[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NORTHWESTERN UNIVERSITY]

The Structure and Configuration of Cholesterylpyridinium Salts

BY L. CARROLL KING AND M. JEROME BIGELOW

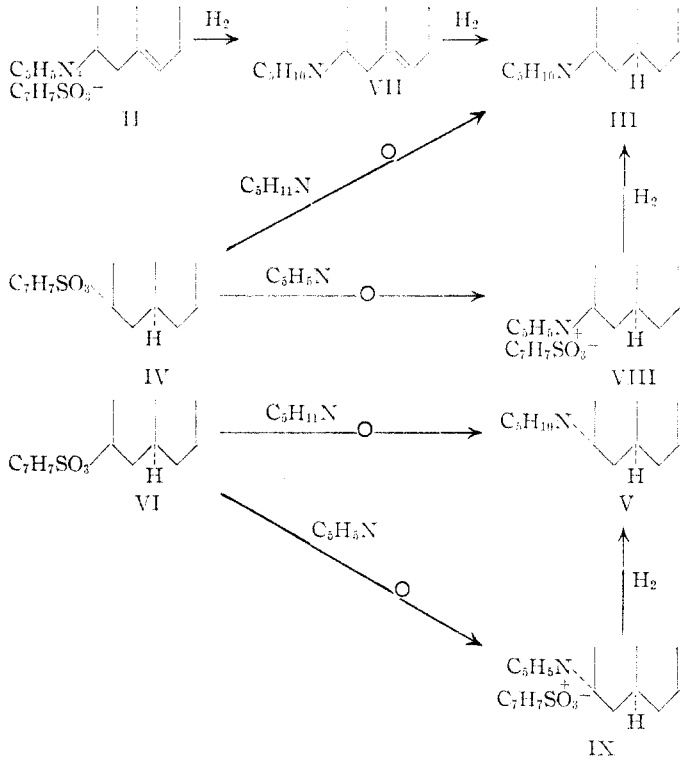
RECEIVED JANUARY 16, 1952

Evidence is presented to support the idea that in cholesterylpyridinium *p*-toluenesulfonate, the nitrogen of the pyridine is attached to the 3-β-position of the steroid group.

The reaction between cholesteryl *p*-toluenesulfonate (I) and pyridine gave a single quaternary salt, cholesterylpyridinium *p*-toluenesulfonate (II) which was not rearranged in the presence of the acid salt of pyridine.[1] On the basis of this observation and the observation that II could be prepared by action of pyridine and *p*-toluenesulfonic acid on *i*-cholesteryl methyl ether, the salt II was assigned the 3-β-configuration.

This paper provides further evidence that II has the 3-β structure as suggested,[1] thus, cholesterylpyridinium *p*-toluenesulfonate (II) was hydrogenated to cholestanylpiperidine (III).[2] Cholestanylpiperidine (III) was also prepared by the reaction of epicholestanyl *p*-toluenesulfonate (IV)[3] with piperidine and the two preparations were shown to be identical. Epicholestanylpiperidine (V) was prepared by the reaction of cholestanyl *p*-toluenesulfonate (VI)[3] with piperidine and V was shown to be different from III. The compounds IV and VI were also allowed to react with pyridine[3]; the quaternary salts VIII and IX produced by these reactions were reduced to III and V, respectively. These relationships are outlined on the chart.

Assuming the conventional structure for cholesterol and that replacement accompanied by inversion of configuration occurs in the reactions of piperidine with cholestanyl *p*-toluenesulfonate (VI) and with epicholestanyl *p*-toluenesulfonate (IV), the configuration of cholesterylpyridinium *p*-toluenesulfonate (II) is established as 3-β.



Experimental[4]

Cholesterylpyridinium *p*-Toluenesulfonate (II).—This compound was prepared as directed in reference 1.

Cholesterylpiperidine (VII).—To 1.27 g. (0.002 mole) of cholesterylpyridinium *p*-toluenesulfonate (II)[1] in 100 cc. of absolute ethanol was added 500 mg. of platinum oxide and a small drop of mercury. This suspension was shaken with hydrogen at atmospheric pressure for 20 minutes. The takeup of hydrogen was 180 cc. (0.007 mole). The suspension was then filtered and 5 cc. of 20% sodium hydroxide solution was added. The solution was evaporated to a volume of about 10 cc. and taken up in ether. The ether solution was washed twice with water, dried with sodium sulfate, and evaporated. The residual white solid was crystallized from acetone; 600 mg. (56%) of white needles, m.p. 157–162°, were obtained. Recrystallization from acetone gave VII, melting at 166–167°; $[\alpha]^{25}D$ −20.8°.

Anal. Calcd. for $C_{32}H_{55}N$: C, 84.83; H, 12.22. Found: C, 84.55; H, 12.41.

The residue from the first recrystallization was repeatedly recrystallized from acetone, then ethanol, to obtain 10 mg. of compound III, m.p. 141–143°.

Cholestanylpiperidine (III) (a) By Reduction of II.—A solution of 1.28 g. of II in alcohol was treated for 45 minutes with hydrogen at atmospheric pressure and in the presence of Adams catalyst. The basic reaction product was recovered and crystallized from acetone; yield 0.9 g. of III; m.p. 146–147°; $[\alpha]^{25}D$ +23°.

Anal. Calcd. for $C_{32}H_{57}N$: C, 84.46; H, 12.62. Found: C, 84.49; H, 12.59.

(b) **By Reduction of VIII.**—An ethanol solution of 30 mg. of cholestanylpyridinium *p*-toluenesulfonate (VIII) and 50 mg. of platinum oxide was hydrogenated at atmospheric pressure for 30 minutes. The basic products on crystallization from acetone–water gave 15 mg. (70%) of brownish crystals, m.p. 137–142°. Recrystallization from acetone gave a product, m.p. 143–145°. The mixed melting point of this substance and that described in (a) above was not depressed.

(c) **By Displacement.**—A solution of 155 mg. of epicholestanyl *p*-toluenesulfonate (IV) in 10 cc. of piperidine was heated on the steam-bath for six hours. The reaction mixture was poured into ether, and the piperidine *p*-toluenesulfonate was separated. The ether solution was washed twice with water, dried with sodium sulfate, and the ether was removed on the steam-bath. The residual solid

(1) L. C. King, R. M. Dodson and L. A. Subluskey, THIS JOURNAL, **70**, 1176 (1948).

(2) When cholesterylpyridinium *p*-toluenesulfonate (II) is reduced both cholesterylpiperidine (VII) and cholestanylpiperidine (III) are produced. Details for preparation of either or separation of the mixture are given in the Experimental part.

(3) In all the replacement reactions carried out on epicholestanyl *p*-toluenesulfonate (IV) or on cholestanyl *p*-toluenesulfonate (VI), some 2-cholestene was obtained.

(4) All rotational data were observed on about 50–100 mg. of sample in 3 cc. of chloroform solution. Melting points were observed on a Fisher–Johns melting point block.

was dissolved in acetone and dry HCl gas was passed in. The powdery hydrochloride salt was converted to the free base with alcoholic sodium hydroxide, and the base was crystallized from acetone; yield 75 mg. (57%) of white needles, m.p. 147–148°. This product was identical with the above preparations.

2-Cholestene.—The acetone-soluble fraction from (c) above, was evaporated to dryness, taken up in ether, and filtered. The ether solution was washed with water, dried with sodium sulfate, and the ether was evaporated. The resulting oil was crystallized from acetone–water. The product, 24 mg. (23%), m.p. 64–66°, was identified as 2-cholestene. Further crystallization gave a sample,[5] m.p. 67–68°; $[\alpha]^{25}D$ +60.3°, $[\alpha]^{25}_{6780}$ +64°, $[\alpha]^{25}_{5460}$ +71°, $[\alpha]^{25}_{4358}$ +120°.

Cholestanyl *p*-Toluenesulfonate (VI).—One and one-half grams of pure cholestanol, m.p. 141–143°, and 1 g. of *p*-toluenesulfonyl chloride were dissolved in 10 cc. of dry pyridine and allowed to stand overnight. To the resulting solution was added 1 cc. of water; an exothermic reaction was observed, and after about 5 minutes excess water was added; the suspension was filtered, and the gummy solid was taken up in ether and washed with water. The ether solution was dried with sodium sulfate and evaporated to dryness. The residue was crystallized from ethanol to obtain 1.9 g. (90%) of white platelets, m.p. 135–136°; reported[6] m.p. 136°.

Epicholestanylpyridinium *p*-Toluenesulfonate (IX).—A solution of 1.9 g. of cholestanyl *p*-toluenesulfonate (VI) in 10 cc. of pyridine was heated overnight on the steam-bath. The pyridine was evaporated in an air stream and the resulting solid mass was recrystallized from acetone to obtain 2 g. of solid, m.p. 160–170°. This was dissolved in chloroform and the solution was washed with a solution of 10% ethanol in water. The chloroform solution was dried with sodium sulfate and the chloroform was removed on the steam-bath. The residue was crystallized from acetone. One gram of IX was obtained, m.p. 165–170°. This product was used directly in the following hydrogenation.

The acetone-soluble fraction from the preparation of the pyridinium salt (IX) was evaporated to dryness, leached with Skellysolve B, and filtered. The filtrate was evaporated to dryness, and the residual oil was crystallized from acetone–water, then from acetone; in this manner 210 mg. (16%) of 2-chlolestene, m.p. 67–68°, was obtained.

Epicholestanylpiperidine (V). (a) By Hydrogenation.—Four hundred mg. of epicholestanylpyridinium *p*-toluenesulfonate (IX) and 100 mg. of platinum oxide in 30 cc. of absolute ethanol were shaken with hydrogen at atmospheric pressure for one hour. The suspension was filtered, and the basic products isolated. On crystallization from acetone–water, 210 mg., m.p. 85–90° (85%), was obtained. After two recrystallizations from acetone–methanol a product was obtained which melted at 96–98°; $[\alpha]^{24}D$ +25.4°.

Anal. Calcd. for $C_{32}H_{57}N$: C, 84.46; H, 12.62; N, 3.07. Found: C, 85.1; H, 13.1; N, 3.65.

(b) By Displacement.—One hundred eighty mg. of cholestanyl *p*-toluenesulfonate (VI) and 5 cc. of piperidine were heated on the steam-bath for two days; then two pellets of sodium hydroxide were added and the solution was steam distilled to remove the excess piperidine. The residue was taken up in ether, washed with water, and the ether solution dried and evaporated. The residue was taken up in 20 cc. of acetone, and the solution was saturated with dry HCl gas and evaporated to dryness. The solid residue was leached with petroleum ether, and filtered; the residue was decomposed with alcoholic sodium hydroxide, taken up in ether, and the ether solution was washed with water, dried and evaporated. After one crystallization from acetone–water, 80 mg. (53%) of product was obtained; m.p. 98–100°, $[\alpha]^{25}D$ +26.2°. Mixed m.p. with the hydrogenation product above was 96–98°.

The petroleum ether soluble fraction was crystallized from acetone–water to give 2-cholestene, m.p. 64–65°. Mixed m.p. with a sample of 2-cholestene showed no depression.

Epicholestanol.—Five hundred mg. of cholestanyl *p*-toluenesulfonate (VI) and 500 mg. of fused potassium acetate were dissolved in 30 cc. of glacial acetic acid and the solution was refluxed for eight hours. The acetic acid was removed in an air stream and the resulting oil was taken up in ether. The ether solution was washed twice with water, dried with sodium sulfate, and evaporated to dryness. A solution of 1 g. of sodium hydroxide in 50 cc. of methanol was then added, and the resulting solution was refluxed for two hours. The methanol was then evaporated nearly to dryness, and the residue was taken up in ether–water. The layers were separated, and the ether layer was washed twice with water, dried, and evaporated. The residual solid was then taken up in 50 cc. of petroleum ether and chromatographed on an activated alumina column 8 inches in length with a holdup of 25 cc.

Fraction	Eluant, cc.	Obtained, mg.	M.p., °C.
1	50 petroleum ether	100	Oil
2	50 petroleum ether	80	
3	50 petroleum ether	2	
4	25 $(C_2H_5)_2O$	140	181–184 (40%)

Fractions 1 and 2 were combined and crystallized from acetone–water to give 2-cholestene, m.p. 64–66°.

Fraction 4 was crystallized from methanol–chloroform to give 80 mg. of epicholestanol, m.p. 183–184°; $[\alpha]^{25}D$ +25°.

Epicholestanyl *p*-Toluenesulfonate (IV).—This substance was prepared by the same method as used for cholestanyl *p*-toluenesulfonate (VI). From 200 mg. of epicholestanol was obtained 160 mg. (57%) of IV, m.p. 138–139°.[7]

Cholestanylpyridinium *p*-Toluenesulfonate (VIII).—A solution of 80 mg. of epicholestanol and 200 mg. of *p*-toluenesulfonyl chloride in 5 cc. of pyridine was allowed to stand overnight, then was heated on a steam-bath for eight hours. The excess pyridine was removed in an air current, and the solid residue was leached with ether, then filtered, and the residue was taken up in chloroform. The chloroform solution was washed twice with ethanol–water, dried with sodium sulfate, and evaporated to dryness. The residue was crystallized from acetone to obtain 30 mg. (32%) of VIII, m.p. 184–185°.

Anal. Calcd. for $C_{39}H_{59}NSO_3$: C, 75.32; H, 9.56; N, 2.25. Found: C, 74.75; H, 8.89; N, 2.56.

The ether-soluble material was recrystallized from acetone–water to obtain 23 mg. (44%) of 2-cholestene, m.p. 64–66°.

EVANSTON, ILLINOIS

(5) The melting point of 2-cholestene has been reported as 68–69°. The rotation as $[\alpha]D$ +64°. Mauthner, *Monatsh.*, **30**, 635 (1909); W. Stoll, *Z. physiol. Chem.*, **246**, 1 (1937).

(6) W. Stoll, *ibid.*, **207**, 147 (1932).

(7) W. Stoll, *ibid.*, **246**, 1 (1937), reported this compound; m.p. 124–125°.