774. *Steroid Sulphates. Part II.* Cholestan-3β-yl Methyl Sulphate and Cholesteryl Methyl Sulphate.*

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Cholestan-3β-yl methyl sulphate (V) and cholesteryl methyl sulphate (XI) were prepared by the action of diazomethane on cholestan-3β-yl (I; Y = C_5H_5NH) and cholesteryl pyridinium sulphate (X; Y = C_5H_5NH) respectively. Strongly nucleophilic agents attacked both esters preferentially at the methyl-carbon atom. With polar solvents, the saturated ester (V) reacted at both the methyl-carbon atom and at the 3-carbon atom, the 5 : 6-unsaturated ester (XI) at the latter position only.

ASYMMETRIC neutral esters of sulphuric acid have been prepared by the interaction of alkyl chlorosulphonates with sodium alkoxides,[1] and of asymmetric ethers with sulphur trioxide,[2] and by oxidation of sulphites.[3] As already briefly reported,[4] a new route to

* Part I, preceding paper.

[1] (*a*) Bushong, *Amer. Chem. J.*, 1903, **30**, 316; (*b*) Bert, *Compt. rend.*, 1924, **178**, 1182; Thayer, *J. Amer. Chem. Soc.*, 1924, **46**, 1044.

[2] Houben and Arnold, *Ber.*, 1907, **40**, 4306.

[3] Garner and Lucas, *J. Amer. Chem. Soc.*, 1950, **72**, 5497.

[4] McKenna and Norymberski, *Chem. and Ind.*, 1954, 961.

this class of compound was found in the action of diazomethane on pyridinium sulphates of steroid alcohols.

Cholestan-3β-yl pyridinium sulphate (I; Y = C_5H_5NH) with diazomethane readily gave cholestan-3β-yl methyl sulphate (V) whose formulation was fully confirmed by the properties now reported. In the same way cholesteryl methyl sulphate (XI) was prepared from cholesteryl pyridinium sulphate (X; Y = C_5H_5NH).

The saturated ester (V) reacted rapidly with sodium iodide in acetone, to give cholestan-3β-yl sodium sulphate (I; Y = Na) in almost theoretical yield. The corresponding potassium salt (I; Y = K) was formed with equal ease by the action of methanolic potassium hydroxide on the ester (II). The ester (V) and pyridine in ether rapidly formed cholestan-3β-yl 1-methylpyridinium sulphate (IV) in which the cation was readily replaced by potassium. The compound (IV) was further characterised by catalytic hydrogenation followed by treatment with alkali, whereupon cholestan-3β-yl sodium sulphate (I; Y = Na) and 1-methylpiperidine (VIII) (identified as its picrate) were isolated. Sodium iodide, methanolic potassium hydroxide, and pyridine reacted with apparently equal ease with cholesteryl methyl sulphate (XI), to give respectively cholesteryl sodium sulphate (X; Y = Na), the potassium salt (X; Y = K), and the 1-methylpyridinium salt (VII). Catalytic hydrogenation of the last compound followed by treatment with alkali gave cholestan-3β-yl sodium sulphate (I; Y = Na) and 1-methylpiperidine (VIII) (identified as its picrate).

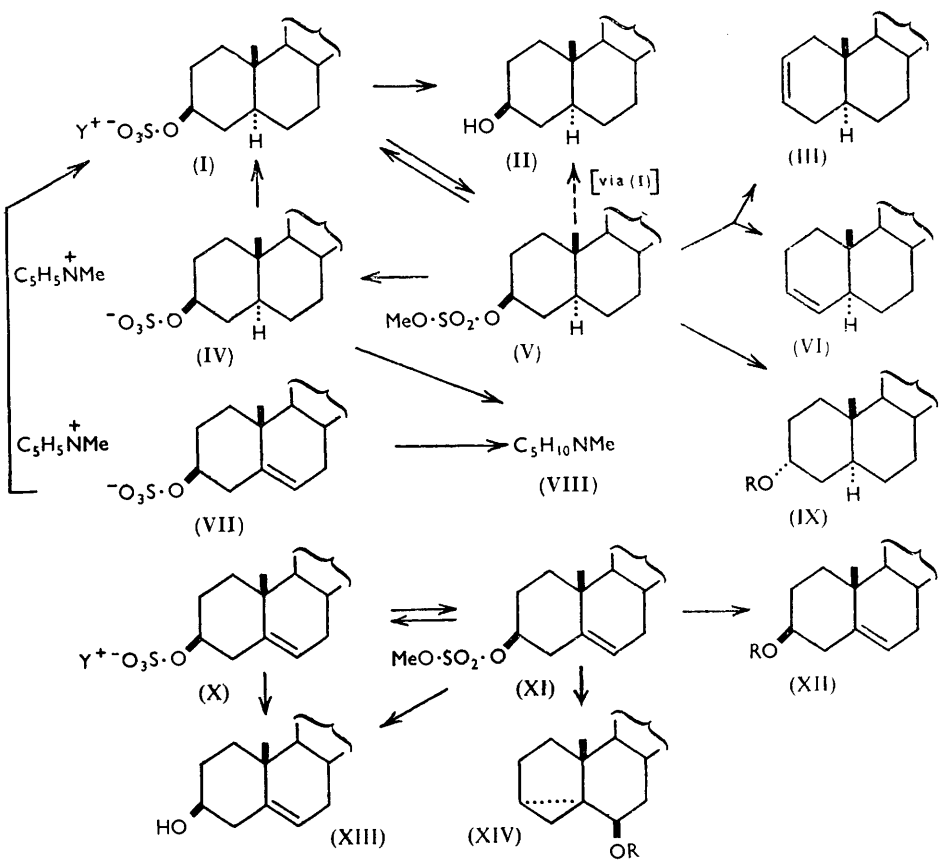
Both esters (V and XI) decomposed on contact with neutralised alumina, though to different extents. Approximately 25% of the former was recovered unchanged from an alumina column; of the remainder *ca.* 15% was readily eluted from the column; the product had m. p. 69—71°, $[\alpha]_D$ +60°, and was identified as a mixture of cholest-2- (III) and -3-ene (VI) by comparison with the 1 : 1 mixture (m. p. 71·5—73·5°, $[\alpha]_D$ +61°) obtained from cholestan-3β-yl toluene-*p*-sulphonate in boiling methanol;[5] a second product (*ca.* 10%) eluted from the column was identified as cholestan-3α-ol (IX; R = H). Cholesteryl methyl sulphate (XI) was completely decomposed on alumina, giving three products: (i) a readily eluted, unidentified oil (*ca.* 5%), probably a mixture of olefins, which, from spectroscopic evidence, contains a substantial proportion of a heteroannular diene (3 : 5-diene ?); (ii) cholesterol (XIII) (25%); (iii) 3 : 5-*cyclo*cholestan-6β-ol (XIV; R = H) (*ca.* 50%). The last had $[\alpha]_D$ +50° whereas $[\alpha]_D$ +24° and +27° were previously reported;[6] a specimen, prepared by us in the usual manner[6a] from cholesteryl toluene-*p*-sulphonate, had $[\alpha]_D$ +49° to +51°. Attempted crystallisation from commercial "absolute" ethanol brought about quantitative conversion into 3β-ethoxycholest-5-ene (XII; R = Et). Since these observations were made, Kosower and Winstein[7] reported $[\alpha]_D$ +50° for 3 : 5-*cyclo*cholestan-6β-ol.

In each of the above experiments the remainder of the material could not be eluted from alumina—an indication that cleavage of the methyl ester linkage occurred, followed by the binding on alumina of the cholestan-3β-yl or cholesteryl sulphate ion formed. Since under comparable conditions the corresponding pyridinium salts (I and X; Y = C_5H_5NH) were almost completely retained on alumina,[8] it follows that the products isolated are derived by initial fission of the $C_{(3)}$–O bond or of the adjacent O–S bond. The latter fission could not lead to elimination, inversion, or 3 : 5-cyclo-rearrangement; that it takes place in the formation of cholesterol (XIII) from (XI) is unlikely since heterolysis of the $C_{(3)}$–O bond occurs much more readily in the cholest-5-ene series than in the cholestane series.

Cholestan-3β-yl methyl sulphate (V) did not react with methanol at room temperature.

[5] Nace, *J. Amer. Chem. Soc.*, 1952, **74**, 5937.
[6] (*a*) Beynon, Heilbron, and Spring, *J.*, 1937, 1459; (*b*) Wallis, Fernholz, and Gephart, *J. Amer. Chem. Soc.*, 1937, **59**, 137.
[7] Kosower and Winstein, *ibid.*, 1956, **78**, 4347.
[8] McKenna and Norymberski, preceding paper.

However, in boiling methanol it readily formed the olefin mixture (III + VI) (10—15%), 3α-methoxycholestane (IX; R = Me) (40—45%), and cholestan-3β-ol (II) (*ca.* 40%). The last compound is thought to be formed by fission of the methyl ester linkage followed by the solvolysis of the cholestan-3β-yl sulphate ion (I).[8] As expected,[8] the latter step was supressed when the reaction was performed in the presence of potassium acetate: cholestan-3β-yl potassium sulphate (I; Y = K) (*ca.* 65%), the olefin mixture (III + VI)



(*ca.* 10%), and 3α-methoxycholestane (IX; R = Me) (*ca.* 20%) were isolated. Two small chromatographic fractions, eluted from alumina after (IX; R = Me), exhibited infrared absorption spectra which indicated the presence of 3α-acetoxy- (IX; R = Ac) and 3α-methoxy-cholestane (IX; R = Me), but failed to reveal the presence of 3β-methoxycholestane.*

In boiling aqueous acetone the ester (V) gave the olefin mixture (III + VI) (*ca.* 15%), cholestan-3α-ol (IX; R = H) (*ca.* 40%), and cholestan-3β-ol (II) (*ca.* 5%). A further quantity (*ca.* 25%) of the last product was obtained by treating with acid the water-soluble reaction product (in all probability cholestan-3β-yl hydrogen sulphate). In the presence of potassium acetate, cholestan-3β-yl potassium sulphate (I; Y = K) was the main product (*ca.* 75%); the olefin mixture (III + VI) and cholestan-3α-ol (IX; R = H) were isolated in small quantities (*ca.* 10% each). Finally, acetolysis of the ester (V) in the presence of potassium acetate, followed by alkaline hydrolysis, gave the usual mixture of (III and VI) (*ca.* 55%), cholestan-3α-ol (IX; R = H) (*ca.* 35%), and cholestan-3β-ol

* For this investigation we are indebted to Dr. A. E. Kellie, Courtauld Institute of Biochemistry.

(II) (*ca.* 5%). The last compound could well arise by initial reaction of the methyl-carbon atom followed by rupture of the O–S bond, as it has been shown [9] that treatment of cholestan-3β-yl sodium sulphate (I; Y = Na) with silver acetate in acetic acid leads to a mixture of the 3β-ol (II) and its acetate.

Reactions of $MeO{\cdot}SO_2{\cdot}OR$.

	Reaction (%) *			
	R = cholestan-3β-yl		R = cholesteryl	
Treatment	at Me	at $C_{(3)}$	at Me	at $C_{(3)}$
NaI–acetone	95	—	100	—
KOH–MeOH–H_2O	90	—	90	—
Pyridine–ether	85	—	100	—
Alumina	75 (?)	25	20 (?)	80
MeOH	40	50—60	—	—
EtOH	—	—	—	100
MeOH–KOAc	65	30	—	90
Acetone–H_2O	30	60	—	85
Acetone–H_2O–KOAc	75	20	—	—
AcOH	—	—	—	95
AcOH–KOAc	5	85	—	90

* Based on isolated products and approximated to the nearest 5%.

Solvolytic reactions of cholesteryl methyl sulphate (XI) proceeded with great ease and, in most instances, with the formation of a single product. The ester (XI) in boiling ethanol gave 3β-ethoxycholest-5-ene (XII; R = Et), in glacial acetic acid at room temperature it gave 3β-acetoxycholest-5-ene (XII; R = Ac), and in boiling aqueous acetone it gave cholesterol (XIII)—all in excellent yield (85—100%). The products of ethanolysis and acetolysis unequivocally indicate that fission of the $C_{(3)}$–O bond occurred primarily, since initial attack on the methyl side would have led, *via* the cholesteryl sulphate ion (X), to cholesterol (XIII).[8] It is assumed that, by analogy with ethanolysis and acetolysis of the ester (XI), its hydrolysis in aqueous acetone is brought about also by initial fission of the $C_{(3)}$–O bond.

The ester (XI) reacted readily with potassium acetate in methanol to give 6β-methoxy-3 : 5-*cyclo*cholestane (XIV; R = Me) in high yield. With potassium acetate in glacial acetic acid at room temperature it gave a mixture which was almost quantitatively eluted with light petroleum from alumina without satisfactory resolution. However, after alkaline hydrolysis, 3 : 5-*cyclo*cholestan-6β-ol (XIV); R = H) (*ca.* 65%) and cholesterol (XIII) (*ca.* 25%) were easily separated. The evidence presented suggests that the cholesterol isolated is derived from hydrolysis of its acetate and hence that here again only fission of the $C_{(3)}$–O bond was demonstrated.

Simple symmetric dialkyl sulphates react with strongly nucleophilic agents in two steps (1) and (2), both of which obey second-order kinetics [10,11] and proceed at rates decreasing in the order Me > Et > Pr^i, in close analogy to the corresponding reactions of alkyl halides, and in conformity with the requirements of the reaction mechanism of a bimolecular nucleophilic substitution (S_N2).[12] Reaction (1) is fast, reaction (2) is slow owing to repulsion of like ions.

$$R{\cdot}O{\cdot}SO_2O{\cdot}R \xrightarrow{X^-} R{\cdot}O{\cdot}SO_3^- + RX \quad . . . . . (1)$$

$$R{\cdot}O{\cdot}SO_3^- \xrightarrow{X^-} SO_4^- + RX \quad . . . . . (2)$$

Under comparable conditions, asymmetric neutral sulphates have been reported [3,13] to undergo reaction (1) more readily at the more electron-deficient of the two α-carbon

[9] Lieberman, Hariton, and Fukushima, *J. Amer. Chem. Soc.*, 1948, **70**, 1427.
[10] Green and Kenyon, *J.*, 1950, 1389, 1589; and references therein.
[11] Foster, Hancock, Overend, and Robb, *J.*, 1956, 2589.
[12] Cf. Ingold, " Structure and Mechanism in Organic Chemistry," G. Bell, London, 1953.
[13] Houben and Arnold, *Ber.*, 1908, **41**, 1565; Lichtenberger and Durr, *Bull. Soc. chim. France*, 1956, 664.

atoms. This is strikingly so in the cases of cholestan-3β-yl methyl sulphate (V) and cholesteryl methyl sulphate (XI), both esters being attacked by strongly nucleophilic agents practically exclusively at the methyl-carbon atom in preference to the highly substituted secondary $C_{(3)}$-atom. Since there is little doubt that these reactions are of the S_N2 type, it follows that, under any reaction conditions, initial substitution at $C_{(3)}$ of (V) or (XI) cannot proceed to any appreciable extent by the S_N2 mechanism.

The solvolytic reactions of cholestan-3β-yl methyl sulphate (V) reveal a common pattern: initial reaction occurred to varying extents at the Me–O bond and at the $C_{(3)}$–O bond (see Table). Cleavage of the Me–O bond led to the cholestan-3β-yl ion (I) which, under appropriate conditions,[8] was further transformed into the 3β-ol (II); cleavage of the $C_{(3)}$–O bond gave invariably cholest-2-ene (III), cholest-3-ene (VI), and the 3-substitution product. The evidence presented strongly suggests that 3-substitution was accompanied by complete inversion of configuration. Since the S_N2 mechanism was essentially blocked by the competitive demand of the methyl group, it follows from the dualistic theory of the mechanism of nucleophilic aliphatic substitution [12] that the above reactions at $C_{(3)}$ proceed by the unimolecular mechanism (S_N1). Relevant to this consideration is the recent kinetic investigation [14] of the methanolysis of cholestan-3β-yl toluene-*p*-sulphonate which is known [5] to proceed with complete inversion at $C_{(3)}$: it was found that the reaction is of first order with respect to the toluene-*p*-sulphonate and that its rate is independent of methoxide concentration; its steric course was attributed to the formation of a solvated intermediate.[15]

The solvolytic reactions of cholesteryl methyl sulphate (XI), in contradistinction to those of the saturated ester (V), gave only products of $C_{(3)}$–O cleavage. The requirement of a unimolecular mechanism for this group of reactions agrees with established views on the mechanism of the corresponding reactions of cholesteryl halides and sulphonates.[16]

It is suggested that methyl sulphates of highly substituted asymmetric alcohols are convenient experimental models for the study of the steric course of substitutions for which the S_N2 mechanism has been virtually ruled out.

Experimental

M. p.s were determined on a Kofler stage. Rotations were measured in $CHCl_3$ (at 13—20°), ultraviolet absorption spectra in EtOH. Specimens for analyses were dried in a high vacuum, for 4—12 hr. at 50—80°. For chromatography Peter Spence's grade H alumina was neutralised as described previously.[17] Reaction products were identified by m. p.s. Deviations from these procedures are indicated.

Cholestan-3β-yl Methyl Sulphate (V).—*Preparation.* Excess of ethereal diazomethane was added to a solution of cholestan-3β-yl pyridinium sulphate (I; Y = C_5H_5NH) (1·5 g.) * in chloroform–ether (2 : 1, v/v; 300 ml.). After 1 min. at room temperature chilled ether (200 ml.) was added and the mixture washed successively with chilled 0·01N-hydrochloric acid (200 ml.), water (100 ml.), 0·01N-sodium hydrogen carbonate (200 ml.), and water (2 × 100 ml.). The extract was dried (Na_2SO_4) and evaporated. The residue was taken up in little ether, some insoluble material being removed; evaporation to dryness furnished *cholestan-3β-yl methyl sulphate* (930 mg.), crystallising from *n*-hexane in needles, m. p. 115·5—116·5°, $[\alpha]_D$ +21° (*c* 1·21) (Found: C, 69·2; H, 10·4; S, 6·9. $C_{28}H_{50}O_4S$ requires C, 69·7; H, 10·5; S, 6·6%. The specimen was dried in a high-vacuum overnight at room temperature). The reaction was carried out in methylene chloride–ether with equal success.

The compound is stable in non-polar solvents. After 15 min. in hot dioxan it was recovered unchanged.

Reaction with sodium iodide. Sodium iodide (90 mg.) was added to the ester (170 mg.) in

* The specimen had an alkali-equivalent of 660 owing to solvation with chloroform; see ref. 8.

[14] Pappas, Meschino, Fournier, and Nace, *J. Amer. Chem. Soc.*, 1956, **78**, 1907.
[15] Cf. Doering and Zeiss, *ibid.*, 1953, **75**, 4733; Streitwieser, *ibid.*, 1955, **77**, 1117.
[16] Shoppee and Westcott, *J.*, 1955, 1891; and references therein.
[17] Brooks and Norymberski, *Biochem. J.*, 1953, **55**, 371.

acetone (40 ml.). A precipitate was formed rapidly and was filtered off after 3 hr. in the dark (164 mg.) and was identified as cholestan-3β-yl sodium sulphate, m. p. 173—175°.

Alkaline hydrolysis. The ester (45 mg.) in ether (2 ml.) was treated with a N-solution of potassium hydroxide in aqueous methanol (90%; 3 ml.) at room temperature. Cholestan-3β-yl potassium sulphate (43 mg.; m. p. 253°) separated immediately.

Reaction with pyridine. On addition of pyridine (1 ml.) to the ester (100 mg.) in ether (20 ml.) a precipitate was formed rapidly and was separated after the mixture had remained overnight at room temperature. Crystallisation of the crude product (105 mg.; m. p. 188—192°) from diethylformamide–ethanol (because of ready solvolysis prolonged heating must be avoided) afforded pure *cholestan-3β-yl 1-methylpyridinium sulphate* in needles, m. p. 175—177° (the m. p. varied within a wide range depending on the mode of crystallisation), $[\alpha]_D$ +19° (*c* 0·98) (Found: N, 2·1; S, 5·7. $C_{33}H_{55}O_4NS$ requires N, 2·5; S, 5·7%). (*a*) Catalytic hydrogenation. Cholestan-3β-yl 1-methylpyridinium sulphate (140 mg.) and platinum oxide (25 mg.) in ethanol (20 ml.) were shaken with hydrogen at atmospheric pressure for 24 hr. The solution, freed from catalyst, was concentrated *in vacuo* to a small volume. Addition of 0·1N-sodium hydroxide (2·5 ml.) precipitated cholestan-3β-yl sodium sulphate (104 mg.), m. p. 173—175°. Addition of picric acid to the filtrate precipitated 1-methylpiperidine picrate (15 mg.), m. p. 208—210° (decomp.). M. p. 222° was reported [18] for this compound. (*b*) Reaction with potassium iodide. Cholestan-3β-yl 1-methylpyridinium sulphate (94 mg.) in water (10 ml.) was treated with excess of aqueous potassium iodide, then cooled to 0°, and the precipitate filtered off and washed with chilled water. Crystallisation from methanol afforded cholestan-3β-yl potassium sulphate (75 mg.), m. p. 234°.

Decomposition on alumina. The ester (96 mg.) was chromatographed on alumina (5 g.) in the usual manner. Light petroleum (b. p. 40—60°; 30 ml.) eluted a mixture (8 mg.; m. p. 68—70°) of cholest-2- and -3-ene which after crystallisation from acetone had $[\alpha]_D$ +60° (*c* 0·64), m. p. 69—71° (authentic specimen prepared by methanolysis of cholestan-3β-yl toluene-*p*-sulphonate [5] had m. p. 71·5—73·5°, $[\alpha]_D$ +61°).

Elution with 9 : 1 (v/v) light petroleum–benzene (30 ml.) gave starting material (26 mg.), m. p. 112—114°. Benzene (20 ml.) eluted cholestan-3α-ol (5 mg.; m. p. 174—185°), m. p. 183—185° after crystallisation from aqueous ethanol. More polar solvents failed to elute any further products.

Methanolysis. The ester (190 mg.) in methanol (25 ml.) was heated under reflux for 12 hr. The solution was concentrated *in vacuo*, and the product (154 mg.) was extracted with ether and chromatographed on alumina (8 g.). Light petroleum (10 ml.) eluted a mixture (15 mg.) (of cholest-2- and -3-ene), m. p. 71—72°, $[\alpha]_D$ +60° (*c* 0·73). The same solvent (10 ml.) eluted next unidentified material (20 mg.; $[\alpha]_D$ +40°), probably a mixture of the preceding and the following fraction. Further elution with light petroleum (10 ml.) gave 3α-methoxycholestane (60 mg.; m. p. 60—64°), which, crystallised from aqueous acetone, had m. p. 64—66°, $[\alpha]_D$ +23° (*c* 1·00). 4 : 1 (v/v) Benzene–ether (75 ml.) eluted cholestan-3β-ol (61 mg.), m. p. 142°, $[\alpha]_D$ +24° (*c* 0·75).

No reaction occurred when a methanolic solution of the ester was left at room temperature for 7 days.

Methanolysis in the presence of potassium acetate. The ester (344 mg.) and anhydrous potassium acetate (520 mg.) in methanol (70 ml.) were heated under reflux for 15 hr. On cooling, cholestan-3β-yl potassium sulphate (230 mg.; m. p. 237—239°) crystallised. From the filtrate more of this salt (10 mg.) and an ether-soluble oil (100 mg.) were isolated. The latter was chromatographed on alumina (5 g.). Elution with light petroleum (20 ml.) gave a mixture (23 mg.) (of cholest-2- and -3-ene), m. p. 68—71°, $[\alpha]_D$ +62° (*c* 1·10). The same solvent (40 ml.) eluted next 3α-methoxycholestane (60 mg.), m. p. 63—64°, $[\alpha]_D$ +22° (*c* 0·80). Exhaustive elution with light petroleum gave oil (6 mg.). This and a corresponding fraction from a second experiment were combined and rechromatographed on alumina (7 g.): two main oily fractions were separated. The infrared spectra of these, in carbon disulphide, were compared with those of pure 3α- and 3β-methoxycholestane. Within the region associated with the ether-stretching vibration, the 3α-epimer had $\nu_{max.}$ 1090 and the 3β-epimer had $\nu_{max.}$ 1103 cm.$^{-1}$ (with shoulder at 1094 cm.$^{-1}$). The more readily eluted of the two chromatographic fractions had $\nu_{max.}$ 1091 and 1732 cm.$^{-1}$ (C=O stretching), the second fraction had $\nu_{max.}$ 1733

[18] Braun, Kühn, and Goll, *Ber.*, 1926, **59**, 2337.

and a triplet band 1257, 1244, 1237 cm.$^{-1}$ characteristic of 3α-acetoxy-5α-steroids,[19] but no significant absorption in the 1150—1050 cm.$^{-1}$ region.

Hydrolysis in aqueous acetone. The ester (115 mg.) in 4 : 1 (v/v) acetone–water (25 ml.) was heated under reflux for 5 hr. Customary working-up gave ether-soluble material (63 mg.) which was chromatographed on alumina (4 g.). Light petroleum (20 ml.) eluted a mixture (14 mg.) (of cholest-2- and -3-ene), m. p. 70—72°, $[\alpha]_D$ +61° (*c* 0·83). Light petroleum–benzene (1 : 1, v/v; 70 ml.) eluted cholestan-3α-ol (39 mg.; m. p. 178—183°), m. p. 185·5—188°, $[\alpha]_D$ +25° (*c* 0·74), after crystallisation from aqueous ethanol. Benzene–ether (19 : 1, v/v; 30 ml.) eluted cholestan-3β-ol (5 mg.), m. p. 138—141°. The aqueous phase and washings from the extraction with ether were combined, acidified to pH 1, and continuously extracted with ether for 24 hr. The dry extract (24 mg.; oil) gave from aqueous ethanol pure cholestan-3β-ol (17 mg.), m. p. 138—143°.

Hydrolysis in aqueous acetone in the presence of potassium acetate. The ester (200 mg.) and anhydrous potassium acetate (260 mg.) in acetone–water (4 : 1, v/v; 50 ml.) were heated under reflux for 13 hr. On cooling, cholestan-3β-yl potassium sulphate (159 mg.; m. p. 236—238°) crystallised. The filtrate was concentrated *in vacuo* and extracted with ether. The ether-soluble product (40 mg.) was chromatographed on alumina (4 g.). Light petroleum–benzene (19 : 1, v/v; 20 ml.) eluted a mixture (16 mg.; oil) (of cholest-2- and -3-ene), m. p. 69—71°, $[\alpha]_D$ +61° (*c* 0·89), after crystallisation from aqueous acetone. Benzene (20 ml.) eluted cholestan-3α-ol (19 mg.; m. p. 182—185°), m. p. 186—188°, $[\alpha]_D$ +29° (*c* 0·82), after crystallisation from aqueous ethanol.

Acetolysis in the presence of potassium acetate. The ester (125 mg.) and anhydrous potassium acetate (115 mg.) in acetic acid (13 ml.) were heated under reflux for 7 hr. The crude product (extracted with ether in the usual manner) was treated with N-potassium hydroxide in aqueous methanol (90%) for 1·5 hr. under reflux. The hydrolysed material (91 mg.) was chromatographed on alumina (5 g.). Light petroleum (40 ml.) eluted a mixture (52 mg.) (of cholest-2- and -3-ene), m. p. 69—71°, $[\alpha]_D$ +60° (*c* 1·13). Light petroleum–benzene (9 : 1, v/v; 30 ml.) eluted a fraction (12 mg.; m. p. 60—81°) which on alkaline hydrolysis (as above) followed by chromatography on alumina (1 g.) gave, in the order of elution, cholestan-3α-ol (9 mg.; m. p. 183—186°) and cholestan-3β-ol (1 mg.; m. p. 139—141°). Light petroleum–benzene (1 : 1, v/v; 40 ml.) eluted cholestan-3α-ol (24 mg.), m. p. 187°, $[\alpha]_D$ +29° (*c* 0·82). Benzene–ether (9 : 1, v/v; 20 ml.) eluted cholestan-3β-ol (4 mg.), m. p. 142°.

Cholesteryl Methyl Sulphate (XI).—*Preparation.* Cholesteryl pyridinium sulphate (X; Y = C_5H_5NH) was treated with diazomethane as described above. The crude *cholesteryl methyl sulphate* (m. p. 99—101°) was obtained in 85% yield. Crystallisation from *n*-hexane gave needles changing spontaneously to prisms, m. p. 103—104° (decomp.; bright red melt), $[\alpha]_D$ −32° (*c* 0·88) (Found: C, 69·7; H, 9·8; S, 6·7. $C_{28}H_{48}O_4S$ requires C, 70·0; H, 10·1; S, 6·7%. The specimen was dried in a high vacuum overnight at room temperature).

A solution of the compound in dioxan became slightly acid after 15 minutes' heating on a boiling-water bath. Unchanged material was recovered in 80% yield.

Reaction with sodium iodide. The ester (50 mg.) was treated with sodium iodide as described above. Cholesteryl sodium sulphate (50 mg.) separated rapidly. It crystallised from methanol in needles, m. p. 184—185°.

Alkaline hydrolysis. The ester (42 mg.) was treated with potassium hydroxide as described above. Cholesteryl potassium sulphate (39 mg.) separated immediately. After crystallisation from ethanol it had m. p. 225—226° (Found: S, 6·25; K, 7·4. Calc. for $C_{27}H_{45}O_4SK$: S, 6·35; K, 7·7%).

Reaction with pyridine. The ester (38 mg.) was treated with pyridine as described above.

Cholesteryl 1-methylpyridinium sulphate (44 mg.) separated immediately as an amorphous precipitate. Since attempted crystallisation was unsuccessful the product was repeatedly washed with ether and dried, then having m. p. 165—167·5°, $[\alpha]_D$ −28° (*c* 0·90) (Found: N, 2·3; S, 5·4. $C_{33}H_{53}O_4NS$ requires N, 2·5; S, 5·7%). (*a*) Catalytic hydrogenation. The 1-methylpyridinium salt (270 mg.) and platinum oxide (124 mg.) in ethanol (35 ml.) were shaken with hydrogen at atmospheric pressure for 24 hr. Treatment as above gave cholestan-3β-yl sodium sulphate (150 mg.) and 1-methylpiperidine picrate (35 mg.). After crystallisation from methanol the former had m. p. 173—175°, $[\alpha]_D$ +17° (*c* 0·60 in EtOH). The picrate,

[19] Jones, Humphries, Herling, and Dobriner, *J. Amer. Chem. Soc.*, 1951, **73**, 3215.

crystallised from ethanol, had m. p. 205—210° (decomp.) (Found: N, 17·1. Calc. for $C_{12}H_{16}O_7N_4$: N, 16·8%). (*b*) Ethanolysis. The 1-methylpyridinium sulphate (120 mg.) in ethanol (8 ml.) was heated under reflux for 4 hr. Extraction with ether gave cholesterol (80 mg.), m. p. 146—147°, $[\alpha]_D$ −40° (*c* 1·59) (from aqueous ethanol).

Decomposition on alumina. The ester (294 mg.) was chromatographed on alumina (15 g.) in the usual manner. Light petroleum (40 ml.) eluted an oil (12 mg.), $[\alpha]_D$ −33° (*c* 0·80), $\lambda_{max.}$ 236 mμ (ε 8150), which was not further investigated. The position of the absorption maximum indicates the presence of a heteroannular diene, possibly cholesta-3 : 5-diene. Benzene (50 ml.) and benzene–ether (9 : 1, v/v; 20 ml.) eluted 3 : 5-*cyclo*cholestan-6β-ol (118 mg.; oil). Crystallisation from aqueous acetone gave needles, m. p. 73—75°, $[\alpha]_D$ +50° (*c* 1·10) (Found: C, 84·3; H, 11·95. Calc. for $C_{27}H_{46}O$: C, 83·9; H, 12·0%). Authentic 3 : 5-*cyclo*cholestan-6β-ol was prepared in 78% yield from cholesteryl toluene-*p*-sulphonate and potassium acetate in aqueous acetone;[6a] after crystallisation from aqueous acetone it had m. p. 73—75°, $[\alpha]_D$ +49° (*c* 1·06). Crystallisation from "absolute" ethanol of "AnalaR" grade yielded 3β-ethoxycholest-5-ene, m. p. 87—90°, $[\alpha]_D$ −37° (*c* 0·87). Crystallisation from purified ethanol (treated with sodium and distilled) and water at between −10° and 0° gave pure 3 : 5-*cyclo*cholestan-6β-ol, m. p. 73—75°, $[\alpha]_D$ +51° (*c* 1·19), without difficulty.

Elution with benzene–ether (1 : 1, v/v; 70 ml.) gave cholesterol (60 mg.), m. p. 145—147°, $[\alpha]_D$ −39° (*c* 1·12).

Ethanolysis. The ester (128 mg.) in ethanol (5 ml.) was heated at reflux for 3 hr. On cooling, 3β-ethoxycholest-5-ene (110 mg.; m. p. 84—87°) crystallised. Recrystallisation from ethanol furnished needles, m. p. 89—90°, $[\alpha]_D$ −39° (*c* 1·01).

When an ethanolic solution of the ester was left at room temperature for 5 days, 3β-ethoxycholest-5-ene was obtained in 60% yield.

Methanolysis in the presence of potassium acetate. The ester (400 mg.) and anhydrous potassium acetate (400 mg.) in methanol (20 ml.) were heated under reflux for 5 hr. Concentration *in vacuo* followed by extraction with ether gave 6β-methoxy-3 : 5-*cyclo*cholestane (306 mg.), plates, m. p. 77·5—80°, $[\alpha]_D$ +53° (*c* 1·71) (from acetone).

Hydrolysis in aqueous acetone. The ester (115 mg.) in acetone–water (4 : 1, v/v; 6 ml.) was heated under reflux for 3 hr. On dilution with water and cooling, cholesterol (77 mg.; m. p. 142—148°) crystallised. Recrystallised from aqueous ethanol it had m. p. 146—148°.

Acetolysis. The ester (73 mg.) dissolved slowly in glacial acetic acid (8 ml.) at room temperature. After 2 days the solution was concentrated *in vacuo* and extracted with ether. The crude product (61 mg.; m. p. 92—110°) gave, from acetone, cholesteryl acetate, m. p. 114—115°, $[\alpha]_D$ −45° (*c* 0·82).

Acetolysis in the presence of potassium acetate. The ester (94 mg.) and anhydrous potassium acetate (130 mg.) in acetic acid (10 ml.) were kept at room temperature for 7 days. The ether-soluble product (81 mg.) was isolated and chromatographed on alumina (5 g.). Practically all material (78 mg.) was eluted with light petroleum (4 × 10 ml.) in the first four fractions without satisfactory resolution. The fractions were combined and treated with N-methanolic potassium hydroxide for 1 hr. under reflux. The product (74 mg.) was chromatographed on alumina (5 g.). Light petroleum (30 ml.) eluted an unidentified oil (9 mg.). Light petroleum–benzene (1 : 1, v/v; 20 ml.) eluted 3 : 5-*cyclo*cholestan-6β-ol (48 mg.; m. p. 63—69°) which crystallised from aqueous acetone in needles, m. p. 72—75°, $[\alpha]_D$ +52° (*c* 1·29). Benzene (20 ml.) eluted cholesterol (18 mg.), m. p. 148—150°.

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