Experiment 8.—A portion (2.8 g.) of the redistilled cyclohexadiene fraction (b. p. 81 to 85°) was analyzed. It consisted of 33% cyclohexene and 67% cyclohexadienes.

Dehydration of *cis*- and *trans*-1,4-Cyclohexanediol.—Pure *cis*-, *trans*- and an equimolar mixture of *cis*- and *trans*-1,4-cyclohexanediol, dissolved in methanol in molal ratio 1:5, was passed over 36 cc. of alumina at a temperature of 275°; the experimental data are given in Table III.

The polymer fractions were composed of oxygen-containing high-boiling condensation products.

TABLE III

DEHYDRATION OF *cis*- AND *trans*-1,4-CYCLOHEXANEDIOL

Experiment		Rate, cc./hr.	Duration, hours	1,4-Epoxycyclohexane	Cyclohexenol	Unreacted diol
trans-	9	36	2.75	73	11.4	0
cis-	10	38	2	28	27	16
Mixture	11	36	2.75	33.5	20.6	17.0

Dehydration of 1,4-Cyclohexanediol in the Absence of Solvent.—Twenty-one grams of 1,4-cyclohexanediols and 15 g. of activated alumina were placed in a round-bottom flask connected to a distilling column and heated to a reflux temperature at 241 to 243° for twenty-one hours. The reaction product, which was distilled off as formed, consisted of 39 mole per cent. epoxycyclohexane, 18% cyclohexenol, and 23% high-boiling products.

Properties of 1,4-Epoxycyclohexane.—The boiling point of the epoxide determined by the Cottrell[20,21] method was 120.1° at 760 mm., dt/dp (770–730 mm.) = 0.080°/mm., d^{20}_4 0.9707, dd/dt (20–30°) = 0.00092/°C.; $n^{20}D$ 1.4477; $M^{20}D$ 27.05 (Lorenz–Lorentz).

(20) Cottrell, THIS JOURNAL, **41**, 721 (1919).

(21) Bruun and Hicks-Brunn, *Bur. Standards J. Research*, **6**, 871 (1931).

Anal.[22] Calcd. for $C_6H_{10}O$: C, 73.47; H, 10.20. Found: C, 74.19; H, 10.14.

1,4-Epoxycyclohexane is soluble in the usual organic solvents, such as methanol, ethanol, acetone, ether and benzene, and is partially soluble in water, with which it forms an azeotropic mixture boiling at 90°. The chemical properties of the 1,4-epoxycyclohexane are those of an ether. It may be dissolved in 40 to 60% sulfuric acid, and recovered unchanged on dilution with water. It does not react with ethylmagnesium bromide nor with metallic sodium.

Hydrobromic Acid.—A mixture of 5 g. of the epoxide and 15 cc. of 48% hydrobromic acid was refluxed for six hours. A lower layer was formed, which crystallized on standing. The material was cooled and filtered, and the solid was recrystallized from hot alcohol. This product melted at 112–113°, corresponding to *trans*-1,4-dibromocyclohexane.

Anal. Calcd. for $C_6H_{10}Br_2$: C, 29.75; H, 4.13. Found: C, 29.67; H, 4.07.

Summary

The dehydration of 1,4-cyclohexanediol to 1,4-epoxycyclohexane was studied with reference to the effect of temperature, solvent, catalyst, and geometric configuration of the diol upon the yield of 1,4-epoxycyclohexane.

An improved method for the separation of the *cis*- and *trans*-1,4-cyclohexanediol was described, and a melting point curve of the mixture of the two isomers given.

(22) Analyses were made by Dr. T. S. Ma, University of Chicago.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Introduction of the 3-Keto-Δ^4-conjugated System in the Desoxycholic Acid Series

BY BYRON RIEGEL AND A. VERN MCINTOSH, JR.

The most physiologically active steroidal hormones from the adrenal cortex possess the same structure in ring A as found in testosterone and progesterone, namely, a 3-keto group and a carbon–carbon double bond at the 4,5-position. For the preparation of corticosterone and its derivatives from desoxycholic acid, the introduction of this conjugated system had to be studied.

In the preparation of methyl 3-keto-12-hydroxy-4-cholenate (XIII) and its *nor* (XIV), *bisnor* (XV), and *etio* (XVI) homologs from the corresponding methyl 3,12-dihydroxycholanates (I, II, III, IV), the 3-keto-12-hydroxy compounds (V, VI, VII, VIII) are necessary intermediates. The usual procedure for making these keto intermediates involves acetylation of both hydroxyl groups, then selective hydrolysis or alcoholysis of the 3-acetoxy group and oxidation of the resulting 3-hydroxyl compound to give the 3-keto derivative. These procedures are illustrated by the synthesis of methyl 3-keto-12-hydroxycholanate (V) by Yamasaki and Kyogoku[1] and methyl 3-keto-12-hydroxy-*etio*-cholanate (VIII) by Lardon and Reichstein.[2] Selective oxidation of the 3-hydroxyl group without protection of the 12-hydroxyl group would provide a simpler synthesis in this series. Gallagher[3] mentioned the preferential oxidation in the Oppenauer reaction of the 3-hydroxyl group in esters of cholic and desoxycholic acids. Ehrenstein and Stevens[4] found that the 3-keto derivative was obtained when 12-acetoxypregnan-3(α),7-diol-20-one was refluxed with cyclohexanone in the presence of aluminum isopropoxide. Recently Fuchs and Reichstein[5] reported the selective oxidation, by means of aluminum phenoxide in acetone, of the 3-hydroxyl group of 3,12-dihydroxy-21-acetoxypregnanone-20. Several examples have been reported where the 3-hydroxyl group has been selectively oxidized in compounds that also contain a 5,6-double bond. These are not considered

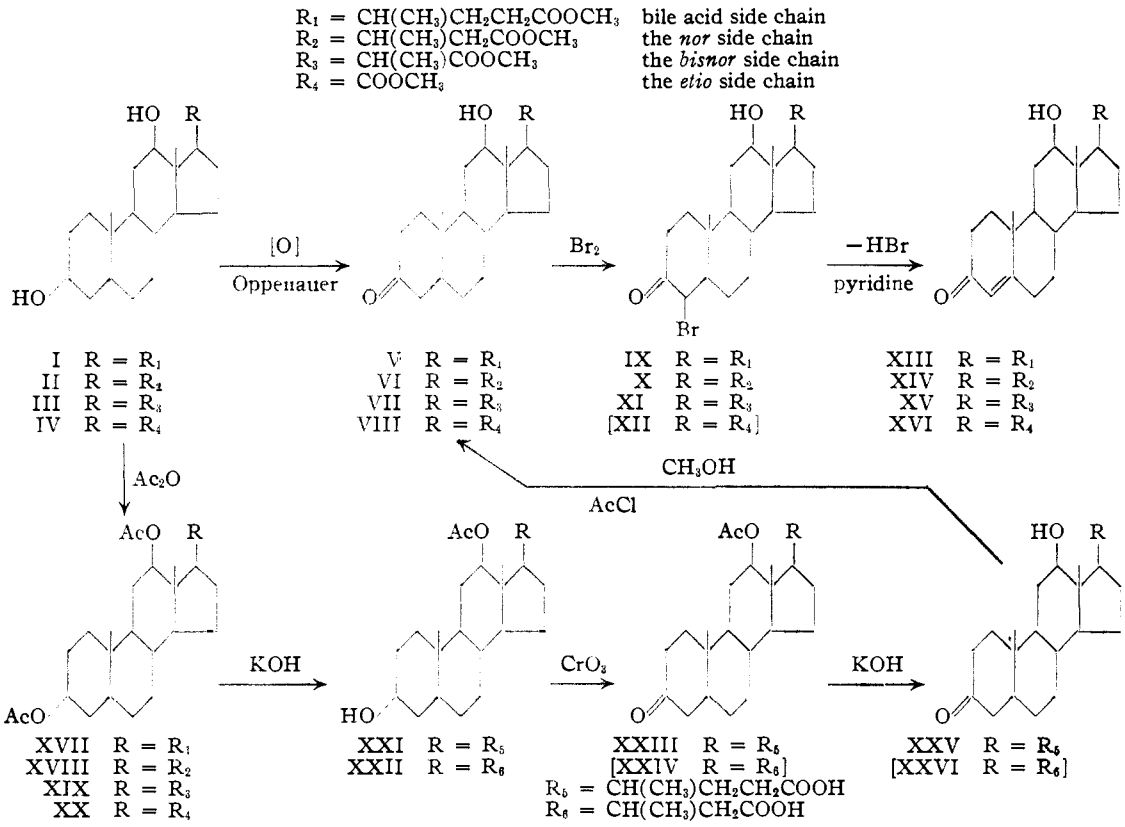
(1) K. Yamasaki and K. Kyogoku, *Z. physiol. Chem.*, **233**, 29 (1939).

(2) A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **26**, 607 (1943).

(3) T. F. Gallagher, *J. Biol. Chem.*, **133**, XXXVI, (1940).

(4) M. Ehrenstein and T. O. Stevens, *J. Org. Chem.*, **5**, 660 (1940).

(5) H. G. Fuchs and T. Reichstein, *Helv. Chim. Acta*, **26**, 511 (1943).



strictly comparable because of the shift of the double bond to form a conjugated system with the resulting 3-keto group.

When methyl desoxycholate (I) was refluxed with cyclohexanone and aluminum *t*-butoxide in a toluene solution, methyl 3-keto-12-hydroxycholanate (V) was obtained. Under the conditions used the optimum reaction time was about two hours. Cleavage of the methyl ester occurred to some extent leading to the formation of an acid fraction which was difficult to purify. The yield of methyl ester was lowered when the heating was prolonged over four hours while the acid fraction increased. Methyl *nor*-desoxycholate (II) and the *bisnor* (III), and *etio* (IV) homologs also gave the 3-keto derivatives (VI, VII, VIII) in the Oppenauer reaction.

As a further check both methyl 3-keto-12-hydroxycholanate (V) and methyl 3-keto-12-hydroxy-*nor*-cholanate (VI) were prepared following the method of Yamasaki and Kyogoku.[1] They were identical with the compounds made by the shorter method. Acetylation of the methyl desoxycholates gave methyl 3,12-diacetoxycholanate (XVII), its *nor* (XVIII), *bisnor* (XIX) and *etio* (XX) homologs which are intermediates for this second method. Partial hydrolysis of XVII and XVIII at room temperature gave 3-hydroxy-12-acetoxycholanic acid (XXI) and the *nor*-acid (XXII). These were in turn oxidized with chromic acid to XXIII and XXIV and then hydrolyzed to remove the 12-acetyl group giving 3-keto-12-hydroxycholanic acid (XXV) and the *nor*-acid (XXVI). Methylation of these by treatment with methanol and acetyl chloride gave compounds V and VI. Further investigations along this line were discontinued because of the obvious advantages of the Oppenauer oxidation. In an attempt to carry out the partial hydrolysis on methyl 3,12-diacetoxy-*etio*-cholanate (XX) both acetyl groups were removed.

The α,β-unsaturated compounds (XIII, XIV, XV, XVI) were prepared by bromination of the 3-keto compounds in glacial acetic acid at room temperature or below, followed by dehydrobromination in boiling pyridine. In a series of experiments carried out on methyl 3-keto-12-hydroxycholanate (V), about 10% excess of bromine gave far higher yields of the bromide (IX) than the use of an equivalent quantity or less.

To help establish the presence of a keto group with α,β-unsaturation, the ultraviolet absorption spectra were used. Steroids containing a 3-keto group and a 4,5-carbon–carbon double bond show a characteristic absorption band at 234 mμ in ether solution, and an extinction coefficient between 15,500 and 17,500. No other absorption band, for this system in other positions of the steroid molecule,[6] is so high. Cholestenone in ether[6] has an absorption maximum at 234 mμ

(6) H. Dannenberg, Der preussischen Akademie der Wissenschaften Jahrgang, 1939, Math.-naturw. Klasse, Nr. 21.

and an extinction coefficient of 16,900 but in alcohol[7] the absorption maximum is shifted to 240 mμ and the extinction coefficient is 14,100. The absorption spectra of methyl 3-keto-12-hydroxy-4-cholenate (XIII) and its *nor* (XIV), *bisnor* (XV) and *etio* (XVI) homologs were measured in 95% ethanol solution. The results are shown in the graph.

The authors wish to thank the Abbott Laboratories, the Glidden Company and the Upjohn Company for the financial aid that made this work possible.

Experimental[8]

Methyl 3-Keto-12-hydroxycholanate (V).—A solution of 15 g. (0.0369 mole) of methyl desoxycholate (I) and 12 g. (0.0487 mole) of aluminum *t*-butoxide in 300 ml. of toluene and 75 ml. (0.725 mole) of freshly distilled cyclohexanone was refluxed for four and one-half hours. The mixture was acidified and shaken with ether. The ether solution was washed with 1 *N* sulfuric acid, and extracted with a 5% sodium carbonate solution, which was saved. Evaporation of the ether solution on the steam-bath, *in vacuo*, gave a yellow liquid, which was dissolved in 150 ml. of high boiling petroleum ether and allowed to stand at −15°. White crystals (m. p. 136–138°) of methyl 3-keto-12-hydroxycholanate formed slowly. The yield was 9.44 g. (63%). This was recrystallized from 75 ml. of 60% ethanol, giving 8.56 g. of white crystals melting at 140.5–142°. The sodium carbonate solution obtained by washing the ether solution on acidifying gave a gummy precipitate. The free acid could not be readily isolated, but a low yield of methyl 3-keto-12-hydroxycholanate was obtained after methylation.

Methyl 3-Keto-12-hydroxy-*nor*-cholanate (VI).—A solution of 2.4 g. (0.0061 mole) of methyl *nor*-desoxycholate (II) in 50 ml. of toluene and 18 ml. of cyclohexanone was distilled until about 5 ml. of distillate had been collected, then 2.0 g. (0.0081) mole of aluminum *t*-butoxide was added and the mixture refluxed for two and a half hours. The reaction mixture was worked up as described above, and from 50 ml. of high boiling petroleum ether was obtained 1.56 g. (65%) of methyl 3-keto-12-hydroxy-*nor*-cholanate (m. p. 139–140°). Several crystallizations from 50% alcohol gave a sample melting at 143–145°.

Anal. Calcd. for $C_{24}H_{38}O_4$: C, 73.81; H, 9.81. Found: C, 74.17; H, 9.59.

Methyl 3-Keto-12-hydroxy-*bisnor*-cholanate (VII).—This compound was prepared by a method similar to that used in making methyl 3-keto-12-hydroxy-*nor*-cholanate. A yield of 78% of white crystals (m. p. 193–196°) was obtained. A sample crystallized several times from alcohol plus water melted at 203–204°.

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.61; H, 9.64.

Methyl 3-Keto-12-hydroxy-*etio*-cholanate (VIII).—A solution of 0.499 g. (0.00143 mole) of methyl *etio*-desoxycholate (IV) in 10 ml. of toluene and 3.5 ml. of freshly distilled cyclohexanone was distilled till 4 ml. of distillate had been collected. The solution was allowed to cool, then 0.40 g. of aluminum *t*-butylate was added and the mixture was refluxed for two hours. The reaction mixture was worked up as described above, and the residue was crystallized from a mixture of acetone (1.5 ml.) and high boiling petroleum ether (3.0 ml.). The yield of crystalline methyl 3-keto-12-hydroxy-*etio*-cholanate (m. p. 135–136°) was 0.1858 g., (37%). After two crystallizations from acetone plus petroleum ether (b. p. 30–60°) a sample melting at 139–141.5° was obtained. The more involved method used by Lardon and Reichstein[2] for the preparation of this compound gave material melting at 144–145°.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.60; H, 9.34.

Methyl 3-Keto-4-bromo-12-hydroxycholanate (IX).—A solution of 0.00525 mole of bromine in 13.6 ml. of glacial acetic acid was poured slowly into a solution of 1.94 g. (0.00478 mole) of methyl 3-keto-12-hydroxycholanate in 20 ml. of glacial acetic acid at room temperature. This addition required about forty-five seconds. The bromine color cleared immediately. After the reaction mixture had been allowed to stand for one minute, it was poured into water and extracted with ether. The ether solution was washed with water and 5% aqueous sodium carbonate. A gummy residue remained on removal of the ether under reduced pressure. When this was dissolved in 5 ml. of ethyl acetate and precipitated by addition of 30 ml. of high boiling petroleum ether an oil was obtained which solidified when freed from solvent. The yield was 1.08 g. (m. p. 118–127°). After two crystallizations from benzene plus petroleum ether, 0.09 g. of white crystals (m. p. 134–134.5°) was obtained.

Anal. Calcd. for $C_{25}H_{39}BrO_4$: C, 62.10; H, 8.13. Found: C, 62.27; H, 7.92.

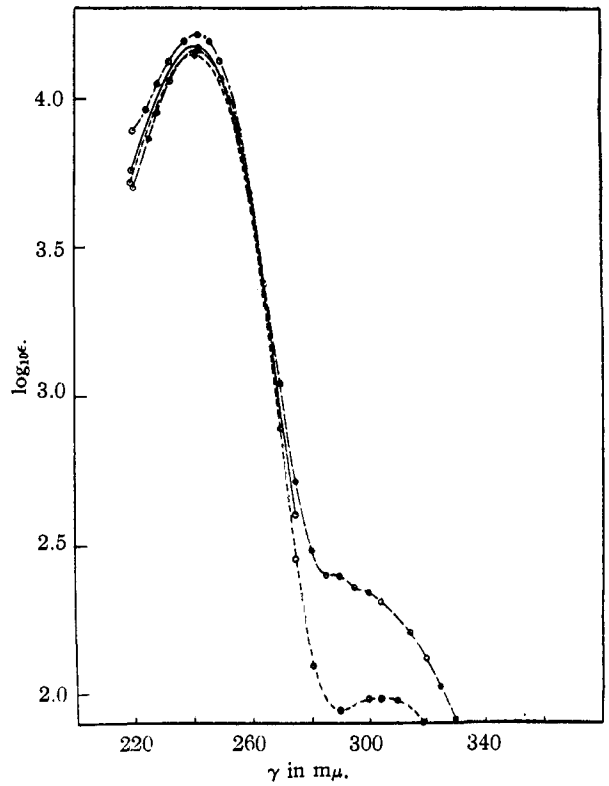


Fig. 1.—Absorption spectra of 3-keto-Δ^4-steroids in 95% ethanol solution.

Compound	λ max. mμ	ϵ max.	M. p., °C.
Methyl 3-keto-12-hydroxy-4-cholenate (– – – –)	241.5	14,470	144 –145
Methyl 3-keto-12-hydroxy-*nor*-4-cholenate (-·-·-·-)	241.5	16,320	136.5–137
Methyl 3-keto-12-hydroxy-*bisnor*-4-cholenate (------)	241	14,100	175 –176
Methyl 3-keto-12-hydroxy-*etio*-4-cholenate (———)	241	14,940	152 –153

(7) H. Mohler, *Helv. Chim. Acta*, **20**, 289 (1937).

(8) All melting points are corrected. Microanalyses by Dr. T. S. Ma, University of Chicago.

Methyl 3-Keto-12-hydroxy-*nor*-cholanate (X).—The reaction mixture from 1.00 g. of methyl 3-keto-12-hydroxy-*nor*-cholanate brominated as described above was poured into a mixture of 20 ml. of chloroform and 50 ml. of ether. The ether–chloroform solution was washed with water, 5% aqueous sodium carbonate, dried, and allowed to evaporate almost to dryness at room temperature The yield of crystalline methyl 3-keto-4-bromo-12-hydroxy-*nor*-cholanate (m. p. 145–160°) was 1.12 g. (92.5%). A sample twice crystallized from chloroform and ether melted at 178.5–180°.

Anal. Calcd. for $C_{24}H_{37}BrO_4$: C, 61.40; H, 7.94. Found: C, 62.01; H, 8.01.

Methyl 3-Keto-4-bromo-12-hydroxy-*bisnor*-cholanate (XI).—Methyl 3-keto-12-hydroxy-*bisnor*-cholanate (2.04 g.) was brominated as previously described and poured into water containing a small quantity of ether. A white crystalline solid formed which was collected by filtration. The yield of methyl 3-keto-4-bromo-12-hydroxy-*bisnor*-cholanate was 1.57 g. (m. p. 206–207°). The melting point remained constant when the compound was recrystallized from ether and chloroform.

Anal. Calcd. for $C_{23}H_{35}BrO_4$: C, 60.65; H, 7.75. Found: C, 60.82; H, 7.72.

Methyl 3-Keto-12-hydroxy-4-cholenate (XIII).—The crude product from brominating 1.0 g. of methyl 3-keto-12-hydroxycholanate was dissolved in 15 ml. of dry pyridine, and refluxed for four and one-half hours. The solution was poured into a mixture of dilute hydrochloric acid, benzene and ether. The ether–benzene solution was washed with 3 *N* hydrochloric acid, 5% aqueous sodium carbonate, dried and evaporated *in vacuo*. The residue was dissolved in 3.5 ml. of ethyl acetate, diluted with 30 ml. of high boiling petroleum ether and allowed to stand at −15°. The yield of crystalline methyl 3-keto-12-hydroxy-4-cholenate (m. p. 126–138°) was 0.35 g. (35%). A sample dissolved in 50% alcohol and treated with activated charcoal gave white crystals melting at 144–145°. Burckhart and Reichstein[9] prepared this compound by a different method and gave its m. p. as 150–152°.

Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51. Found: C, 74.32; H, 9.28.

Methyl 3-Keto-12-hydroxy-*nor*-4-cholenate (XIV).—A solution of 0.102 g. (0.0002 mole) of methyl 3-keto-4-bromo-12-hydroxy-*nor*-cholenate in 3 ml. of dry pyridine was refluxed for four hours. The pyridine was removed by evaporation *in vacuo* on the steam-bath. The yellow residue remaining was extracted with 3 ml. of ethyl acetate, which was then diluted with 10 ml. of high boiling petroleum ether. The solution was concentrated to 7 ml. and allowed to stand at 3°. A precipitate of 0.0138 g. (16%) of white crystals (m. p. 127–131°) was obtained. A sample crystallized from an alcohol–water solution which was treated with activated charcoal gave needles melting at 136.5–137°.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.58; H, 9.51. Found: C, 74.38; H, 9.56.

Methyl 3-Keto-12-hydroxy-*bisnor*-4-cholenate (XV).—Methyl 3-keto-4-bromo-12-hydroxy-*bisnor*-cholanate (1.2 g.) was dehydrobrominated and the reaction mixture was poured into benzene and extracted with dilute hydrochloric acid. The benzene solution was washed with 5% aqueous sodium carbonate, dried over anhydrous sodium sulfate and evaporated *in vacuo* on the steam-bath. The residue was crystallized from a mixture of ethyl acetate and high boiling petroleum ether, giving 0.41 g. (41%) of needles melting at 175–176°. A second crop of 0.11 g. (m. p. 153–161°) was obtained by concentration of the filtrate. Crystallization from an alcohol–water mixture gave a sample melting at 164–167°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.50; H, 8.99.

(9) V. Burckhart and T. Reichstein, *Helv. Chim. Acta*, **25**, 821 (1942.)

Methyl 3-Keto-12-hydroxy-*etio*-4-cholenate (XVI).—Bromination of 0.162 g. of methyl 3-keto-12-hydroxy-*etio*-cholanate gave a yellow resin which could not be easily crystallized. The crude methyl 3-keto-4-bromo-12-hydroxy-*etio*-cholanate was dissolved in 10 ml. of pyridine and the solution was refluxed five hours, then poured into 50 ml. of water and extracted with ether. The ether solution was washed with water, 1 *N* sulfuric acid, and 5% aqueous sodium carbonate. Evaporation of the ether solution *in vacuo* at room temperature gave a yellow residue which was dissolved in benzene and passed through an alumina column, then developed with ethanol in benzene to give a fraction from which 16.6 mg. of white crystals of methyl 3-keto-12-hydroxy-*etio*-4-cholenate (m. p. 148–149°) was obtained. This was crystallized from benzene plus low-boiling petroleum ether to give a sample melting at 152–153°.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 73.18; H, 8.70.

Methyl 3,12-Diacetoxy-*nor*-cholanate (XVIII).—A solution of 5 g. of methyl *nor*-desoxycholate in 15 ml. of acetic anhydride and 30 ml. of pyridine was refluxed for five hours then evaporated to dryness *in vacuo*. The residue was crystallized two times from aqueous ethanol giving 5.35 g. (88%) of methyl 3,12-diacetoxy-*nor*-cholanate melting at 151–152°. A sample crystallized from methanol melted at 153–153.4°.

Anal. Calcd. for $C_{28}H_{44}O_6$: C, 70.55; H, 9.30. Found: C, 70.71; H, 9.08.

Methyl 3,12-Diacetoxy-*bisnor*-cholanate (XIX).—Acetylation of 1.5 g. of methyl *bisnor*-desoxycholate gave 1.42 g. (77.5%) of methyl 3,12-diacetoxy-*bisnor*-cholanate melting at 165–167° after crystallization from 95% ethanol.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 70.17; H, 8.99.

Methyl 3,12-Diacetoxy-*etio*-cholanate (XX).—The preparation of this compound was similar to that described above. An 88% yield of crystals melting at 146–148° was obtained. When recrystallized from methanol it gave a m. p. of 149–150.5°. Lardon and Reichstein[2] gave 149–150° for the m. p. of this compound.

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 69.12; H, 8.75.

3-Hydroxy-12-acetoxycholanic Acid (XXI).—Following the method of Yamasaki and Kyogoku,[1] 1.62 g. of 3-hydroxy-12-acetoxycholanic acid (m. p. 171–172.5°) was obtained from 2.45 g. of methyl 3,12-diacetoxycholanate (XVII) (m. p. 110–113°) by hydrolysis with 0.5 *N* alcoholic potassium hydroxide. The highest yield was obtained after standing two hours. No crystalline product was obtained when aqueous alcoholic potassium carbonate was used, as in the preparation of 3-hydroxy-12-acetoxy-*etio*-cholanic acid by Reichstein and Arx.[10]

3-Hydroxy-12-acetoxy-*nor*-cholanic Acid (XXII).—A solution of 2.0 g. (0.0042 mole) of methyl 3,12-diacetoxy-*nor*-cholanate in 60 ml. of 95% ethanol and 5 ml. of water containing 1.0 g. (0.0178 mole) of potassium hydroxide was allowed to stand seventeen hours at room temperature, then was diluted with 150 ml. of water and extracted with ether. The aqueous layer was separated and made acid with 3 *N* hydrochloric acid, giving 1.46 g. (82.5%) of 3-hydroxy-12-acetoxy-*nor*-cholanic acid as an amorphous solid (m. p. 219–221°). A sample crystallized from acetone melted at 219–221°. When the hydrolysis was interrupted after two and one-half hours, the yield of 3-hydroxy-12-acetoxy-*nor*-cholanic acid was only 26.5%.

Anal. Calcd. for $C_{25}H_{40}O_5$: C, 71.36; H, 9.62. Found: C, 71.31; H, 9.29.

3-Keto-12-acetoxycholanic Acid (XXIII).—By oxidation of 0.85 g. of 3-hydroxy-12-acetoxycholanic acid with chromium trioxide, following the method of Yamasaki and Kyogoku, 0.72 g. of crude 3-keto-12-acetoxycholanic acid was obtained. It was found that the optimum yield was

(10) T. Reichstein and E. V. Arx, *ibid.*, **23**, 747 (1940).

obtained from oxidations allowed to proceed one hour at room temperature.

Methyl 3-Keto-12-hydroxycholanate (V) from 3-Keto-12-acetoxycholanic Acid (XXIII).—Saponification of 0.7 g. of 3-keto-12-acetoxycholanic acid gave 3-keto-12-hydroxycholanic acid. This was dissolved in 10 ml. of methanol to which 1 ml. of acetyl chloride was added. The solution was allowed to stand overnight, poured into water and extracted with ether. The ether solution was washed with water, 5% aqueous sodium carbonate, and evaporated *in vacuo*, leaving an orange resin which was crystallized from an alcohol–water mixture, then an acetone and petroleum ether mixture. The yield of methyl 3-keto-12-hydroxycholanate (m. p. 140–141°) was 0.28 g.

Methyl 3-Keto-12-hydroxy-*nor*-cholanate (VI) from 3-Hydroxy-12-acetoxy-*nor*-cholanic Acid (XXII).—A solution of 2.4 g. of chromium trioxide in 2 ml. of water and 15 ml. of glacial acetic acid was added in four portions at five-minute intervals to a solution of 2.84 g. of 3-hydroxy-12-acetoxy-*nor*-cholanic acid in 25 ml. of glacial acetic acid. The reaction mixture was kept at room temperature, and allowed to stand for two hours, then was poured into 200 ml. of water and extracted with ether. The ether solution was washed with 3 *N* hydrochloric acid, water, and then extracted with 5% aqueous sodium carbonate. Acidifying the alkaline extract gave 2.17 g. of crude 3-keto-12-acetoxy-*nor*-cholanic acid (XXIV), which was dissolved in 80 ml. of 5% aqueous potassium hydroxide and refluxed for two hours. After acidifying the solution 1.67 g. of crude 3-keto-12-hydroxy-*nor*-cholanic acid was obtained.

A 1.17-g. sample of the crude acid was methylated as described above. The product was crystallized from acetone plus petroleum ether, then from 25 ml. of 50% ethanol to give 0.55 g. of methyl 3-keto-12-hydroxy-*nor*-cholanate melting at 146.5–147.5°. The mixture melting point with methyl 3-keto-12-hydroxy-*nor*-cholanate (m. p. 143–145°) prepared from methyl *nor*-desoxycholate by the Oppenauer reaction was 144–144.5°.

3-Keto-12-hydroxy-*bisnor*-4-cholenic Acid.—Saponification of 0.10 g. of methyl 3-keto-12-hydroxy-*bisnor*-4-cholenate (VII) gave 0.09 g. of 3-keto-12-hydroxy-*bisnor*-4-cholenic acid melting at 210–220°.

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.29; H, 8.95. Found: C, 73.09; H, 8.67.

Summary

1. Methyl 3-keto-12-hydroxy-4-cholenate, and the *nor*, *bisnor*, and *etio* homologs have been prepared from the corresponding desoxycholic acids.

2. The methyl 3-keto-12-hydroxycholanates used as intermediates were made by selective oxidation of the 3-hydroxyl group by means of the Oppenauer reaction.

3. Proof of the selective oxidation was obtained for two of these compounds by preparing them using a procedure involving partial hydrolysis.

4. The absorption spectra of methyl 3-keto-12-hydroxy-4-cholenate, and the *nor*, *bisnor*, and the *etio* homologs gave similar curves typical of conjugated unsaturation.

EVANSTON, ILLINOIS RECEIVED MARCH 2, 1944

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL AND COLLOIDAL CHEMISTRY, THE HEBREW UNIVERSITY]

Preparation of *l*-Leucyl-*l*-glutamic Acid Anhydride and its Behavior toward Proteinases

BY N. LICHTENSTEIN

When *d,l*-α-bromoisocaproyl bromide and *l*-glutamic acid are coupled, a reaction product is recovered in over 70% yield. When this product is aminated with aqueous ammonia and the evaporated amination mixture treated with water and alcohol, *l*-leucyl-*l*-glutamic acid separates, whereas the diastereoisomer, *d*-leucyl-*l*-glutamic acid, is apparently retained, together with ammonium bromide, in the aqueous alcoholic phase. The separated dipeptide possesses a specific optical rotation corresponding to that characteristic of the product prepared by E. Fischer[1] from *l*-glutamic acid and the chloride of *l*-α-bromoisocaproic acid. To confirm the configuration, our dipeptide was hydrolyzed by heating with hydrochloric acid and compared as to optical activity with an equimolecular mixture of *l*-leucine and *l*-glutamic acid similarly heated with hydrochloric acid. The specific rotation of the amino acid hydrochloride mixtures obtained from the two solutions by evaporation was one and the same.

The dipeptide is readily converted into its anhydride by treatment with hot β-naphthol. As has previously been reported[2] a number of dipeptides can be converted into corresponding diketopiperazines by solution in β-naphthol at 135–150°. It has been found that *l*-leucyl-*l*-glutamic acid, in distinction, does not dissolve in β-naphthol at the mentioned temperature. At 170–180°, however, solution is effected and anhydride formation occurs. The C, H and N content and the carboxyl titer of the product correspond in value with the theoretical expectation from a diketopiperazine composed of glutamic acid and leucine. Titration according to Linderstrøm-Lang failed to reveal the presence of a free amino group. Hydrolysis with hydrochloric acid yielded, after evaporation to dryness, a product of corresponding specific rotation to that obtained when solutions of the original dipeptide or equimolecular mixtures of its components are similarly treated. The anhydration product is therefore *l*-leucyl-*l*-glutamic acid anhydride.

According to K. Shibata,[3] leucylglutamic acid anhydride ought to be cleaved by trypsin and papain, which are "carboxy-cyclopeptidases."

(1) E. Fischer, "Untersuchungen über Aminosäuren, etc.," **2**, 465, 466 (1923).

(2) N. Lichtenstein, THIS JOURNAL, **60**, 560 (1938).

(3) K. Shibata, *Acta Phytochim.*, **8**, 173 (1934).