermediate in the biosynthesis of cholesterol and ergosterol from lanosterol.[2] The isolation of zymosterol from the yeast sterol fraction is the main way of obtaining zymosterol [1–3]. However, this method is ineffective, because zymosterol is a rare yeast sterol. A number of methods for the chemical synthesis of this compound have been developed [4, 5], but all of them are multistep (up to 20 steps from ergosterol), laborious, and only a low yield of the product is obtained.



(**I**)

RESULTS AND DISCUSSION

We suggest here a modified method for the synthesis of zymosterol starting from 3-bensoyloxyergosta-8(14),22-dien-15-one (**II**) [6] that reduces the number of stages. The Horner–Emmons reaction with aldehyde (**III**) [4], the ozonolysis product of (**I**), was chosen as the main approach for the partial formation of the zymosterol side chain. The treatment of aldehyde (**III**) with triethyl phosphonoacetate anion proceeds in a high (91%) yield and results in ester (**IV**) with the *E*-geometry of the Δ^{22} bond ($J_{22,23}$ = 15 Hz). The less polar *Z*-alkene was also found in the reaction mixture by ^{1}H NMR, but we did not isolate it because it was a minor product and its reduction, like that of (**IV**), would result in the same product. We successfully avoided the epimerization of the C20 chiral center by performing this reaction at low temperature (–70°C). Similar results (concerning the yield and the isomer ratio) were also obtained in the case of other 22-aldehydes, e.g., upon the synthesis of Δ^{22} analogues of cholic acids [7–10].

According to the literature data, the reduction of the double bond in the side chain of a structurally similar systems is not a serious problem. Various catalysts were suggested for the hydrogenation [11–14], and their use results in high yields. Obviously, the presence of the tetrasubstituted $\Delta^{8(14)}$ bond in (**IV**) should not affect the selectivity of the Δ^{22} bond reduction. However, the use of the Pd/C catalyst turned out to be ineffective. This was likely due to the presence of negligible amounts of phosphonate admixtures that are difficult to remove [15]. The use of magnesium in methanol [10, 15] resulted in the saturation of the conjugated double bond, but was complicated by the significant hydrolysis of benzoate. Raney nickel turned out to be an effective catalyst, as its use helped obtain a high yield of (**V**) (94%). The structure of (**V**) was confirmed by the absence of the signals of olefin protons and the presence of the resonance of 7β proton in a low field (δ 4.14 ppm) of its ^{1}H NMR spectrum. Note also that the presence of resonances of C8 and C14 (δ 140.8 and 151.2 ppm, respectively) in the ^{13}C NMR spectrum of (**V**) confirms the presence of an intact $\Delta^{8(14)}$ double bond in the molecule.

For the formation of the 8(9) double bond, enone (**V**) was transformed into diene (**VII**). This transformation was achieved through the intermediate enol ester (**VI**), which was obtained by treatment of (**V**) with trifluoromethanesulfonic anhydride in the presence of 2,6-di-*tert* -butyl-4-methylpyridine. Subsequent reduction of trifluoromethanesulfonyl enol ester (**VI**) on the Pd-containing catalyst [4] resulted in diene (**VII**) (a yield of 88%). The structure of (**VII**) was confirmed, in particular, by the presence of the signal of the 15-olefin proton at δ 5.38 ppm) in its ^{1}H NMR spectrum. We should note that all attempts to hydroborate enol ester

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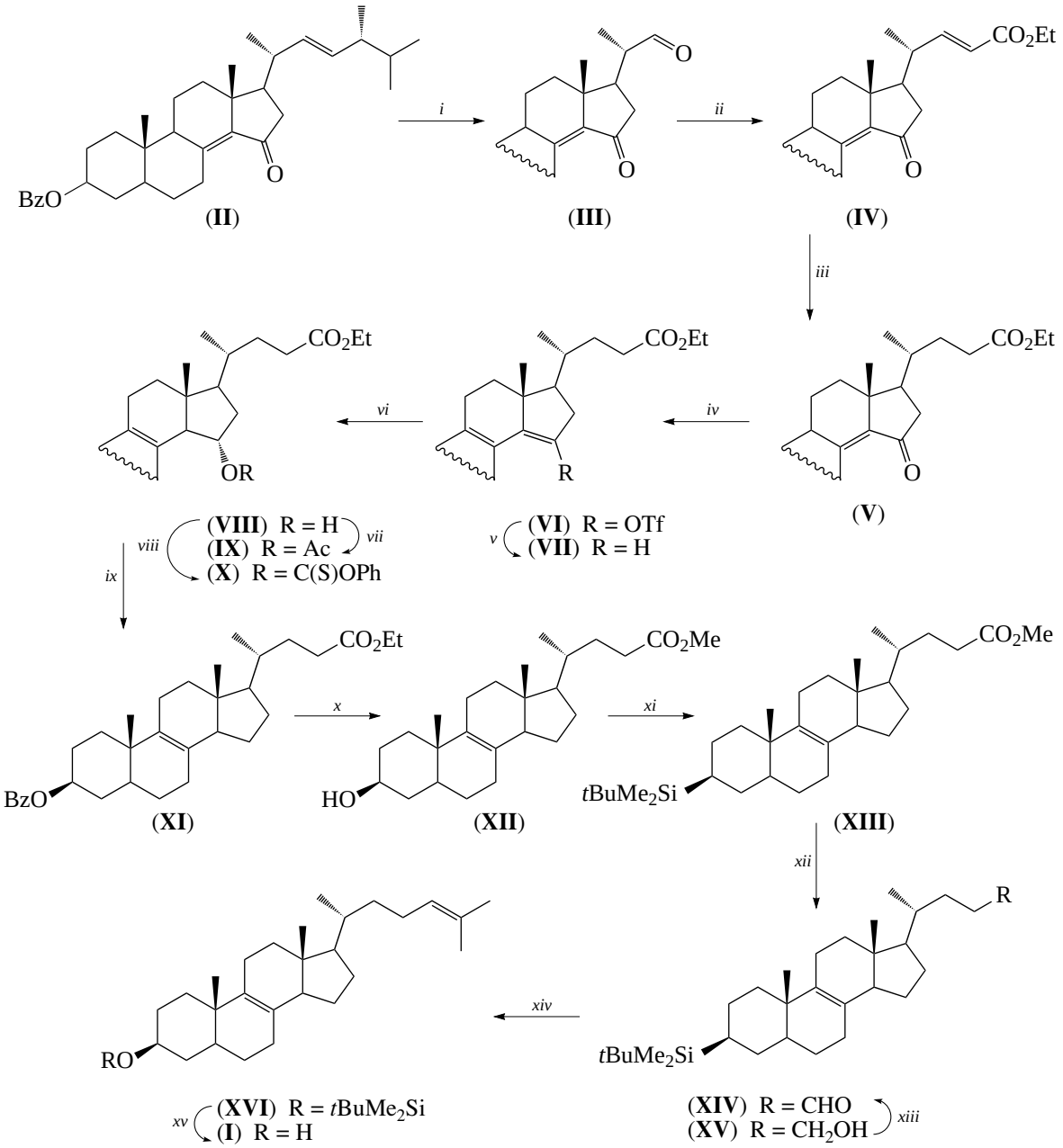
[2] Abbreviations: AIBN, azodiisobutyronitrile; DIBAH, diisobutyl aluminum hydride; and DMAP, 4,4-*N*,*N*-dimethylaminopyridine.



Reagents, conditions, and yields: (*i*) (1) O_3, Sudan III, CH_2Cl_2, –78°C, 75% and (2) Me_2S; (*ii*) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, –78°C, 91%; (*iii*) Ra/Ni, H_2, EtOH, 20°C, 94%; (*iv*) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2, 20°C, 99%; (*v*) $Pd(OAc)_2$, Ph_3P, Bu_3N, HCO_2H, DMF, 70°C, 88%; (*vi*) (1) $BH_3 \cdot Me_2S$, THF, 15°C and (2) Et_3N, H_2O_2, H_2O, THF, 20°C, 48%; (*vii*) Ac_2O, DMAP, CH_2Cl_2, 20°C, 92%; (*viii*) PhOCSCl, DMAP, CH_2Cl_2, 20°C, 99%; (*ix*) Bu_3SnH, AIBN, PhMe, 90°C, 75%; (*x*) KOH, MeOH, THF, 20°C, 86%; (*xi*) *t*$BuMe_2SiCl$, imidazole, DMF, 20°C, 93%; (*xii*) DIBAH, PhMe, –78°C, 87% of (**XIII**); (*xiii*) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, –78°C, 67%; (*xiv*) BuLi, *i*$PrPh_3PI$, THF, 0°C, 87%; and (*xv*) *n*Bu_4NF, THF, 20°C, 97%.

(**VI**) for the subsequent obtainment of 8(9)-ene steroids failed.

The catalytic hydrogenation or hydroboration of (**VII**) followed by treatment with propionic acid led us (and other authors who studied the relative diene structures [4, 5]) to a nearly inseparable mixture of $\Delta^{8(9)}$- and $\Delta^{8(14)}$-steroids in comparable amounts. In this connection, we used a two-step procedure of hydroboration–deoxygenation [4, 16] for the synthesis of the Δ^8-derivative from the 8(9),14(15)-diene (**VII**). As we learned that the ester groups undergo partial hydrolysis upon oxidation of the intermediate boron derivative, we replaced the alkaline hydrogen peroxide with a neutral oxidizer. We used triethylamine oxide generated *in situ*,

which was more effective than trimethylamine oxide under these conditions. The alcohol (**VIII**) was thus obtained in a yield of 48%. The starting diene was also isolated from the reaction mixture, and its conversion was 89%. The signal of the 15β proton (δ 4.11 ppm) appeared in the ^{1}H NMR spectrum of (**VIII**), whereas the band corresponding to the valent stretching oscillations of the hydroxyl group (3550 cm^{-1}) appeared in its IR spectrum. To additionally confirm its structure, (**VIII**) was acetylated with acetic anhydride in the presence of DMAP to give the corresponding derivative (**IX**). This exhibited the signal from the protons of acetyl group in its ^{1}H NMR spectrum, a downfield shift of the signal of 15-proton, and the disappearance of the band corresponding to the oscillations of the hydroxyl group from its IR spectrum.

To perform the Barton deoxygenation, alcohol (**VIII**) was converted into xanthate (**X**), and the latter was subjected to reduction with tri-*n*-butyltin hydride in the presence of AIBN. The standard procedure [17] was ineffective, as diene (**VII**) was the main product; that is, the Chugaev reaction prevailed. After a number of attempts, we found the conditions under which reduction prevailed over elimination. Thus, the addition of substrate (**X**) and the reagents to the reaction flask preliminarily heated to 90°C [18] resulted in a 75% yield of (**XI**). Ester (**XI**) has the completely formed cyclic moiety of zymosterol, which was most clearly proved by its ^{13}C NMR spectrum (the signals of C8 and C9 at 128.3 and 135 ppm).

The completion of zymosterol synthesis required the construction of its side chain by the introduction of isopropenyl moiety. This could be achieved by the reduction of the side chain ester to the corresponding aldehyde followed by the Wittig reaction. However, it was necessary to preliminarily replace the protective group in position 3, because it was impossible to reduce the ester group in the side chain by DIBAH selectively in the presence of the benzoate moiety. In one attempt, steroid (**XI**) gave a mixture of all four possible products upon reduction even at a very low temperature (–78°C). The alkaline hydrolysis of benzoate with potassium hydroxide in methanol was accompanied by a transesterification and resulted in a 86% yield of alcohol (**XII**); some C24-acid was also observed. A similar situation was also retained with sodium methylate or $Ba(OH)_2$ in place of KOH. Alcohol (**XII**) was converted into silyl ester (**XIII**), and the latter was reduced with DIBAH [10, 19]. The reduction resulted in two products, labile aldehyde (**XIV**) (87%) and alcohol (**XV**) (10%). We should mention that 24-alcohols like (**XV**) are often used to obtain 24-aldehydes, which are the starting products in the synthesis of a number of Δ^{24}-sterols (e.g., desmosterol) using the Wittig reaction. Oxidation to the aldehyde is usually achieved by pyridinium chlorochromate [20] or oxalyl chloride in DMSO according to Swern [21–24]. The use of the Swern oxidation allowed us to increase the yield of (**XIV**) from (**XV**) up to 67% and, hence, increase its total yield. The signal of 24-proton (δ 9.74 ppm) as a broadened singlet in the ^{1}H NMR spectrum and the bands of oscillations of the aldehyde carbonyl group at 1750 and 2720 cm^{-1} in the IR spectrum are the most characteristic parameters that confirm the structure of aldehyde (**XIV**).

Aldehyde (**XIV**) readily enters the Wittig reaction [10, 25–27] with the corresponding ylide and gives diene (**XVI**). Its 3*O*-protective group was hydrolyzed with tetrabutylammonium fluoride to give alcohol (**I**), all the physicochemical characteristics of which coincided with those of natural zymosterol [2, 4, 5, 28–31].

Thus, we have developed a new scheme for the synthesis of zymosterol starting from 3-benzoyloxyergosta-8(14),22-dien-15-one, which allowed us to decrease the number of stages in comparison with that suggested previously [4].

EXPERIMENTAL

Melting points were determined on a Kofler hot plate. ^{1}H and ^{13}C NMR spectra were registered on a Bruker A-200 (200 MHz) spectrometer in $CDCl_3$ (unless otherwise specified) using Me_4Si as the internal standard. The values of chemical shifts (δ, ppm) and spin coupling constants (*J*, Hz) are given. IR spectra were taken on a UR-20 instrument in films or in KBr pellets. Mass spectra were measured on a Hewlett–Packard 5890 spectrometer using linear temperature programming from 40 to 280°C at a rate of 10°/min and an accelerating voltage of 70 eV. The solvents were purified according to standard procedures [32]. All reactions were performed in a nitrogen atmosphere. The reactions were monitored by TLC on Silica gel 60 F_{254} precoated plates (Merck). The reaction mixtures were chromatographically separated on Silica gel 60 (40–60 μm, Merck).

The starting (**II**) was synthesized in seven steps starting from ergosterol [6]; mp 174–176°C (MeOH–CH_2Cl_2).

Aldehyde (**III**), mp 182–185°C (methanol), was obtained from (**II**) in 75% yield by the procedure reported in [4].

(22*E*)-3β-Benzoyloxy-15-oxo-5α-chola-8(14),22-dien-24-oic acid ethyl ester (IV). Sodium hydride (80% suspension, 1.75 g, 58 mmol) was added in small portions to a stirred solution of triethyl phosphonoacetate (13.2 g, 11.7 ml, 59 mmol) in THF (70 ml) at room temperature. After the gas liberation ceased, the solution was cooled to –70°C and a solution of aldehyde (**III**) (17.6 g, 39.3 mmol) in THF (150 ml) was added. The resulting solution was stirred for 30 min at –70°C and for 1 h at –20°C and then treated with saturated aqueous NH_4Cl solution (100 ml) and diethyl ether (150 ml). The aqueous phase was separated and extracted with ether. The combined organic extract was washed with water and saturated aqueous NaCl solu-

tion, dried over Na_2SO_4, and evaporated in a vacuum. The resulting oily residue was separated on a silica gel column eluted with 5 : 95 EtOAc–toluene to yield 18.52 g (91%) of ester (**IV**), mp 165–167°C (methanol), α_D +74° (*c* 1.30, $CHCl_3$); ^{1}H NMR: 0.79 (3 H, s, 18-Me), 1.02 (3 H, s, 19-Me), 1.18 (3 H, d, *J* 6.5, 21-Me), 1.29 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 4.14 (1 H, m, H7β), 4.14 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 5.00 (1 H, m, H3α), 5.79 (1 H, d, *J* 15, H23), 6.82 (1 H, dd, *J* 9 and 15, H22), 7.40 (2 H, m, *m*-H in Ph), 7.53 (1 H, m, *p*-H in Ph), and 8.02 (2 H, d, *J* 7.3, *o*-H in Ph); ^{13}C NMR: 13.5 q, 14.9 q, 19.7 q, 20.1 t, 20.5 q, 27.9 t, 28.1 t, 29.7 t, 34.3 t, 36.9 t, 37.5 t, 39.3 s, 39.4 d, 42.9 s, 43.6 t, 44.6 d, 50.6 d, 51.3 d, 60.9 t, 74.3 d, 120.8 d, 128.8 two d, 130.1 two d, 131.4 s, 133.4 d, 140.2 s, 151.6 s, 153.2 d, 166.7 s, 167.2 s, and 207.1 s; IR (ν, KBr, cm^{-1}): 1720, 1630, 1450, 1280, and 1120; MS, *m/z*: 518 $[M]^{+\bullet}$, 472, 429, 396, 251, and 105.

3β-Benzoyloxy-15-oxo-5α-chol-8(14)-en-24-oic acid ethyl ester (V). Raney nickel (6 g) was added to a solution of (**IV**) (47 g, 90.7 mmol) in ethanol (1 l). The flask was evacuated and filled with hydrogen. The mixture was hydrogenated with vigorous stirring for 24 h. The catalyst was filtered off and washed with CH_2Cl_2. The filtrates were combined and concentrated. The resulting oily residue was dissolved in EtOAc and filtered through a silica gel layer to give 44.3 g (94%) of saturated ester (**V**); mp 125–127°C (methanol); α_D +92° (*c* 0.77, $CHCl_3$); ^{1}H NMR: 0.78 (3 H, s, 18-Me), 0.99 (3 H, s, 19-Me), 1.02 (3 H, d, *J* 6.3, 21-Me), 1.26 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 4.12 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 4.14 (1 H, m, H7β), 4.99 (1 H, m, H3α), 7.40 (2 H, m, *m*-H in Ph), 7.52 (1 H, m, *p*-H in Ph), and 8.04 (2 H, d, *J* 7.3, *o*-H in Ph); ^{13}C NMR: 13.5 q, 14.9 q, 19.5 q,19.5 q, 20.2 t, 27.9 t, 28.1 t, 29.7 t, 31.3 t, 31.8 t, 34.3 d, 34.7 t, 36.9 t, 37.5 t, 39.4 s, 42.9 s, 43.5 t, 44.6 d, 51.3 d, 51.3 d, 61.0 t, 74.3 d, 128.7 two d, 130.1 two d, 131.4 s, 133.6 d, 140.8 s, 151.2 s, 166.8 s, 174.4 s, and 208.5 s; IR (ν, KBr, cm^{-1}): 1730, 1720, 1630, 1280, and 1120; MS, *m/z*: 520 $[M]^{+\bullet}$, 475, 383, 251, and 105.

3β-Benzoyloxy-15-trifluoromethylsulfonyloxy-5α-chola-8,14-dien-24-oic acid ethyl ester (VI). A solution of (**V**) (7.8 g, 15 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (4 g, 19.5 mmol) in CH_2Cl_2 (40 ml) was cooled to 0°C and trifluoromethanesulfonic anhydride (4.9 g, 2.9 ml, 17.3 mol) was added under stirring. The resulting solution was stirred for 8 h at room temperature and then diluted with hexane (140 ml). The precipitate was filtered off and washed with hexane. The combined extract was evaporated, and the resulting oily product (10.1 g) was used in the next step without additional purification; ^{1}H NMR: 0.91 (3 H, s, 18-Me), 0.96 (3 H, d, *J* 5.5, 21-Me), 1.07 (3 H, s, 19-Me), 1.27 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 4.14 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 4.97 (1 H, m, H3α), 7.40 (2 H, m, *m*-H in Ph), 7.52 (1 H, m, *p*-H in Ph), and 8.05 (2 H, d, *J* 6.7, *o*-H in Ph).

3β-Benzoyloxy-5α-chola-8,14-dien-24-oic acid ethyl ester (VII). Compound (**VI**) (10.1 g), $Pd(OAc)_2$ (188 mg, 0.84 mmol), triphenylphosphine (438 mg, 1.94 mmol), tributylamine (15.6 ml, 57.2 mmol), and formic acid (1.7 ml) were successively dissolved in DMF (30 ml). The resulting solution was stirred for 40 min at 70°C and then 25 ml of the solvent was removed in a vacuum. The residue was cooled and diluted with water (100 ml). The organic material was extracted with ethyl acetate. The extract was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, dried over Na_2SO_4, and evaporated. The residue was chromatographed on a silica gel column eluted with 5 : 95 EtOAc–toluene to yield 6.65 g (88%) of diene (**VII**), mp 138–140°C (methanol); ^{1}H NMR: 0.83 (3 H, s, 18-Me), 0.96 (3 H, d, *J* 5.5, 21-Me), 1.06 (3 H, s, 19-Me), 1.26 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 4.13 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 4.97 (1 H, m, H3α), 5.38 ((1 H, s, H15), 7.40 (2 H, m, *m*-H in Ph), 7.52 (1 H, m, *p*-H in Ph), and 8.05 (2 H, d, *J* 7.3, *o*-H in Ph); ^{13}C NMR: 15.7 q, 17.2 q, 19.8 q, 20.0 q, 23.3 t, 26.7 t, 28.0 t, 29.3 t, 32.4 t, 32.8 t, 35.2 d, 35.7 t, 36.6 t, 37.3 t, 39.1 t, 39.3 s, 42.3 d, 46.6 s, 58.4 d, 61.7 t, 75.4 d, 118.9 d, 124.7 s, 129.7 two d, 131.0 two d, 132.3 s, 134.2 d, 142.0 s, 152.3 s, 167.6 s, and 175.7 s; IR (ν, KBr, cm^{-1}): 1750, 1730, 1280, and 1120; MS, *m/z*: 504 $[M]^{+\bullet}$, 459, 367, 253, and 105.

3β-Benzoyloxy-15α-hydroxy-5α-chol-8-en-24-oic acid ethyl ester (VIII). A solution of (**VII**) (10.2 g, 20.2 mmol) in THF (60 ml) was cooled to 0°C and treated with 16.2 ml (32.3 mmol) of 2 M borane–methyl sulfide complex in THF under stirring. The resulting solution was stirred for 2 h at 15°C, cooled to 0°C, and carefully treated with water (2 ml). After the gas liberation ceased, triethylamine (5.7 ml) and then 30% H_2O_2 (2.2 ml) were added to the solution. The mixture was stirred for 2 h at 15°C, treated with $Na_2S_2O_3$ and NaCl solutions, the organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The extract was dried over Na_2SO_4 and concentrated in a vacuum. The residue was chromatographed on a silica gel column eluted with 1 : 9 EtOAc–toluene to give 4.7 g (46%) of the starting diene (**VII**) and 5.0 g (48%) of alcohol (**VIII**) as an oil; ^{1}H NMR: 0.64 (3 H, s, 18-Me), 0.92 (3 H, d, *J* 5.5, 21-Me), 1.01 (3 H, s, 19-Me), 1.24 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 4.11 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 4.11 (1 H, m, H15β), 4.96 (1 H, m, H3α), 7.42 (2 H, m, *m*-H in Ph), 7.54 (1 H, m, *p*-H in Ph), and 8.03 (2 H, d, *J* 7.3, *o*-H in Ph); ^{13}C NMR: 12.5 q, 14.2 q, 17.6 q, 18.0 q, 21.4 d, 22.6 t, 25.3 t, 27.1 t, 27.6 t, 30.7 t, 31.1 t, 34.1 t, 34.8 t, 35.3 d, 35.8 s, 37.0 t, 40.3 t, 43.1 s, 52.6 d, 59.4 d, 60.1 t, 71.7 d, 74.0 d, 126.9 s, 128.1 two d, 129.4 two d, 130.8 s, 132.6 d, 135.7 s, 166.0 s, and 174.0 s; IR (ν, film, cm^{-1}): 3550, 1740, 1720, 1610, 1280, and 1120; MS, *m/z*: 504 $[M - H_2O]^+$, 459, 367, 253, and 105.

3β-Benzoyloxy-15α-acetoxy-5α-chol-8-en-24-oic acid ethyl ester (IX). Acetic anhydride (0.02 ml, 0.2 mmol) and DMAP (24 mg, 0.2 mmol) were added to a solution of (**VIII**) (50 mg, 0.095 mmol) in methylene chloride (2 ml). The solution was stirred for 12 h, diluted with methylene chloride, washed with water, $NaHCO_3$ solution, and saturated aqueous NaCl, dried over Na_2SO_4, and evaporated. The residue was chromatographed on a silica gel column. Elution with 5 : 95 EtOAc–toluene gave 49 mg (92%) of (**IX**), mp 126–128°C (methanol); ^{1}H NMR: 0.67 (3 H, s, 18-Me), 0.92 (3 H, d, *J* 5.5, 21-Me), 1.00 (3 H, s, 19-Me), 1.24 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 2.01 (3 H, s, OAc), 4.10 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 4.93 (2 H, m, H3α and H15β), 7.41 (2 H, m, *m*-H in Ph), 7.52 (1 H, m, *p*-H in Ph), and 8.02 (2 H, d, *J* 7.3, *o*-H in Ph); ^{13}C NMR: 13.9 q, 15.7 q, 19.1 q, 19.5 q, 22.8 q, 24.1 t, 26.7 t, 28.1 t, 29.1 t, 32.2 t, 32.8 t, 35.6 t, 36.4 t, 36.8 d, 37.4 s, 38.4 t, 39.2 t, 41.9 d, 43.9 s, 54.3 d, 56.8 d, 61.7 t, 75.5 d, 76.0 d, 127.8 s, 129.7 two d, 131.0 two d, 132.3 s, 134.2 d, 137.7 s, 167.6 s, 172.5 s, and 175.4 s; IR (ν, film, cm^{-1}): 1740, 1720, 1280, and 1120.

3β-Benzoyloxy-15α-phenyloxythiocarbonyloxy-5α-chol-8-en-24-oic acid ethyl ester (X). Phenyl chlorothionoformate (2.36 g, 13.68 mmol, 1.9 ml) was added at 0°C to a stirred solution of (**VIII**) (5.1 g, 9.77 mmol) and DMAP (3.34 g, 27.36 mmol) in methylene chloride (35 ml). The solution was stirred for 12 h and then poured onto ice–diethyl ether. The organic phase was separated; washed with a $CuSO_4$ solution (6 × 40 ml), water, aqueous $NaHCO_3$ (3 × 40 ml), and saturated aqueous NaCl; dried over Na_2SO_4; and then evaporated. The resulting oily product (**X**) (6.4 g) was used at the next stage without additional purification; ^{1}H NMR: 0.73 (3 H, s, 18-Me), 0.95 (3 H, d, *J* 5.5, 21-Me), 1.03 (3 H, s, 19-Me), 1.26 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 4.13 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 4.97 (1 H, m, H3α), 5.39 (1 H, m, H15β), 7.34 (8 H, m, Ar), and 8.05 (2 H, d, *J* 7.3, *o*-H in Ph); IR (ν, film, cm^{-1}): 1750, 1725, 1280, and 1210.

3β-Benzoyloxy-5α-chol-8-en-24-oic acid ethyl ester (XI). A degassed solution of (**X**) (6.4 g, 9.77 mmol) and tributyltin hydride (5.61 g, 19.28 mmol, 5.1 ml) solution in toluene (28 ml) were added in 5-ml portions to a flask heated to 90°C while simultaneously (with each portion) adding AIBN (30 mg, 0.18 mmol). The solution was stirred for 10 min after each addition. After the reduction was over, the solution was cooled, aqueous KF solution was added, and the reaction mixture was stirred for 12 h. The organic layer was separated, washed with saturated solution of NaCl, dried over Na_2SO_4, and evaporated. The residue was chromatographed on a silica gel column. Elution with 5 : 95 EtOAc–toluene gave 3.70 g (75%) of (**X**), mp 137–139°C (methanol); α_D +33° (*c* 0.6, $CHCl_3$); ^{1}H NMR: 0.59 (3 H, s, 18-Me), 0.92 (3 H, d, *J* 6.1, 21-Me), 0.99 (3 H, s, 19-Me), 1.23 (3 H, t, *J* 7.1, $\underline{CH_3}CH_2$), 4.10 (2 H, q, *J* 7.1, $CH_3\underline{CH_2}$), 4.94 (1 H, m, H3α), 7.40 (2 H, m, *m*-H in Ph), 7.52 (1 H, m, *p*-H in Ph), and 8.02 (2 H, d, *J* 7.3, *o*-H in Ph); ^{13}C NMR (C_6D_6): 11.5 q, 14.3 q, 17.8 q, 18.5 q, 23.0 t, 24.0 t, 25.6 t, 27.4 t, 28.0 t, 28.9 t, 31.3 t, 31.4 t, 34.6 d, 35.1 t, 35.9 s, 36.1 d, 37.3 t, 40.7 d, 42.4 s, 52.1 d, 54.9 d, 60.0 t, 74.1 d, 128.3 s, 128.5 two d, 129.9 two d, 131.7 s, 132.7 d, 135.0 s, 165.8 s, and 173.3 s; IR (ν, KBr, cm^{-1}): 1740, 1730, and 1290; MS, *m/z*: 506 $[M]^{+\bullet}$, 461, 384, 255, 213, and 105.

3β-Hydroxy-5α-chol-8-en-24-oic acid methyl ester (XII). Potassium hydroxide (0.4 g, 7.1 mmol) was added to a solution of (**XI**) (5.3 g, 10.47 mmol) in anhydrous 4 : 1 methanol–THF mixture (400 ml). The solution was stirred for 5 h, neutralized with 1 N HCl, and then poured out to water (0.5 l). The precipitate was filtered off, washed with water, dried in a vacuum, and chromatographed on a silica gel column. Elution with 1 : 9 EtOAc–toluene gave 3.49 g (86%) of (**XII**); mp 139–141°C (methanol); α_D +27° (*c* 0.45, $CHCl_3$); ^{1}H NMR: 0.58 (3 H, s, 18-Me), 0.90 (3 H, d, *J* 6.1, 21-Me), 0.92 (3 H, s, 19-Me), 3.59 (1 H, m, H3α), and 3.64 (3 H, s, OMe); ^{13}C NMR: 11.2 q, 17.8 q, 18.3 q, 22.7 t, 23.7 t, 24.5 t, 27.1 t, 28.6 t, 30.9 t, 31.1 t, 31.6 t, 35.1 t, 35.7 s, 35.8 d, 36.9 t, 38.3 t, 40.7 d, 42.1 s, 51.4 q, 51.8 d, 54.5 d, 71.1 d, 128.1 s, 135.0 s, and 174.7 s; IR (ν, KBr, cm^{-1}): 3500, 1730, 1310, and 1110; MS, *m/z*: 388 $[M]^{+\bullet}$, 370, 355, 273, and 213.

3β-*tert*-Butyldimethylsilyloxy-5α-chol-8-en-24-oic acid methyl ester (XIII). *tert*-Butyldimethylsilyl chloride (1.49 g, 9.88 mmol) and imidazole (1.34 g, 19.76 mmol) were added to a solution of (**XII**) (2.95 g, 7.8 mmol) in DMF (25 ml). The solution was stirred for 12 h at room temperature, poured out into water, and the organic material was extracted with hexane. The extract was washed with water and saturated NaCl, dried over Na_2SO_4, and then evaporated. The residue was chromatographed on a silica gel column. Elution with 5 : 95 EtOAc–toluene led to (**XIII**), yield 3.55 g (93%); mp 125–127°C (methanol); α_D +30° (*c* 0.98, $CHCl_3$); ^{1}H NMR: 0.02 (6 H, s, Me_2Si), 0.57 (3 H, s, 18-Me), 0.86 (9 H, s, *t*-BuSi), 0.90 (3 H, d, *J* 6.1, 21-Me), 0.91 (3 H, s, 19-Me), 3.52 (1 H, m, H3α), and 3.64 (3 H, s, OMe); ^{13}C NMR (C_6D_6): –4.3 q, 11.4 q, 18.0 q, 18.3 s, 18.5 q, 23.1 t, 24.1 t, 26.0 t, 26.1 q, 27.6 t, 28.9 t, 31.2 t, 31.3 t, 32.6 t, 35.5 t, 36.0 s, 36.1 d, 37.4 t, 39.4 t, 41.1 d, 42.4 s, 51.0 q, 52.2 d, 54.9 d, 72.2 d, 128.1 s, 135.5 s, and 173.7 s; IR (ν, KBr, cm^{-1}): 1730 and 1110; MS, *m/z*: 502 $[M]^{+\bullet}$, 445, 369, 353, and 213.

3β-*tert*-Butyldimethylsilyloxy-5α-chol-8-en-24-al (XIV) and 3β-*tert*-butyldimethylsilyloxy-5α-chol-8-en-24-ol (XV). A solution of DIBAH (0.206 M, 25.2 ml) in toluene was added over 20 min to a stirred solution of (**XIII**) (2.6 g, 5.18 mmol) in toluene (50 ml) at –78°C. The resulting solution was stirred for an additional 40 min and diluted with ethyl acetate (5 ml). It was then treated with 0.5% acetic acid (10 ml) and the

resulting emulsion was stirred for 1 h at room temperature. The aqueous phase was separated and extracted with ethyl acetate. The extracts were combined, washed with saturated NaCl solution, dried over Na_2SO_4, and evaporated. The residue was chromatographed on a silica gel column eluted with toluene to give 2.12 g (87%) of aldehyde (**XIV**) and 0.25 g (10%) of the more polar alcohol (**XV**).

(**XIV**): mp 120–122°C (hexane); ^{1}H NMR: 0.03 (6 H, s, Me_2Si), 0.58 (3 H, s, 18-Me), 0.86 (9 H, s, *t*-BuSi), 0.91 (3 H, d, *J* 6.1, 21-Me), 0.91 (3 H, s, 19-Me), 3.54 (1 H, m, H3α), and 9.74 (1 H, br. s, H24); ^{13}C NMR (C_6D_6): –4.3 q, 11.4 q, 18.0 q, 18.4 s, 18.5 q, 23.1 t, 24.1 t, 26.0 t, 26.1 q, 27.6 t, 28.0 t, 28.9 t, 32.6 t, 35.6 t, 36.0 d, 36.0 s, 37.4 t, 39.4 t, 40.9 d, 41.1 d, 42.4 s, 52.2 d, 54.9 d, 72.2 d, 128.1 s, 135.5 s, and 200.7 d; IR (ν, KBr, cm^{-1}): 2720, 1750, and 1110; MS, *m*/*z*: 472 $[M]^{+\bullet}$, 445, 415, 387, 339, 255, and 213.

(**XV**): mp 149–151°C (hexane); α_D +21° (*c* 0.62, $CHCl_3$); ^{1}H NMR: 0.02 (6 H, s, Me_2Si), 0.5 (3 H, s, 18-Me), 0.86 (9 H, s, *t*-BuSi), 0.91 (3 H, s, 19-Me), 0.92 (3 H, d, *J* 5.8, 21-Me), and 3.57 (3 H, m, H3α and H24); ^{13}C NMR: –4.6 q, 11.2 q, 17.9 q, 18.2 s, 18.7 q, 22.8 t, 23.7 t, 25.5 t, 25.9 q, 27.2 t, 28.6 t, 29.4 t, 31.8 t, 32.1 t, 35.3 t, 35.7 s, 36.0 d, 37.0 t, 38.8 t, 40.9 d, 42.1 s, 51.9 d, 54.7 d, 63.5 t, 72.1 d, 128.0 s, and 135.2 s; IR (ν, KBr, cm^{-1}): 3500, 1270, 1110, and 1090; MS, *m*/*z*: 474 $[M]^{+\bullet}$, 417, 341, 255, and 213.

Oxidation of alcohol (XV) to aldehyde (XIV). DMSO (215 mg, 2.76 mmol) in methylene chloride (2 ml) was added to a solution of $(COCl)_2$ (175 mg, 1.38 mmol) in methylene chloride (3 ml) at –78°C under stirring. After 5 min, when the solution again became transparent, alcohol (**XV**) (293 mg, 0.62 mmol) in methylene chloride (3 ml) and, after 15 min, triethylamine (368 mg, 3.61 mmol) were added. The resulting solution was stirred for 40 min at –20°C; diluted with ethyl acetate; successively washed with 1% HCl, aqueous $NaHCO_3$, and saturated NaCl solution, dried over Na_2SO_4, and then evaporated. The residue was chromatographed on a silica gel column eluted with toluene to give 196 mg (67%) of aldehyde (**XIV**).

3β-*tert*-Butyldimethylsilyloxy-5α-cholesta-8,24-diene (XVI). A solution of (**XIV**) (2.116 g, 4.48 mmol) in THF (10 ml) was added at –30°C to a stirred solution of ylide prepared from 1.6 N butyllithium (5.4 ml, 8.77 mmol) in hexane and isopropyltriphenylphosphonium iodide(4.55 g, 10.52 mmol) in THF (10 ml). The solution was stirred for 1 h at 0°C, diluted with saturated solution of NH_4Cl, and extracted with hexane. The extract was washed with saturated NaCl and dried over Na_2SO_4. The resulting solution was treated with methyl iodide (1 ml) and stirred for 12 h. The precipitate was filtered off, the filtrate was concentrated, and the residue was chromatographed on a silica gel column. Elution with hexane led to olefin (**XVI**); yield 1.944 g (87%); mp 103–105°C (methanol); α_D +32° (*c* 0.60, $CHCl_3$); ^{1}H NMR: 0.02 (6H, s, Me_2Si), 0.57 (3 H, s, 18-Me), 0.85 (9 H, s, *t*-BuSi), 0.91 (3 H, s, 19-Me), 1.56 and 1.65 (2 × 3 H, 2 s, 26-Me and 27-Me), 3.54 (3 H, m, H3α), and 5.06 (1 H, br. s, H24); ^{13}C NMR: –4.6 q, 11.2 q, 17.6 q, 17.9 q, 18.2 s, 18.6 q, 22.8 t, 23.8 t, 24.8 t, 25.5 t, 25.8 q, 25.9 q, 27.2 t, 28.7 t, 32.1 t, 35.3 t, 35.7 s, 36.0 d, 36.0 t, 37.0 t, 38.8 t, 40.9 d, 42.1 s, 51.9 d, 54.8 d, 72.1 d, 125.2 d, 128.0 s, 130.8 s, and 135.2 s; IR (ν, KBr, cm^{-1}): 1640, 1480, 1270, 1110, 1090, and 1080. MS, *m*/*z*: 498 $[M]^{+\bullet}$, 441, 365, 255, and 213.

3β-Hydroxy-5α-cholesta-8,24-diene (I) (zymosterol). A 1 M solution of tetra-*n*-butylammonium fluoride (7.5 ml) in THF was added to a solution of (**XVI**) (0.935 g, 1.88 mmol) in THF (3 ml). The resulting solution was stirred for 24 h at room temperature, diluted with diethyl ether, washed with water, 0.1 N HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl; dried over Na_2SO_4; and evaporated. The residue was chromatographed on a silica gel column. Elution with toluene led to (**XVI**); yield 0.702 g (97%); mp 107–109°C (methanol); α_D +48° (*c* 0.58, $CHCl_3$) (lit.: mp 107–109°C, α_D +50° [31]; mp 110°C, α_D +49° [30]; mp 110–112°C, α_D +52° [2, 28]); ^{1}H NMR (C_6D_6): 0.69 (3 H, s, 18-Me), 0.91 (3 H, s, 19-Me), 1.04 (3 H, d, *J* 6.1, 21-Me), 1.61 and 1.69 (2 × 3 H, 2 s, 26-Me and 27-Me), 3.04 (1 H, s, OH), 3.40 (3 H, m, H3α), and 5.27 (1 H, br. t, *J* 7.0, H24); ^{13}C NMR (C_6D_6): 11.5 q, 17.7 q, 18.0 q, 18.9 q, 23.2 t, 24.2 t, 25.3 t, 25.9 q, 26.0 t, 27.6 t, 29.1 t, 32.1 t, 35.6 t, 36.0 s, 36.5 d, 36.5 t, 37.4 t, 38.8 t, 41.1 d, 42.4 s, 52.3 d, 55.2 d, 71.0 d, 125.7 d, 128.2 s, 130.8 s, and 135.5 s; IR (ν, KBr, cm^{-1}): 3500, 1640, 1470, and 1380; MS, *m*/*z*: 384 $[M]^{+\bullet}$, 369, 297, 271, and 213.

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