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# 28. Morio Ikehara, Eiko Ohtsuka, and Fumiyoshi Ishikawa : Studies on Coenzyme Analogs. IX.*1 Synthesis of 6-Alkylamino-9-β-D-ribofuranosylpurine 5'-Monophosphate.

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During the course of studies on non-natural purine ribotides as the substrate of several enzymatic systems, such as 5'-nucleotidase of snake venom,[1] polynucleotide phosphorylase,[2] and actomyosin,[3] 6-substituted aminopurines were required. In this paper the synthetic method for AMP,*3 GMP, and 6-dimethylaminopurine ribotide, and the new synthesis of 6-methylaminopurine ribotide are described.

As to the chemical synthesis of natural purine and pyrimidine nucleoside monophosphates, considerable amount of papers have appeared. The use of phosphoryl chloride as the phosphorylating agent was extensively employed by Levene, *et al.*[4] and leading to the reëxamination of its utility by Todd[5] and Khorana[6] in the synthesis of AMP and GMP. Todd's powerful reagent, dibenzylphosphorochloridate, offered a general method for phosphorylation of natural nucleosides[5,7] and it was also applied to the phosphorylation of azathymidine[8] and nebularine,[9] but it sometimes failed to react for unclarified reasons.[10] O-Benzylphosphorous O,O-diphenylphosphoric anhydride was also found by Todd[11] and used widely, because of its moderate reactivity.[10,12] Khorana[6] synthesized GMP by the aid of tetrakis(*p*-nitrophenyl) pyrophosphate quite successfully and this reagent seems to be suitable for general use in the phosphorylation of purine nucleoside. In order to obtain the 5'-phosphoramidate directly from the nucleoside, phenyl phosphorodichloridate, which

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*1 Part Ⅷ. M. Ikehara, E. Ohtsuka : This Bulletin, **9**, 27 (1961).

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*3 Following abbreviations are used : AMP, adenosine 5'-phosphate; GMP, guanosine 5'-phosphate; DCC, dicyclohexylcarbodiimide; DTC, ditoluylcarbodiimide.

1) J. M. Gulland, E. M. Jackson : Biochem. J., **32**, 597 (1938); L. Heppel, R. J. Hilmoe : J. Biol. Chem., **188**, 665 (1957).
2) M. Grunberg-Manago, D. J. Ortitz, S. Ochoa : Biochim. et Biophys. Acta, **20**, 269 (1956).
3) V. A. Engelhardt, M. N. Ljubimova : Nature, **144**, 668 (1939).
4) P. A. Levene, E. T. Stiller : J. Biol. Chem., **104**, 299 (1934); P. A. Levene, R. S. Tipson : *Ibid.*, **106**, 113 (1934); *ibid.*, **111**, 313 (1935); *ibid.*, **121**, 131 (1937).
5) J. Baddiley, A. R. Todd : J. Chem. Soc., **1947**, 648.
6) R. W. Chambers, J. G. Moffatt, H. G. Khorana : J. Am. Chem. Soc., **79**, 3747 (1957).
7) J. Baddiley, A. R. Todd : J. Chem. Soc., **1949**, 2476.
8) R. H. Hall, R. Haselkorn : J. Am. Chem. Soc., **80**, 1138 (1958).
9) D. J. Magrath, G. B. Brown : *Ibid.*, **79**, 3252 (1957).
10) J. M. Andrews, W. E. Barber : J. Chem. Soc., **1958**, 2768.
11) V. M. Clark, G. W. Kirby, A. R. Todd : *Ibid.*, **1957**, 1497; N. S. Corby, G. W. Kenner, A. R. Todd: *Ibid.*, **1952**, 3669, 3675.
12) *a*) B. E. Griffin, A. R. Todd, A. Rich : Proc. Natl. Acad. Sci., U. S. A., **44**, 1123 (1958). *b*) M. Ikehara : This Bulletin, 8, 308 (1960).

had been used widely in the field of phosphatides, was used by Khorana.[13] This reagent may be used as the monophosphorylation agent. Recently, phosphorodichloridic acid anhydride,[14] 2-cyanoethyl phosphate in the presence of DCC,[15] and trichloroacetonitrile[16] were reported as an excellent phosphorylating agent of varied applicability.

In the pyrimidine nucleoside field, phosphorus pentoxide and orthophosphoric acid (polyphosphoric acid)[17] are the most useful reagent, which are easily available in chemical laboratory. Unfortunately, this reagent is not applicable to the purine nucleoside, because of acid-labile nature of the nucleoside-linkage of this compound.*4

These evidences in mind, tetrakis(*p*-nitrophenyl) pyrophosphate was first examined for 2′,3′-O-isopropylideneadenosine (I), in order to find general conditions for phosphorylation of purine nucleoside. The reaction of tetrakis(*p*-nitrophenyl) pyrophosphate (II) with (I) proceeds smoothly (quantitative). Isolated 2′,3′-O-isopropylideneadenosine 5′-(bis-*p*-nitrophenylphosphate) (III) was subjected to alkaline hydrolysis. Treatment with 1*N* lithium hydroxide for 30 minutes at room temperature gave a monosubstituted derivative of AMP (IV), which was followed by further removal of nitrophenyl group by ways of both alkaline and enzymatic hydrolyses. At this stage, a mild alkaline hydrolysis and removal of protective group was not sufficient and gave AMP contaminated with *p*-nitrophenyl ester. More drastic condition should be avoided to prevent hydrolysis of nucleoside linkage and especially in the case of dimethylaminopurine riboside (V), as observed in the case of nebularine (VI),[18] the alkaline cleavage of the imidazole ring will be possible, because in both purines, tautomerism between 6-$NH_2$ and 1-$N_1$ is entirely inhibited. Enzymatic cleavage of second *p*-nitrophenyl group in (IV) was achieved in a low yield of pure AMP and on a limited scale.

Then, the attention was turned to phenyl phosphorodichloridate, which is more easily available than other phosphorylating agents (for instance, O-benzylphosphorous O,O-diphenylphosphoric anhydride or tetrakis(*p*-nitrophenyl) pyrophosphate requires difficult intermediates and tedious handlings). 2′,3′-O-Isopropylideneadenosine was treated with equivalent mole of phenyl phosphorodichloridate[19] (VII) in the presence of the same amount of quinoline. 2′,3′-O-Isopropylideneadenosine 5′-(phenylphosphorochloridate) (VIIIc) thereby obtained was converted directly to the amidate (IXc) (97% yield) by passing dry ammonia and hydrolysis with 1*N* lithium hydroxide at room temperature to remove the last phenyl group. Isopropylidene-AMP-amidate (IXc) thus obtained was heated with 2*N* hydrochloric acid without further purification and neutralization with barium hydroxide afforded AMP (Xc) in 33% yield. By an essentially analogous procedure GMP was obtained from 2′,3′-O-isopropylideneguanosine in 45% yield.

Accordingly, phenyl phosphorodichloridate was proved to be a suitable reagent for phosphorylation of various nucleosides, especially for purine nucleosides.

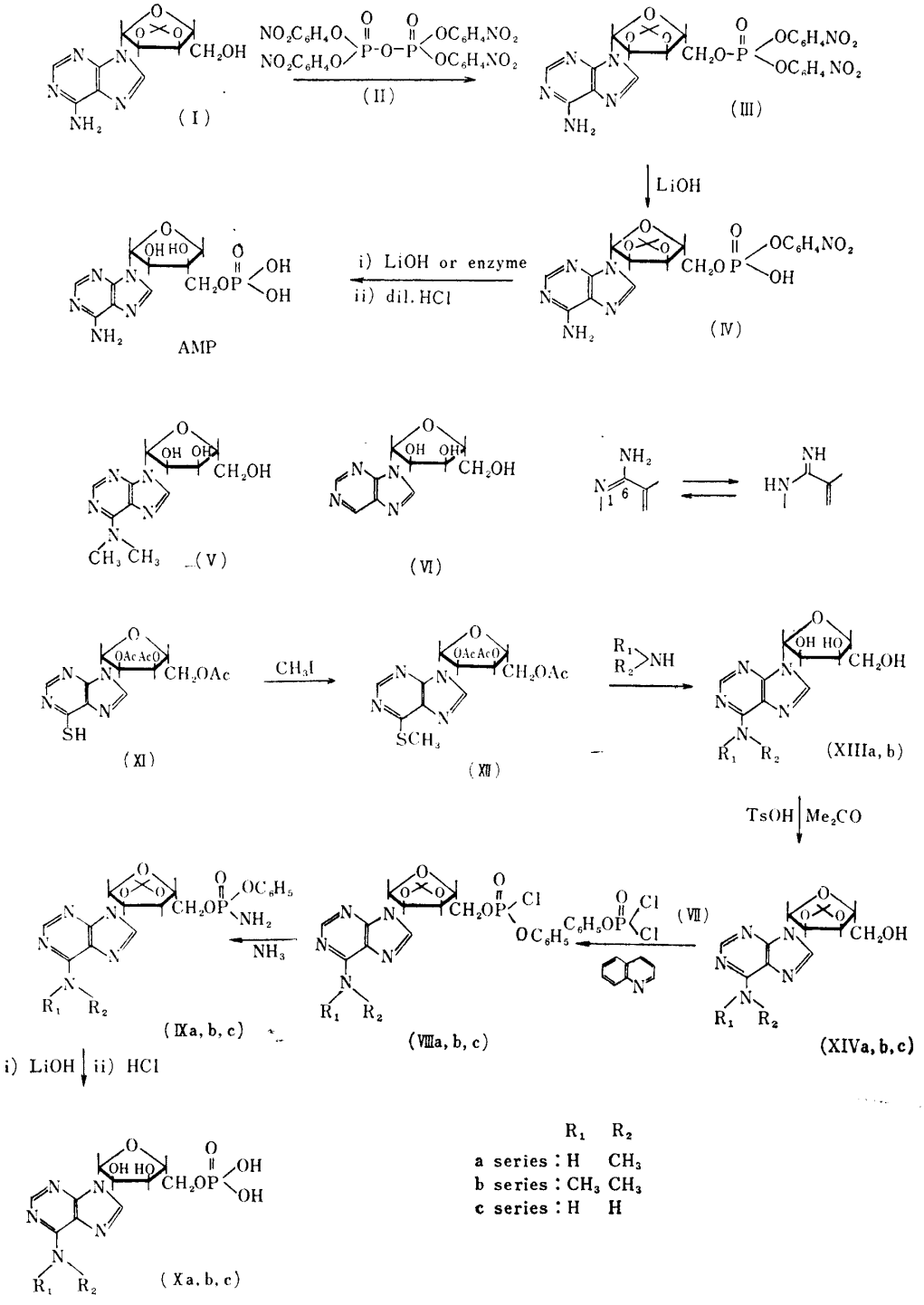
6-Methylamino- and 6-dimethylamino-9-β-D-ribofuranosyl-β-purine were then subjected to phosphorylation by this procedure.

Although the synthesis of 6-dimethylaminopurine riboside 5′-phosphate was reported by Barber,[10] this was not suitable for the present purpose. 2′,3′,5′-Triacetylthioinosine[20]

*4 This method was also used to phosphorylate 6-deoxyuridine,[12b] 9-(2′-hydroxyethyl)adenine,*1 and benzimidazole riboside (unpublished data).

13) R. W. Chambers, H. G. Khorana : J. Am. Chem. Soc., **80**, 3749 (1958).
14) H. Grunze, W. Koransky : Angew. Chem., **71**, 407 (1959).
15) P. T. Gilham, G. M. Tenner : Chem. & Ind. (London), **1959**, 542.
16) F. D. Cramer, G. Weimann : *Ibid.*, **1960**, 46.
17) R. H. Hall, H. G. Khorana : J. Am. Chem. Soc., **77**, 1871 (1959); A. M. Michelson : J. Chem. Soc., **1958**, 1957.
18) M. P. Gordon, V. S. Weliky, G. B. Brown : J. Am. Chem. Soc., **79**, 3245 (1957).
19) E. Baer, H. C. Stancer : *Ibid.*, **75**, 4510 (1953).
20) M. Ikehara : This Bulletin, 8, 367 (1960).

(XI) was methylated with methyl iodide to 6-methylthio-9-(tri-O-acetyl-β-D-ribofuranosyl)-β-purine (XII) and (XII) was reacted with methylamine or dimethylamine to obtain 6-methylamino- or 6-dimethylamino-9-β-D-furanosyl-β-purine (XIIIa and XIIIb), which was converted to the 2′,3′-O-isopropylidene derivatives (XIVa and XIVb) by reaction with *p*-toluenesulfonic acid and acetone.[21] In order to obtain the 5′-monophosphate, phosphorylation of (XIV) with phenyl phosphorodichloridate was carried out in almost similar manner as described for AMP and GMP. The yield of 6-methylamino- and 6-dimethylamino-9-β-D-ribofuranosyl-β-purine 5′-phosphate (Xa, Xb) was 37.4% and 18.5%, respectively. The structure of both




21) A. Hampton, D. I. Magrath : J. Am. Chem. Soc., **79**, 3250 (1957).

compounds was confirmed by elementary analysis, ultraviolet absorption spectra, chromatographic behavior, and other properties.

The activity of the compounds as the substrate of snake venom 5′-nucleotidase will be reported elsewhere. Further phosphorylation of both compound to di- and tri-phosphates is now under way.

## Experimental

**Paper Chromatography**—All on Toyo Roshi No. 51 A. Solvent system : (A) iso-PrOH:1% $(NH_4)_2SO_4$=3:2; (B) BuOH:$H_2O$=86:14; (C) saturated $(NH_4)_2SO_4$:$H_2O$:iso-PrOH=79:19:2; (D) iso-PrOH (75 cc.)-$H_2O$ (25 cc.)-$CCl_3COOH$ (5 g.)-$NH_4OH$ (0.25 cc.); (E) PrOH:$NH_4OH$:$H_2O$=60:30:10. Ascending technique, except (B).

**Synthesis of AMP with Tetrakis(*p*-nitrophenyl) Pyrophosphate**—i) Tetrakis(*p*-nitrophenyl) pyrophosphate[13] (prepared from 4.2 g. of bis(*p*-nitrophenyl) phosphate and 1.2 g. of DTC) in 20 cc. of dioxane was added with 1 g. of 2′,3′-O-isopropylideneadenosine[22] and set aside for 2 days at room temperature. Paper chromatography (solvent B) showed a main spot (Rf 0.85) of 2′,3′-O-isopropylideneadenosine 5′-bis(*p*-nitrophenyl phosphate). Urea was removed by filtration, washed with dioxane, filtrate and washings were combined, and evaporated. The residue was taken up in 30 cc. of $CHCl_3$, washed with 1*M* AcOLi buffer (pH 6.5) to remove *p*-nitrophenyl phosphate and with $H_2O$, and $CHCl_3$ was evaporated (yield quantitative).

This residue was taken up in 15 cc. of dioxane, added with 12 cc. of 1*N* LiOH, and 12 cc. of $H_2O$ was added after 10 min. The white precipitate that appeared was dissolved by addition of dioxane and the solution was kept at room temperature for 30 min. A small amount of urea was filtered off, the filtrate was adjusted to pH 7~8 with 2*N* HCl, and evaporated under a reduced pressure. Residual glass was washed with 20 cc. of 1*N* LiOH into a polyethylene tube and heated on a boiling water bath for 1.5 hr. *p*-Nitrophenol was extracted with $Et_2O$ until no yellow color appeared with alkali. The mixture was adjusted to pH 8.5 with 4*N* LiOH, added with 4 cc. of 2*M* $(AcO)_2Ba$, and the precipitate that appeared was removed by centrifugation. Supernatant was added with 2 volumes of EtOH to precipitate Ba-salt, which was collected by centrifugation and washed with EtOH, $Me_2CO$, and $Et_2O$. Dried ($P_2O_5$, 2 mm. Hg at room temp.) material weighed 1.2 g. (70%). Test on paper chromatography (solvent A) showed 2 spots at Rf 0.32 and 0.54. The latter was adenosine 5′-mono-(*p*-nitrophenyl phosphate) ($\lambda_{max}^{H_2O}$ 260, 310 mμ) and the former corresponded to AMP. Ratio of AMP to *p*-nitrophenyl ester was 77:23 as estimated spectrophothometrically.

ii) In the second run from 0.18 g. of isopropylideneadenosine, removal of the second phenyl group was achieved by crude snake venom on one-half the initial amount. 2′,3′-O-Isopropylideneadenosine 5′-mono-(*p*-nitrophenyl phosphate) (3 cc.) was evaporated *in vacuo*, dissolved in 1.5 cc. of tris-buffer (pH 8.95), followed by the addition of 1.25 cc. of 0.3*M* $MgCl_2$, and 4.5 g. of the venom.*5 Incubation at 37° for 3 hr. gave a spot of the starting material (Rf 0.37, solvent (B)) on paper chromatogram. Since further 1 hr.'s incubation gave no significant decrease of this spot, the mixture was treated with Amberlite IR-120 ($NH_4^+$) resin and added with 1.4 cc. of AcOH, which made the solution pH 2. In order to remove isopropylidene group, the reaction mixture was heated for 1 hr. at 100°. After cool, the pH was adjusted to 8.5 with 4*N* LiOH and added with 0.2 cc. of EtOH. Precipitated AMP-Ba was removed by analogous manner as described above (Yield, 37 mg.). This material was tested by paper chromatography (Solvent A), which showed 2 spots at Rf 0.42 and 0.65. The latter corresponded to adenine confirmed by ultraviolet absorption spectrum and calculated as 12% contamination. Ba-AMP was freed by the addition of calculated amount of $H_2SO_4$, $BaSO_4$ was removed, supernatant was concentrated, and cooled. Crystalline AMP was obtained in a yield of 20 mg.

**Synthesis of AMP with Phenyl Phosphorodichloridate**—2′,3′-O-Isopropylideneadenosine (179 mg., 0.58 mmole) was dissolved in 3 cc. of dry dioxane containing 75 mg. (0.58 mmole) of quinoline. The turbid solution was added with 135 mg. (0.64 mmole) of phenyl phosphorodichloridate dissolved in 1.5 cc. of dehyd. dioxane during 10~15 min. White precipitate, initially appeared, converted to a yellow oil at the end of 4 hr.'s reaction at room temperature. After setting aside overnight, dry $NH_3$ was bubbled through the reaction mixture at 0°, $NH_4Cl$ was removed by centrifugation, and the supernatant was concentrated to a heavy syrup, which solidified to a white powder by trituration with dehyd. $Et_2O$. This crude 2′,3′-O-isopropylideneadenosine 5′-(phenyl phosphoramidate) weighed 255 mg. (97%).

*5 This was a gift from Dr. D. Mizuno of the National Institute of Health, Tokyo, to whom the authors' thanks are due.

22) P. A. Levene, R. S. Tipson : J. Biol. Chem., **121**, 131 (1937); M. A. Stevens, D. J. Magrath, H. W. Smith, G. B. Brown : J. Am. Chem. Soc., **80**, 2755 (1958).

A solution of this material in 1 cc. of dehyd. dioxane and 1.5 cc. of 1*N* LiOH was set aside at room temperature for 1 hr. After washing with $Et_2O$ (3 times), whole was adjusted to pH 2.4~2.6 with 2*N* HCl, and washed well with $Et_2O$ to remove PhOH. Aqueous layer was heated at 100° for 1 hr. and adjusted to pH 6.5~6.8 with $Ba(OH)_2$ solution after cooling. The precipitate was removed by centrifugation and the supernatant was concentrated to ca. 2 cc. Addition of 2 volumes of EtOH precipitated AMP as Ba salt, which was collected, washed, and dried as described above. Yield, 90 mg. (33.2% as mono-Ba salt). Paper chromatography showed a spot of AMP at Rf 0.36 and a trace of spot at Rf 0.57 (monophenyl ester).

**Synthesis of GMP with Phenyl Phosphorodichloridate**—To a suspension of 323 mg. of isopropylideneguanosine[6] in 3 cc. of dioxane, added with 0.12 cc. of quinoline, a solution of phenyl phosphorodichloridate (231 mg., 1.1 mmoles) in 2 cc. of dioxane was gradually added at 0° with vigorous stirring. After 2 hr., initially appeared white precipitate converted into a vitreous substance. Hydrolysis of protective groups by alkali and HCl was carried out as described for AMP-synthesis. Final alkaline solution was adjusted to pH 8.5 and 1.3 cc. of 2*M* $(AcO)_2Ba$ was added. The white precipitate was collected by filtration and added with 2 volumes of EtOH. After 1 hr.'s standing in a refrigerator, precipitated GMP-Ba was collected, washed, and dried. Yield, 225 mg. (45%). Paper chromatography (solvent A) showed this material was slightly contaminated with guanine (Rf 0.57).

**6-Methylthio-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (XII)**—To a solution of 12.5 g. of triacetylthioinosine[20] dissolved in 60 cc. of 0.4*N* NaOH, 3 g. of MeI was added dropwise. After 30 min. of vigorous shaking, further 30 cc. of 4*N* NaOH and 1.5 g. of MeI were added. A gummy solid that separated out was collected by decantation, washed with a small amout of $H_2O$, and dried (Yield, 7.5 g.). This material was hygroscopic and used for the next step without further purification. UV $\lambda_{max}^{EtOH}$ mμ : 282, 288 (shoulder).

**6-Methylamino-9-β-D-ribofuranosyl-β-purine (XIIIa)**—7.5 g. of above compound was fused in a glass tube with 50 cc. of monomethylamine (40% aqueous solution) and heated in an oil bath for 4 hr. at 120~130°. Whole was evaporated *in vacuo* to a syrup containing solid material, both were taken up in 20 cc. of $H_2O$, treated with charcoal, and evaporated *in vacuo*. Recrystallization from MeOH gave crystals of m.p. 132~135°. Yield, 2.8 g. (46.7%). UV : $\lambda_{max}^{H_2O}$ 264 mμ. These properties are identical with those in the literature.[23]

**6-Dimethylamino-9-β-D-ribofuranosyl-β-purine (XIIIb)**—5.0 g. of (XII) was reacted with 35% aqueous solution of dimethylamine at 130~140° for 5 hr. and treated as described above. Recrystallization from $Me_2CO$-$H_2O$ gave crystals of m.p. 174~175°. Yield, 1.6 g. (64.0%). UV : $\lambda_{max}^{EtOH}$ 273.5 mμ. These data are identical with those in the literature.[24]

**6-Methylamino-9-(2,3-O-isopropylidene-β-D-ribofuranosyl)purine (XIVa)**—2.0 g. of (XIIIa) (dried over $P_2O_5$ at room temp., 2 mm. Hg for 8 hr.) was dissolved in 200 cc. of $Me_2CO$ containing 20 g. of *p*-toluenesulfonic acid. After 2 hr.'s vigorous stirring, a clear solution was obtained, which was poured into aqeous solution of 20 g. of $NaHCO_3$ and evaporated to one-half its volume. $CHCl_3$ extraction of this solution gave 1.5 g. of glassy isopropylidene derivative. Paper chromatography (Solvent B) showed one spot at Rf 0.70, which did not have the *cis*-glycol system as tested by $IO_4^-$ spray. UV : $\lambda_{max}^{H_2O}$ 265 mμ. *Anal.* Calcd. for $C_{14}H_{19}O_4N_5$ : C, 52.31; H, 5.92; N, 21.80. Found : C, 51.85; H, 6.14; N, 20.66.

**6-Dimethylamino-9-(2,3-O-isopropylidene-β-D-ribofuranosyl)purine(XIVb)**—Barber's procedure[10] was slightly modified and $CuSO_4$ was omitted from the reaction catalysis. The procedure was essentially the same as above. From 2.3 g. of dimethylaminopurine riboside (XIIIb), 1.7 g. (65.2%) of crystalline isopropylidene derivative was obtained, m.p. 175~176°. UV : $\lambda_{max}^{EtOH}$ 274 mμ.

**6-Methylamino-9-β-D-ribofuranosylpurine 5'-Monophosphate (Xa)**—The solution of 1.3 g. of the above isopropylidene derivative (XIVa) and 0.49 cc. of quinoline dissolved in 13 cc. of dehyd. dioxane, 10 cc. of dioxane containing 0.95 g. of phenylphosphorodichloridate was added dropwise at 0° with vigorous stirring. After 30 min., an amorphous substance precipitated out, which converted into an oily mass during 3.5 hr. Reaction was stopped by bubbling dry $NH_3$ at 0° until saturation (5 min.). The mixture was set aside for 3 hr., resulting white precipitate ($NH_4Cl$) was filtered off, and the filtrate and washings were evaporated to ca. 1 cc. This was diluted to 12 cc. with dioxane. 8 cc. of 1*N* LiOH was added, and the mixture was kept at room temperature overnight. The whole was adjusted to pH 2.5 with 2*N* HCl and heated at 100° for 1 hr. After cool, the reaction mixture was extracted with $Et_2O$, and neutralized with $Ba(OH)_2$ to pH 6.5. The precipitate was filtered off and extracted thoroughly with $Et_2O$. Aqueous layer was concentrated to 7 cc., added with aqeous $Ba(OH)_2$ until pH 7.5, and finally 2 volumes of EtOH was added. Resulting precipitate was collected by centrifugation, washed with EtOH and $Et_2O$, and dried (750 mg., 37.4% as Ba-salt). *Anal.* Calcd. for $C_{11}H_{14}O_7N_5BaP$: P, 6.25. Found : P, 6.69. Purity estimated spectrophotometrically (ε of 6-methylaminopurine riboside

23) J. A. Johnson, Jr., H. J. Thomas, H. J. Shaeffer : J. Am. Chem. Soc., **80**, 700 (1958).
24) H. M. Kissman, C. Pidacks, B. R. Baker : *Ibid.*, **77**, 18 (1955).

at 266 mμ is $15.9\times10^3$), 92.6%. The ratio of the base to total P=0.87:1.00. Rf 0.51 (solvent A), 0.20 (solvent C).

**6-Dimethylamino-9-β-D-ribofuranosylpurine 5′-monophosphate (Xb)**—All procedure was essentially analogous to that described in the previous section. From 1.8 g. of the above isopropylidene derivative (XIVb), 500 mg. (18.5%) of monophosphate Ba-salt was obtained. *Anal.* Calcd. for $C_{12}H_{16}O_7N_5BaP$ : P, 6.08. Found : P, 6.42. Paper chromatography gave only one spot in two solvent systems at Rf 0.37 (solvent E) and 0.29 (Solvent A). Purity calculated on the basis of ultraviolet absorption (ε of dimethylaminopurine riboside at 268 mμ, $18.3\times10^3$), 89.7%. The ratio of the base to total P=0.85:1.00.

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## Summary

The phosphorylation of isopropylidene derivatives of adenosine and guanosine using tetrakis(*p*-nitrophenyl) pyrophosphate and phenyl phosphorodichloridate was examined to establish a general method for monophosphorylation. With the aid of the latter reagent, 6-methylamino- and 6-dimethylamino-9-β-D-ribofuranosyl-β-purine 5′-monophosphates were synthesized.

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**29. Takuo Okuda** : Studies on the Components of *Coriaria japonica* A. GRAY. XIV.[1] Two New Compounds isolated from Old Stem and Seed.

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In the course of studies on the components of *Coriaria japonica*, it sometimes happened that leaves of this plant did not produce the toxic principle by the regular method of extraction.[1] This may partially be due to decomposition of the toxic principles which took place during drying of the leaves. However, it is also considered that leaves of *C. japonica* contain the toxic principles only in certain periods of the year.

In order to determine the amount of toxic principles in the plant at all seasons and also to determine if the toxic principles are contained in other parts of the plant, every part of the plant above the ground was collected every month from a colony,[*2] quickly dried, and extraction was carried out by the regular method. The plant of this colony carries flowers at the beginning of May and the leaves appear a little later. Leaves and young stems are fully grown by the end of August and wither by the beginning of October. In March and April, old stems covered by brown bark and small buds are only part of the plant seen on the ground. Although no compound which is considered to be any of the toxic principles was extracted from this part, a pale yellow crystalline component was newly isolated.

As it is probable that this compound is a derivative of ellagic acid (I), which was formerly isolated from leaves of this plant by Kariyone, *et al.*,[2] properties of this new product

*1 Yoshida-konoe-cho, Sakyo-ku, Kyoto (奥田拓男).

*2 The plant was collected from a colony on Kitahira Pass, Mt. Hira, Shiga-ken.

1) Part XIII : This Bulletin, **2**, 185 (1954).

2) T. Kariyone, K. Kashiwagi, S. Mizutani : Yakugaku Zasshi, **57**, 800 (1937).