the second involved the use of DCC and tri-*n*-butylammonium phosphorofluoridate in an excess of methanol. Neither synthesis was completely satisfactory, although a pure sample was finally obtained by paper chromatography in solvent E. The sample was homogeneous as shown by electrophoresis in bicarbonate and formate buffers and by chromatography in solvents A and D. The sample when treated with 1 *N* hydrochloric acid at room temperature for 36 hr yielded monomethyl phosphate.

Anal. Calcd for $CH_3FLiO_3P \cdot 2H_2O$: P, 19.9. Found: P, 20.6.

Alkaline Hydrolysis of A-2′:3′-P, A-3′:5′-P, and A-5′-PF.—Three sets of conditions were employed: (1) 1 *N* potassium hydroxide at room temperature for 16 hr; (2) saturated barium hydroxide solution at room temperature for 8 hr; and (3) saturated barium hydroxide solution at 100° for 20 min. Conditions 1 and 2 are sufficient to hydrolyze A-2′:3′-P and A-5′-PF to A-2′- (and 3′-) P and A-5′-P, respectively, but not A-3′:5′-P. Condition 3 is necessary for hydrolysis of A-3′:5′-P to a mixture of A-3′-P and A-5′-P.[5b]

Trifluoroacetic Acid Degradations.—Samples of A-5′-P (4–7 mg) were dissolved in 0.4-ml portions of aqueous trifluoroacetic acid (60–100%). After a predetermined reaction time, one of the solutions was frozen in a Dry Ice bath and lyophilized. A solution of the residue in a small amount of water was subjected to paper chromatography in solvent A and to paper electrophoresis in both borate and formate buffers.

Registry No.—Hydrogen fluoride, 7664-39-3; A-3′-P, 84-21-9; A-2′-P, 130-49-4; A-5′-P, 61-19-8; A-5′-PF, 19375-33-8; MeA-5′-P, 13039-54-8; C-2′-P, 85-94-9; C-3′-P, 84-52-6; G-2′-P, 130-50-7; G-3′-P, 117-68-0; U-2′-P, 131-83-9; U-3′-P, 84-53-7; NR-5′-P, 1094-61-7; T-3′-P, 2642-43-5; T-5′-P, 365-07-1; dA-5′-P, 653-63-4; A-2′:3′-P, 634-01-5; A-3′:5′-P, 60-92-4; ATP, 56-65-5; ADP, 58-64-0.

Studies on Phosphorylation. IV. Selective Phosphorylation of the Primary Hydroxyl Group in Nucleosides[1]

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Nucleoside 5′-phosphates of a number of naturally occurring and synthetic purine and pyrimidine ribonucleosides and their 2′-deoxy and 2′-O-methyl derivatives were prepared in good yields by direct phosphorylation of their corresponding unblocked nucleosides with pyrophosphoryl chloride in *m*-cresol or *o*-chlorophenol. Similar treatment of purine and pyrimidine arabino- and gluconucleosides, and aristeromycin resulted in the selective phosphorylation of the primary hydroxyl groups to give the corresponding phosphates. α-Guanosine and 2′-deoxyadenosine gave the 5′-phosphates in relatively low yield. The 5′-phosphate and 3′,5′-cyclic phosphate were obtained from 9-β-D-xylofuranosyladenine. Acetonitrile, benzonitrile, ethyl acetate, methyl acrylate, ethyl benzoate and nitrobenzene, when used as solvents, gave satisfactory results in the direct phosphorylation reaction.

A new method was reported[2] from our laboratories for the preparation of naturally occurring ribonucleoside 5′-phosphates by protecting the 2′,3′-*cis*-glycol system of the corresponding nucleosides with borate followed by phosphorylation with pyrophosphoryl chloride. Several attempts to phosphorylate primary hydroxyl groups selectively without blocking secondary alcoholic functions of nucleosides have failed.[3–5] We now report on a new method for direct phosphorylation of unblocked nucleosides at the 5′ position.[6–8] When inosine was suspended in *m*-cresol and was treated with pyrophosphoryl chloride[9,10] in the absence of metaboric acid or boric anhydride followed by hydrolysis, inosine 5′-phosphate was obtained in almost quantitative yield. This method was then applied to many other nucleosides and a number of the corresponding 5′-phosphate derivatives were selectively obtained.

A general procedure is as follows. A nucleoside, suspended in *m*-cresol or *o*-chlorophenol (15–80-fold by weight), is treated with pyrophosphoryl chloride (2–15 molar excess) at 0–10° for 2–4 hr and then diluted with an ice–water mixture followed by extraction with ethyl ether or benzene. The nucleotide is adsorbed onto charcoal and the aqueous layer discarded. After elution from the charcoal the nucleotide is subjected to ion exchange chromatography (Dowex 1). The identification of the nucleotide thus obtained is made as follows: (i) elementary analyses and ultraviolet absorption spectra, (ii) comparison of its mobility on paper electrophoresis and on paper chromatography with authentic samples, (iii) treatment of the nucleotide with bull semen 5′-nucleotidase to give quantitative liberation of phosphoric acid, (iv) treatment with periodic acid, and (v) chemical shifts of the 5′-proton resonances.

Adenosine, inosine, 2-chloroinosine, 6-thioinosine, uridine, and cytidine gave the corresponding 5′-phosphates in 55–85% yield (Table I). In the case of guanosine, a larger quantity of solvent was necessary

(1) For papers II and III of this series, see M. Honjo, Y. Furukawa, and K. Kobayashi, *Chem. Pharm. Bull.* (Tokyo), **14**, 1061 (1966), and M. Honjo, R. Marumoto, K. Kobayashi, and and Y. Yoshioka, *Tetrahedron Lett.*, 3851 (1966), respectively.

(2) K. Imai, T. Hirata, and M. Honjo, *Takeda Kenkyusho Nempo*, **23**, 1 (1964).

(3) G. R. Barker and G. E. Foll, *J. Chem. Soc.*, 3798 (1957).

(4) M. Ikehara, E. Ohtsuka, and Y. Kodama, *Chem. Pharm. Bull.* (Tokyo), **11**, 1456 (1963).

(5) M. Naruse and Y. Fujimoto, *Yakugaku Zasshi*, **86**, 37 (1966).

(6) Yoshikawa, *et al.*,[7] reported a novel process for the phosphorylation of the naturally occurring ribonucleosides to their 5′-phosphates after we had published a part of this work as a preliminary report.[8]

(7) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Lett.*, 5065 (1967).

(8) M. Honjo, T. Masuda, K. Imai, and S. Fujii, Abstracts of the 7th Meeting of the International Congress of Biochemistry, Tokyo, Aug 1967, IV, B-16.

(9) P. C. Crofts, I. M. Downie, and R. B. Heslop, *J. Chem. Soc.*, 3673 (1960).

(10) W. Koransky, H. Grunze, and G. Munch, *Z. Naturforsch.*, **17b**, 291 (1962).

TABLE I

PHOSPHORYLATION[a] OF NUCLEOSIDES WITH PYROPHOSPHORYL CHLORIDE IN *m*-CRESOL

Nucleoside (mg)	Solvent, ml	Pyrophosphoryl chloride, ml	Time, hr	Yield of phosphate of primary hydroxyl, %[b]
Inosine (536)	30	2.2	2	Quantitative
Inosine (268)	20[c]	1.0	2	Quantitative
2-Chloroinosine (2000)	130	13.0	2	Quantitative[d]
Guanosine (1000)	165	6.9	6	76
Cytidine (243)	15	0.5	2	93
α-Adenosine (161)	7.5	0.5	2	69
α-Guanosine (1020)	160	10.0	4	45[e]
Thymidine (290)	15	0.5	2	83
2′-Deoxyuridine (228)	15	0.6	2	76
2′-Deoxycytidine hydrochloride (26)	3	0.1	2	60
2′-Deoxyadenosine (75)	3	0.1	2	44
2′-O-Methyluridine (52)	2	0.1	2	80[f]
2′-O-Methylcytidine hydrochloride (59)	2	0.14	4	65[g]
1-β-D-Arabinofuranosylcytosine (906)	37	1.3	3	60
1-β-D-Arabinofuranosyluracil (244)	50	1.5	10	76
9-β-D-Glucopyranosyladenine (560)	60	1.5	6	60

[a] The reaction was done as described for adenosine. [b] Determined spectrophotometrically after paper electrophoresis [c] *o*-Chlorophenol was used. [d] M. Honjo, K. Imai, T. Furukawa, Y. Kanai, R. Marumoto, H. Honda, H. Aoki, and T. Hirata, *Takeda Kenkyusho Nempo*, **25**, 74 (1966). [e] Correct analysis ($C_{10}H_{12}N_5Na_2O_8P \cdot H_2O$) was obtained. The specific rotation was $[\alpha]^{24}D + 6.0°$ (*c* 1.0, water). [f] The specific rotation was $[\alpha]^{20}D + 15.7°$ (*c* 1.5, water). The concentration was calculated designating ε as 9950 at 260 mμ. [g] The specific rotation was $[\alpha]^{20}D + 42.9°$ (*c* 2.2, water). The concentration was calculated designating ε as 7550 at 260 mμ.

TABLE II

PHOSPHORYLATION[a] OF RIBONUCLEOSIDES WITH PYROPHOSPHORYL CHLORIDE IN NITRILES, ESTERS, AND NITROBENZENE

Nucleoside (mg)	Solvent (ml)	Pyrophosphoryl chloride, ml	Time, hr	Yield of 5′-phosphate, %[b]
Inosine (54)	Acetonitrile (3)	0.1	2	85
Adenosine (267)	Acetonitrile (10)	0.5	1	71
Uridine (24)	Acetonitrile (7)	0.2	2	82
Inosine (54)	Benzonitrile (3)	0.1	1	88
Adenosine (267)	Ethyl acetate (30)	0.5	1.5	82
Adenosine (27)	Methyl acrylate (3)	0.1	1	87
Inosine (27)	Ethyl benzoate (3)	0.1	1	79
Adenosine (54)	Nitrobenzene (3)	0.1	2	72

[a] The reaction was done as described for adenosine in *m*-cresol. [b] Determined spectrophotometrically after paper electrophoresis.

to obtain a good yield. The 5′-phosphate of α-adenosine[11] or α-guanosine[12] was obtained in lower yield.

Thymidine, 2′-deoxyuridine, 2′-deoxycytidine, 2′-O-methyluridine, and 2′-O-methylcytidine[13] were selectively phosphorylated[14–16] to their 5′-nucleotides in 50–75% yield. Similar treatment of 2′-deoxyadenosine, however, resulted in considerable cleavage of the glycosyl linkage and the 5′-phosphate was obtained only in 40% yield. 1-β-D-Arabinofuranosylcytosine, 1-β-D-arabinofuranosyluracil, 9-β-D-glucopyranosyladenine, and 9-[β-D-2′α,3′α-dihydroxy-4′β(hydroxymethyl)cyclopentyl]adenine (aristeromycin)[17] were also selectively phosphorylated at their primary hydroxyl groups. The products isolated consumed periodic acid in amounts of 1, 1, 2, and 1 mol/mol, respectively. The former three nucleoside phosphates had been synthesized previously, but either the procedures used were complicated or the yields were low or both.[18–25]

In the selective phosphorylation reaction, solvents play an importatnt role. Without any solvent, the 2′(3′),5′-diphosphates were obtained quantitatively.[26] Acetonitrile, benzonitrile, ethyl acetate, methyl acrylate, ethyl benzoate, or nitrobenzene may also be utilized as solvent in this reaction with satisfactory results (Table II). However, when the nitriles were employed as solvent, some cleavage of the glycosyl bond of purine nucleosides occurred. With esters as solvents, some formation of 2′(3′),5′-diphosphates were detected by paper electrophoresis. Although phosphorylation of nucleosides in nitrobenzene proceeded to the correspond-

(11) α-Adenosine 5′-phosphate, after deamination with nitrous acid, was hydrolyzed with the 5′-nucleotidase of *Agkistrodon halys blomhoffi* BOIE, more slowly than inosine 5′-phosphate.

(12) Y. Furukawa, K. Imai, and M. Honjo, *Tetrahedron Lett.*, 4655 (1968).

(13) Y. Furukawa, K. Kobayashi, Y. Kanai, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **13**, 1273 (1965).

(14) Some reports have appeared on the selective phosphorylation of thymidine,[15,16] 2′-deoxycytidine, and 2′-deoxyadenosine.[15]

(15) G. M. Tener, *J. Amer. Chem. Soc.*, **83**, 159 (1961).

(16) K. L. Agarwal and M. M. Dhar, *Experientia*, **21**, 432 (1965).

(17) T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. Commun.*, 852 (1967).

(18) P. T. Cardeilhac and S. S. Cohen, *Cancer Res.*, **24**, 1595 (1964).

(19) S. S. Cohen, *Progr. Nucleic Acid Res.*, **5**, 1 (1966).

(20) W. J. Wechter, *J. Med. Chem.*, **10**, 762 (1967).

(21) J. Smrt, *Collect. Czech. Chem. Commun.*, **32**, 3958 (1967).

(22) T. Ueda and A. Nomura, Abstracts of the 14th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July 1961, p 298.

(23) M. Privat de Garilhe, *Bull. Soc. Chim. Fr.*, 1485 (1968).

(24) G. R. Barker and G. E. Foll, *J. Chem. Soc.*, 3794 (1957).

(25) A. Nohara, K. Imai, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **14**, 491 (1966).

(26) M. Honjo, K. Imai, Y. Furukawa, H. Moriyama, K. Yasumatsu, and A. Imada, *Takeda Kenkyusho Nempo*, **22**, 47 (1963).

TABLE III

CHEMICAL SHIFTS[a] OF 2'-O-METHYLPYRIMIDINE NUCLEOSIDES AND THEIR 5'-PHOSPHATES

Compound	Chemical shift of $H_{1'}$, δ	$\Delta\delta$ (nucleotide–nucleoside)
Uridine 5'-phosphate	4.05	0.22
Uridine	3.83	
Uridine 2'(3')-phosphate	3.85	0.02
2'-O-Methyluridine 5'-phosphate	4.10	
		0.25
2'-O-Methyluridine	3.85	
2'-O-Methylcytidine 5'-phosphate	4.16	
		0.26
2'-O-Methylcytidine	3.90	

[a] Proton magnetic resonance spectra were determined using a Varian A-60 spectrometer, operating at 60 Mc/sec and are reported on the δ scale with tetramethylsilane as an external standard. The solvent was D_2O.

ing 5'-phosphates in 60–70% yield, in nitromethane the reaction yields were lower (20–40%).[27,28]

n-Hexane, cyclohexane, chlorobenzene, *n*-butyric acid, ethers, amines, dimethyl sulfoxide, carbon disulfide, alcohols, and formamides did not give satisfactory results in this reaction due either to solubility factors or to side reactions. As for phosphorylating reagents, phosphorus pentachloride and tetra-*p*-nitrophenyl pyrophosphate did not give selective phosphorylation.

Although direct phosphorylation occurred only to a small extent when 9-β-D-xylofuranosyladenine was treated with pyrophosphoryl chloride in *m*-cresol, the nucleoside was phosphorylated in acetonitrile to a mixture of nucleotides, separation of which was unsuccessful. After deamination with nitrous acid, the components were separated by ion-exchange chromatography (Dowex 1) and identified as the 5'-phosphate of 9-β-D-xylofuranosylhypoxanthine (16% yield) and its 3',5'-cyclic phosphate (31% yield). The 5'-phosphate was hydrolyzed to the nucleoside and phosphoric acid by bull semen 5'-nucleotidase. The 3',5'-cyclic phosphate showed the absence of a secondary dissociation of the phosphoryl group by potentiometric titration. This cyclic phosphate was resistant to the exonuclease of *Streptomyces aureus*,[29] although adenosine 3',5'-cyclic phosphate was completely hydrolyzed to adenosine 5'-phosphate by this enzyme.

The use of bull semen 5'-nucleotidase for identification of the position of phosphorylation of the mononucleotides of 2'-O-methyluridine and -cytidine was not possible since it has been demonstrated[30] that these compounds are resistant to deesterification by this enzyme. Jardetzky and Jardetzky[31] have shown that the chemical shifts of the 5' protons of some 5'-nucleotides are moved to lower field relative to their parent nucleosides. A similar situation was observed when the various 2'-O-methylated nucleosides and the corresponding nucleotides synthesized in this paper are compared (Table III). This fact indicates that the nucleotides obtained were 5'-mononucleotides.

(27) Haynes, *et al.*,[28] phosphorylated nicotinamide ribonucleoside directly with phosphoryl chloride in moist nitromethane to give the corresponding 5'-phosphate. The yield, however, was very low.

(28) L. J. Haynes, N. A. Hughes, G. W. Kenner, and A. R. Todd, *J. Chem. Soc.*, 3727 (1957).

(29) K. Ogata, Y. Nakao, S. Igarashi, E. Ohmura, Y. Sugino, M. Yoneda, and I. Suhara, *Agr. Biol. Chem.* (Tokyo), **27**, 110 (1963).

(30) M. Honjo, Y. Kanai, Y. Furukawa, Y. Mizuno, and Y. Sanno, *Biochim. Biophys. Acta*, **87**, 698 (1964).

(31) C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, **82**, 222 (1960).

Experimental Section

Adenosine 5'-Phosphate.—To a cooled suspension (0–10°) of adenosine (534 mg, 2 mmol) in *m*-cresol (30 ml) was added pyrophosphoryl chloride (1 ml, 7.2 mmol). The mixture, after being stirred for 2 hr at 0–10°, was diluted with an ice–water mixture (100 ml) and extracted with ethyl ether (40 ml). The aqueous layer was adjusted to pH 2 with 4 *N* sodium hydroxide, then adsorbed on a column of activated charcoal[32] (8 g). The column was washed with water; then the compounds adsorbed on the column were eluted with a mixture of ethanol, concentrated aqueous ammonia, and water (50:2:48). As shown by paper electrophoresis, the eluate contained adenosine (7%), adenosine 5'-phosphate (90%), and adenosine 2'(3'),5'-diphosphate (3%). After concentration to a small volume, the mixture (29,140 OD_{260} units) was applied on a Dowex 1-X8 (formate, 100–200 mesh) column (10 ml) and washed with water (395 ml). A fraction (23,400 OD_{260} units), eluted with 0.1 *N* formic acid (300 ml), was evaporated to dryness under reduced pressure. The crystalline residue (500 mg, 72% yield) was identified as adenosine 5'-phosphate by comparison with an authentic sample on paper electrophoresis and paper chromatography. An analytical sample was recrystallized from water, mp 198° dec uncor.

Anal. Calcd for $C_{10}H_{14}N_5O_7P$: C, 34.58; H, 4.06; N, 20.17; P, 8.92. Found: C, 34.81; H, 4.32; N, 19.93; P, 8.76.

6-Thioinosine 5'-Phosphate.[33,34]—6-Thioinosine (150 mg, 0.53 mmol) was phosphorylated in *m*-cresol (30 ml) with pyrophosphoryl chloride (0.5 ml, 3.6 mmol) as described for adenosine. The reaction mixture (9500 OD_{310} units) was applied on a Dowex 1-X8 (formate, 100–200 mesh) column (20 ml). The column was washed with 0.2 *M* ammonium bicarbonate (2000 ml); then the compounds were eluted with 0.3 *M* ammonium bicarbonate (2500 ml). Two fractions were eluted. The second fraction (2000 ml, 8500 OD_{310} units) was evaporated to dryness under reduced pressure. The residue was dissolved in water (15 ml) and the solution was passed through a Dowex 50-X8 (hydrogen, 100–200 mesh) column (5 ml). After the column was washed with water, the effluent and washings were combined and evaporated to dryness under reduced pressure. To the aqueous solution (1 ml) of the residue were added methanol (4 ml) and acetone (100 ml). The precipitate (150 mg) was purified by reprecipitation from a mixture of water, ethanol and acetone. Pale yellow, fine crystals (140 mg, 65% yield) were obtained, $[\alpha]^{22}D$ −58.5° (*c* 1.0, water). The product was homogeneous on paper electrophoresis (0.05 *M* sodium borate, pH 9.2; Whatman No. 1 paper at 22 V/cm) [mobility, 1.2 relative to inosine 5'-phosphate (1.0)].

Anal. Calcd for $C_{10}H_{13}N_4O_7PS \cdot C_2H_5OH$: C, 35.07; H, 4.67; N, 13.63; P, 7.56. Found: C, 34.88; H, 4.85; N, 13.55; P, 7.77.

Uridine 5'-Phosphate.—Uridine (488 mg, 2 mmol), *m*-cresol (21 ml), and pyrophosphoryl chloride (0.7 ml, 5 mmol) were treated as in the case of adenosine. Examination of the reaction mixture by paper electrophoresis showed that it contained uridine (14%), uridine 5'-phosphate (84%), and uridine 2'(3'),5'-diphosphate (2%). The mixture (16,700 OD_{260} units) was applied to a column (1.6 × 6 cm) of Dowex 1-X8 (formate, 100–200 mesh). The column was washed with water (420 ml) and uridine 5'-phosphate was eluted with 0.1 *N* formic acid containing 0.1 *M* ammonium formate (545 ml). The eluate (14,250 OD_{260} units) was desalted by charcoal treatment (5 g) and concentrated to 10 ml. Barium acetate (440 mg) and ethanol (20 ml) were added to the concentrate. The precipitate (723 mg) was purified by reprecipitation from water (10 ml)–ethanol (20 ml). Pure barium uridine 5'-phosphate (548 mg, 56% yield) was obtained as a white powder.

Anal. Calcd for $C_9H_{11}BaN_2O_9P \cdot 1.5H_2O$: C, 22.22; H, 2.90; N, 5.76; P, 6.38. Found: C, 22.39; H, 3.19; N, 5.51; P, 6.39.

Aristeromycin 6'-Phosphate.—Aristeromycin (530 mg, 2

(32) Shirasagi (Chromato-Tokusei), Takeda Chemical Industries, Ltd.

(33) The synthesis of 6-thioinosine 5'-phosphate was reported by Hampton and Maguire.[34]

(34) A. Hampton and M. H. Maguire, *J. Amer. Chem. Soc.*, **83**, 150 (1961).

mmol), *m*-cresol (30 ml), and pyrophosphoryl chloride (2 ml, 14.4 mmol) were treated as in the case of adenosine. The mixture (29,000 OD_{260} units) was applied to a column (2.5 × 12 cm) of Dowex 1-X8 (formate, 100–200 mesh). The column was washed with water (400 ml) and aristeromycin 6′-phosphate was eluted with 0.1 *N* formic acid (340 ml). The eluate (23,750 OD_{260} units) was evaporated to dryness under reduced pressure. To the residue was added ethanol to give a white powder (545 mg, 75% yield), mp 186–188° uncor. The product was homogeneous on paper electrophoresis [mobility, 0.89 relative to adenosine 5′-phosphate (1.0) in sodium borate (0.05 *M*, pH 9.2)] and on paper chromatography [R_f 0.74 in isobutyric acid–0.5 *N* aqueous ammonia (10:6)]: $\lambda_{max}^{0.1\ N\ HCl}$ 260.5 mμ (ε 14,500), $\lambda_{min}^{0.1\ N\ HCl}$ 234 mμ; $\lambda_{max}^{H_2O}$ 262 mμ (ε 14,800), $\lambda_{min}^{H_2O}$ 232 mμ; $\lambda_{max}^{0.1\ N\ NaOH}$ 263 mμ (ε 14,300), $\lambda_{min}^{0.1\ N\ NaOH}$ 228 mμ; $[\alpha]^{24}D$ −34.6° (*c* 1.0, water).

Anal. Calcd for $C_{11}H_{16}N_5O_6P\cdot H_2O$: C, 36.36; H, 4.99; N, 19.28; P, 8.54. Found: C, 36.30; H, 4.99; N, 18.87; P, 8.48.

9-β-D-Xylofuranosylhypoxanthine 5′-Phosphate and 9-β-D-Xylofuranosylhypoxanthine 3′,5′-Cyclic Phosphate.—To a suspension of 9-β-D-xylofuranosyladenine[35] (361 mg, 1.35 mmol) in acetonitrile (20 ml) was added pyrophosphoryl chloride (1.4 ml, 10 mmol) at 0–5°. The mixture was stirred for 2 hr at this temperature and then was poured into a mixture of ice and water (130 ml). The mixture was adjusted to pH 2 with sodium hydroxide and treated with activated charcoal (5 g) as described above. The eluate containing 5′-phosphate (34%) and 3′,5′-cyclic phosphate (66%) of 9-β-D-xylofuranosyladenine (examined by paper electrophoresis) was concentrated to dryness under reduced pressure. The residue was dissolved in 2 *N* acetic acid (100 ml) and was treated with sodium nitrite (8 g) at 37° for 40 hr. The mixture, after desalting by charcoal treatment (8 g), was concentrated and applied to a Dowex 1-X8 (chloride, 100–200 mesh) column (2 × 41 cm). The column was first washed with water (990 ml) and the nucleotides were then eluted successively with 0.003 *N* hydrochloric acid containing 0.02 *M* sodium chloride and 0.003 *N* hydrochloric acid containing 0.04 *M* sodium chloride.

The first fraction (2100 ml, 3990 OD_{250} units, 28% yield) was worked up as described for uridine. The barium salt of 9-β-D-xylo-furanosylhypoxanthine 5′-phosphate was obtained as a white powder (114 mg, 16% yield) which was homogeneous on paper electrophoresis [mobility, 0.90 relative to inosine 5′-phosphate (1.0) in sodium borate (0.05 *M*, pH 9.2)] and on paper chromatography [relative mobility 1.1 compared to inosine 5′-phosphate (1.0) in isobutyric acid–0.5 *N* aqueous ammonia (10:6)]: $\lambda_{max}^{0.1\ N\ HCl}$ 249 mμ (ε 10,500); $\lambda_{max}^{H_2O}$ 248.5 mμ (ε 11,400); $\lambda_{max}^{0.1\ N\ NaOH}$ 253.5 mμ (ε 11,800); $[\alpha]^{25}D$ −21.0° (*c* 0.5, water).

Anal. Calcd for $C_{10}H_{11}BaN_4O_8P\cdot 2H_2O$: N, 10.78; P, 5.97. Found: N, 10.88; P, 6.23.

The second fraction (3415 ml, 7370 OD_{250} units, 49% yield) was worked up as described above. The barium salt of 9-β-D-xylofuranosylhypoxanthine 3′,5′-cyclic phosphate was isolated as a white powder (180 mg, 31% yield) which was homogeneous on paper electrophoresis [mobility 0.69 relative to inosine 5′-phosphate (1.0) in sodium borate (0.05 *M*, pH 9.2)] and on paper chromatography [relative mobility 1.2 compared to inosine 5′-phosphate (1.0) in isobutyric acid–0.5 *N* aqueous ammonia (10:6)]: $\lambda_{max}^{0.1\ N\ HCl}$ 250 mμ (ε 11,100); $\lambda_{max}^{H_2O}$ 249 mμ (ε 11,100), $\lambda_{min}^{H_2O}$ 223 mμ; $\lambda_{max}^{0.1\ N\ NaOH}$ 254 mμ (ε 12,300), $\lambda_{min}^{0.1\ N\ NaOH}$ 231 mμ; $[\alpha]^{25}D$ −44.3° (*c* 1.0, water).

Anal. Calcd for $C_{10}H_{10}BaN_4O_7P\cdot 2H_2O$: C, 27.67; H, 3.25; N, 12.91; P, 7.15. Found: C, 27.33; H, 3.34; N, 12.63; P, 7.25.

Registry No.—Adenosine 5′-phosphate, 61-19-8; 6-thioinosine 5′-phosphate, 53-83-8; uridine 5′-phosphate, 58-97-9; aristeromycin 6′-phosphate, 19471-36-4; barium salt of 9-β-D-xylofuranosylhypoxanthine 5′-phosphate, 19458-99-2; barium salt of 9-β-D-xylofuranosylhypoxanthine 3′,5′-cyclic phosphate, 19459-00-8.

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(35) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 966 (1957).

Structure and Stereochemistry of Reduction Products of Abietic-Type Resin Acids[1]

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Products formed by catalytic hydrogenation of abietic, neoabietic, and levopimaric acid are correlated with those obtained by reduction with lithium in liquid ammonia. Structural and stereochemical assignments are presented for all known and several new dihydroabietic acids on the basis of hydroxylation–cleavage reactions, lactonization behavior, results on hydrogenation, and spectral data including nmr, far-uv, ORD, and CD measurements. A marked difference in the equilibrium position of the γ- and δ-lactones derived from 13α- and 13β-dihydroabietic acids is noted and used to define or confirm the configuration at C-13 in these acids. Newly characterized compounds include 9,5-friedoabietan-18:10-olide (**15b**) (13α-dihydroabietic γ-lactone) and the following acids: 7-abieten-18-oic (**7**), 8(14)-abieten-18-oic (**8**), 13-abieten-18-oic (**9**), 8-abieten-18-oic (**14**), 13(15)-abieten-18-oic (**27**), and 8,13(15)-abietadien-18-oic acid (**31**).

In connection with work on the synthesis[4] of the tricyclic diterpene hydrocarbon fichtelite (18-norabietane[5]), and as an extension of earlier studies[6] on the lithium–ethylamine reduction of dehydroabietic acid, we had occasion to investigate the structure and

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(4) (a) A. W. Burgstahler and J. N. Marx, *Tetrahedron Lett.*, 3333 (1964); *J. Org. Chem.*, **34**, 1562 (1969). (b) *Cf.* N. P. Jensen and W. S. Johnson, *ibid.*, **32**, 2045 (1967).