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The Synthesis of Nucleoside and Nucleotide Analogs

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During the course of our studies of the preparation of polymers containg nucleic acid bases,[1] we attempted to synthesize nucleoside and nucleotide analogs, replacing the sugar part of the nucleoside or the nucleotide with an aliphatic diol. In the present study, the preparation of nucleoside and nucleotide analogs, such as Ia, IIa, Ia-p, and IIa-p, was investigated. These nucleotide analogs may be useful for the study of the physicochemical properties of nucleic acids when the polymers of these nucleotide analogs are prepared. Moreover, these compounds might be of biological and chemotherapeutical interest because of their structual similarity to Cordycepin, Aristeromycin,[2] etc.

Adenine (I) was chosen as the base part of the nucleoside model. When the sodium salt of I, prepared without isolating from I and sodium hydride, was allowed to react with glycerol α-monochlorohydrin, 3- and 9-(2′,3′-dihydroxypropyl)adenines (Ia and Ib) were obtained. These products were easily separated by fractional crystallization. Both compounds were chromatographically pure. The structures of Ia and Ib were determined by studing their ultraviolet spectra.[3]

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[1] K. Kondo, H. Iwasaki, N. Ueda, K. Takemoto, and M. Imoto, *Makromol. Chem.*, **120**, 21 (1968); K. Kondo, K. Takemoto, and M. Imoto, *ibid.*, **125**, 42 (1969); K. Kondo, H. Iwasaki, N. Ueda, K. Takemoto, and M. Imoto, *ibid.*. **125**, 298 (1969).

[2] E. Waiton, F. N. Nutt, S. R. Jenkins, and F. W. Hally, *J. Amer. Chem. Soc.*, **86**, 2952 (1964); Y. F. Shealey and J. D. Clayton, *ibid.*, **88**, 3885 (1966).

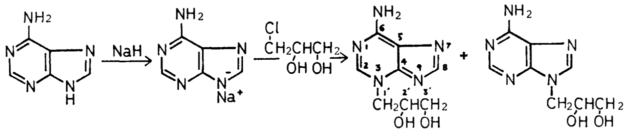
[3] It is known that 3-alkyladenines and 9-alkyladenines show λ_{max} at 273 ± 2 mμ (pH: 7) and 260 ± 2 mμ (pH: 7) respectively. J. A. Montgomery and H. H. Thomas, *J. Org. Chem.*, **28**, 2304 (1963); J. A. Montgomery and H. J. Thomas, *J. Heterocycl. Chem.*, **1**, 115 (1964); N, J. Leonard and T. Fujii, *J. Amer. Chem. Soc.*, **85**, 3719 (1963).

Table 1. The melting points, R_f values, UV data, and elemental analyses

Compd.	Mp (°C)	R_f a)	$\lambda_{max}^{H_2O}$ mμ (pH, ε)	Analyses (%) Calcd C	H	N	Found C	H	N
Ia	299—301	0.67	276 (2, 14000) 275 (7, 13500) 274 (12, 14500)	45.93	5.30	33.48	46.09	5.35	33.22
Ib	205—206	0.76	259 (2, 12200) 261 (7, 11600) 262 (12, 12000)	45.93	5.30	33.48	45.86	5.13	32.78
IIa	260 (decomp.)	0.72	265 (7, 11200)	45.71	4.80	26.66	45.50	4.76	26.59
IIb	251—252	0.80	251 (7, 10800)	45.71	4.80	26.66	45.42	5.01	26.38
Ia-p	241—242	0.48	275 (7, 15800)	33.25	4.18	24.22	33.11	4.25	23.89
Ib-p	224—225	0.62	259 (7, 13800)	33.25	4.18	24.22	33.18	4.28	23.97
IIa-p	238—239	0.60	265 (7, 12600)	33.11	3.82	19.31	33.31	3.62	19.21
IIb-p	203—204	0.55	250 (7, 12300)	33.11	3.82	19.31	32.82	3.58	19.01

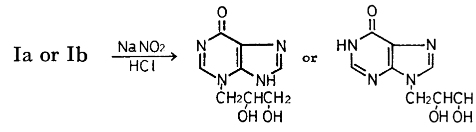
a) Solvent systems used were: water satulated with *n*-butanol - concd. NH_4OH (15:10) for Ia, Ib, IIa, IIb, and ethanol - conc. NH_4OH - water (15:10:1) for Ia—p, Ib—p, IIa—p, and IIb—p.

In this experiment, 7-(2′,3′-Dihydroxypropyl)adenine was not obtained.



I Ia Ib

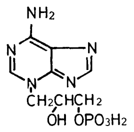
When compounds Ia and Ib were treated with concentrated hydrochloric acid and sodium nitrite, the corresponding hypoxanthine derivatives (IIa and IIb) were produced in 70 and 75% yields respectively.

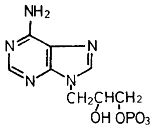


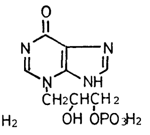
IIa IIb

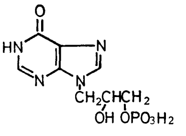
These compounds were purified by recrystallization. The UV and IR spectra supported the structures.[4]

The phosphorylation of the above compounds was carried out using phosphorus oxychloride in trimethyl phosphate by a modification of the procedure of Yoshikawa, Kato, and Takenishi.[5] By this method, compounds Ia, Ib, IIa, and IIb were phosphorylated to give the corresponding nucleotide analogs, Ia-p, Ib-p, IIa-p, and IIb-p, in 52, 39, 36, and 42% yields respectively. The phosphates were identified by paper chromatography and by elemental analysis. In these experiments, the 2′-phosphates were not obtained since the 2′-position of hydroxyl groups on the nucleoside analogs are sterically hindered.


Ia-p


Ib-p


IIa-p


IIb-p

Pharmacological studies and a study of the polymerization reaction of these phosphates are now under way.

Experimental

The melting points are uncorrected. The infrared spectra (IR) were run on a JASCO Model IR-G Spectrometer. The ultraviolet spectra (UV) were measured by a Hitachi Recording Spectrometer Model EPS-3T. Paper chromatography was carried out by the ascending technique using Toyo Roshi No. 50 paper. The results of the elemental analysis, the UV data, the R_f, and the mp of the reported compounds are listed in Table 1.

3-(2′,3′-Dihydroxypropyl)adenine (Ia) and 9-(2′,3′-Dihydroxypropyl)adenine (Ib). A mixture of adenine (0.67 g, 5 mmol) and sodium hydride (0.25 g, 50%, 5.2 mmol) in anhydrous *N,N*-dimethylformamide (200 m*l*) was stirred at room temperature for 1 hr to give a milky solution of the sodium salt of adenine. To this solution glycerol α-monochlorohydrin (0.55 g, 5.5 mmol) was added, and the mixture was heated at 80—85°C with stirring for 24 hr. The sodium chloride thus precipitated in the cooled reaction mixture was filtered out, and the filtrate was concentrated as much as possible. The resulting solid was filtered and recrystallized from water to afford Ia (0.22 g (21%)) as colorless plates.

The mother liquid was concentrated to dryness. A yellow solid thus obtained was crystallized from ethanol. Recrystallization from water gave 0.47 g (45%) of Ib as colorless needles.

IR of Ia (KBr): 3350(s), 3200(s), 2980(s), 1960(s), 1628(s),

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[4] J. H. Lister and G. M. Timmis, *J. Chem. Soc.*, **1960**, 327.

[5] This method is known to be useful for the selective phosphorylation of the 5′-hydroxyl group of nucleoside (M. Yoshikawa, T. Kato, and T. Tadenishi, *Tetrahedron Lett.*, **1967**, 5065); the selective phosphorylation of nicotinamide nucleoside and riboflavin has also been reported by this method. In the case of riboflavin, as intermediate, 4′, 5′-cyclic riboflavin phosphate was isolated; it was exclusively hydrolized to riboflavin 5′-phosphate. Propanediol-1,2-cyclic phosphate and glycerol-1,2-cyclic phosphate produced the corresponding 1-phosphates almost quantitatively under acidic conditions. From these results, it seems that the reaction of diols with phosphorus oxychloride proceed through an intermediate, five-membered cyclic phosphate, which is selectively hydrolyzed into primary phosphates. L. H. Yaynes, N. A. Hughs, G. W. Kener, and A. R. Todd, *J. Chem. Soc.*, **1957**, 3727; H. S. Torrest and A. R. Todd, *ibid.*, **1950**, 3295; T. Ukita, K. Nagasawa, and M. Irie, *Pharm. Bull* (Tokyo), **5**, 127 (1957); E. Baer, ans M. Kates, *J. Biol. Chem.*, **175**, 79 (1948).

1560(w), 1415(m), 1225(w), 1180(w), 1010(m), 890(w), and 710(w) cm^{-1}.

IR of Ib (KBr): 3300(s), 3150(s), 2950(w), 1650(s), 1600(s), 1480(m), 1416(w), 1330(m), 1295(m), 1105(m), 1020(m), 900(m), and 730(s) cm^{-1}.

3-(2′,3′-Dihydroxypropyl)hypoxanthine (IIa). Into a solution of Ia (1.0 g, 4.8 mmol) in concentrated hydrochloric acid (20 m*l*), sodium nitrite (1.2 g, 17.1 mmol) in water (40 m*l*) was slowly with vigorously stirring over a 1-hr period. The temperature was maintained at 0°C. After having been stirred at room temperature for an additional 6 hr, the reaction mixture was neutralized with sodium bicarbonate and evaporated to dryness under reduced pressure. The residue was crystallized from water to give IIa (0.7 g (70%)) as colorless plates.

IR (KBr): 3000(s), 2900(s), 2850(m), 1630(s), 1595(m), 1545(s), 1430(s), 1380(w), 1200(m), 1120(m), 1010(m), 940(w), and 790(w) cm^{-1}.

9-(2′,3′-Dihydroxypropyl)hypoxanthine (IIb). Compound Ib(1.0 g, 4.8 mmol) was treated with sodium nitrite (1.2 g, 17.1 mmol) in a manner similar to that mentioned above. The reaction mixture was neutrallized with concentrated ammonium hydroxide and concentrated. Crude IIb was crystallized from ethanol. Recrystallization from water afforded IIb (0.75 g (75%)) as colorless needle crystals.

IR (KBr): 3200(s), 2900(s), 1690(s), 1620(w), 1590(m), 1550(m), 1520(m), 1420(m), 1380(w), 1227(s), 1120(s), 1060(m), 1015(m), 870(w), and 770(m) cm^{-1}.

3-(2′,3′-Dihydroxypropyl)adenine 3′-Phosphate (Ia-p). Compound Ia (0.5 g, 2.4 mmol) was dissolved in trimethyl phosphate (5 m*l*) and cooled to 0°C. To this solution phosphorus oxychloride (0.4 g, 2.6 mmol) was added slowly over 6-hr period. Water was then added, and the mixture was stirred for 20—30 more min. A white precipitate was then filtered out and crystallized from water. The phosphate, Ia-p (0.36 g (52%)), was obtained as colorless needle crystals.

IR (KBr): 3100(m), 3000(s), 1680(m), 1630(s), 1410(m), 1200(s), 1100(s), 1060(s), 970(s), and 770(w) cm^{-1}.

9-(2′,3′-Dihydroxypropyl)adenine 3′-Phosphate (Ib-p). Compound Ib (0.5 g, 2.4 mmol) was treated with phosphorus oxychloride (0.4 g, 2.6 mmol) in trimethyl phosphate (4 m*l*) in a manner similar to that described above. Water added to the reaction mixture and it was stirred for an additional hour. After the reaction mixture had then been concentrated under reduced pressure at below 50°C, the residue was treated with ethanol (20 m*l*). The white precipitate was crystallized from water to give Ib-p (0.26 g (39%)) as colorless needles.

IR (KBr): 3200(s), 3050(s), 1690(s), 1610(m), 1415(m), 1220(m), 1120(s), 990(s), 930(s), and 760(m) cm^{-1}.

3-(2′,3′-Dihydroxypropyl)hypoxanthine 3′-Phosphate (IIa-p). Compound IIa (0.5 g, 2.4 mmol) was treated with phosphorus oxychloride (0.4 g, 2.6 mmol) in trimethyl phosphate (5 m*l*) as has been described above. Water the then added to the reaction mixture, and it was stirred for an additional hour. After the evaporation of water below 50°C, a mixture of 20 m*l* of ethanol and 10 m*l* of ether was added. The white precipitate was crystallized from water to give IIa-p (0.25 g (36%)) as colorless crystals.

IR (KBr): 3250(m), 3100(m), 1680(s), 1550(m), 1230(m), 1110(s), 770(w), and 610(w) cm^{-1}.

9-(2′,3′-Dihydroxypropyl)hypoxanthine 3′-Phosphate (IIb-p). IIb-p was prepared in the way described in the case of IIa-p; IIb (0.5 g, 2.4 mmol) was treated with phosphorus oxychloride (0.4 g, 2.6 mmol) and trimethyl phosphate (5 m*l*). IIb-p (0.29 g (42%)) was obtained and crystallized from a mixture of ethanol and water (8: 2).

IR (KBr): 3400(m), 3150(s), 1700(s), 1570(m), 1530(w), 1370(s), 1210(s), 1100(s), 990(m), and 910(m) cm^{-1}.