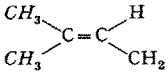
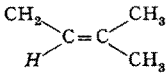
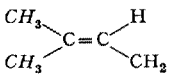
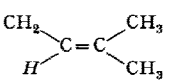
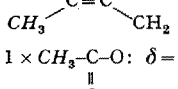
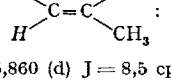
Helvolinsäure-methylester (vgl. Figur)

$3 \times CH_3$–C–: $\delta = 0{,}916$ (s); 1,182 (s); 1,450 (s); $1 \times CH_3$–C–H: $\delta =$ 1,275 (d) J = 6,5 cps; $1 \times$  : $\delta = 1{,}600$ (d) J ≅ 2 cps; 1,685 (d) J ≅ 2 cps; $2 \times CH_3$–C(=O)–O–: $\delta = 1.950$ (s); 2,100 (s); $1 \times$ CH_3–O–C(=O)–: $\delta = 3{,}633$ (s); $1 \times$  : $\delta = 5{,}120$ (bt) J = 7 cps; $2 \times H$–C(–O–Ac)–: $\delta = 5{,}210$ (bs); 5,840 (d) J = 8,5 cps; $1 \times$ –C(=O)–CH=CH–: $\delta = 5{,}840$ (d) J = 9 cps; 7,300 (d) J = 9 cps.

Cephalosporin P_1-methylester

$3 \times CH_3$–C–: $\delta = 1{,}050$ (s); 1,181 (s); 1,181 (s); $1 \times CH_3$–C–H: $\delta =$ 0,900 (d) J = 6,5 cps; $1 \times$  : $\delta = 1{,}600$ (d) J ≅ 1 cps; 1,685 (d) J ≅ 1 cps; $2 \times CH_3$–C(=O)–O: $\delta = 1{,}952$ (s); 2,064 (s); $2 \times$ H–C–OH: $\delta = 3{,}500$ (s); 3,670 (bm); $1 \times CH_3$–O–C(=O)–: $\delta = 3{,}630$ (s); $1 \times$  : $\delta = 5{,}130$ (bt) J = 7 cps; $2 \times H$–C(–O–Ac)–: $\delta =$ 4,570 (d) J = 9,5 cps; 5,840 (d) J = 8,5 cps.

Fusidinsäure-methylester

$3 \times CH_3$–C–: $\delta = 0{,}917$ (s); 0,984 (s); 1,392 (s); $1 \times CH_3$–C–H: $\delta =$ 933 (d) J = 7 cps; $1 \times$  : $\delta = 1{,}601$ (d) J ≅ 1 cps; 1,685 (d) J ≅ 1 cps; $1 \times CH_3$–C(=O)–O: $\delta = 1{,}982$ (s); $2 \times H$–C(–OH)–: $\delta = 3{,}750$ (m); 4,360 (m); $1 \times CH_3$–O–C(=O)–: $\delta = 3{,}630$ (s); $1 \times$  : $\delta = 5.120$ (bt) J = 7 cps; $1 \times H$–C(–O–Ac)–: $\delta =$ 5,860 (d) J = 8,5 cps.

(s) = Singlett; (d) = Doublett; (t) = Triplett; (m) = Multiplett; (b) = breites Signal.

Bildung von γ-Lactonen aus Helvolinsäure[9] und Cephalosporin P_1[4] ist damit in bestem Einklang. Durch Integration der früheren Ergebnisse[3,4] mit den hier beschriebenen Resultaten ergeben sich sowohl für Helvolinsäure wie auch für Cephalosporin P_1 Konstitutionsformeln, die wegweisend für weitere Untersuchungen sein können.

Summary. On the basis of nuclear magnetic resonance measurements and in agreement with recently reported mass-spectrometric investigations it is shown that the molecular formulae of Helvolic acid and Cephalosporin P_1 must be revised to $C_{33}H_{44}O_8$ and $C_{33}H_{50}O_8$ respectively, and that the additional carbon atom is present as an angular methyl group.

A comparison with the data obtained with fusidic acid points to a similarity among the three substances which is closer than hitherto suspected.

A. MELERA

Varian AG Research Laboratory, Zürich (Schweiz), 22. Juli 1963.

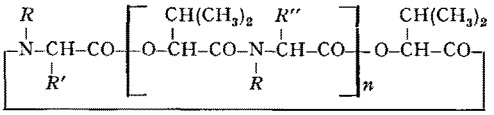
1 J. F. LYNCH, J. M. WILSON, H. BUDZIKIEWICZ und C. DJERASSI, Exper. *19*, 213 (1963).
2 S. A. WAKSMANN, E. S. HORNING und E. L. SPENCER, J. Bact. *45*, 233 (1943). – E. CHAIN, H. W. FLOREY, M. A. JENNINGS und T. I. WILLIAMS, Brit. J. exp. Path. *24*, 108 (1943). – A. E. O. MENZEL, O. WINTERSTEINER und J. C. HOOGERHEIDE, J. biol. Chem. *152*, 419 (1944).
3 N. L. ALLINGER und J. L. COKE, J. org. Chem. *26*, 4522 (1961).
4 B. M. BAIRD, T. G. HALSALL, E. R. H. JONES und G. LOWE, Proc. chem. Soc. *1961*, 257.
5 T. G. HALSALL, E. R. H. JONES und G. LOWE, Proc. chem. Soc. *1963*, 16.
6 W. O. GODTFREDSEN und S. VANGEDAL, Tetrahedron *18*, 1029 (1962).
7 D. ARIGONI, W. VON DAEHNE, W. O. GODTFREDSEN, ANDRÉE MARQUET und A. MELERA, Exper. *19*, 521 (1963).
8 Die Interpretation eines früher aufgenommenen[3] NMR-Spektrums des Helvolinsäure-methylesters wurde durch niedriges Signal/Rausch-Verhältnis und schlechtere Auflösung erschwert. Unsere Spektren wurden in $CDCl_3$-Lösung mit Tetramethylsilan als interne Referenz aufgenommen, wobei die chemische Verschiebung in δ-Einheiten angegeben wird. Die Spektren von Cephalosporin P_1- und Fusidinsäure-methylester wurden bei 60 MH_z aus einem A-60-Spektrometer erhalten; das Spektrum von Helvolinsäure-methylester stammt aus einem HR-100-Spektrometer der Firma Varian Ass., Palo Alto (Californien).
9 H. S. BURTON, E. P. ABRAHAM und H. M. E. CARDWELL, Biochem. J. *62*, 171 (1956).

The Structure–Antimicrobial Relation of Depsipeptides[1]

In the course of studies in depsipeptide chemistry, we have prepared a number of natural cyclodepsipeptides and their analogues. In particular, the structure was established and total synthesis carried out of two cyclohexadepsipeptide antibiotics, namely, enniatin B (I) and enniatin A (II)[2–5]. We also prepared the closely related compounds: cyclotetradepsipeptides (III)–(IX)[6,7], cyclohexadepsipeptides (X) and (XI)[5], cyclooctadepsipeptides (XII)–(XIV) and the cyclododecadepsipeptide (XV)[5,7]. Furthermore, the two naturally occurring cyclohexadepsipeptides sporidesmolide I (XVI) and sporidesmolide II (XVII)[8–10] and a number of closely related compounds (XVIII)–(XXIII) were synthesized[7].

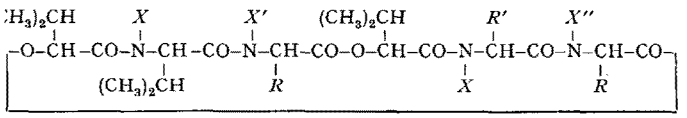
Since, as is well known, enniatins A and B are highly active against various mycobacteria[11], we deemed it of interest to test the antimicrobial activity of (I)–(XXIII) against bacteria, yeasts and fungi, which could have given some insight into the effect of the structure (nature, configuration and sequence of the hydroxy and amino acid residues, ring size, etc.) upon the biological action of depsipeptides. Owing to the slight solubility of the cyclodepsi-

peptides in water, the compounds were first dissolved in dimethylformamide and then diluted with distilled water. The main results of the biological tests are presented in the Table.



I $n=2$ (LDLDLD), $R=CH_3$, $R'=R''=CH(CH_3)_2$;
II $n=2$ (LDLDLD), $R=CH_3$, $R'=R''=CH(CH_3)CH_2CH_3$;
III $n=1$ (LDLD), $R=CH_3$, $R'=R''=CH(CH_3)_2$;
IV $n=1$ (LDLD), $R=CH_3$, $R'=R''=CH(CH_3)CH_2CH_3$;
V $n=1$ (LDLD), $R=CH_3$, $R'=R''=CH_2CH(CH_3)_2$;
VI $n=1$ (LDLD), $R=CH_3$, $R'=CH(CH_3)_2$, $R''=CH(CH_3)CH_2CH_3$;
VII $n=1$ (LDLD), $R=CH_3$, $R'=CH(CH_3)_2$, $R''=CH_2CH(CH_3)_2$;
VIII $n=1$ (LDLD), $R=CH_3$, $R'=CH(CH_3)CH_2CH_3$, $R''=CH_2CH(CH_3)_2$;
IX $n=1$ (all D), $R=CH_3$, $R'=R''=CH(CH_3)_2$;
X $n=2$ (LDLDLD), $R=CH_3$, $R'=CH(CH_3)_2$, $R''=CH(CH_3)CH_2CH_3$;
XI[a] $n=2$ (all L), $R=H$, $R'=R''=CH(CH_3)_2$;
XII $n=3$ (LDLDLDLD), $R=CH_3$, $R'=R''=CH(CH_3)_2$;
XIII $n=3$ (all D), $R=CH_3$, $R'=R''=CH(CH_3)_2$;
XIV $n=3$ (LDLDLDLD), $R=H$, $R'=R''=CH(CH_3)_2$;
XV $n=5$ (LDLDLDLDLDLD), $R=H$, $R'=R''=CH(CH_3)_2$.

[a] Cyclodepsipeptide (XI) was synthesized in our laboratory by E. I. VINOGRADOVA.



XVI (LLLLDD), $X=X''=H$, $X'=CH_3$, $R=CH_2CH(CH_3)_2$, $R'=CH(CH_3)_2$;
XVII (LLLLDD), $X=X''=H$, $X'=CH_3$, $R=CH_2CH(CH_3)_2$, $R'=CH(CH_3)CH_2CH_3$;
XVIII (all L), $X=X'=X''=CH_3$, $R=CH_2CH(CH_3)_2$, $R'=CH(CH_3)_2$
XIX (all L), $X=H$, $X'=X''=CH_3$, $R=CH_2CH(CH_3)_2$, $R'=CH(CH_3)_2$;
XX (LDLLDL), $X=H$, $X'=X''=CH_3$, $R=CH_2CH(CH_3)_2$, $R'=CH(CH_3)_2$;
XXI (DDLDDL), $X=H$, $X'=X''=CH_3$, $R=CH(CH_3)CH_2CH_3$, $R'=CH(CH_3)_2$;
XXII (DLLDLL), $X=H$, $X'=X''=CH_3$, $R=CH(CH_3)CH_2CH_3$, $R'=CH(CH_3)_2$;
XXIII (LDDLDD), $X=X'=X''=H$, $R=CH_2CH(CH_3)_2$, $R'=CH(CH_3)_2$.

First of all one can note a very definite dependence of the antimicrobial activity of cyclodepsipeptides upon the size of the ring. The cyclotetradepsipeptides (III)–(IX) are practically inactive against any of the organisms we have tested[12]. The highest activity and broadest spectrum belong to the cyclohexadepsipeptides of the enniatin group with regularly alternating amino and hydroxy acid residues (the natural enniatins (I) and (II) and also the synthetic analogue (*X*)). Of considerable interest is the fact that their activity is not only little dependent upon the nature of their N-methylamino acid residues, but that they also retain their activity (although it falls to a certain extent) when these residues are replaced by corresponding amino acids, and moreover that change in configuration is also permissible (compound XI). On the contrary, cyclohexadepsipeptides with irregular sequence of the hydroxy and amino acid residues (sporidesmolides and

[1] Communication XXVII in the Series *Studies in Depsipeptide Chemistry*.
[2] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, A. A. KIRYUSHKIN, and V. T. IVANOV, Izv. Akad. Nauk SSSR, Otdel. khim. Nauk *1963*, 579.
[3] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, A. A. KIRYUSHKIN, and V. T. IVANOV, Tetrahedron Letters 885 (1963).
[4] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, A. A. KIRYUSHKIN, and V. T. IVANOV, Izv. Akad. Nauk SSSR, Otdel. khim. Nauk *1963*, 1148.
[5] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, A. A. KIRYUSHKIN, and V. T. IVANOV, Izv. Akad. Nauk SSSR, Otdel. khim. Nauk, in press.
[6] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, V. T. IVANOV, and A. A. KIRYUSHKIN, Tetrahedron *19*, 581 (1963).
[7] YU. A. OVCHINNIKOV, V. T. IVANOV, A. A. KIRYUSHKIN, and M. M. SHEMYAKIN, Dokl. Akad. Nauk SSSR *19*, 995 (1963).
[8] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, V. T. IVANOV, and A. A. KIRYUSHKIN, Izv. Akad. Nauk SSSR, Otdel. khim. Nauk *1962*, 1699.
[9] YU. A. OVCHINNIKOV, A. A. KIRYUSHKIN, V. T. IVANOV, and M. M. SHEMYAKIN, Izv. Akad. Nauk SSSR, Otdel. khim. Nauk *1963*, 770.
[10] M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, V. T. IVANOV, and A. A. KIRYUSHKIN, Tetrahedron, *19*, 995 (1963).
[11] E. GÄUMANN, S. ROTH, L. ETTLINGER, PL. A. PLATTNER, and U. NAGER, Exper. *3*, 202 (1947).
[12] According to PLATTNER et al.[13], the activity of cyclotetradepsipeptide (III) against *Staphylococcus aureus* ATCC 6538 is about 10% that of enniatin B.
[13] PL. A. PLATTNER, K. VOGLER, R. O. STUDER, P. QUITT, and W. KELLER-SCHIERLEIN, Exper. *19*, 71 (1963).

Compound	Minimal growth inhibiting concentration (γ/ml)											
	Staph. aureus 209-P	*Staph. aureus* UV-3	*B. mycoides*	*B. subtilis*	*Sarcina lutea*	*E. coli*	*Cand. albicans*	*Sacch. cerevisiae*	*M. phlei*	*Mycobact. B-5*	*M. tuberculosis* H_{37}	*M. avium*
I	75–100	9	25–37	37–50	18	>100	37	37	9–12	18	4,5–6	75
II	9	1,5	4,5	6	2	>100	9	6	1,5–2,5	2,5	0,75	6
III–IX	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
X	12–18	2	9	9	4,5	>100	9	9	1,5–2	3	1,5	12
XI	>100	30	30	>100	30	>100	>100	>100	30	–	–	–
XII	>100	1,5–3	>100	>100	3–4,5	>100	>100	>100	18–25	50	4,5	>100
XIII	> 25	> 25	> 25	> 25	> 25	> 25	> 25	> 25	> 25	–	–	–
XIV–XXIII	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

their analogues XVI–XXIII) are completely devoid of antimicrobial activity. Further increase in ring size causes a fall in biological activity of the depsipeptide molecule. Thus, the cyclooctadepsipeptide (XII) built up similarly to enniatin B (I), has a narrower antimicrobial spectrum than the latter. The activity is completely lost when the *L*-N-methylvaline residues of this compound are replaced by *L*-valine residues (compound XIV) or when their configuration is changed. One of the closest cyclopolymer homologues of compound (XII), namely cyclododecadepsipeptide (XV) is also without antimicrobial activity.

It is noteworthy that all linear depsipeptides corresponding to the cyclodepsipeptides (I)–(XXIII) are entirely inactive.

We have thus found that of the depsipeptide cyclopolymer homologues we have studied, the highest activity is manifested by cyclohexadepsipeptides with regularly alternating hydroxy and amino acid residues. This is apparently due to the fact that such cyclohexadepsipeptides possess the most appropriate conformation, sterically complementary to the active centre of the corresponding enzymes.

Zusammenfassung. Die Beziehungen zwischen Struktur und antimikrobieller Aktivität in der Reihe der Depsipeptide wurden untersucht. Cyclotetradepsipeptide sind praktisch inaktiv. Die grösste Aktivität besitzen die Cyclohexadepsipeptide mit regulärer Oxy- und Aminosäurensequenz. Eine weitere Vergrösserung des Depsipeptidringes führt zu einem Abfall der biologischen Aktivität. Cyclohexadepsipeptide mit irregulärer Oxy- und Aminosäurensequenz sind völlig inaktiv.

M. M. SHEMYAKIN, YU. A. OVCHINNIKOV,
V. T. IVANOV, A. A. KIRYUSHKIN,
G. L. ZHDANOV, and I. D. RYABOVA

Institute for Chemistry of Natural Products, USSR Academy of Sciences, Moscow (USSR),
July 22, 1963.

Influence of Diglycyl-glycine on the Radiation Sensitivity of Catalase

Recently we have shown[1] that glycerol added to catalase solution prior to irradiation provides excellent protection to the enzyme. A maximum protective effect was obtained with very small amounts of glycerol (0.0004 vol %). The protective factor was about 13 as compared with controls containing no glycerol, and at higher glycerol concentrations the protective effect was found to decrease slightly. In order to explain this protective effect, we proposed a chelating mechanism by which glycerol forms a complex with the metal-ions present in the catalase molecule. These complexed metal-ions consequently inhibit the decomposition of radiation produced H_2O_2 into reactive radicals. Metal-ions, such as Cu^{2+} or Fe^{3+}, catalyse this destruction as is well known[2].

There is also additional radiation damage produced by radicals which originate from the decomposition of radiation produced H_2O_2 in solution. Because of their short lifetime, they will, preferably, undergo competitive reactions such as radical combinations and reactions with H_2O_2 rather than reactions with the enzyme molecules. This depends, however, on the degree of dilution[3].

If this mechanism is correct, a modification of the radiation response should be expected when other organic substances, acting as a radical scavenger, are added to the catalase solution.

In our investigations we used diglycyl-glycine as a radical scavenging agent. Diglycyl-glycine (Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in the enzyme solution before irradiation. Concentrations which were used are mentioned in Figure 1 and are expressed in mg/ml. Catalase solution ($8 \times 10^{-8} M$) was prepared by dissolving 2 mg of lyophilized beef liver catalase (Worthington Biochemical Corp., Freehold, New Jersey) in 100 ml of a 0.05 *M* phosphate buffer, pH 7.0. Hydrogen peroxide ($5 \times 10^{-3} M$) was prepared by diluting 0.15 ml of a 30% H_2O_2 solution (Superoxol, Merck & Co., Rahway, New Jersey) with 25 ml of 0.05 *M* phosphate buffer, pH 7.0.

The catalase activity was determined spectrophotometrically using the decrease in optical density (of H_2O_2) at 240 mμ as function of time after mixing enzyme and substrate (method of BEERS and SIZER[4]). A Cary 14 Spectrophotometer (Applied Physics Corp., Monrovia, California) was used for the determination. 1 ml of H_2O_2 was rapidly injected into 2 ml of catalase solution. All measurements and irradiations were done at room temperature. 2 ml samples of catalase solution were irradiated in Lucite containers with different doses (0 to 2.7×10^6 *r*). The irradiations were done with a beryllium-window X-ray tube (100 kV, 12 mA, HVL 0.064 mm Al; Philips Electronics Inc., Mount Vernon, New York). The dose rate was about 9×10^4 *r*/min. The X-ray tube was calibrated with an air-wall ionization chamber.

The modification of the radiation effect on catalase by diglycyl-glycine is shown in Figure 1. As can be seen, diglycyl-glycine protects catalase very well. The 50%-

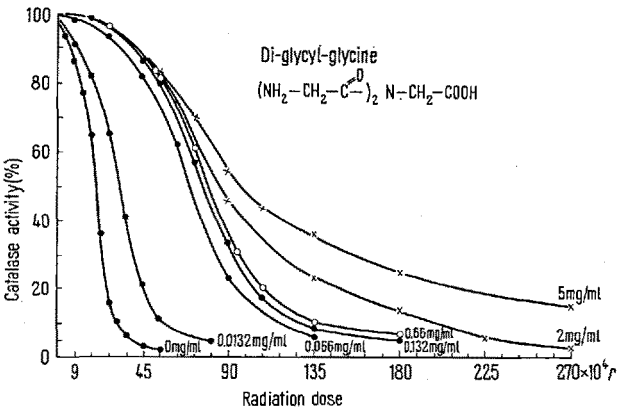


Fig. 1. The influence of different concentrations of diglycyl-glycine (mg/ml) on the radiation sensitivity of catalase.

[1] W. LOHMANN, A. J. MOSS JR., W. H. PERKINS, and C. F. FOWLER, Biophysik, in press.
[2] H. BAXENDALE, Adv. Catalys. *4*, 31 (1952).
[3] To be published.
[4] R. F. BEERS and I. W. SIZER, J. biol. Chem. *195*, 133 (1952).