latter type is presumably involved in the stannic chloride-catalyzed polymerization of dienone III in ether.

(3) National Science Foundation Predoctoral Fellow, 1953–1955, 1956–1957.

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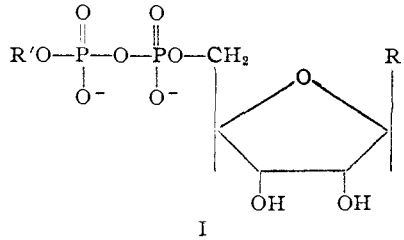
THE PREPARATION OF NUCLEOSIDE 5'-PHOSPHORAMIDATES AND THE SPECIFIC SYNTHESIS OF NUCLEOTIDE COENZYMES

Sir:

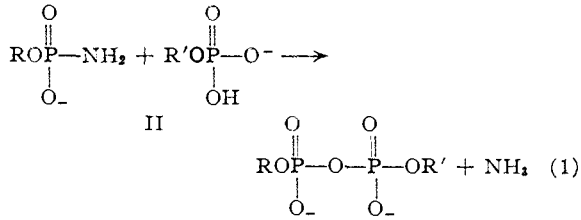
While the carbodiimide method[1] has been applied successfully to the syntheses of certain nucleotide coenzymes[2] (General Formula I) in good yield, the method is often unsatisfactory owing to its lack of specificity in effecting condensation between two dissimilar phosphate esters and frequently, to competing intramolecular cyclic phosphate formation.[3] In seeking to devise more specific methods for the synthesis of compounds of the type I, we have recently investigated[4] the use of phosphoramidates in reactions represented by equation 1. The application of this promising



I

R = Purine or Pyrimidine
R' = Sugar, etc.

approach to the practical synthesis of nucleoside



R = nucleoside
R' = H, sugar, etc.

pyrophosphate derivatives (I) was limited, however, by the relative inaccessibility of the key intermediates, nucleoside 5'-phosphoramidates[4] (II) and more direct methods for the preparation of these derivatives were, therefore, sought. In the present communication we outline a convenient one-step preparation of these compounds and record their use in highly improved syntheses of the unsymmetrical pyrophosphates, adenosine 5'-diphosphate (I, R = adenine; R' = H; ADP) and uridine diphosphate glucose[5,6] (I, R = uracil; R' = glucose; UDPG).

The direct preparation of nucleoside 5'-phosphoramidates by the condensation of readily available nucleoside 5'-phosphates with ammonia in the presence of an excess of dicyclohexyl carbodiimide (DCC) was examined and it was found that by the proper choice of reaction conditions, quantitative conversion of the nucleotides to the corresponding amidates could indeed be realized. Under the reaction conditions used, 1,3-dicyclohexylguanidine also is formed and the nucleoside 5'-phosphoramidates finally were obtained as the guanidinium salts. The general procedure may be illustrated by the preparation of adenosine 5'-phosphoramidate (AMP–NH_2): a mixture of adenosine 5'-phosphoric acid (3 mmoles), ammonia (7.5 ml. of 2*N*), formamide (5 ml.), *tert*-butyl alcohol (18 ml.) and DCC (3.0 g.) was heated at 80° for three hours. Paper chromatography at this stage showed a single strong spot corresponding to AMP–NH_2.[4] After a simple work up, which included removal of dicyclohexyl urea, ether extraction and subsequent evaporation, AMP–NH_2 was obtained as the crystalline dicyclohexylguanidinium salt (m.p. 240–241°, dec.) from formamide–acetone in 92% yield. *Anal.* Calcd. for $C_{23}H_{40}N_9O_6P\cdot 1H_2O$: C, 47.05; H, 7.21; N, 21.46; P, 5.27. Found: C, 47.46; H, 6.98; N, 21.92; P, 5.20; adenine phosphorus, 1.02. The addition of lithium hydroxide to an aqueous solution of the salt liberated the theoretical amount of free dicyclohexylguanidine (m.p. 181–182°; reported m.p. 182°).[7] Acidification of the above salt with hydrochloric acid liberated dicyclohexyl guanidinium hydrochloride; m.p. 296–297°, dec.

By the same procedure we have prepared the dicyclohexylguanidinium salts of the amidates derived from uridine 5'-phosphoric acid (75%) and monophenylphosphoric acid (68%).

In the previously reported experiments[4] on the synthesis of pyrophosphates from the phosphoramidic acids, formamide solutions of the free acids were used. In the present work, AMP–NH_2 reacted with orthophosphoric acid in *o*-chlorophenol to give 60% yield of ADP, which is easily separated from the other products of the reaction, AMP and orthophosphate, by ion exchange chromatography.[8] This new procedure should lead to more satisfactory syntheses of all the nucleoside 5'-diphosphates.[9] For the synthesis of the nucleoside pyrophosphate esters, it has now been found that pyridine solutions may be employed. In model experiments, dicyclohexylguanidium salts of uridine 5'- and adenosine 5'-phosphoramidates reacted in anhydrous pyridine with monophenylphosphoric acid to give, respectively, 87 and 70% yields of the corresponding pyrophosphates (I, R = uracil or adenine; R' = phenyl). The application of this technique

(1) H. G. Khorana, THIS JOURNAL, **76**, 3517 (1954).

(2) E. P. Kennedy, *J. Biol. Chem.*, **222**, 185 (1956).

(3) H. G. Khorana, G. M. Tener, R. S. Wright and J. G. Moffatt, THIS JOURNAL, **79**, 430 (1957).

(4) R. W. Chambers and H. G. Khorana, *Chemistry and Industry*, 1022 (1956).

(5) R. Caputto, L. F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.*, **184**, 333 (1950).

(6) For earlier syntheses of this substance see: (a) G. W. Kenner, A. R. Todd and R. F. Webb, *J. Chem. Soc.*, 2843 (1954); (b) A. M. Michelson and A. R. Todd, *ibid.*, 3459 (1956).

(7) U. S. Patent, *Chem. Abs.*, **37**, 540 (1953).

(8) In a recent paper, Clark, *et al.*, describe the use of monobenzylphosphoramidic acid in the synthesis of ADP and ATP: V. M. Clark, G. W. Kirby and A. R. Todd, *J. Chem. Soc.*, 1497 (1957).

(9) Experiments in the uridine and cytidine series are in progress

to the synthesis of true nucleotide coenzymes was demonstrated by the following preparation of UDPG. Dicyclohexylguanidiniumuridine 5′-phosphoramidate (1 equiv.) and tri-*n*-octylammonium hydrogen glucose-1-phosphate[6] (4 equiv.) were allowed to react for three days in dry pyridine at room temperature and the products were then separated by ion exchange chromatography. UDPG was the major ultraviolet absorbing product (66%) and was isolated readily as the amorphous dilithium salt. This material, which was homogeneous by paper and ion exchange chromatography, had uridine to phosphorus to glucose ratios of 1:1.95:1.01 and was enzymatically active.[10] Its behavior to acid and alkali paralleled that reported for the naturally occurring substance.[11]

This work is being extended to the synthesis of other nucleotide coenzymes.

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(10) H. M. Kalckar, "Methods in Enzymology," Vol. II, p. 676, Academic Press, Inc., New York, N. Y., 1955.

(11) A. C. Paladini and L. F. Leloir, *Biochem. J.*, **51**, 426 (1952).

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THE STABILITY OF POTASSIUM BOROHYDRIDE IN ALKALINE SOLUTIONS[1]

Sir:

In connection with an undergraduate research program to investigate some properties of KBH_4, it became necessary to determine its stability toward hydrolysis in alkaline solutions. It is generally stated in the literature that aqueous borohydride solutions are stable at a *p*H greater than 9,[2] but quantitative data on this point are meager. Pecsok[3] has presented a study on the hydrolysis of $NaBH_4$ solutions buffered at various *p*H values between 7.7 and 9.5. Jensen[4] has determined the stability of $NaBH_4$ in solutions buffered at *p*H 9.6 and 10.1 and of $NaBH_4$ dissolved in 1.00, 0.25 and 0.10 *N* sodium hydroxide.

Experimental.—0.10 *M* solutions of KBH_4 were prepared by dissolving the commercially available material[5] in a saturated $Ca(OH)_2$ solution[6] and placed in a water-bath thermostated at 25.0 ± 0.1°. At intervals of time, aliquot portions were analyzed for borohydride content by determining the volume of hydrogen liberated upon acidification.

Results and Discussion.[7]—A plot of the logarithm of borohydride content *vs.* time proved to be linear, indicating that the hydrolysis is first order with respect to borohydride at a constant *p*H. Likewise, it can be shown that Jensen's data fit the relation

$$-d(BH_4^-)/dt = k'(BH_4^-) \qquad (1)$$

where k' is a function of *p*H.

The relation between log k' and *p*H is shown in Fig. 1, indicating that the reaction is also first order in hydronium ion. The results shown in Fig. 1 support Pecsok's conclusion that the rate of hydrolysis of borohydride solutions is controlled by the formation of $HBH_4{\cdot}xH_2O$. This intermediate hydrolyzes immediately to an aquated borine radical which also hydrolyzes rapidly. It would appear from Fig. 1 that the rate of hydrolysis of the BH_4^- ion at any given *p*H is independent of the presence of Na^+, K^+, or Ca^{++} cations.

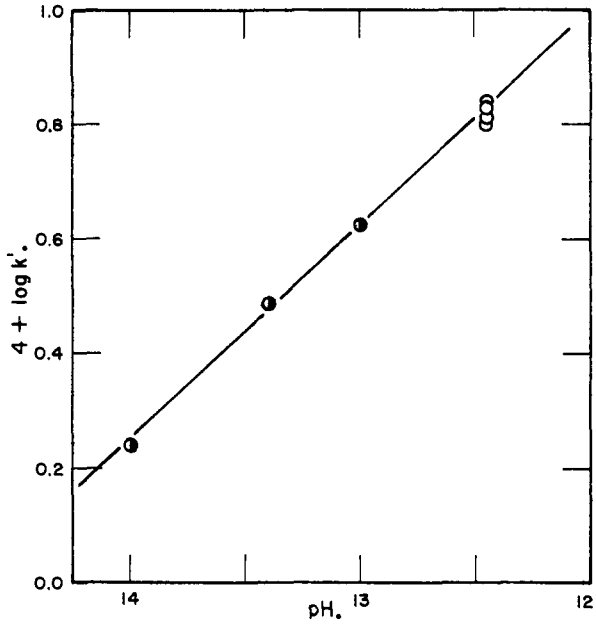

Fig. 1.—Rate constant of equation (1) as a function of *p*H: ○, KBH_4 in $Ca(OH)_2$ solutions at 25°; ◑, $NaBH_4$ in NaOH solutions at 24°.

Extrapolation of the line of Fig. 1 to lower *p*H values does not fit the data of Jensen or of Pecsok for the hydrolytic stability of the borohydride ion in buffered solutions. Nor do Jensen's data with the borate buffer lie on the same line as do Pecsok's

(1) This investigation was supported by a grant from the Denison University Research Foundation.

(2) H. I. Schlesinger, *et al.*, THIS JOURNAL, **75**, 215 (1953); H. R. Hoekstra, A.E.C.D. 2144 (June 1947).

(3) R. L. Pecsok, THIS JOURNAL, **75**, 2862 (1953).

(4) E. H. Jensen, "A Study on Sodium Borohydride with Special Reference to its Analytical Application in Organic Chemistry," Nyt Fordisk Forlag, Arnold Busch, Copenhagen, 1954, pp. 38–48.

(5) Metal Hydrides, Inc., Beverly, Mass., Lot No. K-12, Purity 97+%.

(6) Saturated $Ca(OH)_2$ at 25° has a *p*H of 12.45 and has been recommended as a buffer solution for defining the National Bureau of Standards standard *p*H scale; R. G. Bates, V. E. Bower and E. R. Smith, *J. Research Natl. Bur. Standards*, **56**, 305 (1956).

(7) More complete details will soon be published in *J. Sci. Labs., Denison Univ.*