Shoji Hara : On the Structure of Cholenic Acid.

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Wieland[1] obtained lithobilianic acid and isolithobilianic acid by the nitric acid oxidation of lithocholic acid. Lithobilianic and isolithobilianic acids were required as starting materials for the syntheses of azacholane derivatives,[2] and the method of Wieland was employed for the preparation of these acids. It was observed that along with 60% of lithobilianic acid, formation of a mixture of complicated carboxylic acids occurred and the yield of the separable iso acid was very low.

On the other hand, the known method[6] for the preparation of Δ^2-cholestene (II) calls for the decomposition of cholestanyl chloride,[3] cholestanyl tosylate,[4] or potassium cholestanyl sulfate[5](I). The elimination reaction occurs with ease in the case of epicholestanyl tosylate,[4] where the 3-tosyloxy group is in the α-configuration, and forms Δ^2-cholestene. In the series of hydroxycholanic acids where the A/B ring junction differs, the position of the double bond in the unsaturated compound formed, whether Δ^{2-} [6,7,8] or the alternative Δ^3-position,[9,10] has not been proved experimentally. In an effort to obtain Δ^2-cholenic acid for its conversion to the desired isolithobilianic acid, a study of the dehydration reactions of various bile acids was initiated.

Methyl lithocholate was treated with tosyl chloride in pyridine and converted to methyl lithocholate *p*-toluenesulfonate (IIIa), scaly crystals, m.p. 117~118°. This sulfonate was boiled in pyridine for three hours and methyl cholenate (scales, m.p. 71~73°) thereby obtained was submitted to ozonolysis, followed by chromic acid oxidation, and finally esterified with diazomethane to the trimethyl ester (needles, m.p. 110~112°). This ester corresponded to trimethyl lithobilianate (Va) and the desired trimethyl isolithobilianate was not obtained. Dieckmann condensation of this ester followed by decarboxylation yielded pyrolithobilianic acid (VI), and thus it is clear that the foregoing methyl cholenate is Δ^3-cholenate (IVa) and not Δ^2-cholenate.

Methyl 12α-hydroxycholenate (IVb), first obtained by Reichstein[9] as an intermediate in the synthesis of 12α-hydroxycholanic acid, was also converted to trimethyl desoxybilianate by the oxidation procedures described for methyl Δ^3-cholenate. The position of the double bond in this case must also be in the 3-4 position, contrary to the assumption of Fieser.[6]

Based on these facts, the writer is of the opinion that in steroids where the A/B ring is in the *cis*-configuration, Δ^3-steroids are mainly formed by the elimination of the tosyl ester of the α-hydroxyl function in the 3-position.

Wieland[11] also obtained "α- and β-lithocholenic acid" in a 9 : 1 ratio by the pyrolysis of lithocholic acid and thought the "α-acid" to be Δ^2-cholenic acid by the nature of the

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1) Wieland, *et al.*: Z. physiol. Chem., **211**, 261(1932).
2) Ochiai, Hara : Paper read before the Monthly Meeting of the Pharmaceutical Society of Japan, September, 1953.
3) Mauthner : Monatsh., **30**, 635(1909).
4) Stoll : Z. physiol. Chem., **246**, 1(1937).
5) Sobel, *et al.* : J. Am. Chem. Soc., **63**, 3536(1941).
6) L. F. Fieser, M. Fieser : "Natural Products Related to Phenanthrene," Reinhold Pub. Corp., New York, 3rd Ed., 127, 246(1949).
7) Sarett : J. Biol. Chem., **162**, 591(1946).
8) Naha : J. Pharm. Soc. Japan, **73**, 1199(1953).
9) Reichstein, *et al.* : Helv. Chim. Acta, **21**, 926(1938).
10) Webb, *et al.* : J. Chem. Soc., **1949**, 2164.
11) Wieland, *et al.* : Z. physiol. Chem., **241**, 47(1936).

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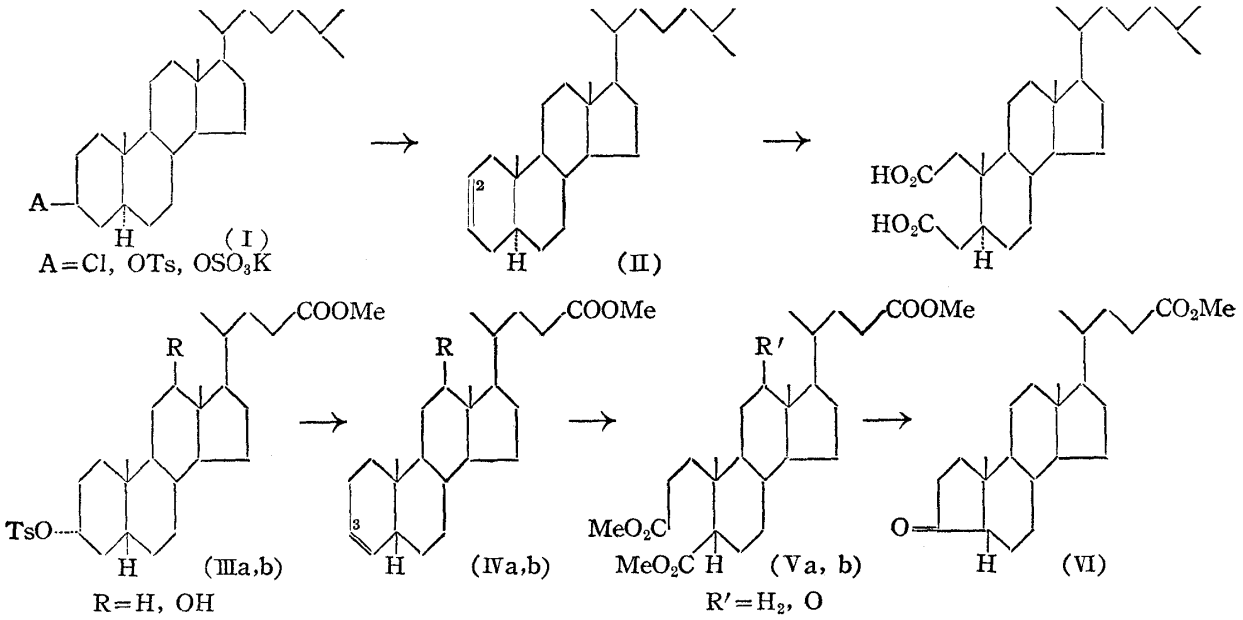
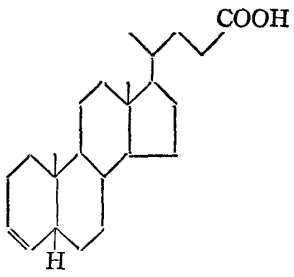
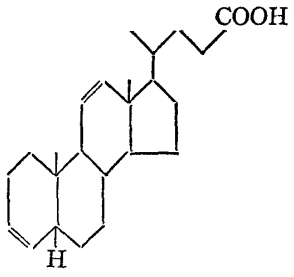


products obtained from the selenium oxide oxidation. This method was also investigated for the preparation of isolithobilianic acid and a cholenic acid (prismatic crystals of m.p. 153~155°) corresponding to the above "α-acid" was obtained. Oxidation and esterification were carried out under the same conditions but the ester obtained was that of lithobilianic acid, showing a marked depression on admixture with trimethyl isolithobilianate. It was thereby concluded that the "α-acid" is Δ^3-cholenic acid and that Wieland's structural assignment had been erroneous.

As for the structure of the unsaturated acids obtained by the pyrolysis of bile acids, Barton[12,13] disproved the presence of a double bond in 7-8 position, as had heretofore been assumed, by the consideration of its reaction mechanism and showed the double bond to be in the 6-7 position. However, he assumed that Wieland's structural assignment for the position of the double bond in ring A was correct. The present writer is of the opinion that these error should be corrected and the structure of cholenic and choladienic acids formed as the chief products of the pyrolysis of bile acids are better represented by the expressions shown.



Cholenic Acid



Choladienic Acid

As described in the foregoing, the writer was not able to obtain isolithobilianic acid in good yield but was unexpectedly able to confirm the structure of cholenic acid. It has been found that the direction of the dehydration reaction of the hydroxyl function in the 3-position in cholestanol and hydroxycholanic acid is reversed. This is thought to be due to the difference in the steric configuration of the A/B ring. In general, bromination and sulfonation of 3-ketosteroids occur chiefly in the 2-position when A/B

12) Barton : J. Chem. Soc., **1946**, 1116.
13) Barton : *Ibid.*, **1949**, 2174.

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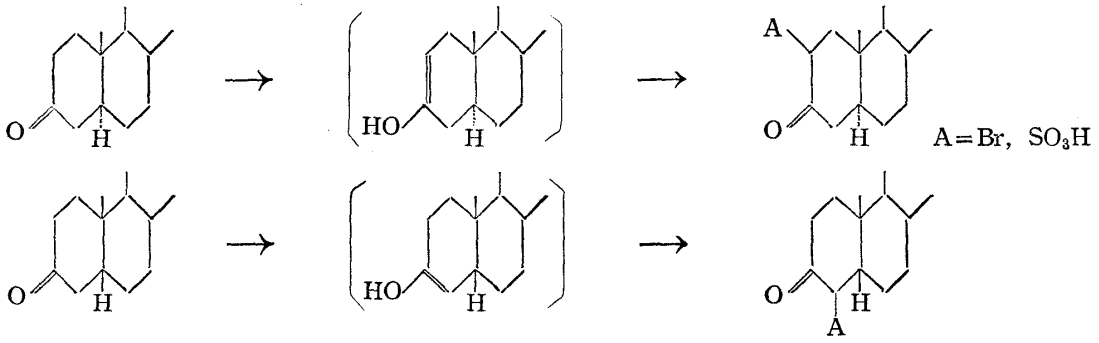
is *trans* and in the 4-position when A/B is *cis*, and hence the dehydration reactions of the 3α-hydroxyl function proceeds in an analogous direction with the enolization reactions of 3-ketosteroids.



The writer takes this opportunity to express his deep gratitude to Prof. Eiji Ochiai for his kind guidance, and to thank Dr. M. Tanabe (Riker Lab.) for his kind advice and the members of the Central Analysis Room of this Institute for the elemental analyses.

Experimental

Methyl Lithocholate *p*-Toluenesulfonate (IIIa)—A solution of 0.7 g. of methyl lithocholate and 0.45 g. of tosyl chloride dissolved in 1.5 cc. of pyridine was allowed to stand at a room temperature for 1.5 days. This was poured into ice water, extracted with ether, and the ether extract was washed consecutively with 5% HCl, 5% NaOH, and water. After drying over Na_2SO_4, ether was evaporated and 0.85 g. of the residue crystallized. This residue was recrystallized from ether–MeOH mixture to 0.7 g. of scales, m.p. 116~118°. *Anal.* Calcd. for $C_{32}H_{48}O_5S$ (Methyl lithocholate *p*-toluenesulfonate): C, 70.60; H, 8.80; S, 5.90. Found: C, 70.33; H, 8.79; S, 6.03.

Methyl Δ^3-Cholenate (IVa) (Decomposition of the Sulfonate)—A solution of 600 mg. of the sulfonate dissolved in 2.5 cc. of pyridine was refluxed for 3 hrs., pyridine was distilled off under a reduced pressure, and the residue was extracted with ether. The ether extract was washed consecutively with 5% HCl, 5% NaOH, and water, and dried. Removal of ether left 160 mg. of a crystalline residue which was recrystallized from ether–MeOH mixture to 100 mg. of scales, m.p. 71~73°, which colored with tetranitromethane. *Anal.* Calcd. for $C_{25}H_{40}O_2$ (Methyl cholenate): C, 80.70; H, 10.80. Found: C, 80.78; H, 11.12.

The portion (400 mg.) insoluble in ether finally solidified but would not crystallize and was assumed to be a pyridinium salt.

Oxidation of Methyl Cholenate—Ozone was passed through an ice-chilled solution of 100 mg. of methyl cholenate dissolved in a mixture of 5 cc. AcOH and 4 cc. $CHCl_3$ until bromine is no longer discolored. After addition of a few drops of water, $CHCl_3$ was distilled off under a reduced pressure, and 2 cc. of a solution of 160 mg. of CrO_3 dissolved in 20 cc. AcOH was added to the original AcOH solution. After allowing the mixture to stand over night, the excess of CrO_3 was decomposed with SO_2, the mixture was poured into water, and the precipitate was collected by filtration. The precipitate was washed with water, dissolved in ether, and extracted with 5% NaOH. The alkali extract was acidified with HCl, extracted again with ether, and the ether was removed from the extract after washing with water and drying over Na_2SO_4, providing 80 mg. of a residue which solidified. A small amount of a neutral substance was obtained from the former ether extract. The solid residue was esterified with ether solution of CH_2N_2 and the crystals of m.p. 105~106° thereby obtained were recrystallized from MeOH to 50 mg. of needles, m.p. 110~112°, showing no depression on admixture with trimethyl lithobilianate. Fusion of this substance with trimethyl isolithobilianate (prisms, m.p. 104~106°) caused depression, melting at around 90°.

Pyrolithobilianic Acid (Dieckmann Condensation)—A mixture of 100 mg. of trimethyl lithobilianate, obtained by the oxidation of methyl cholenate, and 135 mg. of MeONa in 4 cc. of benzene was refluxed for 2 hrs. After allowing the mixture to cool, 0.2 cc. AcOH was added and poured into a mixture of ether and water. The aqueous layer was extracted with ether and ether was distilled off after washing and drying. The oily residue was refluxed in a mixture of 4 cc. AcOH, 2 cc. conc. HCl, and 0.4 cc. H_2O for 20 mins. Removal of AcOH left 70 mg. of a crystalline residue which was recrystallized from MeOH to 60 mg. of scales, m.p. 199~201°, undepressed by admixture with pyrolithobilianic acid obtained by the pyrolysis of lithobilianic acid.

Oxidation of Methyl 12α-Hydroxycholenate—Oxidation was carried out as in the case of methyl Δ^3-cholenate and subsequent esterification yielded prisms, m.p. 120~121°, identical with trimethyl

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desoxybilianic acid.

Pyrolysis of Lithocholic Acid—Following the method given in the literature,[11] 200 mg. of cholenic acid, m.p. 152~153°, obtained from 250 mg. of lithocholic acid, was recrystallized from MeOH to 150 mg. of prisms, m.p. 153~155°. After esterification, 100 mg. of the ester was submitted to oxidation as in the case of methyl Δ^3-cholenate and 80 mg. of an acid portion and 15 mg. of a neutral portion were obtained. The acid portion was esterified and recrystallized from MeOH to 45 mg. of needles, m.p. 109~111°, identical with trimethyl lithobilianate.

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