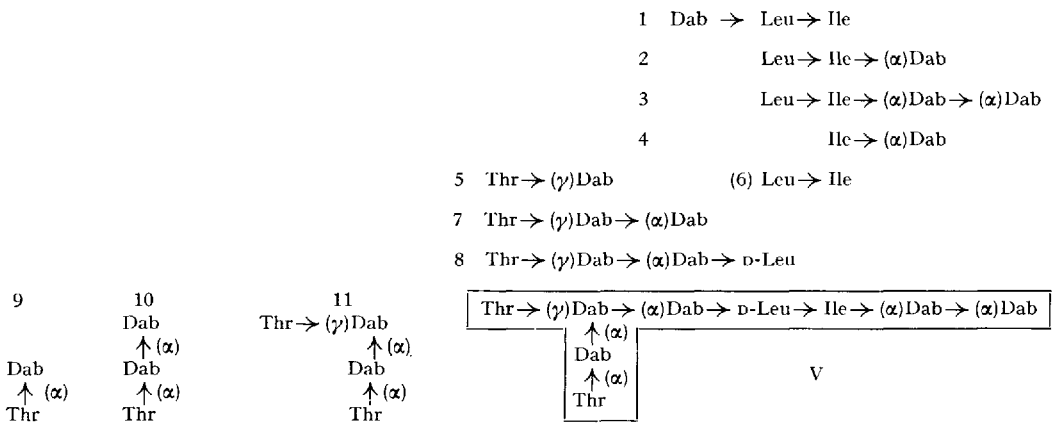


Fig. 2

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.

K. FUJIKAWA, Y. SUKETA,
K. HAYASHI, and T. SUZUKI

Institute for Protein Research, Osaka University, Osaka (Japan), April 1, 1965.

[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

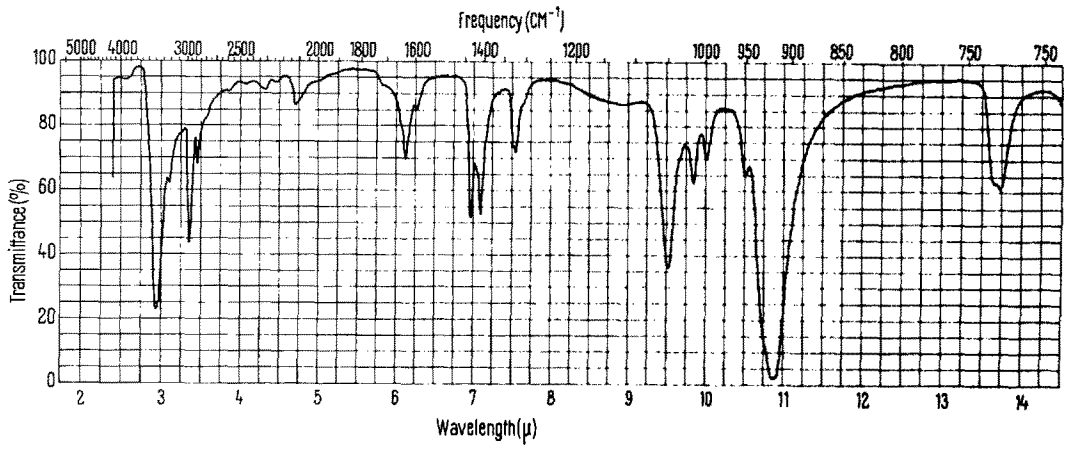
Der Reaktionsmechanismus beruht vermutlich auf der Bildung eines Adduktes aus zwei Molekülen Dimethylsulfoxid und einem Molekül Halogencyan und anschliessendem Zerfall der labilen Additionsverbindung unter Abspaltung von CO_2 (siehe Formeln Seite 309).

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

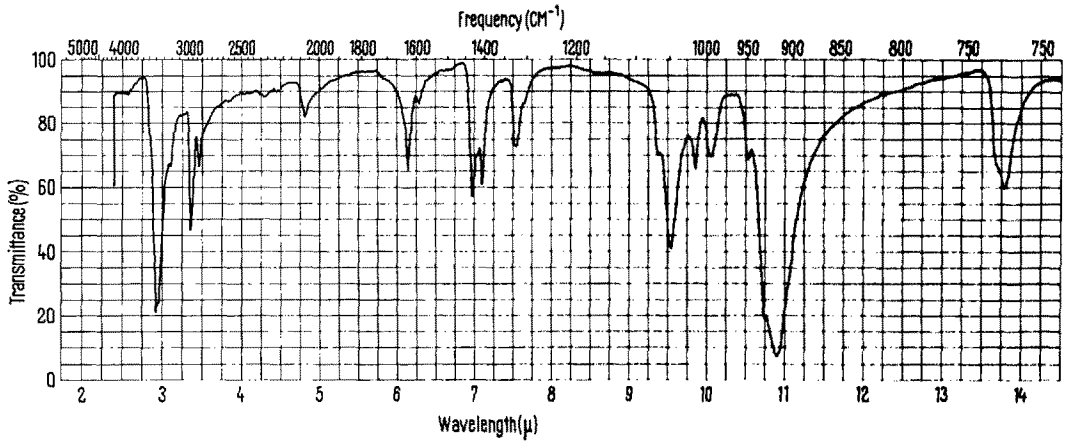
[1] Den Herren K.-O. ALT und Dr. H. WAGNER und ihren Mitarbeitern danken wir für Spektren und Analysen.

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

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

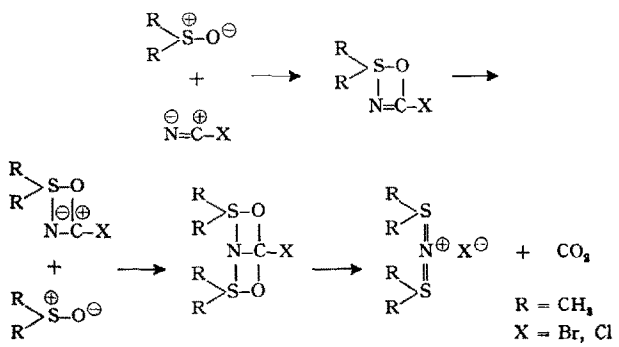


$[(CH_3)_2SNS(CH_3)_2]^{\oplus}Cl^{\ominus}$



$[(CH_3)_2SNS(CH_3)_2]^{\oplus}Br^{\ominus}$

Infrarot-Spektren von substituierten Dischwefel-Nitriden (KBr-Presslinge/Perkin-Elmer 221).



sulfoxid zu Sulfuryl-sulfiminen unter Entwicklung von CO_2.

Die Umsetzung von Halogencyan mit Dimethylsulfoxid wird unter Feuchtigkeitsausschluss bei einer Reaktionstemperatur von 20–30° in Gegenwart oder Abwesenheit eines Lösungsmittels (Benzol, Acetonitril) durchgeführt. Der Verlauf der Reaktion kann auf Grund der CO_2-Entwicklung leicht verfolgt werden. Die Salze I sind hygroskopisch und zersetzen sich bei höherer Temperatur. Sie sind löslich in Wasser, Alkoholen, Dimethylsulfoxid, N-Methylformamid und Acetonitril, unlöslich in Aceton, Benzol und Petroläther. Ihr Molekulargewicht wurde nach Verseifung mit alkoholischem Alkali durch Rücktitration mit HCl gegen Phenolphtalein bestimmt. Bei der Hydrolyse zerfallen sie in Dimethylsulfoxid und Ammoniumhalogenid:

$$[(CH_3)_2SNS(CH_3)_2]^{\oplus}X^{\ominus} \xrightarrow{2\,HOH} 2(CH_3)_2SO + NH_4X$$

I

Bei der katalytischen Hydrierung entstehen Dimethylsulfid und Ammoniumhalogenid:

$$[(CH_3)_2SNS(CH_3)_2]^{\oplus}X^{\ominus} \xrightarrow[Pd/C(CH_3COOH)]{H_2} 2(CH_3)_2S + NH_4X$$

I

Das IR-Spektrum (Figur) weist eine starke, charakteristische Absorptionsbande bei ca. 10,8–10,9 μ auf. Im Einklang mit der vorgeschlagenen Struktur zeigt das 60 MHz-Protonenresonanzspektrum eine einzige Linie bei $\tau = 6{,}94$ (in $CDCl_3$) für das Chlorderivat und bei $\tau = 7{,}16$ (in $(CD_3)_2SO$) für das Bromderivat.

Chlorid des Tetramethyl-dischwefel (IV)-nitrids: In einer Apparatur, die mit einem auf – 5° bis 0° gekühlten Rückflusskühler versehen ist, werden unter starkem Rühren bei 22–28° etwa 90–120 g gasförmiges Chlorcyan in 156 g Dimethylsulfoxid eingeleitet. Man rührt solange bei der

angegebenen Temperatur weiter, bis das Reaktionsgemisch zu einem Kristallbrei erstarrt ist, saugt ab und wäscht die Kristalle mit Aceton. Das Rohprodukt kann aus Acetonitril/Aceton, Äthylenchlorid oder Petroläther/Äthanol umkristallisiert werden. Farblose Nadeln. F. ca. 170–180°, Zers. (Kofler Heizbank). Rohausbeute: 120–145 g.

$C_4H_{12}ClNS_2$	Ber.	C 27,65	H 6,96	Cl 20,41	N 8,06	S 36,91%
	Gef.	„ 27,55	„ 7,08	„ 20,39	„ 8,09	„ 36,36%
MG	Ber.	173,73				
	Gef.	174				

Bromid des Tetramethyl-dischwefel (IV)-nitrids: 31,2 g Dimethylsulfoxid werden mit 15,9 g Bromcyan versetzt und mit einem Vibromischer während 120 h bei 26–30° intensiv durchmischt. Die entstandenen Kristalle werden abfiltriert und mit Aceton und Äther gewaschen. Umkristallisieren aus Acetonitril/Aceton. F. ca. 170–180°, Zers. (Kofler Heizbank). Rohausbeute: 11–13 g.

$C_4H_{12}BrNS_2$	Ber.	Br 36,63	N 6,42%
	Gef.	„ 36,57	„ 6,40%
MG	Ber.	218,18	
	Gef.	219	

Résumé. Lorsqu'on fait agir des halogénures de cyanogène sur le diméthylsulfoxide, on observe le dégagement d'acide carbonique et la formation de nitrures de soufre substitués.

P. Y. BLANC

Forschungslaboratorien der J. R. Geigy AG, Basel (Schweiz), 1. März 1965.

On the Biosynthesis of Testicular Steroids in vitro and its Inhibition

In the course of our studies on the selective inhibition of various steps in the adrenal biosynthesis of steroids by 3- and 4-substituted pyridine derivatives[1–3], further questions have arisen, including in particular the following: Is it possible to demonstrate similar examples of inhibition in other species and in other types of endocrine tissue, such as the gonads or the placenta? Are there any substances capable of blocking preferentially the biosynthesis of androgens and/or oestrogens without interfering with the adrenal 17α- and 11β-hydroxylases? In other words, are there any substances which directly inhibit either the side-chain splitting of 17α-hydroxylated C_{21} steroids into C_{19} steroids or the aromatisation of 19-hydroxylated C_{19} steroids into oestrogens, thereby exerting an influence on fertility?

Described below are the results of experiments in which an attempt was made to inhibit the side-chain splitting of appropriate radioactive C_{21} steroids in rat testicular tissue in vitro[4].

Methods. For a series of 12 incubations, 13 g of fresh testes taken from normal rats (aged 2 months) were homogenized for 4 min at 0°C in 220 ml buffer-saline solution[5]; for each incubation, 17 ml (corresponding to 1 g tissue) were then added in a special flask to 17 ml incubation solution[5] together with the radioactive precursor. After the test substances, dissolved in 0.25–0.5 ml water or ethanol, had been added, the incubate was gassed for 2 h at 35°C with O_2/CO_2 (95/5%; 0.5 l/min) at pH 7.1–7.25. The composition of each incubate was as follows: vol 34 ml, fresh tissue 1 g, NADP 14.5 μmoles, 49.3 m*M* NaCl, 14.7 m*M* KCl, 5.9 m*M* Na_2HCO_4, 10 m*M* $NaHCO_3$, 20 m*M* K-fumarate, 4 m*M* $MgSO_4$, 59 m*M* sucrose, 0.12–2.0 μC steroid precursor, i.e. for routine procedure 0.12 μC 4-^{14}C-17-hydroxyprogesterone, sp. act. 35.9 mC/mmole, weight ratio steroid/tissue $2.6 \cdot 10^{-6}$. After having been worked up as described in[5], the acetone-soluble portion of the $CHCl_3$ extract was paperchromatographed either free or following acetylation; for routine procedure 1/40 of the acetylated extract was run on strips in the 'Decanit' system[5]; the paper chromatograms were quantitated either in the Packard TRICARB liquid scint. counter Model 314 Ex or in the Packard Radiochromatogram scanner Model 7201. If the net radioactivity (NRA) of the peaks of androstenedione and testosterone in % of the NRA of the toal strip = U, and the remaining percentage of the NRA of the precursor = V, then the degree of the side-chain split D corresponds to $(U-V)/U \cdot 100$ (D = 100 in the control experiments without addition of blockers). A significant inhibition is thought to have a value of 25–50, and is expressed as the 'effective concentration' in μg substance/ml incubate: EC_{25-50}.

At a weight ratio to tissue of 10^{-6}, 17-hydroxyprogesterone was completely used up, yielding 50–80% testosterone and 5–25% androstenedione, as well as 11-deoxycortisol, its 20-dihydro derivative (20α) and pregn-4-ene-17,20α-diol-3-one in a yield of approx. 0.2–2%; no 11β-hydroxylated derivatives or testololactone were found. Qualitatively similar results were obtained from the transformation of 4-^{14}C-progesterone, which yielded 17-hydroxyprogesterone in addition. When tritiated pregnenolone, 17-hydroxypregnenolone, and androst-5-ene-3β,17β-diol were incubated, the main product obtained was again testosterone, with some androstenedione and dehydroepiandrosterone; by-products consisting partly of 20-dihydro derivatives (20α) and partly of Δ^4-3-keto analogues occurred in very much lower concentrations; for their identification see [5]. To this extent our results were in accordance with those reported in the literature[6–8] and indicated that in our set-up the testicular

[1] F. W. KAHNT and R. NEHER, Exper. *18*, 499 (1962).
[2] R. NEHER and F. W. KAHNT, Internat. Pharmacol. Meeting, Prague (August 1963), in R. ČAPEK, Ed., *Drugs and Enzymes* (Pergamon Press, 1965).
[3] F. W. KAHNT and R. NEHER, in preparation.
[4] A short account of some of these results was given at the 2nd Internat. Congress of Endocrinol., London (August 1964).
[5] R. NEHER, F. W. KAHNT, G.-D. ROVERSI, and A. BOMPIANI, Acta endocrinol., in press (1965).
[6] R. I. DORFMAN, E. FORCHIELLI, and M. GUT, Rec. Progr. Hormone Res. *19*, 251 (1963).
[7] H. F. ACEVEDO, L. R. AXELROD, E. ISHIKAWA, and F. TAKAKI, J. clin. Endocr. Metab. *23*, 885 (1963). – J. M. ROSNER, S. HORITA, and P. H. FORSHAM, Endocrinee *75*, 299 (1964).
[8] L. L. GROSSO and F. UNGAR, Steroids *3*, 67 (1964).