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Synthesis and Antiviral Activity of Certain 5'-Monophosphates of 9-D-Arabinofuranosyladenine and 9-D-Arabinofuranosylhypoxanthine

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A number of 5'-phosphates of 9-D-arabinofuranosyladenine and 9-D-arabinofuranosylhypoxanthine were prepared and tested against a variety of DNA viruses in tissue culture. The syntheses of the antiviral agent 9-β-D-arabinofuranosylhypoxanthine 5'-monophosphate (**6**) and a series of related nucleotides, 9-β-D-arabinofuranosyladenine 5'-*O*-methylphosphate (**3**), 9-β-D-arabinofuranosylhypoxanthine 5'-*O*-methylphosphate (**7**), 9-β-D-arabinofuranosylhypoxanthine cyclic 3',5'-phosphate (**13**), and 9-α-D-arabinofuranosylhypoxanthine 5'-monophosphate (**17**), are described. The concepts underlying the development of these antiviral agents are discussed. Comparison of the anti-DNA viral activity is made with 9-β-D-arabinofuranosyladenine (ara-A). Reproducible antiviral activity against three DNA viruses in vitro at nontoxic dosage levels is demonstrated by **3**, **6**, and other related nucleotides.

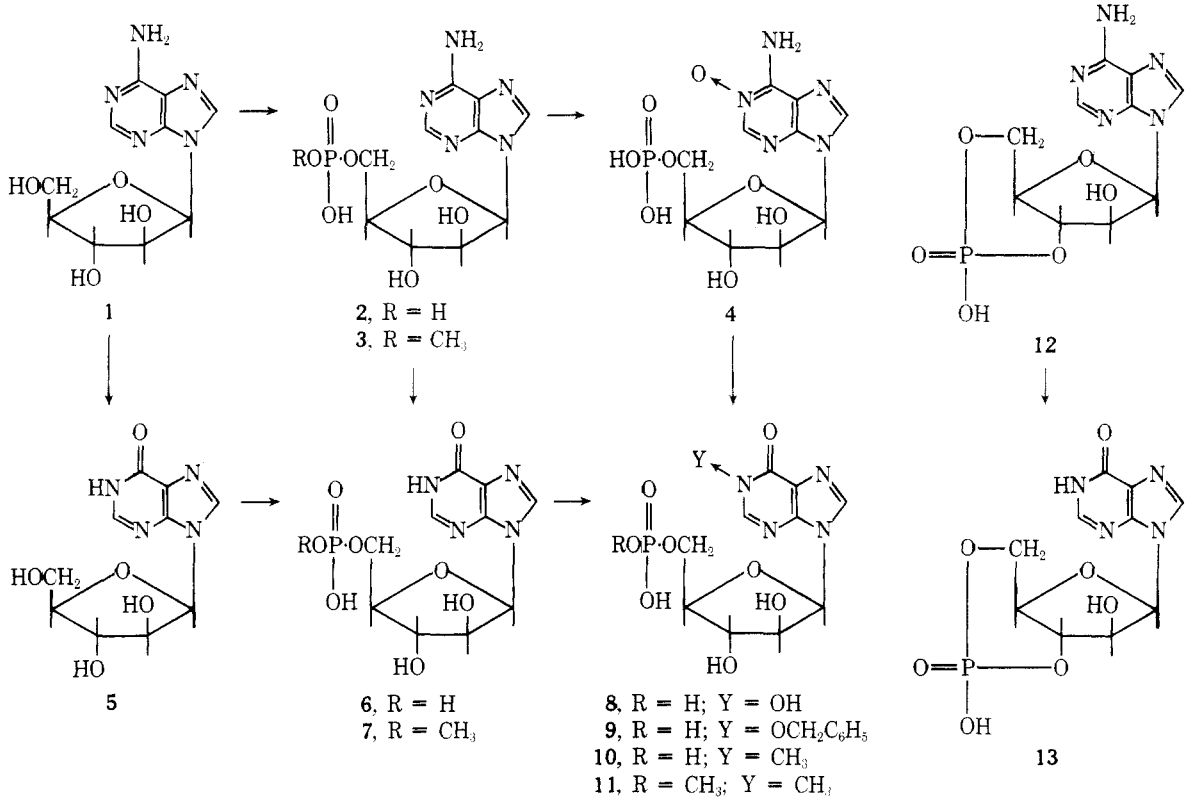
The last decade has witnessed the recognition of nucleoside analogs as potential clinically useful antitumor and antiviral agents. Among the presently known synthetic nucleosidic antiviral agents, some of the more active analogs are 5-iodo-2'-deoxyuridine (IUDR),[1-3] 1-β-D-arabinofuranosylcytosine (ara-C),[4-10] 9-β-D-arabinofuranosyladenine (ara-A),[11-14] and the broad spectrum antiviral agent 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin).[15,16] Of these antiviral agents, only IUDR is currently available as a prescribed drug; however, its low solubility and high toxicity make its use somewhat limited. Ara-A is presently undergoing extensive clinical evaluation against a variety of diseases primarily caused by the Herpes viruses.[17] While efficacy in humans has been established,[14,18,19] the nucleoside has certain disadvantages which may preclude its overall usefulness. These include a relative insolubility in water (0.5 mg/ml at 25°, 1.8 mg/ml at 37°) and a moderate toxicity, manifested predominantly as mild nausea, central nervous system involvement, and leucocyte chromosome breakage.[14,19] It is pertinent to note also the marked antiviral activity[20] of 9-β-D-arabinofuranosylhypoxanthine (ara-Hx), the apparent major breakdown product of ara-A.[21,22]

Numerous derivatives of ara-A have been described. Recent studies[23] with several 2-substituted ara-A derivatives (2-chloro, 2-methoxy, 2-benzyloxy, 2-methylthio) indicated 2-Cl-ara-A had antiviral efficacy in vitro, but its in vivo activity was inferior to ara-A. Replacement by N of the 8-CH moiety of α-ara-A (8-aza-α-ara-A) resulted in in vitro antiviral activity approximately equal to ara-A, although the compound was relatively ineffective and quite toxic in vivo.[24] Similar efficacy in vitro but loss of activity in vivo was also reported by Renis et al.[25] using the 5'-benzoyl and 5'-palmitoyl esters of ara-A. 9-β-D-Arabinofuranosyladenine 5'-monophosphate (ara-AMP)[26] and 9-β-D-arabinofuranosyladenine cyclic 3',5'-phosphate (cyclic ara-AMP),[27,28]

both relatively water soluble, have marked in vitro and in vivo activity against a host of DNA viruses.[29,30] Topical application of ara-AMP or cyclic ara-AMP appeared superior to ara-A in Herpes keratitis in rabbit or Herpes virus induced cutaneous lesions in tails of mice.[29]

We have continued our search for nucleoside or nucleotide analogs which are capable of effectively inhibiting the development of viral infections and which also possess superior solubility and less toxicity than the presently known antiviral agents. Since the antiviral efficacy of ara-A in vivo may be due to the activity of its principal breakdown product, ara-Hx, considerable effort was exerted on producing water-soluble derivatives of that compound as well. The present report describes the synthesis and initial in vitro antiviral activity of 11 new derivatives of both 9-D-arabinofuranosyladenine and 9-D-arabinofuranosylhypoxanthine. The synthetic approaches followed are shown in Scheme I.

Scheme I



Ara-A (1) was phosphorylated according to the reported procedure[26,31] giving 9-β-D-arabinofuranosyladenine 5′-monophosphate (2). Treatment of 2 in aqueous acetic acid with sodium nitrite at ambient temperature provided crystalline 9-β-D-arabinofuranosylhypoxanthine 5′-monophosphate (6) in over 90% yield, isolated as free acid. Alternatively, the deamination of 2 with liquid nitrosyl chloride in *N,N*-dimethylformamide under anhydrous conditions provided a 62% yield of 6. The direct phosphorylation of 9-β-D-arabinofuranosylhypoxanthine (5)[32] with phosphorus oxychloride in the presence of trimethyl phosphate also furnished 6. Compound 6 was characterized by its ultraviolet absorption spectrum which is typical of 9-β-D-arabinofuranosylhypoxanthine.

Phosphorylation of ara-A with methyl phosphorodichloridate using trimethyl phosphate as the solvent at ambient temperature provided a 48% yield of 9-β-D-arabinofuranosyladenine 5′-*O*-methylphosphate (3), the structure of which was assigned on the basis of its ^{1}H NMR (determined in D_2O, doublet centered at δ 3.65 with POCH coupling of 12 Hz)[33] and ultraviolet absorption spectra and by the elemental analysis. Deamination of 3 with sodium nitrite in aqueous acetic acid solution furnished crystalline 9-β-D-arabinofuranosylhypoxanthine 5′-*O*-methylphosphate (7). Compound 7 demonstrated homogeneity in several thin-layer systems and on paper electrophoresis (phosphate buffer, pH 7.3, and borate buffer, pH 9.2). The structure of 7 was also confirmed by its characteristic inosine-like ultraviolet absorption spectrum, ^{1}H NMR spectrum, and elemental analysis.

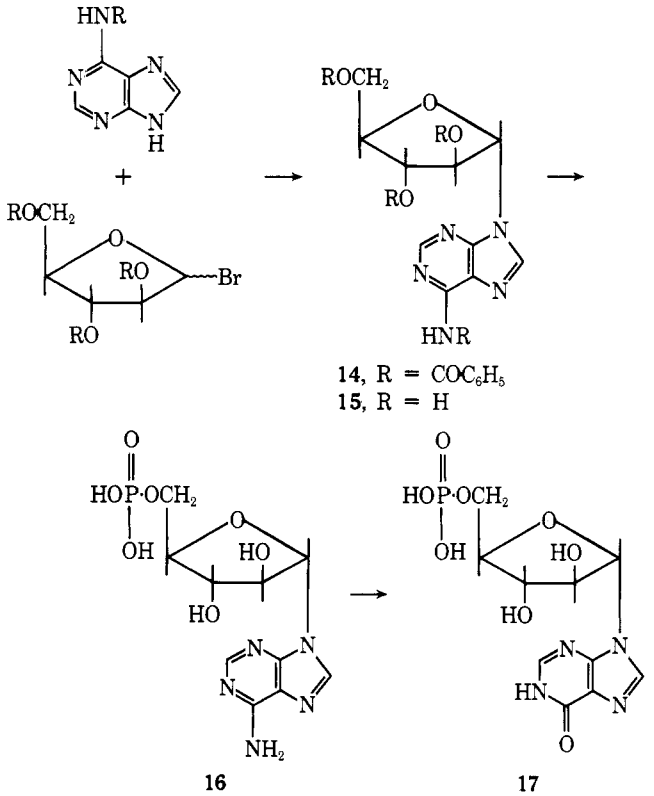
The observation that the 1-oxide[34] and various 1-alkyl and aralkyloxy[35] derivatives of purine nucleosides exert considerable antiviral activity induced us to prepare a number of 1-substituted ara-HxMP analogs as potential antiviral agents. Oxidation of ara-AMP (2) with *m*-chloroperbenzoic acid in a buffered two-phase system provided crystalline 9-β-D-arabinofuranosyladenine 5′-monophosphate 1-oxide (4).[36] Deamination of 4 with aqueous nitrous acid furnished 9-β-D-arabinofuranosyl-1-hydroxyhypoxanthine 5′-monophosphate (8) in 67% yield. Compound 8 was treated with benzyl bromide in anhydrous dimethyl sulfoxide solution containing 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) to provide 9-β-D-arabinofuranosyl-1-benzyloxyhypoxanthine 5′-phosphate (9). Treatment of 6 with methyl iodide in dimethyl sulfoxide containing DBU gave a compound which was identified as 9-β-D-arabinofuranosyl-1-methylhypoxanthine 5′-*O*-methylphosphate (11) on the basis of its ^{1}H NMR spectrum and elemental analysis. However, acetylation of 6 with acetic anhydride in dry pyridine at room temperature provided syrupy 9-(2,3-di-*O*-acetyl-β-D-arabinofuranosyl)hypoxanthine 5′-phosphate, isolated as the triethylammonium salt. Treatment of this blocked nucleotide with sodium hydride, followed by methyl iodide in dimethyl sulfoxide, and subsequent deacetylation with methanolic ammonia furnished 9-β-D-arabinofuranosyl-1-methylhypoxanthine 5′-phosphate (10). The structure of 10 was confirmed by its characteristic 1-meth-

ylinosine-like ultraviolet absorption spectrum[37] and by elemental analysis.

The evidence that the cyclic 3',5'-monophosphate moiety does not inhibit and may, indeed, enhance the antiviral activity of purine and pyrimidine nucleosides[29,38-42] suggested the synthesis of 9-β-D-arabinofuranosylhypoxanthine cyclic 3',5'-phosphate (13) as a potential antiviral agent. The synthesis of 9-β-D-arabinofuranosyladenine cyclic 3',5'-phosphate (12)[27] has been accomplished according to the reported procedure[28,30] and was subsequently deaminated with aqueous nitrous acid to furnish 13 in over 89% yield. Compound 13 moved as a homogeneous ultraviolet-absorbing component in several thin-layer systems and the structure was confirmed by its ultraviolet absorption spectrum, elemental analysis, and ^{1}H NMR spectrum (determined in D_2O–NaOD) in which the anomeric proton was a doublet located at δ 6.5.

The synthesis of the α anomer of 6 was accomplished by the direct glycosylation of 6-benzamidopurine with 2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl bromide in acetonitrile at room temperature. The major product, 9-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-6-benzamidopurine (14), was isolated in 51% yield and was subsequently debenzoylated with sodium methoxide in methanol at ambient temperature to furnish 9-α-D-arabinofuranosyladenine (15). The identity of 15 was confirmed by rigorous comparison of the physicochemical data reported for α-ara-A.[43,44] This alternate method of synthesis of α-ara-A is relatively simple and could be prepared in laboratory quantities with overall yields comparing favorably with those of the reported method.[43,44] Phosphorylation of 15 with phosphorus oxychloride in trimethyl phosphate followed by ion-exchange resin column chromatography provided a 69% yield of 9-α-D-arabinofuranosyladenine 5'-monophosphate (16), the structure of which was confirmed by ^{1}H NMR, ultraviolet absorption spectra, and elemental analysis. Deamination of 16 by the conventional method gave good yield of 9-α-D-arabinofuranosylhypoxanthine 5'-monophosphate (17) isolated as the free acid. The characteristic shift in ultraviolet absorption maximum (λ max) and decreased chromatographic mobility as compared to 16, was observed with 17 (Scheme II).

Scheme II



16 17

Antiviral Evaluation. Antiviral activity was determined by observing the inhibition of virus-induced cytopathic effects (CPE). In this system, cultures of human carcinoma of nasopharynx (KB) or rabbit kidney (RK-13) cells were grown in disposable plastic microplates.[45] Monolayers (18–24 hr) of cells were exposed to 320 $CCID_{50}$ of virus and a concentration of each compound ranging from 1000 to at least 1 μg/ml was added within 15 min. The cells were observed for CPE development after a 72-hr incubation at 37°. Degree of CPE inhibition and compound cytotoxicity were scored numerically and used in calculating a virus rating (VR) as described previously.[45] Significance of activity in terms of VR's has been assigned as follows: <0.5, slight or no activity; 0.5–0.9, moderate activity, and ≥1.0, marked activity. Viruses used in this study were types 1 and 2 Herpes simplex and vaccinia. Data are expressed as the average of 1–5 tests with ara-A and ara-AMP being shown for comparison. Of the compounds tested, ara-A, ara-AMP (2), ara-HxMP (6), and ara-A 5'-*O*-methylphosphate (3) had approximately equal, marked antiviral activity. Moderate antiviral activity was seen with the cyclic 3',5'-phosphate of ara-Hx (13), α-ara-A (15), and α-ara-AMP (16). Ara-Hx 5'-*O*-methylphosphate (7) was less effective than 3 but retained partial activity. α-Ara-A and α-ara-AMP both demonstrated moderate activity against type 1 Herpes simplex and vaccinia virus but were less active than ara-A. α-Ara-HxMP (17) was not active in this system. Slight activity was seen with 1-hydroxy-ara-HxMP (8). Essentially no activity was observed with 1-benzyloxy-ara-HxMP (9) or 1-methyl-ara-HxMP (10) (see Table I).

These data indicate that two of the compounds (3 and 6) tested had antiviral activity comparable to ara-A and ara-AMP. Both these compounds were water soluble and consequently possess therapeutic advantages over ara-A. Little in vivo work has yet been completed with 9-β-D-arabinofuranosyladenine 5'-*O*-methylphosphate (3) or the other nucleotides described in the present report. 9-β-D-Arabinofuranosylhypoxanthine 5'-monophosphate is quite active in vivo in animals[46] and extensive reports of these data will be published elsewhere.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Proton magnetic resonance (^{1}H NMR) spectra were obtained at 60 MHz on a Hitachi Perkin-Elmer R-20A spectrometer in D_2O using DSS as an internal reference. Ultraviolet spectra were recorded on a Cary Model 15 spectrometer and infrared spectra on a Perkin-Elmer 257 spectrophotometer (KBr pellets). Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich. Evaporations were carried out under reduced pressure with bath temperature below 30°. Detection of components on silica gel F-254 (EM Reagents) was by ultraviolet light and with 10% sulfuric acid in methanol spray followed by heating. Chromatography solvent mixtures were by volume and the following systems were used: A, isopropyl alcohol–concentrated ammonium hydroxide–water, 7:1:2; B, isopropyl alcohol–concentrated ammonium hydroxide–water, 5.5:1.0:3.5; C, 1-butanol–acetic acid–water, 5:2:3; and D, acetonitrile–0.2 *M* aqueous ammonium chloride, 7:3.

9-β-D-Arabinofuranosylhypoxanthine 5'-Monophosphate (6). Method 1. To an ice-cooled suspension of 9-β-D-arabinofuranosyladenine 5'-monophosphate[31] (2, 2.0 g, 0.0057 mol) in water (15 ml) and glacial acetic acid (3.0 ml) was added sodium nitrite (2.5 g, 0.036 mol). The flask was loosely stoppered and stirred for 2–3 hr in the ice bath. The stirring was continued overnight at room temperature. The clear solution was evaporated to dryness;

Table I. Comparative in Vitro Antiviral Activity of Ara-A, Ara-HxMP, and Related Derivatives

Compd		Virus ratings[a]		
		Type 1 Herpes	Type 2 Herpes	Vaccinia
1	Ara-A	1.0	0.5	0.9
2	Ara-AMP	0.9	0.8	0.8
6	Ara-HxMP	0.8	0.8	0.9
3	Ara-A 5′-*O*-methylphosphate	1.0	0.8	0.8
7	Ara-Hx 5′-*O*-methylphosphate	0.5	0.0	0.4
8	1-Hydroxy-ara-HxMP	0.4	0.6	0.2
9	1-Benzyloxy-ara-HxMP	0.1	0.0	0.0
10	1-Methyl-ara-HxMP	0.0	0.0	0.0
11	1-Methyl-ara-Hx 5′-*O*-methylphosphate	0.2	0.0	0.0
13	Cyclic 3′,5′-phosphate of ara-Hx	0.6	[b]	0.7
15	α-Ara-A	0.6	0.1	0.6
16	α-Ara-AMP	0.5	0.0	0.6
17	α-Ara-HxMP	0.0	0.0	0.0

[a]The virus rating (VR) was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE value (0–4) assigned to T for each drug level was subtracted from that of C, and the differences (C − T) were totaled. If partial toxicity was evident at any drug level, the C − T of that level was divided by 2. The sum of all C − T values was then divided by ten times the number of test cups used per drug level. [b]Not determined.

the residue was dissolved in water (20 ml) and then carefully neutralized with solid $KHCO_3$. The neutral solution was applied to a column containing 50 ml of Dowex 50 (H^+) ion-exchange resin. The column was washed with water and the fractions containing uv-absorbing material were pooled and concentrated to about 10 ml. Ethanol (50 ml) was added to the aqueous solution and chilled overnight. The solid was collected and crystallized from water as needles to yield 1.82 g (90.6%): mp 204–205° dec; $[\alpha]^{25}D$ +9.9° (*c* 1.0, H_2O); ^1H NMR (D_2O–NaOD) δ 6.39 (d, *J* = 5.5 Hz, $C_1'H$), 8.22 (s, 1), 8.50 (s, 1); uv λ max (pH 1) 249 nm (ε 12,700); λ max (pH 7) 248 nm (ε 13,600); λ max (pH 11) 252 nm (ε 14,400).

Anal. Calcd for $C_{10}H_{13}N_4O_8P$ (348.20): C, 34.48; H, 3.74; N, 16.10. Found: C, 34.45; H, 3.80; N, 15.99.

Method 2. To a stirred suspension of **2** (2.0 g, 0.0057 mol) in dry DMF (40 ml) at 0° was added 2.0 ml of liquid NOCl, under anhydrous conditions. After 15 min another 1.2 ml of NOCl was added. Gas evolution was observed as long as solid remained suspended in the reaction mixture and after 10 min a clear orange solution was obtained. The solution was allowed to warm to room temperature over an 80-min period and then cooled again in an ice–salt bath. Water (40 ml) was added and the colorless solution was carefully neutralized with $KHCO_3$. The mixture was evaporated to dryness. The residue was dissolved in water (25 ml) and passed through a Dowex 50 (H^+) column (50 ml). The column was washed with water and the appropriate fraction was collected. The volume was reduced to about 10 ml and 50 ml of ethanol was added. The product that separated after cooling was collected and crystallized from water to yield 1.30 g (64.7%): mp 203–204° dec.

Method 3. 9-β-D-Arabinofuranosylhypoxanthine[32] (**5**, 10.72 g, 0.04 mol) was added with stirring to a precooled (0–5°, ice bath) mixture of trimethyl phosphate (100 ml) and phosphorus oxychloride (12.3 g). After the solid had dissolved (~10 min), it was stored for 4 hr at 0° and then poured slowly into ice–water (150 ml) containing 25 g of $NaHCO_3$. The aqueous solution was allowed to stand for 1 hr to stabilize the pH at 6. The solution was extracted with ether (3 × 10 ml) to remove trimethyl phosphate. The volume of the aqueous phase was reduced until salts began to crystallize. Enough water was added to dissolve the crystals and the solution was applied to a Barneby Cheney charcoal[47] column (700 g). The column was washed with water to remove salts, and then the product was eluted with 50% aqueous methanol containing 10% NH_4OH. The eluent was reduced to a small volume and the pH was adjusted to 2. Ethanol was added until the solution became turbid and then stored at 5°. The crystals were collected and dried to yield 6.50 g (46.5%) of **6**: mp 203–204° dec.

9-β-D-Arabinofuranosyladenine 5′-*O*-Methylphosphate (3). Methyl phosphorodichloridate (10.0 g, 0.067 mol) in freshly distilled trimethyl phosphate (100 ml) was cooled to 0–5° in an ice bath. The ice bath was removed as 9-β-D-arabinofuranosyladenine (10.0 g, 0.037 mol, dried at 80° for 5 hr) was added. The temperature was monitored between 5 and 20°. After 2 hr a clear solution was obtained, which was stored overnight at 4°. The solution was then poured into ice–water (400 ml) containing $NaHCO_3$ (6.0 g). Additional $NaHCO_3$ was added periodically until the pH was stable at 5–6, ca. 1 hr. Trimethyl phosphate was removed by extraction with ether (4 × 150 ml). Dissolved ether and excess water were removed by evaporation until salts began to crystallize. Enough water was added to achieve solution and the pH was checked (6–7) before application to the top of a Dowex 1 X2 column (formate form, 100–200 mesh, 250 ml). The column was washed with water until no further uv-absorbing species were detected in the eluent. Gradient elution (water to 0.1 *M* formic acid) gave the product as a thick band. The appropriate fractions were evaporated to about 100 ml. The remaining solution was frozen and lyophilized to obtain a white, fluffy solid weighing 6.50 g (48.0%): mp 170–190° dec; $[\alpha]^{25}D$ +48.7° (*c* 1.0, H_2O); uv λ max (pH 1) 257 nm (ε 14,200); λ max (pH 7) 258 nm (ε 13,000); λ max (pH 11) 258 nm (ε 13,000).

Anal. Calcd for $C_{11}H_{16}N_5O_7P$ (361.25): C, 36.57; H, 4.47; N, 19.39. Found: C, 36.42; H, 4.26; N, 19.19.

9-β-D-Arabinofuranosylhypoxanthine 5′-*O*-Methylphosphate (7). To an ice-cooled solution of **3** (2.0 g, 0.0055 mol) in water (15 ml) containing glacial acetic acid (3.0 ml) was added sodium nitrite (2.25 g, 0.032 mol). The flask was loosely stoppered and stirred for 2–3 hr in an ice bath. After stirring for 15 hr at room temperature, the solution was evaporated to dryness and treated in the same way as described in **6** to yield 1.65 g (82.3%) after recrystallization from aqueous ethanol: mp 165–180° dec; $[\alpha]^{25}D$ +55.7° (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 6.46 (d, *J* = 5.5 Hz, $C_1'H$), 8.30 (s, 1), 8.85 (s, 1); uv λ max (pH 1) 248 nm (ε 10,300); λ max (pH 7) 248 nm (ε 9900); λ max (pH 11) 251 nm (ε 12,000).

Anal. Calcd for $C_{11}H_{15}N_4O_8P$ (362.23): C, 36.48; H, 4.17; N, 15.46. Found: C, 36.22; H, 4.39; N, 15.49.

9-β-D-Arabinofuranosyl-1-hydroxyhypoxanthine 5′-Phosphate (8). To a cooled suspension of 9-β-D-arabinofuranosyladenine 5′-monophosphate 1-oxide[36] (**4**, 2.0 g, 0.0055 mol) in water (15 ml) containing glacial acetic acid (3.0 ml) was added sodium nitrite (2.5 g, 0.036 mol). After stirring overnight at room temperature, the reaction mixture was evaporated to dryness. The residue was dissolved in water, neutralized with $KHCO_3$, and treated in the same way as described in **6** to yield 1.35 g (67.3%): mp >150° dec; ^1H NMR (D_2O–NaOD) δ 6.33 (d, *J* = 5.5 Hz, $C_1'H$), 8.33 (s, 1), 8.42 (s, 1); uv λ max (pH 1) 251 nm (ε 11,650); λ max (pH 7) 255 nm (ε 11,650), 285 (sh) (3300); λ max (pH 11) 255 nm (ε 12,000), 285 (sh) (3300).

Anal. Calcd for $C_{10}H_{13}N_4O_9P$ (364.20): C, 32.98; H, 3.59; N, 15.38. Found: C, 32.82; H, 3.62; N, 15.19.

9-β-D-Arabinofuranosyl-1-benzyloxyhypoxanthine 5′-Phosphate (9). Compound 8 (1.12 g, 0.030 mol) was dissolved in dry DMSO (10 ml) and 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU, 0.5 g) was added. The mixture was stirred at room temperature.

The gelatinous precipitate which initially formed dissolved after 30 min with rapid stirring. The mixture was treated with benzyl bromide (0.65 g, 0.0038 mol) and stirring was continued at room temperature overnight. The mixture was then poured into a cold ethanol–ether mixture (1:1, 500 ml). The mixture was filtered and the residue was washed thoroughly with anhydrous ether. The hygroscopic solid was dissolved in minimum volume of water and cautious addition of ethanol caused the product to precipitate as white crystals. The mixture was chilled overnight and the product was collected, washed with ethanol, and dried to yield 0.80 g (57.4%): mp >175° dec; uv λ max (pH 1) 250 nm (ε 13,600); λ max (pH 7) 255 nm (ε 14,500); λ max (pH 11) 255 nm (ε 14,500).

Anal. Calcd for $C_{17}H_{19}N_4O_9P$ (454.33): C, 44.91; H, 4.21; N, 12.33. Found: C, 45.18; H, 4.43; N, 12.61.

9-β-D-Arabinofuranosyl-1-methylhypoxanthine 5'-Phosphate (10). 9-β-D-Arabinofuranosylhypoxanthine 5'-phosphate (**6**, 3.0 g, 0.0086 mol) was dissolved in dry pyridine (90 ml) containing triethylamine (2.25 g) and acetic anhydride (5.7 g) and the solution was stirred at room temperature for 3 hr. The pale yellow solution was evaporated and the oily residue mixed with about 50 g of ice. It was again evaporated to dryness. This process was repeated three times with 25-g portions of ice. The residue was dissolved in 100 ml of water and extracted with ether. The clear, aqueous solution was frozen and lyophilized to obtain 5.0 g of syrupy 9-(2,3-di-*O*-acetyl-β-D-arabinofuranosyl)hypoxanthine 5'-phosphate triethylammonium salt.

The triethylammonium salt (2.5 g) was dissolved in anhydrous DMSO (50 ml) and sodium hydride (350 mg, 57% oil dispersion) was added to it. The mixture was stirred at room temperature with the exclusion of moisture for 2 hr. The clear mixture was then treated with methyl iodide (5.0 ml) and the stirring continued for 4 hr. The resulting brown mixture was then poured into a cold ethanol–ether mixture (1:3, 600 ml) and chilled overnight. The solid was collected, dissolved in a small volume of water, and applied to a Dowex 1 X2 column (formate form, 100–200 mesh, 75 ml). The product was eluted with water and the appropriate fractions were evaporated to give colorless syrup (2.0 g). The product was deacetylated with methanolic ammonia (50 ml, presaturated at 0°) at room temperature for 15 hr. The solid was collected, dissolved in water (10 ml), and passed through a Dowex 50 X8 (H^+) column (25 ml). The eluate was concentrated to about 5 ml and ethanol was added. The product that separated after cooling was collected and crystallized from aqueous ethanol to yield 0.30 g: mp >125° dec; uv λ max (pH 1) 253 nm (ε 7000); λ max (pH 7) 253 nm (ε 7200); λ max (pH 11) 261 nm (ε 7200).

Anal. Calcd for $C_{11}H_{15}N_4O_8P$ (362.23): C, 36.48; H, 4.17; N, 15.47. Found: C, 36.28; H, 4.36; N, 15.32.

9-β-D-Arabinofuranosyl-1-methylhypoxanthine 5'-*O*-Methylphosphate (11). A solution of **6** (1.0 g, 0.0028 mol) in dry DMSO (10 ml) was treated with DBU (0.5 g) followed by methyl iodide (2.0 ml). The reaction was allowed to proceed for 15 hr at room temperature and treated in the same way as described in **9** to yield 0.40 g (37%): mp 162–165° dec; uv λ max (pH 1) 253 nm (ε 10,050); λ max (pH 7) 253 nm (ε 9500); λ max (pH 11) 263 nm (ε 7450).

Anal. Calcd for $C_{12}H_{17}N_4O_8P$ (376.26): C, 38.31; H, 4.55; N, 14.89. Found: C, 38.14; H, 4.68; N, 14.65.

9-β-D-Arabinofuranosylhypoxanthine Cyclic 3',5'-Phosphate (13). To an ice-cooled suspension of 9-β-D-arabinofuranosyladenine cyclic 3',5'-phosphate[28] (**12**, 2.0 g, 0.0060 mol) in water (15 ml) containing glacial acetic acid (3.0 ml) was added sodium nitrite (2.5 g, 0.036 mol). The flask was loosely stoppered and stirred for 3 hr at ice bath temperature. The reaction was allowed to proceed 20 hr. After evaporation and neutralization with $KHCO_3$, it was treated in the same way as described in **6** to yield 1.80 g (89.7%): mp 240° dec; $[\alpha]^{25}D$ −49.5° (*c* 1.0, H_2O); ^1H NMR (D_2O–NaOD) δ 6.50 (d, *J* = 6 Hz, $C_{1'}H$), 8.21 (s, 1), 8.24 (s, 1); uv λ max (pH 1) 247 nm (ε 12,100); λ max (pH 7) 247 nm (ε 12,400); λ max (pH 11) 251 nm (ε 13,000).

Anal. Calcd for $C_{10}H_{11}N_4O_7P$ (330.19): C, 36.40; H, 3.35; N, 16.97. Found: C, 36.35; H, 3.39; N, 16.87.

9-(2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranosyl)-6-benzamidopurine (14). To 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranose (34.5 g, 0.068 mol) in dry dichloromethane (250 ml) at −20° was added a solution of anhydrous dichloromethane (originally 250 ml) which had been saturated at −20° with dry hydrogen bromide gas. The mixture was protected from moisture with a drying tube and allowed to warm to 5°. The solvent was evaporated and the resulting syrup was coevaporated twice with dry toluene (100 ml). The residual white foam was dissolved in "nanograde" acetonitrile[48] (200 ml) and was added to the suspension of 6-benzamidopurine (32.5 g, 0.136 mol) in acetonitrile (200 ml). The stoppered reaction flask was stirred at room temperature for 44 hr and then filtered to remove the hydrobromide salt of 6-benzamidopurine (21.5 g). The filtrate was evaporated to dryness, the residual foam dissolved in chloroform (750 ml), and the chloroform phase was washed with cold aqueous saturated $NaHCO_3$ solution (2 × 150 ml), followed by water (2 × 150 ml), and dried over anhydrous $MgSO_4$. The chloroform was evaporated to dryness and the residual foam was chromatographed on a silica gel column (5 × 100 cm) using $CHCl_3$–H_3CCOCH_3 (8:2) as the eluting solvent. The appropriate fraction was evaporated to dryness to yield a homogeneous white foam: 24.0 g (51.3%); $[\alpha]^{25}D$ +26.8° (*c* 1.0, $CHCl_3$); uv λ max (pH 1) 285 nm (ε 32,800); λ max (pH 7) 287 nm (ε 30,100); λ max (pH 11) 285 nm (ε 28,400).

Anal. Calcd for $C_{38}H_{29}N_5O_8$ (683.65): C, 66.76; H, 4.28; N, 10.25. Found: C, 66.56; H, 4.20; N, 9.97.

9-α-D-Arabinofuranosyladenine (15). Compound **14** (20.0 g, 0.029 mol) was dissolved in 0.1 *M* sodium methoxide (in methanol, 250 ml) and the solution was stirred at room temperature for 24 hr and then allowed to stand at 0° overnight. The solid that separated was collected, washed with cold methanol, and dried. The combined filtrate and washings were evaporated to dryness; the residue was dissolved in water (50 ml) and neutralized with glacial acetic acid. The neutral solution was extracted with chloroform (4 × 75 ml), and the aqueous phase was concentrated to about 20 ml, chilled overnight. The solid was collected and the combined solids were crystallized from water to yield 7.5 g (95.9%): mp 208–210° (lit.[43,44] mp 208°); $[\alpha]^{25}D$ +69.0° (*c* 1.0, H_2O); ^1H NMR (DMSO-d_6) δ 5.95 (d, *J* = 5.5 Hz, $C_{1'}H$), 7.33 (s, 2), 8.26 (s, 1), 8.40 (s, 1); uv λ max (pH 1) 257 nm (ε 14,500); λ max (pH 7) 259 nm (ε 15,600); λ max (pH 11) 259 nm (ε 15,600).

Anal. Calcd for $C_{10}H_{13}N_5O_4$ (257.24): C, 44.94; H, 4.90; N, 26.21. Found: C, 44.90; H, 4.79; N, 26.03.

9-α-D-Arabinofuranosyladenine 5'-Monophosphate (16). 9-α-D-Arabinofuranosyladenine (**15**, 1.5 g, 0.0056 mol) was phosphorylated with phosphorus oxychloride (1.8 g) using trimethyl phosphate (20 ml) as the solvent. It was treated as described in **3** to yield 1.35 g (69.2%): mp >200° dec; $[\alpha]^{25}D$ +39.1° (*c* 1.0, H_2O); ^1H NMR (DMSO-d_6–D_2O) δ 5.97 (d, *J* = 5.5 Hz, $C_{1'}H$), 8.30 (s, 1), 8.46 (s, 1); uv λ max (pH 1) 256 nm (ε 9800); λ max (pH 7) 257 nm (ε 10,400); λ max (pH 11) 257 nm (ε 10,600).

Anal. Calcd for $C_{10}H_{14}N_5O_7P$ (347.22): C, 34.58; H, 4.06; N, 20.17. Found: C, 34.46; H, 4.21; N, 20.27.

9-α-D-Arabinofuranosylhypoxanthine 5'-Monophosphate (17). Compound **16** (0.4 g, 0.00115 mol) was deaminated according to the procedure as described in **6** (method 1) to yield 250 mg (62.3%): $[\alpha]^{25}D$ +21.4° (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 6.34 (d, *J* = 5.0 Hz, $C_{1'}H$), 8.43 (s, 1), 9.26 (s, 1); uv λ max (pH 1) 248 nm (ε 8400); λ max (pH 7) 249 nm (ε 8400); λ max (pH 11) 253 nm (ε 8800).

Anal. Calcd for $C_{10}H_{13}N_4O_8P$ (348.20): C, 34.48; H, 3.74; N, 16.10. Found: C, 34.23; H, 3.96; N, 15.89.

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(47) Acid-washed AU-4 charcoal, purchased from Barneby-Cheney, Columbus, Ohio.
(48) Distilled in glass, purchased from Mallinckrodt Chemicals, St. Louis, Mo.

4-Hydroxy-3-nitro-2-quinolones and Related Compounds as Inhibitors of Allergic Reactions

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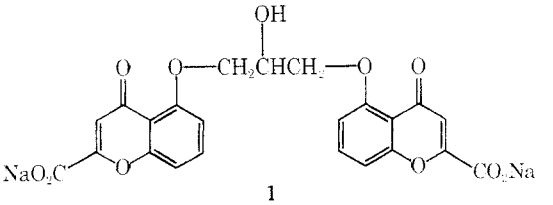
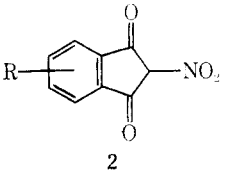
The synthesis and biological activity of a number of 4-hydroxy-3-nitro-2-quinolones are discussed and compared with their related hydroaromatic analogs. Antiallergic activity has been assessed by their ability to inhibit the homocytotropic antibody–antigen induced passive cutaneous anaphylaxis reaction in the rat.

In 1967 disodium cromoglycate (**1**) was introduced as a treatment for asthma and it was shown that it could inhibit the release of spasmogens induced by antigen challenge of tissue sensitized with immunoglobulin E.[1] Since the introduction of compound **1**, a variety of compounds have been claimed to possess a similar activity. Many of these possess an acidic function attached to a carbon atom linked by ethylenic conjugation to a carbonyl group. This acidic function is often a carboxyl group although this can be replaced by the tetrazolyl group.[2] We have described compounds which show a similar type of activity to disodium cromoglycate and yet are of a somewhat different type in that they possess the α-nitro-β-dicarbonyl moiety, a system which again confers acidity.[3]



1



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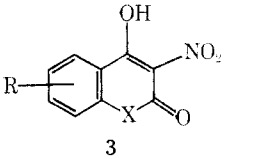

3

Following the observation that activity was shown by 2-nitro-1,3-indandiones (**2**)[3,4] and to a lesser extent in their reduced derivatives,[5] we have embarked on a program of ring-expanded systems of type **3**. As part of this program we have reported on the activity of 4-hydroxy-3-nitrocoumarins[6] (**3**, X = O) and currently wish to report our work on the analogous nitrogen compounds (**3**, X = NR).

Compounds have been compared with disodium cromoglycate for antiallergic activity by assessing their relative