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The synthesis and in vitro activity of some $\Delta^{7,9(11)}$-lanostadienes

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The synthesis of $\Delta^{7,9(11)}$-lanostadiene derivatives functionalized at C(32) starting from 3β-acetoxy-7α,32-epoxylanostan-11-one has been presented. The $\Delta^{7,9(11)}$ moiety was efficiently introduced in three steps in 71% yield by the regioselective abstraction of allylic 8β hydrogen. The formyl group of the key intermediate, 3β-benzoyloxylanosta-7,9(11)-dien-32-al, has been stereoselectively alkylated into (32S) derivative, whereas its oxidation unexpectedly afforded 3β-benzoyloxy-7-oxolanost-8-ene-32,11α-lactone and not the corresponding acid. $\Delta^{7,9(11)}$-lanostadienes possessing HC(32)=O, C(32)≡N, HC(32S)CH$_3$OH, H$_2$C(32)OH, as well as some 11-keto lanostenes, were tested in vitro against several purified cholesterogenic enzymes showing moderate activity, with most the active aldehyde **16** *having IC_{50} = 86 μM.* (Steroids **62**:709–718, 1997) © *1997 by Elsevier Science Inc.*

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Introduction

Because certain lanosterol metabolites (e.g., oxysterols **1–6**; Figure 1) were found to possess the feedback effect on its biosynthesis,[1–3] they are interesting as potential cholesterol-lowering agents in mammals. A number of steroidal compounds were synthesized and tested as potential suppressors of lanosterol 14-demethylase activity[3–7] and downregulators of HMG-CoA reductase (HMGR) activity,[8–10] as well as inhibitors of fungal ergosterol biosynthesis.[11,12] Most of the synthesized compounds were 24,25-dihydrolanosteryl (DHL) derivatives that, beside various functional groups attached to C(32), contained Δ^7-double bond instead of the natural Δ^8 bond.[13] Analysis of available data[1,3–11] suggests that the potential lanostane-based inhibitor should possess (a) sp^2 hybridized C(8), (b) functionalized C(32), (c) 15α substituent or heteroatom in position 15, and (d) not to possess the axial β-hydroxy group.

Although some other targets are currently also under investigation,[14–16] finding a specific inhibitor of HMGR or lanosterol 14α-methyl demethylation process seems promising in the controlling of cholesterol levels in mammals. As the binding sites for oxysterols in corresponding enzymes are not yet known, we decided to gather further information on their structural demand by the synthesis of flattened lanostene $\Delta^{7,9(11)}$ system functionalized at C(32) and test them against several purified enzymes, such as HMGR, sterol 14-reductase, sterol 24-reductase, sterol 7-reductase, and sterol 8-isomerase.

Experimental

Chemistry

General

Melting points (m.p.) were determined on a Mikro-Heiztisch Boetius PHMK apparatus and were not corrected. Specific rotations were measured on a Karl Zeiss Polamat A and Perkin-Elmer 141 MC polarimeters at the given temperatures. Ultraviolet (UV) spectra were recorded on a Beckman DU-420 spectrophotometer. Infrared (IR) spectra were recorded on Perkin-Elmer spectrophotometer FT-IR 1725X. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM-600, Bruker AM-250, Varian Gemini-200, and Varian FT-80A (at 600, 250, 200, and 80 MHz, respectively) spectrometers in $CDCl_3$ using TMS as internal standard. Chemical shifts were expressed as ppm (δ) values and coupling constants (*J*) in Hz. Mass spectra were taken on a Finnigan-MAT 8230 spectrometer as indicated below.

1. 7α,32-Epoxylanostane-3β,11β-diol (8)

3β-Acetoxy-7α,32-epoxylanostan-11-one (**7**; 2 g, 4 mmol) was dissolved under argon in dry toluene (100 mL) at room temperature (r.t.). Then, diisobutylaluminium hydride (DIBAH; 8.8 mL;

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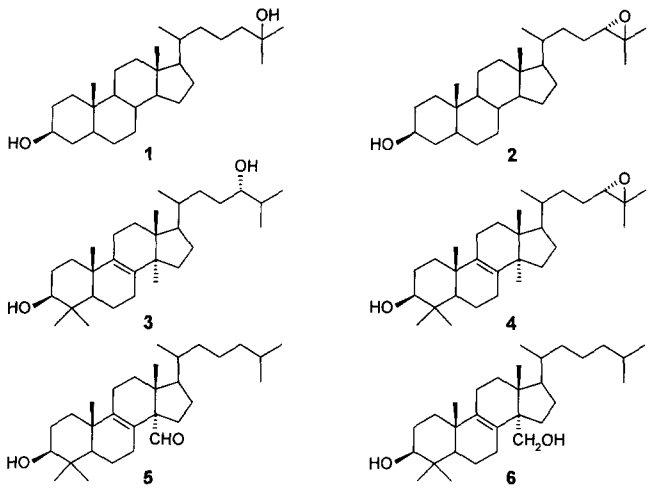


Figure 1 Some of isolated metabolic oxysterols (for details, see reference 1).

1.5 M in toluene) was added dropwise within 15 min, and the reaction mixture was stirred at r.t. for 4 h. The reaction mixture was then cooled to 0°C, and the resulting aluminate was destroyed with 25 mL of MeOH:H_2O (1:1; dropwise addition). After the addition was completed, the reaction mixture was stirred for additional 3 h and filtered through a Büchner funnel. Precipitate was thoroughly extracted with $CHCl_3$ (4 × 10 mL), and extracts were mixed with filtrate evaporated previously to dryness. Mixed extracts were dried over Na_2SO_4, filtered over short pad of SiO_2, evaporated to dryness, and crystallized to give diol **8** (1.71 g; 93%). 7α,32-epoxylanostane-3β,11β-diol (**8**): m.p. 248–255°C (colorless plates; acetone). $[\alpha]_{546}^{23}$ = +34.5; $[\alpha]_{578}^{23}$ = +31.0 (c = 0.58; chl.). IR (KBr): 3467m, 3349s, 3000s, 2953s, 2937s, 2897s, 2871s, 1385m, 1369m, 1068m, 1016m, 986m, 973m cm^{-1}. ^1H NMR (600 MHz; $CDCl_3$): 4.32 [br.s., H-C(11 or 7)]; 4.27 [br.s., H-C(7 or 11)]; 3.80 [d,*J* = 7.4 Hz, H-C(32)]; 3.31 [d,*J* = 7.4 Hz, H-C(32)]; 3.24–3.21 [m, H-C(3)]; 1.08 (s, CH_3); 1.06 (s, CH_3); 0.93 (s, CH_3); 0.86 [d,*J* = 6.5 Hz, H_3C-C(20)]; 0.845 [d,*J* = 6.5 Hz, H_3C-C(25)]; 0.839 [d,*J* = 6.5 Hz, H_3C-C(25)]; 0.79 (s, CH_3). MS-EI (70 eV, m/z): 460 (M^+, 14); 442 (10); 429 (17); 411 (15); 315 (22); 305 (27); 220 (32); 135 (25); 107 (24); 95 (32); 57 (47); 43 (100). Analysis calculated for $C_{30}H_{52}O_3$ (460.74): C78.21; H11.38. Found: C78.46; H11.35.

2. 7α,32-Epoxylanost-9-en-3β-ol 3-benzoate (9)

Diol **8** (2 g; 4.3 mmol) was dissolved in pyridine (30 mL) followed by the addition of BzCl (2 mL); the reaction mixture was stirred at r.t. until completed (ca. 3 h), poured onto acidified (HCl) ice/water mixture, and left overnight. The mixture was then filtered, precipitate dissolved in EtOAc (100 mL), washed with saturated $NaHCO_3$ solution (4 × 40 mL) and H_2O (3 × 20 mL), dried over Na_2SO_4, and evaporated to dryness. The crude monobenzoate **A** was then dissolved in pyridine at r.t. with dimethylaminopyridine (DMAP; 150 mg) added, and treated with MsCl (2.5 mL). The reaction was completed within 7 h (TLC), and the reaction mixture was poured onto acidified (HCl) ice/water mixture and left overnight. The precipitate was filtered off, dissolved in EtOAc (100 mL), washed with sat. $NaHCO_3$ (4 × 30 mL), water (2 × 50 mL) and dried over anhidrous Na_2CO_3. Subsequent filtration and evaporation afforded 2.3 g of crude **9**. Crystallization of the crude product from EtOH gave 2.02 g (85%) **9** as colorless needles. 7α32-epoxylanost-9-en-3β-ol 3-benzoate (**9**): m.p. 230–231°C. $[\alpha]_{546}^{26}$ = +20; $[\alpha]_{578}^{26}$ = +16 (c = 0.5; chl.). IR (KBr): 3060w, 2949s, 2931s, 2868m, 1711s, 1603w, 1585w, 1278s, 1178w, 1115m, 1069m, 1025m, 976m, 710s cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): 8.05 [d,*J* = 7.6 Hz, H-C(2′) H-C(6′)]; 7.55 [t,*J* = 7.1 Hz, H-C(4′)]; 7.44 [t,*J* = 7.4 Hz, H-C(3′), H-C(5′)]; 5.34 [br.s, H-C(11)]; 4.75–4.71 [m,H-C(3)]; 4.27 [br.s, H-C(7)]; 3.67 [d,*J* = 6.9 Hz, H-C(32)]; 3.36 [d,*J* = 6.9 Hz, H-C(32)]; 1.07 (s, 2 CH_3); 0.92 (s, CH_3); 0.91 [d,*J* = 6.3 Hz, H_3C-C(20)]; 0.873 [d,*J* = 6.1 Hz, H_3C-C(25)]; 0.868 [d,*J* = 6.2 Hz, H_3C-C(25)]; 0.81 [s, H_3C-C(13)]. MS-CI (isobutane; m/z): 547 [$(M+1)^+$, 46]; 546 (M^+, 13); 528 (8); 467 (9); 426 (36); 425 (100); 407 (11); 257 (4); 123 (10). Analysis calculated for $C_{37}H_{54}O_3$ (546.83): C81.27; H9.95. Found: C81.30; H10.02.

3. Lanosta-7,9(11)-diene-3β,32-diol 32-acetate, 3-benzoate (10)

7α,32-Epoxylanost-9-en-3β-ol 3-benzoate (**9**; 1.5 g, 2.74 mmol) was suspended in acetic anhydride (22.5 mL), pyridinium chloride (4.8 g) was added, and the reaction mixture was heated to reflux for 6 h. When cooled to 50°C, the dark brown mixture was poured onto acidified (HCl) ice/water and left overnight. Upon extraction of formed heavy oil with $CHCl_3$ (3 × 40 mL) and drying over an h. Na_2SO_4, the crude product was chromatographed on SiO_2 column (eluent: toluene) to give 1.37 g of **10** as pale yellow solid. Crystallization from ethanol afforded 1.36 g (84%) of **10** as colorless needles. Lanosta-7,9(11)-diene-3β,32-diol 32-acetate, 3-benzoate (**10**) m.p. 142–143°C. $[\alpha]_{546}^{23}$ = +88.1; $[\alpha]_{578}^{23}$ = +80 (c = 0.5; chl.). IR (KBr): 3036w, 2961s, 2927s, 1747s, 1712s, 1601w, 1584w, 1275s, 1254m, 1240m, 1177m, 1117m, 1070m, 1027m, 974m, 716s cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): 8.05 [d,*J* = 7.5 Hz, H-C(2′), H-C(6′)]; 7.55 [t,*J* = 7.3 Hz, H-C(4′)]; 7.44 [t,*J* = 7.7 Hz, H-C(3′), H-C(5′)]; 5.52 [br.s, H-C(7) or H-C(11)]; 5.39 [d,*J* = 5.1 Hz, H-C(11) or H-C(7)]; 4.77 [dd,*J* = 11.7, 4.0 Hz, H-C(3)]; 4.12 [d,*J* = 10.2 Hz, H-C(32)]; 3.85 [d,*J* = 10.2 Hz, H-C(32)]; 1.11 (s, CH_3); 1.04 (s, CH_3); 0.95 (s, CH_3); 0.89 [d,*J* = 6.4 Hz, H_3C-C(20)]; 0.873 [d,*J* = 6.4 Hz, H_3C-C(25)]; 0.869 [d,*J* = 6.4 Hz, H_3C-C(25)]; 0.66 [s, H_3C-C(13)]. MS-CI (isobutane, m/z): 589 [$(M+1)^+$, 5]; 529 (7); 467 (100); 425 (12); 407 (68); 307 (6); 247 (5); 123 (63). Analysis calculated for $C_{39}H_{56}O_4$ (588.87): C 79.55; H 9.58. Found: C 81.00; H 9.60.

4. Lanosta-7,9(11)-diene-3β,32-diol 3-benzoate (12)

Lanosta-7,9(11)-diene-3β,32-diol 32-acetate, 3-benzoate (**10**; 1.2 g, 2.04 mmol) was hydrolyzed with NaOH/EtOH solution (200 mg NaOH, 140 mL EtOH) at r.t. for 72 h. The reaction mixture was then poured onto acidified (HCl) ice/water and filtered to give 1.2 g of crude product. Flash chromatography (SiO_2; toluene) and crystallization afforded 691 mg (62%) of monobenzoate **12** as colorless needles, 290 mg of unreacted **10**, and 90 mg of (**10** + **12**) mixture. Lanosta-7,9(11)-diene-3β,32-diol 3-benzoate **12**: m.p. 217.5–219°C. $[\alpha]_{546}^{23}$ = +100.2, $[\alpha]_{578}^{23}$ = +88.2 (c = 0.5, chl.). IR (KBr): 3548m, 3405m, 3031w, 2966s, 2929s, 1709s, 1601w, 1585w, 1282s, 1177m, 1116m, 1071m, 1047m, 1026m, 975m, 712s. ^1H NMR (600 MHz, $CDCl_3$): 8.05 [d,*J* = 7.4 Hz, H-C(2′), H-C(6′)]; 7.56 [t,*J* = 7.0 Hz, H-C(4′)]; 7.45 [t,*J* = 7.2 Hz, H-C(3′), H-C(5′)]; 5.57 [br.s, H-C(7) or H-C(11)]; 5.47 [br.s, H-C(11) or H-C(7)]; 4.74 [dd,*J* = 11.2, 3.6 Hz, H-C(3)]; 3.39 [br.s, 2H-C(32)]; 1.13 (s, CH_3); 1.06 (s, CH_3); 0.97 (s, CH_3); 0.89 [d,*J* = 6.2 Hz, H_3C-C(20)]; 0.874 [d,*J* = 6.4 Hz, H_3C-C(25)]; 0.869 [d,*J* = 6.2 Hz, H_3C-C(25)]; 0.68 [s, H_3C-C(13)]. MS-CI (isobutane, m/z): 547 [$(M+1)^+$, 100]; 529 (8); 515 (9); 425 (99); 407 (5); 391 (14); 300 (24); 123 (6). Analysis calculated for $C_{37}H_{54}O_3$ (546.83): C 81.27; H 9.95. Found: C 81.45; H 9.84.

5. 3β-Benzoyloxylanosta-7,9(11)-dien-32-al (13)

Lanosta-7,9(11)-diene-3β 32-diol 3-benzoate (**12**; 300 mg, 0.55 mmol) was dissolved in CH_2Cl_2 (30 mL) and treated with pyridinium chlorochromate (PCC; 120 mg) for 1 h at r.t. After the solvent was evaporated under reduced pressure, the left tar was dissolved in toluene and filtered over short pad of SiO_2 to afford the crude **13** (300 mg). Crystallization from EtOH afforded 272 mg (91%) of **13** as colorless prisms. 3β-Benzoyloxylanosta-7,9(11)-dien-32-al (**251**): m.p. 222–223°C. $[\alpha]_{546}^{23} = -16$, $[\alpha_{578}^{23} = -8$ (c = 0.5, chl.). IR (KBr): 3061w, 2973m, 2959s, 2931s, 2728w, 1709s, 1602w, 1282s, 1177w, 1115m, 1071w, 973m, 712m. ^{1}H NMR (600 MHz, $CDCl_3$): 9.41 [s,H-C(32)]; 8.05 [d,*J* = 7.5 Hz, H-C(2′), H-C(6′)]; 7.56 [t,*J* = 7.1 Hz, H-C(4′)]; 7.45 [t,*J* = 7.4 Hz, H-C(3′), H-C(5′)]; 5.61 [br.s, H-C(7) or H-C(11)]; 5.6 [br.s, H-C(11) or H-C(7)]; 4.74 [dd,*J* = 11.6, 3.7 Hz, H-C(3)]; 1.13 (s, CH_3); 1.09 (s, CH_3); 0.95 (s, CH_3); 0.89 [d,*J* = 6.2 Hz, H_3C-C(20)]; 0.863 [d,*J* = 6 Hz, H_3C-C(25)]; 0.858 [d,*J* = 6 Hz, H_3C-C(25)]; 0.71 [s, H_3C-C(13)]. MS-CI (isobutane, m/z): 545 [$(M+1)^+$, 52]; 517 (50); 424 (45); 423 (100); 395 (24); 307 (3); 257 (4); 123 (17). Analysis calculated for $C_{37}H_{52}O_3$ (544.82): C 81.57; H 9.62. Found: C 81.53; H 9.62.

6. 3β-Hydroxylanosta-7,9(11)-dien-32-al (16)

3β-Benzoyloxylanosta-7,9(11)-dien-32-al (**13**; 850 mg, 1.56 mmol) was hydrolyzed with KOH (100 mg) in EtOH (20 mL) at reflux. After usual work up and treating of the crude product with hot MeOH, 618 mg (90%) of **16** was obtained. 3β-Hydroxylanosta-7,9(11)-dien-32-al (**16**), m.p. 126–128°C, was in all respects identical with the one synthesized in reference 42.

7. (32S)-32-Methyllanosta-7,9(11)-diene-3β,32-diol (14)

A stirred solution of 3β-benzoyloxylanosta-7,9(11)-dien-32-al (**13**; 190 mg, 0.35 mmol) in dry THF (30 mL) was cooled under dry argon to −95°C (acetone/liquid N_2), and was treated with MeLi (1.48 M in ether; 0.47 mL; 0.70 mmol). After 30 min, solid NH_4Cl (500 mg) was added to the reaction mixture followed by 0.5 mL of H_2O, and the mixture was left to warm to r.t. overnight. The reaction mixture was then extracted with $Et_2O/CHCl_3$ (5:1; 3 × 10 mL), washed with brine, and dried over Na_2SO_4. Upon chromatography of the crude product (SiO_2, Lobar A column, hexane/EtOAc = 80:20) and crystallization from diisopropyl ether, 130 mg (82%) of diol **14** was obtained. (32*S*)-32-methyllanosta-7,9(11)-diene-3β,32-diol (**14**): m.p. 194–196°C (colorless plates-needles). $[\alpha]_{546}^{26} = +64$; $[\alpha]_{578}^{26} = +56$ (c = 0.5, chl.). IR (KBr): 3368m, 2960s, 2932s, 2889m, 2867m, 1461m, 1088m, 1075m, 1029m cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): 5.46 [d,*J* = 5.8 Hz, H-C(7 or 11)]; 5.42 [d,*J* = 4.9 Hz, H-C(11 or 7)]; 3.88 [bd,*J* = 5.9 Hz, H-C(32)]; 3.23 [dd,*J* = 11.4 Hz, 3.9 Hz, H-C(3)]; 2.51 [d,*J* = 18.2 Hz, 1H); 1.01 [d,*J* = 5.9 Hz, irradiation at 3.88 ppm → s, H_3C-C(32)]; 1.00 (s, CH_3), 0.98 (s, CH_3), 0.91 [d, *J* = 6.5 Hz, H_3C-C(20)]; 0.88 (s, CH_3), 0.863 [d,*J* = 6.6 Hz, H_3C-C(25)]; 0.858 [d,*J* = 6.6 Hz, H_3C-C(25)]; 0.66 [s, H_3C-C(13)]. MS-EI (70 eV, m/z): 457 ([M+1]$^+$, 6); 456 (M^+, 8); 412 (100), 411 (99); 393 (47); 245 (29); 131 (29); 119 (18). Analysis calculated for $C_{31}H_{52}O_2$ (456.75): C 81.52; H 11.48. Found: C 81.02; H 11.13.

8. (32S)-32-methyllanosta-7,9(11)-diene-3β,32-diol 3,32-dibenzoate (15)

(32*S*)-32-Methyllanosta-7,9(11)-diene-3β,32-diol (**14**) was benzoylated in 95% yield to give (32*S*)-32-methyllanosta-7,9(11)-diene-3β,32-diol 3,32-dibenzoate (**15**): m.p. 213.5–215°C, (colorless prisms, 95% ethanol). $[\alpha]_D^{24} = +106.9$ (c = 0.9, chl.). IR (KBr): 3061w, 3032w, 2997s, 2960s, 2946s, 2863s, 1712s, 1673w, 1653w, 1602w, 1585m, 1275s, 1254s, 1115s, 1097s cm^{-1}. ^{1}H NMR (600 MHz, $CHCl_3$): 8.07–7.42 (aromatic); 5.59 [d,*J* = 6.3 Hz, H-C(7 or 11)]; 5.51 [d,*J* = 5.7 Hz, H-C(11 or 7)]; 5.43 [q,*J* = 6.2 Hz, H-C(32)]; 4.78 [dd,*J* = 11.8 Hz, 4.3 Hz, H-C(3)]; 2.51 [d,*J* = 18.3 Hz, 1H); 1.12 (s, CH_3); 1.071–1.066 (m, 2 CH_3); 0.98 (s, CH_3); 0.771–0.747 (m, 3 CH_3); 0.67 [s, H_3C-C(13)]. MS-DEI (70 eV, m/z): 664 (M^+, 11); 542 (54); 515 (43); 429 (25); 401 (10); 393 (23); 374 (15); 285 (11); 185 (27); 105 (100). Analysis calculated for $C_{45}H_{60}O_4$ (664.98): C 81.28; H 9.09. Found: C 81.09; H 8.98.

9. 3β-benzoyloxy-7α-hydroxylanost-8-ene-32, 11α-lactone (21)

3β-benzoyloxylanosta-7,9(11)-dien-32-al (**13**; 50 mg; 0.09 mmol) was dissolved in dioxan (10 mL) and NH_2SO_3H (38 mg) in H_2O (5 mL) was added at 0°C, followed by $NaClO_2$ (9.95 mg; 0.11 mmol). After stirring at 0°C for 5 h, the reaction mixture was worked up as in reference 38. The crude mixture was purified through a SiO_2 column, to afford 11 mg of hydroxylactone **21** as concluded according to the following data: m.p. 192.5–194.5°C (diisopropyl ether). IR (KBr): 3510m, 3060w, 2955s, 2936s, 2911m, 2871m, 1751s, 1723s, 1695m, 1601w, 1584w, 1277s, 1222m, 1202m, 1172m, 1151m, 1113s, 981s, cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): 8.04 [d,*J* = 8.01 Hz, H-C(2′), H-C(6′)]; 7.56 [t,*J* = 7.1 Hz, H-C(4′)]; 7.45 [t,*J* = 7.2 Hz, H-C(3′), 5.12 (bs, H-C(11)]; 4.81 [dd,*J* = 11.9 Hz, 4.5 Hz, H-C(3)]; 4.22 [bs, H-C(7)]; 1.07 (s, CH_3); 1.02 (s, CH_3); 1.01 (s, CH_3); 0.88–0.86 (m, 3 CH_3); 0.74 [s, H_3C-C(13)]. MS-DEI (70 eV, m/e): 532 [$(M-44)^+$, 3]; 514 [$(M-44)^+ - H_2O$, 61]; 401 (93); 377 (9); 279 (42); 209 (10); 105 (100). Analysis calculated for $C_{37}H_{52}O_5$ (576.82): C 77.04; H 9.09. Found: C 77.13; H 8.97.

10. 3β-benzoyloxy-7-oxolanost-8-ene-32,11α-lactone (17)

3β-benzoyloxylanosta-7,9(11)-dien-32-al (**13**; 100 mg; 0.184 mmol) was dissolved in acetone (15 mL) and the Jones reagent (138 μL, 0.368 mmol) was added at 0°C. The reaction mixture was left to warm to r.t. within 2 h, and was poured onto ice/water. The crude product was obtained after extraction with EtOAc (3 × 10 mL), drying the organic solvent over anh. Na_2SO_4, and evaporation to dryness. Chromatography (SiO_2, eluent toluene) and crystallization from acetone afforded pure **17** (75 mg; 71%) as colorless needles. 3β-Benzoyloxy-7-oxolanost-8-en-32,11α-lactone (**17**): m.p. 229–232°C. $[\alpha]_D^{22} = +41$ (c = 0.1, chl.). UV λ_{max}^{MeOH}: 231 nm (11500), 250 nm (5000). IR (KBr): 3069w, 2957s, 2936s, 2868m, 1741s, 1717s, 1672s, 1600w, 1585w, 1278s, 1118m, 710s cm^{-1}. IR (CCl_4): 2958s, 1763s, 1721s, 1680m, 1273s, 1112m cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): 8.04 [d,*J* = 7.7 Hz, H-C(2′), H-C(6′)]; 7.58 [t,*J* = 7.3 Hz, H-C(4′)]; 7.45 [t,*J*-7.6 Hz, H-C(3′), H-C(5′)]; 5.27 [dd,*J* = 2.9 Hz, 1.9 Hz, H-C(11)]; 4.79 [dd,*J* = 11.2 Hz, 4.2 Hz, H-C(3)]; 2.88–2.84 (m, 1 H), 1.23 (s, CH_3), 1.13 (s, CH_3), 0.98 (s, CH_3), 0.88 [d,*J* = 6.6 Hz, H_3C-C(20)]; 0.861 [d,*J* = 6.7 Hz, H_3C-C(25)]; 0.856 [d,*J* = 6.5 Hz, H_3C-C(25)]; 0.75 [s, H_3C-C(13)]. ^{13}C NMR (150 MHz, $CDCl_3$): 195.86, 174.79, 165.94, 164.72, 133.03, 132.79, 130.47, 129.56, 128.44, 79.54, 72.83, 61.32, 52.60, 49.58, 48.07, 41.63, 39.32, 38.04, 37.86, 36.30, 35.62, 35.44, 34.12, 29.08, 27.97, 27.48, 24.00, 23.62, 22.80, 22.49, 21.23, 19.19, 18.48, 16.41. MS-CI (isobutane, 70eV, m/z): 575 [$(M+1)^+$, 8]; 531 (63); 425 (65); 409 (100); 395 (7); 259 (7); 123 (28); 81 (15). Analysis calculated for $C_{37}H_{50}O_5$ (574.80): C 77.32; H 8.77. Found: C 77.04; H 8.54.

11. Hydrolysis of lactone 17

3β-benzoyloxy-7-oxolanost-8-ene-32,11α-lactone (**17**; 150 mg; 0.26 mmol) was treated with KOH (40 mg) in EtOH (100 mL) at reflux for 2 h. The reaction mixture was then cooled to r.t., acidified with AcOH, and evaporated to dryness. Upon column chromatography (SiO_2, eluent toluene, toluene/EtOAc = 9:1) lactone **18** (99.5 mg) and 3β-hydroxy-7-oxolanost-8-en-32-oic acid (**19**; 15 mg) were isolated in 81% and 12% yield, respectively. 3β-Hydroxy-7-oxolanost-8-ene-32,11α-lactone (**18**): m.p. 172–175°C (colorless needles, toluene). $[\alpha]_D^{24}$ = +73.5 (c = 1.0, chl.). UV λ_{max}^{MeOH}:251 nm (9600). IR (KBr): 3475m, 2957s, 2933s, 2871s, 1761s, 1666s, 1599w, 1467m, 1382m, 1342m, 1312m, 1274m, 1033m, 1000m cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): 5.25 [dd,*J* = 3.6, 1.8 Hz, H-C(11)]; 3.31 [dd,*J* = 10.8, 4.5 Hz, H-C(3)]; 2.93–2.78 (m, 1 H); 1.16 (s, CH_3); 1.01 (s, CH_3); 0.90 (s, CH_3); 0.87 [d,*J* = 6.2 Hz, H_3C-C(20)]; 0.858 [d,*J* = 6.6 Hz, H_3C-C(25)]; 0.853 [d,*J* = 6.6 Hz, H_3C-C(25)]; 0.73 [s, H_3C-C(13)]. MS-CI (isobutane, m/z): 471 [(M+1)$^+$, 19]; 427 (100); 409 (28); 392 (7); 296 (10); 210 (3); 123 (3). Analysis calculated for $C_{30}H_{46}O_4 \times 1/2\ H_2O$ (479.70): C 75.12; H 9.88. Found: C 75.33; H 9.53. 3β-Hydroxy-7-oxolanost-8-en-32-oic acid (**19**): m.p. 215–220°C (pale yellow needles; acetone). $[\alpha]_D^{23}$ = −25.9 (c = 0.5, chl.). UV λ_{max}^{MeOH}: 252 nm (7800). IR (KBr): 3640–2470 m br., 2954s, 2933s, 2869s, 1697s, 1661s, 1592m, 1467m, 1374m, 1242m, 1197m cm^{-1}. ^1H NMR (600 MHz, lock $CDCl_3$ at 7.25978 ppm): 3.31 [dd,*J* = 11.6, 4.4 Hz, H-C(3)]; 2.72 (m, 1 H); 1.23 (s, CH_3); (s, CH_3; 0.92 [d,*J* = 6.5 Hz, H_3C-C(20)]; 0.89 (s, CH_3); 0.856 [d,J = 6.6 Hz, H_3C-C(25)]; 0.851 [d,*J* = 6.7 Hz, H_3C-C(25)]; 0.73 [s,H_3C-C(13)]; lock TMS ($CDCl_3$: 7.25809 ppm): 9.38 (m, 0.38 H); 3.50 (br.s, 0.66 H) both exchangeable with D_2O. ^{13}C NMR (DEPT; 50 MHz, $CDCl_3$): 201.19(q), 177.57(q), 171.02(q), 132.48(q), 77.20(*CH*), 59.09(q), 50.23 (*CH*), 48.62 (*CH*), 47.29(q), 40.59 (CH_2), 39.37 (CH_2), 39.03(q), 36.35 (CH_2), 36.10 (CH_2), 36.01 (*CH*), 34.27 (CH_2), 29.76 (CH_2), 29.56 (CH_2), 27.94 (CH), 27.37 (CH_3), 27.25 (CH_2), 24.90 (CH_2), 22.78 (CH_3), 22.49 (CH_3), 18.53 (CH_3), 16.88 (CH_3), 15.41 (CH_3). MS-DCI (isobutane, m/z): 474 [(M+2)$^+$, 24]; 473 [(M+1)$^+$, 70]; 455 (24); 441 (17); 429 (57); 427 (50); 410 (22); 391 (100); 279 (13); 214 (9); 113 (13). Analysis calculated for $C_{30}H_{48}O_4$ (472.71): C 76.23; H 10.24. Found: C 76.15; H 10.31.

X-ray analyses

Crystal measurement and refinement data for compounds **15** and **17** (Figure 2) are summarized in Table 1. Lattice parameters were refined using 20 reflections in the range $10° \leq 2\Theta \leq 50°$. Huber four circle diffractometer with Rigaku rotating anode generator, graphite monochromatized CuKα radiation (λ = 1.54178Å). One standard reflection was checked every 50 reflections, and no significant deviation was observed. Structures solved by direct methods using SHELXS86.[17] Anisotropic least squares refinement (SHELXL93)[18] using F^2. For both structures, all the hydrogen atoms were calculated with AFIX) and refined with a common isotropic temperature factor. The C_8H_{17} side chain is highly agitated in **15**, and restraints on bond lengths were applied (target value for C-C 1.54Å (σ 0.03Å). Two positions were refined for atoms C(26) and C(27); in the end of the refinement, their occupation factors converged to 0.62 (position A) and 0.38 (position B). Atomic scattering factors from international tables for X-ray crystallography (1974, Volume 4). Complete X-ray data have been deposited with the Cambridge Crystallographic Data Centre.

Table 1 Data Collection and Refinement Parameters for **15** and **17**

	15	**17**
Formula	$C_{45}H_{60}O_4$	$C_{37}H_{50}O_5$
Mr	664.93	574.77
System	monoclinic	monoclinic
Space group	$P2_1$	$P2_1$
a(Å)	10.259(2)	6.804(1)
b(Å)	13.984(2)	12.621(1)
c(Å)	14.061(3)	19.092(2)
(β°)	100.46(2)	96.31(1)
V(Å^3)	1983.7(6)	1629.6(3)
Z	2	2
Dx(gcm^{-3})	1.11	1.17
λ(Å)	1.54178	1.54178
F(000)	724	624
μ(mm^{-1})	0.534	0.599
Approximate crystal size (mm)	0.30 × 0.18 × 0.12	0.50 × 0.32 × 0.08
Collection range (sinΘ/λ)max Å^{-1}	0.60	0.60
Range of *hkl*	0≤h≤12 −16≤k≤16 −16≤l≤16	0≤h≤8 0≤k≤15 −22≤l≤22
Indices of standard refl.	210	1-1-6
No. of collected indep refl.	6956	3078
No. of observed refl. (I≥2σ(I))	4717	2270
No. of parameters	463	381
No. of restraints	11	1
U of the H atoms (Å^2)	0.120	0.138
R (I≥2σ(I))	0.063	0.071
(all data)	0.086	0.085
wR_2	0.163	0.217
weight	1/[σ^{2}Fo^2+0.1080P^2]	1/[σ^{2}Fo^2+0.1704P^2]
S	0.956	1.104
Extinction parameter	0.0059	0.01
Shift/esd max	0.21	0.10
(max, min) (e/Å^3)	0.17 − 0.24	0.32 − 0.27

Biochemistry

Materials

The sources of the following drugs or agents are indicated in parentheses: cholestyramine (Lucky Pharmaceutical Co., Seoul, Korea) and lovastatin (Dr. Y-K. Sim, Choongwae Pharmaceutical Co., Suwon, Korea). The following isotopes were purchased from Amersham Intl. Buckinghamshire, U.K.: [2-^{14}C]mevalonolactone (55 mCi/mmol), 3β-hydroxy-3-methyl[3-^{14}C]glutaryl coenzyme A (27.6 Ci/mmol), (*R,S*)-[2-^{14}C]mevalonic acid lactone (56 mCi/mmol), and (*R,S*)-5-^{3}H-mevalonic acid (dibenzylethylenediamine salt, 55 mCi/mmol), HMG-CoA, NADP$^+$, NADPH, mevalonolactone, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase, were from Sigma (Chemical Co. (St. Lous, Missouri, USA). Sterols, including 25-hydroxycholesterol, lanosterol, 7-dehydrocholesterol, and cholesterol were purchased form Steraloids (Wilton, New Hampshire, USA) and recrystallized once before use. Zymosterol (5α-cholesta-8,24-dien-3β-ol) and 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol were exactly the same as described previously.[20,21] All others reagents were of the best grade available.

Animals and diet feeding

Male Sprague-Dawley rats (200–250 g body weight) were maintained on a standard rodent chow supplemented with 5%

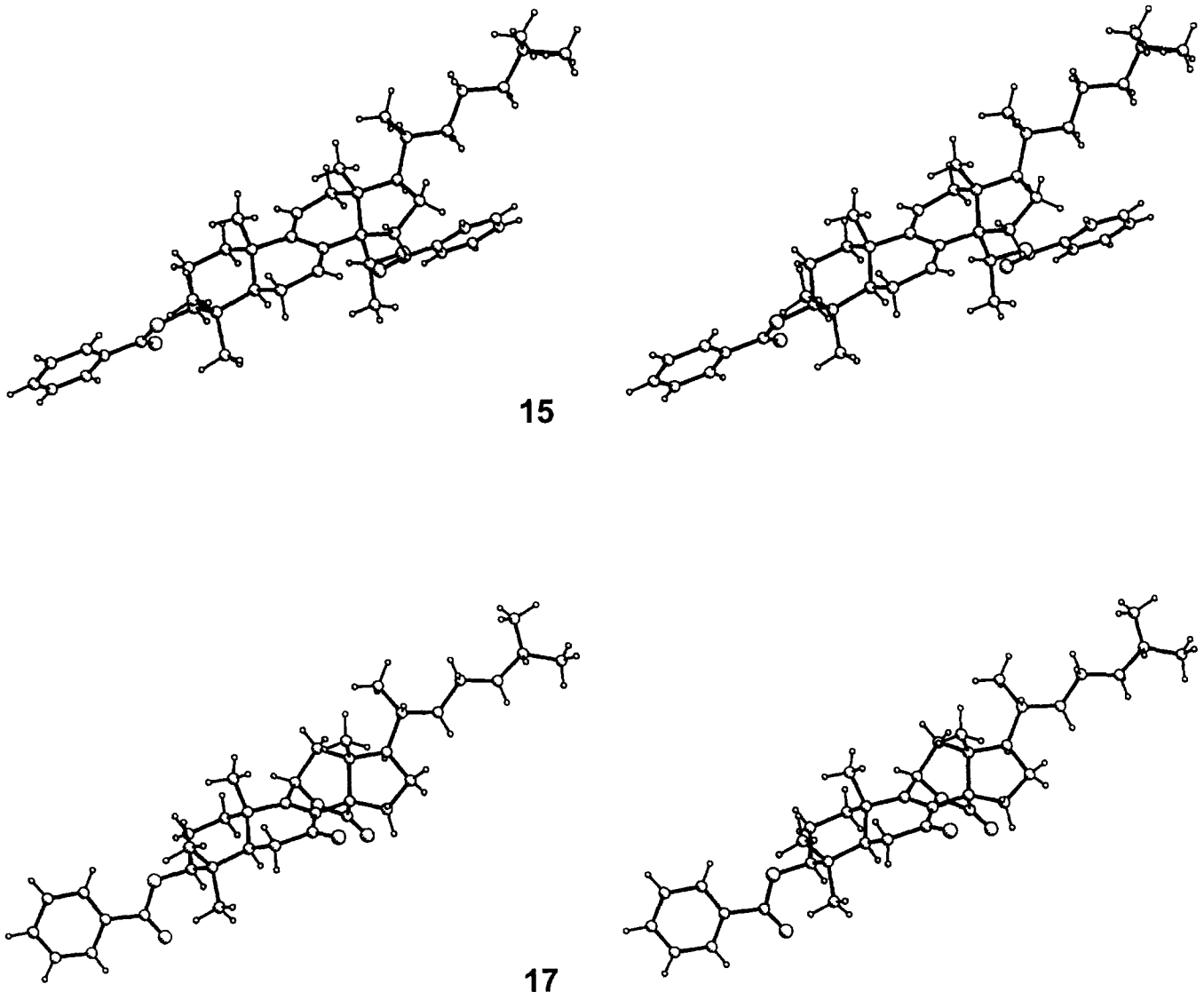


Figure 2 Stereoscopic views of dibenzoate **15** and lactone **17**.[19]

cholestyramine plus 0.1% lovastatin (CL-diet)[22] and various agents under a reverse light cycle (light 6:00 p.m. to 6:00 a.m.; dark 6:00 a.m. to 6:00 p.m.) unless otherwise specified. Food was supplied between 9:00 and 10:00 a.m. every day unless otherwise indicated.

Preparation of microsomes, sterol substrates, and enzyme assays

Rats were killed by decapitation at the midpoint of the dark period, and their livers were excised and processed for microsome preparation as described previously.[22] The sterol 7-reductase assay was performed using 7-dehydrocholesterol as substrate.[21] The assay of other enzymes, such as sterol 8-isomerase,[20] sterol 14-reductase,[21] sterol 24-reductase,[21] and HMGR,[23] was performed as described.[28] Microsmal cholesterol concentration was measured by gas-liquid chromatography (260°C, FID, 3% OV-17 on Chromosorb WHP 120) in Young-In 680 D GC or Hewlett Packard GC 5890 (FID, capillary column: SAC-5, 5% diphenyl/95% dimethylsiloxane, 30 m × 0.25 mm, 0.25 μm ID, flow rate 2.44 mL/min), using 5α-cholestane as a standard.[22] Quantification of protein was performed according to the method of Lowry.[24]

Results and discussion

The unsubstituted $\Delta^{7,9(11)}$ system has been chosen as a structural modification because it provides sp^2 hybridization at C(8) and C(9) and extends the flattening to C(7) and C(11); it also was of interest to explore the effect of the conjugated system with a flattened steroidal ring C on several purified enzymes. The influence of functional groups such as CHROH (R = H; CH_3) and CHO attached to C(14) was envisaged to be screened having in mind that hydroxy and formyl derivatives are formed during demethylation of lanosterol in mammals, and of COOH, which we became interested in upon finding that natural product panasterol[25] exhibits a pronounced antitumor activity (panasterol is 3β-hydroxylanosta-8,24-dien-32-oic acid). The Δ^7 analogue also possessing antitumor activity has been synthesized.[26] In addition, the influence of C(14) cyano group has also been examined ($\Delta^{7,9(11)}$), as well as that of C(11) carbonyl ($\Delta^{8(9)}$ and Δ^6).

The synthesis of $\Delta^{7,9(11)}$ system

Functionalization of C(14) methyl group in lanostane series has usually been performed using the nitrite photocyclization reaction,[11,27] LTA,[28–30] and LTA/I_2 reaction.[26,29] One limitation of this approach is the low yield of the desired C(32) lanostene derivatives due to poor regioselectivity of hydroxy group elimination[31] or tetrahydrofuran (a LTA cyclization product) ring-opening reactions,[29,30,32] providing various mixtures of Δ^6, Δ^7, and Δ^8 isomers.[33] Also, until now the synthetic approach to $\Delta^{7,9(11)}$ system used the Se- and Hg-based methods for the introduction of a double bond.[33,34]

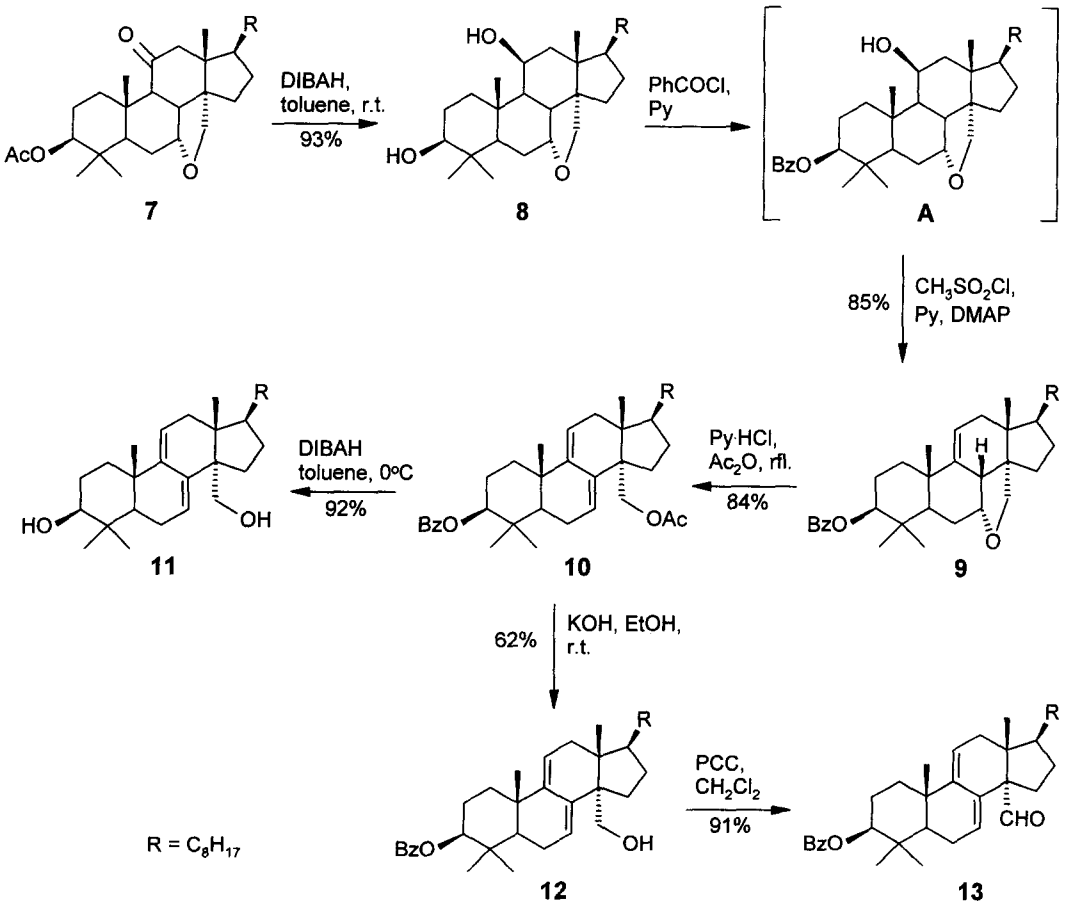
The lanostane derivatives substituted at C(32) along with $\Delta^{7,9(11)}$ pattern were prepared by exploiting an easy and nearly quantitative transformation of HOβ-C(11) into $\Delta^{9(11)}$ double bond,[35] followed by the facilitated abstraction of allylic 8β-hydrogen by a base. We expected to obtain only one lanostadiene and to avoid tedious separation of isomers.

Scheme 1

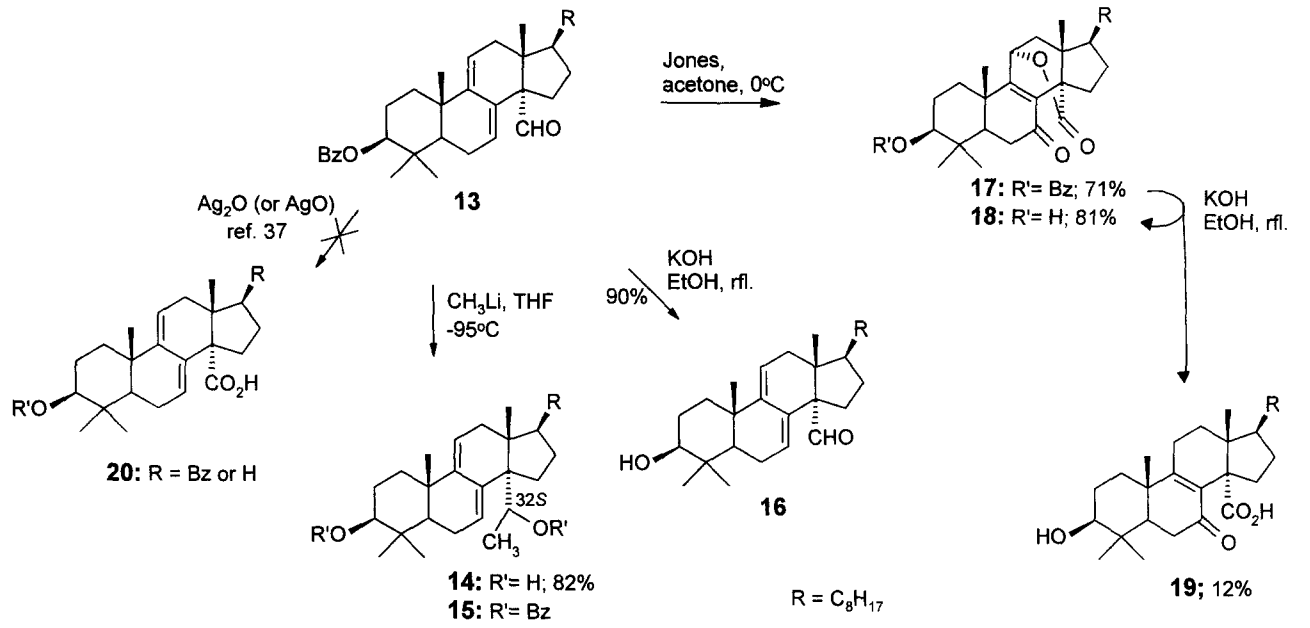
7α,32-epoxylanostane-3β,11β-diol (**8**) was obtained in 93% yield by reducing the 3β-acetoxy-7α,32-epoxylanostan-11-one (**7**; Scheme 1).[36] Diol **8** was then treated with benzoyl chloride in pyridine, and the resulting crude monobenzoate **A** was subsequently treated with mesyl chloride in the presence of DMAP to afford the alkene **9** in 85% yield. The high regioselectivity of the eliminative ring opening was achieved using pyridinium chloride in Ac_2O at reflux (**10**: 84%). Reduction of **10** afforded diol **11** having the same properties as an authentic sample,[36] whereas selective hydrolysis gave monobenzoate **12** in 62% yield. It is interesting to note that the attempts on selective hydrolysis of the corresponding diacetate gave only the mixtures of monoacetates and the diol **11** (in ca. (1:1:1)-ratio). Aldehyde **13**, obtained from **12** by oxidation under anhydrous conditions, was envisaged to be transformed into C(32) monoalkylated alcohols and the corresponding acid **20**.

Scheme 2

The reaction of **13** with CH_3Li at −95°C afforded only **14** (82%); the configuration at secondary C(32) was determined as (32S) by an X-ray analysis of the corresponding dibenzoate **15** (vide supra). It is interesting to note that the observed stereoselectivity was not detected with Δ^7 system (1.5:1 diastereomeric ratio)[8] using Grignard reagent as nucleophilic species. The exclusive attack to the *Si* side of C(14) formyl group of **13** (as compared with both side attack in Δ^7 analogue) is probably facilitated by the absence of Hα-C(9), the hindrance of which competes with that of Hα-C(15) in compound **B** (Figure 3).



Scheme 1



Scheme 2

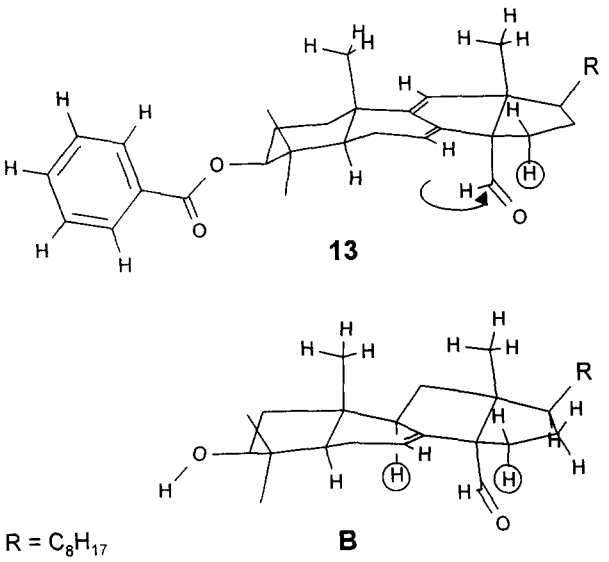


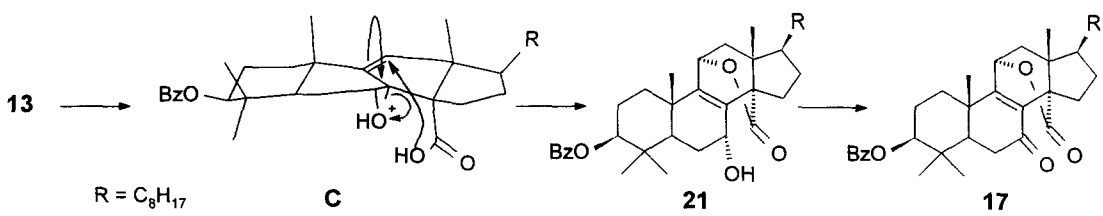
Figure 3 The aldehydes **13** and **B** in their most their stable conformations as calculated by molecular-mechanics calculations. For **B**, the next most stable conformation of formyl group is not given.

The oxidation of aldehydes **13** and **16** into corresponding acid **20** was attempted using (a) silver oxides,[37] (b) $NaClO_2$,[38–40] (c) PDC[41] (**12**), and (d) the Jones reagent (**12** and **13**). An attempt to oxidize **13** using silver oxides (under alkali conditions) resulted only in hydrolyzed product **16** (quant) (alternatively, the aldehyde **16** was synthesized via corresponding nitrile,[42]) whereas the PDC oxidation of alcohol **12** afforded only aldehyde **13** (in DMF also). Oxidation of **13** by Jones reagent gave in good yield (71%) a new compound that was less polar (TLC) than the starting material. The structure of **17** was proposed on the basis of its spectroscopic data (see "Experimental") and was confirmed by an X-ray analysis (vide supra). The lactone **17** was also obtained directly from alcohol **12** in 55% yield. We propose that the lactone formation is driven by an attack of the carboxylic acid on the double bond of the allylic epoxide **C** formed in situ (Scheme 3).

Scheme 3

In our hands, the overoxidation of 7,9(11)-diene system could not be avoided during $NaClO_2$ oxidation of aldehydes **13** and **16** using various reaction conditions and HOCl scavengers (amidosulfonic acid, 2-methyl-2-butene).[38–40] The only isolable product was identified as **21** (from **13**).

Hydrolysis of lactone **17** afforded the corresponding hydroxy compound **18** and was accompanied by the carboxylic acid **19** in ca. 8–13% yield (instead of expected



Scheme 3

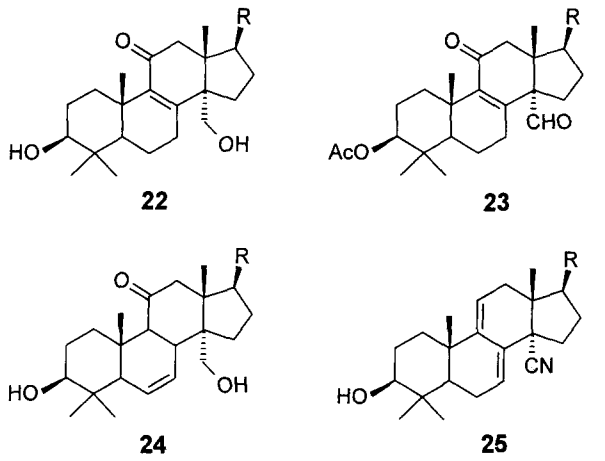


Figure 4 The synthesis of 11-ketones **22–24** was described in reference 36, and that of nitrile **25** in reference 42.

11α-hydroxy acid). The structure of **19** was determined on the basis of its spectral data (The mechanism of its formation is not clear enough. To check whether the acid **19** is the product of the artifact contaminating the sample of its precursor, the hydrolysis was also conducted with (TLC and) ^{1}H NMR (600 MHz) pure sample of **17**, affording **19** in 10% yield).

Biochemistry

To examine the potential cholesterol-lowering effects of lanostene derivatives, we tested the synthesized compounds (**11, 14, 16, 18, 19** and **22, 23, 24, 25**; Figure 4) on several cholesterogenic enzymes for their inhibitory activities.

It is of interest to note that **16** exhibited the widest range of inhibition against cholesterogenic enzymes tested here (Table 2). Aldehyde **16** significantly inhibits the representative enzymes involved both in early pathway (HMGR) and in the post-mevalonate pathway (sterol 7-reductase, sterol 8-isomerase, and sterol 14-reductase), which are all membrane-bound nonoxidative enzymes, with microsomal sterol 14-reductase activity considerably influenced by this compound (IC_{50} = 86 μM).

It is assumed that this potent inhibition may be due to the presence of C(32) aldehyde group. In fact, the C(32) aldehyde group containing intermediate has been proposed as an obligatory intermediate during the C(14) demethylation step, which is catalyzed by lanosterol 14α-methyl demethylase.[43–46] Compound **16** may be a good candidate for a novel cholesterol-lowering agent because it shows significant inhibitory effects on the most of the cholesterogenic enzymes tested. Further experiments are required in order to determine the patterns of inhibition on each enzyme, as well as the corresponding inhibition constants, K_i. In addition to **16**, both **11** and **24** also inhibit HMGR activity. It is not known at present how these derivatives may affect HMGR. In general, increased polarity at C(14) or C(7) of the lanostane derivatives such as **19, 14,** and **18** make them more potent inhibitors of $\Delta^{5,7}$-diene conversion into 5-ene and $\Delta^8 \rightarrow \Delta^7$ isomerization. For example, compound **19** containing both carboxylic acid at C(14) and C(7) oxo group has exhibited the highest inhibitory effect on the sterol 8-isomerase activity.

Reduction of $\Delta^{24(25)}$-double bond (sterol 24-reductase) in comparison with the reactions catalyzed by sterol 7-reductase, sterol 8-isomerase, and sterol 14-reductase appears to be least affected by compounds containing carboxyl, lactone, aldehyde, and the hydroxy groups at C,D rings. Recently, a similar observation was made by Bae and Paik,[47] in which the sterol 24-reductase was not affected at all by AY-9944, a common inhibitor for the sterol 7-reductase, sterol 8-isomerase and sterol 14-reductase.[20,48,49]

Conclusion

The aim of present work was to explore the influence of flattened rings B and C on the in vitro inhibition of several cholesterogenic enzymes. Here, we presented the synthesis of new $\Delta^{7,9(11)}$ lanostadiene derivatives variously functionalized at C(32) in 34–61% yields starting from known ketone **7**. The introduction of $\Delta^{7,9(11)}$ moiety was achieved in high yield (**8** → **10**; 71%) by C(9) = C(11)-directed allylic hydrogen abstraction. It was shown that change of C(11) from sp^3 to sp^2 (conjugated $\Delta^{7,9(11)}$ system, or C(11)

Table 2 Effects of Lanostane Derivatives on Various Cholesterogenic Enzymes

	Inhibition of enzymic activity (%)[a]				
Compound	HMGR at 100 μM	7-reductase at 200 μM	14-reductase at 200 μM	24-reductase at 200 μM	8-isomerase at 200 μM
11	−44.3	−6.4	+3.6	−3.5	−2.7
14	+12.2	−24.3	+2.5	−17.1	−19.9
16	−34.9	−18.0	−70.1[b]	−7.4	−24
25	+14.6	+1.8	−0.6	+18	−22.1
22	+24.5	−17.8	+2.7	−6.3	−17.8
23	−8.2	−20.2	−22.8	−14.3	−6
24	−36.9	−11.6	−11.2	−1.7	−33.1
18	−1.8	−34.2	−6.2	−14.4	−9.1
19	+12.8	−29	−8	−11.4	−45.9

[a]Results represent the mean values of duplicate samples under optimal conditions.
[b]IC_{50} = 86 μM.

= O) exerts moderate effect on the inhibitory activity of HMGR, sterol 14-reductase, sterol 7-reductase, and sterol 8-isomerase. The most active was aldehyde **16**, which partially inhibits enzymes involved both in early pathway (HMGR) and in the postmevalonate pathway, with the lowest IC_{50} = 86 μM for sterol 14-reductase.

Acknowledgment

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