NUCLEOPHILIC SUBSTITUTION REACTIONS IN STEROID SERIES. PART I

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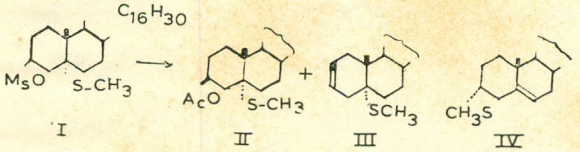
The reaction of tetra-n-propylammonium acetate with 3 β-mesyloxy-5α-methylmercaptocholestane (I) in methyl ethyl ketone gave 3 β-acetoxy-5α-methylmercaptocholestane (II) i.e., with retention of configuration at 3-position, while 3β-tosyloxy-5 α-hydroxycholestane (X) furnished 3 α-acetoxy-5 α-hydroxycholestane (XI) with inversion. The retention of the configuration in (I) at 3-position and the inversion of the configuration in (X) at 3-position are discussed and a mechanism is suggested.

Introduction

In an attempted nucleophilic substitution reaction in methyl ethyl ketone, 3 β-mesyloxy-5 α-methylmercaptocholestane (I) and tetrapropylammonium acetate gave 5α-methylmercaptocholest-2-en (III) and 3 β-acetoxy-5α-methylmercaptocholestane (II) instead of the expected epimeric 3α-acetoxy-5α-methylmercaptocholestane.

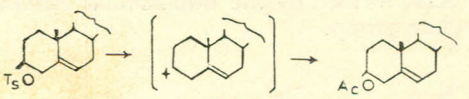


I II III IV

Generally, in most of the substitution reactions in aprotic solvents, there always occurs the formation of an olefine as the second product. The amount of the olefine formed depends upon the orientation of the displaced group[8] and other factors such as anchimeric assistance. In this case the 5α-methylmercaptocholest-2-en (III) was formed in good yield and the rate of elimination as checked by periodic TLC of the reaction mixture was much faster than substitution. It shows that methylmercapto group at position-5 helps in the heterolysis of the mesylate group with the formation of carbonium ion (VIII) which, in turn, gives the elimination product by E_I mechanism.

In order to prove that the olefine formed was 5α-methylmercaptocholest-2-en (III) and not 3α-methylmercaptocholest-5-en(IV),[3] the latter was prepared and its melting point and R_f values were found to be different from those of the elimination product.

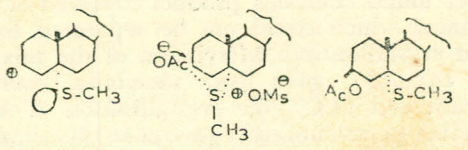
The retention of the configuration at C-3 in (II) finds analogy in the solvolysis of cholesteryl tosylate(V). Cholesteryl tosylate, when solvolysed in the absence of a buffer, usually gives a 3β-substituted cholest-5-en (VII).[1] In this case the carbonium ion (VI) is stabilised by the pi-electrons of the double bond.



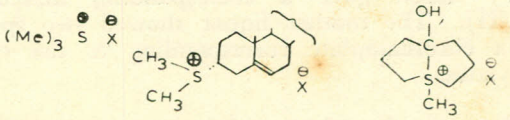
V VI VII

Similarly, the carbonium ion (VIII) formed at C-3 by the heterolysis of the methanesulphonate group in (I) is stabilised by the pair of electrons on sulphur. An alternate explanation for the retention of configuration at C-3 may be that a cyclic sulphonium salt (IX) is formed, which is attacked by acetate ion from the β-side to give 3β-acetoxy - 5α-methylmercaptocholestane with overall retention. Attempts to isolate the sulphonium salt (IX) as perchlorate were unsuccessful, although such type of sulphonium salts are known in some noncyclic and cyclic systems.[2-4]

The formation of the cyclic sulphonium salt (IX) may be regarded as being analogous to the



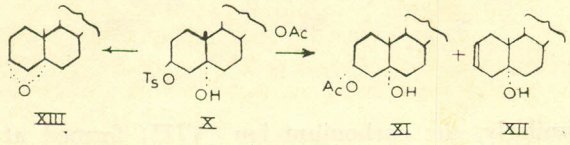
VIII IX II



X = halide, ClO_4^-.

formation of mesomeric cations (VI) by the heterolysis of cholesteryl tosylate. Combination of this mesomeric cation with a nucleophile also leads to a 3β-substituted product with retention configuration.[1]

It is known that 3β-tosyloxy-5α-hydroxycholestane (X) gives 3α,5α-oxidocholestane (XIII) on treatment with strong base.[5] The formation of the oxidocholestane (XIII) is explained by an intramolecular nucleophilic attack of enolate ion at C-5 from the α-side with inversion of configuration at C-3. However, the reaction of the tosylate (X) with acetate ion in methyl ethyl ketone gave 3α-acetoxy-5α-hydroxycholestane (XI) through simple bimolecular substitution reaction accompanied by the inversion of configuration. The other major product was 5α-hydroxycholest-2-en (XII) formed by the bimolecular elimination of tosylate group.

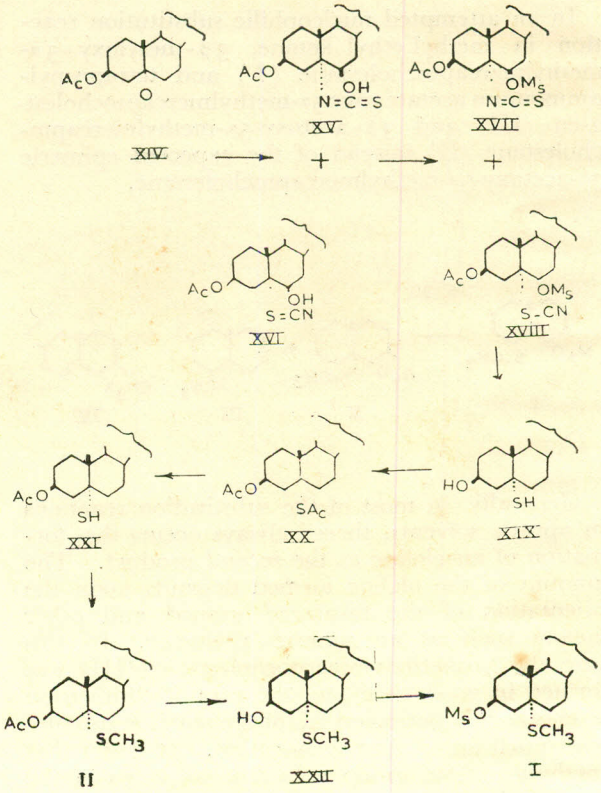


XIII X XI XII

The formation of the acetoxyhydroxysteroid (XI) was very slow, possibly due to the steric hindrance imposed by the hydroxyl group at position-5, preventing the formation of linear transition state for the nucleophilic attack by the acetate ion.

3β-Hydroxy-5α-mercaptocholestane (XIX) was obtained by the method adopted by Komeno *et al.*[6] The reaction of 3β-acetoxy-5β,6β-oxidocholestane (XIV) with thiocyanic acid gave a crystalline product from methanol. The Japanese workers formulated this product as 3β-acetoxy-5α-thiocyanatocholestane-6β-ol (XVI). By employing thin layer chromatography the authors found that this product consisted of two substances which could not be separated by repeated crystallisation. Mesylation of the mixture, m.p. 157-158°, gave two mesylates (infrared spectrum and TLC), the crystallisation of which from ether-petroleum ether gave pure 3β-acetoxy-5α thiocyanato-6β-mesyloxycholestane (XVIII) in 45% yield. The low yield was attributed to the presence of the 5α-isothiocyanato compound (XV) which gave a corresponding mesylate (XVII). The mother liquor showed two spots on a chromatoplate corresponding to the two mesylates.

The reduction of 3β-acetoxy-5α-thiocyanato-6-α β-mesyloxycholestane (XVIII) with lithium aluminium hydride in a refluxing mixture of tetrahydrofuran and ether (1:1) followed by acetylation gave 3β-acetoxy-5α-acetylmercaptocholestane (XX) in 50% yield after the direct crystallisation of the crude product. The 56% yield of diacetate (XX) represents a distinct improvement on the previous yield of 36% obtained by Komeno *et al.*[6] These workers had obtained the diacetate (XX) by the reduction of 3β-acetoxy-5α-thiocyanato-6β-mesyloxycholestane (XVIII) with lithium aluminium hydride in refluxing ether, acetylation of the product and tedious chromatography on alumina.



XIV XV XVII XVI XVIII XXI XX XIX II XXII I

Partial hydrolysis of 3β-acetoxy-5α-acetylmercaptocholestane (XX) with aqueous potassium hydrogen bicarbonate for 14 minutes gave 3β-acetoxy-5α-mercaptocholestane (XXI) in 45% yield after one crystallisation of the crude product from chloroform-methanol. The previous workers[6] had treated the diacetate (XX) with aqueous methanolic potassium hydrogen carbonate for 45 minutes and could not crystallise the 3β-acetoxy-5α-mercaptocholestane (XXI) from the reaction product. They had to separate 3β-acetoxy-5α-mercaptocholestane (XXI) from 5α-mercaptocholestane-3β-ol (XIX) by careful chromatography on the costly florisil.

Methylation of 3β-acetoxy-5α-mercaptocholestane (XXI) with diazomethane in ether proceeded smoothly to give 3β-acetoxy-5α-methylmercaptocholestane (II) in 80% yield after crystallisation. The reductive hydrolysis of 3β-acetoxy-5α-methylmercaptocholestane (II) with lithium aluminium hydride at 0° gave 3β-hydroxy-5α-methylmercaptocholestane (XXII) leaving the mercapto group unchanged. 3β-Hydroxy-5α-methylmercaptocholestane (XXIII) on treatment with methanesulphonyl chloride in pyridine at 0° furnished 3β-methylsulphonyloxy-5α-methylmercaptocholestane (I).

Experimental

All the solvents were dried before use. Merck's silica gel was used for thin layer chromatography (TLC) on glass. The term chromatoplate denotes the employment of the thin-layer chromatography on small plates (2″×7½″).

TETRA-N-PROPYLAMMONIUM ACETATE

Tetra-*n*-propylammonium hydroxide (10% in water) was neutralised with acetic acid. The excess of water was distilled under reduced pressure on a steam bath. It was then kept over phosphorus pentoxide for 24 hours. A crystalline hygroscopic tetra-n-propylammonium acetate was obtained which was used in the later experiments.

3β-ACETOXY-5α-THIOCYANATO-6 β-HYDROXYCHOLESTANE (XVI)

Potassium thiocyanate (4.38 g) was dissolved in water (25 ml) in a separating funnel. To it were added ether (26 ml) and crushed ice, followed by 85 per cent phosphoric acid (3.6 ml), dropwise along with more ice and with vigorous shaking. The dark red ethereal layer was separated and added to a solution of 3β-acetoxy-5β,6β-oxidocholestane (XIV) (5.3 g) in ether (100 ml). After two days at room temperature in the dark, the solution was washed with aqueous sodium carbonate solution, water, and dried over sodium sulphate. Evaporation of the ether afforded a syrup which was crystallised from methanol to give plates (3.2 g), m.p. 157-158°. Thin-layer chromatography on silica showed two spots and no separation could be effected on repeated crystallisation. The Japanese workers have formulated this compound as 3β-acetoxy-5α-thiocyanatocholestane-6β-ol (XIV).[6] Separation of the above product by column chromatography, both on neutral deactivated alumina and neutral activated alumina, was unsuccessful as one of the products decomposed on the column to some extent and the separation was not satisfactory. Chromatography of 118 mg of this impure compound on one silica gel plate afforded two distinct layers, which were separated and then extracted with ether. The fast moving layer furnished 3β-acetoxy-5α-isothiocyanatocholestane-6β-ol (XV) as needles (12 mg), m.p. 182-184°, after crystallisation from methanol, $[\alpha]_D$-6.4, (c 2.33), (Found: C, 71.2; H, 9.7. $C_{30}H_{49}NS$ requires C, 71.5; H, 9.8%). Its infrared spectrum (KBr) showed the following bands: 3450 (OH), 2080 (—N=C=S, broad, strong); 1730 and 1225 cm^{-1} (OAc). The slow moving layer gave pure 3β-acetoxy-5α-thiocyanatocholestane-6β-ol (XVI) as plates (80 mg), m.p. 158-159°, after crystallisation from methanol. Its infrared spectrum (KBr) exhibited the following peaks: 3450 (OH), 2150 (—S—C=N, sharp), 1750 and 1240 cm^{-1} (OAc).

3β-ACETOXY-5α-THIOCYANATO-6β-METHYLSULPHONYLOXYCHOLESTANE (XVIII)

A mixture of 3β-acetoxy-5α-isothiocyanatocholestane-6β-ol (XV), and 3β-acetoxy-5α-thiocyanatocholestane-6β-ol (XVI), (20.5 g), m.p. 157-158° was treated with methanesulphonyl chloride (30 ml) in dry pyridine (100 ml) at 0° for 14 hours. The mixture was poured into ice cold 2N hydrochloric acid, extracted with ether, washed with an aqueous sodium bicarbonate solution and water, and dried (anhydrous Na_2SO_4). Distillation of the ether at room temperature under reduced pressure gave a crystalline mass, which was a mixture of two substances (TLC and IR). Crystallisation from ether–light petroleum, furnished 3β-acetoxy-5α-thiocayanato-6β-methylsulphonyloxycholestane (XVIII) (9.3 g), m.p. 124-126°, which was homogeneous on thin layer chromatography. A portion further recrystallised from ether had a m.p. of 129° (lit., m.p. 129°). Infrared spectrum shows peaks at 2150 (—S—C=N sharp), 1740, 1240 (OAc) and 1180 cm^{-1}. The mother liquor consisted of two isomeric mesylates (XVII, XVIII) as shown by TLC and infrared spectrum.

3β-ACETOXY-5α-ACETYLMERCAPTOCHOLESTANE (XX)

(i) To a solution of lithium aluminium hydride (6 g) in dry ether (200 ml) was added dropwise a solution of 3β-acetoxy-5α-thiocyanato-6β-methysulphonyloxycholestane (XVIII) (8.3 g) in dry tetrahydrofuran (200 ml). After refluxing for two hours, the excess hydride was decomposed with wet ether, and the mixture was then washed with 10% aqueous solution of hydrochloric acid, water, and dried over sodium sulphate. After the evaporation of the ether, the white solid

residue was acetylated with acetic anhydride (20 ml) in pyridine (20 ml) by heating for two hours on the steam bath. The product was worked up *via* ether, to give a semi-solid product (7.5 g), which, on crystallisation from acetone, afforded 3β-acetoxy-5α-acetylmercaptocholestane (XX) as needles (4.5 g), m.p. 142°, ν_{max}, (KBr) 1743 (OAc), 1680 (SAc), 1225 (OAc), 1118 cm^{-1} (SAc) (lit., m.p. 142°).[6] Thin layer chromatography showed that mother liquor contained 3β-acetoxy-5α-acetylmercaptocholestane (XX), 3β-acetoxy-5α,6-α-epithiocholestane and cholesteryl acetate.

(ii) According to the method given by Kameno *et al.*[6] a solution of 3β-acetoxy-5α-thiocyanato-6β-methylsulphonyloxycholestane (XVIII) (1.77 g.) in ether was refluxed with lithium aluminium hydride (500 mg) for 3 hours. After working up the product as usual, a white solid was obtained (1.149 g). It was acetylated with acetic anhydride in pyridine and the product chromatographed on a column of deactivated neutral alumina (30 g). Elution with petroleum ether, and a mixture of petroleum ether–benzene (4:1) afforded cholesteryl acetate (502 mg); elution with petroleum ether–benzene (1:1) gave a mixture (258 mg) of 3β-acetoxy-5α-mercaptocholestane (XXI), 3β-acetoxy-5α,6α-epithiocholestane and 3β-acetoxy-5α-acetylmercaptocholestane (XX) as shown by TLC. Elution with benzene–petroleum ether (4:1) and benzene afforded 3β-acetoxy-5α-acetylmercaptocholestone (XX) (697 mg), m.p. 142-143° (after crystallisation from acetone). Elution with benzene and 10% ether–benzene afforded a mixture (166 mg) containing mostly 3 β-hydroxy-5α-mercaptocholestane (XIX) (chromatoplate).

3 β-ACETOXY-5α-MERCAPTOCHOLESTANE (XXI)

3 β-Acetoxy-5α-acetylmercaptocholestane (XX) (9.713 g) was treated with a solution of potassium hydrogen carbonate (10.4 g in 45 ml of water) in a mixture of methanol–ethanol, (2:1, 800 ml). After 14 minutes under reflux the mixture was worked up *via* ether, and the residue crystallised from chloroform–methanol to give 3β-acetoxy-5α-mercaptocholestane (XXI) (3.840 g, 45%) as plates, m.p. 168-170°, $[\alpha]_D$–5° (lit.[6], m.p. 168-170°).

The mother liquor was reacetylated with acetic anhydride in pyridine and again partially hydrolysed in the above manner, giving more of 3β-acetoxy-5α-mercaptocholestane (XXI) (2.18 g).

3 β-ACETOXY-5α-METHYLMERCAPTOCHOLESTANE (II)

3 β-Acetoxy-5α-mercaptocholestane (3.807 g) in dry ether was treated with an ethereal solution of diazomethane (obtained from 40 g of nitrosomethylurea). After standing at room temperature, the excess of diazomethane was decomposed with water, and the ethereal solution dried, filtered and evaporated. The product was crystallised from chloroform–methanol, affording 3β-*acetoxy*-5α-*methylmercaptocholestane* (II) as plates (3.304 g), double m.p. 114-20/135°. After drying at 80°/0.1 mm, it had m.p. 135-137°, $[\alpha]_D$–12°, (c,1.1.). (Found: C, 75.3; H, 10.6. $C_{30}H_{52}O_2S$ requires, C, 75.52; H, 11.0%).

5α-METHYLMERCAPTOCHOLESTANE-3 β-ol (XXII).

3 β-Acetoxy-5-α-methylmercaptocholestane (770 mg) was treated with lithium aluminium hydride at 0° for 20 minutes, affording the white crystalline compound (600 mg) m.p. 171-174° (after changing the crystalline form at 140°). Crystallisation from ether gave 5 α-*methylmercaptocholestane*-3β-*ol* (XXII), m.p. 181 - 183°, $[\alpha]_D$+8° (c, 2.1). (Found: C, 77.4; H, 11.6. C_{28} H_{50} OS; requires C, 77.1; H,11.3%).

3β-METHYLSULPHONYLOXY-5α-METHYL-MERCAPTOCHOLESTANE (I)

5α-Methylmercaptocholestane-3β-ol (XXII) (500 mg) was treated with methanesulphonyl chloride (0.3 ml) in dry pyridine (2 ml) at 0°, overnight. It was poured into ice cold 2N hydrochloric acid and extracted with ether. The ether extract was washed with a cold aqueous sodium hydrogen carbonate solution, then with cold water, dried over sodium sulphate and the ether was evaporated under reduced pressure at room temperature to give a white crystalline solid m.p. 83-85°. Crystallisation from cold ether–petroleum ether (b.p. 4060°C), gave 3β-*methylsulphonyloxy*-5α-*methylmercaptocholestane* (I), m.p. 111-115° (decomp.), $[\alpha]_D$–3.8° (c, 1.3). (Found: C, 67.3; H, 10.45. $C_{29}H_{59}O_3S_2$ requires C, 67.1; H, 10.45).

TREATMENT OF 3β-METHYLSULPHONYLOXY-5α-METMYLMERCAPTOCHOLESTANE (I) WITH TETRA-n-PROPYLAMMONIUM ACETATE

3β-Methylsulphonyloxy-5α-methylmercaptocholestane (I) (350 mg) was treated with dry tetra-n-propylammonium acetate (300 mg) in boiling dry methyl ethyl ketone (50 ml) overnight. The product was worked up in ether,

washed, and dried, giving a syrup (317 mg). It showed two spots on a chromatoplate, one of them corresponding to 3β-acetoxy-5α-methylmercaptocholestane (II). The product was chromatographed on two silica plates. The fast moving layer afforded 268 mg of compound which on crystallisation from acetone gave 5α-methylmercaptocholest-2-en (III) as needles m.p. 92-98°, $[\alpha_D]$-28 (c, 1.16). (Found: C, 80.4; H, 11.4%. $C_{28}H_{48}S$ requires C, 80.7; H, 11.6%). Thin-layer chromatography showed that this compound had a different R_f value from that of synthetic 3α-methylmercaptocholest-5-en (IV).[3] The slow moving layer gave 3β-acetoxy-5α-methylmercaptocholestane (II) (45 mg), m.p. and mixed m.p. 132-135°, $[\alpha]_D$-8°, and it displayed an infrared spectrum identical with that of an authentic sample.

REACTION BETWEEN 3β-TOSYLOXY-5α-HYDROXYCHOLESTANE (X) WITH TETRA-n-PROPYLAMMONIUM ACETATE

3β-Tosyloxy-5α-hydroxycholestan(X)[5] (350 mg), tetra-n-propylammonium acetate (1 g) and dry methyl ethyl ketone were refluxed for 35 hours. The reaction mixture was poured into water, and extracted with ether. The ethereal extracts were washed with sodium bicarbonate solution, and then dried over sodium sulphate. The ether was evaporated. The product showed four spots by TLC The residue (209 mg) was chromatographed on one silica gel plate in benzene solvent. The four layers were separated and extracted thoroughly with ether. The top layer gave a syrup (10 mg), which is an unidentified hydrocarbon (shown by TLC and IR). The second layer gave 48 mg of 5α-hydroxycholestane-2-en[5] (XII), m.p. 93-94°. The third layer gave 3α-acetoxy-5α-hydroxycholestane (XI) (73 mg), m.p. 132-133° (lit., m. p. 132-138°), and the fourth layer furnished a mixture of two dihydroxy alcohols (17 mg) as shown by TLC.

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