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Studies on Steroids. XXXVIII.[1] A New Preparation of 1α-Hydroxycholesterol[2]

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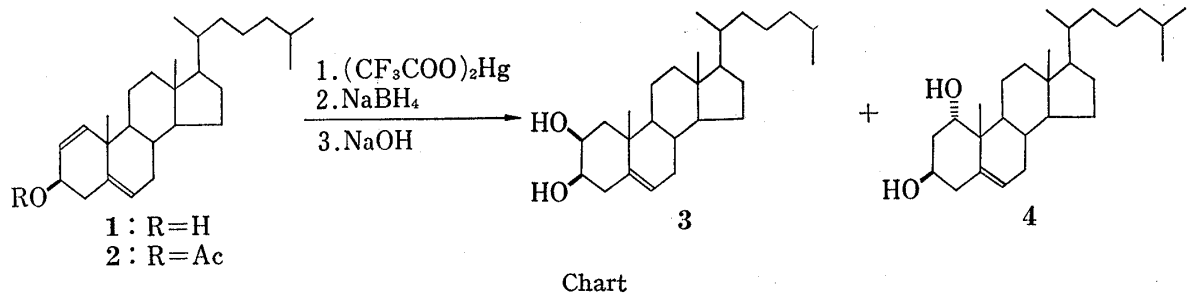
Oxymercuration-demercuration of 3β-acetoxycholesta-1,5-diene(**2**) followed by saponification gave 2β-hydroxycholesterol (**3**) and 1α-hydroxycholesterol (**4**) in 14% and 26% yield, respectively.

Since 1α-hydroxyvitamin D_3 has been found[4] to elicit a comparable biological activity to 1α,25-dihydroxyvitamin D_3, a hormonal active form of vitamin D_3, several research groups[5] including ours have been actively exploring for synthetic route to 1α-hydroxycholesterol (**4**),[6] the immediate precursor of 1α-hydroxyvitamin D_3. Although the method of Kaneko, *et al.*,[5d] involves the short steps for preparation of **4** from cholesterol, the yield of 1α-hydroxylation which was performed by hydroboration-oxidation reaction of cholesta-1,5-dien-3β-ol (**1**), is low (10—15%). We have considered that oxymercuration-demercuration reaction[7] of the dienol **1** or its derivatives would be an alternative for the introduction of 1α-hydroxyl function.

The dienol **1** prepared by the known 4 steps procedures from cholesterol,[8,5d] was converted to the acetate **2** in the usual manner. Reaction of the acetate **2** with mercuric acetate in tetrahydrofuran followed by reduction with alkaline sodium borohydride gave only the starting dienol **1**. However, when mercuric trifluoroacetate was used[7] in place of mercuric acetate, there were obtained after saponification, the less polar diol **3** and the more polar diol **4** in yields of 14% and 26%, respectively, together with the recovered **1** (9%).[2] The trimethylsilyl derivative of the minor product **3** showed a mass ion peak at *m/e* 546, corresponding to the molecular ion of bis-trimethylsilyl ether of cholestenediol. In accordance with this, the nuclear magnetic resonance (NMR) spectrum of **3** indicated besides 3α-H signal at 3.55 ppm, a one proton multiplet at 4.03 ppm, which may be assigned to a hydrogen attached to secondary hydroxy group. This hydroxyl should be located at C-2 position as deduced from a positive periodate oxidation test. All these facts and the marked difference of its physical data from 2α-hydroxycholesterol,[5d] as well as the good agreement of melting point with the published 2β-hydroxycholesterol,[9] we have concluded that this diol is 2β-hydroxycholesterol (**3**). The major product of the oxymercuration-demercuration reaction was identified as 1α-hydroxycholesterol by

1) Part XXXVII: K. Bannai, M. Morisaki, and N. Ikekawa, *J. Chem. Soc., Perkin I*, in press.
2) Presented at The 17th Symposium on the Chemistry of Natural Products, Tokyo, Oct. 1973. Symposium Papers p. 167.
3) Location: *Ookayama, Meguro-ku, Tokyo, 152, Japan.*
4) M.F. Holick, E.J. Semmler, H.K. Schnoes and H.F. DeLuca, *Science*, **180**, 190 (1973).
5) *a*) M. Morisaki, K. Bannai, and N. Ikekawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 1853 (1973); M. Morisaki, A. Saika, K. Bannai, M. Sawamura, J. Rubio-Lightbourn and N. Ikekawa, *ibid*, **23**, 3272 (1975); *b*) D.H.R. Barton, R.H. Hesse, M.M. Pechet and E. Rizzardo, *J. Am. Chem., Soc.*, **95**, 2748 (1973); *c*) A. Fürst, L. Lebler, W. Meier, and K. Pfoertner, *Helv. Chim. Acta*, **56**, 1708 (1973); *d*) C. Kaneko, S. Yamada, A. Sugimoto, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron Letters*, **1973**, 2339; *e*) M.N. Mitra, A.W. Norman and W.H. Okamura, *J. Org. Chem.*, **39**, 2931 (1974).
6) M. Mihailović, L. Lorenc, N. Popov, and J. Klavoda, *Helv. Chim. Acta*, **54**, 2281 (1971).
7) H.C. Brown, P.J. Geoghegan, Jr., G.J. Lynch, and J.T. Kurek, *J. Org. Chem.*, **37**, 1941 (1972).
8) E. Shapiro, L. Weber, E.P. Oliveto, H.L. Herzog, R. Neri, S. Tolksdorf, M. Tanabe, and D.F. Crowe, *Steroids*, **8**, 461 (1966).
9) V. Černý, A. Kasal and F. Šorm, *Coll. Czech. Chem. Comm.*, **35**, 1235 (1970).



Chart

direct comparison (mp, thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and NMR) with an authentic sample.[5a]

The yield of 1α-hydroxylation of the present method appears to be better than that of hydroboration-oxidation reaction.[5d] The applicability of our procedures has recently been exemplified by synthesis of 1α,24-dihydroxyvitamin D_3.[10]

Experimental

Melting points were determined on a hot stage microscope and are uncorrected. NMR spectra were run on a Varian T-60 spectrometer with deuteriochloroform as solvent and with tetramethylsilane as an internal standard. Mass spectra were determined with Shimadzu-LKB 9000S. Column chromatography was effected with Wakogel C-200. Abbreviations used for NMR data: s, singlet; d, doublet; m, multiplet.

3β-Acetoxycholesta-1,5-diene (2)——Cholesta-1,5-dien-3β-ol(1)[5d] (2.7 g) was heated in a mixture of acetic anhydride (7 ml), pyridine (7 ml) and benzene (15 ml) on a boiling water-bath for 2.5 hr. The reaction mixture was poured into ice-water and the aqueous layer was extracted with benzene. The combined benzene layer was washed with dil HCl and saturated $NaHCO_3$. Drying over K_2CO_3 and evaporation of the solvent gave a colorless residue, which was crystallized from methanol to give the acetate **2** (2.75 g) mp 68—70° (from ether-methanol), NMR δ 0.67 (3H, s, 13-Me), 1.09 (3H, s, 10-Me), 2.05 (3H, s, acetyl), 5.2 (1H, m, 3α-H), 5.4 (1H, m, 6-H), 5.4 and 5.8 (2H, a pair of d, J=10 Hz, 1 and 2-H). *Anal.* Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.54; H, 10.87.

Oxymercuration-demercuration of the Acetate (2)——To a stirred mixture of the acetate **2** (170 mg), tetrahydrofuran (2.0 ml) and water (0.54 ml), was added mercuric oxide (476 mg) and trifluoroacetic acid (0.34 ml) under cooling with an ice-bath. Stirring was continued at 3° for 8 hr and then at 15° for 16 hr. Two ml of 3 M NaOH and a solution of $NaBH_4$ (60 mg) in 3 M NaOH (2 ml) were added to the reaction mixture. After stirring 30 min, the mixture was extracted with ethyl acetate, washed with brine, 2 N HCl, sat. $NaHCO_3$ and brine. Evaporation of the solvent gave a colorless crystal, which was heated with a mixture of 3 N NaOH (1 ml) and methanol (10 ml) at 70° for 15 min. The mixture was partitioned between ethyl acetate and brine. The ethyl acetate layer was dried over $MgSO_4$ and evaporated to dryness. The resulting yellow amorphous materials were applied on a column of silica gel (3 g). Elution with benzene–ether (50: 1) gave the recovered **1** (13 mg). Further elution with benzene–ether (10: 1) afforded 2β-hydroxycholesterol (**3**) (22 mg), mp 222—224° (from acetone) (ref.[8] mp 224—226°), NMR δ 0.68 (3H, s, 13-Me), 1.18 (3H, s, 10-Me), 3.55 (1H, m, 3α-H), 4.03 (1H, m, 2α-H) and 5.40 (1H, m, 6-H). Treatment of **3** with trimethylsilylimidazole gave the bis-trimethylsilyl ether, *m/e* 546 (M^+), 531 (M-Me), 456 (M-TMSOH), 441 (M-TMSOH-Me), and 366 (M-2TMSOH). Continued elution on the above chromatography with benzene–ether (5: 1) gave 1α-hydroxycholesterol (**4**) (39 mg), which was identified with an authentic sample[5a] in mp, TLC, GLC, and NMR.

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10) K. Ochi, I. Matsunaga, M. Shindo, and C. Kaneko, The 96th Annual Meeting of the Japanese Pharmaceutical Society, April 1976, Nagoya. Abstract Papers II, p. 183.