[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, THE MAYO FOUNDATION]

The Degradation of Desoxycholic Acid to *etio*-Desoxycholic Acid through *etio*-Desoxycholyl Methyl Ketone

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Although a number of the cholanic acids have been degraded to the corresponding *etio* acids and *etio* ketones,[1] the degradation of desoxycholic acid (3,12-dihydroxycholanic acid) has not been described. Since *etio*-desoxycholic acid was desired in connection with studies of the steroid compounds of the adrenal cortex, this degradation was undertaken.

The procedures for the usual Barbier–Wieland[2] degradation have been described in detail sufficiently by others. We shall refer to those procedures only in a general way but shall describe in detail two improvements which increase our yields very considerably and which should be generally applicable. It has been customary to oxidize the carbinols (such as II) or the diphenylethylenes (such as III) with chromic acid in hot glacial acetic acid. The yields with this procedure are approximately 50% with *nor*- and *bis-nor*-cholanyldiphenylethylenes. We have found that the acetates of *nor*- and *bis-nor*-desoxycholydiphenylethylene were oxidized smoothly with chromic acid at temperatures below 15° with yields of about 70% of the corresponding acids.

The greatest loss in this systematic degradation occurs when the *ter-nor*-cholanyldiphenylethylenes are oxidized to the *etio*-cholanic acids. With chromic acid a variety of products is obtained and much material is irretrievably lost. Our best yield of *etio*-desoxycholic acid obtained by this means was 16%. We have increased the yield of *etio*-desoxycholic acid to approximately 40% based on the ethylene by proceeding in four steps. Ozonolysis of 3,12-diacetoxy-*ter-nor*-cholanyldiphenylethylene (VI) gave 3,12-diacetoxy-*etio*-cholanyl methyl ketone (VII), which was condensed with benzaldehyde in the presence of sodium ethoxide. Analysis of the condensation product indicated a partial removal of the acetate groups. It was reacetylated by refluxing with acetic anhydride and was then ozonized. The glyoxal that was formed was not isolated but was oxidized by periodic acid.[3] Hydrolysis of the resulting acidic product gave the desired *etio*-desoxycholic acid (XII) (Fig. 1).

Dehydro-*nor*-desoxycholic and dehydro-*bis-nor*-desoxycholic acids were prepared from the corresponding dihydroxy acids by oxidation with chromic acid in aqueous acetone. Because of the insolubility of *etio*-desoxycholic acid in aqueous acetone, dehydro-*etio*-desoxycholic acid was prepared from it by oxidation of the methyl ester in this medium with subsequent hydrolysis. *etio*-Desoxycholic acid also was oxidized in glacial acetic acid, but the yield of dehydro acid was much lower than when aqueous acetone was used. Oxidation of *etio*-desoxycholyl methyl ketone with chromic acid in acetic acid did not yield the desired dehydro-*etio*-desoxycholyl methyl ketone. Analysis indicated that the 17-acetyl group had been removed with the formation of 3,12,17-triketo-*etio*-cholane (XIV).

Although ozone has been found to be a convenient reagent for the preparation of *etio*-cholanones and *etio*-cholanyl methyl ketones from the appropriate ethylenic compounds, the possibility of loss by excessive ozonization has not been pointed out. We have found that the amount of 3,12-diacetoxy-*ter-nor*-cholanyl methyl ketone (VII) was lowered considerably when the ethylene (VI) was treated with more than the calculated amount of ozone.

Sixty-seven grams of *nor*-desoxycholic acid was obtained from 100 g. of methyl desoxycholate. This amount of *nor*-desoxycholic acid was converted to 32 g. of *bis-nor*-desoxycholic acid which in turn gave 20 g. of crystalline 3,12-diacetoxy-*ter-nor*-cholanyldiphenylethylene. This ethylene could be converted to 7.3 g. of 3,12-diacetoxy-*etio*-cholanyl methyl ketone or 4.2–4.6 g. of *etio*-desoxycholic acid.

Experimental

General Procedure.—Methyl desoxycholate and *nor*-desoxycholate were prepared by refluxing the acids for two hours in a 1% solution of hydrogen chloride in methyl

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(1) Recent degradation studies are given in papers by (a) Dalmer, v. Werder, Honigmann and Heyns, *Ber.*, **68**, 1814 (1935); (b) Marker, Kamm, McGinty, Jones, Wittle, Oakwood and Crooks, THIS JOURNAL, **59**, 768, 1367 (1937); (c) Morsman, Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 3 (1937); (d) Ruzicka, Oberlin, Wirz and Meyer, *ibid.*, **20**, 1283 (1937); (e) Steiger and Reichstein, *ibid.*, **20**, 1040 (1937).

(2) (a) Barbier and Locquin, *Compt. rend.*, **156**, 1443 (1913); (b) Wieland, Schlichting and Jacobi, *Z. physiol. Chem.*, **161**, 80 (1926).

(3) Clutterbuck and Reuter, *J. Chem. Soc.*, 1467 (1935).

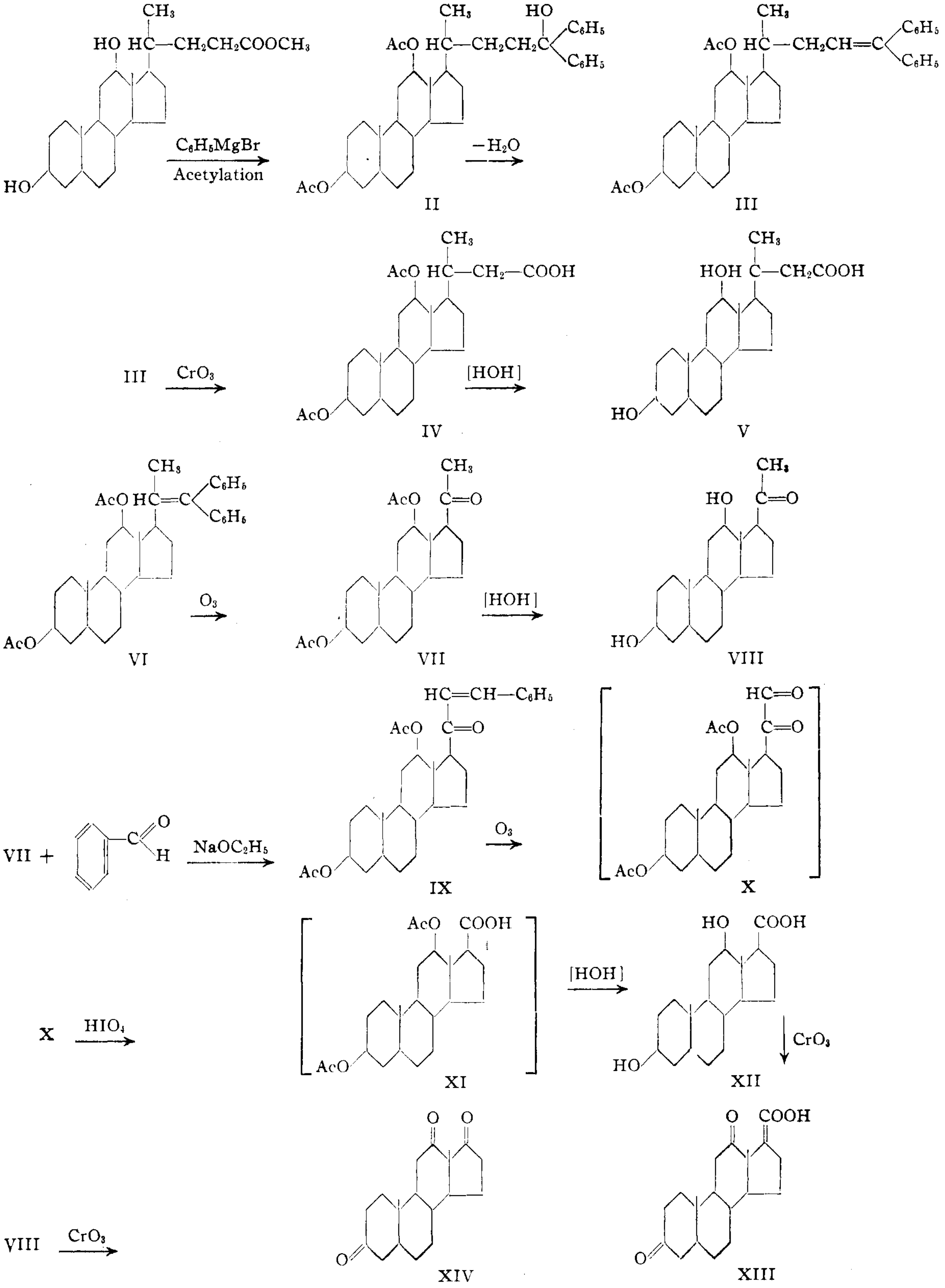

Fig. 1.—Systematic scheme of degradation

alcohol. *bis-nor*-Desoxycholic acid, being more difficult to esterify, was converted to the ester with diazomethane. The esters were not isolated but were added in dry benzene to 16 moles of phenylmagnesium bromide. The mixture was refluxed for three hours. The ether and benzene were distilled as completely as possible; the residue was dissolved in dry benzene and refluxed overnight. Best results were obtained when free magnesium was absent and when the ether used for preparation of the Grignard reagent was free of peroxide. The product was worked up in the usual way. The carbinols were acetylated with acetic anhydride and pyridine at 100° for twenty hours and then dehydrated to the diphenylethylenes in boiling glacial acetic acid.[1c] The acetylated and non-acetylated ethylenes were isolated and characterized.

All melting points are corrected unless otherwise specified. Micro-methods were used for the elementary analysis of the products obtained. All samples for analysis were dried for at least one hour at 110° and 0.1 mm.

3,12-Diacetoxy-*nor*-cholanyldiphenylethylene (III) was obtained in yields of 105 g. (74%) of recrystallized material from 100 g. of desoxycholic acid. It crystallized readily from acetic acid. For analysis a sample was recrystallized from ethyl alcohol. It melted at 160° and $[\alpha]^{25}_{5461}$ +118 ± 2° (0.38% in alcohol).

Anal. Calcd. for $C_{40}H_{52}O_4$: C, 80.48; H, 8.79. Found: C, 80.31; H, 8.87.

***nor*-Desoxycholic Acid** (V).—One-tenth mole (59.6 g.) of crystalline 3,12-diacetoxy-*nor*-cholanyldiphenylethylene was dissolved in 50 cc. of chloroform and 300 cc. of glacial acetic acid was added. This solution was cooled to 15° and a solution of 37.5 g. of chromic acid (0.51 atom of oxygen) in 30 cc. of water and 70 cc. of acetic acid was stirred in at such a rate that the temperature remained at 15° or below. About thirty to forty minutes was required. Immediately the excess chromic acid was destroyed with sulfur dioxide while cooling in an ice-bath. The solution was diluted with 500 cc. of water and distilled *in vacuo* to a volume of 150 cc. After addition of 200 cc. of water the suspension was filtered. The semi-solid mat was washed with water and then dissolved in 0.5 *N* sodium hydroxide. The alkaline solution was washed thoroughly with ether, acidified to congo red with dilute hydrochloric acid, warmed to 35° and filtered. The crude acid was hydrolyzed with 2 *N* potassium hydroxide solution by refluxing for two hours. This alkaline solution was cooled and stirred slowly into dilute hydrochloric acid. The suspension obtained was warmed to 35° and the crystalline acid was filtered out. After drying for twelve hours at 105°, the acid (34–35 g.) was recrystallized from dry acetone. The yield was 30–31.5 g. (69–72%) of acid which contained a molecule of acetone of crystallization. A total yield of 67 g. (63.5%) was obtained from 100 g. of methyl desoxycholate. This amount included acid obtained by oxidation of the material that remained in the mother liquors after removal of the crystalline ethylene. The yield of acid was found to be appreciably lower when the chromic acid was reduced at a later time than that designated in the above procedure. The acetone-containing acid melted at 140–145°, resolidified and melted at 206–210° and $[\alpha]^{25}_{5461}$ + 62 ± 2.5° (0.26% in alcohol).

Anal. Calcd. for $C_{23}H_{38}O_4$: C, 72.95; H, 10.13. $C_{23}H_{38}O_4 \cdot C_3H_6O$: C, 71.50; H, 10.16. Found: C, 71.50; H, 10.14.

Dehydro-*nor*-desoxycholic Acid.—A solution of 5 cc. of 2 *N* potassium dichromate and 2 cc. of 5 *N* sulfuric acid was added to 110 mg. of *nor*-desoxycholic acid dissolved in 25 cc. of acetone. This mixture was allowed to stand two hours. Then the excess chromic acid was reduced with sulfur dioxide and, after dilution with water, the acetone was evaporated *in vacuo*. The precipitate was filtered out, washed with water and recrystallized from aqueous acetone. The dehydro-*nor*-desoxycholic acid melted at 230–232° after it was dried at 110° for one hour; $[\alpha]^{25}_{5461}$ +114 ± 2° (0.32% in alcohol).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.74; H, 9.16. Found: C, 73.67; H, 9.33.

3,12-Diacetoxy-*bis-nor*-cholanyldiphenylethylene was obtained crystalline in yields of 85–90 g. (55–58.5%) from 100 g. of *nor*-desoxycholic acid. A sample for analysis was crystallized from alcohol. It melted at 158–160° and $[\alpha]^{25}_{5461}$ +183 ± 2° (0.32% in alcohol). The ethylene was crystallized from acetic acid for use in the preparation of *bis-nor*-desoxycholic acid.

Anal. Calcd. for $C_{39}H_{50}O_4$: C, 80.36; H, 8.65. Found: C, 80.26; H, 8.70.

***bis-nor*-Desoxycholic acid** was obtained in yields of 48 g. from 100 g. of *nor*-desoxycholic acid. This yield included acid obtained from the oxidation of the mother liquor of the *bis-nor*-ethylene. The acid was crystallized from alcohol. For analysis a sample was precipitated from a bicarbonate solution with dilute hydrochloric acid and allowed to crystallize. The acid was filtered out and washed with water. It was dried for two hours at 110° and 0.1 mm. It then had the composition of a monohydrate and melted at 236–238° after it had melted and resolidified at 195–202°. For the monohydrate $[\alpha]^{25}_{5461}$ +35.8 ± 5° (0.96% in alcohol). A sample of 97.6 mg. of this acid required 2.54 cc. of 0.1 *N* sodium hydroxide solution for neutralization; a sample of 5.743 mg. lost 0.287 mg. when dried to a constant weight at 205°.

Anal. Calcd. for $C_{22}H_{36}O_4$: C, 72.46; H, 9.97; mol. wt., 364. For $C_{22}H_{36}O_4 \cdot H_2O$: C, 69.30; H, 9.95; mol. wt., 382; H_2O, 4.71. Found: C, 69.75; H, 9.93; mol. wt., 384; H_2O, 4.99.

Dehydro-*bis-nor*-desoxycholic Acid.—*bis-nor*-Desoxycholic acid was oxidized by the same procedure used for the preparation of dehydro-*nor*-desoxycholic acid. The acid obtained melted at 275–276° (sintered at 265°) and $[\alpha]^{25}_{5461}$ +98 ± 5° (0.11% in alcohol).

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.29; H, 8.95. Found: C, 73.17; H, 9.20.

3,12-Diacetoxy-*ter-nor*-cholanyldiphenylethylene (VI) was obtained in a yield of 36 g., recrystallized from acetic acid, from 55 g. of *bis-nor*-desoxycholic acid. This ethylene melted at 215–217° and $[\alpha]^{25}_{5461}$ +537 ± 2° (0.1% in ethyl alcohol).

Anal. Calcd. for $C_{38}H_{48}O_4$: C, 80.22; H, 8.51. Found: C, 80.16; H, 8.87.

3,12-Diacetoxy-*etio*-cholanyl Methyl Ketone (VII).—Ozonolysis of 5.68 g. (0.01 mole) of 3,12-diacetoxy-*ter-nor*-cholanyldiphenylethylene in chloroform was carried out at 0° for twenty-five minutes (0.01 mole ozone). Four cc. of acetic acid and 3 g. of zinc dust were added and the mixture was allowed to stand in an ice-bath until a negative starch–iodine test was obtained. After filtration the chloroform solution was washed with water and dilute sodium bicarbonate solution. The chloroform solution was dried over anhydrous sodium sulfate and distilled *in vacuo*. In order to remove all chloroform the residue was dissolved in 20 cc. of absolute alcohol and the solvent removed *in vacuo*. The ketone mixture was redissolved in 40 cc. of absolute alcohol and 5 g. of Girard's reagent[4] and 4 cc. of acetic acid were added. The solution was refluxed for thirty minutes. After removal of the alcohol *in vacuo*, the residue was shaken vigorously with 400 cc. of ice-cold 0.15 *N* sodium hydroxide solution. The "non-ketone" fraction was removed by extraction with ether. Acidification with 30 cc. of 20 *N* sulfuric acid liberated the ketone fraction which was taken into ether. This crude ketone fraction weighed 3.25 g. and, after crystallization from methyl alcohol, yielded 2.1 g. of 3,12-diacetoxy-*etio*-cholanyl methyl ketone which melted at 121–122.5°; $[\alpha]^{25}_{5461}$ +190.4 ± 2.5° (0.125% in alcohol).

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.72; H, 9.16. Found: C, 71.44; H, 9.20.

3,12-Dihydroxy-*etio*-cholanyl Methyl Ketone.—Hydrolysis of the diacetoxy ketone with 2 *N* potassium hydroxide solution yielded 3,12-dihydroxy-*etio*-cholanyl methyl ketone (VIII) which melted at 165–166° after crystallization from a 50% alcohol solution; $[\alpha]^{25}_{5461}$ +165 ± 5° (0.16% in alcohol).

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.39; H, 10.25. Found: C, 75.34; H, 10.42.

3,12,17-Triketo-*etio*-cholane.—To a solution of 320 mg. of 3,12-dihydroxycholanyl methyl ketone in 20 cc. of acetic acid was added 125 mg. of chromic acid in 10 cc. of 90% acetic acid. After one day the mixture was worked up in the usual manner for the neutral fraction. This fraction, crystallized from ethyl alcohol, yielded 150 mg. of material which melted at 189–191° and $[\alpha]^{25}_{5461}$ +235 ± 2.5° (0.16% in alcohol). Analysis indicated this compound was 3,12,17-triketo-*etio*-cholane (XIV) instead of the expected dehydro-*etio*-desoxycholanyl methyl ketone.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.31; H, 9.16. $C_{19}H_{26}O_3$: C, 75.44; H, 8.67. Found: C, 75.53, 75.67; H, 9.09, 9.16.

***etio*-Desoxycholic Acid (XII).**—(a) Three cc. of sodium ethoxide solution (0.2 g. of sodium per cc.) was added to a solution of 655 mg. of 3,12-diacetoxycholanyl methyl ketone (0.00156 mole) and 250 mg. of benzaldehyde in 1 cc. of absolute ethyl alcohol. This mixture was allowed to stand one day and was then digested with 100 cc. of ether and dilute hydrochloric acid solution. The ether solution was washed with water and dilute sodium bicarbonate solution and was then dried over anhydrous sodium sulfate. After distillation of the ether, the residue was refluxed in acetic anhydride for one hour. Excess acetic anhydride was removed; the material was dissolved in 30 cc. of dry chloroform and ozonized at −10° (0.0032 mole of ozone). A vacuum was applied to remove the ozone in solution and then 2 cc. of acetic acid and 600 mg. of zinc dust were added. The mixture was allowed to remain in an ice-bath until a negative starch–iodine test was obtained. After filtration the chloroform solution was washed with water and dilute sodium bicarbonate solution and dried over anhydrous sodium sulfate. The chloroform was distilled *in vacuo*. The residue, in 30 cc. of alcohol, was oxidized overnight with 5 cc. of 4 *N* periodic acid. After removal of alcohol the aqueous residue was extracted with ether. The ether solution was washed with water and then with dilute sodium hydroxide solution. Acidification of the alkaline solution yielded crude acid, which was hydrolyzed at 100° for two hours with 2 *N* sodium hydroxide solution. Recrystallization of the crude *etio*-desoxycholic acid from dry acetone yielded 230 mg. which melted at 283–286°, and $[\alpha]^{25}_{5461}$ +102 ± 1.5°.

Anal. Calcd. for $C_{20}H_{32}O_4$: C, 71.38; H, 9.60. Found: C, 71.49; H, 9.88.

By the use of the crude product of the ozonolysis of VI for the condensation with benzaldehyde it was found that yields of 36–42% of the theoretical amount (based on VI) of *etio*-desoxycholic acid were obtained. Further studies are in process to determine the most favorable conditions for the preparation of the intermediate benzal ketone (IX).

(b) A solution of 2.84 g. of 3,12-diacetoxy-*ter-nor*-cholanyldiphenylethylene in 100 cc. of acetic acid was cooled to 15° and 17 g. of chromic acid in 15 cc. of 60% acetic acid was added. After standing for one day at room temperature the mixture was worked up in the usual way for the acid fraction. A yield of 0.65 g. of crude acid was obtained which, after the usual alkaline treatment, gave the dihydroxy acid. The 3,12-dihydroxy-*etio*-desoxycholic acid was dried at 110° and recrystallized from dry acetone. The yield of crystalline acid was 250 mg. or 15% of the theoretical based on the ethylene used. The acid possessed the same properties as that obtained by the procedure (a). A mixture of this acid with that from (a) melted at 283–286°.

Dehydro-*etio*-desoxycholic Acid.—To a solution of 400 mg. of *etio*-desoxycholic acid in 10 cc. of acetic acid was added slowly 160 mg. (0.00205 atom of oxygen) of chromic acid in 5 cc. of 90% acetic acid. This mixture was allowed to stand at room temperature overnight. After destruction of the slight excess of chromic acid with sulfur dioxide it was diluted with an equal volume of water and evaporated *in vacuo* to about 20 cc. The acid was filtered out, washed with water and dissolved in 0.2 *N* sodium bicarbonate solution. This solution was filtered and acidified. The acid, which soon crystallized, weighed 160 mg. or 40% of theory after drying at 110°. When the procedure employed for the preparation of dehydro *nor*- and dehydro-*bis-nor*-desoxycholic acids was used, the oxidation of methyl *etio*-desoxycholate with subsequent hydrolysis gave a yield of 61% of dehydro-*etio*-desoxycholic acid. The acid melted at 177–178.5° and $[\alpha]^{25}_{5461}$ +166 ± 4° (0.106% in alcohol).

Anal. Calcd. for $C_{20}H_{28}O_4$: C, 72.46; H, 8.49. Found: C, 72.19; H, 8.59.

Summary

The use of cold chromic acid for oxidation of the acetylated diphenylethylenic intermediates of the Barbier–Wieland degradation of desoxycholic

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[4] Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936).

acid has given improved yields of *nor*- and *bis-nor*-desoxycholic acids. The yield of *etio*-desoxycholic acid also has been improved by the following steps: (1) ozonolysis of 3,12-diacetoxy-*ter-nor*-cholanyldiphenylethylene; (2) condensation of the resulting 3,12-diacetoxy-*etio*-cholanyl methyl ketone with benzaldehyde; (3) ozonolysis of the condensation product; and (4) oxidation of the resulting glyoxal with periodic acid to give 3,12-diacetoxy-*etio*-cholanic acid, followed by hydrolysis.

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A Molecular Rearrangement Induced by Ultrasonic Waves

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Acid azides of the type $RCON_3$ are converted, through the Curtius reaction, into nitrogen and isocyanates.[1] Some other azides, including those derived from sulfonic acids, yield nitrogen, when heated, but do not, as a rule, undergo the rearrangement.[2]

Benzazide, $C_6H_5CON_3$, yields nitrogen and phenyl isocyanate at measurable rates when heated in the solid state or in any solvent and we selected this compound for treatment with ultrasonic vibrations. The rate of the thermal decomposition of the compound in the solid state has been measured by Vasilevskii and others.[3]

We present in this paper the rates of rearrangement of benzazide in benzene solution and in aniline solution at 25° with and without the application of vibrational energy.

The vibrator—a magnetostriction oscillator—is so constructed that the energy input may be varied between 100 and 1500 watts. The efficiency of the machine, however, is less than 5%. The vibrating unit is a nickel tube, 2.7 cm. in diameter, which is closed at the upper end. Tubes of various lengths are used for frequencies ranging from 10,000 to 50,000 cycles per second. The lower end of the nickel tube is surrounded by concentric coils of insulated wire. One coil is part of the oscillating circuit to which power is supplied, at the resonance frequency of the rod, by two self-exciting vacuum tubes. Adjustment to resonance is made by a variable condenser which, with the coil, controls the frequency of the oscillations. The vacuum tube plate voltage used in most of this work was 1500 v. and the plate current 300 m. a. The outside coil carries a direct current to produce a steady magnetic field which polarizes the rod. A current of 3.5 amp. from a 110-v. d. c. supply was used in this coil. A polarizing field is necessary to prevent the flux in the rod from reversing and causing the rod to vibrate feebly at double the fundamental frequency.

The benzene solution of benzazide was placed in a glass cell covering the upper end of the nickel tube as shown in Fig. 1. The evolved nitrogen was measured in a slender buret. It had been determined by other experiments that the thermal decomposition of benzazide is not catalyzed by nickel.

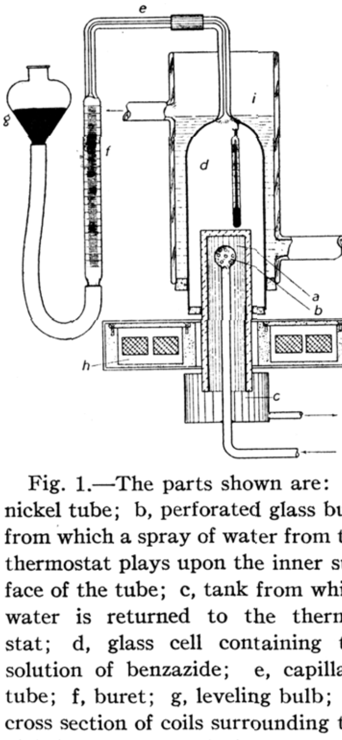

Fig. 1.—The parts shown are: a, nickel tube; b, perforated glass bulb from which a spray of water from the thermostat plays upon the inner surface of the tube; c, tank from which water is returned to the thermostat; d, glass cell containing the solution of benzazide; e, capillary tube; f, buret; g, leveling bulb; h, cross section of coils surrounding the nickel rod; i, water jacket surrounding the cell.

The vibrational energy imparted to the liquid surrounding the upper half of the nickel tube was estimated calorimetrically and also by measuring the amplitude and frequency of the vibrations. Several measurements were made and values ranging from 10 to 15 watts were obtained. The average corresponds to a vibrational energy of 12 watts. The amplitude is 0.00015 cm. in the 16-cm. tube and the vibration frequency in this tube is 16,000 cycles per sec. (The tube is a 0.5 wave length oscillator and the velocity of sound in nickel is 4800 m. per sec.)

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[1] Curtius and Darapsky, *Ber.*, **35**, 3229 (1902).

[2] Alfred Bertho, *J. prakt. Chem.*, **120**, 89 (1928).

[3] Vasilevskii, Blotshtein and Kustrya, *Khim. Zhur.*, *Ser. A*, **5**, 1652 (1935).