

often failed²; on the other hand there appear to be favorable nucleophiles and solvents which facilitate the steps a-b of eq. 1. For example, $C_6H_5C\equiv CP-(C_6H_5)_3^+Br^-$ and $C_6H_5C\equiv CP(C_4H_9)_3^+Br^-$ have been prepared from reactions in ether at room temperature. We are investigating the scope and rationale of these displacements involving nucleophiles containing sulfur, nitrogen, phosphorus, oxygen, *etc.*, in detail.

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NEUTRON ACTIVATION AS A METHOD FOR LABELLING THE PHOSPHORUS OF NUCLEOTIDES¹

Sir:

Ribonucleotides and deoxyribonucleotides labelled with phosphorus-32 have been of great value in exploring the biochemistry of nucleic acids. The preparation of such compounds tends to be complex, however, involving as it does either biological methods or multi-step synthetic procedures with chromatographic purification of the final product.² These problems are particularly unfortunate, since the half-life of phosphorus-32 is comparatively short (14.3 days).

It was found recently that neutron activation could be used as a method for the preparation,³ from "cold" selenium-containing organic compounds, of the corresponding substances labeled with selenium-75; under these conditions only negligible decomposition occurred. It was of interest, therefore, to determine whether neutron activation could be applied to the phosphorus of nucleotides.

Samples (100 to 200 mg.) of 5'-adenosine monophosphate (AMP), 5' - adenosine diphosphate (ADP), 5'-adenosine triphosphate (ATP), 3'-adenosine monophosphate (3'-AMP), and 5'-deoxyadenosine monophosphate (dAMP) were 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. After discharge, the samples were permitted to stand for 150 hours to permit decay of sodium-24 in those samples (ADP, ATP) that had been submitted as sodium salts. Gamma spectrometry showed traces of arsenic-76 and antimony-122 in dAMP, traces of antimony-122 in 3'-AMP, and traces of residual sodium-24 in ADP and ATP. All other radioactivity was attributable to phosphorus-32.

Seventeen days after discharge from the reactor,

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(2) For instance, G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961); J. M. Lowenstein and R. L. Metzberg in "Biochemical Preparations," Vol. 7, John Wiley & Sons, Inc., New York, N. Y., 1960, p. 5.

(3) K. P. McConnell, H. G. Mautner, and G. W. Leddicotte, *Biochim. Biophys. Acta*, in press.

the radioactivity of the samples was determined using a low background automatic counter (Nuclear-Chicago) with a counting efficiency of 45%. Aliquots of sample solutions were plated on stainless steel planchettes. Recovery of samples after activation was quantitative; no purification was carried out prior to counting.

These results were obtained:

Compound	Counts/ μ mole/min.
AMP	2.4×10^6
ADP	4.9×10^6
ATP	8.0×10^6
3'-AMP	2.0×10^6
dAMP	2.2×10^6

Chromatography of the activated compounds (isobutyric acid:concd. ammonium hydroxide: water; 66:1:33) yielded well-defined spots with R_f values identical with those of control material. Neutron activation did not reduce the ability of the ATP sample (Pabst Laboratories, lot no. 131A) to induce luminescence in the luciferin-luciferase assay which specifically requires the triphosphate.⁴ This assay was carried out in quadruplicate.

Use of a strip counter showed only negligible radioactivity outside the spots. On the basis of these findings, neutron activation appears to be a useful tool for the labelling of the phosphorus of nucleotides and presumably of other phosphorus-containing compounds.

(4) 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.

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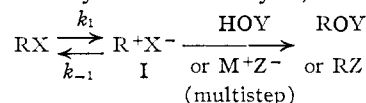
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THE QUESTION OF INTIMATE ION PAIRS¹

Sir:

Ion pairs (I) are intermediates in substitution reactions of triphenylmethyl (trityl) or benzhydryl compounds with hydroxylic reagents (solvolysis, hydrolysis, alcoholysis or acetolysis) or salts.³



Since certain rearrangement or racemization reactions of these compounds proceed even faster than substitution and also show a very large (but not identical) dependence of rate on solvent,⁴ these

(1) Supported in part by the Atomic Energy Commission. We are grateful to Dr. W. v. E. Doering for information² in 1959 on trityl benzoate-carbonyl-O¹⁸.

(2) W. v. E. Doering, K. Okamoto and H. Krauch, *J. Am. Chem. Soc.*, **82**, 3579 (1960).

(3) R. F. Hudson and B. Saville, *Chem. and Ind.*, 1423 (1954); C. G. Swain and M. M. Kreevoy, *J. Am. Chem. Soc.*, **77**, 1122 (1955); E. D. Hughes, C. K. Ingold, S. F. Mok, S. Patai and Y. Pocker, *J. Chem. Soc.*, 1220, 1230, 1238, 1256, 1265 (1957); C. G. Swain and E. E. Pegues, *J. Am. Chem. Soc.*, **80**, 812 (1958).

(4) S. Winstein and J. S. Gall, *Tetrahedron Letters*, **2**, 31 (1960); S. Winstein, M. Hojo and S. Smith, *ibid.*, **22**, 12 (1960); S. Winstein, J. S. Gall, M. Hojo and S. Smith, *J. Am. Chem. Soc.*, **82**, 1010 (1960); Y. Pocker, *Proc. Chem. Soc.*, 140 (1961); S. Winstein, A. Ledwith and M. Hojo, *Tetrahedron Letters*, **10**, 341 (1961); H. L. Goering and J. F. Levy, *ibid.*, **18**, 644 (1961).