ISOLATION AND CHARACTERIZATION OF A NOVEL POLYETHER ANTIBIOTIC OF THE PYRROLETHER CLASS, ANTIBIOTIC X-14885A

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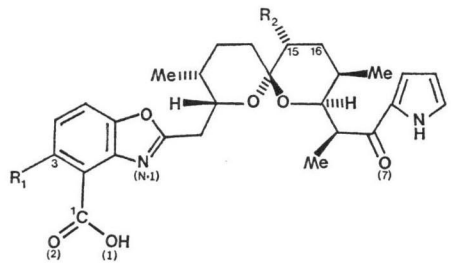
Antibiotic X-14885A is a polyether antibiotic belonging to the class of these natural acid ionophores known as pyrrolethers. The structure of the antibiotic was elucidated by X-ray crystallographic analysis of the hydrated sodium salt, which crystallized as a tetramer containing four antibiotic and water molecules and four atoms of sodium. Antibiotic X-14885A differs from the most well-known member of the class, A-23187, in two respects: the aromatic *N*-methylamino group present in the latter is replaced by a phenolic hydroxyl, and one of the four aliphatic methyls is replaced by a proton. Antibiotic X-14885A is active against Gram-positive bacteria and the spirochete, *Treponema hyodysenteriae*.

The polyether antibiotics fall into four broad classes[1]. The antibiotic reported here, X-14885A (**1**), belongs to the pyrrolether class, whereas most of these naturally occurring acid ionophores are either monovalent or divalent polyethers with only two examples so far of the fourth class, the acyl tetronic acids.



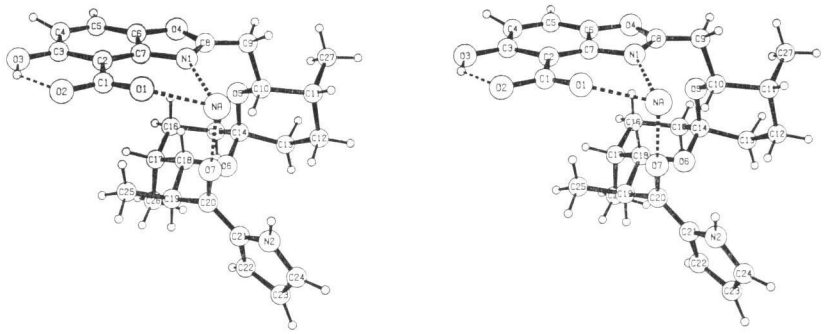
1 X-14885A R_1=OH, R_2=H
2 A-23187 R_1=NHMe, R_2=Me
3 Cezomycin R_1=H, R_2=Me

One pyrrolether antibiotic, X-14547A[2], was isolated from *Streptomyces antibioticus*, but the others reported earlier are all elaborated by different strains of *S. chartreusis*. The most well-known antibiotic in the class is antibiotic A-23187 (**2**)[3] which, in addition to its antimicrobial activity against Gram-positive bacteria, has potent pharmacological activities including cardiovascular and renal effects[4]. ABBOTT *et al.*[5] have prepared *N*-demethylated A-23187, 16-hydroxy-*N*-demethyl A-23187 and 16-hydroxy A-23187 by microbial transformation of the methyl ester of **2** with different strains of *S. chartreusis*. Another strain of this microorganism has recently been reported[6] to produce cezomycin (**3**), the demethylamino derivative of A-23187.

As part of our search for novel antimicrobial agents active *inter alia* against *Treponema hyodysenteriae*, fermented cultures of a newly discovered microorganism designated *Streptomyces* sp. X-14885 were extracted with an equal volume of ethyl acetate at pH 10 and the crude extract partitioned between *n*-hexane and acetonitrile. The acetonitrile layer was concentrated and the residue dissolved in methylene chloride and washed sequentially with 1 N HCl, saturated Na_2CO_3 and water. After drying over Na_2SO_4, concentration of the solution with addition of diethyl ether yielded the crystalline sodium salt of antibiotic X-14885A, which on recrystallization from aqueous acetone gave the pure salt as a mono-

Fig. 1. A stereoscopic drawing of the anion of antibiotic X-14885A in which the coordination of the sodium ion by O(1), N(1) and O(7) is indicated by dashed bonds.

The carboxylate oxygen O(1) also coordinates a second Na in the dimer and a third in the tetramer.



hydrate, $C_{27}H_{31}N_2O_7Na \cdot H_2O$ (calcd.: C 60.44, H 6.19, N 5.22, Na 4.28, H_2O 3.36; found: C 60.72, H 5.92, N 5.20, Na 4.30, H_2O 2.87, mol. wt. 536.57), mp 264~266°C, $[\alpha]_D$ +177° (*c* 1, $CHCl_3$), IR ν_{max} $CHCl_3$ 3140 (phenolic OH), 1640 (C=C–C=O) and 1610 (CO_2^-) cm^{-1} and UV max in ethanol, 204 (ε 30,690), 257 (ε 15,130) and 306 nm (ε 21,840).

The structure of antibiotic X-14885A (**1**) was determined from a single crystal X-ray analysis of the aforementioned hydrated sodium salt and found to differ from antibiotic A-23187 (**2**) at C-3, where the methylamino substituent is replaced by a phenolic hydroxyl, OH (3) and at C-15, where the methyl group in A-23187 is replaced by a proton. The absolute configuration of antibiotic X-14885A (**1**) has been established as illustrated, by EVANS and CRIMMINS[7] in an unequivocal synthesis of the natural product.

In the crystal of the monohydrated sodium salt of X-14885A, the antibiotic molecules are part of a tetramer consisting of four X-14885A anions, four sodium ions and four water molecules. The unique portion of the unit cell consists of half the tetramer, a dimer, which is related to the other one in the cell by a crystallographic 2-fold rotation axis. Each tetramer contains four structurally similar units. The coordination of a sodium ion by an antibiotic molecule is illustrated in Fig. 1. The sodium ion, located about 0.4 Å from the plane of the benzoxazole ring, is coordinated by the carboxylate oxygen O(1) and the benzoxazole nitrogen N(1) in that same plane, and by the carbonyl oxygen O(7) perpendicular to the plane. Also coordinating this same sodium ion are the water molecule O(11) and the carboxylate oxygen O(1′) of the other molecule present in the dimer (Fig. 2). Note that both oxygens O(1) and O(1′) coordinate both Na and Na′ in the dimer. The sixth and final ligand of the sodium ion Na is O(1) of the other dimer in the tetramer. These ligands are significantly longer (2.63~2.76 Å) than the other cation binding ligands in the tetramer (2.32~2.48 Å).

The complete tetramer is shown in Fig. 3. The formation of a tetramer from two dimers can be visualized with the aid of Fig. 2. The 2-fold axis which relates the two dimeric halves of the tetramer passes diagonally beneath the dimer in this view, parallel to a line from the lower left to upper right. Upon rotation of the dimer through 180° about this axis, the sodium at the left in the drawing will end up beneath O(1′) and the other sodium will be beneath O(1). Thus, at the center of the tetramer is a distorted cube with four sodium ions at one set of tetrahedral corners and four O(1) atoms [two O(1) and two O(1′)] at the other set of tetrahedral corners. The two dimers which form the tetramer are also joined by four inter-dimer hydrogen bonds: two O(11)–H···O(2) and two O(11′)–H···O(2′). Again, note that the O···O distances (2.96 and 3.01 Å) in these hydrogen bonds are greater than the lengths of the

Fig. 2. A stereoscopic drawing of the two independent molecules of X-14885A showing how they form a dimer *via* the coordination of two Na ions.

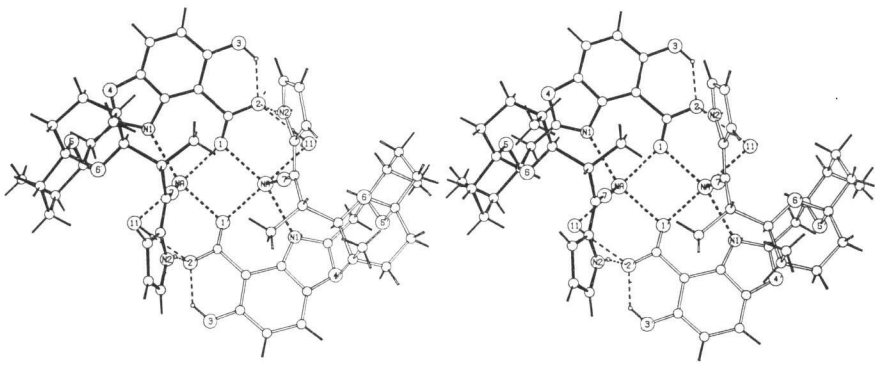
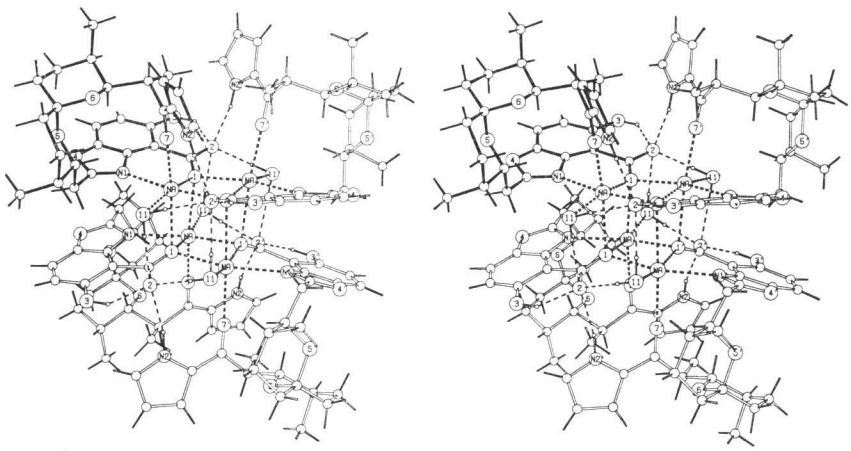


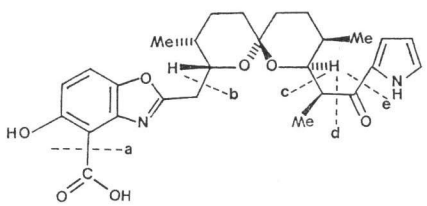
Fig. 3. A stereoscopic drawing of the full tetramer of the sodium salt of X-14885A.
The eight links, four Na···O coordination bonds and four O(11)-H···O(2) hydrogen bonds which join the two halves (see Fig. 2) of the tetramer run vertically in this view.



corresponding intra-dimer hydrogen bonds O(11)–H···O(2′) and O(11′)–H···O(2), which are 2.78 and 2.81 Å.

Electron impact mass spectrometry of antibiotic X-14885A yielded a number of fragment ions as a result of the bond cleavages indicated in Fig. 4. As expected for an aromatic *ortho*-hydroxy acid, facile decarboxylation is observed (a) to yield peaks at *m/z* 44 (CO_2) and 452 ($C_{26}H_{32}N_2O_5$). Subsequent cleavage at (b) results in a major peak at *m/z* 149 ($C_8H_7NO_2$) and a minor one at 303 ($C_{18}H_{25}NO_3$). Fragmentation c at the other end of the molecule gives rise to a peak at *m/z* 123 (C_7H_9NO) and a less intense peak at 311 ($C_{19}H_{21}NO_3$) due to a combination of a and c and the loss of water. Further evidence for the pyrrole carbonyl moiety characteristic of the pyrrolether class of polyether antibiotics is provided by the base peak at *m/z* 94 (C_5H_4NO) due to cleavage d and a peak at *m/z* 66 (C_4H_4N) from cleavage e.

Fig. 4. Mass spectral fragmentation of antibiotic X-14885A.



Antibiotic X-14885A exhibits *in vitro* activity at concentrations less than 1 μg/ml against such Gram-positive bacteria as *Staphylococcus aureus* and *Bacillus subtilis* and the spirochete responsible for swine dysentery, *T. hyodysenteriae*.

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