# Alkylation of the Purine Nucleus by Means of Quaternary Ammonium Compounds. I. Tetraalkylammonium Hydroxides

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The alkylation of various purines was carried out by heating a mixture of the purine and the tetraalkylammonium hydroxide under reduced pressure. 9-Methyl adenine (IIIa), -kinetin (IIIb), -guanine (IIIc), 9-ethyladenine (IIId), 9-*n*-propyladenine (IIIe), and caffeine (VI) were obtained in excellent yield and in one step from the readily available purine.

Purines are associated intimately with all living systems which have been studied. For the most part the natural purines, as they occur in the nucleic acids, puromycin, the vitamin $B_{12}$ analogs, and the coenzymes, are substituted in the 9-position of the nucleus by a carbohydrate moiety attached through its anomeric carbon.[3] Purine derivatives substituted in the 9-position may be regarded as structural analogs of these compounds and, therefore, are of interest as potential inhibitors of biological processes.

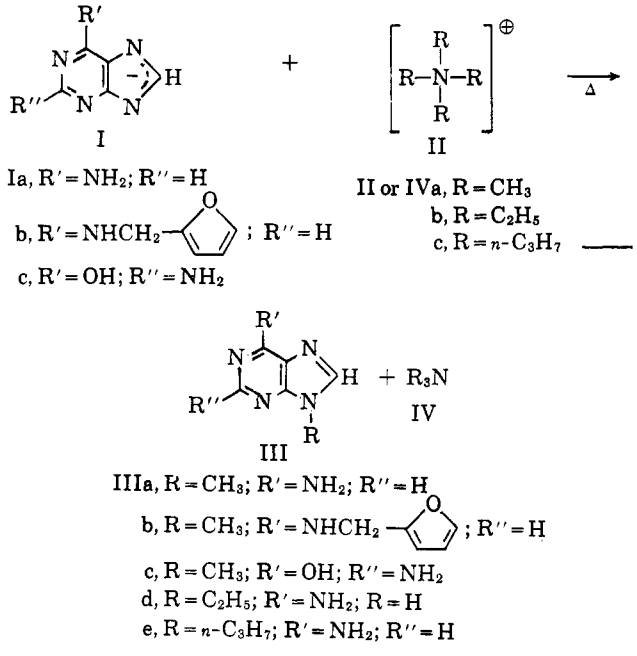
Syntheses of 9-substituted purines have been performed in general, either by the cyclization procedures which are modifications of the classical synthesis, *i.e.*, condensation of a substituted 4,5-diaminopyrimidine with a one carbon unit,[4] or by alkylation of the 9-position of a purine. This procedure involves the reaction of alkyl halides with alkali metal salts or chloromercuri salts of the purine and results usually in mixtures of the 7- and 9-substituted purines.[5]

The development of a facile method involving the direct alkylation of the purine nucleus at the 9-position was desirable since such a method would yield alkylpurines in one or two steps from readily available purines. In the search for suitable procedures a number of quaternary ammonium salts have been investigated as alkylating agents. The present paper concerns the use of tetraalkylammonium hydroxide in the alkylation of several purines.

N-Methylpurines in particular have been synthesized by a variety of methods, and the synthesis of 9-methylpurines has been reviewed by Robins.[6] The best synthetic method to date involves cyclization of substituted diaminopyrimidines, a procedure that has a number of disadvantages: several steps are required to obtain the desired pyrimidine and yields are not always high.

In the present work methylation was carried out by heating under reduced pressure the solid residue left after lyophilization of a solution of equimolar quantities of tetramethylammonium hydroxide and the purine to be methylated. When the temperature of the reaction mixture reached *ca.* 170° in the case of adenine (Ia), or *ca.* 260° in the case of guanine (Ic), the product sublimed from the mixture, in many cases in homologeneous form as determined by paper chromatography.

CHART I




This procedure was employed successfully to prepare 9-methyladenine (IIIa), 9-methylkinetin (IIIb), and 9-methylguanine (IIIc). The identity of the products was confirmed by analysis and by comparison with authentic samples. The infrared spectra[7] of 9-methyladenine (IIIa) and 9-methylkinetin (IIIb) were identical in all respects with those of authentic samples.[8] Paper chromatography[9] of 9-methylguanine (IIIc), prepared by alkylation, and an authentic sample[8] in two solvent systems gave identical results. In the case of the guanine a second product was obtained that exhibited an ultraviolet absorption spectrum similar to that of guanine (Ic) but migrated with a different $R_f$ in one of the chromatographic solvents employed.

(1) U. S. Public Health Service Fellow 1956–1960. From a thesis submitted by L. D. Z. in partial fulfillment of the requirements for the Ph.D. degree, University of Illinois, 1961.

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(6) R. K. Robins and H. H. Lin, *ibid.*, **79**, 490 (1957).

(7) Infrared spectra determinations were done by J. Broder or P. McMahon, University of Illinois, Urbana, Ill.

(8) Graciously donated by R. K. Robins.

(9) Paper chromatograms were run by the descending technique on Whatman no. 1 paper in 1-butanol–0.6 *M* ammonium hydroxide (6:1) (solvent A) or in 95% ethanol–1 *M* ammonium acetate pH 7.5 (7:3) (solvent B). The compounds were located by visual examination with the use of an ultraviolet lamp.

TABLE I

ALKYLATION OF THE PURINE NUCLEUS WITH VARIOUS QUATERNARY AMMONIUM COMPOUNDS

| Reactants | Product | Reaction | | Recrystallization solvent | % Yield crude sublimate |
|---|---|---|---|---|---|
| | | Temp., °C. | Time, hr. | | |
| Ia + IIa | 9-Methyladenine (IIIa) | 170–200 | 6 | 95% ethanol | 77 |
| Ib + IIa | 9-Methylkinetin (IIIb) | 190–200 | 5 | Acetone | 85 |
| Ic + IIa | 9-Methylguanine (IIIc) | 260 | 5.5 | ... | 50 |
| Ia + IIb | 9-Ethyladenine (IIId) | 150–160 | 2 | Methyl ethyl ketone | 74 |
| Ia + IIc | 9-*n*-Propyladenine (IIIe) | 195 | ... | Methyl ethyl ketone | 71 |
| V + IIa | Caffeine (VI) | 150 | ... | ... | 85 |
| VII + IIa | Caffeine (VI) | 210 | ... | ... | 87 |

TABLE II

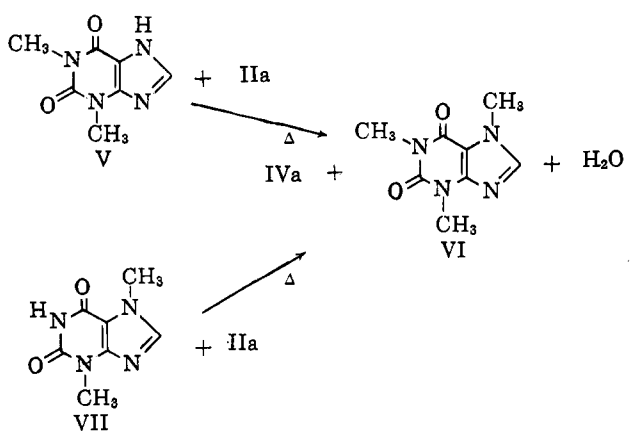
PHYSICAL CONSTANTS AND ANALYSES OF ALKYLPURINES

| Alkylpurine | M.p., °C.[a] | Ultraviolet spectra or chromatographic identity[b] | Formula | Analyses, %[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Carbon | | Hydrogen | | Nitrogen | |
| | | | | Calcd. | Found | Calcd. | Found | Calcd. | Found |
| 9-Methyladenine (IIIa) | 301–302[c] | Ultraviolet absorption maxima, 258 mμ | $C_6H_7N_5$ | 48.31 | 47.81 | 4.73 | 4.75 | 46.96 | 47.31 |
| 9-Methylkinetin (IIIb) | 176.5–177.5 | ... | $C_{11}H_{11}N_5O$ | 57.64 | 57.47 | 4.63 | 4.94 | 30.57 | 30.49 |
| 9-Methylguanine (IIIc) | ... | $R_f$ (solvent A) 0.14[a]<br>$R_f$ (solvent B) 0.66<br>Identical to those of authentic sample[d]<br>A second ultraviolet absorbing material was detected in crude sublimate<br>$R_f$ (solvent A) 0.26<br>$R_f$ (solvent B) 0.55 | | | | | | | |
| 9-Ethyladenine (IIId) | 192–194[e] | ... | $C_7H_9N_5$ | 51.53 | 51.35 | 5.56 | 5.56 | 42.92 | 42.90 |
| 9-*n*-Propyladenine (IIIe) | 168 | $R_f$ (solvent A) 0.87 | $C_8H_{11}N_5$ | 54.23 | 54.00 | 6.26 | 6.10 | 39.52 | 39.44 |
| Caffeine (VI) | ... | $R_f$ (solvent A) 0.55<br>$R_f$ (solvent B) 0.84<br>Identical to those of sample | | | | | | | |

[a] See ref. 12. [b] See ref. 9. [c] Melting point determined in a sealed tube. [d] See ref. 8. [e] Reported m.p. 194–195° (Ref. 4c).

This product was not identified but is believed to be a methylated guanine. The yields in the preparation of 9-methyladenine (IIIa) and 9-methylkinetin (IIIc) were about 80% based on the starting purine.

The methylation of other purines was then investigated. Theophylline (V) and theobromine (VII) were methylated using tetramethylammonium hydroxide (IIa) to form chromatographically pure caffeine (VI) in *ca.* 80% yield.




If quaternary ammonium hydroxides containing alkyl groups higher than methyl are employed, Hofmann degradation with alkene formation might be expected to compete with or even to abolish N-alkylation.[10] However, tetraethylammonium hydroxide (IIb) served to prepare 9-ethyladenine (IIId) in 74% yield. The product was characterized by melting point and elemental analysis; its infrared spectrum resembles that of 9-methyladenine. Similarly, 9-*n*-propyladenine (IIIe) was obtained in 71% yield by use of tetra-*n*-propylammonium hydroxide (IIc). The identity of the propyl residue as *n*-propyl, and not isopropyl, was determined by n.m.r. spectroscopy.

The reaction is believed to proceed as formulated in Chart I. The residue after lyophilization is assumed to be the tetraalkylammonium salt of the purine. Salts of this type have been isolated when the purine has been theophylline.[11] The intermediate salt decomposes on heating with alkylation of the purine nucleus and production of free trialkylamine. From the reaction of tetra-*n*-propylammonium hydroxide (IIc) with adenine (Ia), tri-*n*-propylamine (IVc) was isolated in 85% yield and characterized as its picrate and methiodide.

Further work is in progress on the use of other quaternary ammonium compounds as alkylating agents.

## Experimental[12]

**Starting Materials.**—The tetraalkylammonium hydroxides solutions were prepared from the corresponding tetraalkylam-

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monium halides (Eastman) by treatment of aqueous solutions of the salts with powdered silver oxide. Purines were obtained from commercial sources and used without further purification.

**Alkylpurines.**—The compounds were prepared as described for 9-methylkinetin (IIIb). Reaction time and temperature as well as recrystallization solvent are given in Table I. Physical constants and analysis of the products are given in Table II.

**9-Methylkinetin.**—A solution of 0.22 g. (2.38 mmoles) of tetramethylammonium hydroxide (IIa) and 0.50 g. (2.32 mmoles) of kinetin (Ib) in 5 ml. of water was lyophilized. The hydroscopic residue, in a sublimation apparatus, was heated slowly under oil pump vacuum (*ca.* 0.05 mm.) to a temperature of 190–200°. At this temperature a sublimate began to collect on the condenser. After 5 hr. 0.38 g. of sublimed product was collected. An additional 0.07 g. of product was collected on continued heating at 200°. The combined sublimates yielded 0.45 g. (85%) of crude product. A portion of the crude product was recrystallized twice from acetone to give white crystals, m.p. 176.5–177.5°, undepressed when admixed with an authentic sample of 9-methylkinetin.[8]

(12) Melting points were taken on a Kofler hot-stage microscope and are uncorrected. The analyses were carried out at Micro-Tech Laboratories, Skokie, Ill. The ultraviolet absorption spectra were measured in aqueous solution on a Beckman DU spectrophotometer.

# The Reaction of Nitric Oxide with Dialkyl Phosphonates

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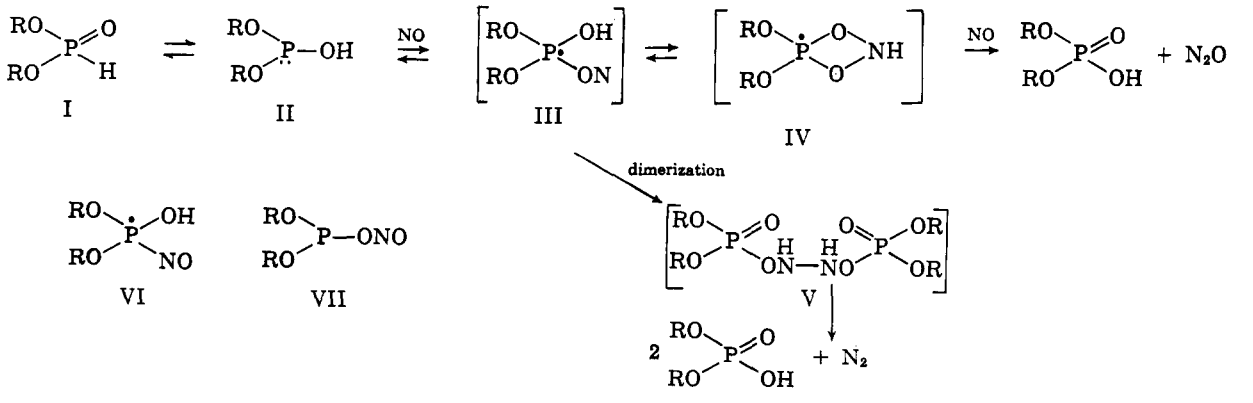
Nitric oxide reacts with dialkyl phosphonates to give the corresponding dialkyl phosphates in high yield. Both tracer experiments with oxygen-18 and the gaseous reaction products indicate that the reaction proceeds *via* two mechanisms.

A number of reactions between derivatives of trivalent phosphorus and various oxides of nitrogen and related compounds have been reported and recently been reviewed.[1]

It appears that nitric oxide is a particularly efficacious reagent for the oxidation of triphenylphosphine[2] and methylphosphines[3] to the phosphine oxides and of triethyl phosphite to triethyl phosphate.[4] In connection with our studies on the properties of the P–H bond,[5] we have examined the reaction of nitric oxide with dialkyl phosphonates and found that the corresponding dialkyl phosphates are formed readily in excellent yield. The reaction occurs at room temperature both with and without a solvent. The gaseous product obtained is mainly nitrogen with some nitrous oxide. The proportion of the latter component is increased on increasing dilution of the phosphonate in benzene solution. In order to elucidate the mechanism, reaction was run using nitric oxide with ~78 atom % $O^{18}$ and diethyl phosphonate. Both the recovered unchanged nitric oxide, and the product nitrous oxide had a considerably reduced $O^{18}$ content (of the order of 57%) indicating that at some stage the oxygen of nitric oxide is in a reversible equilibrium with a normal oxygen atom which in this case can only be the "phosphoryl" oxygen atom of the phosphonate. The following mechanism is proposed on the basis of the products and in order to account for these isotopic results. The isomerization I ⇄ II is well known[6] and, although no physical evidence for the existence of appreciable concentrations of II has been obtained, many reactions of dialkyl phosphonates are assumed to proceed *via* this intermediate.[5] The intermediate III was not isolated but has a radical type structure formed by attack of oxygen on phosphorus and is analogous to one of the structures proposed by Kuhn[4] for the reaction of nitric oxide with triethyl phosphite. His alternative suggestion of compound with a P–N bond (VI) is not likly in this case since the reversible steps II ⇄ III ⇄ IV are required in order to account for the equilibration of isotopic oxygen in the products. In a control experiment nitric oxide was found not to exchange oxygen with diethyl phosphate. The exact structure of IV is not known, but the alternative reaction involving abstraction of hydrogen and attack on *oxygen* by nitric




oxide to form compound VII is excluded by the observation that the intermediate in this case, prepared directly from the phosphorochloridite and silver nitrite is known[7] to form tetralkyl pyrophosphate in high

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