Communications to the Editor

THE STRUCTURE AND ABSOLUTE CONFIGURATION OF THE 18-MEMBERED MACROLIDE LACTONE ANTIBIOTIC X-4357B (CONCANAMYCIN A)

Sir:

The structure of the first 18-membered lactone, borrelidin, was described in 1967 by KELLER-SCHIERLEIN[1]. In recent years, four more antibiotics have been added to this class. Concanamycins A, B and C[2,3], produced by *Streptomyces diastatochromogenes* S45, inhibit the proliferation of mouse splenic lymphocytes stimulated by concanavalin A. The fourth antibiotic, virustomycin A[4,5] also produced by a *Streptomyces* sp. (AM-2604A), is active against trichomonads and both DNA and RNA viruses. In 1978, a new antibiotic X-4357B, was reported in the patent literature[6] to possess fungicidal, larvicidal and cytotoxic activities. In this communication, the structure determination of this 18-membered macrolide antibiotic by X-ray analysis of its diacetate derivative is described.

The molecular formula of antibiotic X-4357B (**1a**) was deduced by microanalysis and fast-atom-bombardment mass spectrometry to be $C_{46}H_{75}NO_{14}$ (866.12). Although the antibiotic crystallized from methylene chloride - ethanol, the crystals were not suitable for X-ray analysis. Conversion of antibiotic X-4357B to a diacetate (**1b**) which crystallized from ether - hexane, yielded acceptable crystals for X-ray analysis. The diacetate ($C_{50}H_{79}NO_{16}$) has a mp 169~171°C.

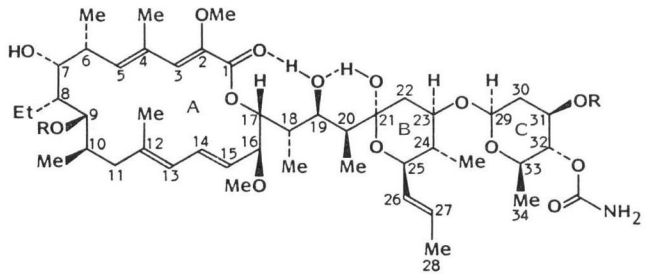
The crystal data of **1b** are listed in Table 1*. The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered CuKα radiation, $\theta-2\theta$ scans, pulse-height discrimination). The size of the crystal used for data collection was approximately $0.30\times0.35\times0.40$ mm; the data were not corrected for absorption. Of the 5,778 accessible reflections for $\theta<76°$, 5,033 were considered to be observed [$I>3.0\sigma(I)$].

Table 1. Crystal data for **1b**.

Crystal system	Monoclinic
Space group	$P2_1$
a	13.375 (4) Å
b	18.871 (7) Å
c	11.317 (7) Å
β	106.39 (4)°
Z	2
d_{calcd}	1.152 g cm^{-3}
μ(CuKα)	7.1 cm^{-1}

The structure of **1b** was solved by a multiple-solution procedure and was refined by block-diagonal least squares in which the matrix was partitioned into two blocks. Seven reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R=0.040 and wR=0.049 for the remaining 5,026 observed reflections. The final difference map has no peaks greater than ±0.2 eA^{-3}.

Stereoscopic pictures of antibiotic X-4357B diacetate (**1b**) are shown in both the ball-and-stick type presentation (Fig. 1) and as a space-filling model (Fig. 2). The macrocyclic ring



* The list of atomic parameters has been sent to Cambridge Crystallographic Data Center.

Fig. 1. A stereoscopic ball-and-stick drawing of antibiotic X-4357B diacetate (**1b**). The dashed bonds indicate intramolecular hydrogen bonding.

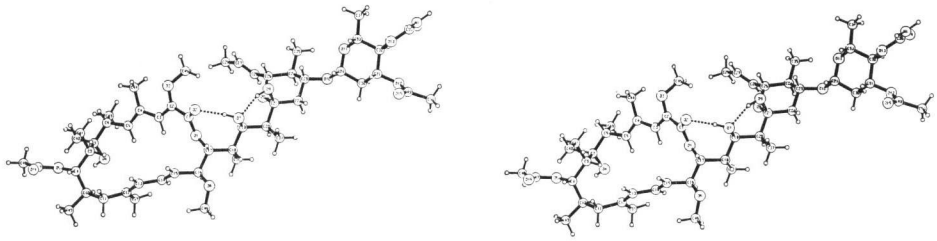


Fig. 2. A stereoscopic space-filling drawing of antibiotic X-4357B diacetate with the same orientation as in Fig. 1.

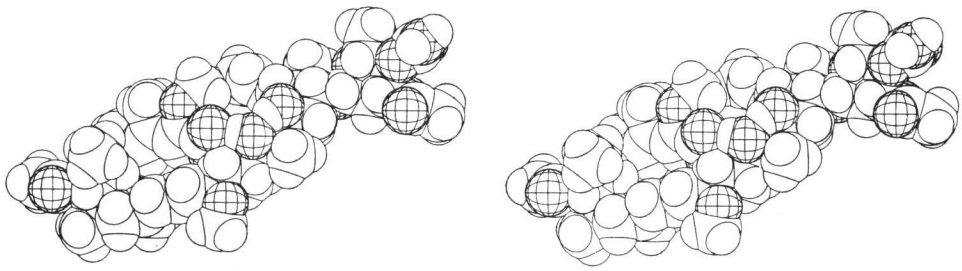


Table 2. Minimum inhibitory concentration (MIC) values for antibiotic X-4357B (**1a**).

Organism	MIC (μg/ml)
Candida albicans 155 (NRRL 477)	1.11
Saccharomyces cerevisiae 90 (ATCC 4226)	3.33
Paecilomyces varioti M16 (ATCC 26820)	3.33
Penicillium digitatum 0184 (ATCC 26821)	3.33

cavity which is so prominent in the ball-and-stick representation of **1b** is virtually nonexistent in the space-filling model.

The absolute configuration of antibiotic X-4357B (**1a**) is based on the isolation and identification of 2-deoxy-D-rhamnose as a degradation product from the alkaline degradation[7] of concanamycin A. Ring C of **1a** is therefore 4-*O*-carbamyl-2-deoxy-D-rhamnose, an isomer of 3-*O*-carbamyl-2-deoxy-D-rhamnose which has been found in venturicidin A[8]. These results are of interest as the opposite enantiomeric form of 2-deoxy-D-rhamnose, namely L-olivose, has been reported[9] for another macrolide antibiotic, *O*-demethyloleandomycin and as the 3-*O*-methyl derivative (L-oleandrose) in oleandomycin.

Antibiotic X-4357B (**1a**) exhibits antifungal activity against *Candida albicans*, *Penicillium digitatum*, *Saccharomyces cerevisiae* and *Paecilomyces varioti* as shown in Table 2.

The antibiotic **1a** is also active against *Piricularia oryzae* (rice-blast disease), *Puccinia* and *Uromyces* species (cereal-rust disease), *Botrytis cinerea* (grey-mold disease) and *Rhizoctonia solani* (damping-off disease) on several plants. As a larvicidal agent, **1a** has exhibited excellent activity against such species as the Colorado potato beetle, army worms, housefly, bean beetle and mosquito larvae amongst others. As a cytotoxic agent, the compound has exhibited activity in the KB cells (from baby-hamster kidney) monolayer test at an ID_{50} of 0.0045 μg/ml. Antibiotic X-4357B (**1a**) has a 24-hour acute toxicity (LD_{50}) in mice of 21 mg/kg (po) and 2.45 mg/kg (ip).[6]

The structure of antibiotic X-4357B (**1a**) as elucidated by the X-ray analysis of the diacetyl ester of the antibiotic (**1b**) is virtually identical to that proposed by KINASHI *et al.* for concanamycin A, on the basis of ^{1}H NMR analyses of that antibiotic and its ozonolysis products. However, in the case of concanamycin A, the absolute configuration of only ring C was proposed, leaving the seven asymmetric centers in ring A, the four in ring B and the three centers at C-18, C-19 and C-20 unassigned. All fourteen of these chiral centers are assigned for the first time in this communication.

After this work was completed, we received a sample containing concanamycins A, B and C from Dr. HARUYASU KINASHI of the Mitsubishi-Kasei Institute of Life Sciences. Comparison by thin-layer chromatography revealed the same mobility for both concanamycin A[2,3] and antibiotic X-4357B.

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References

1) KELLER-SCHIERLEIN, N.: Stoffwechselprodukte von Mikroorganismen. 55. Uber die Konstitution des Borrelidins. Helv. Chim. Acta 50: 731~753, 1967
2) KINASHI, H.; K. SOMENO & K. SAKAGUCHI: An antimitogenic agent of mouse lymphocytes produced by a streptomycetes S-45. Abstract Papers of Annual Meeting of Agric. Chem. Soc. Japan, p. 515, Fukuoka, April 4, 1980
3) KINASHI, H.; K. SOMENO, K. SAKAGUCHI, T. HIGASHIJIMA & T. MIYAZAWA: Structure of concanamycin A. Tetrahedron Lett. 22: 3861~3864, 1981
4) ŌMURA, S.; K. OTOGURO & H. TANAKA: The mode of action of a novel 18-membered macrolide, virustomycin A (AM-2604 A), on *Trichomonas foetus*. J. Antibiotics 36: 1755~1761, 1983
5) ŌMURA, S.; N. IMAMURA, K. HINOTOZAWA, K. OTOGURO, G. LUKACS, R. FAGHIH, R. TOLMANN, B. H. ARISON & J. L. SMITH: The structure of virustomycin A. J. Antibiotics 36: 1783~1786, 1983
6) WESTLEY, J. W.: Antibiotic X-4357B. U.S. 4,076,802, Feb. 28, 1978
7) KINASHI, H.; K. SOMENO & K. SAKAGUCHI: Alkaline degradation products of concanamycin A. Tetrahedron Lett. 22: 3857~3860, 1981
8) BRUFANI, M.; W. KELLER-SCHIERLEIN, W. LOFFLER, I. MANSPERGER & H. ZÄHNER: Über das Venturicidin B, das Botrycidin und die Zuckerbausteine der Venturicidine A und B. Helv. Chim. Acta 51: 1293~1304, 1968
9) CELMER, W. D.: Stereochemical problems in macrolide antibiotics. Pure & Appl. Chem. 28: 413~453, 1971