773. *Steroid Sulphates. Part I. Some Solvolytic Reactions of the Salts of Steroid Sulphates.*

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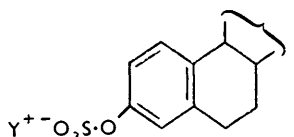
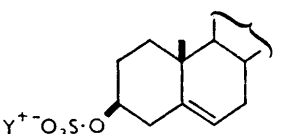
The pyridinium and potassium sulphate of cholesterol and cholestan-3β-ol were converted into their parent alcohols by solvents containing a hetero-atom with a relatively readily available lone pair of electrons. Within a group of ethers, increasing basicity of the solvent facilitated the reaction. Solvolysis in ethers was accompanied by the release of one equivalent of acid; ethanolysis proceeded without change of pH. In dioxan, œstrone pyridinium sulphate was much more readily solvolysed than the corresponding derivatives of cholesterol and cholestan-3β-ol.

HYDROLYSIS of steroid hydrogen sulphates by aqueous acids [1,2] and by enzymes [2] has received considerable attention, largely in connection with problems attaching to the isolation of steroids from biological materials. Little information is available concerning solvolytic cleavage of compounds of this class. Butenandt and Hofstetter [3] noted decomposition of œstrone sodium sulphate (I; Y = Na) in hot organic solvents. Grant and Beall [4] made the more specific observation that, on dissolution in dioxan, the salt (I; Y = Na) is rapidly converted into œstrone and that the solvolysis is inhibited by excessive amounts of water or alcohol. Cohen and Oneson [5] extended the reaction with dioxan to the cleavage of sodium sulphates of non-phenolic steroid alcohols.


(I)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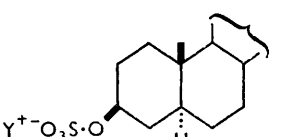

(III)

The pyridinium sulphates of œstrone, cholesterol, and cholestan-3β-ol (I; II; and III severally; Y = C_5H_5NH) were prepared from the parent alcohols with pyridine–sulphur trioxide in chloroform at room temperature, *i.e.*, by a modification of Sobel and Spoerri's procedure.[6] In a series of preliminary experiments, a selection of which is given in Table 1, the pyridinium salts (II and III; Y = C_5H_5NH) and the corresponding potassium salts [6] were treated with various solvents with and without heating. Although these experiments were not carried out under strictly comparable conditions, they nevertheless reveal some gross differences in reactivity.

Conversion of the salts (II and III) into their parent alcohols proceeded most readily in dioxan: although cholestan-3β-yl potassium sulphate (III; Y = K) remained unchanged in dioxan at room temperature, quantitative conversion into cholestan-3β-ol was effected by heating the suspension for a few minutes. At completion of the reaction one equivalent of acid was liberated: potassium hydrogen sulphate was isolated from the potassium salts (II and III; Y = K). Although dry dioxan was used in these experiments, no precautions were taken to exclude traces of water. It is clear that at least an equimolar amount of water was present. In contrast to the ready solvolysis of the salts (II and III), their

[1] Katzman, Straw, Buehler, and Doisy, *Recent Progr. Hormone Res.*, 1954, **9**, 45; and references therein.
[2] Roy, *Biochem. J.*, 1956, **62**, 41; and references therein.
[3] Butenandt and Hofstetter, *Z. physiol. Chem.*, 1939, **259**, 222.
[4] Grant and Beall, *Recent Progr. Hormone Res.*, 1950, **5**, 307.
[5] Cohen and Oneson, *J. Biol. Chem.*, 1953, **204**, 245.
[6] Sobel and Spoerri, *J. Amer. Chem. Soc.*, 1941, **63**, 1259.

fully covalent derivatives, cholesteryl methyl sulphate and cholestan-3β-yl methyl sulphate, are stable in dioxan under comparable conditions.[7]

Among the solvents listed in Table 1, *NN*-diethylformamide was, next to dioxan, most effective in bringing about the cleavage of cholesteryl potassium sulphate (II; Y = K).

TABLE 1. *Some solvolytic reactions of the pyridinium and potassium sulphates of cholestan-3β-ol and cholesterol.*

		Reaction (%) * of			
		cholestan-3β-yl		cholesteryl	
Solvent	Condns.†	C_5H_5NH sulphate ‡	K sulphate ‡	C_5H_5NH sulphate ‡	K sulphate ‡
Dioxan	A	70 §	0 §	100 §	95 §
NN-Diethylformamide	A	—	—	—	85
Acetone	A	5	5 §	25	5 §
Pyridine	A	0	0	10	0
	B	95	95	100	100
Ethanol	B	90	0 §	95	65 §
Water	B	10—30	60	30	55
	C	20—75	90	90	80
Chloroform	B	0	0 §	0	0 §

* Based on the weight of the ether-soluble product and approximated to the nearest 5%.
† A, 20—24 hr. at room temperature; B, 8—10 hr. at b. p. or at 100°, whichever lower; C, as B but for 19 hr.
‡ 0·005—0·03M-solutions or suspensions.
§ Suspensions (at least initially).

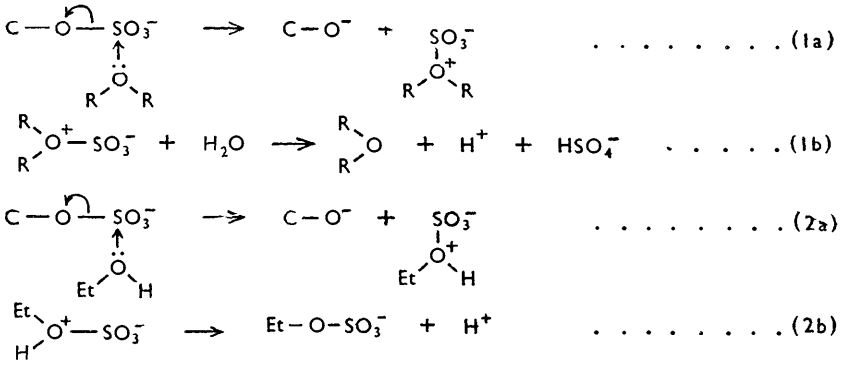
Chloroform proved completely inert towards the salts (II and III) even under vigorous conditions. In acetone, in pyridine, and in ethanol little if any reaction occurred at room temperature, but on heating for 8—10 hours complete solvolysis was effected in most instances. In contradistinction to the solvolysis in dioxan, ethanolysis and methanolysis of the salts (II and III) proceeded without change of the pH, *i.e.*, in the case of potassium salts in a neutral medium. This is consistent with a transesterification mechanism and in analogy to the transesterification[8] between potassium ethyl sulphate and higher aliphatic alcohols.

Aqueous solutions of the salts (II and III) were stable at room temperature. On heating, however, reaction occurred in all instances and apparently more readily with the neutral potassium salts than with the acidic pyridinium salts (see Table 1), an indication that cleavage was not primarily due to acid hydrolysis. Cleavage of salts (II and III) in organic solvents gave essentially pure parent alcohols; in hot water the pyridinium and potassium sulphate of cholestan-3β-ol likewise gave pure cholestan-3β-ol, the corresponding derivatives of cholesterol gave however a mixture of products containing only *ca.* 50% of cholesterol. It is therefore likely that fission of the $C_{(3)}$–O bond occurred in the latter reaction. The yields of cholestan-3β-ol obtained from cholestan-3β-yl pyridinium sulphate in hot water varied within a wide range when the reaction was repeated under apparently identical conditions. Even more surprisingly, it was found that irrespective of the yield of the ether-extractable product the reaction was accompanied by the liberation of 0·8—1·0 equivalent of acid.

Comparison of the ease of solvolysis of cholestan-3β-yl pyridinium sulphate (III; Y = C_5H_5NH) by ethers in chloroform (see Table 2) established the following order of decreasing reactivity: dioxan and tetrahydrofuran > diethyl ether > di*iso*propyl ether > anisole, *i.e.*, the order of decreasing availability of the lone pair of electrons.[9] A

[7] McKenna and Norymberski, *J.*, following paper.
[8] Brodersen and Quaedvlieg, G.P. 606,083.
[9] Brown and Adams, *J. Amer. Chem. Soc.*, 1942, **64**, 2557; Brown and Horowitz, *ibid.*, 1955, **77**, 1731.

hypothetical reaction mechanism accounting for the part played by the ethers is represented by equations (1a) and (1b). Ethanolysis is similarly represented by (2a) and (2b).



Œstrone pyridinium sulphate (I; Y = C_5H_5NH) reacted in dioxan–chloroform much more rapidly than the corresponding derivatives of cholesterol and cholestan-3β-ol (Table 3). This is consistent with the reaction step (1a) since it can be expected that the displacement $\overset{\curvearrowleft}{O}—S$ is facilitated by the attachment of the oxygen atom to an aromatic nucleus.

TABLE 2. *Solvolysis of cholestan-3β-yl pyridinium sulphate by ethers in chloroform.**

Ether	Reaction (%) † after 18 hr.	40 hr.	140 hr.	Ether	Reaction (%) † after 40 hr.	140 hr.
Dioxan	72	—	—	Di*iso*propyl ether	0	8
Tetrahydrofuran	69	—	—	Anisole	0	<1
Diethyl ether	—	21	71			

* 0·005M-Solutions in ether–chloroform (1 : 3; v/v) at 21° ± 1°.
† Based on wt. of ether-soluble product.

TABLE 3. *Solvolysis of the pyridinium sulphates of œstrone, cholesterol, and cholestan-3β-ol in dioxan–chloroform.**

Pyridinium sulphate of	Reaction (%) † after 2·5 hr.	4 hr.	5 hr.	Pyridinium sulphate of	Reaction (%) † after 2·5 hr.	4 hr.	5 hr.
Œstrone	100	—	—	Cholestan-3β-ol	8	11	14
Cholesterol	16	23	28				

* 0·007M-Solutions in dioxan–chloroform (1 : 4; v/v) at 25° ± 1°.
† Based on wt. of the ether-soluble product.

Finally, two observations are noted which are important for the interpretations of some reactions of cholesteryl methyl sulphate and cholestan-3β-yl methyl sulphate.[7] (i) Cleavage of cholesteryl potassium sulphate (II; Y = K) by boiling ethanol was almost completely suppressed by the addition of potassium acetate; (ii) the pyridinium salts (II and III) were tenaciously retained on neutral alumina.

EXPERIMENTAL

Unless otherwise specified, m. p.s were determined on a Kofler stage, rotations in $CHCl_3$ at 15—20°.

Cholesteryl Pyridinium Sulphate (II; Y = C_5H_5NH).—Cholesterol (200 mg.) in chloroform (5·0 ml.; distilled from P_2O_5) was shaken with pyridine–sulphur trioxide (500 mg.) for 2 hr. at room temperature. Surplus reagent was filtered off and washed with a little chloroform. The filtrate and washings were combined, cooled to 0°, and freed from any precipitated material.

Hot light petroleum (b. p. 60—80°) was added until a cloudines appeared; on cooling, cholesteryl pyridinium sulphate crystallised in prisms, m. p 158—160° (175—178° in a capillary tube), $[\alpha]_D$ −27° (*c* 1·16). Sobel and Spoerri [6] reported m. p. 179°, $[\alpha]_D$ −24°.

The product gave a positive test for halogen. It had an alkali equivalent of 664 (calc. for $C_{32}H_{51}NSO_4,CHCl_3$: 665) which decreased very slowly when the compound was kept in a vacuum-desiccator. For analysis, agsample was recrystallised from methylene dichloride–acetone, and dried in a high vacuum for 100 hr. at room temperature (Found: S, 5·3; N, 3·2%; equiv., 550. Calc. for $C_{32}H_{51}O_4NS$: S, 5·9; N, 2·6%; equiv., 546).

In subsequent preparations it was found advantageous to work up the mixture by filtering it through a column of cellulose powder.

Cholesteryl Potassium Sulphate (II; Y = K).—This compound was prepared from the corresponding pyridinium salt in the usual manner.[6] It had m. p. 226—227° (decomp.) (Found: S, 6·8; K, 7·8. Calc. for $C_{27}H_{45}O_4SK$: S, 6·35; K, 7·7%). Sobel and Spoerri [6] recorded m. p. 210° and 239°.

Cholestan-3β-yl Pyridinium Sulphate (III; Y = C_5H_5NH).—Cholestan-3β-ol, treated with pyridine—sulphur trioxide as described above, gave *cholestan-3β-yl pyridinium sulphate*. A sample dried in a high vacuum for 24 hr. at room temperature had m. p. 165—169° (178—180° in a capillary tube), $[\alpha]_D$ +17° (*c* 0·90) (Found: C, 59·3; H, 8·2; N, 2·35; S, 4·8. $C_{32}H_{53}O_4NS,CHCl_3$ requires C, 59·4; H, 8·2; N, 2·1; S, 4·8%).

Cholestan-3β-yl Potassium Sulphate (III; Y = K).—This compound was obtained from the pyridinium salt in the usual manner.[6] It had m. p. 234—235° (decomp.) (Found: K, 7·9. Calc. for $C_{27}H_{47}SO_4K$: K, 7·7%). Sobel and Rosen [10] recorded m. p. 236°.

Œstrone Pyridinium Sulphate (I; Y = C_5H_5NH).—This compound was prepared from œstrone by the method given above but in 25 ml. of chloroform. Crystallised from chloroform–*n*-hexane it had m. p. 170—175°, $[\alpha]_D$ +84° (*c* 0·96); it gave a positive test for halogen (Found: N, 2·6; S, 5·4. Calc. for $C_{23}H_{37}O_5NS,CHCl_3$: N, 2·55; S, 5·8%). Butenandt and Hofstetter [3] recorded m. p. 173—175°, $[\alpha]_D$ +84°.

Solvolytic Reactions.—Solvents were purified by distillation preceded by the following treatments. Chloroform: kept over phosphoric oxide, distilled, and refluxed with anhydrous potassium carbonate. Acetone: refluxed with potassium permanganate, distilled, and refluxed with anhydrous potassium carbonate. Ethanol and dioxan: refluxed with sodium. Tetrahydrofuran, diethyl ether, di*iso*propyl ether, and anisole: kept over calcium hydride. Pyridine: kept over potassium hydroxide. The pyridinium salts of the steroid sulphates were kept in a vacuum-desiccator. Owing to their varying content of solvent of crystallisation, the composition of each salt was determined before each series of experiments by titration with 0·01N-sodium hydroxide and/or by measuring the quantity of the parent alcohol obtained by complete cleavage of the salts with hot dioxan. For each experiment 50—100 mg. of the appropriate salt were taken. Unless otherwise indicated, the reaction mixtures were worked up by extraction with ether. The yields were calculated on the basis of the established composition of the salts and of the weight of the ether-soluble products. The products were identified by m. p.s and mixed m. p.s. A few examples are given below.

Cleavage of Cholesteryl Pyridinium Sulphate.—(i) *In acetone.* The compound (60 mg.) dissolved in acetone (25 ml.) when shaken for 20 min. The solution was left for 4 days at room temperature then worked up. Cholesterol (31 mg., 89%), m. p. 146—148°, was isolated. When the solution was heated for 5 hr. cholesterol was obtained in theoretical yield. (ii) *In ethanol.* The compound (138 mg.) in ethanol (6 ml.) was heated under reflux for 3 hr. The solution was cooled, diluted with water (5 ml.), and titrated with 0·01N-sodium hydroxide (phenolphthalein). The alkali equivalent was identical with that of unchanged material. The usual working up gave cholesterol (76 mg., 95%), m. p. 146—147·5°.

Cleavage of Cholesteryl Potassium Sulphate.—(i) *In dioxan.* The compound (200 mg.) dissolved rapidly in hot dioxan (10 ml.), an amorphous precipitate being formed. After 10 minutes' heating under reflux, the mixture was diluted with light petroleum (40 ml.), and filtered through sintered glass. The precipitated potassium hydrogen sulphate (52 mg.) had m. p. and mixed m. p. 192—194° (Found: S, 23·2; K, 27·95%; equiv., 136. Calc. for $KHSO_4$: S, 23·5; K, 28·7%; equiv., 136). The filtered dioxan–light petroleum solution was evaporated to dryness. The residue (153 mg., 100%) was identified as pure cholesterol, m. p. 146—148°. (ii) *In tetrahydrofuran.* The compound (50 mg.) and tetrahydrofuran (20 ml.) were heated

[10] Sobel and Rosen, *ibid.*, 1941, **63**, 3536.

under reflux for 10 min. The precipitated potassium hydrogen sulphate was filtered off and the filtrate evaporated *in vacuo*. The oily residue was chromatographed on neutral alumina. Benzene–ether (9 : 1, v/v) eluted cholesterol (35 mg., 92%), m. p. 146—147°. (iii) *In ethanol.* The compound (60 mg.) and ethanol (10 ml.) were heated under reflux for 36 hr. Worked up in the usual manner the neutral solution gave cholesterol (43 mg., 100%), m. p. 145—147°. The same experiment, but in the presence of potassium acetate (75 mg.), gave only a trace of ether-soluble product. (iv) *In water.* The compound (60 mg.) and water (10 ml.) were heated on a boiling-water bath for 19 hr. The mixture required for neutralisation a quantity of 0·01N-alkali corresponding to one equivalent of acid. The ether-extract (36·5 mg.) was chromatographed on neutral alumina; benzene–ether (9 : 1, v/v) eluted cholesterol (20 mg.), m. p. 147—149°.

Cleavage of Cholestan-3β-yl Pyridinium Sulphate.—(i) *In ethanol.* The compound (100 mg.) and ethanol (5 ml.) were heated under reflux for 4 hr. The solution was concentrated *in vacuo* and cooled. Cholestan-3β-ol (52 mg., 90%) crystallised in plates, m. p. 140—142°. In a similar experiment the mixture was diluted with water and neutralised with 0·01N-alkali (phenolphthalein). The equivalent was identical with that of starting material. Extraction with ether gave pure cholestan-3β-ol in 85% yield. (ii) *In methanol.* The compound (60 mg.) and methanol (10 ml.) were heated under reflux for 20 hr. The mixture required for neutralisation the same amount of alkali as the starting material. The usual working up gave cholestan-3β-ol (27 mg., 86%), m. p. 137—141°. (iii) *In water.* The compound (60 mg.) and water (10 ml.) were heated on a boiling-water bath for 19 hr. Titration with 0·01N-alkali (phenolphthalein) detected the liberation of 1·0 equivalent of acid. Extraction with ether gave cholestan-3β-ol (6·4 mg., 18%), m. p. 124—127° (139—142° after drying at 100°). In a further experiment cholestan-3β-ol was obtained in 74% yield. When in an identical experiment the reaction was stopped after 4 hr., only 6% of ether-extractable material was isolated, although 0·8 equivalent of acid was liberated.

Cleavage of Cholestan-3β-yl Potassium Sulphate.—(i) *In dioxan.* The compound (65 mg.) and dioxan (10 ml.) were heated under reflux for 10 min. Neutralisation required alkali corresponding to 1 equivalent of acid. Extraction with ether gave cholestan-3β-ol (50 mg., 100%), m. p. 139—142°. (ii) *In ethanol.* The compound (60 mg.) and ethanol (10 ml.) were heated under reflux for 94 hr. and gave cholestan-3β-ol (43 mg., 95%), m. p. 139—142°.

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