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181. Morio Ikehara and Eiko Ohtsuka : Studies on Coenzyme Analogs. XVI.*1 Synthesis of 9-D-Erythrityladenine and its Phosphates.

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In the course of the studies on the interaction of synthetic ATP*3-analogs with myosin systems,[1,2] it was found that analogs (I) having aliphatic chain instead of ribofuranose ring was ineffective for contraction of myofibril, whereas they were considerably hydrolyzed by myosin adenosine triphosphatase. In order to obtain the information about the influence of hydroxyl groups, which lacked in compound (I), the synthesis of 9-D-erythrityladenine 4'-triphosphate (II) was undertaken.

The key intermediate 9-D-erythrityladenine was obtained by a method, which was developed in our laboratory for the synthesis of 9-alkanoladenines.[1,3] 2,4-O-Ethylidene-D-erythritol[4] (III) was selectively tosylated on the primary hydroxyl group with 1 mole of tosyl chloride in pyridine to afford 1-O-tosyl-2,4-O-ethylidene-D-erythritol (IV), m.p. 72~73°. Compound (IV) was caused to react with ammonia and the resulting tosylate salt was made free by the passing through a column of Dowex 1 ion-exchanger. The over-all yield from III was 50%. 2,4-O-Ethylidene-D-erythritylamine (V) was then condensed with 4,6-dichloro-5-aminopyrimidine[5] (VI) in refluxing butanol in the presence of triethylamine as acid acceptor. 4-(2',4'-O-Ethylidene-D-erythritylamino)-5-amino-6-chloropyrimidine (VII) was obtained in a yield of 51%. The structure of VII was confirmed by ultraviolet absorption (λ_{max} 262, 270 mμ), compared with that of 4-alkanolamino-5-amino-6-chloropyrimidine, and by elementary analysis. Cyclization of compound (VII) was carried out by the heating with acetic anhydride and ethyl orthoformate. Reaction extent was estimated by the shift of ultraviolet absorption maxima to 264 mμ. 6-Chlorine of resulting purine was ammonolyzed without isolation in order to avoid hydrolysis during recrystallization. 9-(2',4'-O-Ethylidene-D-erythrityl)adenine (VIII) was obtained as a crystalline substance, m.p. 245~246°. Ethylidene group of VIII was removed by reflux in hydrochloric acid in ethanol at pH 1. On the paper chromatogram, a spot of Rf 0.58 (solvent B, see Experimental) diminished within 1 hour and another spot appeared

*1 Part XV. M. Ikehara, E. Ohtsuka : This Bulletin, **11**, 961(1963).

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*3 Abbreviations : ATP, adenosine 5'-triphosphate; tosyl, *p*-toluenesulfonyl; DMF, N,N-dimethylformamide; AMP, adenosine 5'-monophosphate; trityl, triphenylmethyl.

1) M. Ikehara, E. Ohtsuka, S. Kitagawa, K. Yagi, Y. Tonomura : J. Am. Chem. Soc., **83**, 2679 (1961).
2) N. Azuma, M. Ikehara, E. Ohtsuka, Y. Tonomura : Biochim. Biophys. Acta, **60**, 104 (1962).
3) M. Ikehara, E. Ohtsuka : This Bulletin, **9**, 27 (1961).
4) R. Baker, D. L. MacDonald : J. Am. Chem. Soc., **82**, 2301 (1960).
5) D. J. Brown : J. Appl. Chem., **4**, 72 (1952).

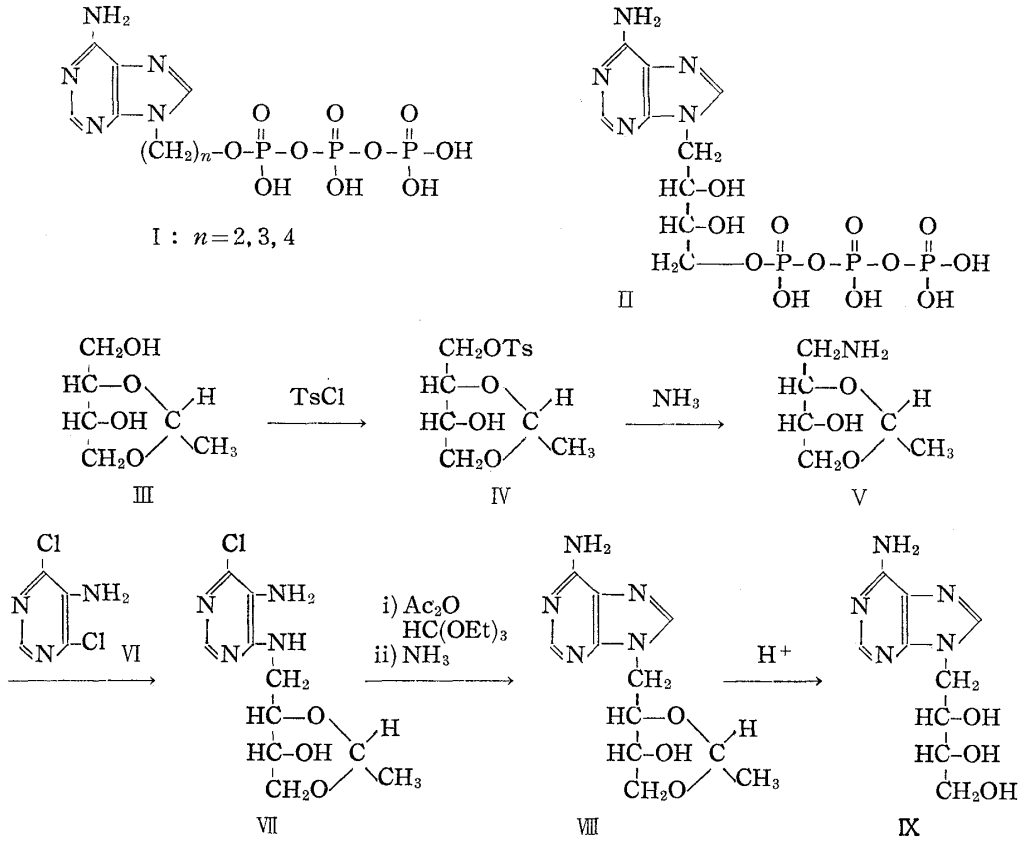

Chart 1.

at Rf 0.11. 9-D-Erythrityladenine (IX) was obtained as crystal, m.p. 217~218°, in an over-all yield of 59% from pyrimidine (III). The structure of IX was confirmed from its ultraviolet absorption spectra and elementary analyses. From $[\alpha]_D$ value (−32.4°), it was deduced that in sugar moiety no big configurational change had occurred. When compound (IX) was treated with nitrous acid, λ_{max} shifted to 250 mμ, which indicated the

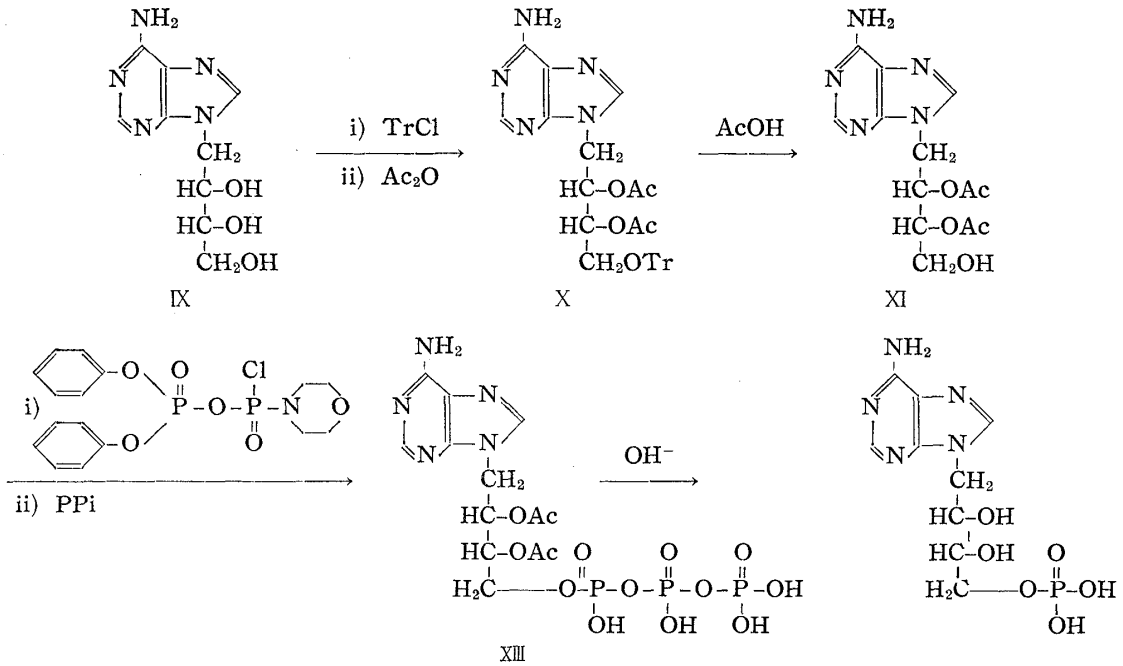

Chart 2.

existence of adenine not substituted in 6-position. By the treatment of IX with 6*N* hydrochloric acid at 100°, no remarkable hydrolysis was observed.[6]

In order to obtain a suitable intermediate for the phosphorylation with P-diphenyl P′-morpholino pyrophosphorochloridate,[7] the synthesis of 2′,3′-di-O-acetyl derivative of IX was attempted. Reaction of IX with trityl chloride in refluxing pyridine-dioxane mixture followed by acetylation with acetic anhydride gave 9-(2′,3′-di-O-acetyl-4′-O-trityl-D-erythrityl)adenine (X). When this compound was refluxed with 80% acetid acid for 8 minutes, a crystalline substance, m.p. 247~248°, was obtained. In contrary to our expectation, it was 2′,3′,4′-tri-O-acetyl derivative and not desired 2′,3′-di-O-acetyl compound. To avoid this acetylation on primary hydroxy group with refluxing acetic acid,[8] X was hydrolyzed with ethanol-hydrochloric acid mixture at 42°. 2′,3′-Di-O-acetyl-9-D-erythrityladenine (XI) was obtained in a crystalline form, m.p. 196~198°, and elementary analysis data showed the correctness of the structure. The yield of this 3-step synthesis was fairy improved by the following procedure. Tritylation was achieved in refluxing pyridine by the successive addition of trityl chloride until a spot (Rf 0.08) of starting material (IX) changed totally to that of Rf 0.90 (about 20 hours were required). After acetylation with acetic anhydride, trityl-diacetyl derivative was hydrolyzed with 60% acetic acid at room temperature for 2 days. Compound (XI), thus obtained, showed single spot on paper chromatograms in two solvent systems. 2′,3′-Di-O-acetyl-9-D-erythrityladenine was then phosphorylated with two equivalents of P-diphenyl P′-morpholino pyrophosphorochloridate as described in preceding report.[7] Analysis of reaction mixture after 48 hours at room temperature by paper electrophoresis showed 70% reaction occurred and afforded 4′-morpholinophosphorochloridate (XII). Reaction of XII with excess bis(tributylammonium)pyrophosphate in pyridine solution for 15 hours gave 24% of 2′,3′-di-O-acetyl-9-D-erythrityladenine 4′-triphosphate (XIII). When compound (XIII) was treated with *N* lithium hydroxide solution for deacetylation, a spot corresponding to XIII changed to a product having Rf of 0.44 on paper chromatography. This assumed to be caused by the conversion of XIII to 3′,4′-cyclic phosphate (XIV). This type of cyclic phosphate formation was observed also in the case of flavine mononucleotide[9~11] and alkaline degradation of ATP.[12] The acidic hydrolysis of XIV and separation by ion-exchanger chromatography gave a fraction eluted with 0.003*N* hydrochloric acid and 0.15 *M* lithium chloride. Monophosphate of 9-D-erythrityladenine, thus obtained, showed single spot on paper chromatograms of two solvent systems and having R_{AMP} 1.00 on paper electrophoresis. From the elementary analyses, optical behavior and metaperiodate consumption,[13] the structure of 9-D-erythrityladenine 4′-monophosphate was established unambiguously.

In order to circumvent this degradation, triphosphate was isolated as the form of diacetyl derivative. Hasselbach's experiment showed that the existence of acetyl group on 2′ and 3′ hydroxyl group of ATP caused no significant change of the behavior of this compound against myosin. Therefore, it is reasonable to use compound (XIII) as the substrate of interaction with myosin systems.

6) In reference 3), it was reported that 9-alkanol group was cleaved by acidic treatment. However, repeated experiments denied such a lability and the results in 3) has to be corrected.
7) M. Ikehara, E. Ohtsuka: This Bulletin, **10**, 997 (1962).
8) R.B. Duff: J. Chem. Soc., **1957**, 4730.
9) R. Kuhn, H. Rudy: Chem. Ber., **68**, 383 (1935).
10) R. Kuhn, H. Rudy, F. Weygand: *Ibid.*, **69**, 1543 (1936).
11) H.S. Forest, A.R. Todd: J. Chem. Soc., **1950**, 3295.
12) D. Lipkin, R. Markham, W.H. Cook: J. Am. Chem. Soc., **81**, 6075 (1959); *Ibid.*, **81**, 6198 (1959).
13) J.S. Dixon, D. Lipkin: Anal. Chem., **26**, 1092 (1954).
14) W. Hasselbach: Biochim. et Biophys. Acta, **20**, 355 (1956).

Phosphorylation of diacetyl-9-D-erythrityladenine was carried out as described above. After the column chromatography, triphosphate fractions were collected and neutralized with tributylamine[15] and evaporated. Ethanol-acetone precipitation gave a lithium salt of 2',3'-di-O-acetyl-9-D-erythrityladenine 4'-triphosphate as decahydrate. The structure was confirmed by optical behavior, elementary analyses, paper chromatography and paper electrophoresis.

Interaction between this compound and myosin systems is now under investigation and will be reported in a separate communication.

Experimental

Paper Chromatography——Solvent A, iso-PrOH-1%$(NH_4)_2SO_4$ (2:1); solvent B, iso-PrOH-1%$(NH_4)_2SO_4$ (3:2); solvent C, BuOH-H_2O (86:14); solvent D, EtOH-*M* $AcONH_4$ (7:3 (pH 7.5)); solvent E, sat. $(NH_4)_2SO_4$-iso-PrOH-H_2O (79:2:19).

Paper Electrophoresis——0.05*M* triethylammonium bicarbonate, pH 7.5, 20 v./cm., 1 hr. Toyo Filter Paper Co., No. 51 A was used.

1-O-Tosyl-2,4-O-ethylidene-D-erythritol——2,4-O-Ethylidene-D-erythritol[4] (3.6 g., 0.024 mole) was dissolved in anhyd. pyridine and into this solution 5 g. (0.026 mole) of tosyl chloride was added portionally. After standing at room temperature for 24 hr., reaction mixture was poured onto ice and extracted with $CHCl_3$. $CHCl_3$ was dried over anhyd. Na_2SO_4 and evaporated under reduced pressure. Residue was codistilled several times with MeOH to remove trace of pyridine. A crystalline residue was recrystallized from MeOH and needles, m.p. 72~73°, were obtained. *Anal.* Calcd. for $C_{13}H_{18}O_6S$: C, 51.55; H, 6.01. Found: C, 51.97; H, 5.28.

Mother liquor of above recrystallization was evaporated and used for next reaction.

2,4-O-Ethylidene-D-erythritylamine——1-O-Tosyl-2,4-O-ethylidene-D-erythritol obtained above was dissolved in 100 ml. of anhyd. MeOH previously saturated with NH_3 at 0° and heated at 100° for 10 hr. in an autoclave. Solvent was evaporated and the residue was recrystallized from MeOH. Tosylate salt of 2,4-O-ethylidene-D-erythritylamine, m.p. 185~187° (from MeOH) was obtained. This was dissolved in MeOH-H_2O (1:1, v/v) and applied to a column of Dowex 1-X 8 (OH' form, 100~200 mesh, 2×7 cm.). MeOH elution gave 1.8 g. of crystalline 2,4-O-ethylidene-D-erythritylamine (over-all yield 1-O-tosyl-2,4-O-ethylidene erythritol was 50%), m.p. 136~138°. *Anal.* Calcd. for $C_6H_{13}O_3N$: C, 48.97; H, 8.91; N, 9.52. Found: C, 49.53; H, 8.92; N, 9.56.

4-(2',4'-O-Ethylidene)-D-erythritylamino-5-amino-6-chloropyrimidine——2,4-O-Ethylidene-D-erythritylamine (6.9 g., 0.047 mole), triethylamine (3.5 g., 0.035 mole) and 4,6-dichloro-5-aminopyrimidine (6.7 g., 0.041 mole) was refluxed in BuOH (60 ml.) for 19 hr. After cooling, triethylamine hydrochloride (m.p. 242~243°) was removed by filtration and solvent was evaporated under reduced pressure. Residue was triturated with H_2O to induce crystallization and recrystallized from H_2O. 4-(2',4'-O-Ethylidene-D-erythritylamino)-5-amino-6-chloropyrimidine, m.p. 103~105° (effervescent), was obtained (yield 5.9 g., 51%). When this material was heated at 60° in 3 mm./Hg, it effervesced and solidified after cool, m.p. 103~105°. UV: $\lambda_{max}^{H_2O}$ 262, 290 mμ; $\lambda_{min}^{H_2O}$ 272 mμ. *Anal.* Calcd. for $C_{10}H_{15}O_3N_4Cl \cdot H_2O$: C, 41.01; H, 5.86; N, 19.14. Found: C, 40.97; H, 5.98; N, 18.94.

9-(2',4'-O-Ethylidene)-D-erythrityladenine——4-(2',4'-O-ethylidene-D-erythritylamino)-5-amino-6-chloropyrimidine (3.5 g.) was heated in a mixture of 20 ml. of Ac_2O and 20 ml. of $HC(OEt)_3$ at 130° (bath temperature) for 3 hr. An aliquot was examined by UV absoption spectra (λ_{max} 264 mμ, single peak) and whole was evaporated *in vacuo*. Residue was codistilled with EtOH and dissolved in 200 ml. of EtOH previously saturated with NH_3 at 0°. Reaction at 120° for 16 hr. in an autoclave and evaporation of NH_3 gave a crystalline material, m.p. 245~246° (from EtOH). *Anal.* Calcd. for $C_{11}H_{15}O_3N_5$: C, 49.76; H, 5.71; N, 26.39. Found: C, 50.07; H, 6.01; N, 26.40.

Mother liquor of EtOH recrystallizaton was evaporated and used also for the next reaction.

9-D-Erythrityladenine——Above 9-(2',4'-O-ethylidene)-D-erythrityladenine was dissolved in 100 ml. of EtOH-H_2O (1:1, v/v), adjusted to pH 1 with 2*N*HCl and refluxed for 1 hr. An aliquot examined by paper chromatography (solvent C) showed two spots of Rf 0.58 (starting material) and 0.11 (product). Refluxing was continued until all of the spots having Rf 0.58 converted to that of 0.11. Whole was taken into dryness under reduced pressure, dried azeotropically with benzene and neutralized with NH_3. Recrystallization from EtOH-H_2O and H_2O gave a crystalline material, m.p. 218~219° (yield 1.8 g., over-all yield from ethylideneerythritylamino-5-amino-6-chloropyrimidine was 59%). *Anal.* Calcd. for $C_9H_{13}O_3N_5$: C, 45.18; H, 5.49; N, 29.29. Found: C, 45.55; H, 5.72; N, 29.06. UV: λ_{max}^{pH7} 261 (ε 15.0×10^3), λ_{min}^{pH7} 228 mμ; $\lambda_{max}^{0.1N HCl}$ 259 (ε 14.5×10^3), $\lambda_{min}^{0.1N HCl}$ 233 mμ; $\lambda_{max}^{0.1N HCl}$ 260 (ε 15.1×10^3), $\lambda_{min}^{0.1N NaOH}$ 225 mμ. $[\alpha]_D^{17}$ −32.4° (c=1.05, *N*HCl).

15) R. Okazaki, T. Okazaki, J. L. Strominger, A. M. Michelson: J. Biol. Chem., **237**, 3014 (1961).

When this sample was heated at 100° in 6*N*HCl for 5 hr., UV absorption did not change in 0.1*N* NaOH. This indicated the stability of linkage at N^9-$C^{1'}$. This sample showed a shift of λ_{max} to 250 mμ after set aside overnight at room temperature in *N*HCl+$NaNO_2$. Both these facts showed that adenine ring in this compound was not substituted other than at 9-position.

9-(4'-O-Trityl-D-erythrityl)adenine and 9-(4'-O-Trityl-2',3'-di-O-acetyl-D-erythrityl)adenine——i) Above 9-erythrityladenine (0.6 g.) and trityl chloride (0.8 g.) was dissolved in pyridine (10 ml.) and DMF (5 ml.) and heated at 100° for 5 hr. Solvent was removed by vacuum distillation, and residue was washed successively with hot hexane and hot H_2O. Resulting solid was tested by paper chromatography (solvent C), which showed the existence of 80% of tritylerythrityladenine (Rf 0.86) and 20% of starting material (Rf 0.11).

ii) Erythrityladenine (0.25 g.) and tritylchloride (0.5 g.) was heated at 70~90° for 17 hr. in 20 ml. of pyridine. Resulting crystalline precipitate, which appeared after cooling, has m.p. 219~220°(decomp., starting material). Residue was containing mainly tritylerythrityladenine (Rf 0.86).

iii) Erythrityladenine (0.085 g.) and tritylchloride (0.11 g.) was refluxed for 5 hr. with 5 ml. of dioxane and 32 μl. of pyridine. 70% of substance having Rf 0.86 (trityl derivative) was obtained. Samples obtained in i~iii) was combined, washed with hot hexane and Et_2O, dissolved in BuOH saturated with H_2O, and applied to a column of cellulose powder[16] (3×25 cm.). A fraction obtained at first by the elution of same buffer was evaporated to a hard syrup (yield 1.2 g.). This was dissolved in 15 ml. of pyridine and acetylated with 2.5 ml. of Ac_2O overnight at room temperature. Pyridine solution was poured into ice-H_2O(300 ml.) and 1.1 g. of white solid was collected by filtration. This substance shrinked at 80~90° and melted at 105° translucently. Recrystallization from MeOH gave m.p. 152°(shrinked at 110~128°).

Attempted Detritylation of (Diacetyl-tritylerythrityl)adenine——i) Above diacetyl-trityl derivative (0.9 g.) was dissolved in *N*HCl(1 ml.) and EtOH(9 ml.). After 2 hr. at 42°, a crystalline material appeared, m.p. 83~85°, which was assigned as triphenylmethane (containing no N). Evaporation of the filtrate gave a residue, which was washed with Et_2O and taken up in EtOH. Concentration of EtOH solution gave 0.1 g. of crystal, m.p. 185~200°. Recrystallization from EtOH gave m.p. 196~198°. *Anal.* Calcd. for $C_{13}H_{17}O_5N_5$: C, 48.29; H, 5.31. Found: C, 48.86; H, 5.51.

Starting material was recovered as insoluble solid in EtOH. In the mother liquor of above crystallization partially deacetylated compound was obtained.

ii) After treating with 60% AcOH 2 days at room temperature, AcOH was evaporated at 20~25° under reduced pressure. Residue was washed with hot hexane and Et_2O, and recrystallized from EtOH. A crystalline material, m.p. 175~185°, was obtained. This was identical with the sample obtained in procedure i) on paper chromatogram (see following section).

9-(2',3'-Di-O-acetyl-D-erythrityl)adenine——One step procedure from erythrityladenine. Tritylchloride (1.4 g.) was dissolved in pyridine (25 ml.) and added portionally into a suspension of 1.1 g. of erythrityladenine in 30 ml. of pyridine. Addition took 2.5 hr. under reflux. At this stage no desired product was observed on paper chromatogram. Tritylchloride (1.5 g.) was added and refluxed further for 3 hr.(25% was tritylated, Rf 0.90, solvent C). Tritylchloride (1.5 g.) was added again and refluxed for 6 hr. The spot of starting material (Rf 0.08) totally diminished. Pyridine was evaporated under reduced pressure and the residue was washed with hexane and Et_2O. H_2O (ca. 50 ml.) and $CHCl_3$ (100 ml.) was added and shaken vigorously. $CHCl_3$-layer was washed with 5% $NaHSO_4$ and dried over anhyd. $MgSO_4$. Chloroform was removed *in vacuo* and residue was evaporated once with pyridine. Residual hard oil was disolved in 15 ml. of pyridine, added with 2.5 ml. of Ac_2O, and set aside overnight at room temperature under exclusion of moisture. Whole was poured into 500 ml. of ice-H_2O, precipitate was collected on a funnel, and washed with H_2O. This material was dissolved in 60% AcOH and was kept in standing for 2 days at room temperature. Analysis by paper chromatography showed 75% of detritylation (Rf 0.55, solvent C). Precipitated tritylalcohol was removed by filtration, solvent was removed *in vacuo* and residue was taken up in Me_2CO. Acetone-insoluble was treated again with 60% AcOH as described above. Whole AcOH solution was evaporated under reduced pressure and taken up in Me_2CO. A small amount of insoluble material, m.p. 215~240°(paper chromatography, Rf 0.57(solvent C), trityl-acetyl derivative) was removed by filtration and the mother liquor was evaporated to a half of its volume. Resulting crystal was recrystallized from EtOH and a crystalline material, m.p. 155~175°, was obtained (yield 0.24 g.). From the mother liquor 0.7 g. of hard syrup was obtained. Both sample showed same Rf 0.55 (solvent C) and 0.70 (solvent D). These values were identical with those revealed by the sample obtained in preceding section. Discrepancy in melting point would probably be attributed to a slight contamination with solvent of recrystallization. Residue from the mother liquor of Me_2CO recrystallization (0.46 g.) was slightly contaminated with a substance having Rf 0.35 (solvent C) and Rf 0.60 (solvent D).

16) Purchased from Toyo Filter Paper Co., 100~200 mesh.

9-(2′,3′,4′-Tri-O-acetyl-D-erythrityl)adenine——Erythrityladenine (0.42 g.) and tritylchloride (0.53 g.) was heated in a mixture of pyridine (5 ml.) and DMF (3 ml.) for 3 hr. at 100°. Heating was continued for additional 3 hr. after the addition of 0.5 g. of tritylchloride. After cool, 5 ml. of Ac_2O was added and whole was kept in standing overnight at room temperature. Reaction mixture was poured into 300 ml. of ice-H_2O, and resulting solid material was collected by filtration. This was dissolved in AcOH (4 ml.) and H_2O (1 ml.) and refluxed for 8 min. Precipitated tritylalcohol was removed by filtration and the filtrate was taken into dryness *in vacuo*. Residue was recrystallized from methylcellosolve, m.p. 247~248°. *Anal.* Calcd. for $C_{15}H_{19}O_6N_5$: C, 49.45; H, 5.20; N, 19.18. Found: C, 50.21, H, 5.38; N, 18.51.

Phosphorylation of 9-(2′,3′-Di-O-acetyl-D-erythrityl)adenine——Di-O-acetylerythrityladenine (323 mg., 1 m mole) was added into a dioxane solution (5 ml.) of freshly prepapred P-diphenyl P′-morpholino pyrophosphorochloridate[7] (2 m mole, prepared from morpholino phosphorodichloridate[17] (2 m mole), diphenyl phosphate (2 m mole) and 2,6-lutidine (6 m mole)). Tightly stoppered flask was stored in a desiccator at room temperature for 48 hr. Analysis of an aliquot by paper electrophoresis and paper chromatography showed the reaction extent of 70% (calculated from the extract of spot, R_{AMP} 0.53, Rf 0.60 (solvent D)). Dioxane was evaporated under diminished pressure and a pyridine solution of 5 m mole of bis(tributylammonium)pyrophosphate[18] was added into residue. Pyridine was evaporated once to remove trace of H_2O and 10 ml. of pyridine was added. Reaction was carried out at room temperature for 15 hr. in a stoppered flask. 2 ml. of H_2O was added and solvent was removed under reduced pressure. The residue was taken up in 10 ml. of H_2O and extracted with Et_2O (20 ml. × 5). Precipitated starting material was removed from the mixture. H_2O-layer became pH 4~5 after this extraction. Analysis of this solution by paper electrophoresis showed following results: starting material 28%, 4′-morpholidate 24%, 4′-diphosphate 24%, 4′-triphosphate 24%. On the paper chromatogram following spots were detected: (solvent D) Rf 0.74 (4′-morpholidate + starting material), 0.37 (unidentified substance), 0.15 (4′-diphosphate) and 0.04 (4′-triphosphate). H_2O solution was adjusted to pH 12.0 with *N* LiOH and extracted with Et_2O thoroughly. H_2O-layer was concentrated under reduced pressure to a half of its volume and examined on paper chromatogram (solvent D). It showed a main spot of Rf 0.44 and a thin spot of Rf 0.15. The former corresponded to erythrityladenine 3′,4′-cyclic phosphate. Whole solution was adjusted to pH 5.0 with *N* HCl and absorbed on activated charcoal[19] (10g.). After the H_2O wash, charcoal was eluted with 800 ml. of 50% EtOH containing 2% NH_3. Eluant was concentrated to ca. 100 ml., adjusted to pH 8.5 and applied to the column of Dowex 1-X 8 (Cl^- form, 100~200 mesh, 2 × 8 cm.). Results of chromatography was as follows:

	(%)	Peak	R_{AMP}[a] Main	R_{AMP}[a] Trace
H_2O-washing	3	unidentified		
0.003*N* HCl + 0.015*M* LiCl	59	I	1.0	0.59
〃	8	II	1.0	0.59 1.20

a) Paper electrophoretic migration.

No other UV absorbing material was eluted even though a higher concentration of eluting buffer was used. Fraction of Peak (I) were collected, neutralized with *N* LiOH and concentrated to a small bulk in a rotary evaporator. Precipitation by the addition of 2 volumes of EtOH gave a Li-salt. Reprecipitation from a small amount of H_2O and 2 volumes of EtOH gave a sample having single spot on paper electrophoresis (R_{AMP} 1.0) and paper chromatography (Rf 0.42 (solvent E), 0.50 (solvent A)). *Anal.* Calcd. for $C_9H_{12}O_6N_5P \cdot Li_2H_2O$: P, 8.89. Found: P, 8.84. $\varepsilon(P) = 15.0 \times 10^3$. UV: $\lambda_{max}^{H_2O}$ 260, $\lambda_{min}^{H_2O}$ 228 mμ.

IO_4^--consumption[13]: in 0.01*M* $NaIO_4$, at 20° for 82 hr., 0.98 equivalent of IO_4^- was consumed (estimated photometrically).

9-(2′,3′-Di-O-acetyl-D-erythrityl)adenine 4′-Triphosphate——i) 2′,3′-Di-O-acetylerythrityladenine (323 mg., 1 m mole) was phosphorylated as described in the preceding section. Resulting acidic H_2O solution containing diacetylerythrityladenine triphosphate was extracted with Et_2O in order to remove diphenylphosphate. H_2O-layer was adjusted to pH 7.5~8.0 with *N* LiOH and extracted again with EtOH thorougthly to remove amines. At this stage pH was 5.0~5.5. This was treated with active charcoal as described above and applied to a column of Dowex 1-X 8 (condition was same as above):

17) M. Ikehara, E. Ohtsuka: This Bulletin, **10**, 536, 539 (1962).
18) J. G. Moffatt, H. G. Khorana: J. Am. Chem. Soc., **83**, 639 (1961).
19) R. W. Chambers, H. G. Khorana: *Ibid.*, **79**, 3752 (1957).

	Peak	(%)
H_2O-washing		5.2
0.003N HCl+0.015M LiCl	I	17
〃	II	33
0.003N HCl+0.05M LiCl	III	3
0.003N HCl+0.15M LiCl	IV	10
0.003N HCl+0.25M LiCl	V	4
N HCl	VI	10

Peak (IV) was neutralized with N LiOH to pH 0.5 and concentrated to a small bulk in a rotary evaporator under 20°(pH became 9~10). Residual syrup was dissolved in 2 volumes of MeOH and Li-salt was precipitated with 20 volumes of Me_2CO. Precipitate was collected by centrifugation, washed twice with Me_2CO and Et_2O, and dried over P_2O_5 at 3 mm./Hg. This sample showed a spot of Rf 0.44, R_{AMP} 1.13 on paper chromatogam (solvent B) and a spot of R_{AMP} 1.54 by paper electrophoresis as the main product. However, it contained also 4′-mono- and/or tri-phosphate, which were detected also by paper chromatography and electrophoresis. Partial degradation of 4′-triphosphate seems to occur during the concentration of Peak (IV).

ii) Charcoal treatment was omitted from the procedure described in i). After Et_2O extraction resulting H_2O solution was adjusted to pH 8.5 and applied to a column of Dowex 1-X 8 (Cl' form, 100~200 mesh, 4×16 cm.).

	Peak	(%)
H_2O-washing		36
0.003N HCl+0.015M LiCl	I	7.2
〃	II	6.0
0.003N HCl+0.05M LiCl	III	9.0
0.003N HCl+0.075M LiCl	IV	3.8
0.003N HCl+0.1M LiCl	V	3.4
0.003N HCl+0.15M LiCl	VI	1.3
0.003N HCl+0.25M LiCl	VII	6.0
N HCl	VIII	4.3

Fraction (V) was neutralized with tri-butylamine and concentrated to a small bulk. Residual syrup was evaporated twice with EtOH and into the residue Me_2CO was added. Li-salt of diacetyl-erythrityladenine 4′-triphosphate was collected by centrifugation. Precipitate was washed with Me_2CO, Et_2O and dried over P_2O_5 at 3 mm./Hg for 5 hr. (yield 14 mg.). *Anal.* Calcd. for $C_{13}H_{16}O_{14}N_5P_3Li_4 \cdot 10H_2O$: total P, 12.1; labile P, 8.1. Found : total P, 12.3; lablie P, 8.0. $\varepsilon(P_3)=15.2\times10^3$.
Paper chromatography : Rf 0.32, R_{AMP} 1.09 (solvent B). Paper electrophoresis R_{AMP} 1.38.

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Summary

9-D-Erythrityladenine was synthesized from 4,6-dichloro-5-aminopyrimidine and 2,4-O-ethylidene-erythritylamine, followed by the cyclization with acetic anhydride and ethyl orthoformate. The phosphorylation of erythrityladenine was achieved by the general method using P-diphenyl P′-morpholino pyrophosphorochloridate described in earlier report. 2′,3′-Di-O-acetylerythrityladenine 4′-triphosphate was obtained as decahydrate in a pure state.

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