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# FORMATION OF 3α,12α,15α-TRIHYDROXYCHOLANIC ACID FROM DEOXYCHOLIC ACID BY FERRO-ASCORBATE SYSTEM

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In 1965, B. Matkovics et al.[1] reported the in vitro transformation of deoxycholic acid (3α, 12α-dihydroxy-5β-cholanic acid, I) into cholic acid (3α,7α,12α-trihydroxy-5β-cholanic acid, II) with ferro-ascorbate system containing ethylenediaminetetraacetic acid (EDTA). We have now succeeded in obtaining the trihydroxycholanic acid (III, m.p. 322-325° (decomp.) ), instead of II as well as pythocholic acid[2] (3α,12α,16α-trihydroxy-5β-cholanic acid, IV, m.p. 186-187°; methyl ester: m.p. 146-148°), from the reactions of I using the same systems both with and without EDTA in absolutely aqueous solutions.

Into the solution of I (1.0 g., 2.55 m moles), $FeSO_4 \cdot 7H_2O$ (5.0 g., 18.0 m moles) and ascorbic acid (100 g., 567 m moles) in 0.4 M phosphate buffer (pH 6.8, 2500 ml.), oxygen was bubbled for 3 hrs. at 37°C. Another 100 g. of ascorbic acid was then added to the reaction mixture which was adjusted again at pH 6.8 and kept at the same conditions as above for another 3 hrs. The products were extracted at pH 3.0 with ether and the chloroform-soluble substances from the ether extracts were then methylated. The methyl esters obtained were submitted to chromatography on silica gel by eluting with the mixture of benzene and ethyl acetate (EtOAc). The eluates by 70% EtOAc-benzene and 100% EtOAc gave the colourless needles (V): yield 3.3%; m.p. 256-259° (EtOAc-MeOH); Calcd. for $C_{25}H_{42}O_5$: C 71.06 H 10.01, Found: C 71.35 H 10.15.

The fragmentation pattern of mass spectrum (Fig. 1) was considerably similar to that of methyl cholate: m/e 386 (M-2x18), 368 (M-3x18), 271 [M-(2x18+115), base peak ], 253 [M-(3x18+115)]; 115 mass units may correspond to the intact side chain. The presence of three nuclear oxygen substituents may thus be elucidated.[3] Chromate oxidation of V in acetic acid gave a triketo ester (VI); m.p. 176-177°, $\nu_{CHCl_3}$ 1739, 1712 $cm^{-1}$, $M^+$ 416 (Fig. 2); that is indicative of the secondary charactor of the third hydroxyl group. Fragmentation (2) of 12-keto steroids as shown in Fig. 2 can be operative with hydrogen transfer[4] and methyl 3,12-diketocholanate derived from I gave the base peak of m/e 247 as expected. Fission of ring C occurs in 15-ketosteroid, affording the stable peak of ketonic ring D with the side chain[5], to which the m/e 211 ion from VI accompanied by hydrogen transfer may reasonably be corresponded (Fig. 2). Free acid of VI afforded the m/e 197 (= 211-14) instead and also 247 ions, both in higher intensity. The fission

(3) can not be operative in 16-ketosteroid.[6] The new oxygen function introduced in this hydroxylation reaction may, therefore, be situated at $C_{15}$.

In NMR studies on steroids, signal shifts of the angular methyl groups due to the hydroxyl groups in the various positions have been reported.[7] The chemical shifts in six known bile acids as well as V and in some of those reported are shown in Table I. Contrary to the remarkable downfield shift (-0.45 p.p.m.) of the signal peak of $C_{18}$-Me caused by $C_{15}$-βOH, the effect of $C_{15}$-αOH was as smaller as -0.10 p.p.m. (Table II). The configuration of the third hydroxyl group in V may thus be rather of alpha.

Hydroboration-oxidation[8] of methyl $\Delta^{14}$-3α,12α-dihydroxycholate, m.p. 89° (free acid: m.p. 259°), derived from methyl ester of II by the method of K. Yamasaki et al.[9], yielded the trihydroxy derivative which was identical with V giving the free acid III as a hydrolysate and the triketo ester VI on chromate oxidation.

Consequently, the chemical structure of the product III from I by the ferro-ascorbate system may reasonably be elucidated as 3α,12α,15α-trihydroxycholanic acid.

Table I. Chemical Shifts of Angular Methyl Protons

| Methyl ester | Site of hydroxyl group | $C_{18}$-H (τ) | $C_{19}$-H (τ) |
|---|---|---|---|
| Cholanate | - | 9.39 | 9.08 |
| Lithocholate | 3α | 9.38 | 9.09 |
| Chenodeoxycholate | 3α, 7α | 9.32 | 9.04 |
| Ursodeoxycholate | 3α, 7β | 9.34 | 9.07 |
| Deoxycholate | 3α, 12α | 9.30 | 9.07 |
| Cholate | 3α, 7α, 12α | 9.23 | 9.03 |
| Compound V | 3α, 12α, 15α | 9.20 | 9.03 |

Spectra were taken with a Hitachi Model H-6013 (60 MC) spectrometer, in pyridine solution containing tetramethylsilane as an internal standard.

Table II. Substituent Effect of Hydroxyl Group on the Chemical Shifts of Angular Methyl Protons

| Site of hydroxyl group | Difference in p.p.m. from parent compound | |
|---|---|---|
| | $C_{18}$-H | $C_{19}$-H |
| 3α | -0.01 (-0.01) | +0.01 (-0.01) |
| 7α | -0.06 (-0.06) | -0.05 (-0.09) |
| 7β | -0.04 (-0.02) | -0.02 (-0.01) |
| 12α | -0.08 (-0.12) | -0.02 (-0.03) |
| 15α | -0.10 (-0.07) | -0.04 (-0.03) |
| 15β | - (-0.45) | - (-0.07) |

Numbers in the brackets are those reported.[7]

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Fig.1

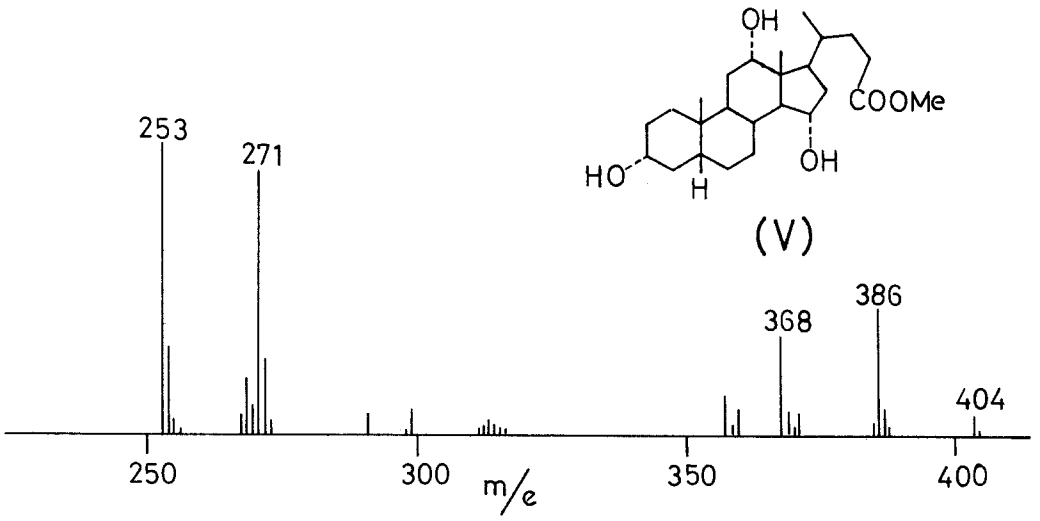
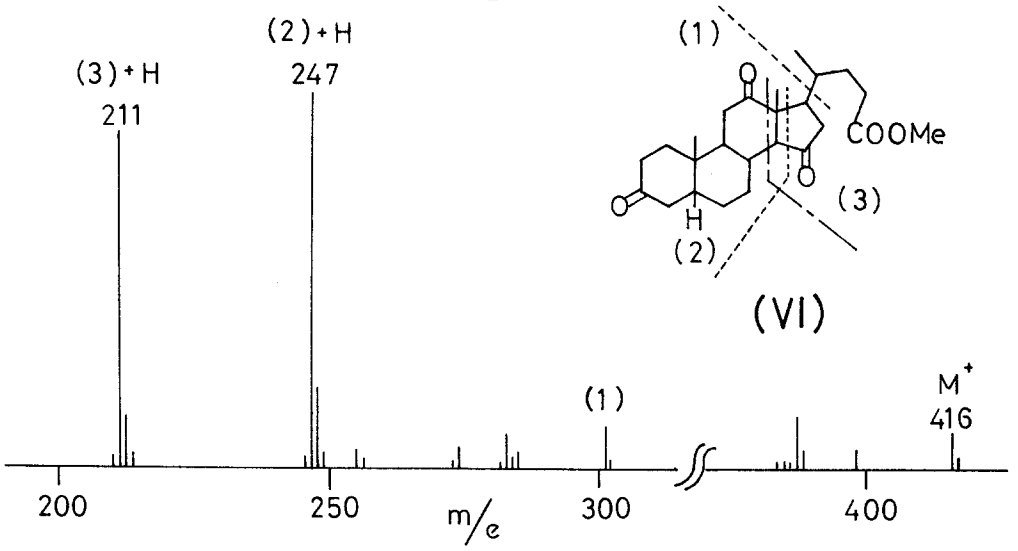

OH
COOMe
HO
OH
H
(V)
253
271
368
386
404
250
300
350
400
m/e

Fig. 2


(3)+H
211
(2)+H
247
(1)
COOMe
(3)
H
(2)
(VI)
(1)
M+
416
200
250
300
400
m/e