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A Simplified Synthesis of 32-Oxygenated Lanosterol Derivatives

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A simplified synthesis of lanost-8-ene-3β,32-diol, lanost-7-ene-3β,32-diol, 3β-hydroxylanost-8-en-32-al, and 3β-hydroxylanost-7-en-32-al, in which 3β-acetoxylanostan-7α-ol prepared by the hydrogenation of 3β-acetoxylanost-8-en-7-one is the key compound, is described.

Keywords——32-oxygenated sterol; 3β-acetoxylanostan-7α-ol; lanosterol 14-demethylation; cholesterol biosynthesis

The biosynthesis of cholesterol from lanosterol requires the removal of the three methyl groups at carbons 4 and 14. The initial step in the removal of these methyl groups has been considered to be the 14-demethylation,[1] which is a complex process, and many aspects of the overall mechanisms remain unclear. A probable intermediate is the lanosterol derivative with a 14-hydroxymethyl, 14-aldehyde or 15-hydroxy group.

Recently, it has been shown that 24(*S*),25-epoxycholesterol[2] is produced in the liver by way of a branch in the sterol biosynthetic pathway, beginning with the formation of squalene 2,3(*S*); 22(*S*),23-dioxide, and the dioxide is known to accumulate in cultured cells that have been treated with oxidosqualene cyclase blocking agents. The squalene dioxide is converted first to 24(*S*),25-oxidolanosterol and finally to 24(*S*), 25-epoxycholesterol, which has been shown to be present in the liver. We have reported[3] that 24(*S*),25-oxidolanosterol and 24(*S*),25-epoxycholesterol inhibited cholesterol biosynthesis from 24,25-dihydrolanosterol *in vitro*. Further, we recently reported[4] that 15-oxygenated lanosterol derivatives inhibit cholesterol biosynthesis from 24,25-dihydrolanosterol *in vitro*. On the other hand, the 32-hydroxylated lanosterol derivatives[5] have been shown to inhibit sterol biosynthesis in animal cells in culture. Such naturally occurring oxygenated steroids may be important in regulating sterol biosynthesis.

With the intention of investigating the effects of the natural precursors on cholesterol biosynthesis from 24,25-dihydrolanosterol, we studied the synthesis of the 32-oxygenated derivatives of 24,25-dihydrolanosterol. This report describes a simplified synthesis of lanost-8-ene-3β,32-diol (**6**), lanost-7-ene-3β,32-diol (**7**), 3β-hydroxylanost-8-en-32-al (**9**), and 3β-hydroxylanost-7-en-32-al (**10**).

For the synthesis of 32-oxygenated compounds, 3β-acetoxylanostan-7α-ol (**2**) was used as the key compound. Barton and Thomas[6] obtained **2** by the reduction of 3β-acetoxylanost-8-en-7-one with lithium in liquid ammonia, followed by catalytic reduction, though in unspecified yield. On the other hand, Parish *et al.*[7] obtained **2** by the reduction of a mixture of 7α, 8α- and 8α, 9α-epoxylanostan-3β-ols, followed by selective acetylation, but the yield was low and the procedure troublesome. In this study, catalytic hydrogenation of 3β-acetoxylanost-8-en-7-one (**1**) in the presence of platinum dioxide in acetic acid afforded **2**, which was identical with an authentic sample synthesized by the method of Parish *et al.*,[7] in 26% yield. The other products were separated by column chromatography, affording 3β-

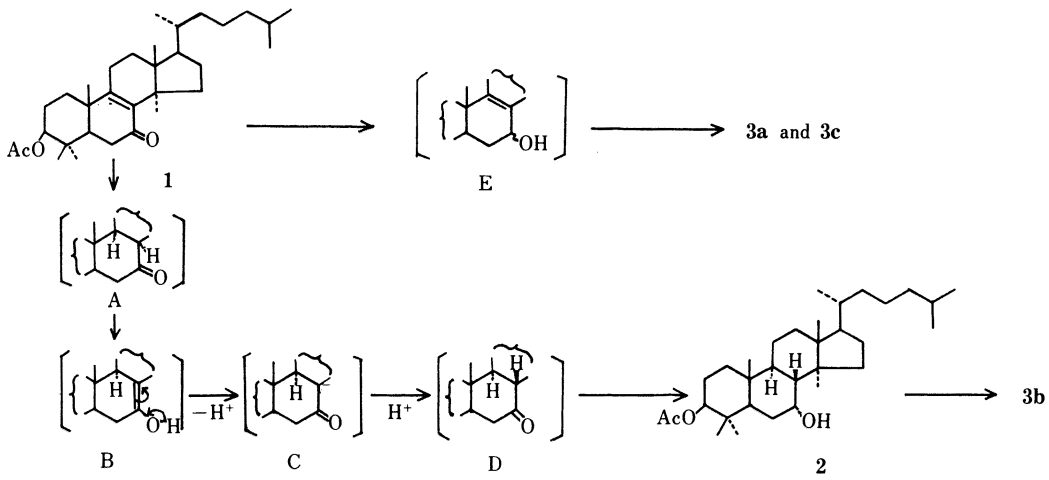

Chart 1

TABLE I. Isotopic Analysis of Deuterated Products

Deuterium content (%)	Deuterated compound			
	2	**3a**	**3b**	**3c**
$^2H_0$	3.8	1.5	1.2	7.1
$^2H_1$	15.1	13.8	7.0	29.3
$^2H_2$	21.4	46.9	27.6	33.2
$^2H_3$	42.8	28.4	43.3	10.6
$^2H_4$	14.8	9.4	18.6	10.1
$^2H_5$	2.2	0	2.3	9.7

acetoxylanost-8-ene (**3a**), 3β-acetoxylanost-7-ene (**3b**), and 3β-acetoxylanosta-7,9 (11)-diene (**3c**). The reaction probably proceeds as shown in Chart 1. The *cis* addition of H_2 from the α-side forms A, which is transformed to **2** *via* the enol compound (B), through deprotonation and protonation of intermediates (C and D, respectively), followed by hydrogenation of the latter. Here, **2** would be transformed to **3b** by dehydration. Compounds **3a** and **3c** would be formed *via* the 7α-hydroxy compound (E). In an attempt to clarify the mechanism of this reaction, **1** was catalytically hydrogenated in the presence of platinum dioxide in acetic acid-$^2H_1$ ($CH_3COO^2H_1$). The products contained unanticipated species ($^2H_0$- to $^1H_5$-compounds) as determined by mass spectrometric analysis (Table I). It is thought that this result is due to exchange between gas-phase hydrogen and the deuterium atom in acetic acid-$^2H_1$.[8] Although the distribution of deuteriums was not fully established, one deuterium at C-7 in **2**, **3a**, **3b** and **3c** and also one deuterium at C-8 in **2** were confirmed by comparison of the proton and/or carbon-13 nuclear magnetic resonance (^{1}H- and/or ^{13}C-NMR) spectra with those of undeuterated samples; these findings are consistent with the steps (A—E) in Chart 1. Now, **2** was reacted with lead tetraacetate according to the procedure of Parish *et al.*[7] to yield the 7,32-oxide (**4**) and 3β-acetoxy-7-oxolanostane (**5a**). Upon hydrolysis, **5a** gave 7-oxolanostan-3β-ol (**5b**). The circular dichroism spectrum of **5b** showed the same negative Cotton effect as that of 18-acetoxylanostan-3β-ol-7-one.[9] This result clearly indicated that the stereochemistry of the B/C ring in **2** and **5a** must be *trans* as in the natural product. Treatment of **4** with pyridinium hydrogen chloride–acetic anhydride followed by alkaline hydrolysis gave

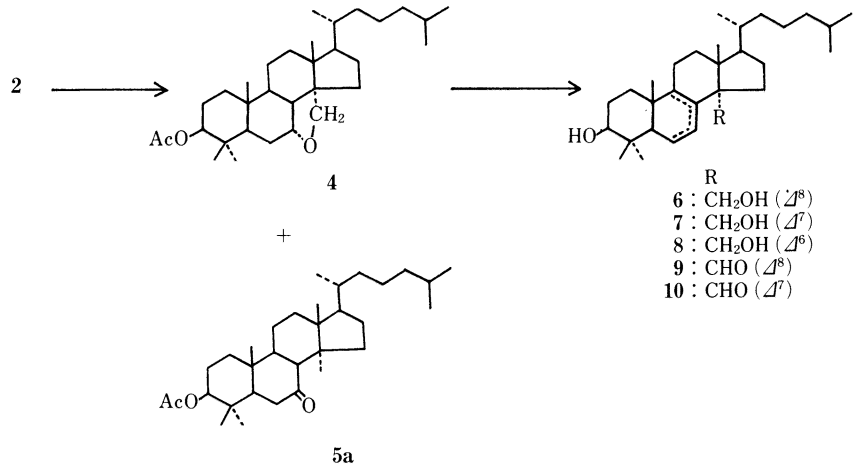

Chart 2

a mixture of 3,32-diols (**6**, **7**, and **8**). These compounds were separated by high-performance liquid chromatography (HPLC) on a μBondapak-NH_2 column using $CHCl_3$–*n*-hexane as the eluent. The Δ^6-3,32-diol (**8**) was eluted first, followed by Δ^7-3,32-diol (**7**) and finally the Δ^8-3,32-diol (**6**). Compound **6** was oxidized with Jones reagent followed by reduction with KBH_4 to give the Δ^8-32-al (**9**), and **7** was also converted to **10** in a similar manner.

Materials and Methods

^{1}H- and ^{13}C-NMR spectra were obtained on JEOL FX-200 NMR machines. Mass spectra (MS) were recorded on a JEOL D-100 spectrometer at 75 eV ionizing potential. HPLC was done on a μBondapak-NH_2 column (7.8 mm × 30 cm), using a Waters pump (model 510) and a Waters detector (model 480 spectrophotometer, set at 247 nm). Chloroform–*n*-hexane (1 : 1, v/v) was used as an eluent (flow rate 2.0 ml/min, pressure 100 kg/cm^2). Optical rotations were measured on a JASCO DIP-SL automatic polarimeter with a cell of 1 cm light path length, and circular dichroism (CD) spectrum was taken in 0.5 mm cell at room temperature (24—25 °C) in chloroform on a JASCO J-20 recording spectropolarimeter.

3β-Acetoxylanostan-7α-ol (2)——A solution of 3β-acetoxylanost-8-en-7-one[10] (**1**, 2 g) in AcOH (100 ml) was shaken under a stream of hydrogen in the presence of PtO_2 (0.5 g) at room temperature, absorbing 340 ml of hydrogen. After removal of the catalyst by filtration, the filtrate was poured into water and extracted with methylene chloride. The organic layer was washed with water, sat. $NaHCO_3$, and water, then dried (Na_2SO_4), and concentrated *in vacuo*. The residue was chromatographed on silica gel (50 g). Elution with benzene gave a solid (1.2 g), which was a mixture of 3β-acetoxylanost-8-ene (**3a**), 3β-acetoxylanost-7-ene (**3b**), and 3β-acetoxylanosta-7,9(11)-diene (**3c**). **3a**, **3b** and **3c** were separated by HPLC (yield; 40%, 8%, and 8%, respectively). Further elution with methylene chloride gave a solid (0.65 g), which was recrystallized from MeOH to give colorless needles of **2** (yield, 26%), mp 208—209 °C, undepressed on admixture with an authentic specimen.[7] ^{1}H-NMR δ (ppm): 0.74 (3H, s, 18-CH_3), 0.86 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.87 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.95 (3H, s, 19-CH_3), 1.08 (3H, s, 14-CH_3), 2.04 (3H, s, 3β-$OCOCH_3$), 4.06 (1H, m, 7β-H, $W_{1/2}$=8 Hz), 4.53 (1H, m, 3α-H). MS *m/z*: 488 (M^+), 470 ($M^+ - H_2O$), 455 ($M^+ - CH_3$, H_2O), 395 ($M^+ - CH_3$, H_2O, base peak). $[\alpha]_D^{19}$: +13.3 (c=1.0, $CHCl_3$) (lit.,[7] +13.9).

Hydrogenation of 1 in Acetic Acid-$^2H_1$——The reaction was carried out by the same procedures as described above except for the use of acetic acid-$^2H_1$ ($CH_3COO^2H_1$).

Lanost-8-ene-3β,32-diol (6), Lanost-7-ene-3β,32-diol (7), and Lanost-6-ene-3β,32-diol (8)——Treatment of 3β-acetoxylanostan-7α-ol (**2**, 0.5 g) with lead tetraacetate (2.5 g) followed by alumina chromatography of the crude reaction product gave 3β-acetoxylanostan-7α,32-oxide (**4**, mp 201—202 °C, yield, 72%) and 3β-acetoxylanostan-7-one (**5a**, mp 170—172 °C, yield, 7%). Treatment of **4** (0.25 g) with pyridinum hydrogen chloride (0.5 g) and acetic anhydride (50 ml) followed by hydrolysis gave a mixture of **6**, **7**, and **8**, which were separated by HPLC. The first eluted product was lanost-6-ene-3β,32-diol (**8**) (15% yield), mp 191—192 °C (lit.,[7] 191.5—192.5 °C). The next eluted product was lanost-7-ene-3β,32-diol (**7**) (42% yield), mp 206—207 °C (lit.,[7] 207—208.5 °C). The last eluted product was lanost-8-ene-3β,32-diol (**6**) (20% yield), mp 173—174 °C (lit., 161—163 °C[7] and 174—175 °C[5]). The melting

points of **6**, **7**, and **8** were undepressed on admixture with the corresponding authentic specimens.

7-Oxolanostan-3β-ol (5b)——**5a** was hydrolyzed with 5% methanolic KOH under reflux for 1 h. After usual work-up, the residue was recrystallized from MeOH to give colorless needles of **5b**, mp 169—170 °C (lit.,[6] 171—173 °C). ^{1}H-NMR δ (ppm): 0.75 (3H, s, 18-CH_3), 0.83 (3H, s, 4β-CH_3), 0.85 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.86 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.90 (3H, s, 19-CH_3), 0.95 (3H, s, 4α-CH_3), 1.08 (3H, s, 19-CH_3), 2.27—2.41 (3H, m, 6-H_2 and 8-H), 3.26 (1H, m, 3α-H). MS m/z: 444 (M^+), 429 ($M^+ - CH_3$), 411 ($M^+ - CH_3$, H_2O), 393 ($M^+ - CH_3$, $2H_2O$), 304, 222 (base peak). CD (c=2.55, methanol) $[\theta]^{24}$ (nm): −1915 (294) (negative maximum).

3β-Hydroxylanost-8-en-32-al (9)——Jones oxidation of lanost-8-ene-3β,32-diol (**6**, 15 mg) gave the 3,32-dioxo compound (9 mg), which was partially reduced with KBH_4 (3 mg) to give **9** (4 mg), mp 159—160 °C, (lit.,[11] 160—161 °C). ^{1}H-NMR δ (ppm): 0.75 (3H, s, 18-CH_3), 0.82 (3H, s, 4β-CH_3), 0.85 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.86 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.97 (3H, s, 4α-CH_3), 1.05 (3H, s, 19-CH_3), 3.23 (1H, m, 3α-H), 9.44 (1H, s, 32-CHO). MS m/z: 413 ($M^+ - CHO$, base peak), 395 ($M^+ - CHO$, H_2O).

3β-Hydroxylanost-7-en-32-al (10)——Jones oxidation of 3β-hydroxylanost-7-ene-3β,32-diol (**7**, 30 mg) gave the 3,32-dioxo compound (20 mg), which was partially reduced with KBH_4 (6 mg) to give **10** (7 mg), mp 119—120 °C (lit.,[5] 121—122 °C). ^{1}H-NMR δ (ppm): 0.73 (3H, s, 18-CH_3), 0.85 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.86 (3H, d, 26 or 27-CH_3, J=6.6 Hz), 0.89 (6H, s, 4β and 19-CH_3), 0.98 (3H, s, 4α-CH_3), 3.24 (1H, m, 3α-H), 5.44 (1H, m, 7-H), 9.62 (1H, s, 32-CHO). MS m/z: 413 ($M^+ - CHO$, base peak), 395 ($M^+ - CHO$, H_2O).

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References

1) N. B. Myant, "The Biology of Cholesterol and Related Steroids," Willium Heinemann Medical Books Ltd., London, 1981, pp. 163—213.
2) S. E. Saucier, A. A. Kandutsch, F. R. Taylor, T. A. Spencer, S. Phirwa, and A. K. Gayen, *J. Biol. Chem.*, **260**, 14571 (1985).
3) Y. Sato, Y. Sonoda, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.*, **32**, 3305 (1984).
4) M. Morisaki, Y. Sonoda, T. Makino, N. Ogihara, N. Ikekawa, and Y. Sato, *J. Biochem.* (Tokyo), **99**, 597 (1986).
5) G. F. Gibbons, C. R. Pullinger, H. W. Chen, W. K. Cavence, and A. A. Kandutsch, *J. Biol. Chem.*, **255**, 395 (1980).
6) D. H. R. Barton and B. R. Thomas, *J. Chem. Soc.*, **1953**, 1842.
7) E. J. Parish and G. J. Schroepfer, Jr., *J. Lipid Res.*, **22**, 859 (1981).
8) M. L. Eidinoff, J. E. Knoll, D. K. Fukushima, and T. F. Gallagher, *J. Am. Chem. Soc.*, **74**, 5280 (1952).
9) G. G. Habermehl, J. H. Kirsch, and K. J. Reibstein, *Heterocycles*, **17**, 183 (1982).
10) J. T. Pinky, B. J. Ralph, J. J. H. Simes, and M. Wootton, *Aust. J. Chem.*, **23**, 2141 (1970).
11) G. F. Gibbons, K. A. Mitropoulos, and C. R. Pullinger, *Biochem. Biophys. Res. Commun.*, **69**, 781 (1976).