contains 6-methyloctanoic acid. By applying polymyxin acylase[5], which was isolated from soil bacteria, to circulin B, deacyl circulin B was obtained. From the acid hydrolysate of DNP-derivative of deacyl circulin B, di-DNP-Dab was detected as the *N*-terminal amino acid, and the molar ratios of free amino acids in the acid hydrolysate of DNP-derivative of circulin B were found to be Thr:Leu:Ile:Dab = 2:1:1:1. These results suggested that circulin B does not have the structure proposed by KOFFLER and KOBAYASHI[2].

Then, using our technique with Nagarse[3] (subtilo peptidase A, EC 3.4.4.16), we obtained the following fragments: (I) Ioa → (α)Dab → Thr, (II) Ioa → (α)Dab → Thr → (α)Dab, (III) Dab, (IV) a cyclic heptapeptide having the molar ratios of amino acids of Thr:Leu:Ile:Dab = 1:1:1:4. Applying the SANGER's DNP method to IV we detected α-DNP-Dab, which could not be found in the completely dinitrophenylated circulin B. The AKABORI's hydrazinolysis method[6] failed to find the carboxy terminal amino acid of peptide IV. The Rf value of IV was identical with that of the cyclic heptapeptide obtained from circulin A by the enzymatic hydrolysis using Nagarse, by comparison with their Rf values in paper chromatography. In addition, considering the chemical structures of other polymyxin series antibiotics[4], the amino acid sequence of peptide IV was found to be cyclo(γ)Dab → (α)Dab → Leu → Ile → (α)Dab → (α)Dab → Thr →.

On the basis of these results, circulin B has a similar structure to circulin A as shown in Scheme 2 and only differs from circulin A in having Ioa in place of Moa.

Zusammenfassung. Es wird über die Strukturaufklärung von Circulin B, einem Antibiotikum aus *Bacillus circulans* ATCC 14040, berichtet, dem die Formel des Schemas 2 zukommt.

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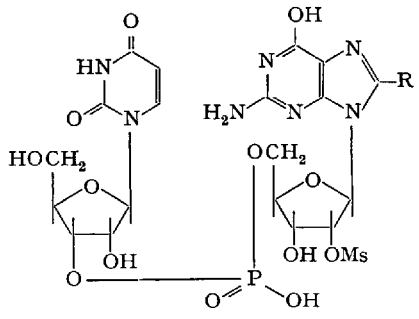
The Synthesis of a Natural Dinucleoside Phosphate Derivative with the Aid of a Purine Cyclonucleoside

In a previous communication[1] we described the use of an 8,5′-*o*-purine cyclonucleoside for the synthesis of a dinucleoside phosphate. As this procedure yielded a product having a hydroxyl group at position 8 of the purine moiety, it was of interest to study the opening of the anhydro linkage of an 8,5′-S-purine cyclonucleoside by the attack of a nucleoside phosphate anion. Such an approach would lead to the formation of a dinucleoside phosphate with a mercapto group at position 8 of the purine nucleus, which could be removed by treatment with Raney Ni, thus resulting in a product having no unnatural substituents.

8,5′-Anhydro-8-mercapto-2′-*o*-mesylguanosine[2] (200 mg) was, therefore, refluxed with 1.2 equivalents of tri-*n*-butyl ammonium 3′-uridylate in dry DMF for 14 h. The dinucleoside phosphate, uridyl-(3′-5′)-8-mercapto-2′-*o*-mesylguanosine (I), was isolated by preparative paper chromatography on Whatman 3 mm paper[3] in 41% yield. Rf, 0.37; λ_{max}, 0.1 *N* HCl-265.5, 295 (sh) nm; 0.1 *N* NaOH 268, 285 (sh) nm. Formic acid hydrolysis of this product gave uracil and 8-mercaptoguanine. Paper chromatographic analysis of the snake venom phosphodiesterase hydrolysate showed that uridine and another substance, presumably 8-mercapto-2′-*o*-mesylguanosine-5′-phosphate, were formed. That the methyl sulphonate group at the 2′ position in the purine cyclonucleoside was not displaced (or displaced only to a very small extent) by the phosphate anion was shown by the presence of absorption at 1170 cm^{-1} (Nujol) in the IR-spectrum of I. This is consistent with an observation made earlier that it is difficult to remove this group[2].



I. R = SH; II, R = H

Uridyl-(3′-5′)-8-mercapto-2′-*o*-mesylguanosine (I) was refluxed with Raney Ni in aqueous ethanol (1 :1) for 3.5 h. Filtration of the catalyst and evaporation of the solvent yielded uridyl-(3′-5′)-2′-*o*-mesylguanosine (II) in 75% yield, Rf 0.40[3]; λ_{max}, 0.1 *N* HCl-260, 0.1 *N* NaOH-262 nm. This product contained sulphur, had mesyl absorption in the IR and yielded guanine and uracil on formic acid hydrolysis[4].

Zusammenfassung. Die Behandlung von 8-5′-Anhydro-8-mercapto-2′-*o*-mesylguanosin mit (1,2 Äquivalent) Tributylammonium-uridylat in siedendem DMF lieferte das Dinucleosidphosphat Uridyl-(3′-5′)-8-mercapto-2′-*o*-mesylguanosin. Weitere Behandlung mit Raney Ni führte zum Uridyl-(3′-5′)-2′-*o*-mesylguanosin.

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Central Drug Research Institute, Lucknow (India), 4 March 1968.

[1] K. L. NAGPAL and M. M. DHAR, Tetrahedron Lett. *47* (1968).

[2] M. IKEHARA, H. TADA and K. MUNEYAMA, Chem. pharm. Bull., Tokyo *13*, 639 (1965).

[3] Descending paper chromatography, solvent: *n*-butanol-acetic acid-water (4:1:5).

[4] Communication No. 1252 from the Central Drug Research Institute.