448. *Nucleotides. Part XLVI.*[1] *A New Method for the Preparation of Nucleoside Phosphites.*

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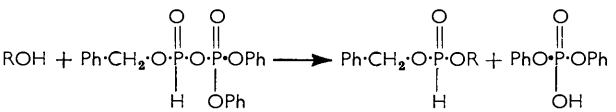
Nucleoside phosphites can be prepared in good yield by reaction of nucleosides with phosphorous acid and stoicheiometric proportions of di-*p*-tolylcarbodi-imide in dry pyridine; no esterification occurs under these conditions when monobenzyl phosphite is used in place of phosphorous acid. The mechanism of the reaction is discussed. Preparation of deoxyadenosine-5′ phosphite by this method followed by permanganate oxidation gives deoxyadenosine-5′ phosphate in an overall yield of 40%.

Most of the recorded monoalkyl esters of phosphorous acid have been prepared by treating the appropriate alcohol with phosphorus trichloride and hydrolysing the resulting phosphorochloridite. Although many monoalkyl phosphites have been prepared in this way,[2] the method is not very suitable for preparing the phosphites of relatively sensitive substances such as nucleosides. Nucleoside benzyl phosphites (II; R = nucleoside residue) have, however, been prepared by reaction of a suitably protected nucleoside with the mixed anhydride (I) obtained by treating monobenzyl phosphite with diphenyl phosphorochloridate; the simple nucleoside phosphite could then be obtained from the product by anionic debenzylation and removal of protecting groups.[3] The yields obtained by this method are usually indifferent, particularly when stoicheiometric amounts of reagents are employed. Recently Tanaka[4] has described the preparation of both riboflavin-5′ phosphite and pyridoxin phosphite using as reagent either diphenyl phosphorochloridite or the cyclic phosphorochloridite prepared from salicylic acid and phosphorus trichloride; protecting groups were in each case removed by hydrolysis and the products were then converted into the corresponding phosphates by oxidation with potassium permanganate. In view of the potential interest of the nucleoside phosphites in various studies relating to nucleic acid chemistry, there is clearly a need for better preparative procedures.



(I) (II)

We have now shown that treatment of an alcohol with phosphorous acid and di-*p*-tolylcarbodi-imide in dry pyridine solution yields the corresponding monoalkyl phosphite directly. The reaction appears to be generally applicable and it gives high yields even when stoicheiometric amounts of reactants are employed. By this method the 2′,3′-*O*-isopropylidene derivatives of adenosine and uridine were converted into the corresponding 5′-phosphites in yields of approximately 78% and 74% respectively. When 5′-*O*-trityl-thymidine was treated in the same way and the product heated for a short time with 80% acetic acid to remove the trityl group, thymidine-3′ phosphite was isolated (as its ammonium salt) in *ca.* 40% yield. The relatively low yield in this instance is undoubtedly due to hydrolytic removal of the phosphite group during the acid treatment; model experiments have shown that the phosphite is completely hydrolysed in 1 hr. by hot 80% acetic acid.

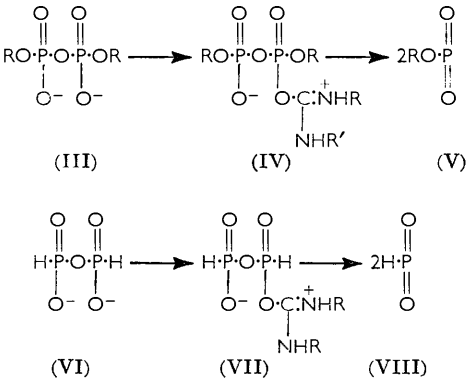
Treatment of thymidine itself with phosphorous acid and di-*p*-tolylcarbodi-imide gave a mixture of the 3′-phosphite (43%) and 5′-phosphite (24%), while deoxyadenosine gave a

[1] Part XLV, Baluja, Chase, Kenner, and Todd, *J.*, 1960, 4678.
[2] Kosolapoff, "Organophosphorus Compounds," Wiley, New York, 1950, p. 201.
[3] Hall, Todd, and Webb, *J.*, 1957, 3291.
[4] Tanaka, *J. Pharm. Soc. Japan*, 1959, **79**, 437, 721, 1301.

larger proportion of the 5′-phosphite (42%) than of the 3′-phosphite (29%). The isomeric deoxyadenosine phosphites could be separated, on a preparative scale, by chromatography on a column of ECTEOLA cellulose. Attempts to oxidise the thymidine phosphites to the corresponding phosphates with potassium permanganate gave only traces of the desired products; paper chromatography showed that destruction of the heterocyclic nucleus had occurred and the main product was a phosphorus-containing material showing no ultraviolet absorption. Similar destruction of the nucleus occurred when thymidine-5′ phosphate and deoxycytidine-5′ phosphate were treated with permanganate under the same conditions. Jones and his co-workers [5] have shown that the ease of oxidation of the bases occurring in deoxyribonucleic acid is in the order cytosine and thymine > guanine ⋙ adenine. In accordance with this 2′,3′-*O*-isopropylideneadenosine-5′ phosphite and the isomeric deoxyadenosine phosphites were oxidised by permanganate to give the corresponding phosphates in good yield.

The fact that nucleosides are converted into monoesters of phosphorous acid so smoothly by the method described prompted us to examine again their conversion into nucleoside benzyl phosphites. Reaction of monobenzyl phosphite with either diphenyl phosphorochloridate or toluene-*p*-sulphonyl chloride in presence of a stoicheiometric amount of 2′,3′-*O*-isopropylideneadenosine and 3 mols. of base gave 2′,3′-*O*-isopropylideneadenosine-5′ benzyl phosphite in only *ca.* 30% yield. When the same nucleoside was treated in stoicheiometric proportion with monobenzyl phosphite and di-*p*-tolylcarbodi-imide in pyridine solution only traces of phosphite were formed.

This striking difference between the preparation of mono- and di-esters of phosphorous acid recalls the marked difference between the reactivity towards alcohols of the mono- and di-esters of phosphoric acid in presence of carbodi-imides. One of us [6] has sought to explain the latter difference by postulating that reaction of a monoester of phosphoric acid with a carbodi-imide yields first the P^1P^2-diester of pyrophosphoric acid (III), which then reacts with a further molecule of carbodi-imide to yield an isouronium pyrophosphate represented in its protonated form by (IV). This product (IV) then breaks down, yielding



2 mols. of the hypothetical monomeric metaphosphate (V) which is the true phosphorylating agent and reacts with an alcohol to yield a diester of phosphoric acid. This explains why monoesters of phosphoric acid are readily converted into diesters by this means,[7] whereas diesters of phosphoric acid, which cannot generate metaphosphate in this way, react with alcohols in presence of carbodi-imides only with difficulty unless the alcohol is

[5] Bayley and Jones, *Trans. Faraday Soc.*, 1959, **55**, 492; Benn, Chatamra, and Jones, *J.*, 1960, 1014.

[6] Todd, *Proc. Acad. Nat. Sci. U.S.A.*, 1959, **45**, 1389.

[7] Gilham and Khorana, *J. Amer. Chem. Soc.*, 1958, **80**, 6212; 1959, **81**, 4647; Tener, Khorana, Markham, and Pol, *ibid.*, 1958, **80**, 6223.

present in overwhelming excess (*e.g.*, as solvent for the reaction). By analogy with this scheme, the reaction of phosphorous acid with a carbodi-imide should yield pyrophosphorous acid (VI) which would presumably react further to give the protonated intermediate (VII). Disruption of the latter would give 2 mols. of (VIII) which is the phosphite (or phosphonate) analogue of the metaphosphate (V) and should react readily with alcohols to yield monoesters of phosphorous acid. Monoesters of phosphorous acid cannot yield a compound of type (VIII) and hence should be, as observed, very indifferent reagents towards alcohols in presence of carbodi-imides. It is interesting that, when thymidine was treated with phosphoric acid and di-*p*-tolylcarbodi-imide in pyridine under the conditions used for phosphorous acid, no phosphorylated material could be detected by paper chromatography. On the mechanism suggested above, metaphosphoric acid should be produced in this reaction; however, in pyridine solution it would almost certainly be in the form of its anion which would not be a favourable phosphorylating agent for alcohols.

Experimental

Materials for analysis were dried at 80°/10^{-2} mm. for 8 hr.

Paper Chromatography.—Unless otherwise stated, data given refer to ascending chromatograms on Whatman No. 1 paper in the solvent system propan-2-ol–ammonia (*d* 0·88)–water (7 : 1 : 2). In the text, solvent system A refers to butan-1-ol–acetic acid–water (5 : 2 : 3) and system B to propan-2-ol–ammonia (*d* 0·88)–acetic acid–water (4 : 1 : 2 : 2).

2′,3′-O-*Isopropylideneadenosine-5′ Phosphite.*—A mixture of crystalline phosphorous acid (28 mg., 0·33 mmole) and 2′,3′-*O*-isopropylideneadenosine (102·3 mg., 0·33 mmole) was thoroughly dried by thrice dissolving it in dry pyridine and evaporating the solution at 10^{-2} mm. The dried mixture was then dissolved in dry pyridine (8 ml.), di-*p*-tolylcarbodi-imide (80 mg., 0·37 mmole) was added, and the mixture left for 72 hr. at room temperature, moisture being excluded. Paper chromatography showed the product to contain a little 2′,3′-*O*-isopropylideneadenosine (R_F 0·93) and phosphorous acid (R_F 0·13) with a new substance (R_F 0·72) as the main constituent. The mixture was poured into water (20 ml.) and filtered, and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in a little water, and the solution streaked on four sheets (11 cm. width) of Whatman No. 3 paper. The chromatographs were developed in the usual way and the main band cut out, eluted with dilute ammonia, and lyophilised to give *ammonium* 2′,3′-O-*isopropylideneadenosine-5′ phosphite* (101·5 mg., 78%) as a slightly yellowish glass. This material was dissolved in a minimum of ethanol, the solution centrifuged to remove a trace of insoluble material, and the product reprecipitated with ether as a white amorphous powder (Found: P, 8·1. $C_{13}H_{17}N_5O_6P,NH_4$ requires P, 8·0%).

The above product (5·8 mg.) was dissolved in 0·015% aqueous sodium hydrogen carbonate (1 ml.), and 0·155% aqueous potassium permanganate (1 ml.) was added dropwise during 1 hr. The mixture was set aside for 6 hr., then evaporated to dryness. The residue was taken up in a little water, Amberlite IR-120 (pyridinium form) added, and the whole filtered. Paper-chromatographic examination of the filtrate showed that it contained a product identical in R_F (0·29) with 2′,3′-*O*-isopropylideneadenosine-5′ phosphate.

2′,3′-O-*Isopropylideneuridine-5′ Phosphite.*—A mixture of 2′,3′-*O*-isopropylideneuridine (95 mg., 1 mol.) and phosphorous acid (28 mg., 1 mol.), dried as above by thrice evaporating it with pyridine, was dissolved in pyridine (5 ml.), and di-*p*-tolylcarbodi-imide (80 mg., 1·1 mol.) was added. After 24 hr., paper chromatography showed the presence of small amounts of unchanged starting material (R_F 0·58) and phosphorous acid (R_F 0·24), with a new substance (R_F 0·87) as the major component. Worked up as in the previous experiment *ammonium* 2′,3′-O-*isopropylideneuridine-5′ phosphite* (91·4 mg., 74%) was obtained as a white amorphous powder (Found: P, 8·2. $C_{12}H_{16}N_2O_8P,NH_4$ requires P, 8·5%).

Thymidine-3′ Phosphite.—A mixture of 5′-*O*-tritylthymidine (121 mg., 1 mol.) and phosphorous acid (20·5 mg., 1 mol.) was dried in the usual way, then dissolved in dry pyridine (5 ml.). Di-*p*-tolylcarbodi-imide (70 mg., 1·2 mol.) was added and the solution set aside for 20 hr. at room temperature, moisture being excluded. The mixture was then evaporated and the residue heated in aqueous 80% acetic acid (5 ml.) at 70° for 15 min. to remove the trityl

group. This solution was next evaporated under reduced pressure, and a solution of the residue in a little pyridine (5 ml.) was poured into water (20 ml.), filtered from the precipitated di-*p*-tolylurea and triphenylmethanol, and concentrated to small bulk. Paper chromatography of a sample showed three phosphorus-containing compounds: (*a*) R_F 0·62 (main product), (*b*) R_F 0·46 (trace), and (*c*) phosphorous acid, R_F 0·27. The remainder of the solution was streaked on two sheets (13 cm. width) of Whatman No. 3 paper and after chromatographic development the main band was cut out and eluted with aqueous ammonia. The eluate was lyophilised and the product purified by dissolution in ethanol and reprecipitation with ether. *Ammonium thymidine-3′ phosphite* (33·8 mg., 40%) was obtained as a white amorphous powder (Found: P, 8·8. $C_{10}H_{14}N_2O_7PNH_4,H_2O$ requires P, 9·1%).

Thymidine-3′ and Thymidine-5′ Phosphite from Thymidine.—A dried mixture of thymidine (81 mg., 1 mol.) and phosphorous acid (35 mg., 1·3 mol.) was dissolved in pyridine (3 ml.) and, after addition of di-*p*-tolylcarbodi-imide (100 mg., 1·4 mol.), was left at room temperature for 3 days, moisture being excluded. In addition to some unchanged starting materials the product contained two major phosphorus-containing and ultraviolet-absorbing spots (R_F 0·71 and 0·53). The mixture was worked up as before by preparative chromatography on Whatman No. 3 paper. Elution of the faster-moving band (R_F 0·71) and isolation of the white amorphous salt gave ammonium thymidine-3′ phosphite (49 mg., 43%), indistinguishable by paper chromatography from the material prepared above from 5′-*O*-tritylthymidine (Found: P, 9·5. Calc. for $C_{10}H_{14}N_2O_7P,NH_4,H_2O$: P, 9·1%).

Worked up in the same fashion, the slower-moving band (R_F 0·53) gave *ammonium thymidine-5′ phosphite* (27 mg., 24%) (Found: P, 9·45%).

Deoxyadenosine Phosphites.—Deoxyadenosine (85 mg., 1 mol.) was treated with phosphorous acid (38 mg., 1·4 mol.) and di-*p*-tolylcarbodi-imide (100 mg., 1·4 mol.) in pyridine (3 ml.), exactly as was thymidine in the above experiment, and the products were worked up in the same way, yielding as major product, *ammonium deoxyadenosine-5′ phosphite* (52 mg., 42%) (Found: P, 8·95. $C_{10}H_{13}N_5O_5P,NH_4,H_2O$ requires P, 8·9%). This product had R_F 0·59 and on oxidation with permanganate gave deoxyadenosine-5′ phosphate identified by comparison with an authentic specimen. The lesser product (R_F 0·46) from the reaction was isolated as *ammonium deoxyadenosine-3′ phosphite* (36 mg., 29%), oxidised by permanganate to deoxyadenosine-3′ phosphate (Found: P, 8·6%).

In a further experiment the reaction mixture (from deoxyadenosine, 0·425 g.), after removal of di-*p*-tolylurea by filtration, was stirred with charcoal (15 g.) for 1 hr. and then filtered. The charcoal was washed with water and extracted with boiling ethanolic ammonia (2 × 100 ml.). The combined extracts were evaporated to dryness and the residue was dissolved in methanol (25 ml.). Addition of ether precipitated a white solid (362·5 mg.) which was separated by centrifugation.

A portion (200 mg.) of this material was dissolved in water (10 ml.) and was added to a column (27 × 2 cm.) of ECTEOLA cellulose (chloride form). Elution with 0·025M-lithium chloride gave fraction A, which was evaporated to dryness. The residue was dissolved in the minimum amount of 95% ethanol. Addition of excess of acetone precipitated *lithium deoxyadenosine-5′ phosphite* (158·9 mg.) as a white powder (Found, in material dried for 15 hr.: P, 9·9. $C_{10}H_{13}LiN_5O_5P$ requires P, 9·7%).

Further elution of the column with 0·05M-lithium chloride gave fraction B, which was worked up as above to give crude lithium deoxyadenosine-3′ phosphite (55·2 mg.) as a white powder.

Deoxyadenosine-5′ Phosphate.—The lithium salt of deoxyadenosine-5′ phosphite (64 mg.), prepared as described above, was dissolved in water (5 ml.) containing sodium hydrogen carbonate (20 mg.). The solution was cooled in ice, and 0·155% aqueous potassium permanganate (13 ml.) was added during 45 min. with stirring. The mixture was filtered, and inorganic cations were removed with Amberlite IR-120 (pyridinium form). The resulting solution was evaporated to dryness, and the residue evaporated once with dilute ammonia, dissolved in water (10 ml.), and put on a column (20 × 2 cm.) of ECTEOLA cellulose (chloride form). Elution was carried out by a linear gradient technique with water (500 ml.) in the mixing vessel and an equal volume of 0·1M-lithium chloride. The main fraction was evaporated and the residue dissolved in a minimum of water. Addition of ethanol (5 ml.) followed by acetone (30 ml.) precipitated *dilithium deoxyadenosine-5′ phosphate* (48·6 mg.) as a white powder (Found: P, 8·6. $C_{10}H_{12}Li_2N_5O_6P$ requires P, 9·0%). The nucleotide obtained in this way

was identical in chromatographic behaviour with authentic deoxyadenosine-5′ phosphate, running as a single spot (R_F 0·16) in propan-2-ol–ammonia (*d* 0·88)–water, R_F 0·23 in system A and R_F 0·63 in system B. Treatment with rattlesnake venom [8] (*C. atrox*) for 3 hr. at 37 converted the product smoothly into deoxyadenosine (R_F 0·7).

Deoxyadenosine-3′ Phosphate.—The crude lithium salt of deoxyadenosine-3′ phosphite, prepared as above, was oxidised and the product worked up as was the 5′-compound. Paper chromatography showed the presence of two compounds (R_F 0·11 and 0·02). The faster-running compound was identical with authentic deoxyadenosine-3′ phosphate; treatment with rattlesnake venom had virtually no effect, only a trace of deoxyadenosine being produced. The slower-moving compound is probably deoxyadenosine-3′,5′ diphosphate.

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[8] Michelson and Todd, *J.*, 1953, 951.