

Communications to the Editor

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**Several 2,3-Cyclic Phosphates of D-Ribofuranoside and their Properties
as Substrate of Ribonuclease**

In the hydrolysis of phosphodiester bonds involved in ribonucleic acid by pancreatic ribonuclease (RNase-I), the cleavage occurs specifically at the 5'-O-P linkage of inter-nucleoside phosphate bond which binds 3'-hydroxyl group of pyrimidine nucleoside to 5'-position of the other nucleosides.

The initial products of this reaction consist of pyrimidine 2',3'-cyclic nucleotides and oligonucleotides containing similar nucleotides as their end groups, and on further digestion by the same enzyme, these 2',3'-cyclic phosphate groups are specifically hydrolyzed to 3'-phosphates.^{1,2)}

The interesting substrate specificity of this enzyme was also confirmed using several synthetic substrates. Thus Todd and Brown³⁾ reported that the only nucleoside 3'-benzylphosphate containing naturally occurring pyrimidine bases, but not purine bases, could be attacked by the enzyme to give corresponding 2',3'-cyclic nucleotide which is hydrolysed specifically to 3'-nucleotide by further incubation with the same enzyme.

These observations, together with further reports that both apurinic acid⁴⁾ and the type-b specific substance of *Haemophilus influenza*, a polymer of 1,1'-ribosylriboside combined intermolecularly by phosphate linkages at their 3-5 and 3'-5' hydroxyl groups, are active as a substrate for this enzyme,⁵⁾ lead to an assumption that minimum structural requirement for the compounds hydrolyzed by this enzyme might be a phosphodiester type of ribofuranoside-3-phosphate containing no purine base in its 1-position¹⁾ or of alkyl group which contains a vicinal hydroxyl capable of forming a cyclic phosphate with neighboring O-phosphate group.^{6,7)}

Under these circumstances, it seemed interesting to test several five-membered cyclic phosphates for their properties as a substrate of RNase-I.

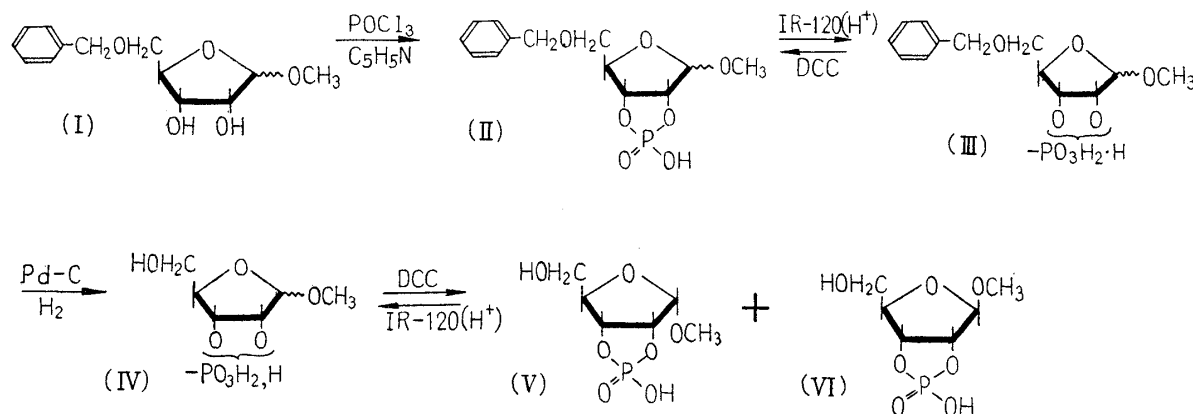
Ethylene glycol cyclic phosphate, glycerol 1,2-cyclic phosphate, and hydrobenzoin cyclic phosphate, the syntheses of which have already been reported,⁸⁻¹⁰⁾ were incubated with RNase-I under the conditions reported by Todd, *et al.*³⁾ However, no hydrolytic cleavage of the cyclic phosphate ring was observed in any of the cases, and these results prompted the syntheses of simple riboside-2,3-cyclic phosphate in order to test their properties as a substrate for RNase-I.

This communication describes the syntheses of methyl 5-O-benzyl-D-ribofuranoside 2,3-cyclic phosphate and methyl α - and β -D-ribofuranoside 2,3-cyclic phosphates, and their properties including their activity as a substrate of RNase-I.

Methyl 5-O-benzyl-D-ribofuranoside (I) was phosphorylated with POCl₃ in pyridine. On paper

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chromatographic test,* the reaction mixture gave a major phosphate spot (Rf 0.87) with two minor spots (Rf 0.94 and 0.01). After removal of pyridine and subsequent hydrolysis with Amberlite IR-120(H⁺), the major product, methyl 5-O-benzyl-D-ribofuranoside 2,3-cyclic phosphate (II), was converted into a mixture of methyl 5-O-benzyl-D-ribofuranoside 2- and 3-phosphates (III) which gave Rf of 0.66. This mixture was separated from the contaminating compound (Rf 0.94) by use of cellulose column chromatography eluting with a mixed solvent of *iso*-PrOH and 5*N* NH₄OH (2:1). (III) was converted into dicyclohexylammonium salt and crystallized in needles (*Anal.* Calcd. for C₂₅H₄₅O₈N₂P·H₂O : C, 54.55; H, 8.55; N, 5.09; P, 5.63. Found : C, 54.19; H, 8.18; N, 4.73; P, 5.37).



After decationization, (III) was treated with dicyclohexylcarbodiimide (DCC) in acetonitrile to obtain a mixture of anomers, methyl 5-O-benzyl- α - and - β -D-ribofuranoside 2,3-cyclic phosphates (II), which was isolated and purified as Na salt by precipitation with *iso*-PrOH from syrupy MeOH solution (*Anal.* Calcd. for C₁₈H₁₆O₇PNa·H₂O : C, 43.82; H, 5.06. Found : C, 43.95; H, 4.85). On electrometric titration, (II) showed no secondary dissociation between pH ranges of 2 and 10.

The dicyclohexylammonium salt of (III) was catalytically debenzylated with Pd-C and the reaction mixture revealed a new phosphorus spot with Rf value of 0.3 on paper chromatogram with simultaneous disappearance of that of the starting material (Rf 0.66). From the reaction mixture the product, a mixture of methyl D-ribofuranoside 2- and 3-phosphates (IV), was isolated as dicyclohexylammonium salt which was recrystallized to needles from EtOH-Et₂O (*Anal.* Calcd. for C₁₈H₃₉O₈N₂P· $\frac{1}{2}$ H₂O : C, 47.89; H, 8.86; N, 6.20; P, 6.87. Found : C, 48.18; H, 8.94; N, 6.34; P, 6.26).

The dicyclohexylammonium salt of (IV) was decationized and dehydrated with DCC in a mixed solvent of pyridine and acetonitrile and the products were converted into NH₄ salts which, on paper chromatogram, gave two spots of main products (Rf 0.66 and 0.64). These two main products, separated from each other on a sheet of paper chromatogram, were extracted with water and isolated to give NH₄ salts of methyl α - and β -D-ribofuranoside 2,3-cyclic phosphates, (V) and (VI), which gave Rf values of 0.66 and 0.64, respectively. (V) was converted into Na salt and crystallized from acetone to needles (*Anal.* Calcd. for C₆H₁₀O₇PNa : C, 29.03; H, 4.03. Found : C, 29.23; H, 4.12. $[\alpha]_D^{20} +93.6^\circ$ ($c=1.0$, H₂O)). (VI) also gave a Na salt which was precipitated from MeOH solution with Et₂O to give crystals (*Anal.* Calcd. for C₆H₁₀O₇PNa : C, 29.03; H, 4.03. Found, C, 29.10; H, 4.11. $[\alpha]_D^{20} -34.2^\circ$ ($c=1.0$, H₂O)).

Both (V) and (VI) were easily hydrolysed with Amberlite IR-120(H⁺) in aqueous solution at room temperature to give the products with the same Rf values of 0.30 which was identified with (IV) on paper chromatogram. Further, (V) and (VI) were alcoholized with BuOH and catalytic amount of CF₃-COOH to the compounds giving Rf values of 0.85 and 0.84, respectively. (V) and (VI) were also detected on paper chromatogram of the products obtained by catalytic hydrogenation of (II) with an additional spot (Rf 0.23) of an unknown compound, whose structure is being examined.

On incubation of (II), (V), (VI), and ammonium cytidine 2',3'-cyclic phosphate with pancreatic RNase according to the conditions described by Todd, *et al.*, the enzymatic hydrolysis was observed only for cytidine 2',3'-cyclic phosphate.

Further studies on syntheses of derivatives of D-ribofuranoside 2,3-cyclic phosphate having a more closely related structure with those of naturally occurring pyrimidine nucleotides are now in progress.

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* Solvent system and paper used for paper chromatography were *iso*-PrOH:5*N* NH₄OH (2:1) and Toyo Roshi No. 53.

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Faculty of Pharmaceutical Sciences
University of Tokyo,
Hongo, Tokyo

Tyunosin Ukita (浮田忠之進)
Masachika Irie (入江昌親)

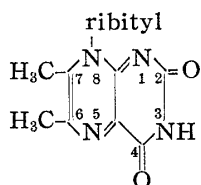
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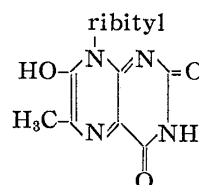
On the Nomenclature of the Fluorescent Substances produced in the Culture of *Eremothecium ashbyii*

One of the writers (Masuda) discovered in the culture broth and mycelium of *Er. ashbyii* a green fluorescent (G-compound) and a violet fluorescent substances (V-compound) in addition to riboflavin, flavin adenine dinucleotide (FAD), and others.¹⁾ These fluorescent substances were isolated in a pure state and their properties and structures were studied. First of all, a photodecomposition product of G-compound was confirmed to be identical with 6,7-dimethyl-2(1*H*),4(3*H*)-pteridinedione and this fact suggested the structure of 6,7-dimethyl-8-ribityl-2(3*H*),4(8*H*)-pteridinedione (I) for the compound.²⁾ The compound was further found to undergo condensation *in vitro* with diacetyl or with acetoin to give riboflavin.³⁾ The V-compound was assumed to be 6-methyl-7-hydroxy-8-ribityl-2(3*H*),4(8*H*)-pteridinedione (II) as its photodecomposition product was in complete agreement with the synthesized 6-methyl-7-hydroxy-2(1*H*),4(3*H*)-pteridinedione.⁴⁾

However, there was a fear that the names G- and V-compounds might cause confusion because such names are also used for groups of other compounds, and it is proposed to call the G-compound 6,7-dimethylribolumazine and the V-compound 6-methyl-7-hydroxyribolumazine.⁵⁾ Since in this case the ribolumazine means the attachment of a ribityl group to N-8 of lumazine, both compounds could be clearly distinguished by the new nomenclature.



(I) (G-Compound)
6,7-Dimethylribolumazine



(II) (V-Compound)
6-Methyl-7-hydroxyribolumazine

Research Laboratories,
Takeda Pharmaceutical Ind., Ltd.,
Juso-nishino-cho, Higashiyodogawa-ku
Osaka, Japan.

Satoru Kuwada (桑田 智)
Toru Masuda (増田 亨)
Toyokazu Kishi (貴志豊和)
Mitsuko Asai (浅井満子)

April 20, 1958

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- 5) Proposal made at the symposium held on February 28, 1958, under the auspices of the Vitamin B Committee.