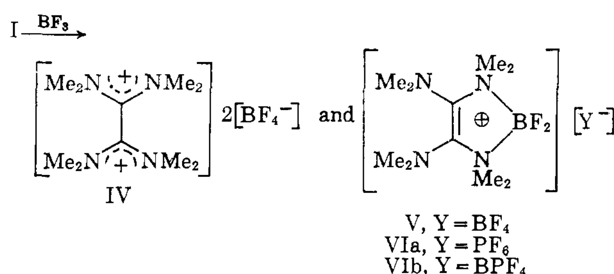

The following data proved the structure V: (1) Infrared- and Raman *Spectra:* besides the strong broad BF_4^--band at 1050 cm.$^{-1}$, a sharp $\nu_{C=C}$-band appears at 1708 cm.$^{-1}$ (infrared: medium; Raman: strong). (2) Ultraviolet *spectrum:* $\lambda_{max}^{H_2O}$ = 259 mμ (ϵ = 2970): (tetramethylformamidinium 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	$NaBO_2$	−0.5 triplet	25	$>BF_2$	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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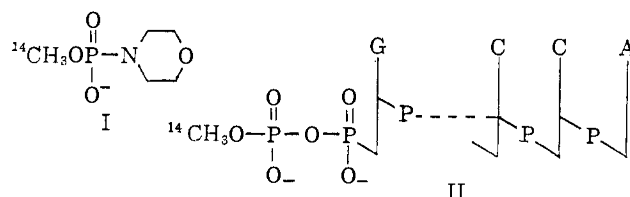
A NEW METHOD FOR THE LABELLING OF 5′-PHOSPHOMONOESTER END GROUPS IN AMINO ACID ACCEPTOR RIBONUCLEIC ACIDS

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.



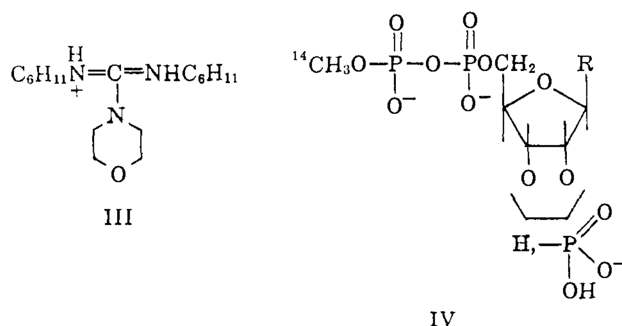
The new method is simpler in operation and at the leve of radioactivity so far used has proved to be completely selective in introducing the label at the phosphomonoester terminus. Furthermore, the pyrophosphate linkage has the desired stability to acidic, neutral and alkaline conditions, any or all 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-

(1) R. K. Ralph, R. J. Young and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 1490 (1962).

(2) G. M. Tener, *ibid.*, **83**, 159 (1961).

(3) J. G. Moffatt and H. G. Khorana, *ibid.*, **83**, 649 (1961).



ture with water, removal of the resin and extraction with ether, the labelled nucleic acids were isolated by chromatography on a DEAE-cellulose column using a linear gradient of sodium chloride in 0.1 *M* tris-hydrochloride buffer. The labelled amino acid acceptor ribonucleic acid was eluted, as expected, at 0.4-0.45 molar salt concentration, the recovery after alcohol precipitation being about 80% of the material applied to the column. The specific activity of the product obtained in different labelling experiments was 30-50 c.p.m./optical density unit (260 mμ).

The labelled product (300 optical density units at 260 mμ) was hydrolyzed in 1 *N* potassium hydroxide for 25 hr. at room temperature and the hydrolysate after neutralization with sulfonic acid ion exchange resin was chromatographed for 3 days on a 25-cm. wide strip of Whatman paper 1 in isopropyl alcohol–ammonia–water (7–1–2) solvent mixture. Scanning of a portion of the strip in a radioactivity scanner showed that 80-85% of the total radioactivity was in a nucleotide travelling just ahead of guanosine-2′(or 3′),5′-diphosphate. The remainder of the radioactivity was present in a band travelling a little faster than the major radioactive band. After further purification by paper electrophoresis in pH 5 ammonium acetate buffer, the major radioactive product was identified as IV (R = guanine). The characterization was accomplished by absorption spectrum, specific activity, degradation with crude snake venom to C^{14}-methyl phosphate and guanosine-2′(3′),5′-diphosphate and, finally, degradation with bacterial alkaline phosphomonoesterase to a compound presumed to be P^1-guanosine-5′,P^2-C^{14}-methyl pyrophosphate. Although an insufficient amount of the minor radioactive product has been available, this has been tentatively identified, by similar techniques, as the adenine analog of IV. This result is in agreement with that reported previously[1] that a few amino acid acceptor ribonucleic acid chains end in adenosine-5 phosphate groups.

The extent of the reaction with the end groups has been in the range of 50% in the experiments carried out so far. The principles for subsequent degradations so as to recognize the sequences near the labelled terminus have been outlined previously.[1]

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Neutron Activation as a Method for Labelling the Phosphoros of Nucleotides

A CORRECTION

It was reported recently that neutron activation could be used to label the phosphorus of nucleotides,[1] the production of phosphorus-32 being proportional to the phosphorus content of the samples. There was no gross decomposition which could be detected by physical or biological means. For instance, 5′-adenosine triphosphate (ATP) irradiated in the water-cooled compartment of a graphite reactor at a neutron flux of 6.5×10^{11} neutrons/cm.^{2}/sec. for 62 hours, retained undiminished ability to induce luminescence in the luciferin-luciferase assay, which specifically requires the triphosphate.[2] The infrared and ultraviolet spectra of all samples were unaltered by the activation procedure.

While all the above findings could be duplicated, it was found on repetition of these experiments that there is extensive decomposition localized exclusively in the radioactive molecules. This decomposition, which is due presumably to recoil reactions, could be detected by the non-correspondence of ultraviolet absorbing spots and radioactivity after paper chromatography or paper electrophoresis. Since the "cold" molecules, making up the bulk of the irradiated nucleotides, were intact no gross decomposition could be found.

Repetitions of the chromatographic procedure coupled with strip counting reported in the original communication,[1] showed that only a small fraction of the radioactive phosphorus is associated with authentic nucleotides. Radioautography provided a more convenient method for obtaining the same results.

Purification of activated 5′-adenosine monophosphate by the successive use of Dowex formate[3] and Ecteola cellulose columns[4] and further analysis of the fractions containing radioactive ultraviolet absorbing materials indicated that not more than 1% of the phosphorus-32 was attached to adenosine, while there was no gross decomposition of the nucleotide.

The chromatography of "activated" ATP with or without treatment with hexokinase and glucose, followed by radioautography, showed that the distribution of radioactivity was not affected by this reaction, although ADP and glucose-6-phosphate were formed normally.[5]

It seems, therefore, that while neutron activation can label the phosphorus of nucleotides, it is not a useful method for labelling nucleotides.

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(1) H. G. Mautner, B. Donnelly, C. M. Lee and G. W. Leddicotte, *J. Am. Chem. Soc.*, **84**, 2021 (1962).

(2) B. L. Strehler and J. R. Totter in D. Glick, "Methods of Biochemical Analysis," Vol. I, Interscience Publishers, New York, N. Y., 1954, p. 345.

(3) G. R. Bartlett, *J. Biol. Chem.*, **234**, 459 (1959).

(4) G. M. Tener, H. G. Khorana, R. Markham and E. H. Pol, *J. Am. Chem. Soc.*, **80**, 6223 (1958).

(5) B. C. Pressman, C. Stroebel and P. D. Boyer "in Biochemical Preparations," Vol. 7, John Wiley & Sons, New York, N. Y., 1960, p. 18.

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