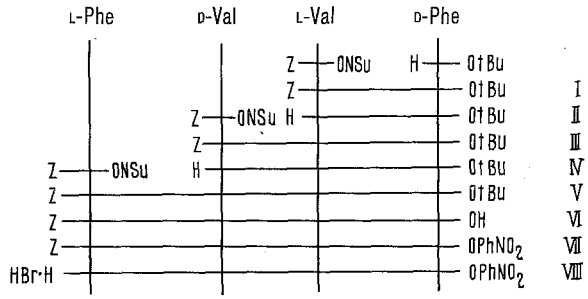
Synthesis and Structure of Fungisporin

In 1952 SUMIKI and MIYAO[1] reported on Fungisporin, a cyclooctapeptide isolated from spores of several species of *Penicillium* and *Aspergillus* as a crystalline sublimate by destructive distillation. Acid and alkaline hydrolysis yielded DL-valine and DL-phenylalanine in equimolar amounts. From the absence of terminal groups, the IR-spectrum, the solubility characteristics and the molecular weight of 980, determined by isothermic distillation in trifluoro acetic acid, MIYAO[2,3] was able to propose the empirical formula cyclo-(Phe-Val)$_4$. Sequence studies and enzymatic experiments on peptide fragments, obtained by partial hydrolysis of fungisporin showed that the formula would most probably be cyclo-(D-Val-L-Val-D-Phe-L-Phe-)$_2$[3].

For the synthesis of this compound the tetrapeptide Z-L-Phe-D-Val-L-Val-D-Phe-OtBu V was prepared via the stepwise elongation method using the N-Hydroxysuccinimidesters of the corresponding Z-Aminoacids (reaction scheme).

Treatment of V with trifluoroacetic acid yielded the acid VI, which was transformed into the activated ester VII with di-(*p*-nitrophenyl) sulfite[5]. After the removal of the benzyloxycarbonyl group with HBr/acetic acid the resulting tetrapeptide *p*-nitrophenylester VIII was submitted to cyclization under high dilution in pyridin[6].



Reaction scheme[4]

From the residue obtained after evaporation of the solvent a crystalline, highly insoluble compound could be isolated by sublimation. The data obtained were in good agreement with the ones published for natural fungisporin with the exception of the molecular weight, which was found to be 482 by mass spectrometry. This is half the value found for natural fungisporin by the isothermic distillation method. A redetermination of the molecular weight of the natural compound[7] by mass spectrometry reveiled, however, also a molecular weight of 482. Fungisporin is therefore a cyclotetrapeptide and identical with cyclo-(L-Phe-D-Val-L-Val-D-Phe-).

Zusammenfassung. Die Struktur von Fungisporin, eines Zyklopeptides aus Sporen verschiedener Spezies von *Penicillium* und *Aspergillus*-Arten, wird durch Synthese und Vergleich mit dem Naturprodukt als die eines Zyklotetrapeptides, cyclo-(L-Phe-D-Val-L-Val-D-Phe-), bewiesen.

R. O. STUDER

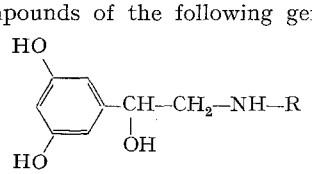
Chemical Research Department,
F. Hoffmann-La Roche and Co. AG,
CH-4002 Basel (Switzerland), 18 June 1969.

[1] Y. SUMIKI and K. MIYAO, J. agric. Chem. Soc. Japan *26*, 27 (1952).
[2] K. MIYAO, Bull. agric. chem. Soc. Japan *19*, 86 (1955).
[3] K. MIYAO, Bull. agric. chem. Soc. Japan *24*, 23 (1960).
[4] Abbreviations: Amino-acids and peptides are abbreviated as recommended by the committee on Nomenclature which reported at the 5th European Peptide Symposium, Oxford, 1962, Proceeding (Ed. G. T. YOUNG, Pergamon Press, 1963). In addition: Z = benzyloxycarbonyl; OtBu = tertiary butylester; ONSu = N-hydroxysuccinimidester; OPhNO$_2$ = *p*-nitrophenylester.
[5] B. ISELIN and R. SCHWYZER, Helv. chim. Acta *43*, 1760 (1960).
[6] R. SCHWYZER and P. SIEBER, Helv. chim. Acta *40*, 624 (1957).
[7] We kindly thank Dr. K. MIYAO for supplying us with a sufficient amount of natural fungisporin for this comparison.

Two New Groups of Selective Stimulants of Adrenergic β-Receptors

Sympathomimetic agents acting on the adrenergic β-receptors are widely used in the treatment of bronchial asthma. Since both bronchodilatation and excitation of cardiac muscle are mediated by stimulation of the adrenergic β-receptors, bronchodilatation is often accompanied by tachycardia and palpitations. However, recent observations by LANDS et al.[1-3] indicate that the adrenergic β-receptors in the heart are different from those in the lung.

We have synthetized and tested pharmacologically a series of compounds of the following general formula:



where R is a branched alkyl group or cycloalkyl group, equal to *t*-butyl, *t*-pentyl, *t*-hexyl, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The compounds were tested for bronchospasmolytic effect in vitro on the isolated guinea-pig trachea and the effect on heart muscle on the right guinea-pig auricle (spontaneously beating). The effect on the tracheal muscles and on the heart muscle did not run parallel within the alkyl and the cycloalkyl series as can be seen from Figures 1 and 2. In the alkyl series maximal effect on the tracheal muscles was obtained for R = *t*-butyl, with an effect corresponding to 0.8 that of (−)-adrenaline. For R = *t*-pentyl and *t*-hexyl the effect on the trachea decreased. The effect on the right guinea-pig auricle was most pronounced for R = isopropyl (0.4 × (−)-adrenaline). With increasing size of the substituent, the effect on this preparation decreased.

[1] A. M. LANDS, G. E. GROBLEWSKI and T. G. BROWN JR., Arch. int Pharmacodyn. *161*, 68 (1966).
[2] A. M. LANDS, F. P. LUDUENA and H. J. BUZZO, Life Sci. *6*, 2241 (1967).
[3] A. M. LANDS, A. ARNOLD, J. P. MCAULIFF, F. P. LUDUENA and T. G. BROWN JR., Nature *214*, 597 (1967).