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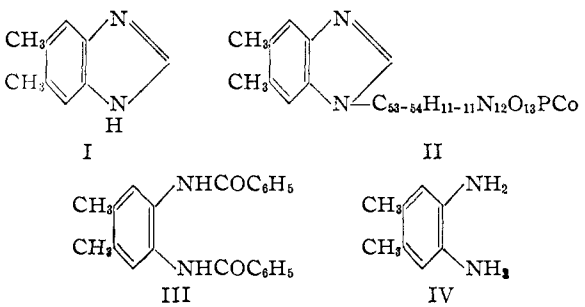
Vitamin B_{12}. VIII. Vitamin B_{12}-Like Activity of 5,6-Dimethylbenzimidazole and Tests on Related Compounds

BY GLADYS EMERSON, NORMAN G. BRINK, FREDERICK W. HOLLY, FRANK KONIUSZY, DOROTHEA HEYL AND KARL FOLKERS

The degradation of vitamin B_{12} by acid hydrolysis to give a new basic compound which was identified by its reactions and by synthesis as 5,6-dimethylbenzimidazole (I) has been described.[1] It was concluded that 5,6-dimethylbenzimidazole is a component of vitamin B_{12} and is terminal and linked through nitrogen as in the provisional formula (II). Further degradation of 5,6-dimethylbenzimidazole with benzoyl chloride and alkali gave 1,2-dibenzamido-4,5-dimethylbenzene (III). Thus, 1,2-diamino-4,5-dimethylbenzene (IV) may be considered a



degradation product of 5,6-dimethylbenzimidazole or vitamin B_{12}, and it may be contemplated as a "precursor" of vitamin B_{12}.

In another laboratory,[2] spectroscopic evidence and paper chromatographic data indicated 5,6-dimethylbenzimidazole as an acid degradation product of vitamin B_{12}.

In addition to its high therapeutic activity for pernicious anemia and other anemias, vitamin B_{12} has shown APF ("animal protein factor") activity[3] when fed at a level of 0.063 μg./day to rats on a diet devoid of animal protein and containing 0.25% of thyroid powder. It is considered important to determine whether parts of the large vitamin B_{12} molecule, which may be readily synthesizable, show useful biological activity. Therefore, 5,6-dimethylbenzimidazole and 1,2-diamino-4,5-dimethylbenzene, as such degradation products, have been tested for APF activity or vitamin B_{12}-like activity. Since this test gave a positive and interesting result, several alkyl-substituted benzimidazoles were prepared and tested to gain knowledge of the specificity of the vitamin B_{12}-like activity of 5,6-dimethylbenzimidazole and its related diamine. It was considered possible that an investigation of benzimidazoles and diamines might lead to the syntheses of vitamin B_{12} antagonists which are desired for various biological problems. A summary of these combined chemical and biological results is as follows.

The data in Table I show that 5,6-dimethylbenzimidazole and 1,2-diamino-4,5-dimethylbenzene have vitamin B_{12}-like activity when fed to rats on the above described diet.[3] Daily quantities of 2–5 mg. of 5,6-dimethylbenzimidazole and 2–3 mg. of 1,2-diamino-4,5-dimethylbenzene were comparable to 0.125–0.250 μg. of vitamin B_{12}. Although these milligram-amounts of 5,6-dimethylbenzimidazole and 1,2-diamino-4,5-dimethylbenzene are approximately 10,000–20,000 times greater than the microgram quantities of vitamin B_{12} necessary to produce comparable growth responses in the test animals, they show

TABLE I

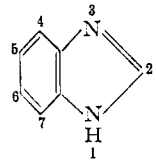
VITAMIN B_{12}-LIKE ACTIVITY DATA

Substance	Quantity fed daily	No. of rats	Wt. increment g.-15 days
(Negative controls)	....	57	28
Vitamin B_{12}	0.0625 μg.	50	54
Vitamin B_{12}	0.125 μg.	20	64
Vitamin B_{12}	0.250 μg.	10	72
5,6-Dimethylbenzimidazole	2.0 mg.	10	48
5,6-Dimethylbenzimidazole	5.0 mg.	10	84
1,2-Diamino-4,5-dimethylbenzene	2.0 mg.	9	60
1,2-Diamino-4,5-dimethylbenzene	3.0 mg.	9	78

that these compounds compare on a weight-basis with certain vitamins such as vitamin E, pantothenic acid and choline in their respective tests. The vitamin requirements of the rat per 100 g. of ration are as follows for these substances: vitamin E, 3 mg.; pantothenic acid, 1 mg.; choline, 100 mg.[4] For better comparison per 100 g. of ration, the level of 5,6-dimethylbenzimidazole may be estimated as about 12–30 mg. and that of 1,2-diamino-4,5-dimethylbenzene as 12–18 mg. The extremely potent biological activity of vitamin B_{12} tends to over-shadow the potency of these degradation products at their respective levels.

Benzimidazole (V), four mono-methylbenzimidazoles, and two other dimethylbenzimidazoles were assayed for vitamin B_{12}-like activity in rats

(1) Brink and Folkers, THIS JOURNAL, 71, 2951 (1949).
(2) Holliday and Petrow, *J. Pharm. and Pharmacol.*, 1, 734 (1949).
(3) Emerson, *Proc. Soc. Exp. Biol. Med.*, 72, 892 (1949).
(4) "Vitamins and Hormones," Vol. VII, Academic Press, Inc., New York, N. Y., 1949, p. 187.



V

under comparable conditions,[3] and these results are summarized in Table II.

TABLE II

RESULTS OF TESTS FOR VITAMIN B_{12}-LIKE ACTIVITY

Substituted benzimidazoles	Amounts fed daily	No. of rats	Wt. increment g.-15 days
Negative Controls	...	57	28
Vitamin B_{12}	0.125 μg.	20	64
Benzimidazole	2.0 mg.	8	37
1-Methyl-	2.0 mg.	9	27
2-Methyl-	2.0 mg.	10	29
4-Methyl-	2.0 mg.	7	31
5-Methyl-	2.0 mg.	10	59
2,5-Dimethyl	2.0 mg.	8	17
4,6-Dimethyl-	2.0 mg.	10	37

Benzimidazole, 1-methyl-, 2-methyl-, 4-methyl- and 4,6-dimethylbenzimidazole did not elicit a significantly high response. 2,5-Dimethylbenzimidazole appeared to act as a growth-depressant and may have inhibitor activity. 5-Methylbenzimidazole showed significant growth activity, a fact which is interesting since 6- and 7-methyl substitutions in the 9-(1′-D-ribityl)-isoalloxazine series show low riboflavin activity.[5]

Both 5,6-dimethylbenzimidazole and the corresponding diamine, 1,2-dimethyl-4,5-diaminobenzene were observed to be inactive in the *Lactobacillus lactis* Dorner assay for vitamin B_{12} activity. They were tested at concentrations up to 0.5 mg./ml. by Miss Muriel C. Caswell of our Microbiology Department.

These benzimidazoles were prepared by the reaction of the diamine with the appropriate acid essentially as described in the literature, and the melting points were identical with the published constants: benzimidazole,[6] 1-methylbenzimidazole,[7] 2-methylbenzimidazole,[8] 4-methylbenzimidazole,[9] 5-methylbenzimidazole,[10] 2,5-dimethylbenzimidazole[11] and 4,6-dimethylbenzimidazole.[12]

Summary

5,6-Dimethylbenzimidazole and 1,2-diamino-4,5-dimethylbenzene, which are degradation products of vitamin B_{12}, have been found to show vitamin B_{12}-like growth activity when fed to rats maintained on a diet devoid of animal protein and containing thyroid powder. These two products are active at milligram-levels in contrast to vitamin B_{12} which is active at the microgram-level.

5-Methylbenzimidazole showed significantly high vitamin B_{12}-like activity also. Benzimidazole and four monomethyl and dimethyl derivatives failed to elicit significantly high activity. 2,5-Dimethylbenzimidazole appeared to show growth-depressant or inhibitor properties.

(5) Karrer, V. Euler, Malmberg and Schopp, *Svensk. Kem. Tids.*, **47**, 153 (1935); Karrer and Strong, *Helv. Chim. Acta*, **18**, 1343 (1935).

(6) Pauly and Gundermann, *Ber.*, **41**, 4012 (1908).

(7) Fischer and Veiel, *ibid.*, **38**, 321 (1905).

(8) Hinsberg and Funcke, *ibid.*, **27**, 2189 (1894).

(9) Gabriel and Thieme, *ibid.*, **52**, 1081 (1919).

(10) Ladenburg, *ibid.*, **10**, 1123 (1877); Fischer, *ibid.*, **22**, 644 (1889).

(11) Ladenburg, *ibid.*, **8**, 677 (1875).

(12) Fischer and Rigaud, *ibid.*, **34**, 4205 (1901).

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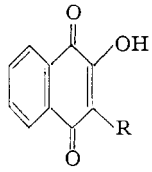
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Hydroxynaphthoquinones. I. Color and Acidity

BY MARTIN G. ETTLINGER[1]

It is well known[2] that 3-substituted 2-hydroxy-1,4-naphthoquinones (I) are acids comparable in strength to carboxylic acids, and that their salts are deeply colored, from yellow to violet. The influence of the substituent (R) on these two properties is the subject of this paper.



I

Absorption spectra[2] of both the free hydroxy quinones (I) and their anions contain several bands in the ultraviolet and visible, whereof those at longest wave lengths are most sensitive to structural change, and therefore interesting. For example, the spectra[3,4] of hydrolapachol (I, R = $(CH_2)_2CH(CH_3)_2$) and α-lapachone (II) are identical below 360 mμ, and hence unaltered in that region by substitution of 2-alkoxyl for hydroxyl, but differ in the position of the band extending into the visible. In an un-ionized hydroxy quinone a more intense peak at 330–335 mμ overlaps the interesting one, but in the anion the latter band is shifted on the order of 100 mμ

(1) Member of the Society of Fellows, Harvard University.

(2) Fieser, Leffler, *et al.*, THIS JOURNAL, **70**, 3151 (1948).

(3) Cooke, Macbeth and Winzor, *J. Chem. Soc.*, 878 (1939).

(4) Ettlinger, Paper II, THIS JOURNAL, **72**, 3090 (1950).