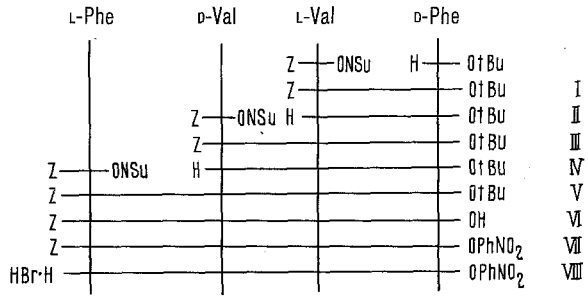
Synthesis and Structure of Fungisporin

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.

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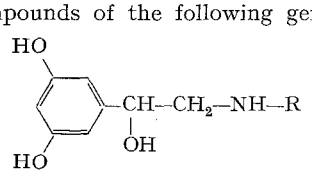
CH-4002 Basel (Switzerland), 18 June 1969.

[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).

The results from this series show that a *t*-butyl group on the nitrogen atom gives maximal effect on the tracheal muscles and the most favourable dissociation between the effect on the tracheal muscles and on the heart muscle.

In the cycloalkyl series maximal effect on the guinea-pig trachea was obtained for R = cyclobutyl. The effect of this compound was about 0.8 that of (−)-adrenaline. In comparison with (−)-adrenaline, the cyclopropyl compound was more effective on the auricle (0.19 × (−)-adrenaline) than on the trachea (0.06 × (−)-adrenaline). For the cyclobutyl and cyclopentyl compounds the condition was the opposite. These compounds were, like the *t*-butyl and *t*-pentyl compounds in the alkyl series, in comparison with (−)-adrenaline, more active on the trachea than on the auricle. The effect of the cyclohexyl compound was very weak.

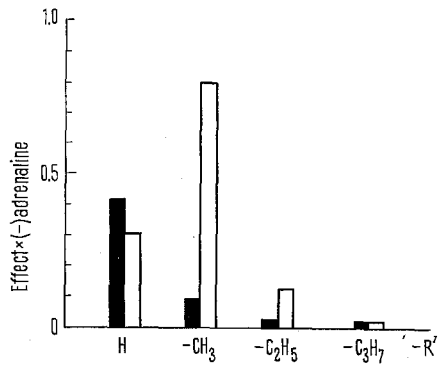
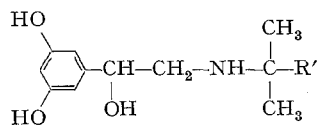


Fig. 1. Effect on isolated guinea-pig trachea and right guinea-pig auricle (spontaneously beating) of compounds of the general formula:



(R' = $-CH_3$ = terbutaline). White bars, effect on the trachea; black bars, effect on the auricle.

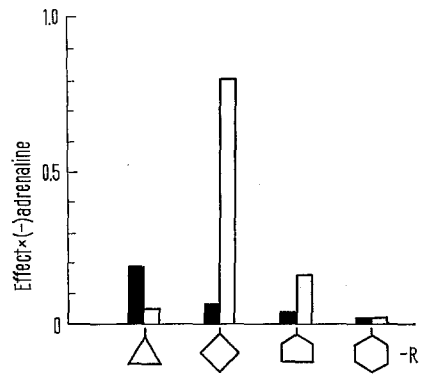
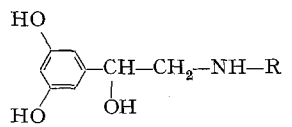


Fig. 2. Effect on isolated guinea-pig trachea and right guinea-pig auricle (spontaneously beating) of compounds of the general formula:



White bars, effect on the trachea; black bars, effect on the auricle.

The results obtained clearly demonstrate a difference in the relation between the effect on the isolated guinea-pig trachea and right auricle for a number of sympathomimetic agents. Such a selective effect has also been reported for another class of adrenergic β-receptor stimulants by HARTLEY et al.[4] and BRITTAIN et al.[5].

One of the compounds[6] in the alkyl series 1-(3,5-dihydroxyphenyl)-2-(*t*-butylamino)-ethanol sulphate (terbutaline sulphate, Prop. I.N.N.) showed such promising properties in the preliminary tests that it was selected for further studies. Its relative bronchospasmolytic activity in different test situations is given in the Table. In vitro, when tested on the isolated guinea-pig trachea, the effect of the racemic compound was slightly less than that of (−)-adrenaline and twice that of orciprenaline. Isoprenaline was 17 times more potent than terbutaline. In the in vivo studies the relation between the different compounds varied according to the route of administration and technique used. After oral administration to conscious guinea-pigs (histamine aerosol), terbutaline was 3 times more active than both orciprenaline and isoprenaline. The duration of action was longer than that of isoprenaline and orciprenaline. The cardiovascular effects of terbutaline are characteristic of those of a β-receptor stimulating agent: depressor effect on the arterial mean pressure, increase in pulse pressure and heart rate. The effect on peripheral resistance assessed by perfusion of the lower abdomen of the cat at constant flow, was $^1/_{20}$ that of isoprenaline and of the order of that of orciprenaline. At equieffective bronchospasmolytic doses the circulatory effects in both cats and dogs were less than those for orciprenaline and isoprenaline.

The acute toxicity in mice of the compound is low and it has no central stimulating effect.

After oral administration to dogs, about 75% of the dose was found in the urine within 48 h after the administration.

The results obtained from the pharmacological studies indicate that terbutaline is a potent bronchospasmolytic agent with an effect predominantly on the tracheal and bronchial muscles. It has an action of long duration and is active after oral administration. Full results will be published in detail later.

Relative bronchospasmolytic effect of terbutaline, orciprenaline and isoprenaline in various test situations

Test	Route of administration	Relative effect Terbutaline	Orciprenaline	Isoprenaline
Guinea-pig trachea		1.0	0.5	17
Anaesthetized guinea-pig (Konzett and Rössler)	i.v.	1.0	0.5	6.7
Anaesthetized cat (Konzett and Rössler)	i.v.	1.0	0.4	1.7
Anaesthetized dog (Konzett and Rössler)	i.v.	1.0	0.7	4.8
Conscious guinea-pig (Histamine aerosol)	i.p.	1.0	0.3	2.0
Conscious guinea-pig (Histamine aerosol)	oral	1.0	0.3	0.3

[4] D. HARTLEY, D. JACK, L. H. C. LUNTS and A. C. RITCHIE, Nature *219*, 861 (1968).

[5] R. T. BRITTAIN, J. B. FARMER, D. JACK, L. E. MARTIN and W. T. SIMPSON, Nature *219*, 862 (1968).

[6] K. I. L. WETTERLIN and L. Å. SVENSSON, Belgium patent No. 704,932.

Zusammenfassung. Verzweigte und zyklische N-Alkylderivate aus 1-(3, 5-Dihydroxyphenyl)-2-aminoäthanol wurden hergestellt und pharmakologisch geprüft. Die Verbindungen sind adrenerge β-rezeptorstimulierende Substanzen mit ausgesprochenem Effektunterschied in ihrer Wirkung auf Herz und Bronchie. Von den untersuchten Verbindungen zeigte 1-(3, 5-Dihydroxyphenyl)-2-(*t*-butylamino)-äthanol (Terbutalin) besonders gute pharmakologische Eigenschaften. Terbutalin ist potenter als Orciprenalin, hat ausserdem eine Wirkung von längerer Dauer als Isoprenalin und Orciprenalin sowie eine höhere selektive Wirkung als diese beiden Verbindungen.

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The X-Ray Analysis of Tolypomycinone Tri-*m*-bromobenzoate

Tolypomycin Y (I)[1], $C_{43}H_{54}N_2O_4$, is a new antibiotic which has been isolated from the culture broth of *Streptomyces tolypophorus*[2]. Mild acid hydrolysis of I afforded a yellow naphthoquinone, tolypomycinone[3], $C_{37}H_{43}NO_{13}$, and a water-soluble aminosugar, tolyposamine[1], $C_6H_{13}NO_2$. The structure of tolypomycin Y and tolypomycinone have been proposed by KISHI et al.[1-3] from the structures of their degradation products and their spectroscopic data. In order to confirm these structures and to establish their stereochemical configurations and conformations, the X-ray analysis was undertaken. A variety of heavy-atom derivatives of tolypomycin Y and tolypomycinone was prepared, but a preliminary study indicated that the tolypomycinone tri-*m*-bromobenzoate (II), $C_{58}H_{52}O_{16}NBr_3$, was the most promising for the X-ray analysis. Small yellow crystals of II mp 201–203° (decomp.), recrystallized from an ethyl acetate solution, were stable enough to make certain amount of data collection possible.

Weissenberg photographs obtained with CuKα radiation ($\lambda = 1.5418$ Å) showed that the crystal is orthorhombic with the unit-cell parameters, $a = 20.51$, $b = 25.60$, and $c = 11.80$ Å. Systematic extinctions of hOO when h is odd, OkO when k is odd and 001 when l is odd, lead uniquely to the space group D_2^4 ($P2_12_12_1$). The calculated density of the crystal, assuming one molecule in an asymmetric unit, is 1.35 g cm^{-3} as compared with the measured value of 1.38 g cm^{-3} (the floatation method).

Reflexion intensities were measured visually from multiplefilm integrating Weissenberg photographs which were recorded at room temperature, rotating around the *c* axis (11 layers, specimens of 0.08×0.05 mm cross-section) and the *a* axis (5 layers, 0.05×0.20 mm cross-section). The crystals were fairly small and were of low reflecting power. Absorption corrections are small and were not applied, nor were the extinction corrections. In all, 3262 independent structure factors were derived from the intensity measurement.

The three-dimensional sharpened peak Patterson function was computed with an over-all temperature factor of 5.5 Å^2. Initial co-ordinates of 2 bromine atoms (0.28, 0.18, 0.84) and (0.50, 0.02, 0.32), were deduced from the 3 Harker sections and vector maps. But the co-ordinates of the third bromine atom, temperature factor of which was later revealed to be very large, were not determined. Each of the locations of the 2 bromine atoms was then used to compute a separate four term minimum function superposition. 2 three-dimensional Fourier syntheses were also evaluated at this stage with phase angles calculated from the co-ordinates of one bromine atom (Br(1)) and from those of 2 bromine atoms (Br(1) and Br(2)), respectively. By careful examination of these maps, 50 peaks were chosen as atomic positions. Instead of calculating a rough electron density distribution with phase angles based on these 50 atoms, least-squares treatment was applied to them in order to ascertain the existence of atoms by their temperature factors[4-5]. Some atoms whose temperature factors diverged in this treatment were discarded. Then the electron density synthesis was computed with phase angles based on the remaining atoms. Further elucidation of the structure was continued by our usual method[4-5], that is, an alternative application of least-squares treatments and Fourier syntheses. 2 benzene rings of the bromobenzoate were clearly seen at an early stage, and the naphthoquinone ring at the next stage. Other parts of the molecule were visualized step by step as the analysis proceeds on. From the map obtained by the electron density synthesis with an R-value of 27.7%, 78 atoms of the whole molecule and 20 other peaks were seen. The latter 20 peaks were revealed to be spurious by the succeeding least-squares treatment, and the present structure was derived without any uncertainty. The chemical identities of all the constituent atoms were determined by the consideration of the temperature factors in the least-squares refinement, the peak values of electron density, bond lengths and angles, together with the chemical evidence. The atomic co-ordinates and the temperature factors were refined through 6 cycles of least-squares calculations. Finally, the R-value decreased to 0.172.

The final atomic co-ordinates and temperature factors are listed in the Table. The structure (II) of tolypomycinone tri-*m*-bromobenzoate is now considered to be established unambiguously. The atomic co-ordinates of the table deviate by a maximum of 0.2 Å from acceptable values for the formula (II).

As the next step, the absolute configuration of the molecule was determined by means of BIJVOET's anomalous dispersion method[6]. Values of $\Delta f'$ and $\Delta f''$ for bromine atoms were taken from the 'International Tables'[7].

[1] T. KISHI, S. HARADA, M. ASAI, M. MUROI and K. MIZUNO, Tetrahedron Letters *2*, 97 (1969).
[2] T. KISHI, H. YAMANA, M. MUROI, S. HARADA, M. ASAI and K. MIZUNO, J. Antibiot., Tokyo, in press (1969).
[3] T. KISHI, M. ASAI, M. MUROI, S. HARADA, E. MIZUTA, S. TERAO, T. MIKI and K. MIZUNO, Tetrahedron Letters *2*, 91 (1969).
[4] Y. TOMIIE, A. FURUSAKI, N. SAKABE, M. NISHIKAWA, K. KAMIYA, H. MATSUDA and I. NITTA, presented at 7th Internat. Cong. and Symp. Crystallography, Moscow, U.S.S.R., July, 1966; Acta Cryst. *21*, A131 (1966).
[5] M. NISHIKAWA, K. KAMIYA, M. TOMITA, Y. OKAMOTO, T. KIKUCHI, K. OSAKI and K. GOTO, J. chem. Soc. *B*, 652 (1968).
[6] J. M. BIJVOET and A. F. PEERDEMAN, Acta Cryst. *9*, 1012 (1956).
[7] *International Tables for X-ray Crystallography* (Kynoch Press, Birmingham 1962), vol. III.