hydrofuran. The mixture was stirred for an hour and poured into water. The tetraphenylsuccinonitrile that separated was collected; yield 3.6 g. (95%).

Reaction of Sodium Diphenylcyanomethide with Ethyl Nitrate.—The same method described above for acetone cyanohydrin nitrate was followed. From 19.3 g. (0.1 mole) of diphenylacetonitrile, 2.5 g. (0.11 mole) of sodium hydride and 13.7 g. (0.15 mole) of ethyl nitrate there was obtained 15.4 g. (80%) of crude tetraphenylsuccinonitrile.

Reaction of Sodium Tri-(*p*-nitrophenyl)-methide with Acetone Cyanohydrin Nitrate.—Sodium tri-(*p*-nitrophenyl)-methide was prepared under nitrogen by dissolving 3.8 g. (0.01 mole) of tri-(*p*-nitrophenyl)-methane[6] in 25 ml. of absolute alcohol containing 0.23 g. (0.01 g. atom) of sodium. A deep blue solution resulted. Acetone cyanohydrin nitrate (1.3 g., 0.01 mole) was added. After stirring for several hours at room temperature a small amount of dark green material began to separate from solution. Stirring was continued for 12 hours. At the end of this period the whole solution had taken on a dark green color. Upon admission of air, the green color gradually disappeared.

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Vitamin B_{12}. XXV. 3,3-Dimethyl-2,5-dioxopyrrolidine-4-propionamide: A New Degradation Product

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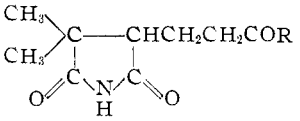
Vitamin B_{12} was degraded by stepwise acid hydrolysis and chromate oxidation to 3,3-dimethyl-2,5-dioxopyrrolidine-4-propionic acid (I).[1] Since this acid was not obtained as an oxidation product of unhydrolyzed vitamin B_{12}, it was suggested[1] that the free carboxyl group of the acid I is present as an amide in the vitamin B_{12} molecule. We now have evidence that the amide interpretation is correct since 3,3-dimethyl-2,5-dioxopyrrolidine-4-propionamide (II) has been obtained as an oxidation product of unhydrolyzed vitamin B_{12}.



I, R = OH; II, R = NH_2

Vitamin B_{12} was subjected to oxidation by sodium chromate in acetic acid. The crude product was divided into neutral and acidic chloroform-soluble fractions. The neutral material was subjected to countercurrent distribution between water and ether. The fraction more soluble in the aqueous phase yielded an optically active neutral compound, m.p. 150–151°. Infrared analysis showed absorption at 2.92, 3.12, 3.70, 6.0 and 6.2 μ, compatible with an imide-amide structure. This substance, when hydrolyzed with acid, was converted to 2-methylpentane-2,3,5-tricarboxylic acid.[1,2]

A sample of synthetic DL-3,3-dimethyl-2,5-dioxopyrrolidine-4-propionic acid was converted to the acid chloride by treatment with thionyl chloride. The crude acid chloride was treated with aqueous ammonia to yield DL-3,3-dimethyl-2,5-dioxopyrrolidine-4-propionamide, m.p. 151–152°. The melting point of a mixture of this synthetic DL-amide and the optically active degradation product was not depressed. This melting point behavior indicates that the synthetic amide is a racemic solid solution. The infrared absorption spectrum of the synthetic amide is the same as that of the amide obtained by degradation, a result of greater significance than the melting point behavior.

As indicated previously, the oxidation of acid hydrolyzed vitamin B_{12} yields DL-3,3-dimethyl-2,5-dioxopyrrolidine-4-propionic acid.[1] However, none of this acid was isolated when unhydrolyzed vitamin B_{12} was similarly oxidized, only the corresponding amide was obtained. From these data, it is concluded that the amide group of II exists in the vitamin B_{12} molecule. The carboxyl group of I corresponds to the amide group of II with the potential formation of ammonia in all vitamin B_{12} degradation products containing the skeletal moiety of amide II. Furthermore, the carboxyl group of I is not formed during the oxidative degradation.

Experimental

Isolation of 3,3-Dimethyl-2,5-dioxopyrrolidine-4-propionamide.—A solution of 5 g. of vitamin B_{12} and 17.4 g. of sodium chromate in 300 ml. of glacial acetic acid, after standing for three hours at room temperature, was heated on the steam-bath overnight. The acetic acid was then removed *in vacuo* and an aqueous solution of the residue, acidified to pH 2 with hydrochloric acid, was extracted continuously with chloroform for sixteen hours. The residue obtained, after removal of the chloroform, was redissolved in water, adjusted to *p*H 7, and again extracted continuously with chloroform. The solvent soluble material, freed of acidic impurities, was separated from traces of 5,6-dimethylbenzimidazole by redissolving it in water and continuously extracting this aqueous solution with chloroform at *p*H 2.

The chloroform soluble fraction, 165 mg., consisting of neutral and weakly acidic products, was subjected to a 39 transfer countercurrent distribution. The system ether-water was used in the proportion of 10 ml. of water and 15 ml. of ether in each tube. The combined residues from tubes 16 to 23, when recrystallized from a mixture of chloroform, ether and petroleum-ether yielded 12 mg. of 3,3-dimethyl-2,5-dioxo-4-hydroxypyrrolidine-4-propionic acid lactone.[1] The residue of tubes 0 and 1 yielded, when dissolved in acetone–ether, 25.6 mg. of crystalline 3,3-dimethyl-2,5-dioxopyrrolidine-4-propionamide. After sublimation *in vacuo* and recrystallization from acetone to constant melting point, the amide melted at 150.5–151°, $[\alpha]^{25}D$ −10° (*c*, 2.8 in water).

Anal. Calcd. for $C_9H_{14}N_2O_3$: C, 54.54; H, 7.12; N, 14.14. Found: C, 54.16; H, 6.50; N, 13.62.

A second preparation of 3,3-dimethyl-2,5-dioxopyrrolidine 4-propionamide in which particular care was taken to prevent racemization by avoiding elevated temperatures, the product was recrystallized to constant rotation, $[\alpha]^{25}D$ −42° (*c*, 0.85 in water). This material, m.p. 150.5–151°, as in the case of the first preparation, did not depress the melting point of the synthetic amide. Furthermore the compounds were shown to be identical by a comparison of their infrared absorption spectra in the solid state.

Hydrolysis of 3,3-Dimethyl-2,5-dioxopyrrolidine-4-propionamide.—A solution of 3.5 mg. of the amide, in 20 ml. of 6 *N* hydrochloric acid, was refluxed for 20 hours. The acid was removed *in vacuo* to yield a dry residue which was separated from the ammonium chloride by trituration with acetone. The acetone-soluble material, when recrystallized from ether, yielded 2.2 mg. of an acid, m.p. 151–152°. This acid, upon admixture with 2-methylpentane-2,3,5-tricarboxylic acid,[2] m.p. 152–153°, melted at 151–152°.

Synthesis of 3,3-Dimethyl-2,5-dioxopyrrolidine-4-propionamide.—3,3-Dimethyl-2,5-dioxopyrrolidine-4-propionic acid[1] (30 mg.) was suspended in thionyl chloride (1 ml.) and the mixture was heated on a steam-bath for 5 minutes. The resulting solution was evaporated to dryness under reduced

(1) F. A. Kuehl, Jr., C. H. Shunk and K. Folkers, THIS JOURNAL, **77**, 251 (1955).

(2) Perkin and Thorpe, *J. Chem. Soc.*, **85**, 128 (1904).

pressure, and 1 ml. of ammonium hydroxide (sp. gr. 0.90) was added to the residue. After 15 minutes at room temperature, the solution was diluted with 2 ml. of water and subjected to continuous extraction with chloroform for 2.5 hours. Evaporation of the chloroform under reduced pressure gave 17 mg. of an oil which was evaporatively distilled at *ca.* 140° (1 mm.). The distillate crystallized from acetone–ether giving 10 mg. of material melting at 125–127°. Recrystallization from the same solvents raised the melting point to 151–152°.

Anal. Calcd. for $C_9H_{14}N_2O_3$: C, 54.54; H, 7.12; N, 14.14. Found: C, 54.24; H, 7.01; N, 14.29.

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Specificity and Mode of Action of Rattlesnake Venom Adenosinetriphosphatase[1]

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Zeller[2] observed that several snake venoms liberate 1 molecule of inorganic orthophosphate when incubated with ATP.[3] He suggested two possible pathways for the reaction: (1) the terminal phosphate is split off, giving orthophosphate and ADP; (2) inorganic pyrophosphate is split off, leaving AMP, which is then hydrolyzed to adenosine and orthophosphate by the specific 5'-phosphomonoesterase present in snake venoms. Zeller concluded that the conditions of the reaction favored one or the other of the two pathways.[4] In 1953 Johnson, *et al.*,[5] showed that when ATP was incubated with cobra venom the second pathway prevailed. Because we wanted to use whole as well as fractionated rattlesnake venom (*Crotalus ademanteus*) for the determination of the structure of nucleotides and related compounds, it was important to ascertain its mode of action on the nucleoside polyphosphates.

In order to detect the formation of pyrophosphate, the substrates were incubated with whole snake venom in the presence or absence of purified yeast inorganic pyrophosphatase (which by itself has no action on ATP) and the amount of inorganic orthophosphate produced was determined. Table I shows that the venom by itself liberated 2 μmoles of phosphorus per μmole of ADP and 1 μmole of phosphorus per μmole of ATP. In the presence of both venom and pyrophosphatase nearly the theoretical amount of 3 μmoles of phosphorus per μmole of ATP was released. It is of interest that the same results were obtained if UTP was substituted for ATP in these experiments, and that GDP was degraded in the same manner as ADP. The specificity of the enzyme, therefore, seems to be directed only toward the pyrophosphate moiety of the substrates.

TABLE I

LIBERATION OF INORGANIC ORTHOPHOSPHATE FROM NUCLEOSIDE DI- AND TRIPHOSPHATES BY SNAKE VENOM IN THE PRESENCE OR ABSENCE OF YEAST INORGANIC PYROPHOSPHATASE (SEE TEXT FOR EXPERIMENTAL CONDITIONS)

Subtrate 4 μmoles	Enzyme	P, μmole Present	P, μmole Released
ADP	Venom	8	7.75
GDP	Venom	8	7.7
ATP	Venom	12	4.8
ATP	Venom and pyrophosphatase	12	11.0
UTP	Venom	12	4.5
UTP	Venom and pyrophosphatase	12	11.8
ATP	Pyrophosphatase	12	0.1

The production of inorganic pyrophosphate during the incubation of ATP with the venom was further confirmed by the separation of the reaction products on a Dowex-1 (chloride) column. The eluted fractions were analyzed for inorganic ortho- and pyrophosphate and their optical density at 260 mμ determined. Figure 1 shows that the ultraviolet absorbing material (adenosine) appeared in the first tubes together with the inorganic phosphate, whereas the inorganic pyrophosphate was eluted later (tubes 9–15).

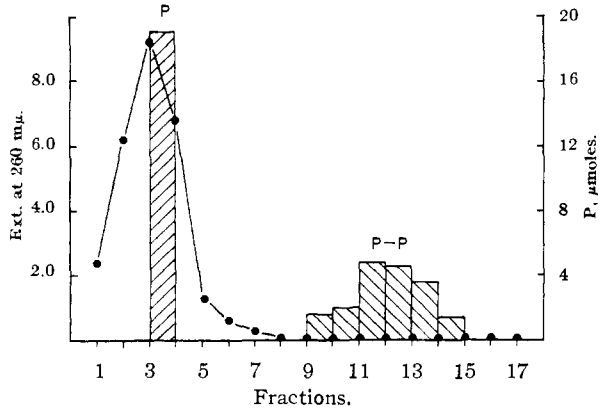

Fig. 1.—Separation of inorganic ortho- and pyrophosphate on a Dowex-1 (chloride) column after incubation of ATP with snake venom. The shaded areas represent the inorganic ortho- and pyrophosphate, respectively; the solid circles the optical density of the fractions at 260 mμ. See text for experimental conditions.

These results show that the ATP-ase of rattlesnake venom specifically splits the pyrophosphate linkage between the phosphate group attached to the ribose and the next phosphate. In this manner AMP and inorganic orthophosphate are produced from ADP; and AMP and inorganic pyrophosphate from ATP. The specific 5'-monoesterase of the venom then splits off the phosphate from the AMP that had been produced.

Experimental

Incubation of Nucleoside Polyphosphates with Snake Venom.—The incubation mixture was made up of 4 μmoles of substrate in 2 ml. of water and 1 ml. of 0.5 *M* glycine buffer *p*H 8.0 (containing Mg^{++} at a concentration of *M*/

(1) Supported by grants from the Damon Runyon Memorial Fund for Cancer Research, Inc., and the National Cancer Institute, National Institutes of Health, United States Public Health Service.

(2) E. A. Zeller, *Experientia*, **4**, 194 (1948); *Arch. Biochem.*, **28**, 138 (1950).

(3) The following abbreviations are used: AMP = adenosine-5-phosphate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; GDP = guanosine diphosphate; UTP = uridine triphosphate. These materials were obtained from Pabst Laboratories, Milwaukee, Wisconsin.

(4) E. A. Zeller, "The Enzymes," Vol. 1, part 2, Academic Press, Inc., New York, N. Y., 1951, p. 1010.

(5) M. Johnson, M. A. G. Kaye, R. Hems and H. A. Krebs, *Biochem. J.*, **54**, 625 (1953).