

Technical Papers

Vitamin B₁₂: The Identification of Vitamin B₁₂ as a Cyano-Cobalt Coordination Complex

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Vitamin B₁₂ contains one cyano group bound coordinatively to the cobalt atom. Vitamin B_{12a}, however, does not contain this group.

During an oxidation of vitamin B₁₂ in dilute sulfuric acid solution at 0° C with potassium permanganate, the characteristic odor of hydrogen cyanide was noted. The solution was distilled, and the distillate examined for the presence of cyanide, using the copper-benzidine (2) and the ferrous sulfate tests (8). Both were positive. In another oxidation, carried out in a closed system, a solution of 49.1 mg of vitamin B₁₂ in 5 ml of 0.75 *N* sulfuric acid was stirred in an ice bath during the dropwise addition of 3.5 ml of 2% potassium permanganate solution. The reaction mixture was warmed to 75°, and nitrogen was bubbled through the solution. The exit gases were passed into dilute sodium hydroxide solution. A volumetric determination of the hydrogen cyanide evolved (5) gave a value of 0.66 mg, corresponding to 0.67 mole/mole of vitamin B₁₂.

Hydrogen cyanide was also liberated readily upon heating solutions of vitamin B₁₂ in hydrochloric or aqueous oxalic acid. In the presence of saturated aqueous oxalic acid, 0.96 mole of hydrogen cyanide was liberated per mole of the vitamin when the solution was boiled under reflux for 5 hr. Due to simultaneous hydrolysis of the cyanide, its yield was somewhat lower when hydrochloric acid was employed. No cyanide could be detected when vitamin B₁₂ was warmed with dilute sulfuric acid under the same conditions used for its liberation by hydrochloric or oxalic acid.

Since chloride and oxalate are known to coordinate readily with cobalt, while sulfate shows only a slight tendency to enter in coordination complexes with this metal (7), it seems probable that the cyanide group in vitamin B₁₂ is a member of the cobalt coordination complex, and that it may be displaced from the coordination sphere by anions with strong coordination tendencies.

No cyanide could be detected when pure vitamin B_{12a}

was treated with oxalic acid solution. Vitamin B₁₂ possesses an absorption band at 4.69 μ in the infrared region, which may be ascribed to the presence of a carbon-nitrogen triple bond; but this band does not appear in the absorption spectrum of vitamin B_{12a}. Vitamin B_{12a} and vitamin B_{12b} are apparently identical (4); and the absence in the infrared spectrum of vitamin B_{12b} of a band that is present at 2,140 cm⁻¹ in the spectrum of vitamin B₁₂ has been observed by others (1). It has been reported (3) that vitamin B_{12a} does not differ greatly in its composition from vitamin B₁₂. One significant difference is the presence of a coordinatively bound cyano group only in the latter compound.

The extreme lack of toxicity of vitamin B₁₂ (6) indicates that the cyano group is tightly bound within the coordination complex. Dose levels of 1,600 mg/kg, both intraperitoneally and intravenously in mice, produced no deaths or toxic manifestations. This dose level corresponds to 112,000,000 times the daily human dose of 1 μ g of vitamin B₁₂ listed in the *U. S. Pharmacopeia*.

References

1. BROCKMAN, J. A., JR., *et al.* *J. Am. Chem. Soc.*, 1950, **72**, 1042.
2. FEIGL, F. *Qualitative analysis by spot tests* (3rd Eng. ed.). New York: Elsevier, 1940. P. 208.
3. KACZKA, E. A., WOLF, D. E., and FOLKERS, K. *J. Am. Chem. Soc.*, 1949, **71**, 1514.
4. *Ibid.* In press.
5. SCHULEK, E. *Z. anal. Chem.*, 1923, **62**, 337.
6. WINTER, C. A., and MUSHETT, C. W. *J. Am. Pharm. Assoc. (Sci. ed.)*, 1950, **39**, 360.
7. *Gmelins Handbuch der anorganischen Chemie*, Teil 58 (B), 192, 235, 285.
8. *Scott's standard methods of chemical analysis* (5th ed.). N. H. Furman (Ed.), New York: Van Nostrand, 1939. Vol. 1, p. 660.

Vitamin B₁₂: Reactions of Cyano-Cobalamin and Related Compounds

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It has been found that a cyano group (1) is present in the vitamin B₁₂ cobalt coordination complex, but not in

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