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THE INTRODUCTION OF DOUBLE BONDS INTO STEROIDS BY THE USE OF THE HOFMANN DEGRADATION[1]

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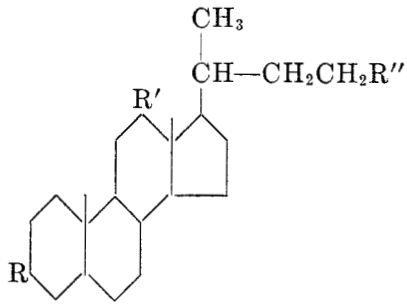
During the past few years various methods for the conversion of desoxycholic acid to compounds of the cortical hormone type have been investigated in these Laboratories. Some of the syntheses had steps which required the introduction of double bonds into the steroid molecule and it was thought that the Hofmann degradation (1) might be useful for this purpose.

Since a number of the important cortical hormones contain an oxygen function at C-11, the synthesis of such compounds is of considerable interest. One of the principal methods used for the introduction of this group starts with steroids containing a Δ^{11}-double bond, and therefore, we first applied the Hofmann degradation to the preparation of this type of unsaturation. 3(α)-Hydroxy-12-aminocholanic acid (I) was made by reducing the corresponding 12-oximino compound (II).[2] The amino acid thus obtained was esterified and the amino group methylated and quaternized to give ethyl 3(α)-hydroxycholanate 12-trimethylammonium iodide (III). After Hofmann degradation the reaction product, isolated as the acetate methyl ester, proved to be methyl 3(α)-acetoxy-Δ^{11}-cholenate (XIII). This series of reactions thus provides a new method for the introduction of the Δ^{11}-double bond.

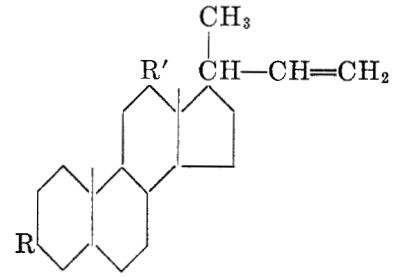
The Hofmann degradation was next investigated as a method for the preparation of Δ^{20}-pregnenes. Compounds of this type are important since they may be used as starting material for the synthesis of the ketol side chain characteristic of the cortical hormones. 3(α),12(α) - Diacetoxy - 20 - aminopregnane (XXIII) was prepared from 3(α),12(α)-diacetoxybisnorcholanic acid (XXII) through the Curtius reaction (2).[2] The amine was methylated and quaternized with methyl iodide and potassium carbonate to yield 3(α)-hydroxy-12(α)-acetoxypregnane 20-trimethylammonium iodide (XXIV), partial saponification having taken place during this reaction. When the Hofmann degradation was carried out with this compound the product, isolated as the diacetate, was shown to be 3(α),12(α)-diacetoxy-Δ^{20}-pregnene (XXV) by oxidation with chromium trioxide to the corresponding etiocholanic acid. After this work had been reported[1] Julian, Meyer, and Printy (3) published essentially the same synthesis of this compound using somewhat different reaction conditions.

[1] Presented in part before the Division of Medicinal Chemistry at the 112th Meeting of the American Chemical Society, New York City, September 17, 1947.

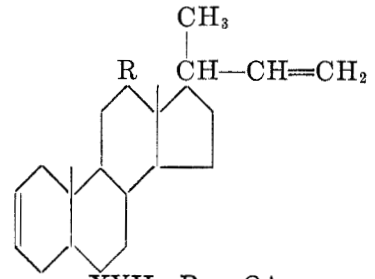
[2] Some of the amines described in this paper were first synthesized by Sarett [Merck Reports, Adrenal Cortical Problem, Committee on Medical Research of the Office of Research and Development] either by reduction of the oxime or by the Curtius method. They were further converted by means of nitrous acid [*cf.* reference (9)] to the corresponding unsaturated compounds which were identical with the corresponding products obtained in this work by means of the Hofmann degradation.



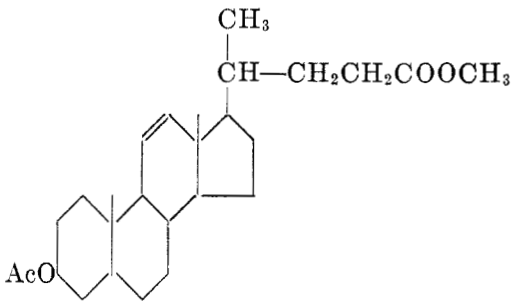
I R = OH, R′ = NH_2, R″ = COOH
II R = OH, R′ = NOH, R″ = COOH
III R = OH, R′ = $N(CH_3)_3I$, R″ = $COOC_2H_5$
IV R = OAc, R′ = OAc, R″ = NH_2
V R = OAc, R′ = OAc, R″ = COOH
VI R = OH, R′ = OAc, R″ = $N(CH_3)_3I$
VII R = H, R′ = OAc, R″ = $NH_2 \cdot HCl$
VIII R = H, R′ = OAc, R″ = COOH
IX R = H, R′ = OAc, R″ = $N(CH_3)_3I$
X R = OAc, R′ = H, R″ = NH_2
XI R = OAc, R′ = H, R″ = COOH
XII R = OAc, R′ = H, R″ = $N(CH_3)_3I$



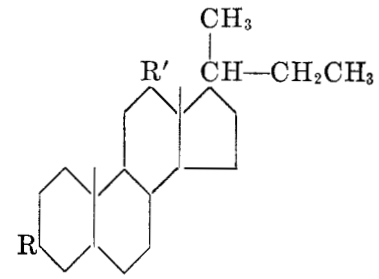
XIV R = OAc, R′ = OAc
XV R = H, R′ = OAc
XVI R = OAc, R′ = H



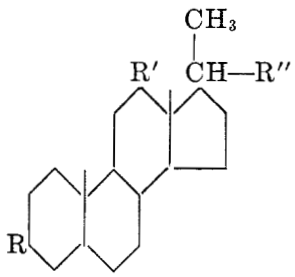
XVII R = OAc
XVIII R = H



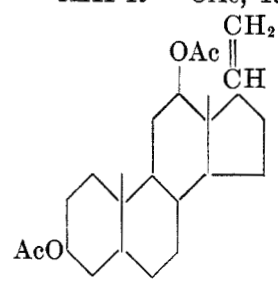
XIII



XIX R = H, R′ = OAc
XX R = H, R′ = H
XXI R = OAc, R′ = H



XXII R = OAc, R′ = OAc, R″ = COOH
XXIII R = OAc, R′ = OAc, R″ = NH_2
XXIV R = OH, R′ = OAc, R″ = $N(CH_3)_3I$



XXV

At the time this research began the long and cumbersome Barbier-Wieland procedure (4) was the only method available for the degradation of the bile acid side chain. In an attempt to find a better method 3(α),12(α)-diacetoxy-23-aminonorcholane (IV) was prepared from 3(α),12(α)-diacetoxycholanic acid (V) *via* the Curtius reaction. The amine was methylated and quaternized to give 3(α)-hydroxy-12(α)-acetoxynorcholane 23-trimethylammonium iodide (VI), which upon Hofmann degradation and acetylation of the product yielded 3(α),12(α)-diacetoxy-Δ^{22}-norcholene (XIV). Oxidation of this compound with chromium trioxide produced the bisnorcholanic acid XXII, which was identified as the methyl ester. Therefore, this series of reactions together with that described above provide a new approach to the side chain degradation. It cannot compare in yield, however, with the "N-bromosuccinimide method" of Meystre, Frey, Wettstein, and Miescher (5).

In addition to the norcholene XIV another product was isolated from the reaction mixture after the Hofmann degradation of the quaternary salt VI. Analytical data showed that this second compound had an additional double bond due to the loss of one of the hydroxyl groups during the reaction and consequently it must be either 3(α)-acetoxy-$\Delta^{11,22}$-norcholadiene or 12(α)-acetoxy-$\Delta^{2,22}$-norcholadiene (XVII).[3] To prove its structure both double bonds were catalytically hydrogenated and the completely reduced product was shown to be identical with 12(α)-acetoxynorcholane (XIX) and different from 3(α)-acetoxynorcholane (XXI). Therefore the second Hofmann degradation product was the diene XVII, the hydroxyl group at C-3 having been eliminated. The quaternary salt XII reacted in a similar manner yielding a small amount of the norcholadiene XVIII in addition to the main reaction product XVI. We encountered this side reaction only in the degradation of the 23-quaternary salts where the higher temperature necessary for their cleavage was responsible for the formation of the by-products.

EXPERIMENTAL[4]

INTRODUCTION OF THE Δ^{11}-DOUBLE BOND

Ethyl 3(α)-hydroxycholanate 12-trimethylammonium iodide (III). Ten grams of 3(α)-hydroxy-12-ketocholanic acid was refluxed for three hours in 80% aqueous alcohol with 1.1 moles of hydroxylamine hydrochloride and sodium acetate. On cooling and diluting with water the oxime crystallized. After recrystallization from methanol 10.1 g. (97%) of 3(α)-hydroxy-12-oximinocholanic acid (II) was obtained, m.p. 201–203°.[2]

Anal. Calc'd for $C_{24}H_{39}NO_4$: C, 71.07; H, 9.69; N, 3.45.
Found: C, 70.84; H, 9.69; N, 3.40.

The oxime II (7.0 g.) was reduced with 9.1 g. of sodium in 100 cc. of boiling isoamyl alcohol and yielded 3.0 g. (44%) of 3(α)-hydroxy-12-aminocholanic acid (I), m.p. 227–228°.[2] A 6.5-g. sample of the acid was esterified by refluxing it for three hours with 100 cc. of absolute ethanol containing 3 cc. of concentrated sulfuric acid. The alcoholic solution

[3] The second double bond has been placed in the Δ^2-position by analogy to the dehydration products of the bile acids, *cf.* Wieland, Kraus, Keller, and Ottawa, *Z. physiol. chem.*, **241**, 47 (1936).

[4] The microanalyses were carried out by Mr. Joseph Alicino, Metuchen, N. J. and Mr. George Stragand, Microchemical Laboratory, University of Pittsburgh, Pittsburgh, Pa. All melting points are corrected.

was concentrated, diluted with water, made alkaline with 10% sodium carbonate solution and the amino ester was extracted with ether. The extract was washed, dried, and the solvent removed leaving 6.7 g. of crude oily ethyl 3(α)-hydroxy-12-aminocholanate. This was not further purified but was used directly for the next step.

The quaternary salt was prepared by dissolving the above amino ester in 250 cc. of absolute ethanol and boiling the solution under reflux for forty-eight hours with the gradual addition of 50 cc. of methyl iodide and 50 g. of anhydrous potassium carbonate according to the directions of Woodward and Doering (6). At the end of this time the inorganic salts were filtered and the alcoholic solution concentrated to dryness *in vacuo*. The residue was taken up in chloroform, filtered, and the solvent was removed. The material remaining was dissolved in acetone and hexane was added. On standing, 1.5 g. of product crystallized, m.p. 158–160°; after solidification it remelted at 290°. The mother liquor fraction was re-methylated and quaternized and yielded 500 mg. of additional material raising the final yield of the quaternary salt III to 2.0 g. (22%).

Anal. Calc'd for $C_{29}H_{52}INO_3$: C, 59.21; H, 8.89; N, 2.38; I, 21.53.
Found: C, 59.20; H, 9.20; N, 2.30; I, 21.46.

Methyl 3(α)-acetoxy-Δ¹¹-cholenate (XIII). Two grams of quaternary salt III was subjected to the Hofmann degradation using essentially the same method as described by Woodward and Doering (6). The material was mixed with 3 cc. of water and 3 cc. of a solution of 5.0 g. of sodium hydroxide in 4 cc. of water was added. This strongly alkaline mixture was heated slowly to 160° in a Woods' metal bath. At that temperature trimethylamine could be detected and heating was continued for an additional half hour. Then the mixture was cooled, dissolved in water and the solution was acidified with concentrated hydrochloric acid. The steroid was extracted with ether and the extract was washed with water and then dried. On evaporation of the solvent 980 mg. of brown oil was obtained. This was esterified with diazomethane and acetylated with acetic anhydride in acetic acid solution using perchloric acid as a catalyst (7). The resulting 900 mg. of oily material was chromatographed in 50% benzene–hexane on 27 g. of acid-washed alumina. The fraction eluted with benzene yielded, after recrystallization from methanol, 200 mg. (35%) of crystalline product, m.p. 117–118° (reported 115–116°) (8).

INTRODUCTION OF THE Δ²⁰-DOUBLE BOND

3(α)-Hydroxy-12(α)-acetoxypregnane 20-trimethylammonium iodide (XXCIV). Twelve grams of 3(α),12(α)-diacetoxybisnorcholanic acid (XXII) was treated with 30 cc. of thionyl chloride at room temperature and the resulting acid chloride was reacted with 5 g. of sodium azide in dilute acetone solution as described by Sarett (9). After the steroid azide had been decomposed with dilute acetic acid the solution was made alkaline and the amine was extracted with ether. The ether solution was washed, dried and on treatment with hydrogen chloride gas 10.5 g. (97%) of amine (XXIII) hydrochloride precipitated.

Six grams of this hydrochloride was methylated and quaternized as previously described yielding 6.1 g. (83%) of crude quaternary salt. A 2.0-g. sample of this material was dissolved in water and the solution made alkaline to *p*H 10–11 with a 10% sodium hydroxide solution. Any incompletely methylated material was removed by extraction with ether. The remaining alkaline aqueous solution was neutralized with 10% hydrochloric acid and concentrated to dryness *in vacuo*. The residue was taken up in chloroform and filtered from the inorganic salts. On removal of the solvent 1.9 g. of material remained which yielded after recrystallization from an acetone–hexane mixture 1.6 g. of pure quaternary salt XXIV, m.p. 238–240°; after solidification it remelted at 285–290°. The presence of a small amount of water in the solvent used for crystallization was necessary since otherwise an amorphous precipitate was formed. The analytical data showed that saponification of one of the acetyl groups, presumably that at C-3, had taken place during the methylation step due to the alkalinity of the potassium carbonate used in this reaction.

Anal. Calc'd for $C_{26}H_{46}INO_3 \cdot H_2O$: C, 55.20; H, 8.50; I, 22.50.
Found: C, 55.23; H, 8.40; I, 22.80.

3(α),12(α)-Diacetoxy-Δ²⁰-pregnene (XXV) (3).[2] A suspension of 6.0 g. of the crude

quaternary salt XXIV in 10 cc. of 50% sodium hydroxide solution was subjected to the Hofmann degradation as previously described. Heating to 180° for a half hour was sufficient to complete the liberation of trimethylamine. After the mixture had cooled water was added and the steroid was extracted with ether. The extract was washed, dried and the solvent removed leaving 1.8 g. of oily material. This was acetylated and the resulting diacetate was recrystallized from methanol, m.p. 177–178°; yield 1.5 g. (35%).

Anal. Calc'd for $C_{25}H_{38}O_4$: C, 74.59, H, 9.51.
Found: C, 74.90; H, 9.25.

Methyl 3(α),12(α)-diacetoxyetiocholanate. A solution of 300 mg. of the pregnene XXV in 6 cc. of 90% glacial acetic acid was allowed to stand overnight at room temperature with 300 mg. of chromium trioxide. The reaction mixture was diluted with water, the excess chromium trioxide was destroyed with sodium bisulfite solution, the steroid was taken up in benzene, and the acid fraction was esterified with diazomethane and acetylated. The diacetate ester was chromatographed on acid-washed alumina yielding 170 mg. (52.5%) of material, m.p. 148–150° after recrystallization from methanol. A mixture with a known sample of methyl 3(α),12(α)-diacetoxyetiocholanate showed no m.p. depression.

INTRODUCTION OF THE Δ^{23}-DOUBLE BOND

3(α)-Hydroxy-12(α)-acetoxynorcholane 23-trimethylammonium iodide (VI) (10). Eighteen grams of 3(α),12(α)-diacetoxycholanic acid (V) was converted to the acid chloride. This was reacted with sodium azide and the azide subjected to the Curtius rearrangement as previously described yielding 14 g. (71.5%) of amorphous 3(α),12(α)-diacetoxy-23-aminonorcholane (IV) hydrochloride.[2]

Six grams of the hydrochloride was methylated and quaternized by the method already mentioned yielding 6.0 g. (82%) of crude material. This was purified and crystallized as described previously giving 4.8 g. of product, m.p. 227–228°.

Anal. Calc'd for $C_{28}H_{50}INO_3 \cdot H_2O$: C, 56.65; H, 8.83; I, 21.38.
Found: C, 56.83; H, 8.48; I, 21.36.

12(α)-Acetoxy-$\Delta^{2,22}$-norcholadiene (XVII) and 3(α),12(α)-diacetoxy-Δ^{22}-norcholene (XIV).[2] The Hofmann degradation was carried out with 13 g. of the crude quaternary salt VI. The material was divided into three portions and each was heated with 10 cc. of 50% sodium hydroxide solution to a temperature of 200–220°. This higher temperature was necessary to effect liberation of the trimethylamine in the case of the C-23-quaternary salts. When the decomposition was complete the batches were combined, diluted with water, and the steroid extracted with ether. After washing and drying the extract, the solvent was removed leaving 3.7 g. of oil. This was acetylated and chromatographed in hexane solution on 90 g. of acid-washed alumina. The fractions eluted with 10% and 20% benzene in hexane gave 650 mg. (8%) of crystals which melted at 143–145° after recrystallization from methanol and were identified as 12(α)-acetoxy-$\Delta^{2,22}$-norcholadiene (XVII).

Anal. Calc'd for $C_{25}H_{38}O_2$: C, 81.03; H, 10.33.
Found: C, 80.62; H, 10.20.

The fractions from the above chromatogram which were eluted with 5% to 25% ether in benzene yielded 1.3 g. (13.8%) of 3(α),12(α)-diacetoxy-Δ^{22}-norcholene (XIV), m.p. 133–134° after recrystallization from methanol.

Anal. Calc'd for $C_{27}H_{42}O_4$: C, 75.49; H, 9.76.
Found: C, 75.60; H, 9.82.

Methyl 3(α),12(α)-diacetoxybisnorcholanate. A 900-mg. sample of the norcholene XIV was oxidized in acetic acid solution with 900 mg. of chromium trioxide as described for the oxidation of the pregnene XXV. The product was similarly purified by esterification, acetylation, and chromatography yielding 470 mg. (49%) of material, m.p. 165–166° after recrystallization from ethanol. A mixture with a known sample of methyl 3(α),12(α)-diacetoxybisnorcholanate showed no m.p. depression.

12(α)-Acetoxynorcholane (XIX) from 12(α)-acetoxy-$\Delta^{2,22}$-norcholadiene (XVII). A suspension of 20 mg. of Adams' platinum oxide catalyst in 5 cc. of glacial acetic acid was pre-reduced and then 118 mg. of the diene XVII in 10 cc. of glacial acetic acid was added.

This mixture was hydrogenated at room temperature under slightly more than atmospheric pressure. About 2.3 moles of hydrogen were absorbed during one-half hour. The catalyst was filtered, the acetic acid removed *in vacuo* and the residue taken up in ether. The ether solution was washed with water and dilute sodium carbonate solution, dried and the solvent evaporated. The residue after recrystallization from methanol yielded 115 mg. (83.5%) of material, m.p. 83–84°.

Anal. Calc'd for $C_{25}H_{42}O_2$: C, 80.15; H, 11.30.
Found:[5] C, 79.74; H, 11.59.

12(α)-Acetoxy-23-aminonorcholane hydrochloride (VII). A 6.5-g. sample of 12(α)-acetoxycholanic acid (VIII) was converted to the acid chloride with thionyl chloride. This was reacted with sodium azide and the steroid azide decomposed with dilute acetic acid, yielding 4.5 g. of crude amine. An ether solution of the amine was treated with hydrogen chloride and 4.5 g. (68%) of amine hydrochloride was precipitated. It was recrystallized from methanol–ether and melted at 272–273°.

Anal. Calc'd for $C_{25}H_{44}ClNO_2$: C, 70.47; H, 10.17; Cl, 8.32.
Found: C, 70.24; H, 10.10; Cl, 8.56.

12(α)-Acetoxy-Δ^{22}-norcholene (XV). Three and one-half grams of the amine hydrochloride VII was methylated and quaternized, yielding 2.5 g. (52.5%) of tan crystalline quaternary salt IX, m.p. 232–240° (dec.). Two grams of this material was heated with 5 cc. of 50% sodium hydroxide solution to 220–230° for one-half hour. The reaction mixture was worked up as previously described and yielded 580 mg. of oily material. This was acetylated and chromatographed in hexane solution on 18 g. of acid-washed alumina. The fraction eluted with 25% benzene in hexane gave, after recrystallization from ethanol, 480 mg. (37%) of compound XV, m.p. 98–99°.

Anal. Calc'd for $C_{25}H_{40}O_2$: C, 80.59; H, 10.82.
Found: C, 81.03; H, 10.81.

12(α)-Acetoxynorcholane (XIX) from 12(α)-acetoxy-Δ^{22}-norcholene (XV). A solution of 120 mg. of the norcholene XV in acetic acid was hydrogenated with Adams' platinum oxide catalyst. About 1.2 moles of hydrogen were taken up and 115 mg. (96%) of product, m.p. 82–83°, was obtained. A mixture of this material with that obtained by the hydrogenation of the diene XVII showed no m.p. depression.

Anal. Calc'd for $C_{25}H_{42}O_2$: C, 80.15; H, 11.30.
Found: C, 79.97; H, 11.54.

3(α)-Acetoxy-23-aminonorcholane (X). When 4.0 g. of 3(α)-acetoxycholanic acid (XI) was converted to the acid chloride, then to the azide, and finally subjected to the Curtius rearrangement as previously described, a yield of 1.2 g. (31%) of the amine X was obtained. After recrystallization from methanol it melted at 121–122°. A somewhat better yield (40%) resulted when the modification of the Curtius reaction described by Hofmann and Bridgwater (11) was used.

Anal. Calc'd for $C_{25}H_{43}NO_2$: C, 77.06; H, 11.12; N, 3.59.
Found: C, 76.95; H, 11.15; N, 3.97.

3(α)-Acetoxynorcholane 23-trimethylammonium iodide (XII). Four grams of the amine X was methylated (3) by refluxing for four hours with 5 cc. of 90% formic acid and 3 cc. of 35% aqueous formaldehyde and the crude dimethylamine obtained by this reaction was quaternized (3) with methyl iodide in benzene solution. After recrystallization from acetone 2.4 g. (42%) of product, XII, m.p. 270–273° (dec.) was thus obtained.

Anal. Calc'd for $C_{28}H_{50}INO_2$: C, 60.09; H, 9.01; N, 2.50; I, 22.68.
Found: C, 60.80; H, 9.60; N, 3.00; I, 21.44.

These analytical results show that the material was impure but they are sufficient to serve as an indication of the compound's identity.

Δ^{2,22}-Norcholadiene (XVIII) and 3(α)-acetoxy-Δ^{22}-norcholene (XVI). A 1.25-g. sample of the salt XII was treated with alkali as already described for the Hofmann degradation,

[5] Some of these low-melting compounds were difficult to burn so that repeated analyses were necessary to obtain check values.

heating to 230° being necessary to eliminate trimethylamine. About 350 mg. of oil was obtained and after acetylation it was chromatographed in hexane solution on 12 g. of acid-washed alumina. The fraction eluted with hexane gave 50 mg. (7.2%) of crystalline diene XVIII, m.p. 100–101.5°, after recrystallization from methanol.

Anal. Calc'd for $C_{23}H_{36}$: C, 88.39; H, 11.61.
Found: C, 88.45; H, 11.91.

The fractions of the above chromatogram which were eluted with 10% and 25% benzene in hexane yielded 60 mg. (7.5%) of the norcholene XVI, m.p. 94–95°, after recrystallization from methanol.

Anal. Calc'd for $C_{25}H_{40}O_2$: C, 80.59; H, 10.82.
Found: C, 80.52; H, 10.76.

Norcholane (XX). Twenty-eight milligrams of the diene XVIII was hydrogenated as previously described and 2.2 moles of hydrogen were absorbed. After recrystallization from methanol 20 mg. (71.5%) of material was obtained, m.p. 105–106° [reported 101–103° (12)].

Anal. Calc'd for $C_{23}H_{40}$: C, 87.26; H, 12.74.
Found: C, 87.56; H, 12.60.

3(α)-Acetoxynorcholane (XXI). Twenty-five milligrams of the norcholene XVI was similarly reduced taking up 1.1 moles of hydrogen. After recrystallization from methanol, 20 mg. (80%) of product was obtained, m.p. 82–83°. A mixture with the norcholane XIX, m.p. 82–83°, obtained by hydrogenating the diene XVII melted at 54–65°.

Anal. Calc'd for $C_{25}H_{42}O_2$: C, 80.15; H, 11.30.
Found: C, 79.87; H, 10.95.

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SUMMARY

1. The Hofmann degradation has been shown to provide a useful method for the introduction of double bonds into the steroid molecule.

2. It has been found that when the 3-hydroxy- and 3-acetoxy-23-quaternary salts of steroids are subjected to the Hofmann degradation, a small amount of material undergoes an additional reaction in which the substituent at C-3 is eliminated with the formation of a $\Delta^{2,22}$-diene.

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