Dehydration of Bile Acids and their Derivatives. IX. Oxidation of Methyl 3α-Acetoxy-Δ⁷-cholenate with Selenium Dioxide

By Ryuzaburo OSAWA

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Previously Fieser et al.[1] reported that the selenium dioxide oxidation of Δ^7-cholestene-3β-ol acetate in benzene-ether or ether-acetic acid affords, along with allylic rearrangement and acetylation, $\Delta^{8(14)}$-cholestene-3β, 7α-diol diacetate. Saucy et al.[2] found later that the same oxidation of $\Delta^{7,22}$-ergostadiene-3β-ol acetate in boiling ether containing a little acetic acid gives, as expected, not only $\Delta^{8(14),22}$-ergostadiene-3β, 7α-diol diacetate, but also $\Delta^{7,22}$-ergostadiene-3β, 9ξ-diol monoacetate. In his preceding paper, Fieser[3] had concluded that among the various steroids tested, 5α- or Δ^5-steroids having a double bond adjacent to the C-14 hydrogen atom give positive results in his selenium dioxide test, while none of the 5β-isomers does. Accidentally Professor Yamasaki has noticed that, contrary to Fieser's conclusion, some of the unsaturated bile acids (5β) give apparently positive results in the selenium dioxide test reported by Fieser[3]. These results are summarized in Table I.

It is interesting to note that not only the bile acids with a double bond adjacent to the C-14 hydrogen atom, but also those having a $\Delta^{8(14)}$ double bond afforded positive results in the selenium dioxide test.

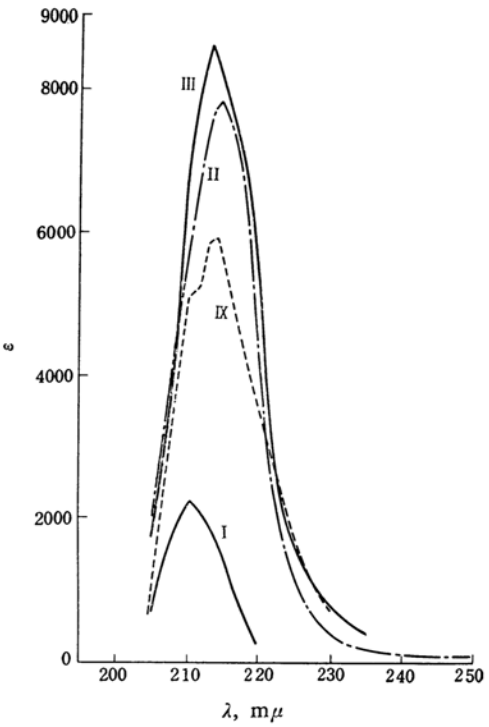

Fig. 1. Absorption spectra of: Methyl 3α-acetoxy-Δ^7-cholenate (I); substance B (II) and its acetate (III); methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate (IX, for reference).

1) L. F. Fieser and G. Ourisson, *J. Am. Chem. Soc.*, 75, 4404 (1953).
2) G. Saucy, P. Geistlich, R. Helbling and H. Heusser, *Helv. Chem. Acta*, 37, 250 (1954).
3) L. F. Fieser, *J. Am. Chem. Soc.*, 75, 4395 (1953).

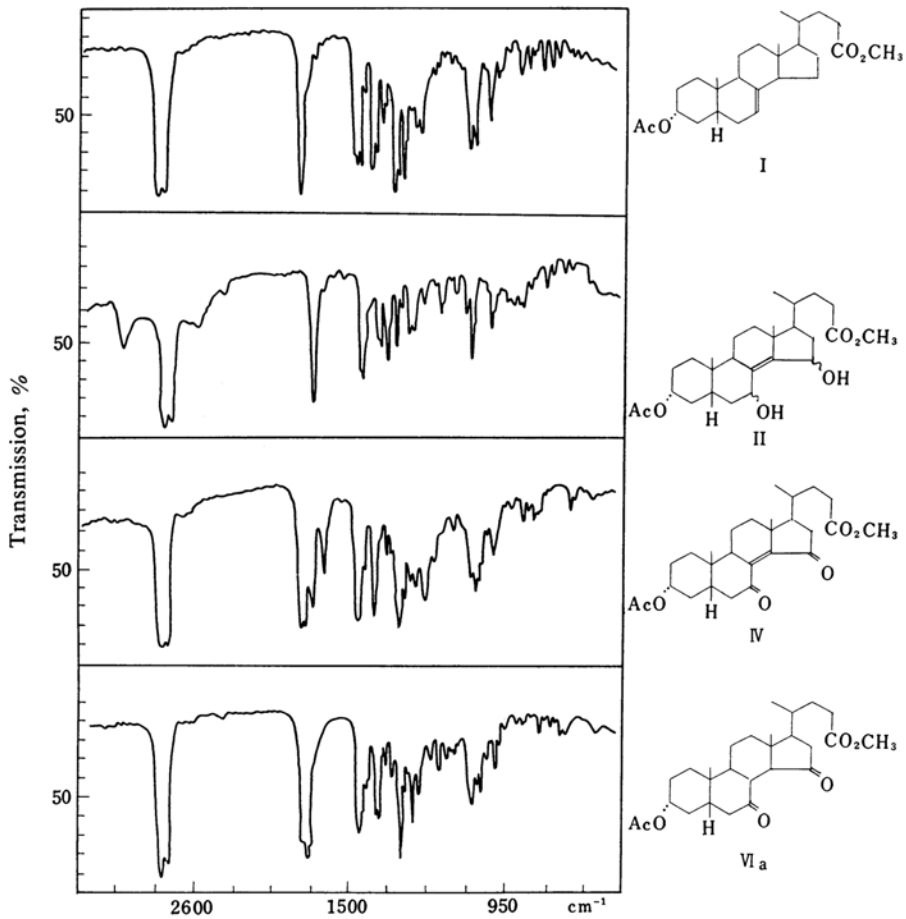

Fig. 2. IR-spectra of: Methyl 3α-acetoxy-Δ^7-cholenate (I); substance B (II); the oxidation product of II (IV); the zinc dust-reduction product of IV (VIa).

TABLE I. SELENIUM DIOXIDE TEST OF SOME BILE ACIDS

Bile acid	SeO_2 test	Ref.
3α-Hydroxy-Δ^7-cholenic acid	++	5
Methyl 3α-acetoxy-Δ^7-cholenate	++	5
Methyl 3α-hydroxy-$\Delta^{8(14)}$-cholenate	++	6
Methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate	++	6
Apocholic acid	++	a
Methyl 3α-acetoxy-Δ^8-cholenate	+	b
Methyl 3α-acetoxy-$\Delta^{9(11)}$-cholenate	—	c
Methyl 3α-acetoxy-Δ^{14}-cholenate	—	5
Methyl 3α-acetoxy-Δ^6-cholenate	—	d
Methyl 3α-acetoxy-$\Delta^{7,9(11)}$-choladienate	++	c

+: Turns yellow within 20 min.
++: Turns yellow within 10~15 min.
The reaction is more intense in acetic acid than in benzene.

a) Fr. Bödecker, *Ber.*, **53**, 1852 (1920).
b) L. F. Fieser, W. P. Schneider and W. Y. Huang, *J. Am. Chem. Soc.*, **75**, 124 (1953).
c) L. F. Fieser and S. Rajagopalan, ibid., **73**, 118 (1951).
d) S. L. Hsia, J. T. Matschiner, T. A. Mahowald, W. H. Elliot and E. A. Doisy, *J. Biol. Chem.*, **226**, 667 (1957).

The present author has sought to isolate the selenium dioxide oxidation products of methyl 3α-acetoxy-Δ^7-cholenate. The crude crystalline oxidation product was dissolved in benzene and chromatographed to give three crystalline substances. The substance eluted first proved to be the recovered starting material (melting point; mixed melting point; the infrared spectrum).

Substance A, eluted immediately after the starting material, was in the form of colorless needles (m. p. 174°C), the infrared spectrum of which indicated that the acetyl group and the double bond of the starting material were eliminated. No further evidence on the structure of this product has yet been obtained, since the amount of the product is too small for further study.

Substance B (II), in the form of colorless prisms (m. p. 114°C), eluted with a mixture of ether-methanol (4:1) showed a high absorption maximum at 214 mμ (ε_{max}^{EtOH} 7804). This characteristic absorption seems to indicate the presence of a tetra-substituted, exocyclic

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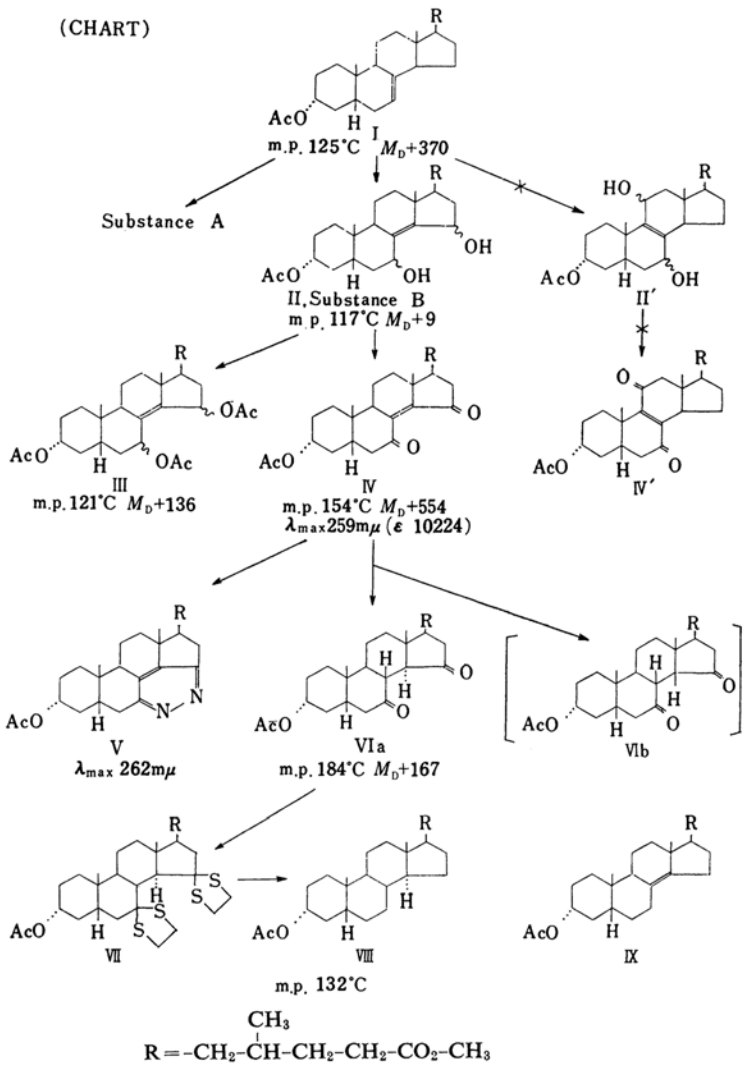

ethylenic linkage in the molecule, as has been shown by Bladon et al.[4] and Osawa et al.[5,6]; indeed, the ultraviolet absorption spectrum of this substance was found to be quite similar to that of methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate[6] (IX) derived from apocholic acid (Fig. 1). From these observations and the structure of the starting material, it is highly probable that substance B has a double bond shifted to the position between C-8 and C-14, just like apocholic acid.

The infrared spectrum of substance B showed a distinct band at 3400 cm^{-1}, indicative of the presence of a hydroxyl group or groups (Fig. 2, II). This band disappeared when substance B was acetylated in the usual way, which indicates that the hydroxyl group or groups are not of tertiary, but probably of secondary nature. Analytical data of this acetylated substance correspond well to a tri-acetate, $C_{25}H_{37}O_5 \cdot (CH_3CO)_3$ (III). From these results it has been concluded that substance B contains no tertiary hydroxyl group unlike the oxidation product of $\Delta^{7,22}$-ergostadiene-3β-ol acetate[2], and that it probably has two acylable hydroxyl groups newly introduced at the positions adjacent to the double bond ($\Delta^{8(14)}$) as formulated as II.

The secondary nature of these hydroxyl groups was confirmed by the fact that chromic acid oxidation gave a diketone IV, which afforded a dioxime.

This unsaturated diketone IV showed an absorption maximum at 259 mμ with a characteristic extinction coefficient (ε_{max}^{EtOH} 10224)

4) P. Bladon, H. B. Henbest anb G. W. Wood, *J. Chem. Soc.*, 1952, 2737.
5) R. Osawa and K. Yamasaki, This Bulletin, 32, 1302 (1959).
6) F. Nakada, R. Osawa and K. Yamasaki, ibid., 34, 538 (1961).

TABLE II

Substance	M. p., °C	α_D	Absorption maximum, λ	Ref.
Methyl 3α-acetoxy-7,11-diketo-Δ^8-cholenate (IV′)	147~149	+ 32° (chf.)	272 mμ* ε_{max}^{EtOH} 5012	10
The oxidation product of substance B (IV)	154~155	+120.4°(chf.)	259 mμ ε_{max}^{EtOH} 10224	—
7,15-Dioxoergosta-$\Delta^{8(14),22}$-diene-3β-ol acetate	180~181	+ 36° (chf.)	259 mμ ε_{max}^{EtOH} 11300	7

* A sample of m. p. 136~143°C showed an absorption maximum ε_{max}^{EtOH} 8139 at 272 mμ[10].

quite similar to that shown by 7,15-dioxo-ergosta-$\Delta^{8(14),22}$-diene-3β-ol acetate[7].

On the basis of these results, it appears highly probable that the resulting diketone IV is methyl 3β-acetoxy-7,15-diketo-$\Delta^{8(14)}$-cholenate. Furthermore, the presence of an ene-1,4-dione system in the diketone was substantiated by the fact that the diketone not only gave a pyridazine derivative, characterized by the absorption maximum at 262 mμ (ε_{max}^{EtOH} 2440)[7,8], but also was reduced at room temperature by zinc and acetic acid like the dioxo-ergostadiene derivative[7]. This reduction product no longer showed such a characteristic absorption at 259 mμ (Fig. 3), and two kinds of crystalls

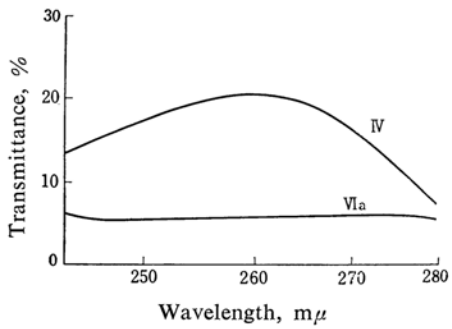

Fig. 3. Absorption spectra of: The oxidation product of substance B (IV) (1/1000% w/v MeOH) and its zinc dust-reduction product (VIa) (1/100% w/v MeOH).

were obtained, the one melting at 184°C and the other at 111°C. The former saturated diketone VIa, the major product, remained unchanged by treatment with alkali followed by esterification and acetylation, and it gave a bisthioketal (VII)[9] which in turn, by desulfuration with Raney nickel, was converted into methyl 3α-acetoxycholanate (VIII) (Identification: melting point; mixed melting point; infrared spectrum). These findings strongly indicate that the C/D ring fusion in this diketone is trans (14α-hydrogen), just like the usual bile acid. The other product of m. p. 111°C had been expected to have the structure of cis C/D fusion, as formulated as VIb, but the amount of the product was too small to elucidate the structure.

Furthermore, not only the unsaturated diketone IV but also the saturated diketone VIa showed characteristic infrared spectra, indicative of α,β-unsaturated (1692 cm^{-1}, 1634 cm^{-1}) and saturated (1732 cm^{-1}, 1717 cm^{-1}) ketonic groups in a five- and a six-membered ring respectively (Fig. 2).

From these experimental results the major product (substance B) of the selenium dioxide oxidation of methyl 3α-acetoxy-Δ^7-cholenate has been proved to be methyl 3α-acetoxy-7,15-dihydroxy-$\Delta^{8(14)}$-cholenate (II).

The fact that substance B (II) and its oxidation product IV showed great absorptions in the ultraviolet region, as indicated above, is consistent with Formulae II and IV, and not with Formulae II′[4] and IV′[10] respectively. The other physical constants of the oxidation product of substance B are also quite different from those of methyl 3α-acetoxy-7,11-diketo-Δ^8-cholenate[10] (IV′) (Table II).

The configurations of the newly introduced hydroxyl groups at C-7 and C-15 of substance B have not yet been established. Since the rear attack of selenium dioxide will be a very probable mode of action, as was the case in the oxidation of Δ^7-cholestene-3β-ol acetate[1], the C-7 hydroxyl group of substance B might be α-oriented (M_D contribution −79). If this were so, the M_D contributions of the hydroxyl and acetyl groups at C-15 could be calculated to be −162 and +30 respectively, indicating that the C-15 OH is α-oriented. Methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate (M_D +250)[6] has here been taken as a point of reference.

7) D. H. R. Barton and G. F. Laws, *J. Chem. Soc.*, **1954**, 52.
8) H. E. Stavely and C. N. Bollenback, *J. Am. Chem. Soc.*, **65**, 1285 (1943).
9) H. Hauptmann, ibid., **69**, 562 (1947); L. F. Fieser, ibid., **75**, 4386 (1953).
10) H. Heusser, K. Eichenberger, P. Kurath, H. R. Dällenbach and O. Jeger, *Helv. Chim. Acta*, **34**, 2106 (1951); C. S. Barnes and D. H. R. Barton, *J. Chem. Soc.*, **1953**, 1419.

Experimental

Oxidation of Methyl 3α-Acetoxy-Δ^7-cholenate.—To a solution of methyl 3α-acetoxy-Δ^7-cholenate (I) (m. p. 126°C; 2 g.) in 50 ml. of absolute ether was added 92 ml. of a solution of 0.1 M selenium dioxide in acetic acid[1], and the mixture was kept at room temperature (18~25°C) overnight. Then the reaction mixture was poured carefully into 1 l. of water and extracted thoroughly with ether. The ethereal extract was washed with water, with sodium carbonate solution and again with water successively, and dried over sodium sulfate. Removal of the solvent gave a crystalline residue, which, dissolved in 4~5 ml. of benzene, was allowed to pass through a Brockmann-alumina column and eluted with petroleum ether, benzene, ether and methanol successively. The first portions (Fraction 1—4) of the eluate contained the starting material (yield: 55% as an average) and substance A of a higher melting point, while substance B of a lower melting point (yield: 15% as an average) was obtained from the fractions eluted with ether-methanol (4:1, v/v). Substance A and B were separately treated and refluxed in acetone with precipitated silver for about 3 hr. to remove colloidal selenium. Removal of the solvent of these solutions gave crystalline residues.

Methyl 3α-Acetoxy-7ξ, 15ξ-dihyroxy-$\Delta^{8(14)}$-cholenate (II).—Substance B was recrystallized repeatedly from methanol. Yield, 230 mg., m. p. 114~117°C, $\alpha_D^{29}=+2.0°$ (chf.); λ_{max} 214 mμ (ε_{max}^{EtOH} 7804). The infrared spectrum of this substance had a distinct hydroxyl absorption band at 3400 cm^{-1}. Substance B showed a melting point depression on admixture with the starting material and no more positive result in the selenium dioxide test.

Found: C, 69.78; H, 9.02. Calcd. for $C_{27}H_{42}O_6$ (462.61): C, 70.10; H, 9.15%.

Methyl 3α, 7ξ, 15ξ-Triacetoxy-$\Delta^{8(14)}$-cholenate (III).—Substance B (200 mg.) dissolved in 2 ml. of pyridine with 1 ml. of acetic anhydride was allowed to stand at room temperature. After 24 hr. the reaction mixture was warmed for 20 min. on a water bath and some concentrated hydrochloric acid was added. The separated solid was then collected. Recrystallized once from methanol, the product was obtained as colorless needles of m. p. 120~121°C, $\alpha_D^{28}=+25.0°$ (chf.); λ_{max} 214 mμ (ε_{max}^{EtOH} 8610). Yield, 188 mg. The hydroxyl absorption at 3400 cm^{-1} was not observed in the infrared spectrum of III.

Found: C, 68.16; H, 8.58. Calcd. for $C_{31}H_{46}O_8$ (546.68): C, 68.10; H, 8.48%.

Methyl 3α-Acetoxy-7, 15-diketo-$\Delta^{8(14)}$-cholenate (IV).—Substance B (400 mg.) in 40 ml. of acetone was oxidized with the chromium trioxide-sulfuric acid mixture (3.2 ml.) according to the method of Bladon et al.[4] After completion of the oxidation (3~4 min.), the excess of the oxidant was reduced with a small amount of methanol. The reaction mixture was diluted with water and extracted thoroughly with ether. The ethereal extract was washed with water, with sodium carbonate solution and again with water successively, dried over sodium sulfate and evaporated. Recrystallization from methanol and then aqueous methanol afforded yellowish crystals of m. p. 154~155°C; $\alpha_D^{28}=+120.4°$ (chf.); λ_{max} 259 mμ (ε_{max}^{EtOH} 10224). The infrared spectrum showed four carbonyl absorption bands, two of which indicated α, β-unsaturated 5- and 6-membered rings (1692, 1634 cm^{-1}).

Found: C, 70.77; H, 8.31. Calcd. for $C_{27}H_{38}O_6$ (458.57): C, 70.71; H, 8.35%.

Oxime of IV.—The diketone (100 mg.) in 20 ml. of methanol was treated with an aqueous solution of hydroxylamine (a mixture of 5 M sodium acetate, 4 ml. and 5 M hydroxylamine hydrochloride, 4 ml.). The mixture was refluxed for 2 hr. and then poured into 100 ml. of water, and the product was collected. The product failed to be crystallized, but repeated precipitations from aqueous methanol afforded a satisfactorily pure sample (87 mg.).

Found: N, 5.46. Calcd. for $C_{27}H_{40}O_6N_2$ (488.33): N, 5.73%.

Pyridazine Derivative of IV.—The diketone IV (200 mg.) in 33 ml. of methanol was refluxed for 6 hr. with 0.6 ml. of hydrazine hydrate (42%). Removal of the solvent left a residue which became crystalline when treated with a small amount of ether. The crystalline material dissolved in benzene was chromatographed (Florisil). M. p. 158°C; yield, 76 mg. λ_{max} 262 mμ (ε_{max}^{EtOH} 2440).

The infrared spectrum of the resulting product indicated that the acetyl group of the diketone IV was removed by the above treatment.

Found: C, 72.20; H, 8.88; N, 6.46. Calcd. for $C_{25}H_{38}O_3N_2$ (414.30): C, 72.42; H, 9.24; N, 6.76%.

Zinc Dust Reduction of IV.—*Methyl 3α-Acetoxy-7, 15-diketo-cholanate (VIa).*—A solution of the $\Delta^{8(14)}$-diketone (IV) (200 mg.) in 20 ml. of acetic acid was stirred with 1 g. of zinc dust for 5 hr. at room temperature. After removal of zinc dust the solution was diluted with water and extracted with ether. The ethereal extract was washed with water, with sodium carbonate and again with water successively and then evaporated to dryness. Recrystallized from methanol, the product was obtained as platelets of m. p. 184°C, $\alpha_D^{16}=+36.4°$ (chf.). Yield, 122 mg.

Found: C, 70.26, 70.40; H, 8.84, 8.73. Calcd. for $C_{27}H_{40}O_6$ (460.59): C, 70.40; H, 8.75%.

The infrared spectrum of the product showed characteristic bands of a saturated ketone: ν_{max}^{Nujol} 1750 cm^{-1} (ester), 1732 cm^{-1} (5-membered ring ketone), 1717 cm^{-1} (6-membered ring ketone); 1254, 1225 cm^{-1} (acetate).

Chromatography of the filtrate gave a further crop (35 mg.) of m. p. 182°C. Total yield, 157 mg. A small amount of another kind of crystals (m. p. 110°C) was also obtained; this yield, however, was not enough for its identification.

A solution of the ketone (50 mg., m. p. 184°C) in 2 ml. of 5% methanolic potassium hydroxide was refluxed for 1 hr. in an atmosphere of nitrogen, and then the solution was neutralized with dilute acetic acid. The precipitate was collected and esterified with diazomethane, followed by the usual acetylation (acetic anhydride and pyridine). Recrystallized from methanol, the product melted at 182°C, showing no melting point depression on admixture with the starting material.

Bisthioketal (VII) of VIa. — The saturated diketone (m. p. 184°C) (200 mg.) was allowed to react with 1 ml. each of ethanedithiol and borofluoride etherate at room temperature. After 72 hr. the reaction mixture was poured into 20 ml. of ice-cold water and extracted with ether. The ethereal extract was washed, dried and evaporated to dryness. After removal of residual ethanedithiol by steam distillation, the product was extracted with ethyl acetate and the solvent removed. Recrystallization from methanol gave needles of m. p. 178°C. Yield, 110 mg.

Found: S, 20.92. Calcd. for $C_{51}H_{48}O_4S_4$ (612.95): S, 21.32%.

Desulfuration of the Bisthioketal (VII).—*Methyl 3α-Acetoxycholanate (VIII).* — A solution of the thioketal VII (100 mg.) in 4 ml. of methanol was refluxed with Raney nickel (1 g.) for 8 hr. on a water bath. Chromatography of the product gave needles of m. p. 132°C (32 mg.). No melting point depression was observed on admixture with an authentic sample of methyl 3α-acetoxycholanate (m. p. 133°C).

Found: C, 75.02; H, 10.03. Calcd. for $C_{27}H_{44}O_4$ (432.626): C, 74.95; H, 10.25%.

Substance A. — Substance A was recrystallized from methanol or acetone-water and melted at 174°C; $\alpha_D^{29.5} = +136°$ (EtOH); λ_{max} 273 mμ; 240 mμ (ε_{max}^{EtOH} 11492, 7681). The substance in acetic acid absorbed bromine to turn dark brown in color. It was found to be resistant to catalytical hydrogenation as well as to chromic acid oxidation at room temperature. (Found: C, 65.04; H, 7.13%).

Summary

Carefully repeated experiments have shown that, contrary to Fieser's report[3], methyl 3α-acetoxy-Δ^7-cholenate and some other unsaturated bile acids (5β) listed in Table I give positive results in the selenium dioxide test. This finding was substantiated by the fact that two oxidation products of methyl 3α-acetoxy-Δ^7-cholenate were isolated, one of which was proved to be methyl 3α-acetoxy-7ξ, 15ξ-dihydroxy-$\Delta^{8(14)}$-cholenate.

It is very interesting to note that, unlike the Δ^7-steroids of the 5α-series, not only C_7-H, but C_{15}-H of the unsaturated bile acid mentioned above was oxidized with selenium dioxide to give hydroxyl groups, both of which remained without being acetylated under the experimental conditions described.

Department of Biochemistry
Tottori University School of Medicine
Yonago & Laboratory of
the Nippon Eiyô Kagaku Co.
Katsushika-ku, Tokyo