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

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(Received February 27, 1961)

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.

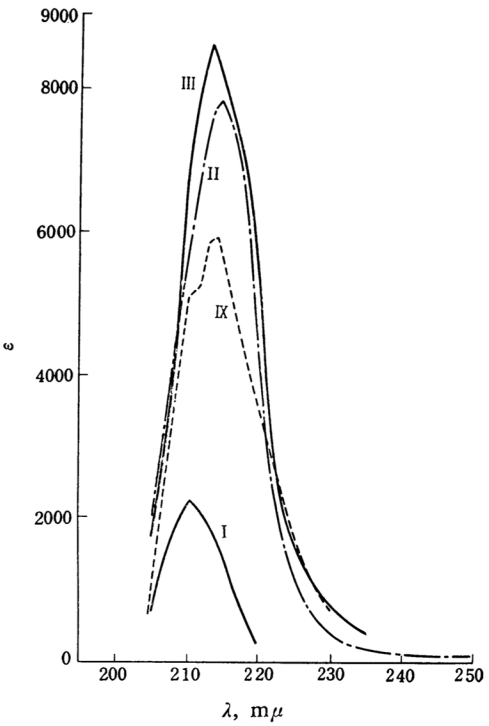
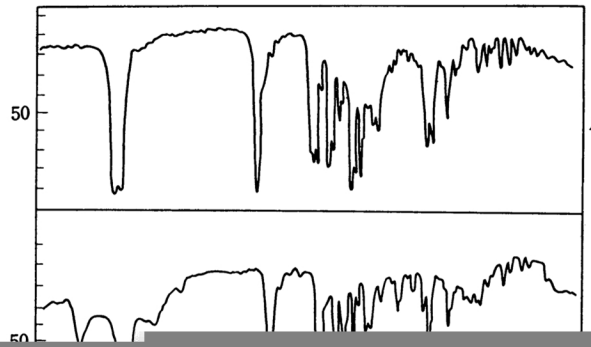
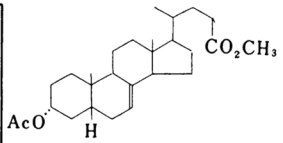


Fig. 1. Absorption spectra of: Methyl 3α-acetoxy-Δ^7-cholenate (I); substance B (II) and its acetate (III); methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate (IX, for reference).

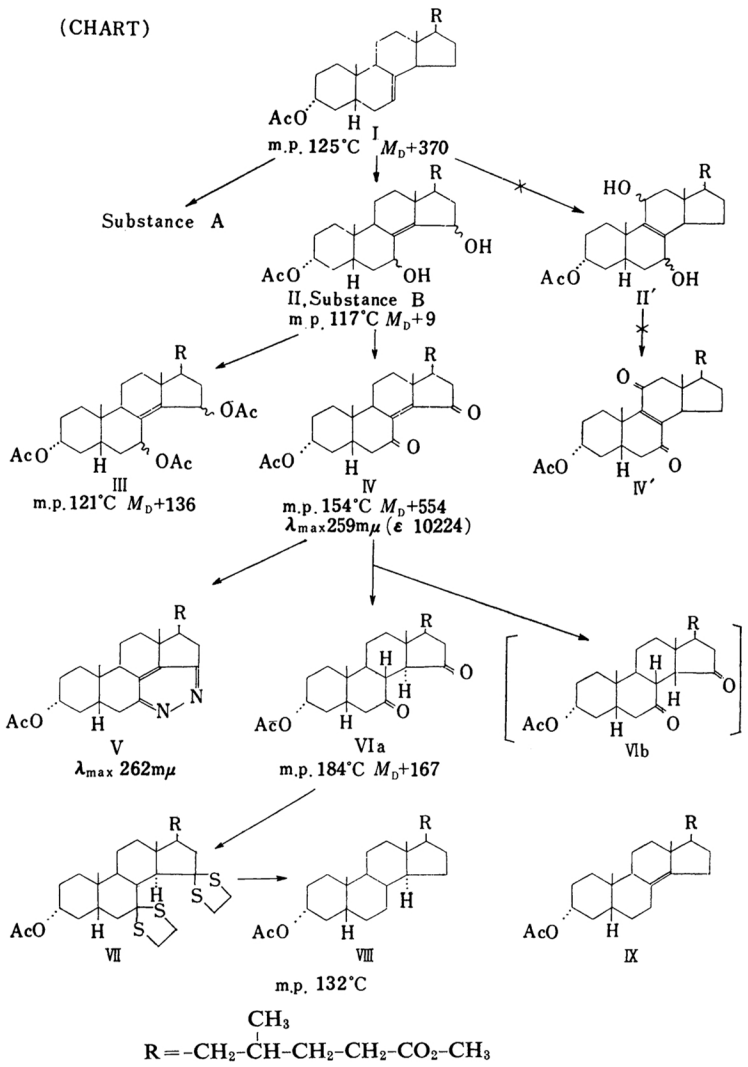
1) L. F. Fieser and G. Ourisson, *J. Am. Chem. Soc.*, 75, 4404 (1953).
2) G. Saucy, P. Geistlich, R. Helbling and H. Heusser, *Helv. Chem. Acta*, 37, 250 (1954).
3) L. F. Fieser, *J. Am. Chem. Soc.*, 75, 4395 (1953).





I

(CHART)



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-dioxoergosta-$\Delta^{8(14),22}$-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

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

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). Subs

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

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.

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