$\Sigma[\mu]^{20}_{D\,obs}$ of (−)-1/2-cyclohexanediol = $(1\beta)\lambda(2\alpha)$ = $-A = -11.73\{(n^2+2)/3\}\zeta^2_{OH} \equiv [M]^{20}_D$(W) of (−)-1/2-cyclohexanediol, −54.0° $\therefore \zeta^2_{OH} = 4.6036\{3/(n^2+2)\}$ (6)

This value is nearly equal to the value in Equation 5. This fact indicates that the values in Equations 5 and 6 are both fairly reliable. The values of A and B for $[\mu]^{20}_{D\,obs}$ in the square brackets in the Note of Table II, can be obtained by multiplying those for $[\mu]^{20}_{D\,calcd}\{3/(n^2+2)\}$ by the value of $\zeta^2_{OH}\{(n^2+2)/3)\}$, 4.7340. Of course, when using Equation 5, new values of $\Sigma[\mu]^{20}_{D\,obs}$ of polyhydroxycyclohexanes of C 1 conformation which are shown in Table I of the previous paper[7] are apparently different from the observed values of $[M]_D$(W). This fact can, however, be explained reasonably by the probable assumption that each of those polyhydroxycyclohexanes exists in the equilibrium mixture of C 1- and 1 C-conformers in its water solution. In this article, both of the series of values of A and B which are given in the Note of Table II, are used. When using these values in Equations 1 and 2, $\Sigma[\mu]^{20}_{D\,obs}$ of (−)-1,2/3,4-cyclohexanetetrol is calculated as 71.8 [or 93.2][9] (C 1 conformation and −141.7 [or −184.3][9] (1 C conformation). The observed value of $[M]^{20}_D$(W), −109.2°, of (−)-1,2/3,4-cyclohexanetetrol indicates the presence of 15.3% [or 27.1%][9] of C 1 conformation and 84.7% [or 72.9%][8] of 1 C conformation in the equilibrium state. From these concentrations, one may calculate[12] the conformational equilibrium constant, K, and from this the free energy difference, $\Delta F°$, between the two conformations (i.e. the strength of the adjacent *trans* hydrogen bonding force between $(OH)^{2\beta}$– and $(OH)^{3\alpha}$– plus the difference in solvation energy between the solvent, water, and each of the two conformers) may be calculated as below:

$$K = [1\,C]/[C\,1] = 84.7/15.3 = 5.54$$
$$[\text{or, } K = 72.9/27.1 = 2.69]$$

$$\Delta F° = -RT\ln K = -2.303\,R\,T\log(5.54) = -1.0 \text{ kcal/mole}$$

$$[\text{or, } \Delta F° = -2.303\,R\,T\log(2.69) = -0.6 \text{ kcal/mole}]$$

This value seems to be comparable with −1.1 kcal/mole[13], the usual value of the corresponding hydrogen bonding force (O–H··O) in CCl_4 solution[8].

Zusammenfassung. Mit Hilfe der PM-Methode ist die molekulare Rotation von (−)-1,2/3,4-Cyclohexantetrol untersucht worden. Daraus geht hervor, dass diese Verbindung in wässeriger Lösung als Gemisch der C 1- und 1 C-Konstellationen vorliegt.

S. YAMANA

Department of Chemistry, Kyoto Gakugei University, Fushimi-ku, Kyoto (Japan), January 5, 1965.

[12] R. A. PICKERING and C. C. PRICE, J. Am. chem. Soc. *80*, 4931 (1958).

[13] The $\Delta\nu$ of the *trans* hydrogen bond in 1/2-cyclohexanediol is 32 cm^{-1} [9], which corresponds to a $-\Delta H$ of 1.1 kcal/mole according to the JOESTEN-DRAGO equation[14] or a $-\Delta F$ of 1.1 kcal/mole, assuming $\Delta S \approx 0$.

[14] M. D. JOESTEN and R. S. DRAGO, J. Am. chem. Soc. *84*, 3817 (1962).

Chemical Structure of Circulin A

In the course of our work in the colistin[1] and polymyxin series[2] we investigated the structure of circulin A[3], another antibiotic exerting high activity against gram negative bacteria. KOFFLER and KOBAYASHI[4] deduced the chemical structure of this antibiotic tentatively as a cyclic decapeptide. This seemed to be very unusual in view of the close biological and chemical relationship between circulin A, the polymyxins and the colistins. The result of our re-examination now proves the structure of circulin A to be that shown in Figure 1[5], confirming that circulin A definitely belongs to the polymyxin family.

Circulin A produced by *Bacillus circulans* ATCC 14040 grown according to NELSON et al.[6] was isolated from the culture fluid mainly by ion exchange chromatography (Amberlite IRC-50, H^+-form) and CRAIG's counter-

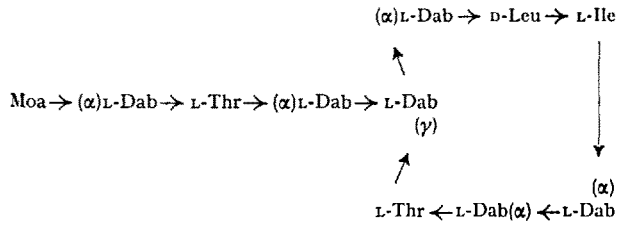

Fig. 1. Full structure for circulin A.

[1] T. SUZUKI, H. INOUYE, K. FUJIKAWA, and Y. SUKETA, J. Biochem. (Jap.) *54*, 25 (1963). – T. SUZUKI, H. INOUYE, K. FUJIKAWA, and S. NAGASAWA, J. Biochem. (Jap.) *54*, 173 (1963). – T. SUZUKI, K. HAYASHI, and K. FUJIKAWA, J. Biochem. (Jap.) *54*, 412 (1963). – T. SUZUKI and K. FUJIKAWA, J. Biochem. (Jap.) *56*, 182 (1964).

[2] T. SUZUKI, K. HAYASHI, K. FUJIKAWA, and K. TSUKAMOTO, J. Biochem. (Jap.) *56*, 335 (1964). – T. SUZUKI and K. HAYASHI, Abstr. of 2nd Symp. on *Peptide Chemistry*, Osaka, Japan (1963), p. 55.

[3] F. J. MURRAY and P. A. TETRAULT, Proc. Soc. Am. Bact. *1*, 20 (1948). – F. J. MURRAY, P. A. TETRAULT, O. W. KAUFMANN, 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).

[4] H. KOFFLER and T. KOBAYASHI, Abstr. Div. Biol. Chem. Ann. Chem. Soc., April 13–18 (1958), San Francisco.

[5] The following abbreviations are used throughout this paper: Moa = (+)-6-methyloctanoyl 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 α-NH_2

[6] H. A. NELSON, C. DEBOER, and W. H. DEVRIES, Ind. Eng. Chem. *42*, 1259 (1950).

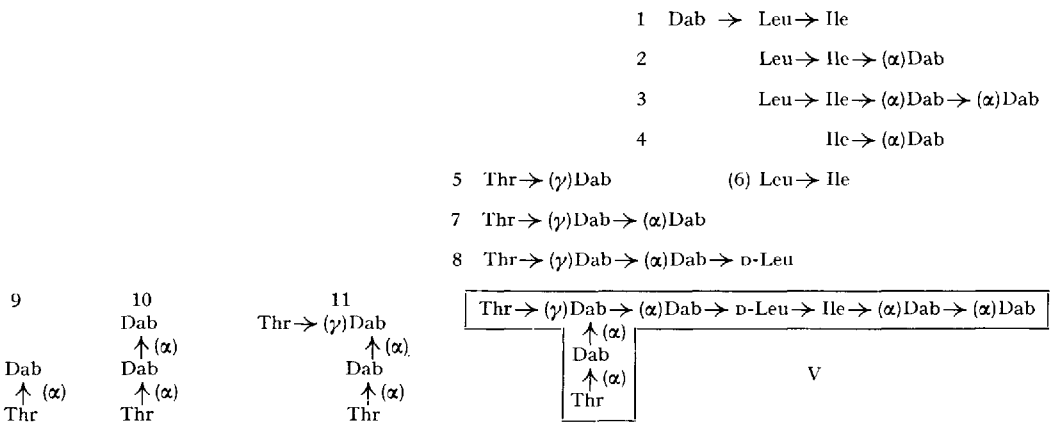

Fig. 2

current distribution using the systems *n*-butanol/sec butanol/2*N* acetic acid 2:1:5 (v/v) and *n*-butanol/2% dichloro acetic acid 1:1 (v/v). We were able to confirm the constituent amino acids L-Dab, L-Thr, L-Ile, D-Leu in the molar ratio 6:2:1:1 already reported[4] as well as the presence of (+)-6-methyl octanoic acid (gas chromatography).

Using our technique with Nagarse[1] (subtilo peptidase A [EC 3.4.4.16]) we obtained the following fragments: (I) Moa → (α) Dab → Thr, (II) Moa → (α) Dab → Thr → (α) Dab, (III) α,γ-diaminobutyric acid and (IV) a cyclic peptide having the amino acid molar ratio of 4:1:1:1 of α,γ-diaminobutyric acid : threonine : leucine : isoleucine. Upon dinitrophenylation of (IV) and hydrolysis of the resulting DNP-cyclic peptide, we detected α-DNP-Dab, which could not be found in completely dinitrophenylated circulin A. Considering the above results, the structure of circulin A was deduced to be Moa → (α) Dab → Thr → (α) Dab cyclic heptapeptide.

The cyclic part was not hydrolysed by Nagarse. However, information on its structure resulted from partial acid hydrolysis of the undegraded antibiotic. The fragments 1–11 (Figure 2) were obtained besides Dab, Moa → (α) Dab and other fragments. From the structure of the peptides 1–11 the existence of the open chain nonapeptide V sequence was deduced.

On the basis of these results circulin A must have the structure as shown in Figure 1.

Thus circulin A has a similar structure to polymyxin B_1 and colistin A. Circulin A differs from polymyxin B_1 in having D-Leu → L-Ile in place of D-Phe → L-Leu and from colistin A in having L-Ile in place of L-Leu[7].

Zusammenfassung. Es wird über die Strukturaufklärung von Circulin A, einem Antibioticum aus *Bacillus circulans* ATCC 14040, berichtet, dem die Formel der Figur 1 zukommt. Somit gehört auch Circulin A zur Polymyxinfamilie.

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[7] According to a personal communication from Dr. K. VOGLER of F. Hoffmann-La Roche & Co. Ltd., Basel (Switzerland), the optical rotatory curve of the purified circulin A is very similar with that of colistin A. This fact also suggested that circulin A had a structure similar to colistin A. We wish to express our thanks to Dr. K. VOGLER for valuable discussions about this study.

Bildung von substituierten Dischwefel-Nitriden aus Halogencyanen und Dimethylsulfoxid[1]

Bei der Einwirkung von Chlorcyan oder Bromcyan auf Dimethylsulfoxid beobachtet man die Entwicklung von CO_2 und es entstehen dabei farblose, kristallisierte Salze. Sie konnten als substituierte Dischwefelnitride (I)[2] identifiziert werden und besitzen die durch folgende Grenzformen umschriebene Struktur:

$$[(CH_3)_2S{=}\underline{N}{-}S^{\oplus}(CH_3)_2]X^{\ominus} \longleftrightarrow [(CH_3)_2S{=}\overset{\oplus}{N}{=}S(CH_3)_2]X^{\ominus}$$

$$\longleftrightarrow [(CH_3)_2\overset{\oplus}{S}{-}\underline{N}{=}S(CH_3)_2]X^{\ominus} \qquad \text{I}$$

X = Br, Cl

Der Reaktionsmechanismus beruht vermutlich auf der Bildung eines Adduktes aus zwei Molekülen Dimethylsulfoxid und einem Molekül Halogencyan und anschliessendem Zerfall der labilen Additionsverbindung unter Abspaltung von CO_2 (siehe Formeln Seite 309).

In ähnlicher Weise verläuft die kürzlich gefundene[3] Reaktion von Schwefelsäure-isocyanaten mit Dimethyl-

[1] Den Herren K.-O. ALT und Dr. H. WAGNER und ihren Mitarbeitern danken wir für Spektren und Analysen.

[2] M. BECKE-GOEHRING und H. P. LATSCHA, Angew. Chemie *74*, 695 (1962).

[3] R. APPEL und H. RITTERSBACHER, Chem. Ber. *97*, 852 (1964).