identified by its conversion to the hydrazo compound, m. p. 188–193° dec.

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Scymnol

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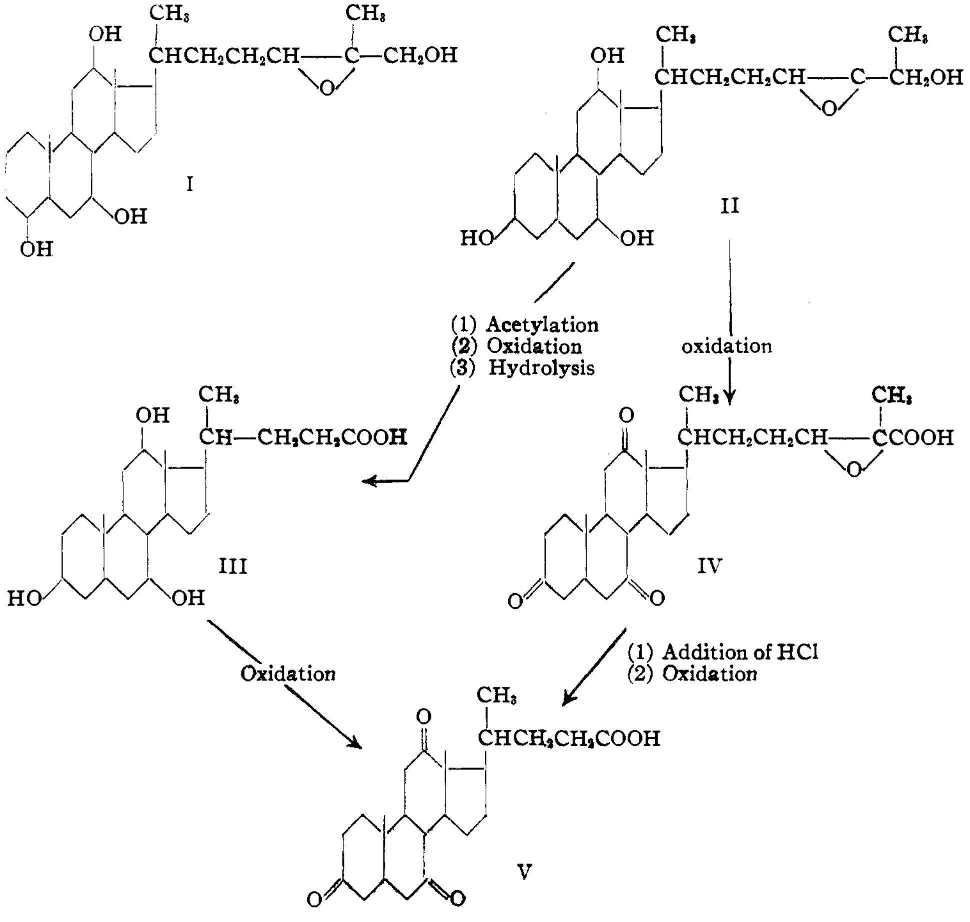
In 1898, Hammarsten¹ showed that the bile of the northern shark, *Scymnus borealis*, lacks the common bile acids, containing in their place the sulfuric acid ester of a polyhydric alcohol, scymnol, $C_{27}H_{46}O_5$. Since then scymnol has been found in the bile of other sharks, and also rays, and it is now regarded² as a typical component of the bile of all elasmobranch fish. Windaus³ and Tschesche⁴ have demonstrated the relation of scymnol to bile acids, and the latter has assigned to it the provisional formula I. In this formula the position of a hydroxyl group on ring A remained uncertain. Tschesche contended that it did not occupy the C_3-position, because degradation of scymnol gave a triketo acid, which was isomeric, but not identical with, 3,7,12-triketocholanic acid. Mild reduction of Tschesche's acid yielded the known 7,12-diketocholanic acid. Because of the apparent ease with which the new triketo acid underwent enolization, Tschesche considered position C_4 the most likely point of attachment of the hydroxyl group. More recently, Asikari⁵ revised Tschesche's formula by attaching the unplaced hydroxyl group to the C_3-position, (II). His evidence favoring this revision was based on the observation that scymnol may readily be degraded to cholic acid (III) by way of the oxidation of its tetra-acetate.



It was one of the objects of the present investigation to prove definitely the location of the hydroxyl group and to explain the contradictory results obtained by Tschesche and Asikari. The scymnol used in the present study was isolated

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(1) Hammarsten, *Z. physiol. Chem.*, **24**, 322 (1898).

(2) Cook, *Nature*, **147**, 388 (1941).

(3) Windaus, Bergmann and König, *Z. physiol. Chem.*, **189**, 148 (1930).

(4) Tschesche, *ibid.*, **203**, 263 (1931).

(5) Asikari, *J. Biochem.* (Japan), **29**, 319 (1939).

from the bile of the tiger shark, *Galeocerdo arcticus*. It was in all respects identical with the scymnol described in the literature. Oxidation of its tetra-acetate gave an amorphous acid, which upon hydrolysis of the acetate groups yielded a nicely crystalline material. This was readily identified as cholic acid (III) by its physical properties, and by its conversion to the methyl ester and 3,7,12-triketocholanic acid (V). These results are therefore in complete agreement with Asikari's observations.

In repeating Tschesche's investigations, scymnol was first oxidized to scymnol-triketo acid[3] (IV), which was then treated with hydrochloric acid, to open the ethylene oxide ring to the corresponding chlorhydrin. Tschesche did not isolate this compound but oxidized the crude product. In the present investigation it was carefully purified until it melted constantly at 225–227°, and analyzed correctly for $C_{27}H_{39}O_6Cl$. Oxidation of this product gave an acid which at first appeared to be identical with Tschesche's compound. Like the latter it melted with decomposition at 216–219° and gave some reaction with alkali. It also gave, however, a Beilstein test for halogen, indicating the presence of impurities, probably unchanged starting material. Upon frequent recrystallization the melting point of the acid rose steadily until it remained constant at 236–238°. The pure material gave no test for halogen, analyzed correctly for $C_{24}H_{34}O_5$ and gave no depression of the melting point when mixed with authentic 3,7,12-triketocholanic acid (IV). These results therefore also favor Asikari's formula, and indicate that Tschesche had been misled by the impurity of his products.

Experimental Part

Oxidation of Scymnol to 3,7,12-Triketocholanic Acid.—Anhydrous scymnol, m. p. 186–188°, was prepared from the bile of *Galeocerdo arcticus* according to the directions of Windaus.[3] It was converted into the tetra-acetate by refluxing it with a mixture of acetic anhydride and pyridine[3]; m. p. 145.5–147°, yield 82%.

Anal. Calcd. for $C_{35}H_{54}O_9$: C, 67.91; H, 8.80. Found: C, 67.93; H, 8.78.

A solution of 45 g. of tetra-acetate in 300 cc. of glacial acetic acid was heated to 90°, and with constant stirring a solution of 75 g. of chromic acid in 750 cc. of 90% acetic acid was added slowly over a period of ninety minutes. The excess chromic anhydride was then reduced by sulfur dioxide, and the solution concentrated *in vacuo* to a viscous oil. This was poured into ice water, and the sticky mass which precipitated was rubbed with water until it turned granular. The product was filtered, dissolved in ether, and the solution extracted with a 10% sodium carbonate solution. Acidification of the alkaline extract gave 15.5 g. of a colorless, amorphous precipitate. It was refluxed for three hours with a 10% solution of potassium hydroxide. Acidification of the solution gave 9.7 g. of an amorphous product, which turned crystalline upon digestion with absolute ethanol. Several recrystallizations from absolute ethanol gave the typical large crystals of the alcoholate of cholic acid, m. p. 193–195°.

Anal. Calcd. for $C_{24}H_{40}O_5.C_2H_5OH$: C, 68.68; H, 10.20. Found: C, 68.94; H, 10.05.

The acid was dried in an Abderhalden apparatus for twenty-four hours at 130° and 2 mm. It now melted sharply at 195.2–195.8° and gave no depression of the melting point when mixed with an authentic sample of cholic acid.

Anal. Calcd. for $C_{24}H_{40}O_5$: C, 70.54; H, 9.88. Found: C, 70.35; H, 10.00.

Treatment of the acid with diazomethane gave a methyl ester of m. p. 153.5–154°, and oxidation with chromic anhydride gave 3,7,12-triketocholanic acid of m. p. 238–239°. Both derivatives gave no depression of the melting point when mixed with authentic samples.

Chlorhydrin of Scymnoltriketo Acid.—Scymnol was oxidized to the triketo acid according to the directions of Windaus.[3] The melting point of the acid, 236°, was the same as that reported in the literature.

Anal. Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.86; H, 8.43.

Three grams of the acid was treated at room temperature for fifteen minutes with a mixture of 15 cc. of glacial acetic acid and 15 cc. of concd. hydrochloric acid. The solution was then poured on ice, and the crystalline precipitate which had formed overnight, was dissolved in glacial acetic acid. Addition of water to the solution gave a small amount of a brown, amorphous precipitate, which was discarded. Upon the addition of more water a copious precipitate of fine, colorless needles was obtained; 2.4 g., m. p. 220°. It was recrystallized from a mixture of ethyl acetate and petroleum ether until the melting point remained constant at 225–227°. *Anal.* Calcd. for $C_{27}H_{39}O_6Cl$: C, 65.50; H, 7.94; Cl, 7.16. Found: C, 64.58; H, 8.00; Cl, 6.85.

3,7,12-Triketocholanic Acid.—A solution of 1.2 g. of chromic anhydride in 12 cc. of 80% acetic acid was added to a solution of 1.2 g. of the chlorhydrin in 10 cc. of glacial acetic acid. The mixture was heated for one hour at 80° and then poured into ice water. The precipitated material was dissolved in ether, the solution extracted with 10% sodium carbonate solution, and the extract acidified. A crystalline precipitate at once appeared which melted at 215° with considerable decomposition. It was recrystallized from ethyl acetate until its melting point remained constant 236–238°. The mixed melting point with an authentic sample of 3,7,12-triketocholanic acid of 238–239° was 236–239°.

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.62; H, 8.51. Found: C, 71.42; H, 8.54.

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