Synthesis of 9-\(\beta-d\)-Arabinofuranosyl)adenine 5'-Phosphate starting from Adenosine 5'-Phosphate

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9-(\(\beta-d\)-Arabinofuranosyl)adenine 5'-phosphate was obtained from adenosine 5'-phosphate via the novel intermediate 8,2'-O-cycloadenosine 5'-phosphate. In contrast to the synthesis of 9-(\(\beta-d\)-arabinofuranosyl)adenine, it was difficult to cleave this compound by hydrogen sulfide directly to 8,2'-O-cycloadenosine 5'-phosphate because of a considerable degree of dephosphorylation. However N-acylated 8,2'-O-cycloadenosine 5'-phosphate was readily cleaved at the cyclo-bond by hydrogen sulfide. Desulfurization of 8-mercapto-9-(\(\beta-d\)-arabinofuranosyl)adenine 5'-phosphate gave the desired pure crystalline product.

**Keywords** — 9-(\(\beta-d\)-arabinofuranosyl)adenine 5'-monophosphate; 8,2'-O-cycloadenosine 5'-phosphate; 2'-O-p-toluene sulfonyl adenosine 5'-phosphate; 8-bromo-2'-O-p-toluene sulfonyl adenosine 5'-phosphate; 8-hydroxy-N',3'-O-diacyetyl-2'-O-p-toluene sulfonyl adenosine 5'-phosphate; 8-mercapto-9-(\(\beta-d\)-arabinofuranosyl) adenine 5'-phosphate; Ara-AMP

9-(\(\beta-d\)-Arabinofuranosyl)adenine has been studied as an antiviral drug against deoxyribonucleic acid viruses for the past ten years. During the course of these studies it has become clear that 9-(\(\beta-d\)-arabinofuranosyl)adenine (Ara-A) is highly insoluble in water and organic solvents. This insolubility leads to studies directed towards more soluble derivative of Ara-A. 9-(\(\beta-d\)-Arabinofuranosyl)adenine (Ara-AMP) has shown high solubility in water and effectiveness against various deoxyribo nucleic acid viruses.

Synthesis of Ara-AMP by phosphorylation of Ara-A with cyanomethylphosphate or more recently, with phosphorus oxychloride has already been reported. In previous publications we reported the synthesis of Ara-A from adenosine or adenosine 5'-phosphate via the novel intermediate 8,2'-O-cycloadenosine. Now, we wish to report here a synthetic

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method applicable to the bulk preparation of Ara-AMP via 8,2'-O-cycloadenosine 5'-phosphate as an intermediate (Chart 1).

The first step is selective $p$-toluenesulfonylation of adenosine 5'-phosphate which is performed by reacting with $p$-toluenesulfonyl chloride in a mixture of dioxane and water containing 3.5 equivalent of sodium hydroxide, a slight modification of the method reported by Ikehara and Uesugi.\(^7\) The reaction solution was worked up as usual and 2'-O-$p$-toluenesulfonyladenosine 5'-phosphate(II) was obtained in a yield of 70%. Although the product of this reaction contained a small amount of 2',3'-di-$O$-$p$-toluenesulfonyladenosine 5'-phosphate, the bromination reaction was carried out without further purification. Crude 8-bromo-2'-O-$p$-toluenesulfonyladenosine 5'-phosphate (III) was obtained quantitatively by purification with a charcoal column after reaction with bromine at pH 4.0. Crystallization of the crude compound (III) with a small amount of water gave pure crystalline compound (III) as the diammmonium trihydrate which decomposed at 181–186°. Nucleophilic substitution of the bromine at the 8-position of III by acetoxy ions in acetic acid and acetic

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anhydride gave 8-hydroxy-N\textsuperscript{6}, 3’-O-diacyl-2’-O-\(\beta\)-toluenesulfonyladenosine 5’-phosphate (IV) after purification with a charcoal column in 85.5\% yield based on its total optical density. The crude compound (IV) can be crystallized from a restricted amount of ethanol and water giving the diammonium trihydrate of IV which decomposed at 159—163°. The cyclization reaction proceeded smoothly upon heating the crude compound (IV) with methanolic ammonia in a steel tube at 65—70° for 20 hr. The crude crystalline 8,2’-O-cycloadenosine 5’-phosphate ammonium salt (V) was obtained on cooling the steel tube at \(-10—-20°\) in ca. 82% yield. The crude ammonium salt of V can be purified by ion exchange column chromatography on Dowex 1\( \times 2 \) (HCOO\textsuperscript{−}) to give pure crystalline 8,2’-O-cycloadenosine 5’-phosphate as a hemihydrate of the free acid which decomposed at 220—225°. The physical properties of compounds (III), (IV) and (V) were identical to those reported.\textsuperscript{8}

In the synthesis of Ara-A, cleavage reaction of the O-cyclo bond of 8,2’-O-cycloadenosine with hydrogen sulfide gave 8-mercapto-9-(\(\beta\)-d-arabinofuranosyl)adenine\textsuperscript{5,6} directly. Under the same conditions, cycloadenosine 5’-phosphate (V) gave only dephosphorylated products. In order to avoid such an undesired reaction, the compound (V) was acetylated to give VIa and VIb. Acetylation of the crude crystalline ammonium salt (V) proceeded readily, but the purified crystalline compound (V) was not acetylated satisfactorily\textsuperscript{8} due to the insolubility of the partially acetylated (V) to organic solvent. On the other hand, the benzoylated product (VIb) was easily obtained from purified crystalline compound (V) using benzoyl chloride.

The cleavage reaction of the acetylated cyclonucleotide (VIa) by hydrogen sulfide in pyridine was readily accomplished by heating the reaction solution at 95—100° for 15 hr to give an 8-mercapto compound (VIIa). After the usual work up and successive deprotection with methanolic ammonia at room temperature for 3 days, the aqueous solution of the resulting crude VIII was refluxed with Raney Ni for desulfurization. The reaction solu-

<table>
<thead>
<tr>
<th>Peak</th>
<th>Fraction No.</th>
<th>A\textsuperscript{a} (derived from VIa)</th>
<th>B\textsuperscript{b} (derived from VIb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>TO(\text{D}^0) (at 260 nm)</td>
<td>UV (\lambda_{\text{max}}) (nm)</td>
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<tr>
<td>I</td>
<td>15—64</td>
<td>65400</td>
<td>H(^+) 257</td>
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<td></td>
<td></td>
<td></td>
<td>OH(^−) 259</td>
</tr>
<tr>
<td>II</td>
<td>139—173</td>
<td>1030</td>
<td>H(^+) 263, 282</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>OH(^−) 280</td>
</tr>
<tr>
<td>III</td>
<td>184—224</td>
<td>3380</td>
<td>H(^+) 273</td>
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<td></td>
<td></td>
<td></td>
<td>OH(^−) 275</td>
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<tr>
<td>IV</td>
<td>246—346</td>
<td>16600</td>
<td>H(^+) 257</td>
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<td>OH(^−) 259</td>
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<tr>
<td>V</td>
<td>396—</td>
<td>7200</td>
<td>H(^+) 263, 282</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>OH(^−) 279</td>
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</table>

\(\text{a})\) Column size: 2.5×2.0 cm; collected each 20 g; elution was performed as follows: 1—20, H\(_2\)O, 75—226: 0.025 HCOOH, 227—395: 0.050 HCOOH, 396—: 0.25 HCOOH.

\(\text{b})\) Column size: 1.3×11 cm; collected each 20 g; elution was performed by linear gradient of H\(_2\)O (1000 ml)—0.5% HCOOH (1000 ml).

\(\text{c})\) TO\(\text{D}^0\)=total optical density.


\text{9}) Acetylation was attempted with the free acid, pyridinium salt and tri-\(\alpha\)-butylammonium salt in pyridine or dimethylformamide-pyridine. In all cases, however, insoluble crystalline product appeared quantitatively after addition of acetylating agent and remained unchanged during further stirring for several days. This insoluble material is considered to be a \(\text{P,F,3’-O-diacyl monopyridinium salt of compound (V)}\) on the bases of its nuclear magnetic resonance (NMR) spectrum.
tion was applied to a Dowex 1×2 (HCOO⁻) column and eluted with dilute formic acid. The elution pattern is shown in Table I (column A). The chromatogram showed five peaks. Fine needles of Ara-A⁵) were obtained from peak I fractions. The ultraviolet (UV) spectrum of peak II fractions shows extrema at 263 nm and 282 nm in acidic medium and at 280 nm in alkaline medium. The structure of the compound in peak II was assigned as 8-hydroxy-9-(β-D-arabinofuranosyl)adenine from its UV spectral and chromatographic behavior.¹⁰) The UV extrema of the peak III were at 273 nm in acidic and 275 nm in alkaline medium. The structure of this compound remains unknown. The fractions of peak IV were collected and lyophilized. The resultant white residue was crystallized from water giving colorless fine needles, which darkened at 208° (205°(l)) and decomposed at 213°(211—213°C). The UV absorption spectrum of this compound showed a maxima at 257 nm in 0.1 n hydrochloric acid, at 258.5 nm in water and at 259 nm in 0.1 n sodium hydroxide, respectively. The NMR spectrum of the compound showed signals at δ 8.53 (1H, s, C-9-H), 8.08 (1H, s, C-2-H) and 6.36 (1H, d, J=2.4, 4.8 cps, C-1'-H).

From these data and the elemental analysis, the structure of the crystals was determined as 9-(β-D-arabinofuranosyl)adenine 5'-phosphate monohydrate (IX). From the UV spectrum and chromatographic behavior of the peak V, it may be considered that these fractions contained an 8-hydroxy adenine derivative having a phosphoric acid residue in its carbohydrate moiety. These 8-hydroxyadenine derivatives contained in peaks III and V may occur by reaction of compound(VIa) with contaminating water as shown in Chart 2. This specula-

![Chart 2](image)

Chart 2

is supported by the fact that addition of small amounts of water during the cleavage reaction of VI with hydrogen sulfide caused formation of 8-hydroxy-9-(β-D-arabinofuranosyl)-adenine and its 5'-phosphate after deprotection with methanolic ammonia. Surprisingly, no 8-mercaptop derivatives could be detected in the reaction mixture.

Benzyolated cyclonucleotide (VIIb) was also converted to 8-mercaptop-N⁶,3'-O-dibenzoyl-9-(β-D-arabinofuranosyl)adenine 5'-phosphate (VIIb) which showed an UV extremum at

326 nm in neutral medium. Its denbeyzolation with manethalic ammonia at room temperature for 3 days gave crude 8-mercapto-9-(β-D-arabinofuranosyl)-adenine 5'-phosphate (VIII). The UV spectrum showed extrema at 307 nm in 0.1n hydrochloric acid, 296, and 303 nm in water and 293 nm in 0.1n sodium hydroxide. Desulphurization of VIII with Raney Ni and successive purification by ion exchange column chromatography (Dowex 1 × 2 (HCOO⁻)) gave the elution profile shown in Table I (column B). The pattern is almost the same as that described above. The total yield of 9-(β-D-arabinofuranosyl)adenine 5'-phosphate from adenosine 5'-phosphate was about 20% in both cases.

Further studies are in progress on the direct application of the hydrogen sulfide cleavage reaction to compound (V) without acylation.

**Experimental**

Melting points were determined using a Yanagimoto melting point apparatus and were uncorrected. NMR spectra were obtained in dimethyl sulfoxide-d₆ (DMSO-d₆) on a Varian T-60 spectrometer using tetramethylsilane (TMS) as an external standard. UV spectra were obtained using an Hitachi Model 200-20 Spectrophotometer. Paper chromatography was performed by the descending technique on Toyo filter paper No. 51A. Solvent A was ethanol: 1n ammonium acetate=1:1; Solvent B was n-butanol: acetic acid: water=5: 2: 3.

2'-O-p-Toluenesulfonyladenosine 5'-Phosphate (II)—To a solution of 150 ml of dioxane and 350 ml of 1n NaOH containing 34.7 g (100 mmol) of adenosine 5'-phosphate (5'-AMP) was added 22.8 g (120 mmol) of finely crushed p-toluenesulfonyl chloride and the solution was stirred.

After 15 hr at 0°, 35 ml of 6n HCl was added and the pH of the solution was adjusted to 4.0. The resulting fine crystals were collected by filtration and washed with acetone to give 46.4 g, of crystalline powder. The yield was 70% based on the total optical density. UV \( \lambda_{\text{max}}^{\text{diox}} \) (e): 258 (15000), \( \lambda_{\text{max}}^{\text{NaOH}} \) (e): 261 (15500), \( \lambda_{\text{max}}^{\text{HCl}} \) (e): 261 (15800). Anal. Calcd. for C₁₉H₂₅O₄N₅S₂PNa·1/10(C₄H₉O₂N₅S₂PNa)·3H₂O: C, 36.04; H, 4.48; N, 11.92; S, 5.95; P, 5.27. Found: C, 35.93; H, 4.45; N, 11.21; S, 6.33; P, 5.38.

8-Bromo-2'-O-p-toluenesulfonyladenosine 5'-Phosphate (III)—To a solution of 2m sodium acetate buffer pH 4.0 (120 ml) containing 8.0 ml of bromine was added 21 g of crystalline II described above at 0°-5° and the solution was stirred for about 18 hr at the same temperature. To the solution was added 12.5 g of NaHSO₃ at 0°-5° and after 15 min stirring, the pH of the solution was adjusted to 4.0 with 5n NaOH (90-100 ml). Water was added to the solution until the total volume of the solution became 1000 ml and the resulting insoluble material was filtered off. The filtrate was applied to a column packed with 100 g of charcoal and the column was washed with 5 l of H₂O. The desired compound (III) was eluted with 50% aqueous EtOH containing 5% of conc. NH₄OH. The eluent was concentrated to dryness under reduced pressure providing 20.2 g of semi-crystalline solid. The yield of the compound was 95.5% based on the total optical density. Although this solid could be used for the next reaction, crystalline compound (III) could be obtained by recrystallization of the solid from a minimum quantity of water. mp 181—186° (dec.). UV \( \lambda_{\text{max}}^{\text{diox}} \) (e): 264 (14900), \( \lambda_{\text{max}}^{\text{NaOH}} \) (e): 265 (13300), \( \lambda_{\text{max}}^{\text{HCl}} \) (e): 265 (13500). Anal. Calcd. for C₁₉H₁₉BrN₅S₂P·3H₂O: C, 31.34; H, 4.33; N, 12.90; P, 4.76; S, 4.92. Found: C, 31.27; H, 4.00; N, 12.34; P, 4.45; S, 5.36.

8-Hydroxy-N³-O-diacetyl-2'-O-p-toluenesulfonyladenosine 5'-Phosphate (IV)—To a solution of 28 ml of acetic acid and 28 ml of acetic anhydride were added 20.2 g of semi-crystalline solid (III) and 6.87 g of NaOAc and the suspension was heated at refluxing temperature for 2 hr. After cooling to room temperature, 30 ml of MeOH was added to the solution and the solvent was evaporated to dryness. After addition and evaporation of EtOH being repeated twice, the resultant residue was dissolved into 1000 ml of water. The solution was applied to a column packed with 100 g of charcoal and the column was washed with 10 l of water and eluted with 50% aqueous EtOH containing 5% of conc. NH₄OH. Eluents were concentrated to dryness under reduced pressure giving a crystalline mass which provided crystalline compound (IV) by recrystallization from a restricted amount of EtOH-H₂O. The yield of the compound (IV) was 85.5% based on the total optical density, mp 159—163° (dec.). UV \( \lambda_{\text{max}}^{\text{diox}} \) (e): 287.5 (12100), \( \lambda_{\text{max}}^{\text{NaOH}} \) (e): 288.5 (11200), \( \lambda_{\text{max}}^{\text{HCl}} \) (e): 304 (12200). Anal. Calcd. for C₂₇H₂₄O₁₄N₅S·3H₂O: C, 37.56; H, 4.80; N, 12.51; S, 4.77; P, 4.61. Found: C, 37.94; H, 4.86; N, 12.45; S, 5.14; P, 4.44.

8,2'-O-Cycloadenosine 5'-Phosphate (V)—The crystalline compound (IV) (18.2 g) was suspended to 100 ml of MeOH in a steel tube and the suspension was saturated with dry NH₃ gas at 0°-5°. The sealed tube was heated at 65—70° for 20 hr with stirring. After cooling to room temperature, the tube was allowed to stand in MeOH—dry ice bath for 2 hr. The resulting crystals were collected by filtration and washed with MeOH providing 10 g of crude compound (V) which could be used in the subsequent acetylation reaction. Purification of the crude compound (V) was accomplished as follow. The crude V (10 g) was dissolved in 250 ml of water and applied to a column packed with 130 ml of Dowex 1 × 2 (HCOO⁻). The column was
washed with 1.51 of water and 3.51 of 0.02N HCOOH. The desired compound (V) was eluted with 0.05N HCOOH. The eluent was collected and lyophilized to give 4.1 g of amorphous solid from which 3.83 g of purified crystalline V was obtained by crystallization from 15 ml of water, mp 220—225° (dec.). UV max, nm (ε) (e) 259 (12300), 235 (13900), 230 (max) nm (ε) 256 (13200), 240 (max) nm (ε) 256 (13200). Anal. Calc. for C_{12}H_{12}O_{5}N_{3}P_{1} 1/2H_{2}O: C, 33.90; H, 4.26; N, 19.76; P, 8.74. Found: C, 33.69; H, 3.83; N, 19.24; P, 8.64.

N^6,3'-O-Diacetyl-8,2'-O-cycloadenosine 5'-Phosphate (VIa)—The crude crystalline compound (V) (10 g) was suspended in 120 ml of pyridine and 60 ml of acetic anhydride was added to the suspension. After stirring for 47 hr at room temperature, 160 ml of pyridine—H₂O (1:1) was added at 0—5° and the mixture was stirred for 5.5 hr at room temperature. After addition and evaporation of EtOH was repeated several times, residual product was dissolved in 145 ml of pyridine and the solution was added dropwise to a mixture of 1000 ml of ether and 500 ml of petroleum ether with vigorous stirring. The resultant powdery product was collected by filtration giving 11.8 g of crude acetylated product (VIa).

N^6,3'-O-Dibenzoyl-8,2'-O-cycloadenosine 5'-Phosphate (VIb)—To a solution of lyophilized 8,2'-O-cycloadenosine 5'-phosphate pyridinium salt (500 mg, 1.18 mmol) in 35 ml of anhydrous pyridine, 3.32 g of benzoyle chloride was added dropwise at 0—5° with stirring. The reaction solution was stirred for 3 hr at room temperature, then 10 ml of water was added. The solution was concentrated to about half of its original volume and 20 ml of CHCl₃ was added. The solution was washed with three 20 ml portions of H₂O and dried over MgSO₄. The solvent was evaporated to dryness under reduced pressure. The residue gummy product was dissolved in 10 ml of anhydrous pyridine and 10 ml of Ac₂O was added. After 24 hr stirring at room temperature, 10 ml of 50% aqueous pyridine was added to the solution. The mixture was stirred for 15 hr at room temperature. The solvent was evaporated to dryness under diminished pressure. The residue was dissolved in 12 ml of pyridine and the solution was added dropwise to 300 ml of a mixture (2:1) of ether and petroleum ether. N^6,3'-O-Dibenzoylated 8,2'-O-cycloadenosine 5'-phosphate (VIb) was obtained as a white powdery pyridinium salt (852 mg). Paper chromatographic Rf value: 0.77 (solvent A), 0.84 (solvent B). UV λ max nm: 280.

8-Mercapto-N^6,3'-O-dibenzoyl-9-(β-d-arabinofuranosyl)adenine 5'-Phosphate (VIIa)—The pyridinium salt of dibenzoylated 8,2'-O-cycloadenosine 5'-phosphate (VIb) (852 mg, 1.18 mmol) was dissolved in 25 ml of anhydrous pyridine and the solution was poured into a steel tube. The reaction vessel was cooled to about —10° and N₂ gas was passed for 2 min, then dry H₂S gas for 20 min. The tube was sealed and heated for 16 hr at 100°. The reaction vessel was cooled to about —10° and N₂ gas was bubbled to remove excess H₂S completely. Pyridine was removed by distillation in vacuo. The residue was dissolved in 12 ml of pyridine and added dropwise to 300 ml of a mixture of ether and petroleum ether (2:1) with stirring. The desired crude product (VIIa) was obtained as the white powdery pyridinium salt (733 mg). Paper chromatographic Rf value: 0.68 (solvent A), 0.85 (solvent B). UV λ max nm: 326.

8-Mercapto-9-(β-d-arabinofuranosyl)adenine 5'-Phosphate (VIII)—The powdery pyridinium salt (VIIIb) (733 mg, 1.1 mmol) was suspended in 50 ml of methanol and the solution was saturated with gaseous ammonia at —10°. The flask was stoppered and the solution was stirred for 3 days at room temperature. The solvent was removed under reduced pressure. The resulting product was dissolved in about 30 ml of water and insoluble material was removed by filtration. The filtrate was extracted with two portions of ether (30 ml) to remove methylbenzoate and the water layer was lyophilized to give crude compound (VIII) (600 mg). Paper chromatographic Rf value: 0.49 (solvent A). UV λ max, nm (ε) (e) 307, 296 (max) nm: 296, 303, 230 (max) nm: 293.

9-(β-D-Arabinofuranosyl)adenine 5'-Phosphate (IX)—From VIIa: The crude powder of VIIa (11.7 g) was dissolved in 117 ml of pyridine. The solution was poured into a steel tube and saturated with dry gaseous H₂S. The sealed tube was heated at 95—100° for 15 hr. The excess H₂S was removed by passing N₂ gas and the resulting yellow solution was concentrated to dryness under reduced pressure. The residual crude compound (VIIa) was dissolved in 200 ml of MeOH and the insoluble material was removed by filtration. The filtrate was saturated with dry NH₃ at 0—5° and allowed to stand for 3 days at room temperature with stirring. The solvent was removed by evaporation under reduced pressure and the residual crude compound (VIII) was dissolved in 500 ml of water. Insoluble material was filtered off. To the filtrate was added 10 ml of Raney Ni (Kawaken Fin Chemical NDT-65) and the solution was refluxed for 2.5 hr. Then a further 3 ml of Raney Ni was added and the solution was refluxed for 0.5 hr. An additional 2 ml of Raney Ni was added to the solution and the reaction mixture was refluxed for another 0.5 hr. The insoluble material was filtered off and the filtrate was applied to an ion exchange column of Dowex 1 x 2 (H⁺—100 ml) (Table I). The column was washed with 21 ml of water and 31 of 0.02N HCOOH. The desired 9-(β-D-arabinofuranosyl)adenine 5'-phosphate (IX) was eluted with 31 of 0.05N HCOOH. The eluent was collected and lyophilized to give 5.0 g of white amorphous solid. The solid was crystallized from 50 ml of H₂O giving 3.5 g of the desired crystalline compound whose physical properties were completely agreement with those of compound (IX) obtained from VIIb.

b) From VIb: The lyophilized 8-mercaptop-Ara-AMP (VIII) (600 mg, 1.1 mmol) was dissolved in 50 ml of water and 1.5 ml of Raney Nickel (Kawaken Fin Chemical NDT-65) was added. The reaction solution was refluxed until the UV absorption at 300 nm disappeared. After completion of the reaction, insoluble material was removed by filtration and the filtrate was applied to a Dowex 1 x 2 column (1.3 x 11 cm, formate form, 100—200 mesh). The column was washed with 300 ml of water. Elution was carried...
out with a linear gradient of water (1 l) to 0.2 M formic acid (1 l) (Table I). Fractions of 20 ml each were collected. Fractions 39—53 were pooled and lyophilized. To the residue was added 7 ml of water and the solution was allowed to stand overnight in a refrigerator. Ara-AMP (monohydrate) was obtained as a fine needles (190 mg). It darkened at 208° (205°340), and decomposed at 213° (211—213°440). Paper chromatographic $R_f$ value: 0.51 (solvent A), 0.28 (solvent B). UV $\lambda_{max}$ R 360 nm (e): 257 (15400), $\lambda_{max}$ R 260 nm (e): 258.5 (15200), $\lambda_{max}$ R 250 nm (e): 259 (15800). NMR $\delta$: in D$_2$O (dissolved by addition of two equivalent of sodium bicarbonate): 8.53 (1H, s, C$_2$–H), 8.88 (1H, s, C$_2$–H), 6.36 (1H, d, $f_{1'--5' 4.8}$ cps, C$_2$–H). Anal. Calcd. for C$_{10}$H$_{14}$N$_2$O$_2$P·H$_2$O: C, 32.88; H, 4.41; N, 19.17; P, 8.46. Found: C, 33.11; H, 4.41; N, 19.20; P, 8.32.