[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ROCHESTER]

β-Erythroidine. II. The Conversion of β-Erythroidine to Derivatives of the Desmethoxy Series[1,2]

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In general drugs exhibiting peripheral curariform activity have in common the structural feature of a quaternary ammonium ion.[3] The behavior of β-erythroidine is anomalous in this regard for it is much more effective as a tertiary base than as a quaternary salt.[4] We have, therefore, initiated an investigation of β-erythroidine in the hope of establishing the structural features responsible for its activity.

Our present knowledge of the chemistry of β-erythroidine is largely due to the work of Folkers and his collaborators. Folkers and Major isolated β-erythroidine from the seeds of *Erythrina americana* Mill and established its molecular formula as $C_{16}H_{19}NO_3$.[5a,5b] Later work showed that β-erythroidine contains two reducible double bonds and that hydrogenation can be controlled to give either the dihydro or tetrahydro derivatives.[6a,6b] The presence of a lactone ring is indicated by the fact that β-erythroidine, which is not acidic, can be converted by alkali to the corresponding salt and this is reconverted by acid to β-erythroidine.[5b]

In addition, Folkers and his co-workers have stated that the nitrogen atom of β-erythroidine is common to two rings,[7a,7b] that indole is produced on fusion of the alkaloid with potassium hydroxide,[8a,8b] and that by certain degradative procedures they have been able to convert β-erythroidine in turn to 3-methoxyphthalic anhydride and to hemipinic anhydride.[8a] However, as yet, no experimental details of this work have been published.

The object of this publication is to report on the behavior of β-erythroidine toward acidic reagents. In 1946 Dietz and Folkers reported on a spectrophotometric method of analysis for β-erythroidine which was based on the fact that the alkaloid, after treatment with concentrated sulfuric acid, gives a purple color with ferric chloride solution.[9] No experimental details regarding the material responsible for the coloration were given. We have now found that, depending on the conditions employed, any one of three different isomeric derivatives of β-erythroidine may be obtained by acid treatment.

When β-erythroidine was treated with anhydrous hydrogen fluoride at room temperature, there was obtained a white solid, m. p. 108–109°, having the empirical formula $C_{15}H_{15}NO_2$. This product differs from β-erythroidine by the elements of methanol, and we have named it desmethoxy-β-erythroidine since it would appear to have been formed by the simple loss of a methoxyl group with introduction of a double bond. This conclusion is based on the fact that β-erythroidine was found to have a methoxyl group by the Zeisel determination, whereas desmethoxy-β-erythroidine gave a negative result. Also, hydrogenation of desmethoxy-β-erythroidine in acid solution gave a hexahydro derivative, indicating that desmethoxy-β-erythroidine has one more double bond than does β-erythroidine.

On the other hand, treatment of β-erythroidine with either phosphoric or sulfuric acid at 120° gave a different product, m. p. 132–132.5°. This material, which is isomeric with desmethoxy-β-erythroidine.[10] The temperature employed is a critical factor in the formation of apo-β-erythroidine for, when β-erythroidine was treated with phosphoric acid at 80°, the product was desmethoxy-β-erythroidine.

The formation of apo-β-erythroidine apparently involves rearrangement as well as loss of a methoxyl group. This view is supported by the fact that desmethoxy-β-erythroidine is converted by phosphoric acid at 120° to apo-β-erythroidine. Additional evidence for a difference in molecular structure lies in the fact that apo-β-erythroidine yielded only a tetrahydro derivative on hydrogenation. Also, bromine titration of apo-β-erythroidine gave an uptake of four bromine atoms.

Attempts to purify apo-β-erythroidine by chromatography over alumina led to a third isomeric product. This product, m. p. 146–147°, is yellow and has been named isoapo-β-erythroi-

(1) Aided by a grant from the National Foundation for Infantile Paralysis.

(2) For the first paper of this series, see Sauvage, Berger and Boekelheide, *Science*, **109**, 627 (1949).

(3) Craig, *Chem. Rev.*, **42**, 285 (1943).

(4) Unna, Kniazuk and Greslin, *J. Pharm.*, **80**, 39 (1944).

(5) (a) Folkers and Major, THIS JOURNAL, **59**, 1580 (1937). (b) See also Folkers and Major, U. S. Patent 2,385,266, Sept. 18, 1945.

(6) (a) Folkers and Koniuszy, U. S. Patent 2,370,651, March 6, 1945. (b) Folkers and Major, U. S. Patent 2,280,837, April 28, 1942.

(7) (a) Folkers and Koniuszy, Abstracts of Papers, 97th Meeting of the American Chemical Society, Baltimore, Maryland, April, 1939, Division of Organic Chemistry, page 17; (b) Folkers and Koniuszy, THIS JOURNAL, **61**, 3053 (1939).

(8) (a) Folkers, Koniuszy and Shavel, Abstracts of Papers, 102nd Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1941, Division of Organic Chemistry, page 30; (b) Folkers, Koniuszy and Shavel, THIS JOURNAL, **64**, 2146 (1942).

(9) Dietz and Folkers, *J. Am. Pharm. Ass., Sci. Ed.*, **35**, 48 (1946).

(10) Dietz and Folkers (ref. 9) indicated that the material responsible for the color in their analytical method had a molecular formula of $C_{15}H_{15}NO_2$ and they referred to it as apo-β-erythroidine. By private communication from Dr. Folkers we have learned that the material to which we have given the name apo-β-erythroidine is undoubtedly the same as that referred to by the same name in their publication.

dine. Apparently isoapo-β-erythroidine differs from apo-β-erythroidine only in the position of a double bond for, on hydrogenation, isoapo-β-erythroidine gave the same tetrahydro derivative as was obtained from apo-β-erythroidine. Likewise the bromine titration of isoapo-β-erythroidine showed an uptake of four bromine atoms. Isoapo-β-erythroidine was found to differ markedly from apo-β-erythroidine, though, in the basicity of its nitrogen atom. Apo-β-erythroidine gave stable ammonium salts, but in the case of isoapo-β-erythroidine these derivatives were too unstable to be isolated.

A summary of the reactions carried out on β-erythroidine is given in Fig. 1. The question of the structural relationships involved in these transformations is not yet clear. However, the evidence at hand does not favor the presence of a benzenoid ring in any of the three desmethoxy derivatives. Although apo-β-erythroidine and isoapo-β-erythroidine both gave a violet coloration with ferric chloride solution, this color is the result of oxidation rather than enolic-complex formation. Both dilute nitric acid and dilute hydrogen peroxide gave the same color reaction. The appearance of color was found to be dependent on *p*H and, at values above 4.2–5.0, the solutions became colorless. A relationship between color formation and the character of the nitrogen atom is indicated by the fact that apo-β-erythroidine methiodide gave no color in this test.

It is of interest that apo-β-erythroidine shows a central action in effecting muscular relaxation that is of fairly long duration. This is in contrast with β-erythroidine which shows predominantly a peripheral action of short duration.[11]

Further work, including ultraviolet absorption spectra studies, is in progress and will be reported shortly.

Acknowledgment.—We are indebted to Merck and Company, Rahway, New Jersey, for the β-erythroidine hydrochloride used in this study.

Experimental[12]

β-Erythroidine.—The free base was obtained by dissolving β-erythroidine hydrochloride (5.0 g.) in 20 ml. of water and then sufficient sodium bicarbonate was added to make the solution slightly basic. The aqueous solution was extracted five times with 100 ml. of benzene per extraction. The combined benzene extracts were concentrated under vacuum causing separation of the free base. In this way a 98% recovery of β-erythroidine, m. p. 97–98°, was obtained.[13]

Anal. Calcd. for $C_{16}H_{19}NO_3$: —OCH_3, 11.35. Found: —OCH_3, 11.10; terminal methyl (Kuhn–Roth), 0.17.

The **methiodide** of β-erythroidine formed readily in alcohol and was obtained, after crystallization from alcohol, as white prisms, m. p. 211°.

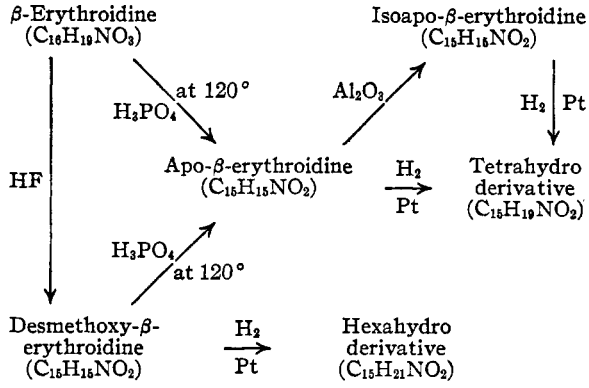

Fig. 1.

Anal. Calcd. for $C_{17}H_{22}NO_3I$: C, 49.16; H, 5.34. Found: C, 49.00; H, 5.29.

Desmethoxy-β-erythroidine. (a) **Hydrogen Fluoride Method.**—To 35 g. of anhydrous hydrogen fluoride in a copper beaker there was added slowly 3.4 g. of β-erythroidine hydrochloride. The hydrogen fluoride was allowed to evaporate and approximately 50 ml. of ice and water was added. After the solution was made basic with sodium bicarbonate, it was extracted five times with 150 ml. of benzene per extraction. The combined extracts were washed with water and concentrated under vacuum. The colorless oil, which remained, crystallized after standing for several days at 5°. The crystals were washed with ether and there was obtained 2.1 g. (80%) of white needles, m. p. 108–109°.

Anal. Calcd. for $C_{15}H_{15}NO_2$: C, 74.66; H, 6.26. Found: C, 74.74; H, 6.06; —OCH_3, trace.

The **methiodide** of desmethoxy-β-erythroidine formed readily in alcohol and, after crystallization from alcohol, was obtained as small white needles, m. p. 166°.

Anal. Calcd. for $C_{16}H_{18}NO_2I$: C, 50.14; H, 4.73. Found: C, 50.22; H, 4.87.

(b) **Phosphoric Acid Method.**—A solution of β-erythroidine hydrochloride (0.50 g.) in 4 ml. of sirupy phosphoric acid was heated at 80° for two hours under a nitrogen atmosphere. After the solution had cooled, it was made basic with aqueous sodium bicarbonate and was extracted five times with benzene. The benzene was removed *in vacuo* leaving 155 mg. of a clear oil, which after it was seeded with crystals of desmethoxy-β-erythroidine, turned to a white solid, m. p. 105–106°. The methiodide was prepared and found to melt at 164–165°. A mixture of the methiodide, thus prepared, and that obtained by the hydrogen fluoride method melted at 165–167°. Pure desmethoxy-β-erythroidine gives no coloration with an acidic ferric chloride solution.

Hydrogenation of Desmethoxy-β-erythroidine.—A solution of 0.27 g. of desmethoxy-β-erythroidine and 0.20 g. of prereduced platinum oxide catalyst in 200 ml. of absolute ethanol containing two equivalents of hydrogen chloride was shaken at room temperature under an atmospheric pressure of hydrogen until hydrogen absorption was complete. The hydrogen absorption corresponded to 3 mole equivalents and the times per mole equivalent were three, seven and one hundred thirty-eight minutes, respectively. The catalyst and solvent were removed, and the residue was treated with a slight excess of aqueous sodium bicarbonate. The aqueous solution was extracted several times with ether, and the ether was removed. There was obtained 190 mg. (70%) of a colorless oil which could not be induced to crystallize. The methiodide of hexahydrodesmethoxy-β-erythroidine was prepared in alcohol and obtained, after crystallization from alcohol, as white prisms, m. p. 246°.

Anal. Calcd. for $C_{16}H_{24}NO_2I$: C, 49.36; H, 6.21. Found: C, 49.54; H, 6.03.

(11) Preliminary results on the pharmacology of the desmethoxy derivatives are given in ref. 2.

(12) Microanalyses by Mrs. G. L. Sauvage and by the Micro-Tech Laboratories.

(13) Folkers and Major (ref. 5a) give 94–96°.

When the hydrogenation of desmethoxy-β-erythroidine was carried out with platinum oxide catalyst in a neutral solution, a mixture of products resulted consisting apparently of both dihydro and tetrahydro derivatives of desmethoxy-β-erythroidine. The nature of their formation is not clear as yet, and further work is in progress to establish these compounds.

Apo-β-erythroidine.—A solution of 4.8 g. of β-erythroidine hydrochloride in 30 ml. of sirupy phosphoric acid (85%) was heated for two hours at 120° under an atmosphere of nitrogen. The solution was then cooled, neutralized with sodium hydroxide solution, and extracted several times with benzene. The benzene extract was washed with water and concentrated, whereupon a white solid separated. After crystallization from alcohol there was obtained 1.50 g. (40%) of white prisms, m. p. 132–132.5°.

Anal. Calcd. for $C_{15}H_{15}NO_2$: C, 74.66; H, 6.26. Found: C, 74.94; H, 6.27.

The **hydrochloride** of apo-β-erythroidine was prepared in benzene with anhydrous hydrogen chloride and was obtained as white needles, m. p. 179°, with dec.

Anal. Calcd. for $C_{15}H_{16}NO_2Cl$: C, 64.86; H, 5.81. Found: C, 64.46; H, 5.63.

The **methiodide** of apo-β-erythroidine formed slowly in ethanol and was obtained as white prisms, m. p. 189°.

Anal. Calcd. for $C_{16}H_{18}NO_2I$: C, 50.14; H, 4.74. Found: C, 49.80; H, 4.69.

Apo-β-erythroidine gave a deep violet color (max. 527 μ) with ferric chloride solution. The same color was produced with dilute nitric acid and dilute hydrogen peroxide. This color was found to be dependent on *p*H and at a *p*H above 5.0 the solution became colorless. It is of interest that apo-β-erythroidine methiodide gave no coloration in this test.

Isomerization of Desmethoxy-β-erythroidine to Apo-β-erythroidine.—When a solution of 0.15 g. of desmethoxy-β-erythroidine in 3 ml. of sirupy phosphoric acid (85%) was heated at 125° for one hour and the product was isolated as given above for the preparation of apo-β-erythroidine, there was obtained a slightly yellow solid, m. p. 128–129°. The methiodide of this product was found to melt at 186–187° and a mixture of it with apo-β-erythroidine methiodide melted at 187–189°. The identity of this material with apo-β-erythroidine is clear.

Hydrogenation of Apo-β-erythroidine.—A solution of 0.59 g. of apo-β-erythroidine and 0.20 g. of prereduced platinum oxide catalyst in 100 ml. of ethanol containing two equivalents of hydrogen chloride was shaken at room temperature under an atmospheric pressure of hydrogen until no further absorption occurred. Hydrogen absorption corresponded to two moles. The first mole was taken up in 40 minutes and the second in 290 minutes. The catalyst was removed and the solution was concentrated causing the precipitation of a solid. This solid was crystallized from alcohol, yielding 0.48 g. (70%) of white prisms, m. p. 211°. This proved to be the hydrochloride of tetrahydroapo-β-erythroidine.

Anal. Calcd. for $C_{15}H_{20}NO_2Cl$: C, 63.93; H, 7.15. Found: C, 64.04; H, 6.88.

The free base was obtained by neutralization of an aqueous solution of the hydrochloride followed by extraction with benzene. The base, after crystallization from alcohol, was obtained as white prisms, m. p. 147°.

Anal. Calcd. for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81. Found: C, 73.58; H, 7.41.

When the hydrogenation of apo-β-erythroidine was carried out in a neutral solution, hydrogenation was very slow but the product was the same as above.

Isoapo-β-erythroidine.—When a solution of 0.50 g. of apo-β-erythroidine in 50 ml. of benzene was passed over a column of activated alumina, a yellow band appeared on the column. The band was developed by elution with alcohol and concentration of the yellow alcoholic solution yielded about 0.20 g. (40%) of yellow needles, m. p. 146–147°.

Anal. Calcd. for $C_{15}H_{15}NO_2$: C, 74.66; H, 6.26. Found: C, 74.81, 74.52; H, 6.39, 6.20.

Isoapo-β-erythroidine appeared to form a hydrochloride and also a methiodide, but attempts to isolate these derivatives gave no useful product. Isoapo-β-erythroidine gave a violet color (max., 530 μ) with ferric chloride solution, dilute nitric acid, and dilute hydrogen peroxide. The color disappeared at a *p*H above 4.2.

Hydrogenation of Isoapo-β-erythroidine.—The hydrogenation was carried out as described for apo-β-erythroidine. The melting point of the free base, thus obtained, and its hydrochloride agreed with that of tetrahydroapo-β-erythroidine and its hydrochloride. Also, a comparison of the two sets of derivatives by mixed melting point determination showed them to be the same.

Bromine Titration of Apo-β-erythroidine and Isoapo-β-erythroidine.—To a weighed amount of apo-β-erythroidine or isoapo-β-erythroidine in 10 ml. of carbon tetrachloride there was added dropwise a solution of 2.68 g. of bromine in 20 ml. of carbon tetrachloride. In each case the point at which persistent coloration occurred corresponded roughly to four equivalents of bromine. A yellow solid separated from solution during addition in each case and the weight of the product corresponded closely to that calculated for a quantitative yield with addition of four bromine atoms. After separation of the solid product, the carbon tetrachloride was washed with water and the washings were titrated with base. No appreciable amount of acid was found.

The yellow solids, obtained in each case, were quite unstable and could not be recrystallized without decomposition involving loss of hydrogen bromide. When a sample of the product from the bromination of apo-β-erythroidine was taken directly from the reaction mixture and analyzed, the presence of four bromine atoms was indicated.

Anal. Calcd. for $C_{15}H_{15}NO_2Br_4$: C, 32.29; H, 2.69. Found: C, 34.59; H, 2.57.

The possibility that a monobromo-perbromide was formed seems unlikely since a solution of the bromination product gave no color on testing with starch–iodide solution.

Action of Acids on Dihydro-β-erythroidine.—The conditions used for converting β-erythroidine to the desmethoxy derivatives were also tried with dihydro-β-erythroidine. With hydrogen fluoride dihydro-β-erythroidine was recovered unchanged. Treatment of dihydro-β-erythroidine with phosphoric or sulfuric acid at 120° caused marked blackening of the solution and no useful products could be recovered.

Summary

The action of acids of β-erythroidine has been found to yield three new derivatives: desmethoxy-β-erythroidine, apo-β-erythroidine, and isoapo-β-erythroidine. These derivatives are of interest in that they help define the structure of β-erythroidine. Also, apo-β-erythroidine has interesting pharmacological properties.

ROCHESTER, NEW YORK RECEIVED AUGUST 31, 1949