Notes

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Phosphorylation of 2′,3′-Isopropylidene Inosine by Heating or Ultraviolet Irradiation in the Presence of Phosphoric Acid and Nitriles

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Usually phosphoro or pyrophosphoro chloridate derivatives have been used for the phosphorylation of nucleosides, but orthophosphoric acid itself rarely is used as a phosphorylating agent, because of the low yields in some cases.

Though the phosphorylation of hydroxyl groups generally can be achieved by heating with polyphosphoric acid or anhydrous phosphoric acid, the application of this method to nucleosides, especially purine nucleosides is very difficult because of the instabilities of them under these reaction conditions. Recently several studies on the phosphorylation of nucleosides with orthophosphoric acid salt[2-4] were reported but in these reactions heatings above 150° were needed. Therefore, some milder phosphorylation methods of nucleosides were sought. This note deals with the phosphorylation using orthophosphoric acid by heating or ultraviolet irradiation in the presence of nitriles.

Cramer and Weimann[5] studied on the effectiveness of trichloroacetonitrile as an agent for esterification of phosphoric acid monoester to diester. On the other hand, recently Symons[6] applied this method to the phosphorylation of nucleosides to 5′-nucleotides but it was not preparative. Considering the reaction mechanism, nitriles carrying electron attracting groups on the α-carbon might be desirable in this reaction. So we attempted malononitrile, acrylonitrile, ethyl cyanoacetate as the agents.

By heating the solution of isopropylidene inosine and tri-*n*-butylammonium dihydrogen phosphate (5 equivalents) in dimethylformamide at 100° for 7 hr, the reaction did not occur, but the addition of malononitrile (3 equivalents) in the reaction media prompted the phosphorylation to give the yield of 12%. Increased amount of the nitrile (9 equivalents) and prolonged reaction time (25 hr) increased the yield and the products were fractionated into 5′-IMP[7] (39%), and 5′-IDP (12%) with ion-exchange chromatography. The reactions were tried in *m*-cresol and dimethylsulfoxide in which the decomposition of inosine was occurred. Acrylonitrile was in effective as a condensing agent in this reaction.

Recently ultraviolet (UV) light has been used as the source of reaction energy in many regions of organic chemistry. In nucleic acid chemistry, as well, it has been used for photolysis[8,9] but not for phosphorylation yet. To avoid the photolysis, we used Toshiba lamp

1) Location: *Juso-Nishino-cho, Higashiyodogawa-ku, Osaka.*
2) C. Ponnamperuma and R. Mack, *Science*, **148**, 1221 (1965).
3) M. Honjo, Y. Furukawa, and K. Kobayashi, *Chem. Pharm. Bull.* (Tokyo), **14**, 1061 (1966).
4) J. Skoda and J. Moravek, *Tetrahedron Letters*, **1966**, 4167.
5) F. Cramer and G. Weimann, *Chem. Ber.*, **94**, 996 (1961); K.E. Pfitzner and J.G. Moffatt, *Biochem. Biophys. Res. Commun.*, **17**, 146 (1964).
6) R.H. Symons, *Biochem. Biophys. Res. Commun.*, **24**, 872 (1966).
7) 5′-IMP, inosine-5′ phosphate; 5′-IDP, inosine-5′ diphosphate.
8) H. Trapmann and M. Devani, *Nature*, **206**, 931 (1965).
9) M. Tomasz and R.W. Chambers, *J. Am. Chem. Soc.*, **86**, 4216 (1964).

SHL–100 UD which generates mixed wave length UV scanty of near 250 mμ light (strong light: 312, 365 mμ). Under the irradiation, the temperature of reaction media elevated to 80—90° by radiant heat and the yield increased to about twice as compared with the case of just heating in the presence of malononitrile. The products resulting from the irradiation reaction for 24 hr were fractionated into 5′–IMP (50%) and 5′–IDP (4%). In this case, the formation of pyrophosphate linkage rarely occurred.

When acrylonitrile was used, ultraviolet irradiation reaction exhibited a striking contrast to heating reaction. The irradiation reaction for 9 hr given phosphorylated products in the yield of 30%. Moreover, the prolongation of irradiation period to 24 hr increased the yield and then the products were fractionated into 5′–IMP (42%) and 5′–IDP (3%) by the chromatography. In this case, the production of pyrophosphate linkage also rarely occurred.

Even in the absence of nitriles, phosphorylated products were given in the yield of 10—20% by ultraviolet irradiation.

Yoshida and Ukita[10] reported that inosine reacted with acrylonitrile at 37° in phosphate buffer pH 8.5 to produced 1–cyanoethylinosine, which was not observed in this case. These results were shown in Table.

It is clarified that some nitriles promote the phosphorylation of nucleosides with *ortho*-phosphoric acid and ultraviolet irradiation does too. Moreover, it was found that less amount of pyrophosphate compound is produced by ultraviolet irradiation than by heating. It is very interesting that acrylonitrile is effective as the agent for the phosphorylation under ultraviolet irradiation but it is not effective under heating.

Table

Solvent	Nitrile (× moles)	Hr	Temp.°C or UV	Yield of phosphorylation	Note
m–Cresol	$CNCH_2CN$(× 3)	7	100	5	destruction
DMSO	$CNCH_2CN$(× 3)	7	100	0	destruction
DMF	$CNCH_2CN$(× 3)	7	100	12	
DMF	$CNCH_2CN$(× 3)	22.5	100	32	
DMF	$CNCH_2CN$(× 9)	25	100	50	
DMF	CH_2=CHCN(× 3)	7	100	trace	
DMF	——	7	100	trace	
DMF	$CNCH_2CN$(×3)	9	UV 80—90	29	
DMF	$CNCH_2CN$(×3)	24	UV 80—90	54	
DMF	CH_2=CHCN(×3)	9	UV 80—90	30	
DMF	CH_2=CHCN(×3)	24	UV 80—90	44, 46	
DMF	CH_2=CHCN(×6)	24	UV 80—90	48	
DMF	——	9	UV 80—90	11	
DMF	——	24	UV 80—90	18	

It is clarified that some nitriles promote the phosphorylation of nucleosides with *ortho*-phosphoric acid and that the ultraviolet irradiation does too. Moreover, it was found that less amount of pyrophosphate compound is produced by ultraviolet irradiation than by heating. It is very interesting that acrylonitrile is effective as condensing agent for the phosphorylation under ultraviolet irradiation but it is not effective by heating.

Experimental

Methods——Paper electrophoresis was carried out on Whatman filter paper No. 1 at 43 V/cm for 20—30 min, using following buffer solutions: (1) 0.05 M borate buffer pH 9.2; (2) 0.1 M ammonium acetate buffer pH

10) M. Yoshida and T. Ukita, *J. Biochem.*, **57**, 818 (1965).

9.1: (3) 0.1 M acetate buffer pH 4.1. Paper chromatography (P.C.) was carried out on the same filter paper as above, using the following solvents by the ascending technique: (A) isobutyric acid–2 N NH_4OH (66:34); (B) *n*-BuOH–AcOH–H_2O (2:1:1); (C) EtOH–0.5 M ammonium acetate (pH 3.8) (5:2). The detection was done using either irradiation with an ultraviolet lamp or coloration with ferric chloride–sulfosalicyclic acid reagent. The nucelar magnetic resonance (NMR) spectra were measured with a Varian A-60 NMR spectrometer at a fixed frequency of 60 Mc using tetramethysilane (TMS) as an external standard.

5′-IMP Synthesis by Heating with Orthophosphoric Acid in the Presence of Malononitrile——In stoppered vessel, isopropylidene inosine 2.0 g (6.49 mmoles), mono–tri–*n*-butylammonium phosphate 9.2 g (6.49×5 mmoles) and malononitrile 1.28 g (6.49×3 mmoles) were dissolved in dimethylformamide (DMF) 40 ml. Malononitrile 2.56 g was added in two portions at interval of 8 hr (total addition of malononitrile: 9 equivalents). The reaction mixture was heated in the boiling water bath for 25 hr, giving a black solution. After dilution with water, the black insoluble material was filtered off. The filtrate was extracted several times with ether (100 ml each) and ether in aqueous layer was evaporated *in vacuo* at room temperature. The eluate desalted as usual with charcoal (40 g) was concentrated to a small volume, then applied to the column of Dowex 1×8 (Cl form, 100—200 mesh, 2.4×27 cm) and washed with water. The nucleotides were eluted in a stepwise manner as follows. Isopropylidene inosine and any other byproducts with water (Fraction I, T.O.D.[11])$_{248.5}$ 46,940), isopropylidene 5′-IMP with 0.01 N HCl+0.02 M NaCl (Fraction II, T.O.D.$_{248.5}$ 30.450; 38.5% of the optical density unit of starting material), isopropylidne IDP with 0.1 N HCl+0.2 M NaCl (Fraction III, T.O.D.$_{248.5}$ 9,420; 11.9%). Unknown product (A) with 2—4 N HCl (Fraction IV, T.O.D.$_{248.5}$ 22,900).

Fraction II was desalted as usual with charcoal. The eluate was concentrated to a small volume, adjusted to pH 1.5 with aqueous hydrochloric acid solution and warmed for 20 min at 70° to remove isopropylidene group at 2′,3′–position of ribose moiety. After desalting, the concentrated solution was passed through Amberlite IR–120 (Na form, 15 ml) to convert 5′–IMP ammonium salt to sodium salt. After concentration to dryness, the residue was crystallized by an addition of H_2O–EtOH to give 880 mg 5′–IMP Na_2 (purity 76.4%, yield 26.5%). UV: $\lambda_{max}^{0.1N\ HCl}$ 248.5 mμ; $\lambda_{max}^{H_2O}$ 248 mμ; $\lambda_{max}^{0.1N\ NaOH}$ 251.5 mμ.

This sample was identical as 5′–IMP from the following evidences. This sample showed the same mobilitieson paper electrophoresis (buffer 1) and thin–layer chromatography (TLC) (cellulose powder, solvent A) as authentic 5′–IMP and inosine was produced by the hydrolysis with purified bull semen 5′–nucleotidase.

After removing isopropylidene group and desalting, the product in Fraction III showed following properties; M 5′-IMP[12]) 0.88 (buffer 2). M 5′-IMP 1.45 (buffer 3) on paper electrophoresis, and *Rf* 0.73 (solvent C) on P.C. UV: $\lambda_{max}^{0.1N\ HCl}$ 249 mμ; $\lambda_{max}^{H_2O}$ 249 mμ; $\lambda_{max}^{0.1NNaOH}$ 253 mμ. Small volume (T.O.D.$_{248}$ about 200) of Fraction III was evaporated to dryness *in vacuo*. The residue was dissolved in 0.5 M Tris–HCl buffer, pH 9.1 (0.2 ml) to which purified pyrophosphatase solution from snake venom (0.1 ml) was added and incubated at 37° overnight. The produced 5′–IMP was identical with an authentic sample on P.C. (solvent C) and paper electrophoresis (buffer 1).

The aqueous solution (1 ml) of $Ba(OAc)_2 \cdot H_2O$ (220 mg) was added to Fraction III (2 ml; T.O.D. *ca.* 5000) to give precipitates as barium salt. Barium salt was collected by filtration and purified with H_2O–EtOH (410 mg). *Anal.* Calcd. for $C_{10}H_{11}O_{11}N_4Ba_{3/2}P_2 \cdot 2H_2O$: C, 18.00; H, 2.27; N, 8.40; P, 9.28. Found: C, 18.31; H, 2.53; N, 8.27; P, 9.13. NMR $\delta_{ppm}^{D_2O}$: H_2 and H_8; 8.48, 8.24. H_1; 6.11 (doublet, J=5 cps).

Fraction IV——After desalting, the eluate was evaporated to dryness. The light brown powder was crystallized from MeOH or MeOH–$CHCl_3$ solution to give colorless needles. mp 163—165°, $FeCl_3$–sulfosalicylic acid reagent (−). UV: $\lambda_{max}^{0.1N\ HCl}$ 253 mμ; $\lambda_{max}^{H_2O}$ 257 mμ; $\lambda_{max}^{0.1N\ NaOH}$ 256 mμ. IR (KBr): 1575, 1400 (–COO^-), 2200 (–CN). NMR $\delta_{ppm}^{D_2O}$: 8.62 (singlet). *Anal.* Found: C, 43.22; H, 4.68; N, 35.51.

The Synthesis of 5′-IMP by Ultraviolet Irradiation in the Presence of Malononitrile and Phosphoric Acid——Isopropylidene inosine 100 mg, mono–tri–*n*-butylammonium phosphate 460 mg (5 equivalents), and malononitrile 52 mg (3 equivalents) were dissolved in DMF 2 ml. The mixture was sealed in the quartz glass tube and irradiated with Toshiba Lamp SHL–100 UD at *ca.* 1 cm distance for 24 hr.

The temperature of reaction mixture elevated to 80—90° by radiant heat. The reaction mixture diluted with water (50 ml) was extracted with ether, and insoluble materials were filtered off. Ether in water layer was evaporated *in vacuo*. The desalted solution (T.O.D.$_{248.5}$ 4,637) was applied to the column of Dowex 1×8 (Cl form, 100—200 mesh, 1.4×10 cm, 15.5 ml) and washed with water. The nucleotides were eluted in a stepwise manner as follows. Hypoxanthine and isopropylidene inosine with water (T.O.D.$_{248.5}$ 2,000, 46% of the total eluate optical density unit), isopropylidne 5′–IMP with 0.01 N HCl+0.03M NaCl (T.O.D.$_{248.5}$ 2,150, 50%), and isopropylidene 5′–IDP with 0.1 N HCl (T.O.D.$_{248.5}$ 160, 4%). The recovery estimated from T.O.D. applied to the column was 95%. By the same procedure as above, 5′–IMP · Na_2 40 mg (purity 90%) was obtained from isopropylidene 5′–IMP fraction.

The Synthesis of 5′-IMP by Ultraviolet Irradiation in the Presence of Acrylonitrile and Phosphoric Adid——Isopropylidene inosine 200 mg (T.O.D.$_{248\cdot5}$ 8,500), mono–tri–*n*-butylammonium phosphate 920 mg (5

11) T.O.D.: Total optical density unit.

12) The ratio of the migration distance of a sample to that of 5′–IMP.

equivalents), and acrylonitrile 104 mg (3 equivalents) were dissolved in DMF 4 ml. The mixture was sealed in a quartz glass tube and irradiated with Toshiba Lamp SHL-100 UD at *ca.* 1 cm distance for 24 hr. The temperature of reaction mixture elevated to 80—90° by radiant heat. The reaction mixture diluted with water (150 ml) was extracted with ether, and insoluble materials were filtered off. Ether in water layer was evaporated *in vacuo*. The desalted solution (T.O.D.$_{248.5}$ 6500) was applied to the column of Dowex 1×8 (Cl form, 100—200 mesh, 2×8 cm, 25 ml) and washed with water. The nucleotides were eluted in a stepwise manner as follows. Hypoxanthine and isopropylidene inosine with water (Fraction I, T.O.D.$_{248.5}$ 3300, 54% of the total eluate optical density unit.), isopropylidene 5′-IMP with 0.01 N HCl+0.03 M NaCl (Fraction II, T.O.D.$_{248.5}$ 2570, 42%), and isopropylidene 5′-IDP with 0.1 N HCl (Fraction III, T.O.D.$_{248.5}$ 190, 3%). The recovery estimated from T.O.D. applied to the column was 94%.

Desalted Fraction II was concentrated to a small volume, adjusted to pH 1.5 with aqueous hydrochloric acid solution and warmed for 20 min at 70°, and desalted with charcoal. The concentrated eluate was passed through a column of IR-120 (Na form, 9 ml) to convert ammonium salt to sodium salt. After concentration to dryness, the residue was recrystallized from H_2O–EtOH to give 60 mg 5′-IMP·Na_2, (purity estimated spectrophotometrically on the weight basis 88.3%). UV: $\lambda_{max}^{0.1N\ HCl}$ 248.5 mμ; $\lambda_{max}^{0.1N\ NaOH}$ 252.5 mμ. This sample was identical with 5′-IMP by paper electrophoresis and NMR.

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Chemistry of Amino Acids. VI.[1] Studies on α-Alkyl-α-amino Acids. X.[1] Unusual Alkyl Migration in the Hofmann Degradation of 5-(2-Dialkylaminoethyl)-5-methylhydantoin Quarternary Ammonium Hydroxide Anhydronium Base

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Previously the chemical correlation of the absolute configuration of (+)-α-methylserine, one of the optically active α-alkyl-α-amino acids from a natural source, with *S*(+)-isovaline, was reported from our laboratory.[3] In this study, 5-methyl-5-vinylhydantoin (I) was selected as a key intermediate and obtained successfully from the thermal decomposition of 5-(2-dimethylaminoethyl)-5-methylhydantoin N-oxide (II). Before this, several unsuccessful attempts to obtain I were undertaken, one of which was the Hofmann degradation of 5-(2-dialkylaminoethyl)-5-methylhydantoin quarternary ammonium hydroxide anhydronuim base (IIIa, and IIIb). In this case, instead of the objective hydantoin (I), 3-alkyl-hydantoin (IVa, and IVb) was obtained in a fairly good yield by the unexpected alkyl migration from the side chain nitrogen to the 3-position of the hydantoin ring.

The materials used in the Hofmann degradation, IIIa and IIIb, were prepared as follows: 5-(2-dimethylaminoethyl)-5-methylhydantoin (Va), prepared from 1-dimethylamino-3-butanone (VIa)[3] using the Bücherer reaction in a 59% yield,[3] was treated with methyl iodide to afford 5-(2-dimethylaminoethyl)-5-methylhydantoin methiodide (VIIa) quantitatively. This product was applied to an ion exchange resin column (Amberlite IRA-400 (OH^- form)),

1) Part V, Part IX: K. Hiroi, K. Achiwa, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 444 (1968).
2) Location: *Hongo, Tokyo.*
3) N. Takamura, S. Terashima, K. Achiwa, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **15**, 1776 (1967).