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The Convenient Synthesis of 25-Hydroxycholesterol[1)]

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The convenient synthesis of 25-hydroxycholesterol (**6**) is described. 5β-Cholestan-3α,25-diol (**2**) derived from lithocholic acid was converted to **6** *via* four steps: oxidation-bromination, dehydrobromination, deconjugation, and reduction. The overall yield of 25-hydroxycholesterol (**6**) starting from **2** was *ca.* 50%.

Keywords——lithocholic acid; hypobromous acid; deconjugation; one step oxidation-bromination; bile acids

In recent studies on the synthesis of active vitamin D_3 analogues, 25-hydroxycholesterol has been recognized as an important synthetic intermediate[3)] and thus, many investigators have reported its synthesis from a variety of starting materials. So far, the following sterols were used as the starting materials; cholesterol,[4)] cholestanol,[5)] stigmasterol,[6)] pregnenolone,[7)] androstenolone,[8)] ergosterol,[9)] and fucosterol (*via* desmosterol).[10)]

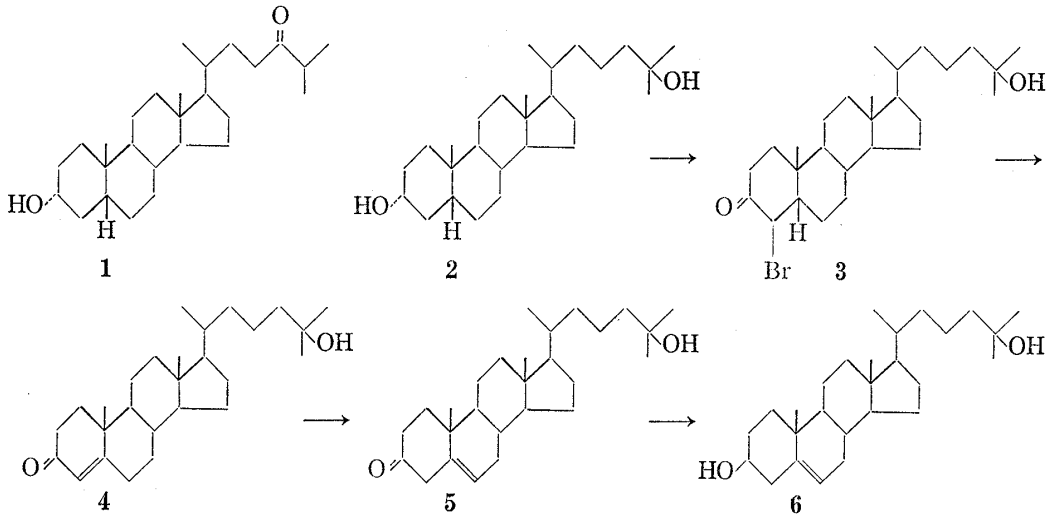

Chart 1

1) This forms Part V of "Synthetic Studies of Vitamin D_3 Analogues from Bile Acids." Part IV, K. Ochi, I. Matsunaga, M. Shindo, and C. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **26**, 2386 (1978) A part of this work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April, 1978.
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In the previous work of this series,[11] 5β-cholestan-3α-ol-24-one (**1**) obtained readily from lithocholic acid with 50% overall yield was converted to 1α,25-dihydroxycholecaiciferol *via* 5β-cholestane-3α,25-diol (**2**).

In this paper, we describe the convenient synthetic method of 25-hydroxycholesterol (**6**) from the same diol (**2**). As shown in Chart 1, the procedure consists of four steps from **2**; i) oxidation-bromination, ii) dehydrobromination, iii) deconjugation, and iv) reduction.

In the first step in the procedure, we have used one step oxidation-bromination reaction of 3-hydroxysteroids discovered originally by two independent groups in the pregnane series.[12] Thus, the 3α,25-diol (**2**) was reacted with N-bromoacetamide in aqueous *t*-butanol containing hydrobromic acid[13] to give 4β-bromo-5β-cholestan-25-ol-3-one (**3**) in 85% yield. The structure of **3** was confirmed by both nuclear magnetic resonance (NMR) (δ 4.97, doublet, $J=12$ Hz, 4α-H) and infrared (IR) spectra ($\nu_{C=O}$, 1730 cm^{-1}) indicating that the bromination occurred in regiospecific manner as in accordance with the greater stability of Δ^3-over Δ^2-isomers in coprostane series.[14]

4β-Bromo-3-one (**3**) was then dehydrobrominated to 25-hydroxycholest-4-en-3-one (**4**) by refluxing for 1 hr in dimethylformamide in the presence of lithium carbonate. The structure of the dehydrobromination product was determined to be the 4-en-3-one (**4**) both by the presence of the absorption maximum at 260 nm in the ultraviolet (UV) spectrum and by the appearance of the characteristic olefinic proton signal at C-4 (δ 5.76, singlet) in the NMR spectrum.

Deconjugation of the 4-en-3-one (**4**) to the 5-en-3-one (**5**) was accomplished by enolization of the former by sodium ethoxide in dimethylsulfoxide followed by the subsequent treatment of the enolate with diluted acetic acid solution.

In the final step in our procedure, the crude **5**, without further purification, was reduced with sodium borohydride in methanol. The crude reduction product was purified by column chromatography over silica gel, and the desired fraction, after recrystallization from acetone, afforded the final product (**6**) whose structure was confirmed by the mixed melting point determination with the authentic sample synthesized from desmosterol obtained in our previous work[15] by the known method.[10] The overall yield of **6** starting from **2** was *ca.* 50%.

Thus, the convenient synthesis of 25-hydroxycholesterol (**6**) from lithocholic acid was successfully accomplished.

Experimental[16]

4β-Bromo-5β-cholestan-25-ol-3-one (3)——To the solution of **2** (1.3 g) in 4% aqueous *t*-butanol (50 ml), N-bromoacetamide (2.1 g) and 47% aqueous hydrobromic acid solution (0.6 ml) were added. The mixture was stirred at room temperature for 48 hr. The reaction mixture was extracted with ether, and the organic layer was washed with H_2O, aqueous $NaHCO_3$ solution, and then with H_2O, and dried over $MgSO_4$ and evaporated. The solid residue was recyrstallized from MeOH to give 1.35 g of **3**, mp 70—75°, IR ν_{max}^{KBr} cm^{-1}: 3440

11) K. Ochi, I. Matsunaga, M. Shindo, and C. Kaneko, *J. Chem. Soc. Perkin I*, **1978**, in press.
12) *a*) A.R. Hanze, G.S. Fonken, A.V. McIntosh, Jr., A.M. Searcy, and R.H. Levin, *J. Am. Chem. Soc.*, **76**, 3179 (1954); *b*) E.B. Hershberg, C. Gerold, and E.P. Oliveto, *J. Am. Chem. Soc.*, **74**, 3849 (1952).
13) The one step oxidation-bromination reaction under these conditions involved hypobromous acid derived from N-bromoacetamide and water as the active reagent, and was found to proceed *via* the corresponding 3-ketosteroids as an isolable intermediate.[12]
14) Bromination of coprostanone was found to give selectively 4β-bromocoprostanone by bromine in acetic acid containing a little hydrobromic acid. Obviously, the direction of enolization played an important role in the reaction. See L.F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Co., New York, 1959, p. 282.
15) K. Ochi, I. Matsunaga, M. Shindo, and C. Kaneko, *Steroids*, **30**, 795 (1977)
16) Melting points are uncorrected. IR spectra were obtained with a Hitachi 285 spectrometer and NMR spectra with a Hitachi Perkin-Elmer R-20A spectrometer using tetramethyl silane (TMS) as an internal standdard. UV spectra were measured with a Hitachi 124 spectrometer.

(OH), 1730 (CO), NMR ($CDCl_3$) δ: 0.68 (3H, s), 1.07 (3H, s), 1.19 (6H, s), and 4.98 (1H, d, J=12 Hz, 4α-H). *Anal.* Calcd. for $C_{27}H_{45}O_2Br$: C, 67.34; H, 9.42. Found: C, 67.18; H, 9.56.

25-Hydroxycholest-4-en-3-one (**4**)——To the solution of **3** (1.3 g) in dry dimethylformamide (20 ml), Li_2CO_3 (250 mg) was added and the mixture was refluxed for 1 hr. The reaction mixture was diluted with ethyl acetate, and the whole was washed with H_2O, dried over $MgSO_4$, and evaporated. The residue obtained after evaporation of the solvent was purified by column chromatography using a short column of silica gel. Elution with $CHCl_3$ afforded 950 mg of **4**, mp 147—148° (from MeOH), UV λ_{max}^{EtOH} nm: 260, IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1660 (CO), 1610 (C=C), NMR ($CDCl_3$) δ: 0.71 (3H, s), 0.96 (3H, s), 1.20 (6H, s), 5.76 (1H, s, 4-H). *Anal.* Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.62; H, 11.23.

25-Hydroxycholesterol (**6**)——To a solution of **4** (102 mg) in dry dimethylsulfoxide (5 ml), freshly prepared NaOEt (1 g) was added, and the mixture was stirred at room temperature for 1 hr under argon atmosphere. The reaction mixture was poured into ice water containing AcOH (1 ml), and the separated oil was extracted with ether. The ether layer was washed with aqueous $NaHCO_3$ solution, and then H_2O, and dried over $MgSO_4$. Evaporation of ether gave the crude 5-en-3-one (**5**) (98 mg).

To the solution of $NaBH_4$ (0.2 g) in MeOH (50 ml), the solution of **5** obtained as above in ether (10 ml) was added dropwise for 10 min under cooling in an ice bath, and the stirring was continued for 30 min. After the excess reagent was decomposed by AcOH, the reaction mixture was evaporated to a small volume and extracted with ether. The ether layer was washed with H_2O, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography using silica gel. Elution with $CHCl_3$ gave 65 mg of **6**, mp 175—177° (from acetone), which was identified with the authentic sample[10] by mixed melting point determination.

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Catalytic Effects of Metal Ions on the Anodic Oxidation of Ascorbic Acid at a Platinum Electrode

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The catalytic effects of several metal ions on the anodic oxidation of ascorbic acid (I) at a platinum electrode were studied by a linear sweep voltammetry in 1 M perchloric acid. Contrary to the air-oxidation of I, Bi^{3+} and Pb^{2+} exhibit a marked effect whereas Cu^{2+} shows a minor one.

Keywords——ascorbic acid; anodic oxidation; platinum electrode; electro-oxidation; catalytic effect; Faradaic adsorption; metal ions; voltammetry

The copper (II)-catalyzed oxidation of ascorbic acid (I) by molecular oxygen to form dehydroascorbic acid has been recognized as an example of model systems in studying enzymatic oxidation processes. The catalytic effect of copper (II) was previously investigated by the present authors,[2] comparing with that of other metal ions.

The electrochemical oxidation of I has been studied on mercury[3] and platinum[4] electrodes. In both cases a two-electron oxidation of I takes place to form dehydroascorbic acid. It seems interesting to investigate if the electro-oxidation of I is also enhanced by the presence of certain metal ions.

This paper is concerned with a comparison of the catalytic effects of several metal ions on the oxidation of I at a platinum electrode in connection with adsorption–desorption of

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