[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Methyl 3(α)-Acetoxy-11-ketocholanate

BY S. ARCHER, T. R. LEWIS, C. M. MARTINI AND MARY JACKMAN

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The Marker–Lawson acid, 3(α)-12(β)-dihydroxy-11-ketocholanic acid, was converted to the corresponding methyl ester 3(α)-formate and then oxidized to methyl 3(α)-formoxy 11,12-diketocholanate. When this diketone was treated with trimethylenedithiol the 3-hydroxy-12-thioketal was obtained. Acetylation followed by desulfuration with Raney nickel afforded the title compound in 31% over-all yield based on desoxycholic acid.

In 1938 Marker and Lawson[1] brominated 3(α)-acetoxy-12-ketocholanate and then hydrolyzed the resulting 11(α)-bromoketone to a ketol acid which contained an oxygen function at C-11. Since that time many workers including Gallagher,[2] Wintersteiner,[3] and Hershberg[4] and their associates have investigated methods for converting this substance, which has become known as the Marker–Lawson acid, to useful 11-oxygenated steroids.

Gallagher[2c] showed that the reaction between Marker and Lawson's bromoketone and alkali was more complicated than originally envisaged[1] in that all four possible Ring C ketols were formed. The predominant isomer (60%) was the 12(β)-hydroxy-11-keto acid (II), but a substantial amount (33%) of the isomeric 11(α)-hydroxy-12-keto acid (VII) was also present contaminated with trace amounts of the other isomers X and XI.

The dimethyl ester of 3(α)-succinoxy-12(β)-hydroxy-11-ketocholanic acid (III) was converted to the corresponding bromoketone (XIII) in 50–70% yield with the aid of phosphorus tribromide.[2d] Gallagher suggested that inversion of configuration had occurred at C-12, but direct comparison of a suitable derivative with methyl 3(α)-acetoxy-12(α)-bromo-11-ketocholanate (XIV)[5] was not made. Reductive removal of the bromine led to the desired 11-ketosteroid (XVI).

In a variant of the above procedure Hershberg[4] converted 3(α)-acetoxy-12(β)-hydroxy-11-keto-24,24-diphenylcholene to the corresponding bromoketone in only 47% crude yield. In view of Gallagher's success[2d] with this reaction in the bile acid series it seemed to us that the presence of the phenyl groups were in some way interfering with the normal course of the replacement. We found that when the ester VI was acetylated under normal conditions the desired monoacetate IV was obtained in poor yield. Probably the formation of the diacetate as a side-product was responsible. The equatorial conformation of the C-12 hydroxyl reduces the selectivity of the acetylation reaction. When the monoacetate IV was allowed to react with phosphorus tribromide there was obtained in poor yield a bromoketone XIV identical in all respects with Kendall's.[5] Thus Gallagher's[2d] original assignment of configuration for the corresponding succinate was confirmed.

It was found subsequently that selective acylation of the ester VI could be carried out in good yield in 90% formic acid. Conversion of this monoformate V to the corresponding bromoketone XV was never accomplished in yields greater than 48% despite much experimentation. In view of this result this line of approach was abandoned.

Recently Djerassi[6] reported in a preliminary note that the ester IV and 11-ketohecogenin were oxidized to the corresponding diketones with bismuth oxide.[7] Treatment of the sapogenin with ethylenedithiol yielded a thioketal which when desulfurated afforded an 11-oxygenated sapogenin. In our hands oxidation of the ester, V, with Rigby's reagent failed and other methods for effecting this transformation were investigated.

When the pure ketol ester V was oxidized with chromic oxide the required diketoformate XX was obtained in 81% yield. Hydrolysis afforded the known acid XXI which was converted to known methyl ester XXII.[3] Formylation of XXII furnished a formate XXIII isomeric with the original ester XX. The presence of a peak at 281 mμ was noted in the ultraviolet absorption spectrum of XXIII, a maximum manifested also by the precursors XXI and XXII. Strong absorption at this wave length seems to be characteristic of such enols.[3] The infrared spectrum of XXIII revealed the presence of three carbonyl groups (bands at 5.76, 5.81 and 5.86 μ), one hydroxyl group and one double bond. Thus all the functionality in the molecule was accounted for.

It was noted that the melting point of the ester XX was always unsharp whether purification was effected by recrystallization or leaching. Our best sample melting at 208–211° was obtained by repeated leaching of crude material with methanol. Recrystallization usually resulted in lower melting points. This difficulty in obtaining a sharp melting product was noted also by Wintersteiner[3] in the case of the corresponding hemisuccinate methyl ester. Part of our trouble resided in the contamination of XX with small amounts of by-products, probably XXI and XII. We believe that the major cause of the wide melting range is the fact that a rapid thermal conversion of the diketone XX to the enol form XXIII occurs just below the melting point. A sample of XX, the absorption spectrum of which showed practically no maximum

(1) R. E. Marker and E. J. Lawson, THIS JOURNAL, **60**, 1334 (1938).

(2) (a) T. F. Gallagher and W. P. Long, *J. Biol. Chem.*, **162**, 521 (1946); (b) T. F. Gallagher and V. P. Hollander, *ibid.*, **162**, 533 (1946); (c) T. F. Gallagher, *ibid.*, **162**, 539 (1946); (d) E. Borgstrom and T. F. Gallagher, *ibid.*, **177**, 951 (1949).

(3) (a) O. Wintersteiner, M. Moore and K. Reinhardt, *ibid.*, **162**, 707 (1946); (b) O. Wintersteiner and M. Moore, *ibid.*, **162**, 725 (1946).

(4) E. B. Hershberg, H. L. Herzog, S. B. Coan, L. Weber and M. Jevnik, THIS JOURNAL, **74**, 2586 (1952).

(5) R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie and E. C. Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

(6) C. Djerassi, H. J. Ringold and G. Rosenkranz, THIS JOURNAL, **73**, 5514 (1951).

(7) W. Rigby, *J. Chem. Soc.*, 793 (1951).

at 281 mμ, was just fused at 210°, cooled immediately and then the spectrum was determined. The extinction coefficient at 281 mμ indicated that about 35% of the enol was present. After five minutes at 210° the enol content rose to 80%.

In the hope that purification of the ester V and subsequent recycling of isomeric material could be avoided[4] we carried out oxidation studies on the mixture of methyl ester formates prepared from the mixture of acids II, VII, X and XI obtained by the hydrolysis of I. The sirupy reaction product so obtained, after chromic oxide oxidation yielded crystalline ester XX in 55% over-all yield. This material was suitable for use in subsequent steps. When potassium chromate[8] was substituted for the chromic oxide the yield of ester rose to 64% but the product was of inferior quality.

It has been shown by Hershberg[9] that 11(α)-hydroxy steroids (hydroxyl equatorial) are not oxidized with N-bromosuccinimide whereas the epimeric alcohols (hydroxyl polar) furnish the corresponding ketones under similar conditions. Thus the relative ease of oxidation of the isomerides is the same as that noted with chromic oxide.[10] It seemed likely then that methyl 3(α)-formoxy-11(β)-hydroxy-12-ketocholanate (XII) would be converted to the diketoformate XX and the epimer VIII would be unattacked. Experimentally, the reverse was found to be true, so that we had at hand a case where the equatorial hydroxyl was more susceptible to oxidative attack than the corresponding polar hydroxyl.

This observation may be rationalized if it can be assumed that the mechanism of N-bromosuccinimide oxidation is similar to the chromic oxide reaction. It was shown by Westheimer[11] that in the latter case a rapid attack on the hydroxyl hydrogen precedes the rate-determining step of abstraction of a hydrogen from the hydroxyl-bearing carbon atom. Now in the case under discussion the difference in susceptibility to attack of the hydrogens at C-11 in the two epimers VIII and XII is diminished by the activating influence of the neighboring carbonyl function. However, due to steric effects the difference in ease of attack on the hydroxyl hydrogen still remains large so that the rate-determining step is the attack on the hydroxyl hydrogen rather than the removal of hydrogen from the carbon atom.[12]

The oxidative behavior of VIII should serve as a test of the above postulate. In this ester the hydroxyl group is equatorial and should be more susceptible to attack than the corresponding polar isomer XII. On the other hand it has been reported[13] that the 11(α)-hydroxyl group is less hindered than the corresponding 12(β)-hydroxyl. On the basis of these considerations one might expect that VIII would be oxidized by N-bromoacetamide even more readily than V. Experimentally it was found that the reaction did indeed occur but the yield of the required diketone XX was lower than in the case of V.

The required ester VIII was prepared by methylation and formylation of 3(α),11(α)-dihydroxy-12-ketocholanic acid. The latter was isolated from the mother liquors resulting from the purification of the Marker–Lawson acid. Upon methanolysis, VII furnished a crude dihydroxy ester which gave the known methyl 3(α),11(α)-diacetoxy-12-ketocholanate.

It is customary to prepare steroid thioketals from ethanedithiol despite the fact that in parallel experiments Hauptmann[14] has shown that better yields of thioketals were obtained with the next higher homolog.

When the diketoformate (XX) was treated with trimethylenedithiol in benzene solution with hydrogen chloride as the condensing agent the desired ester XXV was obtained in minor amounts, the major product being the deformylated product XXIV. Reformylation furnished the ester XXV. Complete solvolysis in the mercaptolization reaction was effected when methanol was used as the solvent. The required ester XXIV was obtained in excellent crude yield but the yield on subsequent acetylation was disappointingly low. Examination of the mother liquors obtained from the purification of the acetate XXVI revealed the presence of an oxygen-free substance, m.p. 83–84°. The analytical data were compatible with the formula $C_{11}H_{20}S_6$.[15] This substance was difficult to remove from the thioketol XXIV and was the principal contaminant responsible for the apparent high yield. In order to minimize the formation of the sulfur-rich *ortho* ester the methyl formate was swept out during the course of the reaction. Although the crude yield of steroid XXIV was reduced this was compensated for by the rise in yield of the acetate XXVI. The carboethoxyoxythioketal (XXVII) and the ethylenemercaptole XXVIII were prepared also. Neither seemed to offer any advantages over the acetate XXVI.

In the present work it was found that the Raney nickel desulfuration of XXVI was best carried out at room temperature for a period of several hours. In this way the conversion to pure methyl 3(α)-acetoxy-11-ketocholanate (XVII) was accomplished in 95% yield when pure mercaptole XXVI was used. The rapidity of the desulfuration reaction at room temperature was demonstrated by the fact that the yield of XVII was 86% when the reaction was run for only 15 minutes. When acetate XXVI of purity indicated in the Experimental part was used the yield of XVII was 90%. Using the latter figure as representative and 55% as the yield of diketoformate XX, then the over-all yield of the ester XVII from desoxycholic acid is 31%.

(8) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **72**, 5533 (1950).

(9) H. L. Herzog, M. A. Jevnik and E. B. Hershberg, *ibid.*, **75**, 269 (1953); E. P. Oliveto and E. B. Hershberg, *ibid.*, **75**, 488 (1953).

(10) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(11) F. H. Westheimer and N. Nicolaides, THIS JOURNAL, **71**, 25 (1949).

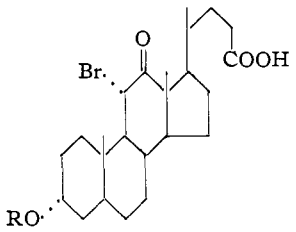
(12) We wish to thank Dr. W. S. Johnson for valuable suggestions concerning this point.

(13) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publ. Corp., New York, N. Y., 1949, p. 659.

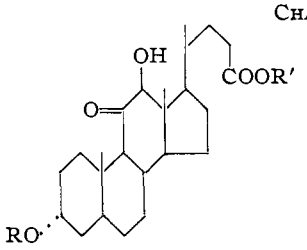
(14) H. Hauptmann and M. Moura Compos, THIS JOURNAL, **72**, 1405 (1950). Recently J. C. Sheehan, *et al.*, *ibid.*, **75**, 6231 (1953) reported a steroid trimethylene thioketal.

(15) It was shown by synthesis that this compound was the trithioorthoformate of trimethylenedithiol.

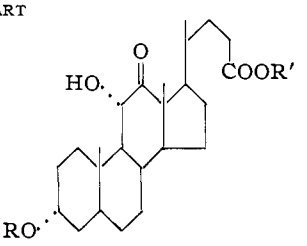
CHART



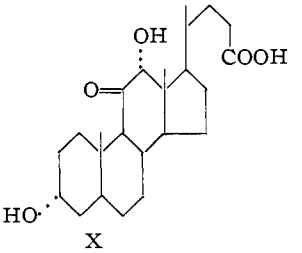
I. R = $HOOC(CH_2)_2CO$



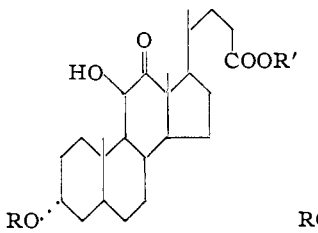
II, R = R′ = H
III, R = $CH_3OOCH_2CH_2CO$, R′ = CH_3
IV, R = CH_3CO, R′ = CH_3
V, R = CHO, R′ = CH_3
VI, R = H, R′ = CH_3



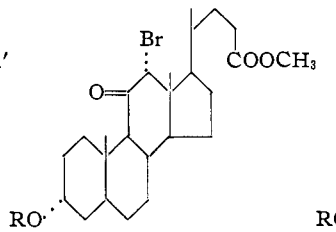
VII, R = R′ = H
VIII, R = CHO, R′ = CH_3
IX, R = H, R′ = CH_3



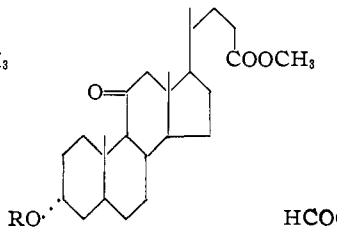
X



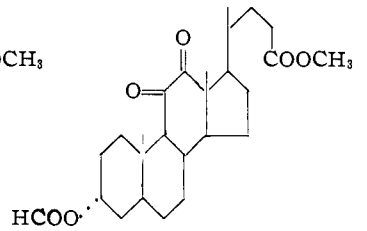
XI, R = R′ = H
XII, R = CHO, R′ = CH_3



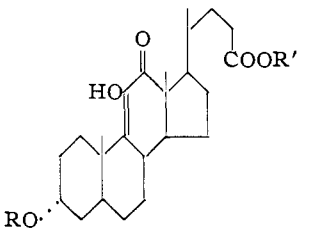
XIII, R = $CH_3OOCH_2CH_2CO$
XIV, R = CH_3CO
XV, R = CHO



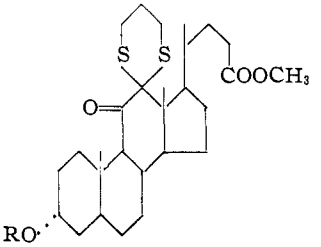
XVI, R = $CH_3OOCCH_2CH_2CO$
XVII, R = CH_3CO
XVIII, R = CHO
XIX, R = H



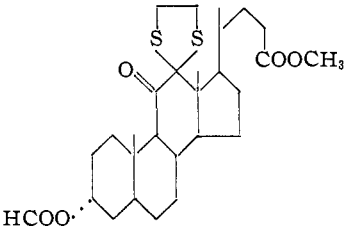
XX



XXI, R = R′ = H
XXII, R = H, R′ = CH_3
XXIII, R = CHO, R′ = CH_3



XXIV, R = H
XXV, R = CHO
XXVI, R = CH_3CO; XXVII, R = C_2H_5OCO



XXVIII

Infrared Studies.[16]—Since the carbonyl band for both the acetate and carbomethoxy functions is found at 5.76 μ and that for the formoxy group is located at 5.81 μ,[17] detection of the latter in the bile acid series is quite easy. No interference from carbonyl bands present in 11-keto, 12-keto or $\Delta^{9,11}$-12-keto steroids was noted. The only case of overlap was with esters such as XX, where carbonyl group interaction caused a shift to 5.81 μ. A change in the relative intensities of the bands at 5.75 and 5.81 μ was noted when the formate was replaced by acetate. The results are summarized in Table I.

TABLE I

WAVE LENGTHS OF CARBONYL ABSORPTION BANDS OF BILE ACID DERIVATIVES

Compound	Wave lengths, μ		
Methyl 3(α)-acetoxy-11,12-diketocholanate	5.76	5.81	
Methyl 3(α)-formoxy-11,12-diketocholanate (XX)	5.76	5.81	
Methyl 3(α)-hydroxy-12-ketocholanate	5.76		5.86
Methyl 3(α)-formoxy-12-ketocholanate	5.76	5.80	5.87
Methyl 3(α)-hydroxy-$\Delta^{9,11}$-12-ketocholanate	5.75		5.95
Methyl 3(α)-formoxy-$\Delta^{9,11}$-12-ketocholanate	5.75	5.81	5.95
Methyl 3(α)-acetoxy-11-ketocholanate(XVII)	5.77		5.87
Methyl 3(α)-formoxy-11-ketocholanate(XVIII)	5.77	5.81	5.87
Methyl 3(α)-acetoxy-12(β)-hydroxy-11-keto-cholanate (IV)	5.76		5.87
Methyl 3(α)-formoxy-12(β)-hydroxy-11-keto-cholanate (V)	5.76	5.80	5.87
Methyl 3(α)-acetoxy-11,12-diketocholanate-12-trimethylenethioketal (XXVI)	5.77		5.88
Methyl 3(α)-hydroxy-11,12-diketocholanate-12-trimethylenethioketal (XXIV)	5.77		5.88
Methyl 3(α)-formoxy-11,12-diketocholanate-12-trimethylenethioketal (XXV)	5.77	5.80	5.88

In addition to the band at 5.81 μ which was attributed to the formyl group there were three bands in the "fingerprint" region which were present in the sterol formates but absent in the corresponding alcohols and acetates. These occurred at 8.53–8.56 μ, 10.30–10.32 μ and 10.84–10.90 μ.

Experimental[18]

Methyl 3(α)-Acetoxy-12(β)-hydroxy-11-ketocholanate (IV).—A solution of 16.8 g. of methyl 3(α),12(β)-dihydroxy-11-ketocholanate in 100 ml. of dry pyridine containing 10 ml. of acetic anhydride was set aside at room temperature for several hours before being poured into ice-water. After 30 minutes the aqueous phase was decanted from the gum. The latter was dissolved in a small amount of methanol and the solution was chilled overnight. The product (6.8 g.)

(16) The infrared spectra were determined in carbon disulfide solution (usually at concentrations of 1% w./v.) using a Perkin–Elmer model 21 recording infrared spectrophotometer.

(17) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2820 (1952).

(18) Analyses were carried out under the supervision of Mr. K. D. Fleischer. Spectra and specific rotations were determined under Dr. F. C. Nachod's direction. All rotations were run as 1% solutions in chloroform unless otherwise specified. Melting points are corrected.

that had separated melted at 105–107°. After several crystallizations from methanol the desired monoacetate IV melted at 115–117°.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 70.37; H, 9.50.

Methyl 3(α)-Formoxy-12(β)-hydroxy-11-ketocholanate (V).—A solution of 34.5 g. of the dihydroxy methyl ester II in 105 ml. of 90% formic acid was heated to 65° and then allowed to stand for three hours. At the end of this time 7 ml. of water was added and the resulting turbid solution was clarified by warming to 45°. The mixture was cooled overnight and the monoformate that had separated was collected and dried; wt. 33.3 g. (91.5%), m.p. 119–122°. The analytical sample, obtained by recrystallization from methanol, melted at 128.5–129.5°; $[\alpha]^{25}D$ +72.0°.

Anal. Calcd. for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 69.30; H, 9.21.

Methyl 3(α)-Acetoxy-12(α)-bromo-11-ketocholanate (XIV).—The monoacetate IV (924 mg.) was dissolved in 15 ml. of dry methylene chloride. The chilled solution was treated with 0.19 ml. of phosphorus tribromide. The following day the mixture was poured into ice-water and the organic layer was separated and freed of acidic matter in the usual way. Removal of the solvent from the dried solution left a residue which weighed 210 mg. after recrystallization from methanol. The bromoketone thus prepared melted at 181–182°, $[\alpha]D$ +7° (*c* 1% acetone). Turner, *et al.*,[5] reported the following properties, m.p. 183.5–185°, $[\alpha]^{25}D$ +8° (1% acetone).

Methyl 3(α)-Formoxy-12(α)-bromo-11-ketocholanate (XV).—A chilled solution of 1.54 g. of methyl 3(α)-formoxy-12(β)-hydroxy-11-ketocholanate in 22 ml. of alcohol-free chloroform was treated with 0.45 ml. of phosphorus tribromide and then allowed to stand at room temperature for six hours. The mixture was processed as directly above. After the removal of the solvent there remained an oil which furnished 850 mg. (47%) of the crystalline bromoketone when leached with 5 ml. of methanol. Recrystallization from ethyl acetate–ligroin furnished pure material, m.p. 182.5–183.5°.

Anal. Calcd. for $C_{26}H_{39}BrO_5$: C, 61.05; H, 7.89. Found: C, 60.71; H, 7.65.

11(α)-Bromo-12-keto-3(α)-succinoxycholanic Acid (I).—This compound was prepared by a slight modification of the procedure described by Hershberg.[4]

Sixty six grams of 3(α)-succinoxy-12-ketocholanic acid (prepared in 91% over-all yield from desoxycholic acid) was dissolved in 920 ml. of acetic acid. The solution was kept at 65° while a solution of 22 g. of bromine in 66 ml. of acetic acid was added. After bromination was complete and the temperature of the reaction mixture had fallen to 50°, 200 ml. of water was added and the whole was left overnight. During this time the major portion of the product crystallized. The suspension was diluted with 3.0 liters of water and the crystalline solid which filtered rapidly was collected and dried; wt. 75.0 g. (98.3%). If the final dilution was performed before the crystallization was allowed to occur then filtration was often very slow.

Methyl 3(α)-Formoxy-11(α)-hydroxy-12-ketocholanate (VIII).—Seventy-eight grams of the above bromoketone was hydrolyzed according to the method of Hershberg[4] to furnish the crude mixed acids in quantitative yield. The dried solid mixture was boiled with 150 ml. of ethyl acetate, chilled and filtered. The insoluble fraction was crude Marker–Lawson acid which was reserved for further work.

The ethyl acetate solution was allowed to evaporate to about half volume. It was then chilled and the crystalline solid which had deposited was filtered and recrystallized from 75 ml. of ethyl acetate. In this way 8.56 g. of crude 3(α),11(α)-dihydroxy-12-ketocholanic acid (VII) was obtained. The acid was esterified with the aid of 90 ml. of dry methanol containing 10 ml. of 7 *N* methanolic hydrogen chloride. The whole was poured into water and the oil which separated was collected in chloroform. The solution was washed in the usual way and concentrated *in vacuo* to leave a sirupy residue. This was dissolved in 30 ml. of 90% formic acid and allowed to stand for two hours. Dilution of the solution caused the required ester VIII to separate; wt. 8.62 g., m.p. 143.5–147°. Crystallization from ethyl acetate furnished 6.53 g. of prisms, m.p. 149–151°. Further crystallization from the same solvent raised the m.p. to 151–152°, $[\alpha]^{25}D$ +91.2°.

Anal. Calcd. for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 70.03; H, 8.51.

Methyl 3(α),11(α)-Diacetoxy-12-ketocholanate from Methyl 3(α)-Formoxy-11(α)-hydroxy-12-ketocholanate.—A suspension of 500 mg. of the above ester in 10 ml. of 1 *N* methanolic hydrogen chloride was shaken for two hours and left overnight. The resulting solution was evaporated to dryness in a stream of nitrogen and the residue was then azeotropically distilled with benzene. The remaining colorless oil was dissolved in 5 ml. of acetic acid, treated with 3 ml. of acetic anhydride and then cooled to 10°. To the cold solution was added 0.5 ml. of 70% perchloric acid. After the solution had stood at room temperature for two hours it was poured into water and worked up in the usual way. The crude material thus obtained was purified by chromatography on alumina followed by crystallization from methanol. The fine mat of needles which separated was dried at 100°; m.p. 149–150°. (Gallagher[2b] reported m.p. 153–154°. Wintersteiner[3a] reported m.p. 150–151°.)

Methyl 3(α)-Formoxy-11(β)-hydroxy-12-ketocholanate (XII).—A suspension of 28.5 g. of 3(α)-succinoxy-11(α)-bromo-12-ketocholanic acid in 77 ml. of methanol was stirred and cooled to 5°. A solution of 150 ml. of 5 *N* potassium hydroxide was added dropwise and the stirring was continued for four hours more. After the reaction mixture had been allowed to stand at room temperature overnight it was kept in the ice-chest for 24 hours before the insoluble potassium salt was filtered and dried; wt. 26.5 g.

The crude salt was suspended in 300 ml. of methanol and then 28.5 ml. of 9 *N* methanolic hydrogen chloride was added dropwise with rapid stirring. The next day the excess acid was neutralized with solid sodium bicarbonate before removal of the inorganic matter by filtration. The filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in 66 ml. of 90% formic acid at room temperature. After two hours a crop of the required formate XII had separated; yield 52%, m.p. 170–173°. The analytical sample was obtained by recrystallization from acetone; m.p. 180.5–182°, $[\alpha]^{25}D$ +119.6°.

Anal. Calcd. for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 70.08; H, 9.13.

Oxidation Experiments[19]

Methyl 3(α)-Formoxy-11,12-diketocholanate (XX). A. **From "Mixed Acids" Using Chromic Oxide.**—Fifty-three grams of the bromosuccinoxy acid I was hydrolyzed as previously described and the resulting "mixed acids" were dissolved in 400 ml. of 1% methanolic hydrogen chloride. After 18 hours the acid was neutralized with 4.0 g. of sodium bicarbonate and the reaction mixture was evaporated to dryness. The residue was warmed to 65° with 125 ml. of 90% formic acid and set aside for three hours. The solution was diluted with water and then was extracted with chloroform. The organic layer was washed with dilute sodium bicarbonate solution and finally with water before being taken to dryness *in vacuo*. The residue was dissolved in 270 ml. of acetic acid and stirred at 24° while a solution of 7.5 g. of chromic oxide in 10 ml. of water was added dropwise. Stirring was continued for another hour during which time the product started to separate from solution. The suspension was diluted carefully with an equal volume of water. The crude diketoformate XX was collected, washed well with water and dried; wt. 34.0 g. The crude ester was recrystallized from dilute formic acid to furnish 22.83 g. of the diketone XX, m.p. 192–200° (over-all yield 55%). Our highest melting material was obtained by repeated leaching of the above product with boiling methanol. The specimen thus obtained melted at 208–211°, $\epsilon\lambda$ 281 = 400, $[\alpha]^{25}D$ +124°.

Anal. Calcd. for $C_{26}H_{38}O_6$: C, 69.92; H, 8.58. Found: C, 69.92; H, 8.69.

The methanol leachings furnished two substances. One of these, m.p. 140.5–145.5°, was probably impure methyl 3(α)-formoxy-11-hydroxy-12-keto-$\Delta^{9,11}$-cholenate (XXIII). The other, m.p. 172–174°, did not depress the m.p. of authentic methyl 3(α)-formoxy-11(β)-hydroxy-12-ketocholanate (XII).

B. **From Pure Methyl 3(α)-Formoxy-12(β)-hydroxy-11-ketocholanate. Using Chromic Oxide.**—Ten grams of the

(19) In the experiments described in this section the identity of the oxidation products was established by mixed melting points and comparison of the infrared spectra.

ester V was dissolved in 80 ml. of acetic acid and oxidized with the aid of a solution of 1.5 g. of chromic oxide in 10 ml. of water. After 90 minutes the suspension was diluted with water to precipitate 9.0 g. of the crude diketo ester. The dried product was boiled for five minutes with methanol. The cooled suspension was filtered. In this way there was obtained 8.1 g. of the purified material, m.p. 196–200°.

C. From Pure Methyl 3(α)-Formoxy-12(β)-hydroxy-11-ketocholanate (V). Using Potassium Chromate.—A solution of 4.49 g. of the methyl ester V in 175 ml. of acetic acid was warmed to 65° before being treated with a solution of 3.0 g. of potassium chromate in 8.0 ml. of water. After one hour the mixture was diluted with five volumes of water. The product was collected and purified as described above to furnish 3.27 g. (73%) of the ester XX, m.p. 199–205°.

When the oxidation was performed at room temperature for 16 hours the yield was reduced slightly and the product seemed to be of inferior quality.

D. From "Mixed Acids" Using Potassium Chromate.—A quantity of 19.2 g. of the "mixed acids" was esterified and formulated in the usual way to afford a sirup which was dissolved in 816 ml. of acetic acid and oxidized as directly above with 14 g. of potassium chromate in 37 ml. of water. The mixture was processed in the usual way. There was obtained 13.5 g. of the diketoformate, m.p. 193–201° (64% over-all).

E. From Methyl 3(α)-Formoxy-11(β)-hydroxy-12-ketocholanate (XII) Using Potassium Chromate.—One-hundredth mole of the ester XII, m.p. 170–173°, was oxidized as described above in C except that the reaction was allowed to proceed at room temperature for 18 hours. The yield was 3.68 g. (72%), m.p. 193–203° after leaching of the crude product with methanol. The yield at 65° was slightly higher (76%) but the quality was very poor.

F. From Methyl 3(α)-Formoxy-12(β)-hydroxy-11-ketocholanate (V) with N-Bromosuccinimide.—One gram of the ester was dissolved in 30 ml. of acetone containing 8 ml. of water. The solution was stirred while 1 g. of N-bromosuccinimide and 1 ml. of water was added. The mixture was allowed to stand overnight and then was diluted with water. The product was filtered and recrystallized from acetone to furnish 440 mg. of the diketoformate XX, m.p. 202–207°.

G. From Methyl 3(α)-Formoxy-11(α)-hydroxy-12-ketocholanate with N-Bromosuccinimide.—A solution of 500 mg. of the ester VIII in 25 ml. of acetone containing 4 ml. of water was oxidized with 500 mg. of N-bromosuccinimide as described immediately above. After the usual work-up there was obtained 150 mg. of a methanol-insoluble material which melted at 197–201° after recrystallization from acetone.

When the 11(β)-hydroxy epimer XII was substituted for VIII in the reaction no appreciable oxidation was noted. One gram of the ester, XII, m.p. 173.5–177.5°, furnished 590 mg. of a product melting at 173.8–177° after recrystallization from acetone. No depression of the melting point was noted when mixed with starting material. No other substance was found in the mother liquor.

Methyl 3(α)-Formoxy-11-hydroxy-12-keto-$\Delta^{9,11}$-cholenate.—Five grams of methyl (α)-formoxy-11,12-diketocholanate (XX) was suspended in a solution prepared from 50 ml. of methanol and 50 ml. 2 *N* sodium hydroxide. The suspension was warmed on the steam-bath for 15 minutes whereupon a clear solution resulted. On careful acidification of the mixture the crystalline 3(α),11-hydroxy-12-keto-$\Delta^{9,11}$-cholenic acid separated which melted at 168–169° after recrystallization from dilute methanol; wt. 3.48 g., $\epsilon_{\lambda 281} = 7{,}200$. (Wintersteiner[3b] reported m.p. 169–171°, $\epsilon_{\lambda 281} = 7{,}000$.)

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.30; H, 8.99. Found: C, 71.27; H, 9.13.

The acid was esterified in methanol with the aid of a catalytic amount of dry hydrogen chloride and the crude ester (2.4 g.) that was isolated in the usual way was dissolved in 7.5 ml. of 90% formic acid at 40°. The solution was warmed to 60°, cooled to room temperature and seeded. The crystalline solid which had separated overnight was collected and dried; wt. 2.00 g. After recrystallization from ethyl acetate it melted at 137.5–139.5°, $[\alpha]^{25}D$ +87.1°.

Anal. Calcd. for $C_{26}H_{38}O_6$: C, 69.92; H, 8.58. Found: C, 69.65; H, 8.05.

Mercaptolization Experiments

Methyl 3(α)-Hydroxy-11,12-diketocholanate-12-trimethylenethioketal (XXIV).—A slow stream of dry hydrogen chloride was passed through a cold solution of 1.0 g. of methanol-slurried diketoformate XX in 4 ml. of toluene and 3 ml. of trimethylene dithiol for two hours. The volatile material was removed *in vacuo* and the residual oil was leached repeatedly with ligroin until the insoluble material turned crystalline; wt. 250 mg., m.p. 154–160°. On cooling the extracts deposited 400 mg. of crystals, m.p. 161–163°. The two crops were combined and recrystallized from a large volume of ligroin. The compound, which proved to be the 3-hydroxythioketal XXIV, melted at 163–164° and separated from isopropyl alcohol as a solvate. It melted at 93–95°, resolidified and then melted at 159–160°. The solvate gave the following analytical figures.

Anal. Calcd. for $C_{28}H_{44}O_4S_2 \cdot 1C_3H_8O$: S, 11.28; loss at 100°, 10.6. Found: S, 11.30; loss at 100°, 11.2.

The solvent-free compound, $[\alpha]^{25}D$ −30.4°, was analyzed.

Anal. Calcd. for $C_{28}H_{44}O_4S_2$: C, 66.10; H, 8.72; S, 12.60. Found: C, 66.53; H, 8.61; S, 12.62.

When the above reaction mixture was subjected directly to chromatography on alumina and the column eluted with benzene–chloroform mixtures the early eluate fractions yielded crystalline material, m.p. 172–174°, after recrystallization first from methanol and then ligroin. This is the 3-formoxy derivative, $[\alpha]^{25}D$ −23.9°.

Anal. Calcd. for $C_{29}H_{44}O_5S_2$: C, 64.89; H, 8.26; S, 11.95. Found: C, 65.20; H, 8.19; S, 11.92.

This formate also was obtained by recrystallizing the 3-hydroxy ester XXIV from formic acid.

The preferred procedure for the preparation of XXIV is as follows: A suspension of 7.5 g. of methyl 3(α)-formoxy-11,12-diketocholanate (purified by a methanol slurry) in 35 ml. of 9 *N* methanolic hydrogen chloride was stirred and cooled. Dry hydrogen chloride was passed through the solution for one hour and after two hours the condenser was removed while dry nitrogen was bubbled through to sweep out methyl formate. The solution was resaturated with hydrogen chloride at 5° and then 3.7 ml. of trimethylenedithiol was added in one portion. The hydrogen chloride stream was maintained for an additional hour. The reaction mixture was allowed to stand for two hours while the ice-bath melted and the temperature rose to 20°.

The red solution was poured into ice-water. The mixture was extracted with ether and the ethereal solution was washed successively with water, dilute sodium bicarbonate and again with water before being concentrated to dryness. The residue was heated on the steam-bath at 0.5 mm. for two hours. During this time it solidified. The material was crystallized from isopropyl alcohol. The first crop, wt. 7.32 g., melted at 91–95°, resolidified and melted at 156–158°. The second crop which was slightly less pure amounted to 510 mg. The total yield of usable solvated thioketal was 82% of the theoretical.

When the reaction was run in the same way except that the sweeping with nitrogen was not done the yield of XXIV was 90–92% but the melting point was quite low. In one experiment in which 10 g. of the diketoformate was used a substance crystallized out soon after the dithiol was added. It was removed by filtration before the usual work-up. This oxygen-free contaminant was crystallized from methanol; wt. 1.86 g., m.p. 80–83°. After purification by chromatography on alumina and recrystallization from ethyl acetate it melted at 81–82°. (See below for further data on this triorthoformate ester.)

Methyl 3(α)-Acetoxy-11,12-diketocholanate-12-trimethylenethioketal (XXVI).—Seven grams of the bulked crops of XXIV obtained in 82% yield as described above was dissolved in benzene and concentrated to dryness to remove the isopropyl alcohol of crystallization. The residue was dissolved in pyridine and treated with 7 ml. of acetic anhydride. The next day the solution was poured into water and set aside for several hours. The crystals were collected and then recrystallized from aqueous acetone to furnish 5.90 g. of the ester, m.p. 216–218° (yield 88%). Further crystallization from acetone raised the m.p. to 221–222°; $[\alpha]^{25}D$ −22.4°.

Anal. Calcd. for $C_{30}H_{46}O_5S_2$: C, 65.41; H, 8.42; S, 11.63. Found: C, 65.72; H, 8.27; S, 11.86.

When the starting material was a sample of the thioketal XXIV prepared in 92% yield, the acetate XXVI was obtained in almost quantitative yield, m.p. 180–190°. Fractional crystallization from ethyl acetate furnished a pure

sample of the trithioörthoformate of trimethylenedithiol; m.p. 80–82°.

Anal. Calcd. for $C_{11}H_{20}S_6$: C, 38.4; H, 5.83; S, 55.8; mol. wt., 344. Found: C, 38.77; H, 5.66; S, 56.7; mol. wt., 342.

The yield of purified acetate, m.p. 221–222°, was 58% in this case but variations from 55 to 75% were noted depending on the amount of contaminant.

Methyl 3(α)-Formoxy-11,12-diketocholanate-12-ethylenethioketal (XXVIII).—A suspension of 2.0 g. of the diketocholanate XX in 9 ml. of methanol was cooled and saturated with dry hydrogen chloride. Then one ml. of ethylene dithiol was added and the hydrogen chloride stream was continued for 1.5 hours. Nitrogen was passed through the solution for one hour and the mixture worked up as above. The yellow oil that was left was formylated in 90% formic acid. Dilution with water threw down the product which was purified by crystallization from dilute acetone and then ligroin; m.p. 128.5–130.5°.

Anal. Calcd. for $C_{28}H_{42}O_5S$: S, 12.27. Found: S, 12.10.

Methyl 3(α)-Carboethoxyoxy-11,12-diketocholanate-12-trimethylenethioketal (XXVII).—Ten grams of the mercaptol XXIV was dissolved in pyridine and then treated with an equal weight of ethyl chlorocarbonate. The ester was isolated in the usual way and then purified by recrystallization from acetone; m.p. 161–163.5°, $[\alpha]^{25}D$ −8°.

Anal. Calcd. for $C_{31}H_{48}O_6S_2$: C, 64.10; H, 8.33. Found: C, 64.20; H, 8.24.

Desulfuration Experiments

Methyl 3(α)-Acetoxy-11-ketocholanate (XVII).—Two grams of the pure thioketal acetate XXVI was suspended in 100 ml. of methanol which contained 10 g. of wet Raney nickel catalyst. The mixture was shaken mechanically for six hours. After the catalyst was removed the filtrate was evaporated to dryness and the residue was recrystallized from dilute acetone to furnish 1.54 g. of the desired substance (yield, 95%); m.p. 131–133°. One further crystallization raised the m.p. to 133–134° $[\alpha]^{25}D$ +70° (Turner, *et al.*,[5] reported m.p. 134–134.5°, $[\alpha]D$ +68 ± 2°).

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.89; H, 9.40.

When the reaction was carried out in refluxing methanol for one hour the yield was 90% of recrystallized ester. When carried out as described above but for only 15 minutes the reaction mixture gave the desired 11-keto ester XVII, m.p. 131–132.4° in 86% yield.

The yield of once recrystallized methyl 3(α)-acetoxy-11-ketocholanate (m.p. 130°) from thioketal acetate, m.p. 216–218°, was 90%. Since the yield of mercaptol was 82% and the acetate XXVI was 88%, the over-all yield of the required ester XVII from methyl 3(α)-formoxy-11,12-diketocholanate was 65%.

Methyl 3(α)-Formoxy-11-ketocholanate (XVIII). A. From Methyl 3(α)-Acetoxy-11-ketocholanate.—A mixture of 1.08 g. of methyl 3(α)-acetoxy-11-ketocholanate, 10 ml. of methanol and 6 ml. of 5 *N* potassium hydroxide was refluxed for one-half hour. The methanol was removed *in vacuo* and the residue was dissolved in water and carefully acidified to *p*H 3. The crystalline acid so obtained weighed 810 mg. and melted at 222–224° after recrystallization from dilute acetone.

The acid was esterified in methanol containing hydrogen chloride. The crude methyl ester melted at 99–102° (Turner[5] reported m.p. 102–103°.) Formylation was accomplished in our usual fashion. The formate was purified by crystallization from acetone; m.p. 142–143.5°, $[\alpha]^{25}D$ + 73.4°.

Anal. Calcd. for $C_{26}H_{40}O_5$: C, 72.18; H, 9.32. Found: C, 71.92; H, 9.53.

B. From Methyl 3(α)-Hydroxy-11,12-diketocholanate-12-trimethylenethioketal (XXIV).—The solvated mercaptol XXIV (2.0 g.) was refluxed in 50 ml. of methanol in which there was suspended 5.0 g. of moist Raney nickel catalyst. After 4 hours the solvent was removed leaving a residue which solidified on cooling. The latter was warmed with 15 ml. of 90% formic acid for one-half hour. The diluted reaction mixture yielded an oil which solidified when triturated with methanol; wt. 1.25 g. After three recrystallizations from acetone the sulfur-free ester melted at 139.5–140.5° and did not depress the m.p. of the sample prepared as described above.

Methyl 3(α)-Carboethoxyoxy-11-ketocholanate.—Three grams of the crude ester XXVII and 10 g. of Raney nickel catalyst were boiled in methanol overnight. The mixture was worked up in the usual way to furnish 1.50 g. of the substance. After recrystallization from acetone and then methanol the desired compound melted at 146–147.5°.

Anal. Calcd. for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31. Found: C, 70.37; H, 9.11.

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[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

The Synthesis of Bis-quaternary Salts of Some 1,7-Phenanthroline Derivatives

BY ALEXANDER R. SURREY, ARTHUR J. OLIVET AND JAMES O. HOPPE

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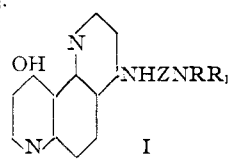
The reaction of 4-chloro-10-hydroxy-1,7-phenanthroline with a variety of primary–tertiary diamines is described. The resulting 4-substituted-amino-10-hydroxy-1,7-phenanthrolines were quaternized with methyl bromide or iodide to give bis-quaternary salts which have been examined for their neuromuscular blocking activity.

As part of a general investigation in these laboratories of potential neuromuscular blocking agents we have prepared a series of bis-quaternary salts (Table IV) of some 1,7-phenanthroline derivatives having the general formula I where Z is $(CH_2)_n$ or $(CH_2)_nO(CH_2)_3$.



I

These 4-substituted-amino-10-hydroxy-1,7-phenanthrolines (Table III) were prepared from 4-chloro-10-hydroxy-1,7-phenanthroline[1] by reaction with the appropriate primary-tertiary diamine in isopropyl alcohol in the presence of hydrogen chloride. On the basis of our previous work in which it was demonstrated that the 1-ring nitrogen is probably involved in hydrogen bonding with the 10-hydroxyl group, we have assumed that quaternization of these bases (I) occurs at the 7-position in the phenanthroline ring and at the terminal tertiary nitrogen in the side chain.

The primary–tertiary diamines employed in the present work were of two types $NH_2(CH_2)_nNR_1R_2$ and $NH_2(CH_2)_3O(CH_2)_nNR_1R_2$. The compounds of the first type have been reported previously. The first two members of this series, where n = 2 and 3, are commercially available. Where n = 4, the diamine 4-diethylaminobutylamine was pre-

(1) A. R. Surrey and R. A. Cutler, THIS JOURNAL, **76**, 1109 (1954).