sandaracopimaric acid (IVa),*3,1d) which was easily rearranged from sandaracopimaric acid (IIIa), isopimaric acid (IIa) and also neoisopimaric acid (Ukita's acid), was carried out and the obtained results are shown in Table I, Fig. 1 and 2.

Based on these observations, we would like to propose herein the following conclusion that cryptopimaric acid and neoisopimaric acid are mixtures consisting mainly of sandaracopimaric acid (IIIa), together with isopimaric acid (IIa) (the ratio of the areas of the peaks in the gas chromatogram are 4:3 for cryptopimaric acid and 3:1 for neoisopimaric acid respectively*4) and therefore pimaric acid (Ia), isopimaric acid (IIa) and sandaracopimaric acid (III a) are only known as the natural pimaric acid type diterpene in pure state.

The gas chromatographic results were also supported by the facts that sandaracopimaric, cryptopimaric and neoisopimaric acid showed the undepressed mixed melting points with each other and had the nearly superimposable infrared spectra.

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A Novel Phosphorylating Agent, P-Diphenyl, P′-Morpholinopyrophosphorochloridate

Recently we have reported[1] the synthesis of morpholinophosphorodichloridate (I) and its use in the synthesis of nucleoside 5′-mono- and -polyphosphates. Because of the relatively weak phosphorylating power of this reagent, the first step of the phosphorylation could not exceed 50～60% extent. This fact would lead to the undesired side reaction, which affected greatly the purification procedure of the products. In order to increase the reactivity of the reagent and to avoid bifunctional reaction, P-diphenyl, P′-morpholinopyrophosphorochloridate (II) was synthesized and tested for the phosphorylation of nucleosides.

The reagent (II) was synthesized from diphenylphosphate (1 mmole) and morpholinophosphorodichloridate (1 mmole) in dioxane solution in the presence of 2,6-lutidine (2 mmole) as acid acceptor. After 15 minutes, reagent (II) was used *in situ* for the phosphorylation of 2′,3′-O-isopropylideneadenosine (IIIa) (0.5 mmole). 40 hours' reaction at 20° showed quantitative conversion of (IIIa) to 5′-(morpholino)phosphorochloridate (IVa) (Migratory distance 6.2 cm, R_{AMP} 0.58)[2] which was proved by paper electrophoresis (0.05*M* triethylammonium bicarbonate, pH 7.5,[3] 20 volt/cm. 1 hour[4]).

(IVa) was hydrolyzed with water (at pH 2.0, 70° for 30 minutes) and extracted with chloroform to remove diphenyl phosphate. After adjustment to pH 8.5 with lithium hydroxide, mixture was extracted again with ether to remove amines. Upon addition

1) M. Ikehara, E. Ohtsuka: This Bulletin, **10**, 536, 539 (1962).
2) This value was identical to that of AMP-morpholidate, which showed the hydrolysis of chloridate residue during electrophoresis.
3) J.G. Moffatt, H.G. Khorana: J. Am. Chem. Soc., **83**, 639 (1961).
4) This condition was used throughout present communication.

of barium acetate to the water layer barium phosphate precipitated,[5] which was removed by centrifugation. To the supernatant was added two volumes of ethyl alcohol and precipitated barium salt of AMP was collected. The nature and the yield of this material were summarized in Table I together with the results obtained in the analogous synthesis of 9-β-D-ribofuranosylpurine 5′-monophosphate,[6] uridine 5′-monophosphate, 6-dimethylamino-9-β-D-ribofuranosylpurine 5′-monophosphate[7] and 9-(4′-hydroxybutyl)-adenine 4′-monophosphate.[8]

Table I. 5′-Monophosphate Barium Salt Obtained from Various Nucleosides

Starting material	Product	Electrophoretic mobility (cm)	Rf[a] A	Rf[a] B	Isolated yield (%)	Purity[b] (%)	$\varepsilon_{260}^{pH\,7}$
Isp.-adenosine	AMP-Ba	10.7	0.41	0.30	60	81	1.5×10^4
Isp.-nebularine	nebularine 5′-MP-Ba	12.0	0.54		54	84	7,350[6]
Isp.-uridine	UMP-Ba	12.2	0.44	0.30	57	71	1.0×10^4
Isp.-$(CH_3)_2$-adenosine	$(CH_3)_2$-AMP-Ba	9.5	0.30		33[c]	—	1.8×10^4
9-(4′-OH-butyl)adenine	9-(4′-OH-butyl)adenine MP-Ba	10.7	0.44		47[c]	—	1.5×10^4

a) descending paper chromatography A; Iso-PrOH:1% $(NH_4)_2SO_4$=3:2
B; Iso-PrOH:conc. NH_3:H_2O=7:1:3

b) calculated photometrically on the weight basis from ε indicated in the last column

c) The yield was estimated in the form of solution in order to subject it to further reaction.

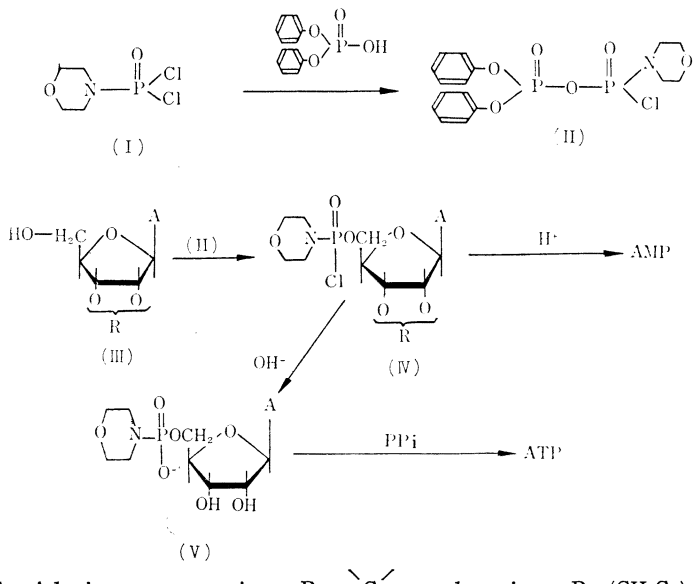

Chart 1.

The synthesis of adenosine 5′-triphosphate (ATP) was carried out as follows. 2′,3′-Di-O-acetyladenosine (IIIb) was phosphorylated as described above except that the molar ratio of reactants was altered to 1:2:6 for nucleoside:reagent (II):2,6-lutidine (Additional

5) When this precipitate contained ultraviolet absorbing substance, it should be dissolved in small amount of water and reprecipitated with ethyl alcohol.
6) A. Hampton, D.I. Magrath : J. Am. Chem. Soc., **79**, 3250 (1957).
7) M. Ikehara, E. Ohtsuka, F. Ishikawa : This Bulletin, **9**, 173 (1961).
8) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, Y. Tonomura : J. Am. Chem. Soc., **83**, 2679 (1961).

2 moles of 2,6-lutidine was used to prevent the degradation of morpholidate with Cl^-, which might be caused by the hydrolysis of phosphorochloridate with the co-existed moisture). In this instance also quantitative phosphorylation was observed by paper electrophoresis as single spot of 5′-(morpholino) phosphorochloridate (IVb) (Migratory distance 6.2 cm, R_{AMP} 0.58). The reaction mixture was treated with methanol containing ammonia and 4 equivalent of water. After 15 minutes the main spot on the paper chromatogram became Rf 0.62 (solvent A), which indicated the hydrolysis of chloridate residue. After standing overnight, examination of an aliquot showed total conversion of (IVb) to AMP-morpholidate (V) (Paper electrophoresis R_{AMP} 0.58, paper chromatography Rf 0.48 (solvent A), Rf 0.45 (solvent B)), which was identical with authentic sample.[3] After evaporation and absolute drying by codistillation with pyridine, the residue was caused to react with 5-equivalents of bis-(tri-butylammonium)pyrophosphate in pyridine. Analysis of aliquots extracted at various reaction intervals were listed in Table II and III. The results obtained in the analogous synthesis of 3′-deoxythymidine[9] 5′-phosphates were also shown in Table III.

Table II.

	Nucleoside+x[a]	AMP-morpholidate	AMP	ADP	ATP
5 hours	13%[b]	34	3	18	29
24 〃	15	30	5	18	32
50 〃	25	25	7.5	27	25
5 days	17	15	23	27	19

a) Unidentified phosphate, which had same mobility with nucleoside, presumably AMP-(morpholino)amidate.
b) Estimated photometrically from the extract of ultraviolet absorbing spot cut out from the paper of electrophoresis.

Table III.

	Nucleoside	MP-Morpholidate	MP	DP	TP	Higher P
10 hours[a]	11%[c]	26	14	13	29	7
24 〃 [a]	10	14	17	11	33	10
20 〃 [b]	35	2	12	27	14	9

a) reaction of diacetyladenosine
b) reaction of 3′-deoxythymidine
c) calculated from the optical density units of separated peak eluted from Dowex-1 ion-exchanger chromatography (Cl^- form, eluted with 0.003*N* HCl and 0.003*N* HCl +0.35*M* LiCl by gradient elution technique). Each fractions were evaporated and tested for their uniformity paper by chromatography and paper electrophoresis.

From these evidences, P-diphenyl, P′-morpholinopyrophosphorochloridate proved to be an useful reagent for the synthesis of various types of nucleoside mono- and polyphospates.

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9) A.M. Michelson, A.R. Todd: J. Chem. Soc., **1955**, 816.