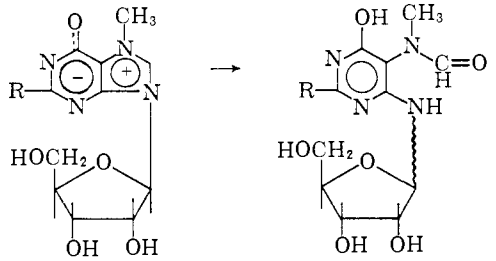
group being used to complete the pyrazine ring. Stuart and Wood[7] have summarized the steps proposed for the biological transformation of guanosine to xanthopterin as follows: (a) ring cleavage of the imidazole ring of guanosine to give a 5-amino-4-ribosylaminopyrimidine. (b) Amadori rearrangement to a 1-deoxypentulose, and (c) cyclization of this ketose to a polyhydroxyalkylpteridine. These authors present support for step (b) and illustrate experimentally the ring closure of step (c). However, the most important step, (a), the controlled ring opening of the guanosine molecule, has not as yet been demonstrated either in the laboratory or in biological systems. The present communication deals with such an observed ring cleavage and thereby adds strong support to the postulations of Weygand.[6] Although 9 B-D-ribofuranosylpurine has been reported to cleave in the imidazole ring in the presence of dilute alkali[8] at room temperature, the common purine ribosides are stable under these conditions. We now have discovered that methylation at position 7 renders guanosine susceptible to ring opening in the imidazole ring under very mild conditions. 7-Methylguanosine (I)[9] is converted to the pyrimidine derivative (II) in dilute aqueous solution after several days. This reaction is considerably accelerated by the presence of aqueous ammonia. One gram of I treated with 14% aqueous ammonia and the solution left to evaporate *in vacuo* at room temperature provided, after purification, 0.6 g. of a white crystalline product which decomposed at 175–180° when placed on the melting point block at 160°; $[\alpha]^{25}D$ +32.45° (*c* 1, H_2O). The compound (II) exhibited $\lambda_{max}^{pH'}$ 270.5 mμ, ε 22,300 and $\lambda_{max}^{pH\ 11}$, 265 mμ, ε 16,300.

Anal. Calcd. for $C_{11}H_{17}N_5O_6 \cdot H_2O$: C, 39.7; H, 5.7; N, 21.0; H_2O, 5.4. Found: C, 39.8; H, 5.9; N, 21.0; H_2O, 5.1 (Karl Fischer titration).



I, R = NH_2
III, R = OH

II, R = NH_2
IV, R = OH

Such base catalyzed ring openings have been observed[10,11] for certain 7,9-disubstituted purines. The opening of the imidazole ring probably is due to the attack of hydroxyl ion at the electrondeficient 8-position.

Methionine has been shown to be the primary precursor of the methyl groups[12–14] of N-methyl-purines, such as 7-methylguanine, which have been isolated from various biological sources. Inspection of formulas I and II strongly suggests the possibility that such derivatives might well be biogenetic precursors of N^5-methyltetrahydrofolic acid,[15,16] a functional form of folic acid. The N^7-methyl group required for the ring opening of guanosine presumably could become the N^5-methyl group of N^5-methyltetrahydrofolic acid (prefolic A). This possibility receives support from the work of Reynolds and Brown[17] who have shown that cell-free extracts of *E. coli* can convert guanosine to derivatives of folic acid.

7-Methylxanthosine (III)[9] similarly opens in the imidazole ring to provide IV as a crystalline solid in 60% yield, dec. 155–160°; $[\alpha]^{25}D$ +20.8° (*c* 1, H_2O); $\lambda_{max}^{pH\ 1}$, 268 mμ ε 20,700; $\lambda_{max}^{pH\ 11}$, 269 mμ, ε 15,000.

N.m.r. spectra[18] of I and III showed a very sharp singlet at 9.6 and 9.4 δ, respectively, due to the C^8 proton of the imidazole ring. N.m.r. of compounds II and IV showed the absence of this band, which was replaced by a new broader absorption band at 8.15 δ characteristic of the C proton of a formylamino group. II and IV exhibited a single spot (absence of starting material) when chromatogrammed in three solvent systems. Acid hydrolysis of II and IV provided D-ribose as one of the products, which was identified by means of paper chromatography.[19] Although the β-configuration and the furanose ring might be inferred in compounds II and IV, the precise structure and configuration of the ribose moiety is presently under investigation.

Neilson and Wood[20] have pointed out that on the basis of present evidence a purine nucleoside is the most likely biogenetic precursor of riboflavin and that cleavage of the imidazole ring to give a compound such as 5-amino-4-ribosylaminouracil probably is the first step in such a transformation. It is of considerable interest that IV bears such close structural relationship to this postulated precursor of riboflavin.

These observations await exploration in biological systems.

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DEPARTMENT OF CHEMISTRY
ARIZONA STATE UNIVERSITY
TEMPLE, ARIZONA
LEROY B. TOWNSEND
ROLAND K. ROBINS

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TETRAKIS-(DIMETHYLAMINO)-ETHYLENE. II.[1] THE REACTION WITH BORON TRIFLUORIDE

Sir:

Carbenes CX_2 with electron donating substituents X (NR_2, OR, F) should be more stable than methylene CH_2.[2] Therefore we considered the possibility of an equilibrium [Ethylene ⇄ 2 Carbene] in the case of tetrakis-(dimethylamino)-ethylene (I)

$$\underset{\text{I}}{(MeN)_2C{=}C(NMe_2)_2} \rightleftarrows \underset{\text{II}}{2(Me_2N)_2\bar{C}} \qquad \underset{\text{III}}{(Me_2N)_2\overset{\oplus}{C} \rightarrow \overset{\oplus}{B}\mp_3}$$

In order to prove the existence of bis-(dimethylamino)-carbene (II) we wanted to trap II in the form of its BF_3 adduct, bis-(dimethylamino)-carbene–boron trifluoride (III).

Boron trifluoride-diethyl-ether was added dropwise to an ethereal solution of I at −20°. A colorless solid formed with evolution of heat. It consisted of a little octamethyloxamidinium-tetrafluoroborate[1] (IV, mechanism of formation unknown) and a large amount of tetrakis-(dimethylamino)-ethylene-difluoroboron-tetrafluoroborate (V) (colorless needles from methanol, m.p. 217° dec., stable to air and water).

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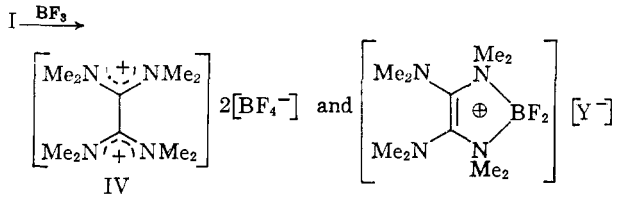

IV

V, Y = BF_4
VIa, Y = PF_6
VIb, Y = BPF_4

The following data proved the structure V: (1) Infrared- and Raman *Spectra:* besides the strong broad BF_4^--band at 1050 cm.$^{-1}$, a sharp $\nu_{C=C}$-band appears at 1708 cm.$^{-1}$ (infrared: medium; Raman: strong). (2) Ultraviolet *spectrum:* $\lambda_{max}^{H_2O}$ = 259 mμ (ε = 2970): (tetramethylformamidinium chloride, however, absorbs at $\lambda_{max}^{H_2O}$ = 224 mμ (ε 16120)). (3) *Nuclear*

TABLE I

	Ext. ref.	Chem. shift (p.p.m.)	J, c.p.s.	Origin	Intensity
^1H	Me_4Si	−2.56 broad	..	$Me_2\overset{\oplus}{N}<$	1:1[a]
		−2.60 sharp	..	Me_2N-	
^{19}F	CCl_3F	151 ... 155 singlet	..	$BF_4^\ominus$	2:1
		159 ... 163 quartet	27	$>BF$	
^{11}B	$NaBO_3$	−0.5 triplet	25	$>B\overset{+}{F}_2$	1:1
	(H_2O)	3.1 singlet	..	$BF_4^\ominus$	

[a] Integration approximate because of partial signal overlap.

Magnetic resonance spectra: (in acetonitrile) see Table I. (4) *Derivatives:* the salt character of V was shown unambiguously by precipitation reactions with a dilute aqueous solution of V. On adding ammonium hexafluorophosphate or sodium tetraphenylborate solutions, insoluble hexafluorophosphate VIa (colorless powder from acetonitrile/ether, m.p. 225° dec.) or tetraphenylborate VIb (colorless plates from acetone/methanol, m.p. 199° dec.) were obtained. The retention of the BF_2-group in VIa,b is easily seen from the conservation of the ^{11}B-triplet and the ^{19}F-quartet in the n.m.r. spectra of these salts.

(5) *Analyses of V, VIa, VIb:*

		C, %	H, %	N, %	P, %	Mol. wt.
V	Calcd.:	35.76	7.20	16.68		336
	Found:	35.73	7.10	16.77		314
VIa	Calcd.:	30.48	6.14	14.22	7.68	
	Found:	30.45	6.02	14.78	6.7	
VIb	Calcd.:	71.80	7.81	9.87		
	Found:	71.87	7.95	9.25		

V is oxidized by $KMnO_4$; boiling V with methanolic potassium hydroxide yields dimethylamine, potassium oxalate, and other products. Concentrated hydrochloric acid removes the dimethylamino groups. Phenylmagnesium bromide does not attack the "BF_2"-cation in V. However, under these conditions, the tetrafluoroborate anion is converted easily to the tetraphenylborate anion (see compound VIb).

V is a new, very stable representative of a class of compounds only recently known in boron chemistry.[3] Its ease of formation and stability may be explained by the formation of a sterically favored five-membered ring. Thus, the reaction also occurs with *o*-bis-(dimethylamino)-benzene and diborane. The corresponding salts have been prepared.[4]

(3) See $[(Me_2NH)_2BCl_2]^+$: H. Nöth and S. Lukas, *Ber.*, **95**, 1505 (1962); H. Nöth, *Angew. Chem.*, **74**, 506 (1962); $[Py_2BI_2]^+$: E. L. Muetterties, *J. Inorg. Nucl. Chem.*, **15**, 182 (1960); $[Py_2BHPh]^+$: J. E. Douglas, *J. Am. Chem. Soc.*, **84**, 121 (1962); $[(H_3N)_2BH_2]^+$: R. W. Parry, *et al.*, *J. Am. Chem. Soc.*, **80**, 4 (1958).

(4) A more detailed report is being prepared.

Therefore, the question posed initially, whether an [Ethylene ⇄ 2 Carbenes] equilibrium does exist here, cannot be answered from these experiments. Either only I is present at −20°, or the dissociation I ⇄ 2II is so slow that III is formed only in traces so far undetected; in the latter case the main reaction would also lead to V.

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INSTITUT FÜR ANORGANISCHE CHEMIE
DER UNIVERSITÄT MÜNCHEN
MUNICH, GERMANY

NILS WIBERG
JOHANN W. BUCHLER

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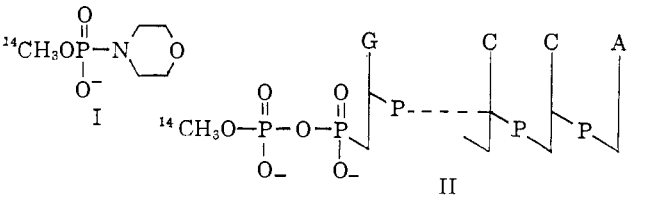
A NEW METHOD FOR THE LABELLING OF 5′-PHOSPHOMONOESTER END GROUPS IN AMINO ACID ACCEPTOR RIBONUCLEIC ACIDS

Sir:

We have recently reported on the conversion of the 5-phosphomonoester end groups in amino acid acceptor ribonucleic acids to the corresponding C^{14}-labelled phosphoroanilidates. The labelling technique facilitated the determination of nucleotide sequences near the ends bearing the 5-phosphomonoester groups. The present communication outlines a new method for the above purpose, which involves the specific reaction of the terminal 5-phosphate end groups with methyl phosphoromorpholidate (C^{14} label in the methyl group) (I) to form the pyrophosphates of the general structure II.



I

II

The new method is simpler in operation and at the leve of radioactivity so far used has proved to be completely selective in introducing the label at the phosphomonoester terminus. Furthermore, the pyrophosphate linkage has the desired stability to acidic, neutral and alkaline conditions, any or all of which may be required for subsequent chemical and enzymic degradations of the labelled polynucleotide chains.

C^{14}-Labelled methyl phosphate, prepared by the phosphorylation of C^{14}-labelled methyl alcohol with a mixture of pyridinium β-cyanoethyl phosphate and dicyclohexylcarbodiimide,[2] was converted quantitatively to the phosphoromorpholidate (I) by the general method previously described.[3] The reagent (I) was isolated, stored and used as the guanidinium (III) salt in which form it is stable over periods of several months. A mixture of pyridinium yeast amino acid acceptor ribonucleic acids (35 mg., *ca.* 1 μmole), tri-*n*-hexylamine (0.025 ml., *ca.* 0.075 mmole), the guanidinium (III) methyl phosphoromorpholidate (I) (0.05 mmole; specific activity about 6×10^4 c.p.m./μmole), and dry pyridinium Dowex-50 (2% cross-linked) ion exchange resin (1 g.) were shaken in freshly distilled dimethylformamide (3 ml.) and dry pyridine (10 ml.) for six days at room temperature. After dilution of the reaction mix-

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