Communications to the Editor

UDC 547.963.3 : 577.155.2

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 internucleoside 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 Brown[3] 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 acid[4] 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-position[1] 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 $POCl_3$ in pyridine. On paper

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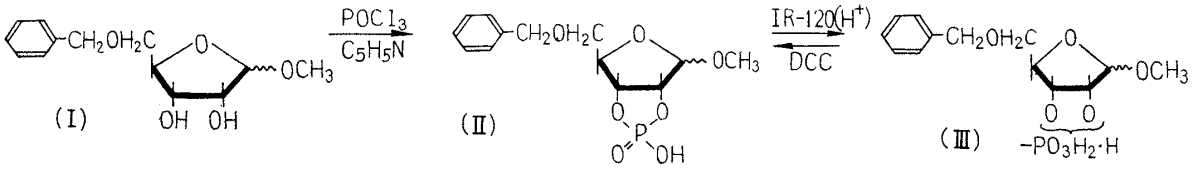
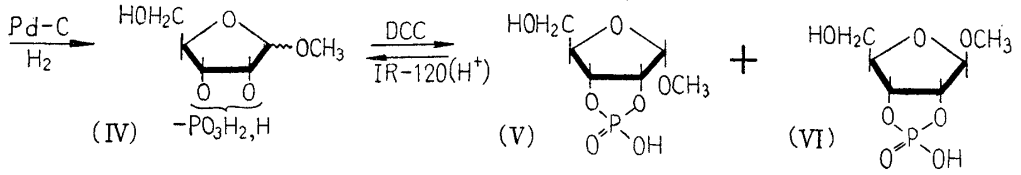
chromatographic test,* the reaction mixture gave a major phosphate spot (Rf 0.87) with two minor spots (Rf 0.94 and 0.01). After removal of pyridine and subsequent hydrolysis with Amberlite IR-120(H^+), the major product, methyl 5-O-benzyl-D-ribofuranoside 2,3-cyclic phosphate (II), was converted into 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 celullose column chromatography eluting with a mixed solvent of *iso*-PrOH and 5*N* NH_4OH (2:1). (III) was converted into dicyclohexylammonium salt and crystallized in needles (*Anal.* Calcd. for $C_{25}H_{45}O_8N_2P \cdot H_2O$: 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 $C_{18}H_{16}O_7PNa \cdot H_2O$: 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-Et_2O (*Anal.* Calcd. for $C_{18}H_{39}O_8N_2P \cdot \frac{1}{2}H_2O$: 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 NH_4 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 NH_4 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 $C_6H_{10}O_7PNa$: C, 29.03; H, 4.03. Found : C, 29.23; H, 4.12. $[\alpha]_D^{20}$ +93.6° (c=1.0, H_2O)). (VI) also gave a Na salt which was precipitated from MeOH solution with Et_2O to give crystals (*Anal.* Calcd. for $C_6H_{10}O_7PNa$: C, 29.03; H, 4.03. Found, C, 29.10; H, 4.11. $[\alpha]_D^{20}$ −34.2° (c=1.0, H_2O)).

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 alcoholyzed with BuOH and catalytic amount of CF_3-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.

The expenses for this research was supplied by Grant in Aid for Scientific research provided

* Solvent system and paper used for paper chromatography were *iso*-PrOH:5*N* NH_4OH (2:1) and Toyo Roshi No. 53.

by the Ministry of Education for which authors wish to extend their gratitude. Thanks are also due to Mr. B. Kurihara, Miss R. Ohta, and Miss S. Ohno for microanalyses.

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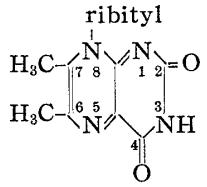
June 4, 1958

UDC 547.854'861.6 : 582.284

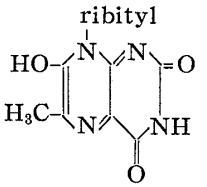
On the Nomenclature of the Fluorescent Substances produced in the Culture of *Eremothecium ashbyii*

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

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April 20, 1958

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1) T. Masuda : This Bulletin, **3**, 434(1955).
2) *Idem.* : *Ibid.*, **4**, 375(1956).
3) *Idem.* : *Ibid.*, **5**, 136(1957).
4) T. Masuda, T. Kishi, M. Asai : *Ibid.*, **6**, 113, 291(1958).
5) Proposal made at the symposium held on February 28, 1958, under the auspices of the Vitamin B Committee.