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175. Tohru Ueda and Eiko Ohtsuka : Chemical Synthesis of Guanosine Diphosphate Mannose (GDPM).*[1]

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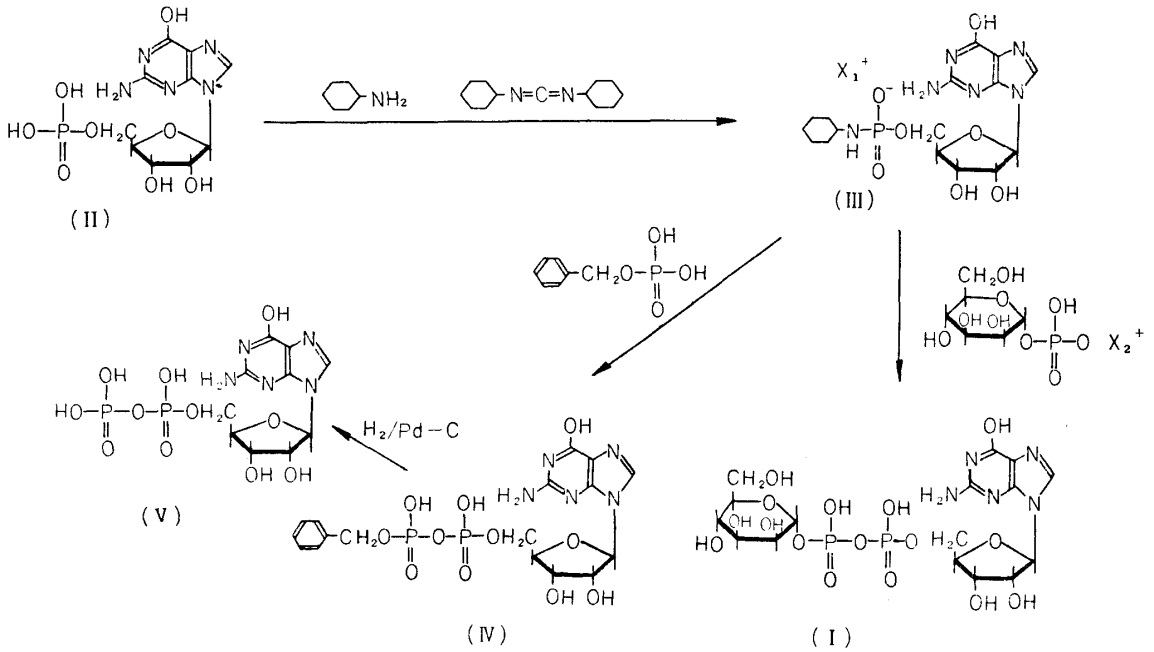
The discovery of uridine diphosphate glucose (UDPG),*[3] a coenzyme of galactowaldenase, in yeast by Leloir and his colleagues[1] was soon followed by the isolation of a number of nucleotides of the UDPG-type from various sources. Among them, GDPM, isolated by Leloir and Cabib from yeast,[2] was found to function as a coenzyme in the synthesis of mannans, at least in yeast. Although the structure of GDPM (I) appeared very likely to be correct by the studies of its chemical hydrolysis[2] and enzymatic synthesis,[3] it seemed necessary to confirm it by chemical synthesis, as has been done in the cases of UDPG[4] and other nucleotide coenzymes. Further, this synthesis is of much value in making available authentic specimen required for the elucidation of the enzymic reaction.

In the chemical synthesis of GDPM, the phosphoramidate method developed by Todd[5] and Khorana[6] has been employed with some improvement. As has been observed



X_1^+=tricyclohexylguanidium ion X_2^+=tri-octylammonium ion

Chart 1.

*1 A preliminary report was made as a Communication to the Editor, This Bulletin, **7**, 389(1959).

*2 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (上田 亨, 大塚英子).

*3 Following abreviations were used : GDPM, guanosine diphosphate mannose; GMP, guanosine 5′-monophosphate; GDP, guanosine 5′-diphosphate; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide.

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4) a) G. W. Kenner, A. R. Todd, R. F. Webb : J. Chem. Soc., **1954**, 2843. b) A. M. Michelson, A. R. Todd : *Ibid.*, **1956**, 3459. c) J. G. Moffatt, H. G. Khorana : J. Am. Chem. Soc., **80**, 3756(1958). d) A. R. Todd, *et al.* : J. Chem. Soc., **1952**, 3665.
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in the synthesis of GDP,[7] the solubility of reactants seriously affects the yield of product. To avoid this drawback N-cyclohexylphosphoramidate was chosen as one of the starting materials.

GMP (II) was allowed to react with cyclohexylamine in the presence of DCC at 70° to yield tricyclohexylguanidinium salt of guanosine 5'-(N-cyclohexylphosphoramidate) (III), in 42% yield. In order to confirm the structure and reactivity, (III) was reacted with benzyl hydrogen phosphate and the product submitted to paper chromatography. The formation of GDP-benzyl (IV) was proved as a moderately moving, UV-absorbing spot. Hydrogenation of the reaction mixture gave GDP which was identical with a sample prepared previously.[7]

The success of the above experiment encouraged undertaking of the synthesis of GDPM (I) by an analogous procedure. Both trioctylammonium salt of *α-d*-mannose 1-phosphate and (III) were homogeneously soluble in pyridine. The mixture was set aside in a sealed tube at room temperature. After 6 days, GDPM increased to the maximum and a small amount of GMP and (III) were also detected on paper chromatogram. Further standing had no favorable effect on the yield of GDPM. Then the reaction mixture was subjected to an ion exchange chromatography to isolate GDPM from contaminants. GDPM was obtained in 44% yield, estimated spectrophotometrically from (III). Owing to technical loss during the purification procedure of reprecipitation, the final yield of isolated GDPCa was 32%. This was homogeneous by the criteria of paper chromatogram with several solvent systems (see Table I) and quite identical with the natural GDPM.*4

Table I.

	R_{ad}		Rf	
Solvent	(4)	(5)	(6)	(7)
GDPM-Ba (natural)	0.46	0.18	0.79	0.80
GDPM-Ca (synthetic)	0.46	0.18	0.79	0.81

Furthermore, acid hydrolysis of synthetic specimen gave a similar result as reported by Leloir and Cabib[2] (see Experimental).

On the basis of these evidences, GDPM obtained by chemical synthesis was confirmed to have the structure of P^1-guanosin-5'-yl P^2-*α-d*-mannopyranos-1-yl pyrophosphate and to be identical with the natural product.

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Experimental

Paper chromatography was carried out with the following solvent systems (a, ascending; d, descending)

(1) *iso*-PrOH:NH_4OH:H_2O (7:1:2), d.[4d] (2) *iso*-PrOH:1% $(NH_4)_2SO_4$ (2:1), d.[4d] (3) *n*-BuOH:AcOH:H_2O (4:1:5), a.[8] (4) EtOH:1*N* AcOH (75:30) adjusted to pH 3.3 with NH_4OH, a.[2] (5) EtOH:1*N* $AcONH_4$ (70:30), a.[2] (6) *iso*-AmOH:5% KH_2PO_4 (0.5:1 cc., a.[9] (7) Satd. $(NH_4)_2SO_4$:H_2O:*iso*-PrOH (79:19:2), d.[10]

*4 This was a gift from Dr. L. F. Leloir, to whom the authors express sincere gratitude.

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Detection: Base by UV absorption. P by $HClO_4$-molybdate spray,[11] and reducing sugar by aniline hydrogen phthalate spray.[12]

Guanosine 5′-(N-Cyclohexylphosphoramidate) (III)—GMP (340 mg.) was dissolved in dimethylformamide (30 cc.) and cyclohexylamine (500 mg. in 30 cc. DMF) was added. Resulting suspension was magnetically stirred and *tert*-BuOH solution of DCC (1.5 g.) was added. The mixture was heated at 70° for 8 hr. in a stoppered flask. After 6 hr., the solution became homogeneous. From the cooled solution, precipitated dicyclohexylurea and unreacted GMP were filtered off and the filtrate was evaporated to a small volume under a reduced pressure, water was added, and extracted with ether. Water layer was evaporated *in vacuo*, the resulting vitreous residue solidified on dropwise addition of dehyd. acetone, and the solid was crystallized from acetonitrile to crystals of m.p. 183~185° (decomp.); yield, 310 mg. (42%). *Anal.* Calcd. for $C_{35}H_{60}O_7N_9P$: C, 56.3; H, 8.08; N, 16.8. Found: C, 55.28; H, 8.40; N, 15.26. Paper chromatography in solvent system (1) showed Rf 0.63.

P^1-Guanosin-5′-yl P^2-Benzyl Pyrophosphate (IV) and GDP (V)—A solution of (III) (15 mg.) and benzyl hydrogen phosphate (35 mg.) dissolved in anhydr. pyridine (2 cc.) was allowed to stand for 3 days at room temperature. Paper chromatography of this solution in solvent (2) showed the spots of GDP-benzyl (IV), unreacted amidate (III), trace of GMP, and GDP at Rf 0.40, 0.52, 0.27, and 0.06, respectively. After pyridine was evaporated, water was added to the residue and adjusted to pH 4.5 with AcOH. When the solution was hydrogenated over Pd-C, GDP was the major product and GDP-benzyl disappeared from the paper chromatogram.

Guanosine Diphosphate Mannose (GDPM) (I)—Trioctylammonium *α-d*-mannos-1-yl phosphate[13] (1.07 g., 1.67 m.moles) was dried azeotropically with anhydr. bezene and dissolved in anhydr. pyridine (20 cc.), (III) (360 mg., 0.48 m.mole) was added to it, and the clear solution was sealed and stored at 20° for 6 days. Paper chromatography in solvent (3) showed the presence of GDPM (R_{ad} 0.24) and a small amount of GMP (R_{id} 0.6) and (III) (R_{ad} 1.2).

Pyridine was evaporated under a reduced pressure and water containing AcONa was added to the residue. The aqueous solution was extracted with ether, adjusted to pH 7.0, and applied to a column (1.8×7 cm.) of Amberlite IRA-400 (Cl-form, 10~200 mesh). After washing with water, the column was eluted with 0.003*N* HCl containing $CaCl_2$. GMP and (III) were eluted with 0.015*N* $CaCl_2$ and GDPM was eluted with 0.1*N* $CaCl_2$ (optical density unit at 260 mμ, 2400, 44%). The GDPM fraction was collected, adjusted to pH 6.5 with $Ca(OH)_2$, concentrated to a small volume, and then freeze-dried. To the resulting solution, dehyd. EtOH and Et_2O (1:1) mixture was added and centrifuged.[14] Precipitated Ca salt of GDPM was washed with a mixture of dehyd. EtOH and Et_2O, and dehyd. Et_2O, and dried *in vacuo*. Reprecipitation with water and EtOH gave 120 mg. (32%) of Ca salt of GDPM. It was 83.5% pure on a weight basis as estimated by spectrophotometry. *Anal.* Calcd. for GDPM-Ca·$7H_2O$: P, 8.05; guanosine: phosphorus: mannose, 1:2:1. Found: P, 7.85; guanosine: phosphorus[15]: mannose,[16] 1.0:1.96:0.97.

The free nucleotide (0.2 m.mole/cc.) was hydrolyzed by its own acidity[2] on heating for 15 min. at 100° in a sealed tube, the products being GDP (Rf 0.03 and R_{id} 0.46 in solvents (3) and (4)) and mannose (Rf 0.3 and R_{ad} 1.2 in solvents (3) and (4)). GMP, guanine, and inorganic phosphate were detected in the 180-min. hydrolyzate (R_{ad} 0.61, 0.65, and 0.95 in solvent (4), respectively). The chromatographic data of natural GDPM-Ba and synthetic GDPM-Ca are shown in Table I.

Summary

Guanosine diphosphate mannose (GDPM), coenzyme of mannose polymerization, was chemically synthesized. GMP and cyclohexylamine were condensed to form N-cyclohexylphosphoramidate in the presence of DCC. The amidate (III) was reacted with benzyl hydrogen phosphate and after hydrogenation led to GDP (V). (III) and *α-d*-mannos-1-yl phosphate were condensed in pyridine and, after isolation and purification by ion-exchange chromatography, 83.5% pure GDPM was obtained in 32% yield. The synthetic GDPM was quite identical with the natural one in all respects.

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