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

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(Received April 13, 1976)

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 D₃ has been found to elicit a comparable biological activity to 1α,25-dihydroxyvitamin D₃, a hormonal active form of vitamin D₃, several research groups including ours have been actively exploring for synthetic route to 1α-hydroxycholesterol (4), the immediate precursor of 1α-hydroxyvitamin D₃. Although the method of Kaneko, et al., 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 of the dienol 1 or its derivatives would be an alternative for the introduction of 1α-hydroxy function.

The dienol 1 prepared by the known 4 steps procedures from cholesterol 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 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%). 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 cholestadienol. 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 4.08 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, as well as the good agreement of melting point with the published 2β-hydroxycholesterol, we have concluded that this diol is 2β-hydroxycholesterol (3). The major product of the oxymercuration-demercuration reaction was identified as 1α-hydroxycholesterol by

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

The yield of $\alpha$-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 $\alpha$,24-dihydroxyvitamin D$_2$.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(146) (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$_2$CO$_3$ 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), 2.65 (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$_{28}$H$_{44}$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% NaOH and a solution of NaBH$_4$ (60 mg) in 3% 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 (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), 2.65 (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 386 (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 sample5b) in mp, TLC, GLC, and NMR.

Acknowledgement We are grateful to Mr. A. Saika and Mrs. M. Matsuura (née, Sawamura) for their skillful technical assistance.