excess of ammonium hydroxide solution was added. The excess was removed by boiling and a slight excess of acetic acid was added. On chilling the solution, a white solid separated in the form of small cubes; after drying, the material weighed 2.39 g. (84% yield). Upon purification by solution and reprecipitation, the **2-(1-hydroxyethyl)-3-isobutylcinchoninic acid** melted at 213.5–214.0° (cor.) (dec.).

Anal. Calcd. for $C_{16}H_{19}NO_3$: neut. equiv., 273.32; C, 70.31; H, 7.01; N, 5.13. Found: neut. equiv., 270.6; C, 70.00; H, 7.00; N, 5.11.

Action of Hydriodic Acid on 3-Isobutyl-2-(1-methoxyethyl)cinchoninic Acid.—As a result of heating 3 g. of this acid with 5 cc. of 57% hydriodic acid in a sealed tube at 160–170° for two hours, a 72% yield of the same hydroxyethyl derivative was obtained.

However, when 2.5 g. of the methoxyethyl derivative and 10 cc. of 57% hydriodic acid were heated in a sealed tube at 160–170° for forty-eight hours, reduction as well as hydrolysis occurred and there resulted 1.55 g. (87% yield) of **2-ethyl-3-isobutylcinchoninic acid**, which melted with decomposition at 206–207° (cor.).

Anal. Calcd. for $C_{16}H_{17}NO_2$: C, 74.67; H, 7.45; N, 5.44. Found: C, 74.51; H, 7.55; N, 5.40.

By heating together 2.06 g. of 3-isobutyl-2-(1-methoxyethyl)-cinchoninic acid, 1 g. of red phosphorus and 10 cc. of 57% hydriodic acid in a sealed tube at 160–170° for forty-eight hours, a further degree of reduction occurred and 1.74 g. (93% yield) of **2-ethyl-3-isobutyl-1,2,3,4-tetrahydrocinchoninic acid** was produced. The latter melts with slight decomposition at 212.5–213.0°; when mixed with 2-(1-hydroxyethyl)-3-isobutylcinchoninic acid, the mixture melted at 184–202°.

Anal. Calcd. for $C_{16}H_{23}NO_2$: C, 73.52; H, 8.78; N, 5.36. Found: C, 73.61; H, 8.81; N, 5.30.

Conversion of Isatin into Anthranilic Acid.—In order to establish the origin of the anthranilic acid isolated in the interaction of potassium isatinate and isoamyl 1-methoxyethyl ketone (page 2097), 10 g. of isatin was dissolved in 62 cc. of 40% potassium hydroxide solution and the solution was boiled under reflux for eight hours. During this period evolution of a small amount of ammonia was noted. The alkaline solution was chilled overnight, causing separation of crystalline material. The latter was dissolved in warm water, the solution was filtered and made barely acidic with hydrochloric acid. White crystals separated, were removed by filtration, and dried; weight 8.5 g. (91% yield) when recrystallized from hot water to melt with decomposition at 145° (corr.).[12] A mixture with an authentic sample of anthranilic acid melted at the same temperature.

Summary

1. In the attempted preparation of 3-isobutyl-2-(1-methoxyethyl)-cinchoninic acid, from interaction of isoamyl methoxyethyl ketone and isatin through the Pfitzinger synthesis, a molecular compound between that acid and anthranilic acid was obtained.

2. Separation of this molecular compound into its two components was accomplished through fractional sublimation.

3. Diazotization of the molecular compound yielded an analogous combination of salicylic acid and 3-isobutyl-2-(1-methoxyethyl)-cinchoninic acid.

4. Whereas hydrolysis and reduction of 3-isobutyl-2-(1-methoxyethyl)-cinchoninic acid proceeded in a normal manner, decarboxylation was not straightforward.

5. Anthranilic acid was prepared in good yield by heating isatin in boiling 40% potassium hydroxide solution.

(12) Lundén [*Z. physik. Chem.*, **54**, 537 (1906)] reported m. p. of 144.3° for anthranilic acid.

AUSTIN, TEXAS RECEIVED JUNE 29, 1944

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF GEORGE A. BREON AND COMPANY]

17-Isopregnan-3(α)-ol-20-one

BY ROBERT BRUCE MOFFETT AND WILLARD M. HOEHN

It has been shown by several workers[1] that steroids with a ketone group at C_{20} are partially isomerized at C_{17} by treatment with alkali. In the present work 17-isopregnan-3(α)-ol-20-one[2] was isolated from the mother liquors from the crystallization of pregnan-3(α)-ol-20-one which had been obtained by the saponification of its acetate by alkali. The acetate of pregnan-3(α)-ol-20-one was obtained by the Barbier–Wieland degradation of lithocholic acid[3] and was saponified by boiling with methanolic sodium hydroxide. After removing the alkali most of the pregnan-3(α)-ol-20-one was obtained by crystallization from cyclohexane. The filtrate was distilled to dryness *in vacuo* leaving an amorphous gum which could not be crystallized. The hydroxyl containing fraction was separated by treating the residue with succinic anhydride in pyridine and separating the acid succinate by its solubility in cold dilute alkali. By saponification of the crude acid succinate an amorphous material was obtained from which a ketone fraction was obtained by treatment with Girard reagent.[4] After decomposing the Girard reagent complex and extracting the "olone" fraction by ether, the ether solution was concentrated, giving a crystalline product. This crystalline material proved to be a mixture which

(1) (a) Butenandt and Mamoli, *Ber.*, **68B**, 1847 (1935); (b) Butenandt and Fleischer, *ibid.*, **70B**, 96 (1937); (c) Butenandt, Schmidt-Thomé and Paul, *ibid.*, **72B**, 1112 (1939); (d) Marker, Wittle and Plambeck, THIS JOURNAL, **61**, 1333 (1939).

(2) This compound has been reported by G. Müller, Danzig, Diss., 1938. "Über isomere Pregnan-ol-one (3, 20)" and abstracted by H. Seyle (Enclopedia of Endocrinology, Section I. The Steroids, Vol. *IV*, p. 579, A. W. T. Franks Publishing Company, Canada) with the remark that the structure is uncertain. The present work indicates conclusively that the compound reported by Müller is not 17-isopregnan-3(α)-ol-20-one.

(3) Hoehn and Mason, THIS JOURNAL, **62**, 569 (1940).

(4) This reagent (acethydrazidepyridinium bromide) was prepared by a procedure similar to that used by Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936), for the corresponding chloride except that ethyl bromoacetate was used in place of ethyl chloroacetate. It is a stable crystalline compound.

could be separated by chromatographic adsorption on alumina. The first few fractions to come off proved to be the normal pregnan-3(α)-ol-20-one. The last few fractions to come off were impure 17-isopregnan-3(α)-ol-20-one. This could not be satisfactorily purified by recrystallization or by rechromatographing on alumina, but was purified by rechromatographing on a mixture of "Darco" activated charcoal and "Hyflo Supercell." The 17-isopregnan-3(α)-ol-20-one fraction came off first and was recrystallized from ether.

In order to show the relationship between the normal and 17-iso-olones a sample of each was refluxed with methanolic sodium hydroxide. The rotation on each at the end of two hours had come to about the same point: $[\alpha]^{30}D$ +70 ± 5°, which is equivalent to an equilibrium mixture of about 71% normal and 29% isopregnanolone. The product from the treatment of the 17-isopregnan-3(α)-ol-20-one with sodium hydroxide was separated by chromatographic adsorption on alumina, and a sample of the normal pregnan-3(α)-ol-20-one was isolated. In a similar manner when samples of the normal and 17-isopregnanolones were refluxed with methanolic hydrogen chloride, the rotations of each came to about the same point: $[\alpha]^{30}D$ +75 ± 5°. This is similar to the isomerization of 17-isoprogesterone to progesterone by Butenandt, Schmidt-Thomé and Paul.[1c] To further characterize the 17-isopregnan-3(α)-ol-20-one its acetate and oxime were made. In order to show that no change in the configuration of the 3-OH group had taken place a sample of the 17-isopregnanolone was tested with digitonin. No precipitate was obtained.

Experimental[5]

Separation of Pregnan-3(α)-ol-20-one and 17-Isopregnan-3(α)-ol-20-one.—To 150 g. of amorphous residue from the crystallization of pregnan-3(α)-ol-20-one[2] was added 150 g. of succinic anhydride and 500 ml. of dry redistilled pyridine. The solution was heated in a water-bath at 60–65° for two hours. After standing overnight the solution was decanted from some crystals of succinic anhydride and poured into a mixture of ice and 600 ml. of hydrochloric acid. The gummy precipitate was extracted with ether and the ether solution was washed with water and then extracted with 1.5 liters of 2.5% ice-cold sodium hydroxide solution in two portions. The basic solution of the acid succinate was washed with ether, heated to boiling to remove dissolved ether and refluxed for two hours. A gummy precipitate separated which, after cooling, was extracted with ether, which was removed by distillation leaving a residue of 105 g. of hydroxyl-containing material.

This was mixed with 100 g. of Girard reagent[4] and 1 liter of 1.520 *N* methanolic acetic acid and refluxed for four hours. After cooling, the solution was poured into a mixture of ice and 526 ml. of 2.315 *N* sodium hydroxide solution and the mixture was repeatedly extracted with ether. The aqueous solution was made strongly acid with sulfuric acid and after standing several hours was extracted repeatedly with ether. After washing with water the ether solution was dried over anhydrous sodium sulfate and then distilled to dryness *in vacuo*. On rubbing the residue with ether it crystallized and was collected, giving 50 g. of nearly white crystalline material melting at 140–143°; $[\alpha]^{30}D$ +54 ± 5°.

A sample of 10 g. of this material was dissolved in 75 ml. of benzene and 25 ml. of petroleum ether (b. p. about 70°) and poured on a column containing 180 g. of Fisher Adsorption Alumina (80–200 mesh). The column was eluted with three 100-cc. portions of benzene, then four 100-cc. portions of 90% benzene–10% ether, then three 100-cc. portions of 80% benzene–20% ether, then three 100-cc. portions of 70% benzene–30% ether, and finally three portions of 60% benzene–40% ether. In all 16 fractions were collected and each was evaporated to dryness, weighed, and the melting point and rotation taken. A total of 9.9 g. of the material was accounted for. Fractions 5 and 6 (combined weight 1.4 grams) each melted at 145–149° and gave a rotation of $[\alpha]^{30}D$ +113 ± 5° in methanol. They were combined and recrystallized from acetone giving 0.98 g. of crystalline pregnan-3(α)-ol-20-one melting at 150–151°, $[\alpha]^{32}D$ +112 ± 5° (24.24 mg. made up to 2 ml. with methanol; *l* = 1 dm., $[\alpha]^{32}D$ +1.36° ± 0.1°).

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.18; H, 10.76. Found: C, 79.79; H, 10.98.

Fractions 14, 15 and 16 gave rotations of −4.1°, −15.5° and −20.4°, respectively, and rechromatographing on alumina failed to give appreciable separations. They were combined (weight 3.16 g.) dissolved in 100 ml. of benzene and poured on a column containing a mixture of 45 g. of "Darco" activated carbon and 45 g. of "Hyflo Super-cel." The column was eluted with four 50-ml. portions of benzene and the fractions were collected, evaporated to dryness, weighed and the rotations taken. Fraction number 3 (the first to contain appreciable material) weighed 1.7 g. and gave a rotation of $[\alpha]^{30}D$ −25° (in methanol). After one recrystallization from ether (cooled in refrigerator) 0.648 g. of 17-isopregnan-3(α)-ol-20-one was obtained, m. p. 142.5–144°; $[\alpha]^{32}D$ −40.9 ± 5° (55.86 mg. made up to 5 cc. with dioxane; *l* = 2 dm.; $[\alpha]^{32}D$ −0.915 ± 0.08°).

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.18; H, 10.76. Found: C, 79.48; H, 11.06.

Pregnan-3(α)-ol-20-one from 17-Isopregnan-3(α)-ol-20-one.—A sample of 204.7 mg. of 17-isopregnan-3(α)-ol-20-one was made up to 20 ml. with 2.5% methanolic sodium hydroxide solution and the rotation taken immediately: $[\alpha]^{30}D$ −27.7 ± 5° ($\alpha^{30}D$ −0.56 ± 0.1°; *l* = 2 dm.). There was no change in rotation on standing at room temperature (30°) for one-half hour. The solution was then refluxed for two hours and the rotation was again taken as $[\alpha]^{30}D$ +75 ± 5° ($\alpha^{30}D$ +1.53°; *l* = 2 dm.).

After cooling, the solution was poured into water and extracted with ether which was washed with water and dried over anhydrous sodium sulfate. The ether was removed *in vacuo* and the residue was dissolved in 20 ml. of 80% benzene and 20% petroleum ether (b. p. 70°) and poured on a column containing 10 g. of Fisher Adsorption Alumina. The column was eluted by 20-cc. portions of benzene and mixtures of benzene and ether in a manner similar to that used above. The first fraction to contain appreciable solid material (20 mg.) proved to be the normal pregnan-3(α)-ol-20-one; $[\alpha]^{35}D$ +112 ± 8° ($\alpha^{35}D$ +1.1 ± 0.1°; 19.6 mg. made up to 2 ml. with methanol; *l* = 1 dm.). After recrystallization from acetone it melted at 148–150° and a mixed melting point with the normal pregnanolone gave no depression, but a mixed melting point with the 17-isopregnanolone melted at 138.5–141.5°.

Treatment of (Normal) Pregnan-3(α)-ol-20-one with Sodium Hydroxide.—A solution of 53.2 mg. of pregnan-3(α)-ol-20-one ($[\alpha]^{35}D$ +114 ± 5°) made up to 5.00 ml. with 2.5% methanolic sodium hydroxide solution was refluxed for one hour. The rotation was then taken: $[\alpha]^{34}D$ +68.5 ± 6° ($\alpha^{34}D$ +1.45 ± 0.1°; *l* = 2 dm.).

Treatment of (Normal) Pregnan-3(α)-ol-20-one with Hydrogen Chloride.—A sample of 0.1135 g. of pregnan-3(α)-ol-20-one was dissolved in 4.3% methanolic hydrogen chloride and made up to 10 ml. The rotation was taken immediately: $[\alpha]^{29}D$ +114.5 ± 5° ($\alpha^{29}D$ +2.6 ± 0.1°; = 2 dm.). After refluxing for three and one-quarter

(5) All melting points are corrected: C, H, and N analyses by Arlington Laboratories.

hours the rotation was again taken: $[\alpha]^{30}D$ +77.2 ± 5° ($\alpha^{30}D$ +1.75 ± 0.1°; l = 2 dm.).

Treatment of 17-Isopregnan-3(α)-ol-20-one with Hydrogen Chloride.—In a manner similar to the above a solution of 44.7 mg. of 17-isopregnan-3(α)-ol-20-one made up to 5 ml. with 4.3% methanolic hydrogen chloride was refluxed for four hours and then the rotation was taken: $[\alpha]^{31}D$ +75.6 ± 5° ($\alpha^{31}D$ +1.35 ± 0.1°; l = 2 dm.).

Acetate of 17-Isopregnan-3(α)-ol-20-one.—A solution of 100 mg. of 17-isopregnan-3(α)-ol-20-one in 1 ml. of acetic anhydride and 0.66 ml. of acetic acid was refluxed for twenty minutes. After standing for three hours the solution was diluted to turbidity with water. The acetate separated in fine needles and was collected, weight 95 mg. After one recrystallization from methanol it melted at 157–159° and mixed melting points with both the normal and 17-isopregnanolones gave about 30° depressions: $[\alpha]^{34}D$ −28.2 ± 8° (24.16 mg. made up to 2 cc. with methanol; l = 1 dm.; $\alpha^{34}D$ −0.34 ± 0.1°).

Anal. Calcd. for $C_{23}H_{36}O_2$: C, 76.61; H, 10.07. Found: C, 76.82; H, 10.32.

Oxime of 17-Isopregnan-3(α)-ol-20-one.—A solution of 100 mg. of 17-isopregnan-3(α)-ol-20-one, 100 mg. of hydroxylammonium chloride, and 150 mg. of sodium acetate (trihydrate) in 2 ml. of methanol and 0.5 ml. of water was refluxed for two hours. After cooling the solution was poured into water, extracted with ether, and the ether solution was dried over anhydrous sodium sulfate. The ether was removed and the residue was crystallized first from dilute acetone and then from methanol giving a white crystalline oxime melting at 192–197° (with decomposition). A mixed melting point with a sample of the oxime of the normal pregnan-3(α)-ol-20-one[6] gave a depression of about 20°.

Anal. Calcd. for $C_{21}H_{35}O_2N$: N, 4.20. Found: N, 4.57.

Test with Digitonin.—To about 1 mg. of 17-isopregnan-3(α)-ol-20-one in a few drops of 90% methanol was added an equal volume of a saturated solution of digitonin in 90% methanol. No precipitate was obtained even after standing for several hours.

Summary

17-Isopregnan-3(α)-ol-20-one has been isolated from the mother liquor from the crystallization of pregnan-3(α)-ol-20-one prepared by alkaline hydrolysis of its acetate, and the acetate and oxime were prepared.

Refluxing normal or isopregnanolone with either methanolic sodium hydroxide or hydrogen chloride solution produced mixtures of these isomers in approximately the same ratio.

(6) Butenandt and Müller, *Ber.*, **71**, 191 (1938).

KANSAS CITY, MISSOURI RECEIVED SEPTEMBER 30, 1944

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

Phosphorescence and the Triplet State

BY GILBERT N. LEWIS AND M. KASHA

1. **Introduction.**—The property of afterglow, or phosphorescence, in spite of its inherent interest and practical importance, has seemed far removed from more commonly studied physical and chemical properties. We propose, however, to show that a spectroscopic study of phosphorescence provides quantitative data concerning an extremely important state of every type of molecule, the triplet state.

Phosphors may be divided into two classes.[1] The first comprises the "mineral phosphors," in which the active centers are regions of physical or chemical inhomogeneity in a crystalline mass. Their activity cannot be ascribed to well-defined molecular species. In phosphors of the other class phosphorescence may be ascribed to a definite substance, whether this substance is in the pure crystalline state, or adsorbed on a foreign surface (including adsorption on the filaments which form the structure of a gel), or finally, dissolved to form a homogeneous transparent solution in a solvent which is usually, through supercooling, in a rigid or glassy state. It is with these homogeneous solutions that the present paper will deal.[2]

As to the cause of phosphorescence, it is supposed in the case of mineral phosphors that light causes the ejection of an electron from some point in the phosphor, and that after a time the electron returns with re-emission of light. This is certainly not the phosphorescent process for an organic substance dissolved in a rigid medium. The ejection of electrons from many such molecules has recently been studied.[3,4,5,6] The energy necessary for electron ejection is higher than that needed for phosphorescence, and the absorption spectrum of the resulting substance is entirely different from that in the phosphorescent state, as illustrated in the case of diphenylamine.[3] Moreover, such a mechanism would be incompatible with the observed[7,8,9,10] strictly exponential decay of an organic phosphor. This first-order rate of decay proves that a single molecule in the phosphorescent state undergoes the process or processes responsible for phosphorescence.

(1) For the earlier literature concerning phosphors we may refer to P. Pringsheim, "Fluorescenz und Phosphorescenz," 3rd ed., Julius Springer, Berlin, 1928, and P. Lenard, F. Schmidt and R. Tomaschek, "Handbuch der Experimental Physik," **23**, Akad. Verlag., Leipzig, 1928.

(2) Many of the substances whose phosphorescence we have studied in rigid solutions give equally characteristic phosphorescence spectra in the pure crystalline state, either at room temperature, or at the temperature of liquid air, usually with a considerable displacement toward the red. It has seemed best to restrict our attention at present to the more easily interpretable dilute solutions.

(3) Lewis and Lipkin, THIS JOURNAL, **64**, 2801 (1942).

(4) Lewis and Bigeleisen, *ibid.*, **65**, 520 (1943).

(5) Lewis and Bigeleisen, *ibid.*, **65**, 2419 (1943).

(6) Lewis and Bigeleisen, *ibid.*, **65**, 2424 (1943).

(7) R. Tomaschek, *Ann. Physik*, **67**, 612 (1922).

(8) Schischlowski and Wawilow, *Physik. Z. Sowjetunion*, **5**, 379 (1934).

(9) Lewschin and Vinokurov, *ibid.*, **10**, 10 (1936).

(10) Lewis, Lipkin, and Magel, THIS JOURNAL, **63**, 3005 (1941).