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.

J. BERGMAN, H. PERSSON
and K. WETTERLIN

Research Laboratories of A B Draco,
S-221 01 Lund (Sweden), 22 May 1969.

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.

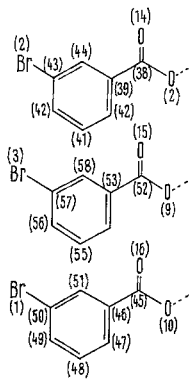
The structure factors, taking the anomalous dispersion into account, were calculated. In about 200 pairs of them, differences in values of F(hkl) and F($\bar{h}\bar{k}\bar{l}$) were obvious. The intensities of these pairs of I(hkl) and I($h\bar{k}l$), which is equivalent to I($\bar{h}\bar{k}\bar{l}$), were compared visually on the same films. Of these, differences of 158 pairs were recognizable. Most of the results (90%) were consistent with the calculated differences based on the atomic parameters in the Table. The resulting stereochemical structure is shown in the Figure. The structure of tolypomycinone is easily derived from (II). The structure is fully consistent with the chemical results on the constitution of tolypomycinone.

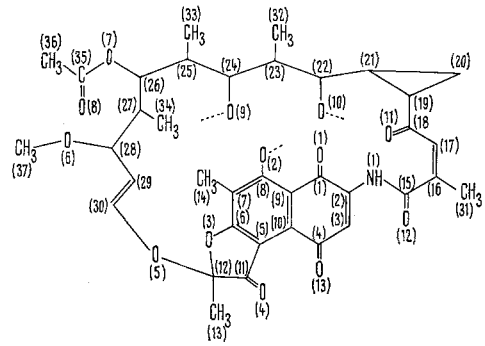
The chemical structure of tolypomycinone is closely related to those of rifamycins and streptovaricins. These molecules consist of naphthoquinone rings and aliphatic macro-rings, that is, so-called ansa-constitution. The absolute configuration of tolypomycinone is also the same as that of rifamycin which was determined chemically[8].

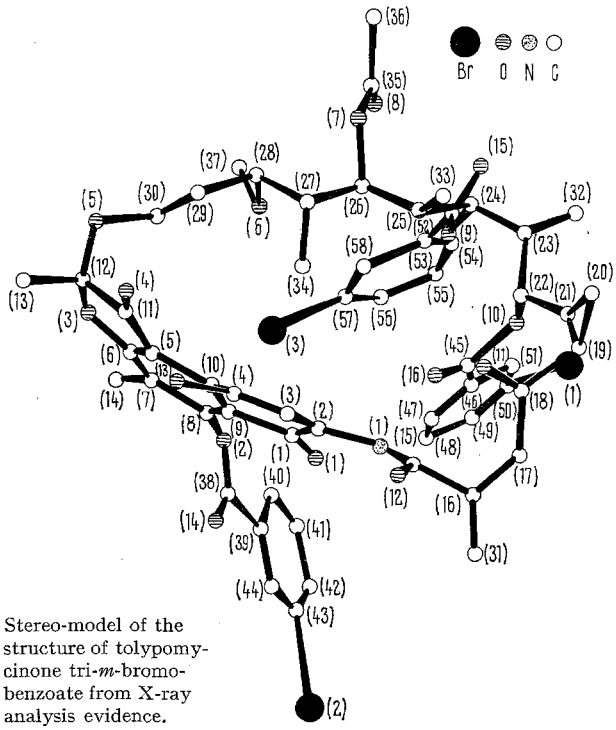
The crystal structures of 2 derivatives of rifamycins[9,10] have been determined by X-ray method. It would be interesting to compare the structural geometry of these compounds. In the structure of tolypomycinone, the methyl and the adjacent double bond of rifamycin B are replaced by the cyclopropane ring and the carbonyl. The configurations of the 2 double bonds are the same. In all cases C(15) and C(18) are *cis* across the C(16)–C(17) double bond. O(5) and C(28) are *trans* across the C(29)–C(30) double bond. In the ester group at C(26) the oxygen atom O(8) is *s-cis* to the alcoholic carbon C(26).

Apart from the differences owing to the changed constitutions described above, one of the most remarkable differences in conformation between the molecules of rifamycin and tolypomycinone is found at the juncture of N(1)–C(15) amide bond. The interplaner angle between the planes of the amide group and of the naphthoquinone ring differs by about 90° in these 2 molecules.

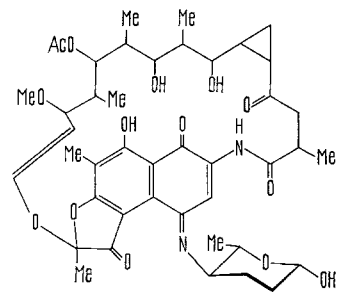
Another important difference exists in hydrogen bond. In the structure of II there are neither intramolecular nor intermolecular hydrogen bonds. As the crystals of II have no solvents of crystallization, the contacts between the main molecule and solvent molecules are impossible, whereas such intermolecular interactions have been reported in the case of rifamycin Y iodoanilide[10]. All the intermolecular distances are greater than the sum of the van der Waals radii of the 2 atoms at the intermolecular contact.







Stereo-model of the structure of tolypomycinone tri-*m*-bromobenzoate from X-ray analysis evidence.



[8] J. LEITICH, W. OPPOLZER and V. PRELOG, Experientia *20*, 343 (1964).

[9] M. BRUFANI, W. FEDELI, G. GIACOMELLO and A. VACIAGO, Experientia *20*, 339 (1964).

[10] M. BRUFANI, W. FEDELI, G. GIACOMELLO and A. VACIAGO, Experientia *23*, 508 (1967).

Final atomic co-ordinates and temperature factors

Atom	x/a	y/b	z/c	b	Atom	x/a	y/b	z/c	b
Br (1)	0.2763	0.1782	0.8309	4.97	C (20)	0.2908	0.1176	0.0950	4.37
Br (2)	0.4980	0.0209	0.3311	8.94	C (21)	0.2868	0.1365	0.2240	1.53
Br (3)	0.4407	0.3539	0.3013	12.31	C (22)	0.2219	0.1185	0.2898	1.43
O (1)	0.5009	0.2357	0.6390	4.43	C (23)	0.2240	0.0575	0.3251	3.66
O (2)	0.3945	0.2495	0.4919	4.02	C (24)	0.1618	0.0345	0.3529	3.99
O (3)	0.2371	0.3610	0.6408	5.42	C (25)	0.1049	0.0415	0.2597	2.45
O (4)	0.2830	0.3778	0.9430	8.13	C (26)	0.0408	0.0170	0.3141	3.77
O (5)	0.2430	0.4448	0.7326	5.92	C (27)	0.4765	0.4707	0.7495	4.72
O (6)	0.4239	0.4623	0.5702	4.46	C (28)	0.4151	0.4926	0.6498	4.53
O (7)	0.4600	0.0450	0.7948	5.31	C (29)	0.3529	0.4728	0.7394	3.96
O (8)	0.4454	0.0543	0.9798	6.18	C (30)	0.3075	0.4511	0.6870	6.63
O (9)	0.1359	0.0621	0.4548	2.11	C (31)	0.1723	0.3564	0.1424	7.41
O (10)	0.2167	0.1459	0.3914	2.53	C (32)	0.2887	0.0432	0.3910	4.02
O (11)	0.1712	0.1827	0.0825	2.88	C (33)	0.1305	0.0249	0.1576	5.99
O (12)	0.0837	0.2790	0.9959	4.60	C (34)	0.4736	0.4077	0.7703	5.38
O (13)	0.3658	0.3005	0.9902	5.83	C (35)	0.4513	0.0736	0.8944	5.39
O (14)	0.3844	0.1733	0.5816	3.37	C (36)	0.4449	0.1386	0.8652	5.72
O (15)	0.1730	0.0075	0.5743	6.84	C (37)	0.4045	0.4936	0.4725	6.53
O (16)	0.1264	0.1903	0.3453	3.27	C (38)	0.3973	0.1975	0.5066	1.52
N (1)	0.0699	0.2572	0.1745	6.70	C (39)	0.4370	0.1663	0.4107	3.02
C (1)	0.4707	0.2555	0.7122	3.54	C (40)	0.4550	0.1950	0.3117	4.68
C (2)	0.5028	0.2614	0.8286	6.23	C (41)	0.4706	0.1683	0.2134	4.87
C (3)	0.4622	0.2749	0.9156	5.18	C (42)	0.4937	0.1150	0.2329	4.39
C (4)	0.4020	0.2895	0.9144	4.61	C (43)	0.4758	0.0907	0.3176	4.15
C (5)	0.3166	0.3233	0.7661	6.06	C (44)	0.4502	0.1143	0.4267	4.26
C (6)	0.2874	0.3294	0.6586	6.44	C (45)	0.1651	0.1788	0.4119	3.91
C (7)	0.3136	0.3029	0.5601	5.66	C (46)	0.1635	0.2012	0.5417	3.88
C (8)	0.3752	0.2735	0.5894	4.14	C (47)	0.1156	0.2327	0.5646	3.99
C (9)	0.3985	0.2742	0.6919	3.44	C (48)	0.1181	0.2469	0.6829	3.27
C (10)	0.3724	0.2981	0.7848	3.65	C (49)	0.1637	0.2292	0.7555	4.73
C (11)	0.2732	0.3696	0.8555	6.16	C (50)	0.2091	0.1980	0.7340	4.28
C (12)	0.2268	0.3925	0.7580	5.19	C (51)	0.2098	0.1810	0.6128	2.89
C (13)	0.1564	0.3895	0.8074	5.46	C (52)	0.1459	0.0437	0.5587	3.82
C (14)	0.2738	0.3039	0.4561	7.56	C (53)	0.1165	0.0728	0.6522	5.55
C (15)	0.1027	0.2757	0.0892	1.80	C (54)	0.1502	0.0703	0.7653	7.52
C (16)	0.1659	0.2953	0.1130	5.27	C (55)	0.1295	0.0992	0.8658	8.10
C (17)	0.2167	0.2651	0.1303	2.59	C (56)	0.0790	0.1157	0.8352	5.94
C (18)	0.2114	0.2016	0.1094	3.14	C (57)	0.0398	0.1158	0.7411	7.69
C (19)	0.2881	0.1744	0.1428	3.64	C (58)	0.0613	0.0902	0.6232	6.37

These facts indicate that the molecules are held together in the crystalline state mainly by van der Waals forces.

Zusammenfassung. Bestimmung der Molekularstruktur des Tolypomycinones durch Röntgenstrukturanalyse seiner *m*-Bromobenzoat-Kristalle. Die auf chemischem Wege aufgeklärte Konstitution wird bestätigt und darüber hinaus Klärung über Konfiguration und Konformation des Tolypomycin Y erreicht.

K. KAMIYA, T. SUGINO, Y. WADA, M. NISHIKAWA and T. KISHI

Research and Development Division, Takeda Chemical Industries, Ltd., Osaka (Japan), 14 April 1969.

Catalytic Properties of Synthetic Pentapeptides

The amino-acid sequences around the active sites of some hydrolytic enzymes have been determined and the functional side chain groups of amino-acids such as serine and histidine have been shown to be essential. Information about the role of these groups has usually been obtained by modifying them and testing the enzymic activity of the modified enzyme[1]. On the other hand many peptides containing in different sequences the amino-acids known to be involved at the active site of hydrolytic enzymes have been synthetized and tested as catalysts in model hydrolytic reactions. The peptide L-threonyl-L-alanyl-L-seryl-L-histidyl-L-aspartic acid (A)[2], which is known to occur at the active site of the enzyme phosphoglucomutase[3] has been shown to exhibit a catalytic activity towards the hydrolysis of *p*-nitrophenyl

[1] Cf. for example the modification of the active L-serines of α-chymotrypsin and subtilisin to dehydroalanine (H. WEINER, W. N. WHITE, D. G. HOARE, and D. E. KOSHLAND JR., J. Am. chem. Soc. *88*, 3851 (1966)) and to L-cysteine (L. POLGAR and M. L. BENDER, J. Am. chem. Soc. *88*, 3153 (1966)) respectively.

[2] Abbreviations follow the tentative rules of IUPAC-IUB Commission on Biochemical Nomenclature, J. biol. Chem. *241*, 2491 (1966).

[3] C. MILSTEIN and F. SANGER, Biochem. J. *79*, 456 (1961).