[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

THE PREPARATION OF 3β-METHOXY-5-METHYL-10-NOR-8(9)-CHOLESTENE, AN ISOMER OF CHOLESTERYL METHYL ETHER

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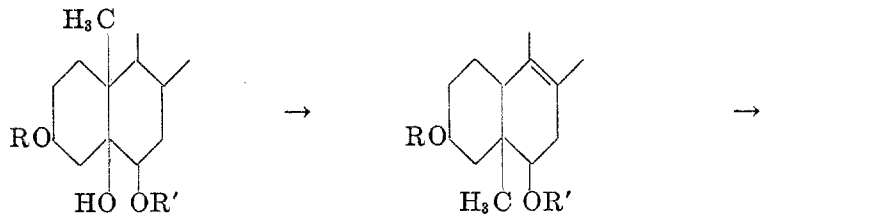
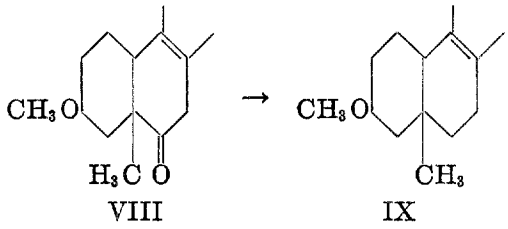
Petrow (1–3) has demonstrated that treatment of 3β,6β-diacetoxy-5α-hydroxycholestane (I) with sulfuric acid in acetic anhydride resulted in dehydration and rearrangement to a molecule formulated as 3β,6β-diacetoxy-5-methyl-10-nor-8(9)-cholestene (IV). More recently, 3β,6β-diacetoxy-5-methyl-10-nor-8(9)-androsten-17-one and certain of its derivatives (4, 5) have been prepared by the rearrangement of 3β,6β-diacetoxyandrostan-5α-ol-17-one. These rearranged androstenes proved to be physiologically inactive, and it was speculated that this lack of activity may be due to the presence of the 6-acetoxy- or 6-keto-grouping in the molecule. 6-Ketotestosterone possesses no androgenic activity (6). In this paper, the preparation of 3β-methoxy-5-methyl-10-nor-8(9)-cholestene (IX), an isomer of cholesteryl methyl ether, is reported. The methods used



I R, R′ = CH_3CO
II R = CH_3; R′ = H
III R = CH_3; R′ = CH_3CO

IV R, R′ = CH_3CO
V R = CH_3; R′ = CH_3CO
VI R = CH_3; R′ = H
VII R = CH_3; R′ = CH_3SO_2



VIII IX

for the preparation of IX should enable one to prepare substituted 5-methyl-10-nor-8(9)-androstenes lacking an oxygen function at C-6 and so to determine whether compounds possessing this rearranged steroid nucleus can possess physiological activity. Also, Petrow (3) has demonstrated that IV can be hydroxylated

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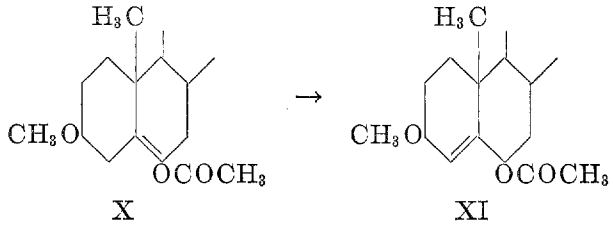
at C-11 with selenium dioxide. Thus, methods are available for the preparation of compounds isomeric with cortisone but possessing a rearranged steroid nucleus.

3β-Methoxy-5α,6β-dihydroxycholestane (II) was prepared in 74% yield by the hydroxylation of cholesteryl methyl ether in carbon tetrachloride with hydrogen peroxide and formic acid. This diol, II, was easily monoacetylated by heating with acetic anhydride (1). When a finely divided suspension of 3β-methoxy-5α-hydroxy-6β-acetoxycholestane (III) in acetic anhydride was treated with a few drops of sulfuric acid at 40°, the suspended material (III) first went into solution and about one minute later 3β-methoxy-5-methyl-6β-acetoxy-10-nor-8(9)-cholestene (V) began to precipitate.

The fact that the substance obtained from this reaction was not one of the simple dehydration products, X or XI, was established in the following way.



X XI

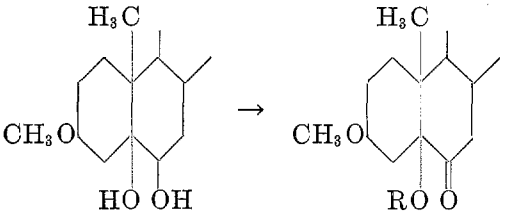
Compound V was readily hydrolyzed to an alcohol (VI) which in turn could be acetylated back to V or oxidized with chromium trioxide to a ketone (VIII). Hydrolysis of a substance of structure X would have yielded 3β-methoxy-6-ketocholestane, m.p. 92° (7), a compound distinctly different from that obtained. While hydrolysis of XI would yield an alcohol, oxidation of that alcohol would give an α,β-unsaturated ketone which should show an absorption maximum at about 240 mμ in the ultraviolet. The ultraviolet absorption spectrum of the ketone (VIII) obtained from this series of reactions did not possess a maximum at 240 mμ and showed no strong absorption in the region 230 to 300 mμ (ϵ = 435 at 230 mμ and decreased with increasing wavelength to 271 mμ.) The presence of a weak absorption band at approximately 286 mμ, ϵ = 37, confirmed the presence of the carbonyl group.

It has been demonstrated (2, 5, 8) that certain 5α-hydroxy-6β-acetoxy-steroids are converted to the corresponding $\Delta^{4,5}$-unsaturated-6β-acetoxy-compounds by treatment with thionyl chloride in pyridine. Treatment of 3β-methoxy-5α-hydroxy-6β-acetoxycholestane (III) in this way produced a crystalline substance, m.p. 94°, which, by analogy with previous work, was formulated as 3β-methoxy-6β-acetoxy-4-cholestene (XI). As expected, this compound gave a blue color with trichloroacetic acid.

3β-Methoxy-5-methyl-10-nor-8(9)-cholestene (IX) was prepared by the reduction of the corresponding 6-ketosteroid (VIII) using the Huang-Minlon (9) modification of the Wolff-Kishner reduction. The compound had a positive rotation, $[\alpha]_D^{27}$ +61.7°, and gave a positive Tortelli-Jaffé test. An attempt to

prepare 3β-methoxy-5-methyl-10-nor-8(9)-cholestene (IX) by the reduction of 3β-methoxy-5-methyl-6β-mesyloxy-10-nor-8(9)-cholestene (VII) with lithium aluminum hydride (10) was unsuccessful; no crystalline material other than a small amount of starting material was isolated from the reaction. The reduction of neopentyl sulfonates with lithium aluminum hydride should probably be studied on simpler molecules.

Since the oxidation of 3β-methoxy-5-methyl-6β-hydroxy-10-nor-8(9)-cholestene (VI) to the corresponding 3β-methoxy-5-methyl-6-keto-10-nor-8(9)-cholestene (VIII) with chromium trioxide in acetic acid was not entirely satisfactory (yield 41%), an attempt was made to prepare the ketone (VIII) by first oxidizing the glycol (II) to the corresponding 5-hydroxy-6-ketone (XII) and then rearranging the hydroxyketone (XII) with sulfuric acid in acetic anhydride. While the oxidation of 3β-methoxy-5α,6β-dihydroxycholestane (II) to 3β-methoxy-



II

XII R = H
XIII R = CH_3CO

5α-hydroxy-6-cholestanone (XII) with N-bromosuccinimide (11) proceeded very well (90% yield), the attempted rearrangement to the corresponding 5-methyl-10-nor compound (VIII) yielded only 3β-methoxy-5α-acetoxy-6-cholestanone (XIII). Apparently the strong inductive effect of the adjacent carbonyl group prevented the acid catalyzed removal of the 5α-hydroxyl group, thus preventing the formation of the carbonium ion necessary for rearrangement; instead the 5α-hydroxyl group was acetylated. These results are in accord with those previously reported (12) on 3β-acetoxy-5α-hydroxy-6-cholestanone.

In the course of this work several attempts were made to rearrange the 5-methyl group of 3β-methoxy-5-methyl-6β-hydroxy-10-nor-8(9)-cholestene (VI) to the adjacent six position. This would remove the angular methyl group and thus enable one to aromatize ring A and/or B of the 6-methyl-steroid. It would also provide further evidence for the structures proposed for these rearranged substances. However, treatment of 3β-methoxy-5-methyl-6β-hydroxy-10-nor-8(9)-cholestene (VI) with acids or heating the corresponding 6β-mesylate (VII) in polar solvents yielded only dark, tarry products. Treatment of VI with thionyl chloride in pyridine yielded bis-[3β-methoxy-5-methyl-10-nor-8(9)-cholesten-6-yl] sulfite. It is highly probable that the 5-methyl group in VI possesses a β-configuration, and thus the methyl group and the 6β-hydroxyl are *cis* to each other. It is possible that the methyl-migration on the corresponding *trans* compound would prove more successful.

EXPERIMENTAL[2]

3β-Methoxy-5α,6β-dihydroxycholestane (II). A solution of 50.0 g. of cholesteryl methyl ether (13) in 250 ml. of distilled carbon tetrachloride was stirred vigorously with 300 ml. of 96% formic acid. To the resulting emulsion at 25° was added 30.0 g. of 30% hydrogen peroxide over a period of one-half hour. The temperature rose slowly and was maintained at 40° for one hour and at 50° for four hours. The mixture was poured into brine, the carbon tetrachloride layer was separated, and the aqueous layer was washed with three additional portions of carbon tetrachloride. The carbon tetrachloride solution was dried over sodium sulfate and freed of solvent by distillation. The residual, light-yellow oil was hydrolyzed by heating it under reflux with 10 g. of potassium hydroxide in 350 ml. of methanol for one hour. The alcoholic solution was poured into water; the product (51.4 g., m.p. 136–148°), which precipitated first as an oil but later solidified, was separated and washed with water. Crystallization of this material from benzene yielded 34.8 g. of colorless needles, m.p. 152.5–153.5°, $[\alpha]_D^{28}$ −5.8°. An additional 5.4 g. of 3β-methoxy-5α,6β-dihydroxycholestane, m.p. 149–151°, was recovered from the mother liquors; total yield 40.2 g. (74%). The compound is reported (1) to melt at 154°, $[\alpha]_D$ −4.8°.

Evaporation of the benzene from the mother liquors and crystallization of the residue from methanol gave 4.0 g. of a by-product, m.p. 81–81.5°, $[\alpha]_D^{28}$ −51.5°, which is presumably a 5,6-oxide of cholesteryl methyl ether. This compound was obtained in greater yield when the proportions of carbon tetrachloride and formic acid relative to cholesteryl methyl ether were reduced. It was not further investigated.

Anal. Calc'd for $C_{28}H_{48}O_2$: C, 80.71; H, 11.61.
Found: C, 80.33; H, 11.63.

3β-Methoxy-5-methyl-6β-acetoxy-10-nor-8(9)-cholestene (V) was prepared by a modification of Petrow's procedure (1, 2). To a suspension of 36.8 g. of finely divided 3β-methoxy-5α-hydroxy-6β-acetoxycholestane (1), m.p. 120–121°, $[\alpha]_D^{28}$ −32°, in 190 ml. of acetic anhydride at 40° was added, with stirring, 12 drops of concentrated sulfuric acid. The mixture became brown immediately, the temperature rose to 43°, the starting material disappeared, and the product began to precipitate within about one minute. The mixture was stirred for five minutes, then cooled in an ice-bath for five minutes, and filtered. The residue was washed with 80 ml. of cold acetic anhydride to remove surface color. From this reaction 18.8 g. (53%) of a white crystalline powder, m.p. 118.5–120.5°, was obtained. Crystallization from methanol afforded pure 3β-methoxy-5-methyl-6β-acetoxy-10-nor-8(9)-cholestene (V),[3] m.p. 121.5–122.5°, $[\alpha]_D^{28}$ +88.6°; 88.2°. This product depressed the melting point of the starting material.

Anal. Calc'd for $C_{30}H_{50}O_3$: C, 78.55; H, 10.98.
Found: C, 78.75; H, 11.1.

3β-Methoxy-5-methyl-6β-hydroxy-10-nor-8(9)-cholestene (VI). A solution of 34.4 g. of 3β-methoxy-5-methyl-6β-acetoxy-10-nor-8(9)-cholestene and 8.4 g. of potassium hydroxide in 550 ml. of methanol was heated under reflux for six hours, then poured into water. The precipitate, 31.0 g. (99%), m.p. 105–107°, was collected and washed with water. Crystallization of the compound from methanol gave m.p. 107–107.5°, $[\alpha]_D^{27}$ +118.5°.

Anal. Calc'd for $C_{28}H_{48}O_2$: C, 80.71; H, 11.61.
Found: C, 80.69; H, 11.71.

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[2] Microanalyses were performed by Messrs. W. C. Cummings, R. E. Kelly, H. W. Turner, L. Errede, and E. L. Wheeler. All melting points were taken on a Fisher-Johns melting-point apparatus. All rotations were taken in chloroform solution using a 2-dcm. tube and are accurate to ±2°.

[3] This compound should be identical with one originally prepared by Petrow (1), before the true nature of the dehydration was realized, and reported to be 3β-methoxy-6β-acetoxy-4-cholestene. However, while the melting points of the two compounds agree, m.p. 121.5–122.5°, the rotation reported by Petrow $[\alpha]_D^{28}$ +166.6°, differs greatly from ours.

This alcohol (VI) could be reconverted to the acetate (V), m.p. 120.5–121.5°, by warming with acetic anhydride.

3β-Methoxy-5-methyl-6β-mesyloxy-10-nor-8(9)-cholestene (VII). To a solution of 5.0 g. of 3β-methoxy-5-methyl-6β-hydroxy-10-nor-8(9)-cholestene (VI) in 80 ml. of dry pyridine was added, with stirring, 4.0 g. of methanesulfonyl chloride over a period of one-half hour. The temperature was held below 35°. After the solution had been stirred at 32° for nine hours, it was poured into water, and the precipitated oil was caused to solidify. The cream-colored powder, 5.75 g. (97%), was thoroughly washed with water. The melting point observed when a sample of this compound was placed on the melting-point block at 102–103° was 106–107.5° (dec.). 3β-Methoxy-5-methyl-6β-mesyloxy-10-nor-8(9)-cholestene is unstable and its melting is dependent on the rate of heating. This compound was analyzed without further purification.

Anal. Calc'd for $C_{29}H_{50}O_4S$: C, 70.40; H, 10.19.
Found: C, 70.40; H, 10.18.

Treatment of the alcohol (VI) with *p*-toluenesulfonyl chloride in pyridine did not furnish a crystalline product.

Bis-(3β-methoxy-5-methyl-10-nor-8(9)-cholesten-6-yl) sulfite. To a solution of 2.0 g. of 3β-methoxy-5-methyl-6β-hydroxy-10-nor-8(9)-cholestene (VI) in 40 ml. of dry pyridine at 5–10° was added 2.0 ml. of thionyl chloride over a period of 15 minutes. The resulting solution was stirred at 5–10° for one-half hour, then poured over ice. Trituration of the crude solid with hot methanol gave 1.72 g. of product, m.p. 186–189°, which after crystallization from benzene-methanol melted at 191–192°, $[\alpha]_D^{27}$ +170°. An elementary analysis showed the presence of sulfur.

Anal. Calc'd for $C_{56}H_{94}O_5S$: C, 76.48; H, 10.77.
Found: C, 76.45; H, 10.86.

When the reaction mixture was allowed to warm to room temperature, the yield of sulfite was lower. Treatment of the alcohol with excess thionyl chloride as solvent (in the absence of pyridine) gave only a dark-brown tar.

3β-Methoxy-5-methyl-6-keto-10-nor-8(9)-cholestene (VIII). To a solution of 10.0 g. of 3β-methoxy-5-methyl-6β-hydroxy-10-nor-8(9)-cholestene in 150 ml. of acetic acid at 32° was added, slowly with stirring, a solution of 1.60 g. of chromium trioxide in 15 ml. of water and 35 ml. of acetic acid. The addition was made over a period of four hours and stirring was continued for an additional four hours. Methanol (10 ml.) was then added to destroy any unreacted chromic acid, and the reaction mixture was poured into an aqueous solution of sodium chloride. The aqueous mixture was extracted with carbon tetrachloride and the organic layer was washed with a saturated sodium bicarbonate solution, dried with sodium sulfate, and freed of solvent. A solution of the residual, orange-colored oil in benzene was chromatographed on a column of alumina (43 × 5 cm.). The ketone, obtained as a colorless oil from the benzene eluate, crystallized on standing. Crystallization of this material from methanol yielded 4.06 g. (41%) of the desired ketone (VIII), m.p. 64.5–65.5°, $[\alpha]_D^{27}$ −7.8°.

Anal. Calc'd for $C_{28}H_{46}O_2$: C, 81.10; H, 11.18.
Found: C, 81.18; H, 11.31.

The *semicarbazone*, m.p. 183.5–185°, was prepared in the usual way and purified by crystallization from ethyl acetate.

Anal. Calc'd for $C_{29}H_{49}N_3O_2$: C, 73.83; H, 10.47.
Found: C, 73.68; H, 10.42.

3β-Methoxy-5-methyl-10-nor-8(9)-cholestene (IX). To 3.1 g. of sodium hydroxide in 140 ml. of diethylene glycol was added 2.0 g. of 3β-methoxy-5-methyl-6-keto-10-nor-8(9)-cholestene and 3.0 ml. of 85% hydrazine hydrate. The suspension was heated at 90–100° for one hour, and the temperature was then gradually raised to 140°. An additional 2.0 ml. of hydrazine hydrate in 20 ml. of diethylene glycol was introduced, and the mixture was maintained at 140° for 2½ hours. At the end of this time, the lower-boiling materials were slowly distilled from the reaction mixture until the temperature reached 195°. The mixture

was then heated at 195–200°, with occasional shaking, for three hours. The solution was extracted with benzene, the glycol layer was poured into water, and the aqueous-glycol mixture was extracted with benzene. The benzene extract was washed with water, dried with sodium sulfate, and distilled. A solution of the residual, light-yellow oil in petroleum ether, b.p. 40–75°, was chromatographed on alumina. Elution with petroleum ether yielded the desired product (IX) as a colorless oil which crystallized when cooled with Dry Ice. Crystallization of this material from ethanol yielded 1.09 g. (56.5%) of colorless needles, m.p. 58–59.5°. A second crystallization of the compound raised its melting point to 59.5–60.5°, $[\alpha]_D^{27}$ +61.7°.

Anal. Calc'd for $C_{28}H_{48}O$: C, 83.93; H, 12.07.
Found: C, 84.15; H, 12.17.

3β-Methoxy-5α-hydroxy-6-cholestanone (XII). To 2.5 g. of 3β-methoxy-5α,6β-dihydroxycholestane in 40 ml. of dioxane and 5 ml. of water was added 1.5 g. of N-bromosuccinimide. The solution was kept at 20–25° by cooling during the first few minutes of the reaction and then was allowed to stand for one hour. After 5 ml. of water had been added, the solution was allowed to stand overnight at 10–15°; a precipitate separated. Further dilution precipitated additional material; upon warming the mixture on the steam-bath the orange color was discharged. The product, 2.25 g. (90%), m.p. 145–146.5°, was separated and crystallized from aqueous dioxane to give colorless needles, m.p. 146–147°, $[\alpha]_D^{23}$ −43.5°. This material, when crystallized from nitromethane, melted at 137.5–138.5°. This lower-melting form melted at 146–147° in admixture with the higher-melting crystals and was reconverted to the latter form by heating above the melting point and allowing the melt to solidify.

Anal. Calc'd for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18.
Found: C, 77.93; H, 11.12.

3β-Methoxy-5α-acetoxy-6-cholestanone (XIII). Two drops of concentrated sulfuric acid was added at 40° to a stirred suspension of 1.0 g. of 3β-methoxy-5α-hydroxy-6-cholestanone in 20 ml. of distilled acetic anhydride. The mixture became brown immediately and the suspended solid dissolved. After the mixture had been stirred at 40° for 10 minutes, it was poured into brine and extracted with carbon tetrachloride, then with benzene. From these extracts 0.45 g. of product, m.p. 123–127°, was isolated. Pure 3β-methoxy-5α-acetoxy-6-cholestanone, m.p. 130.5–131.5°, $[\alpha]_D^{27}$ −21.9°, was obtained by crystallization from methanol.

Anal. Calc'd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62.
Found: C, 75.77; H, 10.48.

3β-Methoxy-6β-acetoxy-4-cholestene (XI). To 1.0 g. of 3β-methoxy-5α-hydroxy-6β-acetoxycholestane in 15 ml. of dry pyridine cooled in an ice-bath was added 0.5 ml. of thionyl chloride. After 10 minutes, the mixture was poured into ice-water. The product, 0.90 g., m.p. 78–84°, was separated, and purified by repeated crystallization from methanol. The pure 3β-methoxy-6β-acetoxy-4-cholestene melted at 94–94.5° and gave a blue color when treated with 90% trichloroacetic acid.

Anal. Calc'd for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99.
Found: C, 78.20; H, 11.05.

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SUMMARY

3β-Methoxy-5-methyl-10-nor-8(9)-cholestene, an isomer of cholesteryl methyl ether, was prepared from cholesteryl methyl ether by a series of reactions involving hydroxylation of the double bond, rearrangement of the C-10 methyl group to C-5, and removal of the oxygen function at C-6.

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