noch nicht beschriebenen 6-Chlor-Campheroxims (II) entspricht. Über chemischen Abbau und Stereochemie dieser Verbindung wird gesondert berichtet.

6-Chlor-Campheroxim entsteht auch als Nebenprodukt beim Einwirken von Nitrosylchlorid auf α-Pinen sowie bei der Reaktion von Chlorwasserstoff mit Pinocarvonoxim. Die hierbei erzielten Ausbeuten sind jedoch nur gering.

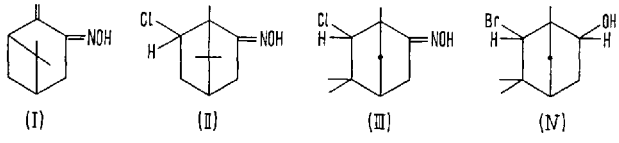
Im Zuge der Konstitutionsaufklärung des 6-Chlor-Campheroxims stellten wir uns zu Vergleichszwecken «Pinocarvonoximhydrochlorid»[1] her.

Das NMR-Spektrum dieser Verbindung zeigt neben den Signalen für die Methylgruppen (1,0, 1,16 und 1,26 ppm) und dem Singulett des Oxim-Protons bei 8,7 ppm ein weiteres Singulett bei 3,7 ppm. Das Auftreten dieses Signals sowie das Fehlen einer charakteristischen Bande für die Partialstruktur $Cl-\overset{|}{\underset{|}{C}}-CH_3$[2] schliesst die von TREIBS[1] vorgeschlagene Struktur eines Pinan-Derivates aus. Eine Camphan-Struktur kommt ebenfalls nicht in Betracht. Dagegen lässt sich das Singulett gut durch das Isofenchan-Grundgerüst erklären. Es entspricht danach dem Proton C_6, das durch den stark negativen Cl-Substituenten am gleichen C-Atom nach tieferem Feld verschoben wird. Der Verbindung von TREIBS[1] muss daher die Struktur des 6-Chlor-Isofenchonoxims (III) zugeschrieben werden. Unsere Ergebnisse ergänzen Feststellungen von HARTSHORN und WALLIS[3], wonach beim Einwirken von Bromwasserstoff auf Pinocarveol ebenfalls ein Derivat des Isofenchans, und zwar der 6-Brom-Isofenchylalkohol (IV) entsteht.

Summary. The nitrosochloride of pinene dissolved in benzene will be transformed quantitatively by adsorbing to SiO_2 to the not yet described 6-chlor-campheroxim. This compound is also a by-product in the reaction of α-pinene with nitrosylchloride.

C. H. BRIESKORN und W. RUSSOW[4]

Institut für Pharmazie und Lebensmittelchemie der Universität, 87 Würzburg (Deutschland), 21. Februar 1968.

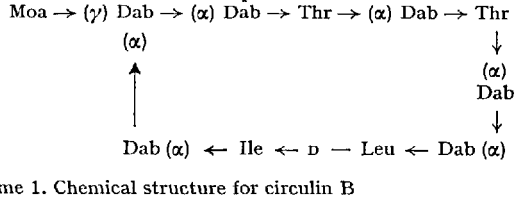


(I) (II) (III) (IV)

[1] W. TREIBS, M. MÜHLSTÄDT, R. MEGGES und J. KLOTZ-HERDMANN, Justus Liebigs Annln Chem. *634*, 118 (1960).
[2] L. M. JACKMAN, *Application of NMR in Organic Chemistry* (London-Oxford-New York-Paris 1959, Pergamon Press 1959).
[3] M. P. HARTSHORN and A. F. A. WALLIS, Chemy Ind. 1878 (1963).
[4] Teil der Dissertation W. Russow, Univ. Würzburg 1967.

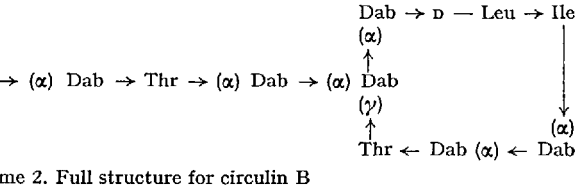
Chemical Structure of Circulin B

In 1949 circulins A and B were isolated from the culture fluid of *Bacillus circulans* Q-19[1]. In 1958 KOFFLER and KOBAYASHI[2] reported that the chemical structures of these antibiotics are cyclic decapeptides, and circulin B has a similar structure to circulin A, with the exception that the Moa[3] is attached through the γ-amino group of the Dab marked by an asterisk in the structure of circulin A (Scheme 1).



Scheme 1. Chemical structure for circulin B by KOFFLER and KOBAYASHI[2]

This seemed to be very unusual in the view of our results obtained in studies of the chemical structures of polymyxin series antibiotics[4]. Namely, colistins A and B, polymyxins B_1 and B_2, or polymyxins D_1 and D_2 differ from each other only in the nature of their fatty acid components. The result of our re-examination now proves the structure of circulin B to be that shown in Scheme 2.



Scheme 2. Full structure for circulin B

Circulin B was isolated from the culture fluid of *B. circulans* ATCC 14040 by ion exchange chromatography (Amberlite IRC-50, H^+-form) and by countercurrent distribution. The amino acid composition of the purified circulin B was confirmed as L-Thr:D-Leu:L-Ile:L-Dab in the molar ratio of 2:1:1:6. The methyl ester derivative of the fatty acid isolated from the acid hydrolysate of circulin B was found to be isooctanoic acid methyl ester by gas chromatography. This result conflicted with the report of KOFFLER and KOBAYASHI[2] that circulin B

[1] F. J. MURREY, P. A. TETRAUL, O. W. KAULMANN, H. KOFFLER, D. H. PETERSON and D. R. COLINGSWORTH, J. Bact. *57*, 305 (1949). – D. H. PETERSON and L. N. REINEKE, J. biol. Chem. *181*, 95 (1949). – J. H. DOWLING, H. KOFFLER, H. C. REITZ, D. H. PETERSON and P. A. TETRAULT, Science *116*, 147 (1952).
[2] H. KOFFLER and T. KOBAYASHI, Abstr. Div. Biol. Chem. Ann. Chem. Soc., San Francisco, April 13–18 (1958).
[3] The following abbreviations are used throughout this paper; Moa, (+)-6-methyloctanoyl residue; Ioa, isooctanoyl residue; Dab, α,γ-diaminobutyric acid residue; DNP, 2,4-dinitrophenyl residue; → =C to N bond in –CO–NH; → (α) Dab = → Dab; → (γ) Dab = → Dab. (γ–NH_2 / NH_2)
[4] T. SUZUKI, H. INOUYE, K. FUJIKAWA and Y. SUKETA, J. Biochem. *54*, 25 (1963). – T. SUZUKI, H. INOUYE, K. FUJIKAWA and S. NAGASAWA, J. Biochem. *54*, 173 (1963). – T. SUZUKI, K. HAYASHI and K. FUJIKAWA, J. Biochem. *54*, 412 (1963). – T. SUZUKI and K. FUJIKAWA, J. Biochem. *56*, 182 (1964). – T. SUZUKI, K. HAYASHI, K. FUJIKAWA and K. TSUKAMOTO, J. Biochem. *56*, 335 (1964). – K. FUJIKAWA, Y. SUKETA, K. HAYASHI and T. SUZUKI, Experientia *21*, 307 (1965). – K. HAYASHI, Y. SUKETA, K. TSUKAMOTO and T. SUZUKI, Experientia *22*, 354 (1966).

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.

K. HAYASHI, Y. SUKETA and T. SUZUKI

Institute for Protein Research, Osaka University, Osaka (Japan), 26 March 1968.

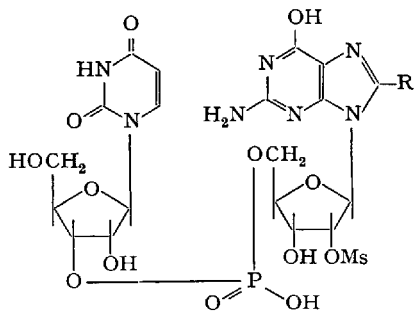
[5] T. SUZUKI, Y. KIMURA and K. IWADARE, Jap. Patent Gazette *12*, 101 (1966).

[6] S. AKABORI, K. OHNO and K. NARITA, Bull. Chem. Soc. Japan *25*, 214 (1952).

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.

P. C. SRIVASTAVA, K. L. NAGPAL and M. M. DHAR

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.