weil der mit der Quaternisierung verstärkte induktive Effekt des Chinuclidinium-Stickstoffs den benachbarten C-3-Wasserstoff beweglicher machen würde.

Pharmazeutische Fakultät,
Universität Tokyo,
Hongo, Tokyo.

Eiji Ochiai (落合英二)
Masayuki Ishikawa (石川正幸)

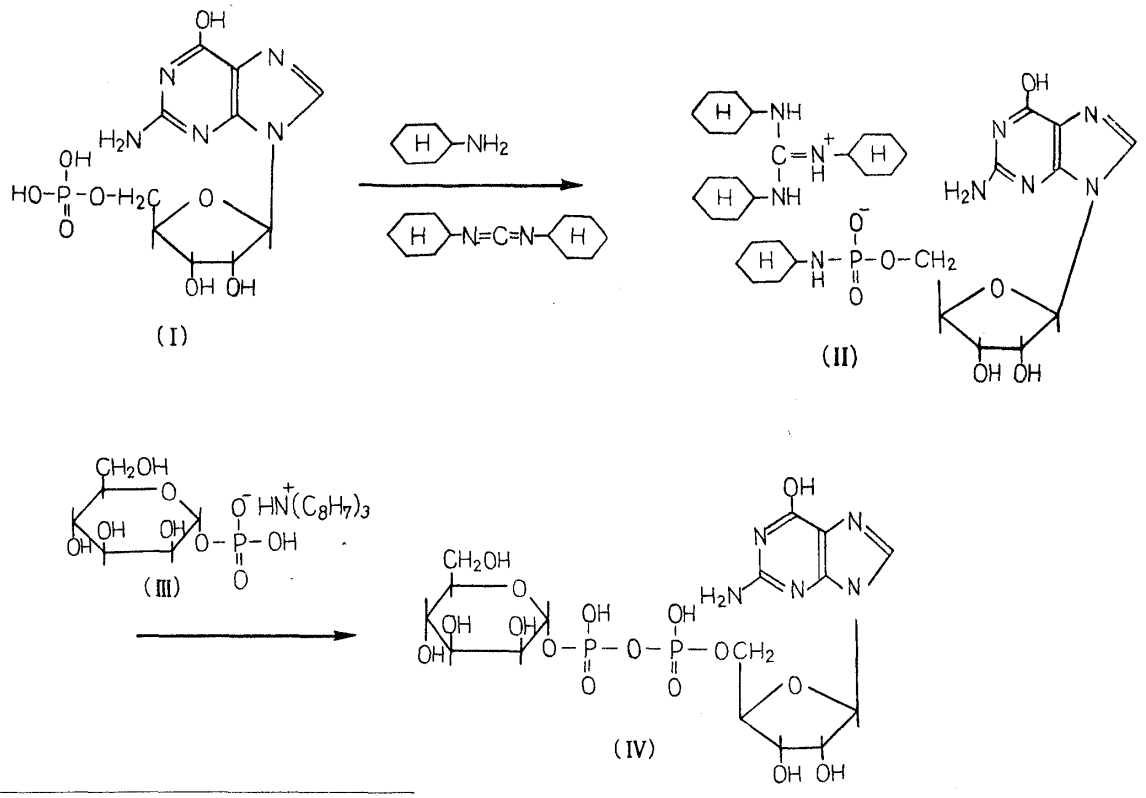
den 13. Januar, 1959

UDC 547.857.7-456-457-118.5.07

Synthesis of Guanosine Diphosphate Mannose

Quite recently, knowledge about coenzymes of the constitution of nucleoside diphosphate sugar has greatly accumulated. Among these, UDPG* was first synthesized by Todd, *et al.* with the aid of DCC method[1] and later by phosphorochloridate method.[2] In 1958, Khorana[3] developed an elegant method for synthesis of substances such as UDPG, utilizing nucleoside phosphoramidate as an important intermediate.

Earlier report[4] from this laboratory showed a new synthesis of GDP by means of



* Following abbreviations are used : UDPG uridine diphosphate glucose, DCC dicyclohexyl carbodiimide, GMP guanosine monophosphate, GDP guanosine diphosphate, GDPM guanosine diphosphate mannose, R_{Ad} $R_{Adenosine}$.

1) G.W. Kenner, A.R. Todd, K.F. Webb : J. Chem. Soc., **1954**, 2843.
2) A.M. Michelson, A.R. Todd : *Ibid.*, **1956**, 3459.
3) J.G. Moffatt, H.G. Khorana : J. Am. Chem. Soc., **80**, 3756(1958).
4) Presented at the 2nd Hokkaido Local Meeting of the Pharmaceutical Society of Japan, October, 1958.

phosphoramidate method. In this communication will be described the first chemical synthesis of GDPM[5] by a method including the use of substituted phosphoramidate.

The condensation of GMP (I) with cyclohexylamine in the presence of excess DCC gave a crystalline tricyclohexylguanidium salt of guanosine 5′-(N-cyclohexylphosphoroamidate)(II), m.p. 183~185°(decomp.), from acetonitrile (*Anal.* Calcd. for $C_{35}H_{60}O_7N_9P$: C, 56.3; H, 8.03; N, 16.8. Found : C, 55.28; H, 8.40; N, 15.26. Rf 0.63 (*iso*-PrOH : NH_3 : H_2O= 7:1:2)).

(II) was reacted with benzyl dihydrogen phosphate in pyridine at room temperature for 3 days. P^1-Guanosin-5′-yl-P^2-benzyl pyrophosphate was detected on paper chromatogram at Rf 0.40 (*iso*-PrOH : 1%$(NH_4)_2SO_4$=2:1). Hydrogenation of the crude product of above reaction in the presence of Pd-C gave GDP, R_{Ad} 0.14 (EtOH : AcOH=75:30, adjusted to pH 3.8 with NH_4OH).[5]

α-D-Mannose 1-phosphate[6] (III) was converted to its trioctylammonium salt and dried over P_2O_5. A solution of 56 mg. of (II) (0.073 m mole) and 270 mg. of the above salt (0.55 m mole) dissolved in 5 cc. of pyridine was set aside in a stoppered flask with exclusion of moisture at room temperature for 4 days. From the reaction mixture GDPM (IV) was detected as an UV-absorbing, organic phosphorus-containing spot, R_{Ad} 0.24 (solvent same with above), 0.27 (EtOH : 1*M* $AcONH_4$ buffer=30:70, pH 7.5),[5] accompanied with the spots proved to be of GMP-cyclohexyl amidate, GDP, and mannose 1-phosphate. The yield of GDPM was 16%, calculated on the basis of UV-absorption.

A part of the reaction mixture was analyzed on the anion exchanger (Amberlite IRA-400, 200~400 mesh, 0.7 cm^2×8 cm) which was eluted by linear gradient of Cl^- concentration with HCl-$CaCl_2$ system. Fractions obtained from Tubes No. 12 to 16 (each 20 cc.), and from No. 22 to 27 were separately collected and lyophilized as its Ca salt. From first portion GMP and GMP-amidate, and from second portion GDP and GDPM were detected on paper chromatogram as the spots having known R_{Ad} values. On hydrolysis of this GDPM-Ca with 0.01*N* HCl at 100° for 15 min.,[5] GDP alone was detected. Mannose was also detected from the hydrolysate by the aniline hydrogen phthalate reagent.[7]

On the basis of these evidences, it was concluded that GDPM thus obtained has the same constitution as the natural coenzyme.

Pharmaceutical Institute,
School of Medicine,
University of Hokkaido,
Sapporo.

Tohru Ueda (上田 亨)
Eiko Ohtsuka (大塚栄子)

January 22, 1959

5) E. Cabib, L. F. Leloir : J. Biol. Chem., **206**, 779(1954).
6) T. Posternak, J. P. Rosselet : Helv. Chim. Acta, **36**, 1614(1952).
7) S. M. Partridge : Nature, **164**, 443(1954).