B–F moment of 1.7 *D*: considerations of orbital following suggest that this is equal to or less than the static bond moment. The structural resemblances between these molecules make it fairly certain that μ_{SiF} should be taken to be 3.3 *D*. It may be noted in passing that there is an approximate correlation between the value of μ in set (1) and the quantity Δr_0 where Δ is the electronegativity difference between the atoms in the bond and r_0 is the bond length.

On the second point, it may be predicted that the static Si–F bond moment is actually less than the vibrational one. The model used is one in which the orbitals of the central atom rehybridize during the vibration so as to continue to point in the direction of the fluorine atoms. In the motion in which three Si–F bonds rotate away from the fourth, orbital following may be achieved by an increase in the p character of the three bonds and a decrease in that of the fourth.

The negative charge on the three atoms will then tend to increase on the three moving fluorine atoms and to decrease on the fourth, resulting in a vibrational bond moment greater than the static one.

TABLE I

		BF_3 (E' class)	CF_4	SiF_4	SF_6
Set (1)	μ	2.6	2.4	3.3	2.7
	$\partial\mu/\partial r$	4.0	3.4	3.7	3.9
Set (2)	μ	0.9	1.1	2.3	0.6
	$\partial\mu/\partial r$	−6.1	4.9	−7.5	−6.6
		(A_2'' class)			
	μ	1.7			
	Δr_0[a]	2.6	2.0	3.4	2.4

[a] Δ = electronegativity difference (Pauling), r_0 = bond length.

It must be emphasized that the bending of bonds may produce considerable electron rearrangement throughout a molecule, particularly where multiple bonds and unshared valency electrons are present,[4] and that in no case yet has the identification of vibrational and static bond moments been justified.

(4) D. F. Hornig and D. C. McKean, Symposium on Bond Moments, 125th National Meeting Am. Chem. Soc., Kansas City, Missouri, 1954.

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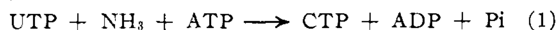
ENZYMATIC AMINATION OF URIDINE TRIPHOSPHATE TO CYTIDINE TRIPHOSPHATE[1]

Sir:

No pathway of synthesis of cytidine nucleotides has been previously elucidated. With an enzyme purified about 45-fold from extracts of *Escherichia coli* B evidence has now been obtained for the amination of UTP[2] to yield CTP in a reaction involving NH_3 and ATP, as illustrated in equation (1).



$$\text{UTP} + \text{NH}_3 + \text{ATP} \longrightarrow \text{CTP} + \text{ADP} + \text{Pi} \quad (1)$$

With this enzyme preparation, uracil, uridine and U5P were totally inactive, and with UDP the reaction rate was approximately half of that with UTP. Whether UDP serves as an amino acceptor without prior phosphorylation to UTP is not yet known. No reaction occurred in the absence of ATP except when ADP replaced it, in which case the rate was 12% of that with ATP. The following nucleotide pairs resulted in no cytosine nucleotide synthesis: A5P plus UTP; ADP plus UDP; and ITP plus UTP.

The stoichiometry with regard to the uracil and cytosine nucleotides was studied with the partially purified enzyme (Table I). The formation of UDP, CDP, and nucleoside monophosphates can be explained by the presence in the enzyme preparation of nucleoside diphosphokinase[3] and mixed myokinase.[4] CTP and CDP were identified by their absorption spectra (peaks at 280 mμ, $\lambda 280/\lambda 260$ = 1.99–2.02, $\lambda 290/\lambda 280$ = 0.74–0.75, at *p*H 2), by the molar ratios of cytosine, pentose,[5] acid-labile P, and total P of 1.00:1.01:1.96:3.06,[6] and 1.00:1.00:0.98:2.10, respectively, and by acid hydrolysis

TABLE I

STOICHIOMETRY OF URACIL AND CYTOSINE NUCLEOTIDES

The reaction mixture (64 ml.) contained 6.5 ml. of glycine (1 *M*, *p*H 8.5), 3.2 ml. of $MgCl_2$ (0.1 *M*), 3.8 ml. of NH_4NO_3 (0.2 *M*), 0.50 ml. of ATP (0.075 *M*), 3.2 ml. of UTP (0.012 *M*), and 9.6 ml. of enzyme fraction IV (containing 3.5 mg. of protein). An aliquot of the reaction mixture (29 ml.) was placed immediately in a boiling water-bath for 3 min., the remainder was incubated at 36° for 90 min. and then heated for 3 min. in a boiling water-bath.

	μMoles 0 min.	90 min.		Δ
UTP[a,b]	16.8	5.54	8.95	−7.85 (−7.46)[e]
UDP[c]	0.00	2.53		
U5P[c]	0.00	0.88		
ATP[b]	17.4	11.3	17.2	−0.20
ADP[b]	0.00	4.48		
A5P[c]	0.00	1.46		
CTP[d]	0.00	4.60	7.68 (7.74)	+7.68 (+7.74)
CDP[d]	0.00	2.39		
C5P[d]	0.00	0.69		

[a] Anion-exchange chromatography of an aliquot of the reaction mixture (29 ml.) yielded UTP, ATP, and ADP free from each other and the other nucleotides; half of the CDP and U5P fractions were free from each other and the other nucleotides, half were mixtures of the two nucleotides; CTP and UDP were eluted together except in the early fractions of CTP; C5P and A5P were eluted together. [b] Estimated spectrophotometrically at 260 mμ. [c] Estimated spectrophotometrically at 260 mμ correcting for the cytosine nucleotide present. [d] Estimated spectrophotometrically at 295 mμ. [e] Values in parentheses were determined by optical density measurements at 250 mμ (uracil nucleotides) and 295 mμ (cytosine nucleotides) before chromatography.

(1) This investigation was supported by a grant from the National Institutes of Health, Public Health Service.

(2) Abbreviations used: uridine-5'-phosphate, U5P; uridine diphosphate, UDP; uridine triphosphate, UTP; adenosine-5'-phosphate, A5P; adenosine diphosphate, ADP; adenosine triphosphate, ATP; cytidine-5'-phosphate, C5P; cytidine diphosphate, CDP; cytidine triphosphate, CTP; inosine triphosphate, ITP; inorganic orthophosphate, Pi.

(3) P. Berg and W. K. Joklik, *J. Biol. Chem.*, **210**, 657 (1954).

(4) I. Lieberman, A. Kornberg and E. S. Simms, THIS JOURNAL, **76**, 3608 (1954); J. L. Strominger, L. A. Heppel and E. S. Maxwell, *Arch. Biochem.*, **52**, 488 (1954); A. Munch-Peterson, *Proc. Swedish Biochem. Society*, June, 1954; *Acta Chem. Scand.*, in press; D. M. Gibson, P. Ayengar and D. R. Sanadi, Absts., 126th Meeting, Am. Chem. Soc., p. 41-C (Sept. 1954); E. Herbert, V. R. Potter and Y. Takagi, *J. Biol. Chem.*, in press.

(5) Unpublished method of C. E. Carter.

(6) Corrected for 7% contamination with UDP.

to C5P which was isolated by cation-exchange chromatography.[7]

Evidence for the source of the amino group was obtained by carrying out the reaction with $N^{15}H_3$.[8] CTP, ATP and UTP were separated by anion-exchange chromatography. The cytosine nucleotide was freed of contaminating UDP by hydrolysis to C5P which was isolated by cation-exchange chromatography.[7] Analysis of the nitrogen derived from the isolated nucleotides after Kjeldahl digestion yielded the following results, expressed in atom per cent. excess: amino N of CTP, 17.3; ATP, 0.00; and UTP, 0.00. The NH_3 in the reaction mixture at zero time contained 17.5 atom per cent. excess N^{15}. With enzyme fraction IV, D,L-aspartate, D,L-glutamate (each 0.02 *M*), L-asparagine, L-glutamine (each 0.01 *M*), and glycine (0.10 *M*) were incapable of satisfying the NH_3 requirement. NH_2OH, on the other hand, could replace NH_3 and two new compounds were formed which could be distinguished from the cytosine nucleotides on the basis of their chromatographic properties on anion-exchange resin, their spectrum (peak at 280 mμ, 280/260 = 1.77, 290/280 = 0.84, at *p*H 2), and their ability to yield color with $FeCl_3$. They have been tentatively identified as N-OH-CTP and N-OH-CDP.

Inorganic phosphate release accompanying CTP formation was studied with ATP labeled with P^{32} in the two terminal phosphate groups. In three experiments the synthesis of 0.044, 0.069, and 0.034 μmole of cytidine nucleotide was accompanied by the release of 0.045, 0.066, and 0.032 μmole of Pi from ATP, respectively. No detectable amination of glutamate or ITP occurred with the partially purified enzyme.

(7) W. E. Cohn, *Science*, **109**, 377 (1949).

(8) N^{15} determinations were carried out by Mrs. G. Shearer, Department of Botany, Washington University, under the supervision of Dr. B. Commoner.

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STRUCTURE AND SYNTHESIS OF KINETIN[1]

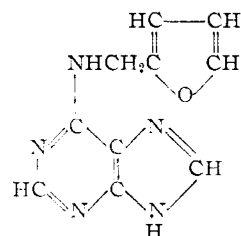
Sir:

Hydrolysis of kinetin, a recently isolated cell division factor,[2] in 2*N* HCl or H_2SO_4 at 120° for two hours caused a shift of its ultraviolet spectrum to one characteristic of adenine. After exhaustive ether extraction of the hydrolysis mixture, picric acid was added to the aqueous layer and a small yield of an insoluble picrate, m.p. 292–294°, resulted. The product was not well crystallized and was difficult to purify. Mixed with known adenine picrate, m.p. 298–299°,[3] it melted at 291–293°. Additional evidence for the presence of adenine was obtained by column chromatography on Dowex 50. The 260 mμ-absorbing material was eluted by 6*N*, but not by weaker, HCl, and behaved exactly as adenine does on this column.[4] Likewise the degradation product showed a single ultraviolet-quenching spot with the same R_f value as authentic adenine on paper chromatograms run in three different solvent systems.

The ether extract from acid-hydrolyzed kinetin was treated with 2,4-dinitrophenylhydrazine, and the amorphous product subjected to paper chromatography in a *n*-butanol–3% aqueous ammonia system. A single spot of the same yellow color and R_f value as that of authentic levulinic acid 2,4-dinitrophenylhydrazone was observed. The yield of this degradation product was also low, and the derivative proved difficult to crystallize. In the Dische test[5,6] kinetin gave a colored product with the same spectrum as that from adenine deoxyriboside, but in only 12% as great an amount.

The pKa_1 and pKa_2 values of kinetin in aqueous solution as determined by spectrophotometric methods were found to lie close to 4 and 10, respectively, and a solution of the factor in 0.05 *N* H_2SO_4 gave an immediate heavy white precipitate with aqueous silver nitrate. The 9-position in the adenine moiety was, therefore, not substituted. Since repeated attempts at acetylation under various conditions led in every case to recovery of unchanged kinetin, it seemed probable that no free hydroxyl or amino group was present in the molecule. Kinetin showed no optical rotation in *N* H_2SO_4 solution (*c*, 1.8), and zero carbon methyl by the Kuhn–Roth method.[7]

From the above and previously published[2] evidence it was concluded that kinetin most probably is 6-furfurylaminopurine, I:



Kinetin, I

This structure accounts for the analytical data and degradation products obtained and also for the marked chemical stability of kinetin.

The correctness of this structure has now been verified by synthesis of I from furfurylamine[8] and 6-methylmercaptopurine[9] by the general method of Hitchings, *et al.*[10] The crude product, in 62% yield, was recrystallized from absolute ethanol, m.p. 266–267° (sealed tube); mixed m.p. with isolated kinetin, 266–267°. The infrared and ultraviolet spectra, Dische color test, and paper chromato-

(1) This work was supported in part by research grants from the American Cancer Society, the National Science Foundation, and the Wisconsin Alumni Research Foundation.

(2) C. O. Miller, F. Skoog, M. H. Von Saltza, and F. M. Strong, THIS JOURNAL, **77**, 1392 (1955).

(3) H. B. Vickery and C. S. Leavenworth, *J. Biol. Chem.*, **63**, 579 (1925). Melting points of this derivative were taken as described by Vickery.

(4) J. S. Wall, *Anal. Chem.*, **25**, 950 (1953).

(5) Z. Dische, *Proc. Soc. Exp. Biol. Med.*, **55**, 217 (1944).

(6) P. K. Stumpf, *J. Biol. Chem.*, **169**, 367 (1947).

(7) Microanalysis by Huffman Microanalytical Laboratories, Wheatridge, Colorado.

(8) Kindly provided by F. N. Peters, Quaker Oats Company.

(9) 6-Mercaptopurine kindly provided by G. H. Hitchings, Wellcome Research Laboratories.

(10) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).