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3β-Acetoxy-20-hydroxy-5-cholenic Acid Lactone, a By-Product of the Oxidation of Cholesterol

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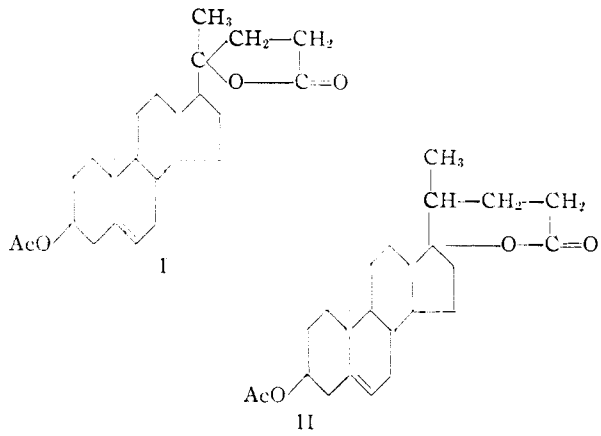
The lactone first isolated by Miescher and Fischer[1] from the products of the oxidation of cholesterol acetate dibromide and given the structure of 3β-acetoxy-17-hydroxy-5-cholenic acid lactone by Billeter and Miescher[3] is shown to be 3β-acetoxy-20-hydroxy-5-cholenic acid lactone. The configuration of the oxygen atom at C_{20} is discussed.

Introduction

Miescher and Fischer[1] isolated a lactone from the neutral products of the oxidation of cholesterol acetate dibromide to which Billeter and Miescher[2] assigned the structure of the 20-hydroxy lactone (I). The location of the oxygen atom at C_{20} was deduced from the following facts: When the allolactone acetate (IV) was opened with phenylmagnesium bromide, a triol was obtained, the 3-acetate of which was readily dehydrated to a $\Delta^{20-22,23}$-diene. The latter, on oxidation with chromic acid, gave 3β-acetoxyallopregnanolone. In a later paper, Billeter and Miescher[3] presented new evidence which indicated that the oxygen atom was attached at C_{17} (II). The allo derivative of Miescher's lactone was treated with methylmagnesium iodide to give a triol whose 3-acetate was partially dehydrated by refluxing with glacial acetic acid. The dehydrated product, which was not isolated, was hydrogenated and the product assigned the structure of 3β-acetoxy-17-hydroxy-24,24-dimethylallocholane in the following way. Billeter and Miescher[3] prepared the two C_{20}-epimers of 3β-acetoxy-20-hydroxy-24,24-dimethylallocholane from 3β-acetoxy-etioallocholanyl chloride. Since neither of the synthesized 20-hydroxy epimers corresponded in properties to the hydroxy compound prepared from the allolactone acetate, Billeter and Miescher[3] concluded that the point of attachment of the lactone ring must be at C_{17}. They stated further that the assignment of a 20-hydroxy lactone structure in their first paper[2] could be explained by the shifting of a 17-20 double bond first formed to a 20-22 double bond to form a conjugated system.

Veer and Goldschmidt[4] also isolated the lactone of Miescher and Fischer but erroneously assumed it to be 3β,17-dihydroxy-5-norcholenic acid lactone. The lactone ring was considered to be closed at C_{17} because oxidation of the 5,6-dibromide of the opened lactone (with phenylmagnesium bromide) produced dehydroepiandrosterone acetate (although only in a yield of less than 1%).

In 1949, it was suggested by Dr. Tarpley of these laboratories that the size of the lactone ring could be established through infrared spectroscopy. It is known from the work of Rasmussen[5] that saturated 6-membered lactone rings have carbonyl absorption bands in the 5.75 micron region, while those of 5-membered lactones occur near 5.65 microns. The carbonyl absorption band of the lactone of Miescher and Fischer used in the present study as well as that of its acetate is found at 5.62 microns. This evidence therefore strongly suggested the formulation of the cholene lactone as a γ-lactone closed at C_{20}.[6] Further support of this was obtained more recently through comparison with the infrared spectrum of a known steroidal γ-lactone (3β-acetoxy-17α-hydroxynor-5-cholenic acid lactone)[7] which also possesses the strong band at 5.62 microns typical of γ-lactones.



The molecular rotational changes obtained by opening the lactone rings with Grignard reagents or alkali showed large differences between the norcholene and cholene lactones. These differences, averaging 150 molecular rotational units, are far greater than would be expected if the difference between the two lactones were only one carbon in the length of the side chain. Barton and Klyne[8] give the molecular rotations for cholanic acid and norcholanic acid as 74 and 78, respectively. These large differences in molecular rotations are shown in Table I.

To avoid any need for protection of the 5-6 double bond, our experimental work was done with the saturated allolactone. The allolactone (III) was readily opened by refluxing with alcoholic potassium hydroxide followed by careful acidification with acetic acid to give 3β,20-dihydroxyallocholanic acid (IX). In a similar manner, the unsaturated lactone (I) could be opened to give 3β,20-dihydroxy-5-cholenic acid. In an effort to follow the

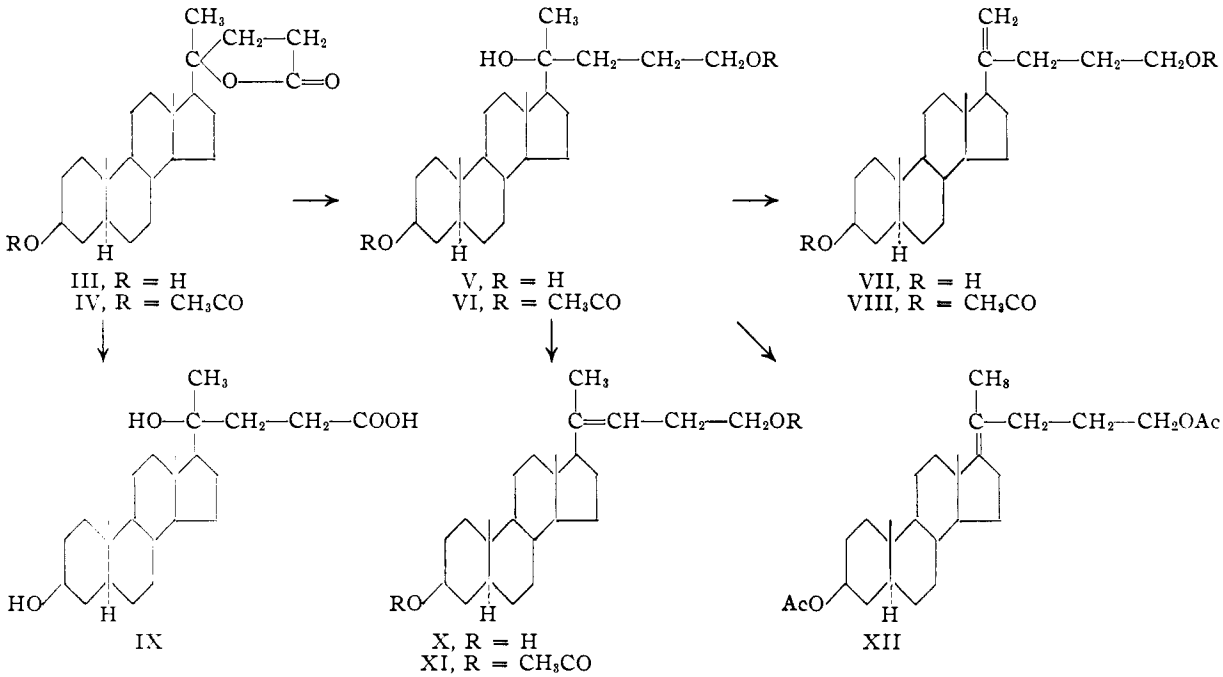
(1) K. Miescher and W. H. Fischer, *Helv. Chim. Acta*, **22**, 155 (1939).
(2) J. R. Billeter and K. Miescher, *ibid.*, **30**, 1409 (1947).
(3) J. R. Billeter and K. Miescher, *ibid.*, **32**, 564 (1949).
(4) W. L. C. Veer and St. Goldschmidt, *Rec. trav. chim.*, **66**, 75 (1947).
(5) R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949).
(6) This observation is in agreement with that of Jones, *et al.*, [R. N. Jones, P. Humphries and K. Dobriner, *ibid.*, **72**, 956 (1950)] whose Δ⁵-20-(spiro-2-oxa-3-oxocyclopentano)-pregnenol-3β-acetate was identified through infrared spectroscopy with that used in this investigation. Private communication from K. Dobriner.
(7) A. I. Ryer and W. H. Gebert, *ibid.*, **74**, 41 (1952).
(8) D. H. R. Barton and W. Klyne, *Chemistry and Industry*, 755 (1948).

TABLE I

Compound (cholane series)	$[\alpha]_D$	M_D-($CHCl_3$)[a]	ΔM_D[b]	Compound (norcholane series)	$[\alpha]_D$	M_D-($CHCl_3$)[a]	ΔM_D[b]
3β,20-dihydroxy-5-cholenic acid lactone	−25.5 $CHCl_3$	− 95		3β,17α-dihydroxynor-5-cholenic acid lactone	−94.4[c] $CHCl_3$	−339	
3β,20-dihydroxy-5-cholenic acid	−38.0 CH_3OH	−178	− 83	3β,17α-dihydroxynor-5-cholenic acid	−55.3[c] CH_3OH	−238	+101
3β,20,24-trihydroxy-24,24-dimethyl-5-cholene	−46[d] C_2H_5OH	−216	−121	3β,17α,23-trihydroxy-23,23-dimethylnor-5-cholene	−85.9[e] pyridine	−335	+4
3β,20,24-trihydroxy-24,24-diphenyl-5-cholene	−38[d] $CHCl_3$	−201	−106	3β,17α,23-trihydroxy-23,23-diphenylnor-5-cholene	−50.5[e] dioxane	−281	+58
3β-acetoxy-20,24-dihydroxy-24,24-diphenyl-5-cholene	−35[d] $CHCl_3$	−200	−105	3β-acetoxy-17α,23-dihydroxy-23,23-diphenylnor-5-cholene	−56.1[e] $CHCl_3$	−312	+27
3β,20-dihydroxyallocholanic acid lactone	+31.3[c] $CHCl_3$	+117		3β,17α-dihydroxyallonorcholanic acid lactone	−16.4[f] $CHCl_3$	−59	
3β,20-dihydroxyallocholanic acid	+ 8.8 CH_3OH	+5	−112	3β,17α-dihydroxyallonorcholanic acid	−1.6[f] CH_3OH	−36	+23

[a] Specific rotations determined in solvents other than $CHCl_3$ were converted to $M_D(CHCl_3)$ following the rules given by D. H. R. Barton and W. Klyne.[8] [b] ΔM_D is the difference between the M_D of the compound formed by opening the lactone ring and the M_D of the lactone itself. [c] A. I. Ryer and W. H. Gebert, unpublished data. [d] J. R. Billeter and K. Miescher, *Helv. Chim. Acta*, **30**, 1409 (1947). [e] A. I. Ryer and W. H. Gebert, THIS JOURNAL, **74**, 41 (1952). [f] A. I. Ryer and W. H. Gebert, *ibid.*, in press.

same line of attack that was used for the structural determination of the norcholenic acid lactone,[7] an attempt was made to esterify the acid (IX) so that dehydration of the 20-hydroxyl could be effected. The silver salt of IX which was easily made by the method of Allen and Wilson,[9] when treated with methyl iodide gave only regenerated lactone (III).



The lactone acetate (IV) was reduced with lithium aluminum hydride to give the triol (V), followed by mild acetylation in pyridine to give the diacetate (VI). The diacetate when dehydrated with phosphorus oxychloride in pyridine produced a mixture of unsaturated products from which 3β,-24-diacetoxy-Δ^{20-22}-allocholene (XI) and 3β,24-diacetoxy-20-allocholene (VIII) were isolated. The diacetate of the Δ^{20-22} compound (XI) was ozonized to give allopregnanolone acetate. The Δ^{20} compound (VIII) when ozonized or oxidized by the method of Bricker and Roberts[10] gave formaldehyde. No 3β,24-diacetoxy-Δ^{17-20}-allocholene (XII) was isolated; however, its presence was revealed in the following manner. The dehydration residue, after removal of most of the two isomers (VIII) and (XI), was also ozonized; only a small additional amount of allopregnanolone acetate was obtained. Although no other product could be isolated, the infrared spectrum of the ozonized mixture after hydrolysis showed the presence of a 17 keto compound [derived presumably from (XII)].

Since dehydration of the triol diacetate (VI) produced 20–22, 20–21 and probably 17–20 unsaturation, further evidence is provided that the lactone ring is closed at C_{20} instead of at C_{17}. The triol diacetate (VI) could also be dehydrated with thionyl chloride, hot acetic acid or acetic anhydride. A sample of the triol diacetate (VI) was easily de-

(9) C. F. H. Allen and C. V. Wilson, *Org. Syntheses*, **26**, 52 (1946).

(10) C. E. Bricker and K. H. Roberts, *Anal. Chem.*, **21**, 1331 (1949).

hydrated at C_{20} with acetic acid using the same procedure that Billeter and Miescher[3] claimed caused dehydration of their dimethyl triol monoacetate at C_{24} only. It is probable that Billeter and Miescher[3] dehydrated the C_{20} instead of the C_{24} hydroxyl and that this product after hydrogenation gave a 24-hydroxydimethylallocholane. This would explain why the two C_{20}-isomers of 3β-acetoxy-20-hydroxy-24,24-dimethylallocholane synthesized by them did not correspond in properties to the compound prepared from the lactone.

The configuration of the oxygen atom at C_{20} is uncertain. Although Fisher–Hirschfelder models of the lactone of Miescher and Fischer can be assembled in which the side chain at C_{20} is either α or β according to the convention of Fieser and Fieser,[11] the C_{20} α-side chain model appears hindered since the C_{20}-methyl group crowds both C_{12} and C_{19}. In a previous communication,[7] we suggested that the side chain is β oriented. This was based on the observation that a Fisher–Hirschfelder model of a 17α-hydroxy-nor-5-cholenic acid lactone could not be constructed with an α-side chain at C_{20} because of the hindrance offered by the C_{13} angular methyl group. If the C_{20}-side chain is β,[12] and if the oxidation of cholenic acid at C_{20} which presumably formed the lactone of Miescher and Fischer proceeded without inversion, then the hydroxyl at C_{20} should possess the α-configuration.

Experimental[13]

3β,20-Dihydroxy-5-cholenic Acid.—A solution of 10 g. of the unsaturated hydroxy lactone (3-hydroxyl of I), m.p. 253–254°, $[\alpha]^{25}D$ −25.5° (2% in $CHCl_3$), in 450 ml. of 5% ethanolic potassium hydroxide and 50 ml. of water was refluxed for two hours, cooled to room temperature and 250 ml. of water added. Dilute acetic acid was added to the solution of the potassium salt until the *p*H was 6.4 and the precipitated free acid filtered, washed neutral and air-dried. The crude acid (10.0 g.) was recrystallized from methanol to give 7.3 g., $[\alpha]^{25}D$ −38.0° (1% in methanol). Heating the acid caused relactonization to take place. Thus, when the melting point capillary was inserted at 240°, the acid immediately melted and bubbled up the tube, resolidified and finally remelted at the melting point of the hydroxy lactone (253.5–254.5°).

Anal. Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81. Found: C, 73.48; H, 10.06.

3β,20-Dihydroxyallocholanic Acid (IX).—The lactone acetate (I) was hydrogenated and the allolactone acetate (IV) isolated following the procedure of Billeter and Miescher.[2] Twenty grams of the allolactone acetate, m.p. 247.2–249.0°; $[\alpha]^{22}D$ +22.6° (2% in $CHCl_3$), was saponified in a manner similar to the saponification of the unsaturated lactone (I) above to give 20.2 g. of crude acid. When the melting point capillary was inserted at 230°, the acid immediately melted and bubbled up the tube, resolidified and finally melted at the melting point of the hydroxy lactone (III) (244.0–244.4°). When titrated in aqueous methanol, the crude hydroxy acid gave a neutralization equivalent of 414.0 instead of the expected 392.5 due to partial relactonization, $[\alpha]^{24}D$ +8.8° (2% in methanol). The acid could not be purified further because recrystallization caused more relactonization.

3β,24-Diacetoxy-20-hydroxyallocholane (VI).—To a refluxing solution of 10 g. of lithium aluminum hydride in 300 ml. of dry, peroxide-free tetrahydrofuran was added 20 g. of the allolactone acetate (IV) dissolved in 700 ml. of warm tetrahydrofuran. The mixture, after refluxing for one hour, was cooled to 20° and 100 ml. of water added cautiously with cooling. Maintaining the temperature below 25°, the complex was decomposed by the addition of 500 ml. of 10% sulfuric acid solution and then poured into 5 l. of water. The precipitate was filtered, washed neutral with water and dried to give 25 g. of crude triol (V) which contained salts and probably some undecomposed complex. The crude triol was acetylated with 225 ml. of dry pyridine and 50 ml. of acetic anhydride by warming at 50–60° with occasional agitation until the sterol dissolved (six hours) and then the mixture was allowed to stand overnight. The inorganic salts which remained undissolved were filtered off and the filtrate poured into water. The product after filtration and drying (21.5 g.) was recrystallized twice from methanol to give 17.5 g. of needles melting at 174.5–175.0°; $[\alpha]^{21}D$ −10.1° (2% in $CHCl_3$).

Anal. Calcd. for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02; sapn. equiv. calcd. for a diacetate, 242. Found: C, 72.86; H, 10.10; sapn. equiv., 242.

3β,20,24-Trihydroxyallocholane (V).—The diacetate (VI) was saponified by refluxing with a solution of potassium carbonate in methanol containing 15% water. The product was recrystallized from methanol to give fine plates of the triol (V) melting at 234.4–235.4°. The optical rotation was not obtained because of its insolubility in common organic solvents.

Anal. Calcd. for $C_{24}H_{42}O_3$: C, 76.14; H, 11.18. Found: C, 76.58; H, 11.36.

3β,24-Diacetoxy-Δ^{20-22}-allocholene (XI).—The procedure used for the dehydration was a modification of that described by Koechlin and Reichstein.[14] The triol diacetate (VI) (22.5 g.) was refluxed for two hours with 670 ml. of dry pyridine and 22.5 ml. of freshly distilled phosphorus oxychloride. The mixture was cooled to 40°, poured into ice water and stirred at 0° until the oily crystals hardened. The crystals were filtered, washed thoroughly with ice-water and air-dried on the filter. The crude product was dissolved in 500 ml. of hot methanol, treated with activated carbon and Adsorptive Magnesia Powder, No. 2642[15] and filtered through a mat of diatomaceous earth. The volume of the filtrate was adjusted to 500 ml., and the solution was placed in a refrigerator for two days. The crystals, which appeared amorphous, were filtered to give 5.5 g., m.p. 89.8–98.0°. (The filtrate was held for the isolation of the other olefins.) The crude product was recrystallized several times from methanol and ethanol to give 3.8 g. of product melting at 103.8–105.4°; $[\alpha]^{27}D$ −4.3° (2% in $CHCl_3$). The Bricker and Roberts[10] test for terminal unsaturation was negative.

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.98. Found: C, 75.32; H, 10.16.

3β,24-Dihydroxy-Δ^{20-22}-allocholene (X).—A 1.0-g. sample of the crude diacetate (XI) melting at 87.5–96.0° was saponified by refluxing with a solution of potassium carbonate in methanol containing 15% water. Three recrystallizations from methanol gave 0.52 g. of fine needles melting at 196.0–197.6°; $[\alpha]^{27}D$ −4.6° (1% in pyridine).

Anal. Calcd. for $C_{24}H_{40}O_2$: C, 79.95; H, 11.18. Found: C, 80.08; H, 11.55.

Ozonization of 3β,24-Diacetoxy-Δ^{20-22}-allocholene (XI) to Give Allopregnanolone Acetate.—A solution of 1.00 g. of the 20–22 unsaturated diacetate (XI) in a mixture of 100 ml. of C.P. chloroform and 6 ml. of pyridine was cooled to 0° and ozonized. After the addition of 14 ml. of acetic acid, the mixture was heated to reflux on a steam-bath. The acetic acid and pyridine were removed by washing the chloroform solution successively with 5% aqueous sodium bicarbonate and 2% hydrochloric acid. The chloroform solution was washed neutral with water, dried over sodium sulfate and finally concentrated to dryness. The resulting oil was refluxed for two hours with 30 ml. of a 7% methanolic solution of semicarbazide acetate and the precipitate filtered and dried to give 0.52 g. of crude allopregnanolone acetate semicarbazone; m.p. 263–264° (with dec.).

(11) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948).

(12) This is in disagreement with the configuration proposed by P. Wieland and K. Miescher, *Helv. Chim. Acta*, **32**, 1922 (1949), and W. Klyne, *Chemistry and Industry*, 426 (1951).

(13) All melting points are corrected. Microanalysis and microrotations by Edwin Conner and staff of these laboratories.

(14) B. Koechlin and T. Reichstein, *Helv. Chim. Acta*, **27**, 549 (1944).

(15) Obtained from Westvaco Chlorine Products Corporation, Newark, California.

The semicarbazone was split with sulfuric acid in dioxane[16] and the free sterol reacetylated with acetic anhydride in pyridine to give, after two recrystallizations from methanol, 0.27 g. of allopregnanolone acetate; m.p. 145.5–147.2°; $[\alpha]^{25}D$ +75.5 (2% in $CHCl_3$). A mixture with a sample of authentic allopregnanolone acetate showed no depression in the melting point; the infrared spectrum was identical with that of a sample of authentic allopregnanolone acetate.

3β,24-Dihydroxy-20-allocholene (VII).—The methanol filtrate remaining after the removal of the crude 20–22 unsaturated diacetate (XI) from the dehydration mixture was concentrated to 280 ml. and placed in a refrigerator overnight. The platelike crystals were filtered to give 6.35 g. of crude diacetate; m.p. 52.8–54.8°. (The methanol filtrate was held for the experiment below.) Since purification of the crude diacetate was difficult, it was saponified with a solution of potassium carbonate in methanol and water to give 4.9 g. of crude diol (VII); m.p. 149–159°. The crude product was dissolved in 380 ml. of hot acetone, cooled slowly to 25° and the needle-like crystals filtered to give an additional yield (1.46 g.) of crude 20–22 unsaturated compound (X); m.p. 181–185°. The acetone filtrate was concentrated to 185 ml., cooled slowly to 25° and filtered to give 1.56 g. of long, fine needles of VII melting at 162.2–164.0°. After recrystallizing twice from acetone and twice from ethyl acetate, the product (1.12 g.) melted at 166.4–167.4°; $[\alpha]^{26}D$ −0.9° (1% in pyridine). The infrared spectrum of VII showed absorption at 11.20 microns which is typical of terminal unsaturation of the type $R_2C{=}CH_2$.[17]

Anal. Calcd. for $C_{24}H_{40}O_2$: C, 79.95; H, 11.18. Found: C, 80.30; H, 11.44.

3β,24-Diacetoxy-20-allocholene (VIII).—A solution of 1.42 g. of the 20-enediol (VII) in 10 ml. of dry pyridine and 4.0 ml. of acetic anhydride was warmed at 50° for one hour, allowed to stand overnight and poured into water. The precipitate was filtered, washed neutral with water and dried, yielding 1.90 g.; m.p. 66.8–68.0°. After recrystallizing from methanol, 1.46 g. of plates melting at 69.8–70.4° was obtained; $[\alpha]^{28}D$ −4.9 (2% in $CHCl_3$).

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.98. Found: C, 75.71; H, 10.12.

Ozonization of 3β,24-Diacetoxy-20-allocholene.—A sample (500 mg.) of the 20–21 unsaturated diacetate (VIII) was ozonized at 0° in a solution of 50 ml. of C.P. chloroform and 3 ml. of pyridine, the exit gases being bubbled through a trap containing 25 ml. of water. Acetic acid (7.0 ml.) was added, the mixture heated to reflux on a steam-bath and cooled. The solution was washed several times with small portions of water and the aqueous washes combined with the water from the trap. The combined water washes were found to contain 23.0 mg. of formaldehyde (theory, 33.7 mg.) when analyzed by the method of Bricker and Roberts,[10] and 14.8 mg. of formaldehyde when isolated as the dimethyldihydroresorcinol derivative (m.p. 189.4–190.5°).[18] The chloroform layer, after washing free of acetic acid and pyridine, was taken to dryness and treated with a methanolic solution of semicarbazide. No insoluble semicarbazones were isolated.

Evidence for the Formation of 3β,24-Diacetoxy-Δ^{17-20}-allocholene (XII).—The methanol mother liquor remaining after the removal of the isomers (VIII) and (XI) was concentrated to dryness and a 4.60-g. portion of the resulting oil ozonized. The oily product was refluxed for two hours with 125 ml. of a 7% solution of semicarbazide acetate to give, after filtration, 1.62 g. of crude allopregnanolone acetate semicarbazone, m.p. 261–262° (with dec.). The filtrate was concentrated to a low volume, poured into water and the sterols extracted with chloroform. The chloroform extracts were evaporated to dryness, split with sulfuric acid in dioxane[16] and then completely saponified with potassium carbonate in aqueous methanol. The oily product after drying in a vacuum oven at 100° over phosphorus pentoxide for several hours was examined for infrared absorption. Two carbonyl bands at 5.75 and 5.84 microns were found. The 5.84 micron absorption is characteristic of side chain carbonyl in steroids. The absence of strong absorption in the 8 micron region which would be found if acetate were present shows that the 5.75 micron carbonyl band is not that of an acetate but is assignable to carbonyl in a 5-membered ring.[19]

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(16) British Intelligence Objectives Sub-Committee, B.I.O.S. Final Report No. 449, Item No. 24, Kennedy, Coppock and White, German Medical Targets, p. 228 (1945).

(17) R. B. Barnes, R. C. Gore, R. W. Stafford and V. Z. Williams, *Anal. Chem.*, **20**, 402 (1948). It is known that the intensity of the C=C stretching bands in the 6.08 micron region of terminally unsaturated compounds such as 1-hexene is relatively strong whereas internal olefinic bonds as in 2-hexene are weak (Catalog of Infrared Spectrograms, A.P.I. Research Project 44, National Bureau of Standards, Washington, D. C.). Internal double bonds in steroids are also weak [R. N. Jones, P. Humphries, E. Packard and K. Dobriner, THIS JOURNAL, **72**, 86 (1950)]. An unusually intense absorption band at 6.08 microns was also found in VII which further substantiates the presence of terminal unsaturation.

(18) F. Wild, "Characterisation of Organic Compounds," Cambridge at The University Press, 1947, p. 135, reports m.p. 189°.

(19) R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, THIS JOURNAL, **70**, 2024 (1948); R. N. Jones, P. Humphries and K. Dobriner, *ibid.*, **71**, 241 (1949).