the reaction completed. After purification a polymer of m. p. 117–121°, inherent viscosity, 0.10 was obtained.

*Anal.* Calcd. for $C_{12}H_{20.4}N_2O_4$: C, 56.14; H, 7.97; N, 10.90. Found: C, 55.72; H, 7.74; N, 10.04.

**cis 90–10 Polyurethan.**—This polymer was formulated to be 80–20, but even though the modified procedure for mixed polyurethans was used, the physical data indicate that the product is closer to 90–10. A mixture of 0.322 g. of *cis*-glycol and 0.332 g. of saturated glycol was allowed to react with 3.10 g. of hexamethylene diisocyanate, and then an additional 0.994 g. of butane-1,4-diol was added dropwise. Upon purification 4.25 g. (89% yield) of polymer was obtained, m. p. 168–170°, inherent viscosity 0.11.

*Anal.* Calcd. for $C_{12}H_{21.8}N_2O_4$: C, 55.83; H, 8.49; N, 10.86. Found: C, 56.22; H, 8.30; N, 10.73.

**trans 20–80 Polyurethan.**—Under the modified procedure for mixed polyurethans, a polymer was prepared from a mixture of 0.955 g. of *trans*-2-butene-1,4-diol and 0.244 g. of butane-1,4-diol with 1.82 g. of hexamethylene diisocyanate. After recrystallization 2.08 g. (69% yield) of product was obtained, m. p. 155–157°, inherent viscosity 0.12.

*Anal.* Found: C, 56.04; H, 8.08; N, 10.53.

**trans 80–20 Polyurethan.**—A mixture of 0.407 g. of *trans*-2-butene-1,4-diol and 1.667 g. of butane-1,4-diol was allowed to react with 3.89 g. of hexamethylene diisocyanate, according to the modified procedure. After purification of the crude product 5.06 g. (85% yield) of polymer was obtained, m. p. 160–162°, inherent viscosity 0.11.

*Anal.* Calcd. for $C_{12}H_{21.6}N_2O_4$: C, 55.88; H, 8.43; N, 10.86. Found: C, 55.76; H, 8.66; N, 10.57.

**Polyesters.**—The apparatus used was that described by Hardy.[13] The polyesters were prepared from sebacic acid and *cis*- or *trans*-2-butene-1,4-diol according to the procedure described for 2-butyne-1,4-diol polymers. A reaction temperature of 170° was maintained by refluxing phenetole vapors, and an oxygen-free nitrogen atmosphere was obtained at all times. The inherent viscosities were measured on a 0.4% solution of the polymer in chloroform.

**Polysebacate of *cis*-2-Butene-1,4-diol.**—From a mixture of 2.213 g. of *cis*-2-butene-1,4-diol and 4.620 g. of sebacic acid there was obtained, after purification, 5.75 g. (90% yield) of polymer, m. p. 58–59°, inherent viscosity 0.56.

*Anal.* Calcd. for $C_{14}H_{22}O_4$: C, 66.13; H, 8.72. Found: C, 65.98; H, 8.45.

**Polysebacate of *trans*-2-Butene-1,4-diol.**—A run made with 2.475 g. of *trans*-2-butene-1,4-diol and 5.160 g. of sebacic acid gave 6.15 g. (86% yield) of polymer, m. p. 68–69.5°, inherent viscosity 0.30.

*Anal.* Found: C, 65.86; H, 8.53.

**Second Order Transition Temperature Determinations.**—The method of Bekkedahl[8] was followed. Isoöctane was employed as the confining liquid and polymer samples of about 5 g. were used. The capillary scale readings were plotted against the temperature as measured by a thermocouple without determining absolute volume changes. Therefore, the measurements may be in error by a few degrees, but the general order of the values is undoubtedly correct.

**Heat and Entropy of Fusion Calculations.**—The melting points used were corrected capillary melting points and these may not represent the precise value for the disappearance of crystallinity. Also there is some difference in molecular weights of the samples as judged by their inherent viscosity, and this introduces a slight element of doubt on exact comparison, but the order of magnitude is the important point in this work.

## Summary

*cis*-2-Butene-1,4-diol has been prepared by the Raney nickel-catalyzed hydrogenation of 2-butyne-1,4-diol.

The *cis* and *trans* isomers of 2-butene-1,4-diol have been converted to polyurethans by reaction with hexamethylene diisocyanate, and to polyesters by reaction with sebacic acid. The capillary melting points of the *cis* polymers are lower than the *trans* isomers, but second order transition temperatures, heat and entropy of fusion calculations, X-ray diffraction and infrared patterns show little difference attributable to *cis–trans* isomerism.

URBANA, ILLINOIS RECEIVED AUGUST 9, 1950

(13) D. V. N. Hardy, *J. Soc. Chem. Ind.*, **67**, 426 (1948).

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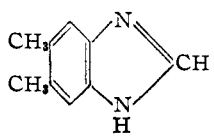
[CONTRIBUTION FROM THE MERCK INSTITUTE FOR THERAPEUTIC RESEARCH AND THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

# Vitamin $B_{12}$. XII. Vitamin $B_{12}$-like Activity of α- and β-Ribazole

BY GLADYS EMERSON, FREDERICK W. HOLLY, CLIFFORD H. SHUNK, NORMAN G. BRINK AND KARL FOLKERS

5,6-Dimethylbenzimidazole (I) has been obtained[1,2] as a degradation product of vitamin $B_{12}$. 1,2-Diamino-4,5-dimethylbenzene (II) may also be considered as a vitamin $B_{12}$ degradation product, since it was obtained as a dibenzoyl derivative[1] by further degradation of 5,6-dimethylbenzimidazole.



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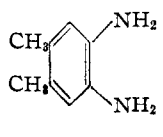


II

It was considered significant to determine whether small fundamental units of vitamin $B_{12}$, which is a compound having a molecular weight of approximately 1300,[3] would possess vitamin $B_{12}$-like activity or APF (animal protein factor) activity. The tests for activity were made by assays on rats which were maintained on a diet devoid of animal protein and containing 0.25% of thyroid powder; it was known that the addition of vitamin $B_{12}$ to this diet counteracted the growth retarding effect of the thyroid powder, and caused a marked increase in the weight gain of the animals.[4]

Daily doses of 2–5 mg. of 5,6-dimethylbenzimidazole or 2–3 mg. of 1,2-diamino-4,5-dimethylbenzene caused weight gains which were comparable to the growth produced by the addition of 0.125–0.25 μg. of vitamin $B_{12}$. Thus, 5,6-dimethylbenzimidazole and 1,2-diamino-4,5-dimethylbenzene show vitamin $B_{12}$-like activity in this test.[5] Even

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

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

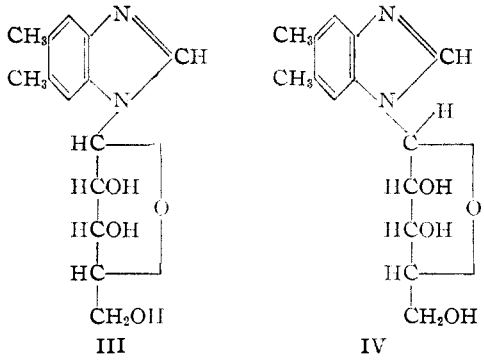
(3) Brink, Wolf, Kaczka, Rickes, Koniuszy, Wood and Folkers, THIS JOURNAL, **71**, 1854 (1949).

(4) Emerson, *Proc. Soc. Exp. Biol. Med.*, **70**, 392 (1949).

(5) Emerson, Brink, Holly, Koniuszy, Heyl and Folkers, THIS JOURNAL, **72**, 3084 (1950).

though the milligram-amounts of these two products are approximately 10,000–20,000 times greater than the microgram-doses of vitamin $B_{12}$, these products have an activity which on a weight basis is somewhat comparable with vitamin E and choline in their respective tests.

The characterization of α-ribazole[6] (III) (1-α-D-ribofuranosido-5,6-dimethylbenzimidazole) by degradation of vitamin $B_{12}$ and by synthesis has shown that the 5,6-dimethylbenzimidazole is attached through a nitrogen atom to D-ribose in the vitamin $B_{12}$ molecule.



III IV

Because of the vitamin $B_{12}$-like activity of 5,6-dimethylbenzimidazole, it was essential to determine whether α-ribazole showed activity also. Samples of both "natural" and synthetic α-ribazole were tested. The synthesis of the anomeric glycoside, β-ribazole (IV) (1-β-D-ribofuranosido-5,6-dimethylbenzimidazole), was also accomplished,[6] and this substance was tested. A summary of the biological results is given in Table I.

TABLE I

VITAMIN $B_{12}$-LIKE ACTIVITY DATA

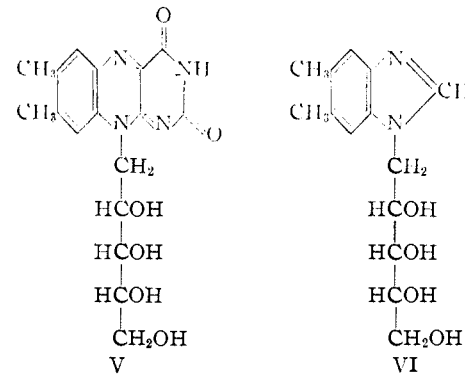
| | Quantity fed daily, μg. | No. of rats | Wt. increment g., 15 days |
|---|---|---|---|
| Controls (undosed) | ..... | 20 | 28 |
| Vitamin $B_{12}$ | 0.062 | 10 | 55 |
| Vitamin $B_{12}$ | 0.125 | 20 | 64 |
| Vitamin $B_{12}$ | 0.250 | 10 | 72 |
| α-Ribazole ("Natural") | 1.0 | 10 | 38 |
| α-Ribazole ("Natural") | 5.0 | 10 | 50 |
| α-Ribazole ("Natural") | 7.5 | 10 | 46 |
| α-Ribazole (Synthetic) | 40.0 | 10 | 59 |
| α-Ribazole (Synthetic) | 100.0 | 10 | 69 |
| β-Ribazole (Synthetic) | 100.0 | 10 | 68 |
| 1-D-Ribityl-5,6-dimethylbenzimidazole | 1.0 mg. | 10 | 43 |
| | 2.0 mg. | 10 | 43 |

It was found that daily quantities of 100 μg. of α-ribazole gave a growth response in the rats which was comparable to that obtained from 0.25 μg. of vitamin $B_{12}$. Thus α-ribazole is 20–50 times as active on a weight basis as 5,6-dimethylbenzimidazole and 1,2-diamino-4,5-dimethylbenzene for vitamin $B_{12}$-like activity, and is approximately one four-hundredth as active as vitamin $B_{12}$ in this particular test. "Natural" α-ribazole was not obtained in amounts which permitted testing at higher levels, but there is no reason to expect any difference in the biological activity of the "natural" and synthetic materials. β-Ribazole, at a daily level of 100 μg., also promoted a growth response which was about equivalent to that resulting from 0.25 μg. of vitamin $B_{12}$. Although a difference in the activities of α- and β-ribazole might be found if each were tested at several lower levels, the fact remains that β-ribazole gave a significant response in this test on rats, and the configuration of the groups about $C_1$ of ribose does not markedly affect this activity.

Although the extremely potent biological activity of vitamin $B_{12}$ may overshadow that of its degradation products, it is noted that the activity of α-ribazole on a weight basis is comparable with that of riboflavin or pantothenic acid; the requirement of these two vitamins for optimal growth of rats is *ca.* 80–100 μg. daily.

The specificity of the number and position of the methyl groups of 5,6-dimethylbenzimidazole for vitamin $B_{12}$-like activity has been discussed with available data.[5]

Since both riboflavin (V) and α-ribazole (III) contain a 1,2-diamino-4,5-dimethylbenzene (II) and a D-ribose moiety, 1-D-ribityl-5,6-dimethylbenzimidazole (VI)[7] was made and tested for vitamin $B_{12}$-like activity. It is apparent from Table I that 1-D-ribityl-5,6-dimethylbenzimidazole does not possess vitamin $B_{12}$-like activity of the order shown by α-ribazole. In fact, the weight gain of the rats treated with this ribitylbenzimidazole derivative, which is so closely related to riboflavin, was less than the gain shown by the rats on an equal level of 5,6-dimethylbenzimidazole.



V VI

## Summary

α-Ribazole (1-α-D-ribofuranosido-5,6-dimethylbenzimidazole), a degradation product of vitamin $B_{12}$, has been found to elicit a marked vitamin $B_{12}$-like response when fed to rats maintained on a diet devoid of animal protein and containing thyroid powder. It is about one four-hundredth as active as vitamin $B_{12}$ in this test, and on a weight basis may be considered comparable to the activity of riboflavin and pantothenic acid in their respective animal tests. β-Ribazole also gave a significant response in the test.

1-D-Ribityl-5,6-dimethylbenzimidazole appears to have less vitamin $B_{12}$-like activity than 5,6-dimethylbenzimidazole.

RAHWAY, NEW JERSEY RECEIVED JULY 5, 1950

(6) Brink, Holly, Shunk, Peel, Cahill and Folkers, THIS JOURNAL, **72**, 1866 (1950).

(7) Holly, Peel, Cahill and Folkers, *ibid.*, **73**, 332 (1951).