ANALOGS OF CARBOHYDRATE-METABOLISM COENZYMES

COMMUNICATION 12. SYNTHESIS OF N_6-METHYLADENOSINE DIPHOSPHATE GLUCOSE AND N_6,N_6-DIMETHYLADENOSINE DIPHOSPHATE GLUCOSE*

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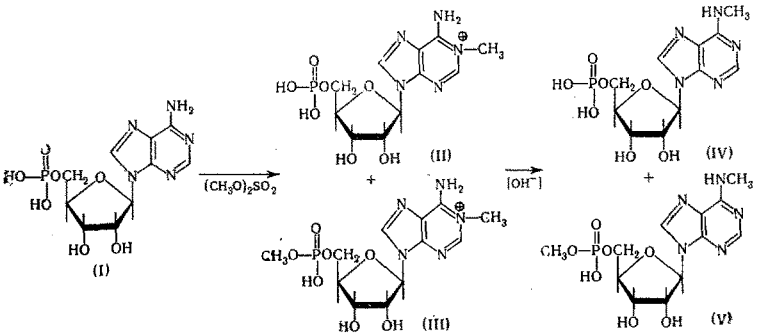
This report is a continuation of work conducted at our laboratory, in conjunction with the Laboratory for Biological Testing, on the influence of changes in the heterocyclic ring of ADPG† on its reactivity in different enzymatic reactions. For this purpose, we studied synthetic nucleoside diphosphate sugar analogs substituted at different positions in the purine ring [2]. This article describes the synthesis of two ADPG analogs substituted at the exocyclic amino group on the C_6 of the adenine ring: N_6-methyl-ADPG and N_6, N_6-dimethyl-ADPG. The behavior of these analogs in biological reactions is of great interest, since it can aid us in determining the significance of the amino group hydrogen atoms in coenzyme biological activity. The initial compounds for synthesis of both ADPG analogs were the corresponding 5'-monophosphates. Synthesis of N_6-methylamino-9-β-D-ribofuranosylpurine-5'-phosphate (N_6-methyl-AMP) (IV) was carried out by regrouping of N_1-methyladenosine-5'-phosphate (II), which was in turn obtained by methylation of adenosine-5'-phosphate:



This synthesis procedure was described by Griffin and Reese [3], but we made several modifications that are described in detail in the experimental section. We obtained N_6-methyl-AMP with a yield of 59%; it was isolated in the form of its triethylammonium salt after chromatography in DEAE-Sephadex (HCO_3^- form). The preparation was homogeneous when subjected to chromatography and paper electrophoresis (Table 1). Its UV spectrum was identical to that given in the literature for derivatives of N_6-methylaminopurine [3-8].

Synthesis of N_6,N_6-dimethylamino-9-β-D-ribofuranosylpurine-5'-phosphate N_6,N_6-dimethyl-AMP) (IX) from 6-mercapto-9-β-D-ribofuranosylpurine (VI) was carried out in two ways: the first procedure consisted in replacement of the mercapto group in 2', 3'-O-isopropylidene-VI (VII) by the dimethylamino group, phosphorylation of the 2', 3'-O-isopropylidene-N_6, N_6-dimethyladenosine (VIII) formed, and removal of the protective groups; in the second procedure, synthesis was employed to introduce the dimethylamino group

*For communication 11, see [1].

†The following abbreviations are used in this article: ADPG) adenosine-5'-diphosphate-α-D-glucopyranose; N_6-methyl-ADPG) N_6-methylamino-9-β-D-ribofuranosylpurine-5'-diphosphate-α-D-glucopyranose; N_6, N_6-dimethyl-ADPG) N_6, N_6-dimethylamino-9-β-D-ribofuranosylpurine-5'-diphosphate-α-D-glucopyranose.

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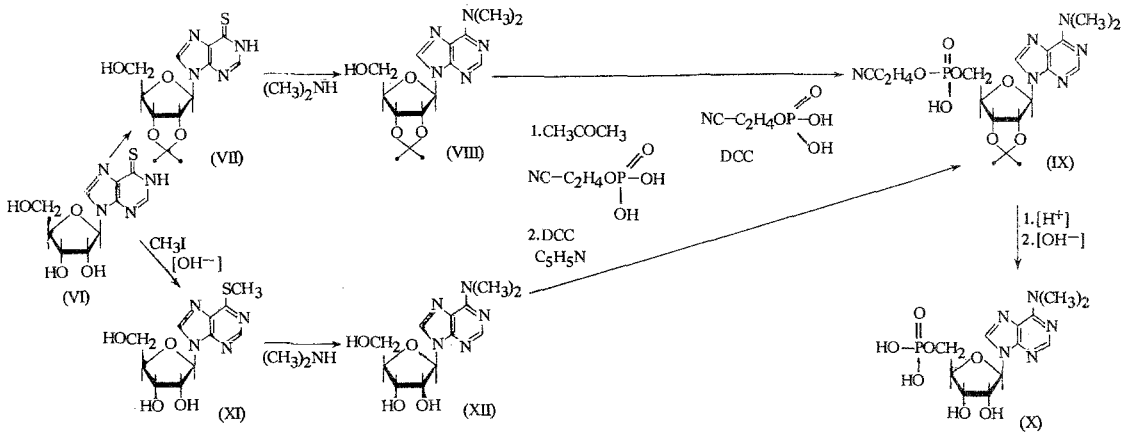
TABLE 1. Chromatographic and Electrophoretic Properties of Nucleotides Obtained

Compound	R_f in solvents			Electrophoretic mobility with respect to AMP
	A	B	C	
Adenosine-5'-phosphate	0.12	0.17	0.28	1.00
Adenosine-5'-phosphomorpholide	0.60	0.65	0.16	0.54
N_6-Methyladenosine-5'-phosphate	0.26	0.38	0.32	0.87
N_6, N_6-dimethyladenosine-5'-phosphate	0.24	0.47	0.25	0.71
N_6-methyladenosine-5'-phosphomorpholide	0.73	–	–	0.58
N_6, N_6-dimethyladenosine-5'-phosphomorpholide	0.69	–	–	0.55
Adenosine biphosphate glucose	0.17	0.21	0.38	0.75
N_6-methyladenosine diphosphate glucose	0.31	0.46	0.68	0.70
N_6, N_6-dimethyladenosine diphosphate glucose	0.39	0.31	0.65	0.64
N_1-methyladenosine-5'-phosphate	0.52	–	0.80	0.60
Methyl adenosine-5'-phosphate	–	0.35	0.20	0.85
Methyl N_1-methyl-AMP	–	0.60	0.89	0.23
Methyl N_6-methyl-AMP	–	–	0.48	0.50

into the purine ring of 6-methylthio-9-β-D-ribofuranosylpurine (IX); the resultant N_6, N_6-dimethyladenosine (XIII) was then converted to N_6, N_6-dimethyladenosine-5'-phosphate (X):



We used the method proposed for natural nucleosides [9] for acetonation of the 6-mercaptopurine riboside. The nucleoside was treated with 2, 2-diethoxypropane in dimethylformamide solution at room temperature overnight, using hydrogen chloride dissolved in dioxane as an acid catalyst. This method was also found to give good results for thionucleosides. Analysis of the reaction mixture by paper chromatography showed that the reaction was quantitative and that no side reactions took place. The resultant isopropylidene derivative (VII) was subsequently used without further purification. In order to produce compound (VIII) from compound (VII), the latter was heated with a saturated methanol solution of dimethylamine for 7 h at 115°. Compound (VIII) was purified by preparative thin-layer chromatography (TLC) in silica gel with a solvent consisting of chloroform and acetone (2 : 1). It was phosphorylated with β-cyanoethylphosphoric acid in absolute pyridene in the presence of dicyclohexylcarbodiimide. The cyanoethyl derivative of 2', 3'-O-isopropylidene-N_6, N_6-dimethyl-AMP (IX) was isolated by ion-exchange chromatography in DEAE-Sephadex (HCO_3^- form); elution was with dilute aqueous solutions of triethylammonium bicarbonate buffer (pH 7.5). The protective groups were removed by treatment first with 70% acetic acid (30 min, 100°) and then with 1 N aqueous KOH (15 min, 100°) [11]. The N_6-methyl-AMP was isolated in the form of its morpholine salt, the yield being 74% with respect to the 2', 3'-O-isopropylidene-N_6, N_6-dimethyladenosine and 40% with respect to the initial 6-mercaptopurine riboside. Preparation (X) was chromatographically and electrophoretically homogeneous (See Table 1). The ultraviolet spectrum of this compound was similar to the spectra given in the literature for derivatives of N_6, N_6-dimethylaminopurine [8, 12-14].

In synthesizing compound (X) by the second procedure, the mercapto group in compound (VI) was methylated with methyl iodide in 0.1 N aqueous NaOH at room temperature for 30 min [15]. In order to prevent oxidation of the mercapto group by atmospheric oxygen, the reaction was conducted in nitrogen; the only

TABLE 2. Chromatographic Properties of Nucleosides Obtained

Compound	Rf in solvents; A	D
Adenosine	0.45	0.15
N_6-methyladenosine	0.58	0.43
N_6, N_6-dimethyladenosine	0.56	0.54
6-Mercaptopurine riboside	–	0.22
6-Methylthiopurine riboside	–	0.64
2', 3'-O-Isopropylideneadenosine	0.80	0.84
2',3'-O-Isopropylidene-6-mercaptopurine riboside	–	0.87
2',3'-O-Isopropylidene-N_6, N_6-dimethyladenoside	0.91	0.78

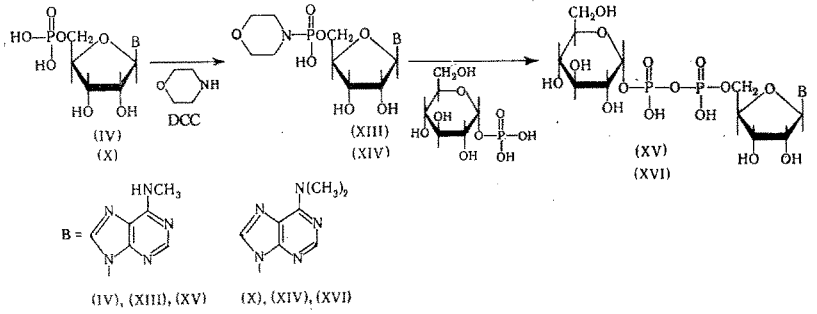
reaction product was 6-methylthiopurine riboside (XI) (with an R_f of 0.64 in a solvent consisting of n-butanol and water, 86:14). Compound (XI) was isolated by preparative paper chromatography in a solvent consisting of n-butanol and water (86 : 14).

Replacement of the methylthio group in compound (XI) by the dimethylamino group was quantitative in aqueous methylamine solution at room temperature overnight.

The only reaction product, N_6, N_6-dimethyladenosine (XII) (R_f = 0.53), was isolated by preparative paper chromatography in a solvent consisting of n-butanol and water (86 : 14). The compound obtained was chromatographically homogeneous and its spectrum agreed well with those given in the literature.

Phosphorylation of compound (XII) was carried out by the method recently proposed by Japanese authors [16], without precipitation of the initially formed isopropylidene derivative. The N_6, N_6-dimethyladenosine was acetonated with absolute acetone in dimethylformamide solution containing β-cyanoethylphosphoric acid as an acid catalyst. After evaporation of the solution and addition of a dicyclohexylcarbodiimide solution in puridine to the residue, phosphorylation was carried in the manner described above. The morpholine salt of N_6, N_6-dimethyl-AMP was isolated with a yield of 71% with respect to the N_6, N_6-dimethyladenosine. The compound was homogeneous when subjected to chromatography and paper electrophoresis and was completely identical to the preparation obtained by the first procedure.

Both ADPG analogs, N_6-methyl-ADPG (XIII), and N_6, N_6-dimethyl-ADPG (XIV), were produced from the corresponding monophosphates by condensation of the nucleoside phosphomorpholide formed from the monophosphate with α-D-glucose-1-phosphate [17].



The phosphomorpholides were produced by the usual method [18] and the product was isolated by ion-exchange chromatography in DEAE-Sephadex (HCO_3^- form). The triethylammonium salt of the corresponding nucleoside phosphomorpholide obtained was employed in pyrophosphate synthesis by the method perfected at our laboratory [19], using dry dimethylsulfoxide as the solvent and conducting the condensation at room temperature. The resultant asymmetric pyrophosphate was separated from the excess glucose-1-phosphate, the unreacted nucleoside phosphomorpholides, and the byproduct nucleoside diphosphate and symmetric pyrophosphate by ion-exchange chromatography in DEAE-cellulose (Cl^- form). The inorganic salt was removed by gel-filtration [20] in Sephadex G-10. The sodium salt preparations of the two nucleoside diphosphate sugars were homogeneous when subjected to chromatography and paper electrophoresis (see Tables 1 and 2) and had ultraviolet spectra similar to those given in the literature. The results of a

determination of the acid-labile phosphorus : total phosphorus : glucose ratio in the preparations confirmed that the compounds obtained had the structure of disodium salts of N_6-methyl- and N_6, N_6-dimethyl-ADPG.

EXPERIMENTAL

The amount of each compound was determined from its optical density at λ_{max}. Chromatography was carried out with "Leningrad" paper (medium) and the following solvent systems: A) isopropanol, saturated ammonia solution, and water (7 : 1 : 2); B) ethanol and 1 M aqueous ammonium acetate solution of pH 7.5 (5 : 2); C) saturated aqueous ammonium sulfate solution, isopropanol, and phosphate buffer of pH 7.2 (79: 2 : 19); D) n-butanol and water (86 : 14). Preparative TLC was conducted with plates of silica gel containing the luminophore "Siligram UU_{254}" with solvent system E, which consisted of $CHCl_3$ and acetone (2 : 1). The paper electrophoresis was carried out in an EFA-1 apparatus with 0.05 M aqueous triethylammonium bicarbonate buffer and a gradient of 23 V/cm. The position of the UV-absorbing spots on the plates and paper was established with a ultrachemiscope. The phosphorus was determined colorimetrically by a modified version of our previous method [19]. The glucose was determined by the method of Park and Johnson [21] after hydrolysis with 0.01 N HCl at 100° for 15 min.

N_6-Methylamino-9-β-D-ribofuranosylpurine-5'-phosphate (IV). A portion of 100 mg [3900 o.u. (260 nm), 0.26 mM] of the disodium salt of adenosine-5'-phosphate was dissolved in 2 ml of water and treated with small portions of 0.197 g of dimethylsulfate under agitation. The latter was added over a period of 2 h, holding the pH of the reaction solution at 4.5-5.0 (pH-meter readings) by addition of 1 M aqueous sodium bicarbonate solution. The reaction mixture was then agitated until the solution pH no longer varied (an additional 3-4 h). The methylation process was monitored by paper chromatography in solvents A and B and by electrophoresis at pH 7.5. When the reaction did not go to completion, methylation was repeated under the same conditions, adding an additional 1.57 mM of $(CH_3)_2SO_4$. When the reaction was complete, the resultant solution was diluted by a factor of 10 with water and passed through a column (2 × 10 cm) of Dowex 1 × 2 (Cl^- form). The column was washed with water until the eluate exhibited no absorption. The solution obtained, which contained 3340 o.u. (260 nm) (85.5%) of a mixture of N_1-methyl-AMP and its methyl ester, was evaporated until dry.

The residue was dissolved in 2.5 ml of water, the solution pH was brought to 10 by addition of concentrated NH_3 solution (7-8 drops), and the solution was left to stand in a water bath at 40° overnight. It was then diluted by a factor of 10 with water and passed through a column (2 × 15 cm) of DEAE-Sephadex (HCO_3^- form). The column was washed with water until the eluate exhibited no absorption and then rinsed with 0.05 M aqueous triethylammonium bicarbonate buffer [yielding 860 o.u. (265 nm), 22% of the methyl ester of N_6-methyl-AMP]; the N_6-methyl-AMP [1960 o.u. (265 nm)] was then eluted with 0.1 M buffer solution. The fractions containing compound (IV) were evaporated until dry and the excess $(C_2H_5)_3N$ was distilled off with water and then with absolute alcohol. The dry residue was dissolved in a small amount of water (1-2 ml) and passed through a column containing the cationite Dowex-50 (H^+ form, 2 × 5 cm). The compound was eluted from the resin with water, whose pH was brought to 10-11 with morpholine. The resultant morpholine salt of N_6-methyl-AMP [1780 o.u. (265 nm), 0.15 mM, 59% with respect to the initial AMP] was chromatographically and electrophoretically homogeneous (see Table 1). Its ultraviolet spectrum had the following characteristics: $\lambda_{max} = 261$ and $\lambda_{min} = 231$ nm at pH 2; $\lambda_{max} = 264$ and $\lambda_{min} = 229$ nm at pH 12.

2', 3'-O-Isopropylidene-N_6, N_6-dimethyladenosine (VIII). A portion of 30 mg of 6-mercapto-9-β-D-ribofuranosylpurine was dissolved in 1 ml of dimethylformamide, 0.5 ml of 2, 2-diethoxypropane and 4-5 drops of saturated HCl solution in dioxane were added to the resultant solution until an acidic reaction was obtained, and the solution was left to stand overnight (the completeness of the reaction was checked by paper chromatography in solvent D). When the reaction was complete, the solution was neutralized with 1 ml of a methanol solution of $(CH_3)_2NH$ and the 2, 2-diethoxypropane was evaporated in a vacuum set up by a water-jet pump. The remaining solution in $HCON(CH_3)_2$ was mixed with 5 ml of a saturated $(CH_3)_2NH$ solution in methanol, transferred to an ampule, sealed, and heated at 115° for 7 h. The solution was then cooled, the ampule was opened, and the reaction solution was evaporated until dry (in a vacuum set up by an oil pump). The residue was dissolved in a minimum amount of $CHCl_3$ (0.3 ml). The 2', 3'-O-isopropylidene-N_6, N_6-dimethyladenosine was separated by preparative TLC on silica gel plates containing the luminophore "Siligram UU_{254}" in solvent D. The zone with an Rf of 0.64 was removed and the compound was eluted on a filter with the same solvent. We obtained 972 o.u. (275 nm) (0.054 mM, 54% with respect to the initial 6-mercaptopurine riboside) of 2', 3'-O-isopropylidene-N_6, N_6-dimethyladenosine. The compound was chromatographically

homogeneous (see Table 2). Its ultraviolet spectrum had the following characteristics: $\lambda_{max} = 268$ and $\lambda_{min} = 233$ nm at pH 2; $\lambda_{max} = 276$ and $\lambda_{min} = 229$ nm at pH 12.

6-Dimethylamino-9-β-D-ribofuranosylpurine (XII). A portion of 30 mg of 6-mercaptopurine riboside in a small conical flat-bottomed flask was dissolved in 1 ml of 0.1 N aqueous NaOH, a weak stream of N_2 was passed through the solution until the flask was filled, the flask was placed in a magnetic mixer, and portions of CH_3I (three 0.1-ml portions) were added; the reaction mixture was then agitated for an additional 30 min (the course of the reaction was monitored by paper chromatography in solvent D). The only reaction product was 6-methylthiopurine riboside ($R_f = 0.34$). When the reaction was complete, a weak stream of N_2 was passed through the solution to remove the excess CH_3I. A portion of 5 ml of saturated aqueous $(CH_3)_2NH$ was added to the solution and it was left to stand overnight at room temperature. The N_6, N_6-dimethylaminopurine riboside was isolated by preparative paper chromatography on Whatman 3 MM paper in solvent D. The zone with an R_f of 0.55 was cut out and the compound was eluted with water. We obtained 1860 o.u. (0.1 mM) of compound (XII). It was chromatographically homogeneous (see Table 2) and its ultraviolet spectrum was identical to that of compound (VIII).

N_6, N_6-Dimethyladenoside-5'-phosphate (X). A) From 2'3'-O-isopropylidene-N_6, N_6-dimethyladenosine: 515 o.u. (275 nm) (0.03 mM) of 2', 3'-O-isopropylidene-N_6, N_6-dimethyladenosine was dissolved in 1 ml of pyridine and 0.12 mM (a fourfold excess) of a 0.1 M β-cyanoethylphosphoric acid solution in aqueous pyridine (1.2 ml) was added. The resultant solution was evaporated until dry and the residue was carefully dried by distillation with anhydrous pyridine (three 2-ml portions) and dissolved in 1 ml of anhydrous pyridine. A tenfold excess (52 mg) of dicyclohexylcarbodiimide was added to the solution. The reaction mixture was heated at 60° for 4 h, carefully preventing moisture from entering. The course of the reaction was monitored by electrophoresis at pH 7.5. After the reaction had gone to completion, the solution was cooled, 0.5 ml of water was added, and the mixture was left to stand for 2 h; it was then evaporated until dry and the traces of pyridine were distilled off with water. The residue was dissolved in a mixture of water and ether (2 : 1, 9 ml). The water layer was extracted with ether (three 1-ml portions) and the ether extracts were rinsed with water (one 2-ml portion). The combined aqueous phases were evaporated until dry. The residue was dissolved in 5 ml of water and the solution was passed through a column (1.5 × 10 cm) of DEAE-Sephadex (HCO_3^- form). The column was washed with water until the eluate exhibited no absorption and 0.05 M aqueous triethylammonium bicarbonate buffer (pH 7.5) was then used to elute the cyanoethyl derivative of 2', 3'-O-isopropylidene-N_6, N_6-dimethyl-AMP [360 o.u. (275 nm), 70%]. The fractions containing the compound were evaporated until dry and the residue was dissolved in 2 ml of 70% CH_3CO_2H and heated at 100° for 30 min; the solution was then evaporated until dry and the traces of CH_3CO_2H were distilled off with water. The residue was dissolved in 2 ml of 1 N KOH and the solution was held in a boiling water bath for 15 min. The alkaline solution was diluted by a factor of 10 with water and passed through a column of the cationite Dowex-50 (H^+ form, 3 × 3 cm). The compound was eluted from the resin with water, whose pH was brought to 10-11 with morpholine. The resultant solution of the morpholine salt of N_6, N_6-dimethyl-AMP [352 o.u. (275 nm), with a yield of 68.5%] was evaporated until dry. The ultraviolet spectrum in water showed $\lambda_{max} = 268$ and $\lambda_{min} = 231$ nm.

B) From N_6, N_6-dimethyladenosine: 870 o.u. (275 nm) (0.02 mM) of N_6, N_6-dimethyladenosine was dissolved in 0.2 ml of $HCON(CH_3)_2$; 0.2 ml of anhydrous acetone, 0.4 ml of 2, 2-diethoxypropane, and 20 mg of a solution of β-cyanoethylphosphoric acid in 0.5 ml of $HCON(CH_3)_2$ were added to the solution. The latter was boiled for 5 h with a reflux condenser (the course of the reaction was monitored by paper chromatography in solvent D). After the reaction had gone to completion, the solution was evaporated in a vacuum set up by a water-jet pump, pyridine was added to the remaining solution in $HCON(CH_3)_2$, and the solution was again evaporated (three 2-ml portions). The solution was then mixed with 0.5 ml of pyridine and 50 mg of dicyclohexylcarbodiimide and the reaction mixture was heated at 60° for 4 h. The subsequent treatment of the reaction mixture was the same as that described above. Ion-exchange separation in a column (1.5 × 10 cm) of DEAE-Sephadex (HCO_3^- form) yielded 274 o.u. (275 nm) of the cyanoethyl derivative of 2', 3'-O-isopropylidene-N_6, N_6-dimethyl-AMP; after removal of the protective groups, we obtained 262 o.u (71%) of the chromatographically homogeneous morpholine salt of N_6, N_6-dimethyl-AMP. The specimen was identical to that obtained by procedure A.

N_6-Methyladenosine-5'-phosphomorpholide (XIII). A portion of 1000 o.u. (265 nm) (0.08 mM) of the morpholine salt of N_6-methyl-AMP was dissolved in 3 ml of 50% aqueous tert-butanol solution and 0.12 ml of morpholine was added. The resultant mixture was heated to the boiling point and a solution of 240 mg of

bicyclohexylcarbodiimide in 3 ml of tert-butanol was added drop by drop. After all the carbodiimide solution had been added (2-3 h), the reaction mixture was boiled for an additional 4 h, checking the course of the reaction by paper electrophoresis at pH 7.5 and by paper chromatography in solvents A and B. When the reaction did not go to completion, an additional 0.12 ml of morpholine and 240 mg of bicyclohexylcarbodiimide were added to the boiling reaction mixture (boiling was continued for 7-8 h). When the reaction was complete, the solvent was distilled off in a vacuum and the residue was dissolved in a mixture of water and ether (5 : 2, 14 ml). The water layer was extracted with ether (three 2-ml portions) and the ether extracts were washed with water (one 2-ml portion). The combined aqueous phases were evaporated until dry. The dry residue was dissolved in 30 ml of water and passed through a column (2 × 15 cm) of DEAE-Sephadex (HCO_3^- form). The column was washed with water. The N_6-methyladenosine-5'-phosphomorpholide was eluted with a 0.05 M triethylammonium bicarbonate solution (pH 7.5) (the unreacted nucleotide was eluted with 0.1 M buffer solution, yielding 120 o.u., or 12%). We obtained 740 o.u. (265 nm) (74%) of the chromatographically pure triethylammonium salt of N_6-methyladenosine-5'-phosphomorpholide (see Table 1). The solution was evaporated until dry and the excess triethylammonium bicarbonate was distilled off with water and then with absolute ethanol. The residue was thoroughly dried by azeotropic distillation with an alcohol–benzene mixture (5 : 2) and with absolute benzene.

N_6,N_6-Dimethyladenosine-5'-phosphomorpholide (XIV). A portion of 290 o.u. (275 nm) (0.016 mM) of the morpholine salt of N_6, N_6-dimethyl-AMP was treated with morpholine and bicyclohexylcarbodiimide as described above. After ion-exchange separation of the reaction mixture in a column (1 × 10 cm) of DEAE-Sephadex (HCO_3^- form), we obtained 265 o.u. (275 nm) (85%) of the triethylammonium salt of N_6,N_6-dimethyladenosine-5'-phosphomorpholide. The unreacted nucleotide from the column amounted to 24 o.u. (8%). The N_6, N_6-dimethyladenosine phosphomorpholide obtained was chromatographically and electrophoretically homogeneous (see Table 1).

N_6-Methylamino-9-β-D-ribofuranosylpurine-5'-diphosphateglucose (XV). A threefold excess of a standard 0.1 M solution of the triethylammonium salt of α-D-glucose-1-phosphate in dry dimethylsulfoxide (1.1 ml) was added to the thoroughly dried triethylammonium salt of N_6-methyladenosine-5'-phosphomorpholide [640 o.u. (265 nm), 0.036 mM]. The resultant mixture was dried by distillation with benzene and the clear solution was left to stand at room temperature for two days. The course of the reaction was monitored by paper electrophoresis at pH 7.5. After the reaction had gone to completion, the solution was diluted by a factor of 100 with water and passed through a column (1.5 × 20 cm) of DEAE-cellulose (Cl^- form). The column was washed with water and then with aqueous NaCl solutions: first with a 0.02 M solution [which yielded 40 o.u. (6%) of unreacted N_6-methyladenosinephosphomorpholide], then with a 0.04 M solution [which yielded 90 o.u. (15%) of N_6-methyl-AMP and removed the excess glucose-1-phosphate], and finally with a 0.08 M solution to elute the N_6-methyl-ADPG [490 o.u. (265 nm), 77%]. The fractions containing the compound were evaporated until dry and the residue was dissolved in a minimum amount of water (about 6 ml) and desalted in a column of Sephadex G-10 (3 × 75 cm). Elution of the N_6-methyl-ADPG began after 185 ml of water had been passed, while the salt appeared in the eluate after 315 ml had been passed.

The fractions containing the N_6-methyl-ADPG (468 o.u., a yield of 70.5%) were evaporated to a small volume and lyophilized. The ultraviolet spectrum showed λ_{max} = 262 nm, ε_{max} = 16,200, λ_{min} = 231 nm, and ε_{min} = 4700 at pH 2; λ_{max} = 264 nm, ε_{max} = 12,900, λ_{min} = 229 nm, and ε_{min} = 3300 at pH 12. The ratio of acid-labile phosphorus to total phosphorus to glucose was found to be 1.06 : 2.05 : 1.00 (calculated: 1 : 2 : 1).

N_6, N_6-Dimethylamino-9-β-D-ribofuranosylpurine-5'-diphosphateglucose (XVI). Using a procedure similar to that described above and proceeding from 265 o.u. (275 nm) (0.015 mM) of the triethylammonium salt of N_6, N_6-dimethyladenosine-5'-phosphomorpholide, we isolated 29 o.u (11%) of unreacted phosphomorpholide (elution with 0.04 M NaCl), 30 o.u. (11%) of N_6, N_6-dimethyl-AMP (elution with 0.08 M NaCl), and 190 o.u. (71.7%) of N_6, N_6-dimethyl-ADPG (elution with 0.12 M NaCl). After desalting a column of Sephadex G-10 (3 × 75 cm), we obtained 168 o.u. (275 nm) (64%) of the chromatographically pure sodium salt of N_6, N_6-dimethyl-ADPG. The ultraviolet spectrum showed λ_{max} = 268 nm, ε_{max} = 18,300, λ_{min} = 234 nm, and ε_{min} = 3500 at pH 2; λ_{max} = 276 nm, ε_{max} = 16,100, λ_{min} = 231 nm, and ε_{min} = 2600 at pH 12. The ratio of acid-labile phosphorus to total phosphorus to glucose was found to be 1.09 : 2.06 : 1.00 (calculated: 1 : 2 : 1).

CONCLUSIONS

We synthesized two analogs of the natural coenzyme adenosine diphosphate glucose: N_6-methyl-ADPG and N_6, N_6-dimethyl-ADPG.

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