[CONTRIBUTION FROM THE WILLIAM G. KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Synthesis of a Biologically Active Nicotinic Acid Precursor: 2-Amino-3-hydroxybenzoic Acid

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Recent investigations[1,2] in this Laboratory have provided evidence that the mold *Neurospora* synthesizes nicotinic acid from tryptophan through the intermediates kynurenine and hydroxyanthranilic acid (2-amino-3-hydroxybenzoic acid). Although it has also been established that higher animals have the capacity for converting tryptophan to nicotinic acid[3,4] it is not yet known whether or not the mechanism is similar to that in *Neurospora*. In order to provide material for further investigations along these lines, it has been necessary to develop methods for synthesis of 2-amino-3-hydroxybenzoic acid. Since this compound has evidently not been previously synthesized, two independent methods of preparation have been devised in order to establish the structure of the products.

A purported preparation of 2-amino-3-hydroxybenzoic acid by degradation of the alkaloid damascenine was reported by Keller in 1908.[5] It is quite evident, however, that the product described by Keller is actually 2-amino-3-methoxybenzoic acid. The data in Table I are in accord with this conclusion.

TABLE I

MELTING TEMPERATURES OF 2-AMINO-3-HYDROXYBENZOIC ACID, 2-AMINO-3-METHOXYBENZOIC ACID, THE COMPOUND OF KELLER AND THE CORRESPONDING HYDROCHLORIDES

	Compound	M. p., °C.
1	2-Amino-3-hydroxybenzoic acid[a]	254–255 (cor.)
2	2-Amino-3-methoxybenzoic acid[a]	171 (cor.)
3	Compound of Keller	164
4	Hydrochloride of 1[a]	227 (cor.)
5	Hydrochloride of 2[a]	205–206 (cor.)
6	Hydrochloride of 3	199–200

[a] Synthesized in this Laboratory.

In addition to the data in Table I, Keller stated that he did not report a carbon analysis because it was too high. He did report a halogen analysis on the hydrochloride but miscalculated the theoretical value and his analysis actually checks very well with the theoretical figure for the hydrochloride of 2-amino-3-methoxybenzoic acid.

The first method for synthesis of 2-amino-3-hydroxybenzoic acid that has been utilized in this Laboratory involves reduction of 2-nitro-3-methoxybenzoic acid followed by demethylation. The nitro compound was previously prepared from 3-methoxybenzoic acid by Ewins[6] and its reduction by chemical means has been reported by several workers.[6,7,8]

In the present work, the reduction was carried out by catalytic hydrogenation and the final demethylation step by treatment of 2-amino-3-methoxybenzoic acid with hydriodic acid.

The second method of synthesis that is described in this paper involves oxidation of 8-methoxyquinoline to give 2-(N-methyl-N-formyl)-amino-3-methoxybenzoic acid.[9] By appropriate treatment of this product with hydriodic acid 2-amino-3-hydroxybenzoic acid was obtained. The products from the two methods of synthesis possessed identical physical properties and the same biological activity for *Neurospora*.[2]

Experimental

2-Nitro-3-methoxybenzoic Acid.—This compound was prepared by a modification of the method of Ewins.[6] A mixture of 10 g. of 3-methoxybenzoic acid and 40 ml. of nitric acid (sp. gr. 1.4) was heated gently in a 250 ml. flask. After the beginning of the exothermic reaction the mixture was maintained at 55° by occasional immersions in cold water. A voluminous precipitate of nitration products appeared as the reaction progressed. After standing three hours at room temperature the precipitate was filtered off, washed with water and treated with three times its weight of boiling ethyl alcohol. The esulting suspension was filtered hot and the undissolved material was crystallized from a minimum amount of boiling ethanol; yield, 0.85 g., 2-nitro-3-methoxybenzoic acid, m. p. 260–263° (cor.). An additional 0.15 g. of the desired product was obtained by two recrystallizations of the material precipitated after cooling of the filtrate of the first hot alcohol extraction.

2-Amino-3-methoxybenzoic Acid.—One-half gram of 2-nitro-3-methoxybenzoic acid was dissolved in 50 ml. of absolute ethanol and hydrogenated at 1 atmosphere pressure and at room temperature, in the presence of 100 mg. of 5% palladium-on-charcoal. Following filtration the solution was evaporated to dryness and the product was crystallized from boiling benzene, yield, 0.4 g., m. p. 170–171° (cor.).

Anal. Calcd. for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.73; H, 5.49; N, 8.54. Absorption spectrum Fig. 1, curve B. The hydrochloride of this compound crystallized from concentrated aqueous hydrochloric acid, m. p. 205–206° (cor.).

2-Amino-3-hydroxybenzoic Acid. Method I.—A mixture of 200 mg. of 2-amino-3-methoxybenzoic acid, 50 mg. of red phosphorus and 4 ml. of hydriodic acid (sp. gr. 1.7) was heated in a sealed tube at 100° for eight hours. The hydriodic acid salt of 2-amino-3-hydroxybenzoic acid crystallized on cooling. After filtration this product, containing phosphorus, was dissolved in 15 ml. of water and again filtered. Solid sodium carbonate was carefully added to the filtrate until the acid reaction to congo red just disappeared. The crude product was obtained as a crystalline powder, m. p. 238–242°. Recrystallization from ethanol yielded 124 mg. of compound, m. p. 254–255° (cor.); absorption spectrum, Fig. 1, curve A.

(1) Beadle, Mitchell and Nyc, *Proc. Nat. Acad. Sci.*, **33**, 155 (1947).
(2) Mitchell and Nyc, *Proc. Nat. Acad. Sci.*, **34**, 1 (1948).
(3) Krehl, Tepley, Sarma and Elvehjem, *Sci.*, **101**, 489 (1945).
(4) Sarett and Goldsmith, *J. Biol. Chem.*, **167**, 293 (1947).
(5) Keller, *Arch. der Pharm.*, **246**, 1 (1908).
(6) Ewins, *J. Chem. Soc.*, **101**, 549 (1912).
(7) Pschorr, *Ann.*, **391**, 27 (1912).
(8) Froelicher and Cohn, *J. Chem. Soc.*, **119**, 1425 (1921).
(9) Kaufmann and Rothlen, *Ber.*, **49**, 578 (1916).

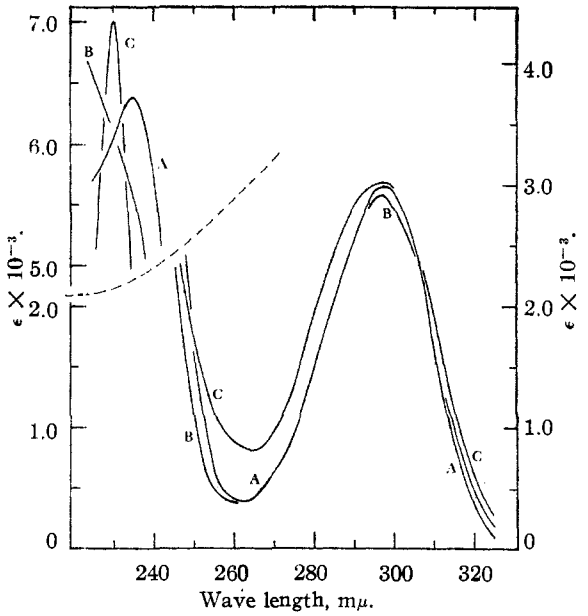

Fig. 1.—Absorption spectra in 0.1 *M* hydrochloric acid: A, 2-amino-3-hydroxybenzoic acid; B, 2-amino-3-methoxybenzoic acid; C, 2-(N-methyl-N-formyl)-amino-3-methoxybenzoic acid.

Anal. Calcd. for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15. Found: C, 55.00; H, 4.83; N, 9.13.

2-Amino-3-hydroxybenzoic Acid Hydrochloride.—This compound crystallized from a hot solution of 2-amino-3-hydroxybenzoic acid in concentrated aqueous hydrochloric acid, m. p. 227° (cor.). *Anal.* Calcd. for $C_7H_8NO_3Cl$: N, 7.39; Cl, 18.70. Found: N, 7.68; Cl, 18.59.

2-Amino-3-hydroxybenzoic Acid. Method II.—2-(N-Methyl-N-formyl)-amino-3-methoxybenzoic acid was prepared according to the procedure of Kaufmann and Rothlen.[9] A mixture of 200 mg. of this compound, 50 mg. of red phosphorus and 4 ml. of hydriodic acid (sp. gr. 1.7) was heated in a sealed tube at 135° for twelve hours. The product was isolated and purified by the procedure described under Method I, yield 57 mg., m. p. 254–255° (cor.), hydrochloride m. p. 227° (cor.).

The products from both methods I and II possessed identical absorption spectra and biological activity on a nicotinic acid requiring mutant of *Neurospora*.[2]

Discussion

Hydroxyanthranilic acid (2-amino-3-hydroxybenzoic acid) has been synthesized by two independent methods each providing in itself nearly conclusive evidence for the structure of the product. It is evident from a consideration of the properties of these products and from the facts given by Keller that the latter investigator did not prepare hydroxyanthranilic acid as reported. No evidence has been found for a synthesis of the compound prior to the present work.

It is to be noted from Fig. 1 that the absorption spectra of some methyl and formyl substituted 2-amino-3-hydroxybenzoic acids are quite similar to that of the parent compound. This is true also for damascenine and damasceninic acid. In the case of the unsubstituted acid considerable variations in spectrum have been observed at wave lengths below 260 mμ. This is evidently due to traces of impurities derived from oxidation of the compound in mildly acid or alkaline solutions. A pure white product can be obtained only from acidic solutions since oxidation is rapid even at neutrality.

Summary

1. Two independent methods have been described for the synthesis of 2-amino-3-hydroxybenzoic acid.

2. Data have been presented to show that the product previously obtained by Keller and reported to be 2-amino-3-hydroxybenzoic acid was actually 2-amino-3-methoxybenzoic acid.

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X-Ray Investigation of Glycerides. VII. Diffraction Analyses of Synthetic 1,3-Dielaidin[1]

BY B. F. DAUBERT AND S. S. SIDHU

In a recent publication by Carter and Malkin,[2] X-ray diffraction data were reported for a series of unsaturated symmetrical 1,3-diglycerides, including 1,3-diolein, 1,3-dierucin, and their *trans* isomers, 1,3-dielaidin and 1,3-dibrassidin.

In our study of the physical properties of synthetic glycerides, an X-ray diffraction study of 1,3-dielaidin had been completed but not reported prior to the publication by Carter and Malkin,[2] although X-ray diffraction data for 1,3-diolein, 1,3-dilinolein, and 1,3-dilinolenin were recently reported by Daubert and Lutton.[3]

The purpose, therefore, of the present communication is to report the X-ray data on 1,3-dielaidin prepared both by direct synthesis and elaidinization of 1,3-diolein.

Experimental

Preparation of 1,3-Dielaidin.—1-Monotrityl glycerol (10 g.) (m. p. 109.5–110.0°) was dissolved, with slight warming, in a mixture of quinoline (15 ml.) and dry chloroform (40 ml.). To this mixture elaidyl chloride (18.0 g.) was added slowly. The mixture, after standing

(1) The generous financial assistance of the Buhl Foundation in support of this investigation is gratefully acknowledged.

(2) Carter and Malkin, *J. Chem. Soc.*, 554 (1947).

(3) Daubert and Lutton, THIS JOURNAL, **69**, 1449 (1947).