[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Vitamin B_{12}. X. 5,6-Dimethylbenzimidazole, a Degradation Product of Vitamin B_{12}

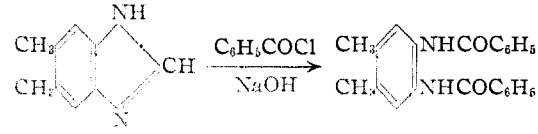
BY NORMAN G. BRINK AND KARL FOLKERS

Acid hydrolysis of vitamin B_{12} yielded a basic product which was identified by degradation and by synthesis as 5,6-dimethylbenzimidazole (I).[1] This observation was recently confirmed by others[2] who based their identification of 5,6-dimethylbenzimidazole on spectrophotometric evidence. It is the purpose of the present communication to describe the details of the isolation and identification of the degradation product.

A solution of vitamin B_{12} in 6 *N* hydrochloric acid was heated at 150° for twenty hours. Continuous chloroform extraction of an alkaline aqueous solution of the products of hydrolysis gave an extract whose content was further purified by removal of an ether-insoluble fraction. The ether-soluble material was heated *in vacuo* at about 140°, yielding a partially crystalline sublimate. From this sublimate, the pure degradation product was obtained by crystallization. The compound is basic to litmus paper, and melts at 205–206°. It is optically inactive. Elementary analyses, a potentiometric titration and a molecular weight determination indicated that the compound is a monoacidic base with the formula $C_9H_{10}N_2$. A crystalline picrate which melts at 273–275° was prepared. A Kuhn–Roth determination gave 1.1 moles of acetic acid per mole of compound, indicating more than one C-methyl group in the degradation product. In 95% ethanol solution in the presence of 0.01 *N* hydrochloric acid, the absorption spectrum showed maxima at 2745 Å. (E_M 7500) and at 2840 Å. (E_M 8100). When the solution was made alkaline by addition of a small excess of sodium hydroxide, maxima were observed at 2470 Å. (E_M 3900), 2775 Å. (E_M 4900), 2810 Å. (E_M 5250) and 2880 Å. (E_M 5700). The change in the absorption with *p*H is reversible.

The molecular formula of the degradation product and its properties indicated that it might be a substituted benzimidazole. A perusual of the literature revealed that the reported properties of 2,5-dimethylbenzimidazole[3] (m. p. 203°) are quite similar to those of the compound obtained from vitamin B_{12}. Accordingly, the 2,5-dimethyl derivative was prepared. Although it was not identical with the degradation product, the close resemblance of the ultraviolet absorption spectra of the two compounds strengthened the proposed benzimidazole formulation.

The cleavage of benzimidazole to 1,2-dibenzamidobenzene by treatment with benzoyl chloride in aqueous alkali was described by Bamberger and Berlé,[4] and was used by Windaus and Knoop[5] as a diagnostic test for imidazoles. When the reaction was applied to the degradation product from vitamin B_{12}, a crystalline product melting at 262–263° was obtained. It was identical with a synthetic sample of the new 4,5-dibenzamido-1,2-dimethylbenzene (II) prepared by benzoylation of 4,5-diamino-1,2-dimethylbenzene. The degradation product was consequently assigned the 5,6-dimethylbenzimidazole structure (I).

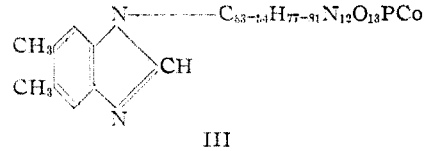


I II

The structure of the degradation product was confirmed by the synthesis of 5,6-dimethylbenzimidazole from 4,5-diamino-1,2-dimethylbenzene and formic acid. The synthetic compound melted at 204–205°, and caused no depression of the melting point when mixed with the degradation product. The absorption spectra of the natural and synthetic products were identical within the limits of experimental error.

The yield of 5,6-dimethylbenzimidazole from vitamin B_{12} was consistently about 70% of one molar equivalent. The stability of the product is such that there is no reason to believe that more than one mole of 5,6-dimethylbenzimidazole per mole of vitamin B_{12} is liberated on hydrolysis. This is in agreement with other observations[2] that vitamin B_{12} contains only one such moiety in its molecule.

It is reasonable to assume that in vitamin B_{12} the 5,6-dimethylbenzimidazole moiety is terminal and is linked to the remainder of the molecule through one of the nitrogen atoms of the imidazole ring. On this basis, a partial formula for vitamin B_{12} may be provisionally represented by structure III.[6]



III

Experimental

5,6-Dimethylbenzimidazole from Vitamin B_{12}.—A sample of vitamin B_{12} weighing 82 mg. was dissolved in 6 ml. of 6 *N* hydrochloric acid and the resulting solution

(1) Brink and Folkers, THIS JOURNAL, **71**, 2951 (1949).

(2) Holliday and Petrow, *J. Pharm. and Pharmacol.*, **1**, 734 (1949); Beaven, Holliday, Johnson, Ellis, Mamalis, Petrow and Sturgeon, *ibid.*, **1**, 957 (1949).

(3) Hobrecker, *Ber.*, **5**, 921 (1872).

(4) Bamberger and Berlé, *Ann.*, **273**, 346 (1893).

(5) Windaus and Knoop, *Ber.*, **38**, 1169 (1905).

(6) *Cf.* Brink, Wolf, Kaczka, Riekes, Koniuszy, Wood and Folkers, THIS JOURNAL, **71**, 1854 (1949).

was heated at 150° for twenty hours. The solution was cooled and filtered to remove insoluble material. The filtrate was evaporated to dryness *in vacuo* to remove excess hydrochloric acid. The residue was dissolved in 12 ml. of water containing 0.3 ml. of 1 *N* hydrochloric acid and the solution was extracted continuously with chloroform for twelve hours. The aqueous solution was then brought to *p*H 10 by the addition of 0.5 ml. of 2 *N* sodium hydroxide and extracted with fresh chloroform for twelve hours.

The chloroform solution from the extraction of the alkaline aqueous solution was concentrated to dryness and the dry residue was extracted with several portions of ether. Removal of the ether from the combined extracts yielded a basic residue which weighed about 7 mg. This residue was sublimed at 140° and a pressure of 3 mm. Traces of oily material were removed from the substantially crystalline sublimate by washing with a mixture of ether and petroleum ether (2:1). The crystalline sublimate, 6 mg., was recrystallized from ether to give 3.5 mg. of pure 5,6-dimethylbenzimidazole, m. p. 205–206° (micro block).

Anal. Calcd. for $C_9H_{10}N_2$: C, 73.94; H, 6.90; N, 19.17; C-methyl, 20.6 (2 moles); mol. wt., 146. Found: C, 74.34; H, 6.47; N, 19.21; C-methyl, 11.1 (1.1 moles); equiv. wt., 144 ±5 (potentiometric titration); mol. wt., 159 (ebullioscopic in acetonitrile).

5,6-Dimethylbenzimidazole Picrate.—Addition of saturated aqueous picric acid solution to an aqueous solution of the 5,6-dimethylbenzimidazole obtained from vitamin B_{12} gave immediately a yellow crystalline precipitate. The picrate was separated, washed with water, and recrystallized from aqueous ethanol, It melted at 272–273° (dec.).

Anal. Calcd. for $C_{15}H_{13}N_5O_7$: N, 18.66. Found: N, 18.76.

4,5-Dibenzamido-1,2-dimethylbenzene.—Synthetic 4,5-diamino-1,2-dimethylbenzene was benzoylated with benzoyl chloride and alkali. The product was recrystallized from ethanol, m. p. 262–262.5°.

Anal. Calcd. for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.14. Found: C, 76.70; H, 6.01; N, 8.25.

4,5-Dibenzamido-1,2-dimethylbenzene from 5,6-Dimethylbenzimidazole.—A solution of 5.1 mg. of 5,6-dimethylbenzimidazole obtained from vitamin B_{12} was cooled to 0° and 90 mg. of benzoyl chloride added. The mixture was stirred in an ice-bath for five hours, and then stored at 2° overnight. The precipitate was collected and dissolved in 2 ml. of boiling ethanol. This solution was filtered and stored at 2° for four hours. The crystals which had separated were then washed with ethanol and water and dried. The yield of 4,5-dibenzamido-1,2-dimethylbenzene, m. p. and mixed m. p. 262–263°, was 5.5 mg.

Synthetic 5,6-Dimethylbenzimidazole.—A solution of 60 mg. of 4,5-diamino-1,2-dimethylbenzene and 60 mg. of 98% formic acid in 3.5 ml. of 4 *N* hydrochloric acid was heated at the reflux temperature for two hours.[7] The reaction mixture was cooled, filtered and neutralized with concentrated ammonium hydroxide solution. A crystalline precipitate of crude 5,6-dimethylbenzimidazole formed in excellent yield. The crystals were separated by centrifugation and dried. A portion was treated with chloroform and filtered to remove insoluble material, and the filtrate was evaporated to dryness. The residue was sublimed at 140° and at a pressure of 3 mm. of mercury. The sublimate was recrystallized from ether to give 5,6-dimethylbenzimidazole, m. p. 204–205°. No depression of melting point was observed when this compound was mixed with the product from vitamin B_{12}. In solution in 95% ethanol which was 0.01 *N* with respect to hydrochloric acid, the synthetic material exhibited maxima at 2745 Å. (E_M 7500) and 2840 Å. (E_M 8100).

Acknowledgment.—The authors wish to thank Miss Janice Mayfield for technical assistance. They are indebted to Dr. N. R. Trenner and Mr. R. P. Buhs for the potentiometric titration, to Dr. J. B. Conn for the molecular weight determination, and to Mr. R. Boos and his associates for the microanalyses.

Summary

5,6-Dimethylbenzimidazole has been isolated from an acid hydrolysate of vitamin B_{12}. Its structure has been established by degradation and by synthesis.

(7) *Cf.* Phillips, *J. Chem. Soc.*, 2393 (1928).

RAHWAY, NEW JERSEY RECEIVED MARCH 29, 1950

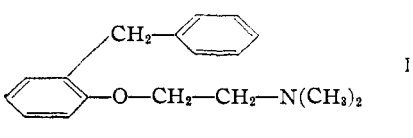
[CONTRIBUTION FROM THE RESEARCH DIVISION, BRISTOL LABORATORIES, INC.]

2-Benzylphenol Derivatives. V.[1] Imidazolines

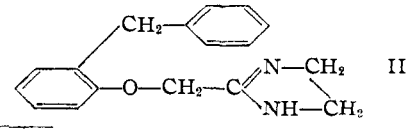
BY WILLIAM B. WHEATLEY, WILLIAM E. FITZGIBBON, LEE C. CHENEY AND S. B. BINKLEY

Numerous compounds containing the imidazoline (4,5-dihydroimidazole) nucleus exhibit interesting physiological properties. Furthermore, it has been observed that replacement of the dialkylaminoethyl by the 2-methylimidazoline group often results in a compound with approximately the same order of physiological activity. For example, both N-phenyl-N-benzyl-N′,N′-dimethylethylenediamine (Antergan) and its imidazoline analog 2-(N-phenyl-N-benzylaminomethyl) imidazoline (Antistin) have been shown to be effective clinically as antihistaminic drugs.[2,3]

The discovery that 2-benzylphenyl β-dimethylaminoethyl ether (I) prevents histamine-induced asthma in guinea pigs[4] made it desirable to prepare and evaluate the imidazoline analog (II). In this



I



II

(1) For the preceding paper in this series, see Wheatley, Fitzgibbon, Cheney and Binkley, THIS JOURNAL, **72**, 1655 (1950).

(2) Halpern, *Arch. Internat. Pharmacodynamie*, **68**, 339 (1942).

(3) Bourquin, *Schweiz. med. Wochschr.*, **76**, 296 (1946); Schindler, *ibid.*, **76**, 300 (1946); Brack, *ibid.*, **76**, 316 (1946).

(4) Cheney, Smith and Binkley, THIS JOURNAL, **71**, 60 (1949).