Notes

Cyclopropylmethyl Dihydrogen Phosphate. Preparation and Use in the Phosphorylation of Nucleosides

Allen M. Schoffstall

Department of Chemistry, University of Colorado, Colorado Springs, Colorado 80907

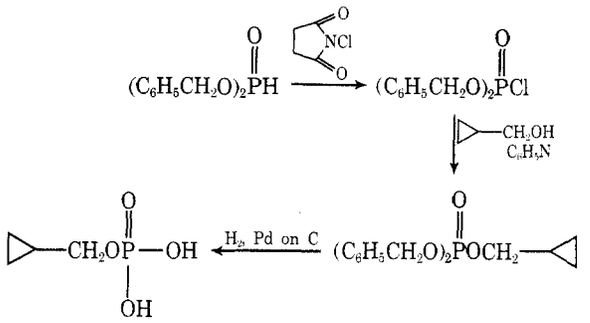
Received April 29, 1975

Many methods have been developed for phosphorylating nucleosides. The main types are condensations brought about by dialkylcarbodiimides and direct use of phosphorus oxychloride or its suitably activated derivatives, such as the phosphorochloridates.[1] The direct use of phosphorus oxychloride in trialkyl phosphate solvent has been shown to yield nucleotides in good yield.[2] A widely used method[3] is that of Tener,[4] who effected phosphorylation of blocked nucleosides with hydracrylonitrile dihydrogen phosphate and dicyclohexylcarbodiimide (DCC) in dry pyridine. The present study offers a convenient alternative to the latter procedure.

Acyclic organophosphodiesters generally undergo hydrolysis and other displacement reactions more slowly than phosphomonoesters and triesters containing similar substituents.[5-7] This behavior is particularly important when considering synthetic routes to nucleotides involving hydrolytic cleavage of ester blocking groups.[8,9] Simple alkyl and aryl substituted phosphorylating agents are rarely used in nucleotide preparations.[10] Even *p*-nitrophenyl nucleotide diesters require strong alkaline hydrolysis to remove the blocking group.[11] A notable advance was made by introduction of the 2-cyanoethyl blocking group[12] and its application to nucleotide syntheses.[4] The 2-cyanoethyl group is very sensitive to alkaline treatment, with 2-cyanoethyl phosphodiesters being more labile than hydracrylonitrile dihydrogen phosphate.

The cyclopropylmethyl blocking group has been employed in the synthesis of phosphomonoesters and nucleotides.[13] Facile hydrolytic removal of the cyclopropylmethyl group from phosphate esters was anticipated, similar to the rapid solvolyses of cyclopropylmethyl benzenesulfonate[14,15] and chloromethylcyclopropane.[16] Bis(cyclopropylmethyl) phosphorochloridate has been used to prepare bis(cyclopropylmethyl) nucleoside phosphates.[13] However, conditions necessary for hydrolysis of both blocking groups to give nucleotides produced considerable glycosidic cleavage. Rearrangement to less reactive cyclobutyl esters was shown to interfere with facile hydrolysis.[13] Therefore, attention was focused on Tener's method, but utilizing the cyclopropylmethyl blocking group instead of the 2-cyanoethyl group.

In this study phosphorylations of blocked nucleosides with cyclopropylmethyl dihydrogen phosphate (1) were carried out in a manner similar to those involving hydracrylonitrile dihydrogen phosphate. Preparation of 1 according to the methods used for hydracrylonitrile dihydrogen phosphate appeared unattractive, however, owing to the aqueous work-ups required.[4] Phosphomonoesters have been prepared directly from an alcohol, orthophosphate, and DCC.[4,17,18] However, the monoesters initially formed underwent further reaction with DCC and gave diesters.[17] The route chosen for the preparation of 1 is shown below.



1 2

Commercial dibenzyl phosphonate was converted with *N*-chlorosuccinimide[19] to bis(benzyl) phosphorochloridate and the product esterified with cyclopropanemethanol, which provided dibenzyl cyclopropylmethyl phosphate (2). Catalytic hydrogenolysis of 2 using 10% palladium on charcoal in ethyl acetate or ethanol, followed by filtration and treatment with pyridine, yielded the pyridinium salt of 1. Addition of gaseous ammonia to a solution of 1 in ethyl acetate gave the crystalline ammonium salt of 1. Since 1 decomposed slowly at room temperature, it was best prepared just before it was to be used in a phosphorylation reaction. Also, it was best converted to the pyridinium salt immediately after the hydrogenolysis reaction.

As illustrations of the use of 1 in nucleotide syntheses, 2′,3′-*O*-isopropylideneuridine, 2′,3′-*O*-isopropylideneadenosine, and 2′,3′-*O*-isopropylideneguanosine were converted to uridine 5′-phosphate (UMP), adenosine 5′-phosphate (AMP), and guanosine 5′-phosphate (GMP). As observed in the case of hydracrylonitrile dihydrogen phosphate,[4] the blocked adenosine nucleoside required a fourfold excess of 1 to give extensive phosphorylation. Hydrolysis of the cyclopropylmethyl and isopropylidene blocking groups was accomplished in refluxing water at pH 2.5–2.8. Data showing yields of nucleotides and reaction conditions are summarized in Table I. The purine nucleotides were hydrolyzed for shorter periods in order to avoid extensive glycosyl cleavage.

Table I
Nucleotides from Cyclopropylmethyl Dihydrogen Phosphate

Nucleoside precursor	Registry no.	Nucleotide	Yield, %	Phosphodiester hydrolysis time, hr
2′,3′-*O*-Isopropylideneuridine	362-43-6	UMP	61	3.0
2′,3′-*O*-Isopropylideneadenosine	362-75-4	AMP	36	1.5
2′,3′-*O*-Isopropylideneguanosine	362-76-5	GMP	35	1.5

The main advantage of the present method, compared with Tener's method,[4] is that the reagent 1 used in the phosphorylations is readily synthesized from 2 in an anhydrous condition. Storage of 2 for over 1 year at room temperature gave little decomposition. If only acid-labile blocking groups are employed, all blocking groups may be removed in a single hydrolysis step.

Experimental Section

NMR spectra were obtained using a Bruker HX-60 spectrometer. All peak positions are in δ (parts per million) from internal tetramethylsilane reference. Pyridine was dried over calcium hydride before use. All evaporations were performed under vacuum below 40°.

Cyclopropanemethanol, obtained commercially (Aldrich Chemical Co.), was fractionated using a Vigreux column. The fraction boiling at 125° was used for syntheses below. Microanalysis was by Galbraith Laboratories, Knoxville, Tenn.

Dibenzyl Cyclopropylmethyl Phosphate (2). Dibenzyl phosphonate was prepared according to the literature[20] and was used undistilled (but free of benzyl chloride) to prepare bis(benzyl) phosphorochloridate by the *N*-chlorosuccinimide method.[19] A solution of 20.1 g of bis(benzyl) phosphorochloridate (68.6 mmol) in 75 ml of ether was added dropwise over a 15-min period to 5.44 g (70 mmol) of cyclopropanemethanol and 5.93 (75 mmol) of pyridine in 100 ml of ether at 0°. Pyridine hydrochloride precipitated from the solution immediately. The mixture was stirred at room temperature for 18 hr. Filtration of the pyridine hydrochloride and evaporation of the ether left 19.9 g of a clear oil (88%): NMR (CCl_4) δ 0.33 (m, 4 H, CH_2CH_2), 1.03 (m, 1 H, CH), 3.73 (dd, 2 H, CH_2), 4.97 (d, 4 H, $ArCH_2$), 7.25 (s, 10 H, ArH). Attempted distillation of **2** at reduced pressure resulted in decomposition.

Cyclopropylmethyl Dihydrogen Phosphate (1). A solution of 1.20 g (3.6 mmol) of **2** was dissolved in 30 ml of dry absolute ethanol and 0.5 g of 10% palladium on charcoal was added. The mixture was hydrogenated for 30 min at 20–30 psi using a Parr hydrogenator. The catalyst was filtered and the solvent evaporated, affording 0.24 g of an oil (91%): NMR (Me_2SO-d_6) δ 0.46 (m, 4 H, CH_2CH_2), 1.17 (m, 1 H, CH), 3.77 (m, 2 H, CH_2), 11.1 (broad s, 2 H, OH).

Treatment of the solution obtained after hydrogenolysis and filtration followed by treatment with ammonia gave the ammonium salt, mp 160–164°.

Anal. Calcd for $C_4H_{12}NO_4P$: C, 28.41; H, 7.15; N, 8.28; P, 18.32. Found: C, 28.57; H, 7.33; N, 8.42; P, 18.40.

Uridine 5′-Phosphate.[21] Cyclopropylmethyl dihydrogen phosphate (from 1.61 g of **2**) was converted to the pyridinium salt by addition of 5 ml of pyridine to the filtered ethanol solution from above. The solution was evaporated and the residue dissolved in 10 ml of pyridine. After addition of 0.284 g (1.0 mmol) of 2′,3′-*O*-isopropylideneuridine, the solution was treated with 2.06 g (10 mmol) of DCC. The mixture was kept at room temperature for 2 days, followed by treatment with 2 ml of water. The mixture was allowed to stand for an additional 1 hr. The solvents were evaporated and the residue treated with 10 ml of water and evaporated to dryness. The residue was treated with 75 ml of water and the mixture was filtered. The filter cake was washed with 50 ml of water. The filtrate and washings were poured through an Amberlite 120 H^+ column. The column was washed with water until the effluent was neutral. The final volume of solution was adjusted to 500 ml and the pH was 2.6. The solution was refluxed for 3 hr. The cooled solution was then reduced to a volume of 50 ml and the pH was adjusted to 7.5–8.0 with saturated barium hydroxide solution. The barium phosphate was removed by centrifugation. The salt was washed well with water and the filtrate and washings (150 ml) were treated with 300 ml of ethanol to precipitate the barium salt of uridine 5′-phosphate. The solid was collected using a centrifuge, washed with water–ethanol, 1:2 (v/v), ethanol, and ether, and dried over P_2O_5 at 0.1 mm for 4 hr. The dry powder was calculated to be the hexahydrate of UMP using uv analysis at 262 nm of a sample dissolved in 0.01 *N* HCl. The product weighed 0.345 g (61%). Chromatographic analysis was performed as reported previously.[13]

Registry No.—**1**, 56599-14-5; 1 NH_3, 56599-15-6; **2**, 56599-16-7; UMP, 58-97-9; AMP, 61-19-8; GMP, 85-32-5; bis(benzyl) phosphorochloridate, 538-37-4; cyclopropanemethanol, 2516-33-8.

References and Notes

(1) E. Cherbuliez in "Organic Phosphorus Compounds", Vol. 6, G. M. Kosalapoff and L. Maier, Ed., Wiley-Interscience, New York, N.Y., 1973, Chapter 15.
(2) (a) M. Yoshikawa, T. Kato, and T. Takenishi, *Bull. Chem. Soc. Jpn.*, **42**, 3505 (1969); (b) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **33**, 2583 (1968).
(3) (a) Y. Fujimoto and Teranishi, *Methods Carbohydr. Chem.*, **6**, 451 (1972); (b) "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Wiley-Interscience, New York, N.Y., 1968, pp 501, 523.
(4) (a) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961); (b) P. T. Gilham and G. M. Tener, *Chem. Ind. (London)*, 542 (1959).
(5) C. A. Bunton, *Acc. Chem. Res.*, **3**, 275 (1970).
(6) J. R. Cox and O. B. Ramsay, *Chem. Rev.*, **64**, 317 (1964).
(7) R. F. Hudson and M. Green, *Angew. Chem., Int. Ed. Engl.*, **2**, 11 (1963).
(8) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest", Wiley, New York, N.Y., 1961, pp 26–33.
(9) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides", Academic Press, New York, N.Y., 1963, pp 110–118.
(10) See, however, T. Hata and K.-J. Chong, *Bull. Chem. Soc. Jpn.*, **45**, 654 (1972).
(11) R. W. Chambers, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3747 (1957).
(12) E. Cherbuliez and J. Rabinowitz, *Helv. Chim. Acta*, **39**, 1461 (1956).
(13) A. M. Schoffstall and H. Tieckelmann, *Tetrahedron*, **22**, 399 (1966).
(14) C. G. Bergstrom and S. Siegel, *J. Am. Chem. Soc.*, **74**, 145, 254 (1952).
(15) E. Tommila and M. Lindholm, *Acta Chem. Scand.*, **5**, 647 (1951).
(16) J. D. Roberts and R. H. Mazur, *J. Am. Chem. Soc.*, **73**, 2509 (1951).
(17) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6204 (1958).
(18) W. Weiss and L. Gladstone, *J. Am. Chem. Soc.*, **81**, 4118 (1959).
(19) G. W. Kenner, A. R. Todd, and F. J. Weymouth, *J. Chem. Soc.*, 3675 (1952).
(20) F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, 382 (1945).
(21) AMP and GMP were prepared similarly except that hydrolysis times were different (see Table I).

Use of Hydrazides of Heterocyclic Carboxylic Acids for the Resolution of Z-DL-Alanine during Papain Catalysis

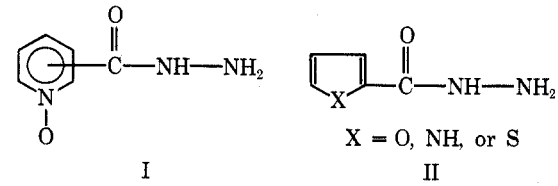
John Leo Abernethy,* David Srulevitch, and Marvin J. Ordway, Jr.

Department of Chemistry, California State Polytechnic University, Pomona, California 91768

Received June 10, 1975

Numerous arenecarboxylic hydrazides have been demonstrated to be effective amino bases for papain-catalyzed reactions with N-blocked amino acids.[1] We have now focused attention on the behavior of a few hydrazides which incorporate a heterocyclic nucleus and a single hydrazide function toward Z-amino acids under papain catalysis.[2] A substantial number of such hydrazides have been prepared in conjunction with a systematic investigation of their antituberculin activity.[3]

The first hydrazides used in the current study contained a pyridine nucleus. These were picolinic hydrazide, nicotinic hydrazide, and isonicotinic hydrazide. When subjected to proper conditions for papain catalysis of reactions with *N*-acylamino acids, all three failed to respond. With the conjecture that the difficulty might be attributed to the basic nature of the heterocyclic nitrogen, this nitrogen was blocked with oxygen. Picolinic *N*-oxide, nicotinic *N*-oxide, and isonicotinic *N*-oxide hydrazides (I) were then examined. In addition, the study was extended to three compounds with representative five-membered heterocycles, namely, 2-furoic, 2-pyrrolecarboxylic, and 2-thiophenecarboxylic hydrazides (II).



I II

When Z-glycine was the *N*-acylamino acid reactant, all six hydrazides yielded the unsymmetrical, achiral N^1,N^2-