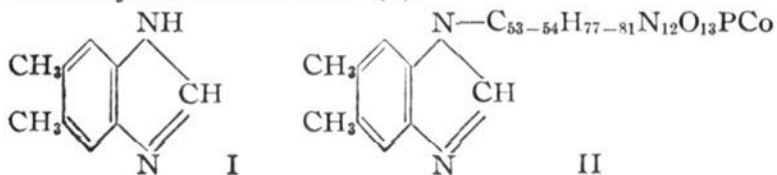


VITAMIN B₁₂. VI. 5,6-DIMETHYLBENZIMIDAZOLE,
A DEGRADATION PRODUCT OF VITAMIN B₁₂

Sir:

Degradation of vitamin B₁₂ by acid hydrolysis has given a new basic compound which has been identified by its reactions and by synthesis as 5,6-dimethylbenzimidazole (I).

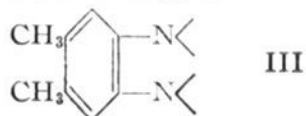


The crystalline product melted at 205–206°. *Anal.* Calcd. for C₉H₁₀N₂: C, 73.94; H, 6.90; N, 19.17. Found: C, 74.36; H, 6.47; N, 19.21. Potentiometric titration showed an equivalent weight of 144 ± 5; calcd., 146. In 95% ethanol solution in the presence of 0.01 *N* hydrochloric acid, the absorption spectrum of the compound was characterized by maxima at 2745 Å. (*E*_M7500) and at 2840 Å. (*E*_M8100). In similar solution in the presence of 0.01 *N* sodium hydroxide, maxima were observed at 2470 Å. (*E*_M3900), 2775 Å. (*E*_M4900), 2810 Å. (*E*_M5250) and 2880 Å. (*E*_M5700). The compound was optically inactive. It gave a crystalline picrate, melting point 273–275°. *Anal.* Calcd. for C₁₅H₁₃N₅O₇: N, 18.66. Found: N, 18.76.

Treatment of the degradation product with benzoyl chloride in aqueous alkali according to the method of Bamberger and Berlé¹ for the cleavage of benzimidazoles to dibenzamidobenzenes afforded a compound, melting point 262–263°, which was identical with a synthetic sample of the new 4,5-dibenzamido-1,2-dimethylbenzene, melting point 262–262.5° (*Anal.* Calcd. for C₂₂H₂₀N₂O₂: C, 76.72; H, 5.85; N, 8.14. Found: C, 76.70; H, 6.01; N, 8.25), prepared by benzoylation of 4,5-diamino-1,2-dimethylbenzene.

The assigned structure of the degradation product was confirmed by the synthesis of 5,6-dimethylbenzimidazole by condensation of 4,5-diamino-1,2-dimethylbenzene with formic acid. The resulting compound had melting point and mixed melting point 204–205°. Its absorption spectrum was identical with that of the natural product, within experimental error. A provisional formula² for vitamin B₁₂ is represented in II, which is based on the assumption that the dimethylimidazole is terminal and linked to a nitrogen.

It is noted that the 1,2-diamino-4,5-dimethylbenzene moiety (III) appears in 5,6-dimethylbenzimidazole and vitamin B₁₂, and also in riboflavin.



Elucidation of the biological implications of this chemical structural relationship will undoubtedly prove of interest.

When a sample of riboflavin was hydrolyzed un-

(1) Bamberger and Berlé, *Ann.*, **273**, 346 (1893).
(2) Brink, *et al.*, *THIS JOURNAL*, **71**, 1854 (1949).

der the same conditions, a similar isolation technique failed to yield any 5,6-dimethylbenzimidazole.

The authors wish to thank Miss Janice Mayfield for technical assistance, Dr. N. R. Trenner and Mr. R. P. Buhs for the potentiometric titration, and Mr. R. Boos and his associates for the microanalyses.

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RECEIVED JULY 6, 1949

ELECTRON MICROGRAPHS OF CRYSTALLINE
EDESTIN

Sir:

Electron micrographs have been obtained showing molecular arrays in crystalline edestin. The protein was recrystallized six times from 5% sodium chloride by cooling slowly from 60 to 4°. Crystals were formed on the specimen screen with collodion film by applying a drop of the protein in 2.5% sodium chloride at 60° and cooling to room temperature. Salt was removed by washing with water or 75% alcohol. Since the crystals are relatively opaque in the electron microscope, a shadow-transfer technique was developed to render the surface structure visible. The specimen was shadowed with uranium or nickel coated normally with an evaporated film of silicon oxide, washed with acetone to remove the collodion and 0.05 *N* hydrochloric acid to remove the protein. In outline, such crystals are mostly equilateral triangles as shown in Fig. 1(a). The molecules on the triangular faces are arranged in a hexagonal pattern as shown in Fig. 1(b). It is con-

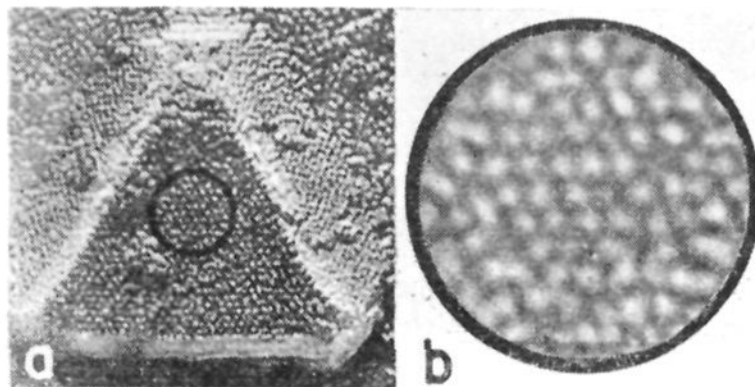


Fig. 1.—(a) Electron micrograph showing surface structure of edestin crystal, × 95,000; (b) circumscribed area of (a) showing molecules in (111) plane, × 396,000.

cluded from the analysis that the triangular faces are the (111) planes of a face-centered cubic lattice. Measurements of the perpendicular distance between rows of molecules parallel to the triangle edges range from about 68 to 72 Å. with an average of 69.7 Å. Magnifications are judged to be accurate to within ±2%. From this measurement it is calculated that *a* = 114 Å. for the unit cell. The conclusion regarding the three-dimensional symmetry of the crystals was suggested by