[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN AND THE DEPARTMENT OF BIOCHEMISTRY OF THE JEWISH HOSPITAL OF BROOKLYN]

The Influence of the 5,6-Double Bond on the Pyridine 7-Sulfates of Cholesterol

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To clarify indications of inertness to replacement of the 7-pyridinium group, the dipyridinium sulfates of 7α- and 7β-hydroxycholesterol and of 7α- and 7β-hydroxycholestanol and their potassium derivatives have been prepared. The 7-pyridinium group of the stanol derivatives was easily replaced while that of the sterol derivatives was inert. It is concluded that resistance to displacement at the 7-position is due to formation of a covalent carbon-to-nitrogen bond.

That the organic cation in pyridine sulfate derivatives of sterols may be replaced by metallic cations (Na^+, K^+, Ca^{++}, Fe^{++}, Al^{+++}, etc.) has been noted many times.[2-5] The facility of this replacement was first pointed out by Sobel and Spoerri.[4] A wide variety of steroid sulfates have undergone this displacement reaction, including the estrogens,[6-9] androgens,[10] bile acids,[11] cholesterol[2-4,12] and ergosterol.[4] However, it was found by Owades,[13] and confirmed and extended by the authors, that treatment of 7α-hydroxycholesteryl phthalate with chlorosulfonic acid in pyridine gave a pyridine sulfate derivative (I) in which the organic base was not replaceable. Prolonged contact with aqueous sodium or potassium chlorides and alcoholic potassium hydroxide solutions did not effect removal of the pyridine. Vigorous saponification hydrolyzed the phthalate, but did not dislodge the pyridine. Since Klyne, *et al.*,[14] had found that many steroid sulfates give insoluble salts with *p*-toluidine, reactions with this amine, as well as with ethylamine, α- and β-naphthylamines, diphenylguanidine, aniline and quinine were tried. In no case was the pyridine replaced.

The apparent inertness of the 7-pyridine was again noticed in the dipyridine sulfate derivative of 7α-hydroxycholesterol (II). The dihydroxy steroid was prepared in two ways, by permanganate oxidation of cholesteryl phthalate,[15] and *via* a route based on the Ziegler bromination of cholesterylbenzoate.[16,17] Treatment of its dipyridine sulfate II with metallic cations (Na^+, K^+) effected replacement of only one pyridine group, as revealed by the ultraviolet absorption spectrum[18] and analysis. Which of the two pyridines had been replaced, was, of course, not revealed by these methods. That the 7-pyridine was immobile was shown, uniquely, by the thermal decomposition of the barium salt III. Sobel[19] had shown that the manner of thermal decomposition of steroid sulfates is a function of the cation—a divalent metal sulfate gives an ether (*e.g.*, calcium cholesteryl sulfate → dicholesteryl ether), whereas a pyridine, or a monovalent metal sulfate abstracts a neighboring hydrogen atom and gives an olefin (*e.g.*, sodium cholesteryl sulfate → 3,5-cholestadiene). Therefore, if the barium salt III were 3-barium 7α-pyridine cholesteryl disulfate (IIIa) it would pyrolyze to di-Δ5,7-cholestadienyl-3-ether (IV), and if it were 3-pyridine-7α-barium cholesteryl disulfate (IIIb) it would yield di-$\Delta^{3,5}$-cholestadienyl-7-ether (V). The compound we obtained from the thermal decomposition of the barium salt had an absorption maximum at 282 mμ, characteristic of a 5,7-diene system. This established the immobile pyridine radical at the 7-position (IIIa).

Analysis of the salt formed from the reaction of the 3,7-dipyridine sulfate derivative of 7α-hydroxycholesterol (II) with potassium hydroxide revealed that one sulfate group had been replaced by hydroxyl VI. It appears likely, therefore, that the 7-pyridine group in the sterols is linked to carbon as a quaternary ammonium salt. This explains its resistance to replacement by metallic ions (IIIa). and the unexpected substitution of hydroxyl for sulfate VI, and is consistent with the pyrolysis to an unsaturated derivative IV. Two factors could be involved in the replacement of the 7-hydroxyl by pyridine: (a) the stereochemistry of the 7-position or (b) the influence of the neighboring 5,6-double bond. The spatial arrangement about the C-7 was eliminated by the finding that one pyridine group was immobile (and therefore quaternary) in the dipyridine derivative of 7β-hydroxycholesterol. The second possibility, the 5,6-double bond, was, however, shown to be significant. Treatment of the dipyridine sulfate derivatives of 7α- and 7β-hydroxycholestanols with alcoholic potassium hydroxide readily gave the dipotassium salts in both cases. The replacement of the 7-hydroxyl by pyridine to give the quaternary compound is a function of the neighboring double bond.

The difference toward sulfation of the 7-hydroxy sterols from the 3-hydroxy sterols and 7-hydroxy stanols may be ascribed to the allylic nature of the 7-position in the sterols. This confers a marked

(1) Part of this work was abstracted from the thesis submitted to the Faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

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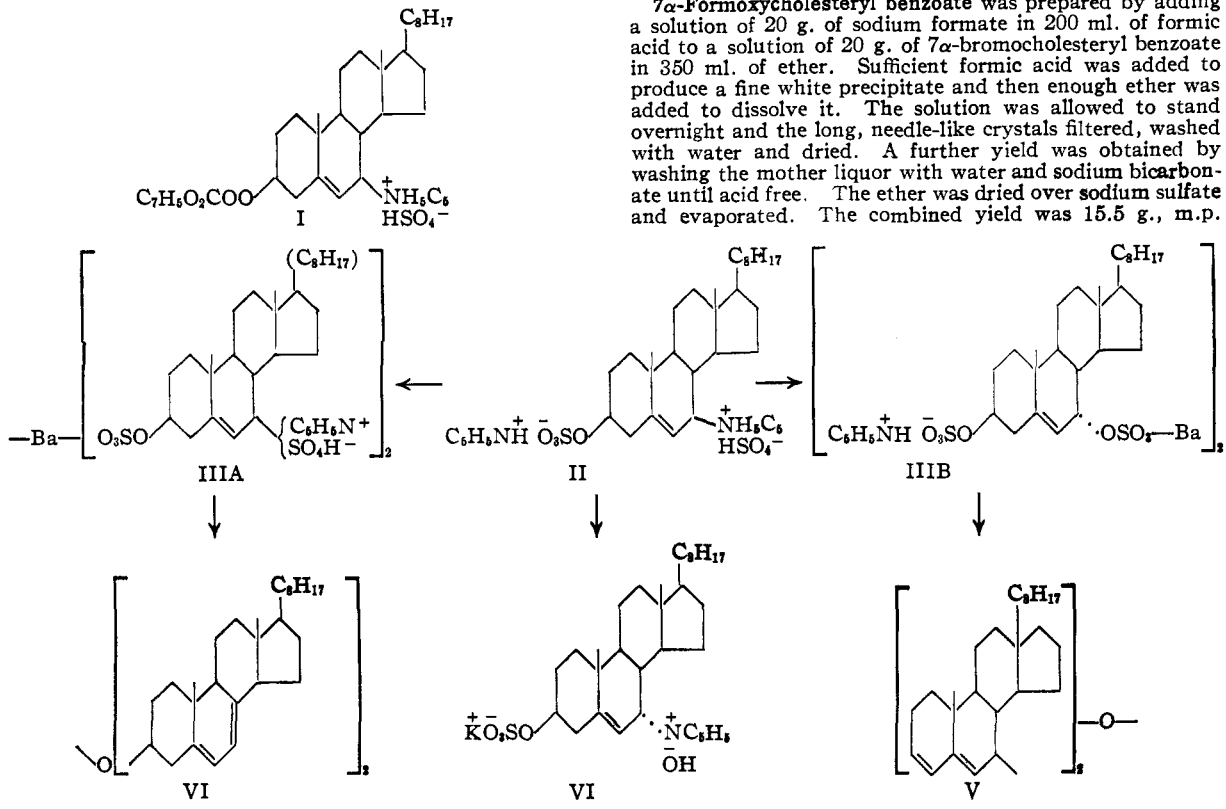
tendency toward carbonium ion formation, with a resultant affinity for nucleophilic reagents, such as pyridine. The formation of a 7-carbonium ion has been invoked, in other connections, previously.[15-18]



A quaternary pyridinium steroid has been reported by King, *et al.*[20a,b] resulting from the action of pyridine on cholesteryl *p*-toluenesulfonate, and of pyridine and *p*-toluenesulfonic acid on *i*-cholesteryl methyl ether. Since these steroids are generally assumed to give carbonium ions (in the conversion to and reversion from the *i*-steroids)[21] the formation of a quaternary salt with pyridine is in accordance with the events reported here.

Experimental

7α-Hydroxycholesteryl Phthalate.—The method of Heilbron, *et al.*,[14] with modifications, was employed. One liter of 0.25 *N* potassium permanganate was added dropwise to a suspension of 10 g. of cholesteryl phthalate in 200 ml. of 1 *N* sodium carbonate during a 2–3 hour period. The mixture was vigorously stirred during the addition and for 24 hours afterward. Many variations in concentration and amount of permanganate and alkali, as well as in time and temperature, effected no improvement in yield. The mixture was clarified with sulfur dioxide, cooled, filtered, washed with water and dried. The insoluble residue, after extraction with ethylene dichloride in a Soxhlet, was recrystallized from hot pyridine to which sufficient water was added to induce precipitation. The yield was 1 g., m.p. 199–201°.

The pyridine sulfate derivative I was prepared by the method of Sobel, Owades and Owades,[5] m.p. 142–144° dec.; λ_{max}^{alc} 256 mμ (ε 5150); cholesterol phthalate λ_{max}^{alc} 256 mμ; (ε 1340).

A.—7α-Hydroxycholesterol. Saponification of 7α-hydroxycholesteryl phthalate and recrystallization from ethanol gave 7α-hydroxycholesterol, m.p. 177–178°, $[\alpha]^{26}D$ −87° (chloroform).

B.—7α-Bromocholesteryl benzoate was prepared by the procedure of Bide, *et al.*,[16] m.p. 138°, $[\alpha]^{25}D$ −172° (chloroform).

7α-Formoxycholesteryl benzoate was prepared by adding a solution of 20 g. of sodium formate in 200 ml. of formic acid to a solution of 20 g. of 7α-bromocholesteryl benzoate in 350 ml. of ether. Sufficient formic acid was added to produce a fine white precipitate and then enough ether was added to dissolve it. The solution was allowed to stand overnight and the long, needle-like crystals filtered, washed with water and dried. A further yield was obtained by washing the mother liquor with water and sodium bicarbonate until acid free. The ether was dried over sodium sulfate and evaporated. The combined yield was 15.5 g., m.p. 116–118°, $[\alpha]^{26}D$ −61° (chloroform). The 7α-formoxycholesteryl benzoate did not precipitate silver bromide when a solution in dioxane was treated with silver nitrate.

Ten grams of 7α-formoxycholesteryl benzoate was refluxed for 4 hours in 200 ml. of 10% methanolic potassium hydroxide. The solution was cooled, diluted with water and extracted with ether. The ether extracts were washed, dried over sodium sulfate and the 7α-hydroxycholesterol precipitated with petroleum ether, yield 5.0 g., m.p. 183–185°, $[\alpha]^{25}D$ −85.5° (chloroform), positive Lifschutz test[22] (in common with the 7-bromo and 7-formoxy compounds).

Pyridine Sulfate Derivative II.—A solution of 10 ml. of pyridine and 10 ml. of chloroform, cooled in an ice-bath, was treated with 3.0 g. of chlorosulfonic acid, followed by a solution of 1.0 g. of 7α-hydroxycholesterol in 20 ml. of chloroform. After 20 minutes in the ice-bath, the solution was refluxed for 2 hours. The solvents were removed *in vacuo* and the residue washed with ether and cold water and dried. The solid was recrystallized thrice by solution in chloroform, filtration and precipitation with petroleum ether (60–80°). It was dried at 100° *in vacuo*, m.p. 125–127° dec., $[\alpha]^{25}D$ −77.5° (chloroform), negative Lifschutz test; λ_{max}^{alc} 256 mμ (ε 8000).

Anal. Calcd. for $C_{37}H_{56}O_8N_2S_2$: C, 61.6; H, 7.83; N, 3.89; S, 8.90. Found: C, 61.3; H, 7.75; N, 3.96; S, 8.92.

The analogous derivative of 7β-hydroxycholesterol was prepared in identical manner; m.p. 154–157° dec., $[\alpha]^{25}D$ +26° (chloroform), λ_{max}^{alc} 256 mμ (ε 7900).

Anal. Found: C, 61.0; H, 7.70; N, 4.06; S, 8.97.

Potassium Sulfate Derivative (VI).—To a solution of pyridinium 3,7α-cholesteryl disulfate was added a 1 *N* methanolic potassium hydroxide solution until the phenolphthalein end-point was just reached. The insoluble material appearing at this point was filtered. The filtrate was treated with absolute ether and another crop of inorganic material was filtered. The methanol–ether solution

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was taken to dryness *in vacuo*, dissolved in chloroform and precipitated with petroleum ether; after several recrystallizations the residue melted at 157–160° dec., λ_{max}^{alc} 256 mμ (ε 3730).

Anal. Calcd. for $C_{32}H_{51}O_6NSK$: N, 2.3; S, 5.3; K, 6.5. Found: N, 2.2; S, 4.7; K, 6.1.

The analogous derivative of 7β-hydroxycholesterol, prepared as above, m.p. 192–197° dec., λ_{max}^{alc} 256 mμ (ε 3850).

Anal. Found: K, 5.9.

Pyridinium 3,7α-Cholestanyl Disulfate.—Prepared as for the sterols; m.p. 132–135° dec., λ_{max}^{alc} 256 mμ (ε 8050).

Anal. Calcd. for $C_{37}H_{59}O_6N_2S_2$: C, 61.4; H, 8.04; N, 3.89; S, 8.81. Found: C, 61.0; H, 7.93; N, 3.92; S, 8.86.

The 3,7β-cholestanol derivative was prepared as above, m.p. 157–162° dec., $[\alpha]^{25}D$ +48° (chloroform), λ_{max}^{alc} 256 mμ (ε 7000).

Anal. Found: C, 60.9; H, 7.75; N, 3.99; S, 8.90.

Potassium 3,7α-Cholestanyl Disulfate.—Prepared in the same manner as the sterol derivative, m.p. 132–135° dec., λ_{max}^{alc} 256 mμ (ε 8050).

Anal. Calcd. for $C_{27}H_{46}O_8S_2K_2$: K, 12.2. Found: K, 13.3.

The 3,7β-cholestanol derivative was prepared similarly, m.p. 178–181° dec.

Anal. Found: K, 12.8.

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Derivatives of Steroids Containing a Small Ring Fused to the D Ring

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Ethyl diazoacetate adds to the conjugated double bond of 3β-acetoxy-5,16-pregnadien-20-one to form the Δ²-pyrazoline carboxylic ester. Pyrolysis of the corresponding free acid yields the 16-methyl-16-dehydro steroid; pyrolysis of the ester gives rise to two cyclopropanecarboxylic esters isomeric at carbon 16a. Alkaline hydrolysis of the latter proceeds with epimerization of one isomer so that the same acid, assigned the 16aβ-configuration, is derived from both esters.

Addition of ethyl diazoacetate to 3β-acetoxy-5,16-pregnadiene-20-one, an extension of the well known addition of diazomethane to 16-dehydro-20-keto steroids,[1,2,3] affords the corresponding pyrazoline carboxylic ester I. The products of diazomethane addition have been assigned Δ¹-pyrazoline structures. Examination of a typical example, XI, revealed the absence of any absorption in the infrared region corresponding to the =N—H stretching but showed a weak band at 6.45 μ probably associated with the —N=N— linkage. These observations further confirm the accepted structure of this adduct.

The diazoacetic ester adduct shows entirely different absorption characteristics. It was assigned the conjugated structure I which is in accord with the classical work in this field[4] and is supported by the infrared spectrum. Thus compound I shows a weak =N—H stretching band at 2.92 μ and a medium band at 6.39 μ due to the conjugated C=N stretching, also present in the spectra of II and III. The same bands occur at 2.93 and 6.39 μ in the model compound ethyl Δ²-pyrazoline-3-carboxylate. The conjugated C=N bond has been assigned at 6.50 μ in a similar model.[5] Additional evidence for the Δ²-pyrazoline structures was obtained from ultraviolet spectra where XI (λ_{max}^{MeOH} 227 mμ (ε 1,275); 335 mμ (ε 239)) shows entirely different absorption from the model, ethyl Δ²-pyrazoline-3-carboxylate (λ_{max}^{MeOH} 293 mμ (ε 9,260)). Compound I (λ_{max}^{MeOH} 290 mμ (ε 7577); 317 mμ (6,777)), is similar to II and III. All of the latter have 290 mμ absorption characteristic of the aforementioned model but not found in XI.

On pyrolytic decomposition these steroid adducts behave according to the predictions of von Auwers based on monocyclic systems,[6] in that 3β-acetoxy-16,17-[3,1-(1-pyrazolino)]-5-pregnen-20-one, yields mainly the 16-dehydro-16-methyl steroid with very little of the 16,17-cyclopropano derivative.[1,3] In pursuing this analogy the acetyl group at C-17 may be considered equivalent to the ester groups discussed by von Auwers. Thus, with two such groups attached, the pyrazoline I led to a mixture nearly half of which consisted of cyclopropane derivatives IV and V. If the pyrolysis of the free acid II involved initial decarboxylation, the resulting monosubstituted pyrazoline would yield little of the cyclopropane derivative. Our recovery of 3β-hydroxy-16-methyl-5,16-pregnadiene-20-one as the only product from a small-scale reaction lends support to this thesis.

Cyclopropane structures were assigned in this series on the basis of the isomerism at C-16a, discussed below, and analogy between their infrared spectra and those of 3β-acetoxy-16,17-cyclopropano-5-pregnen-20-one,[3] *i*-cholestenone and related *i*-steroid ketones.[7] The same 5.90 μ carbonyl band was present in the spectra of V, VI, IX and X and corresponds well with the presence of an adjacent cyclopropane ring. The shift in absorption of the C-20 carbonyl from 5.85 μ in II to its characteristic position in the nitrogen free derivatives was clearly evident and characteristic of the structural changes involved. The carbonyl band for IV could not be resolved but was apparent from the dissymmetry of the larger acetate band.

Configurations about the new centers of asymmetry are still subject to confirmation but may be deduced from the known course of reactions at the positions involved and from study of molecular models. Since epoxidation and catalytic addition of hydrogen, alcohols, mercaptans and other sub-

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