Structure of Antibiotic X-537A

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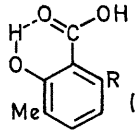
Summary The structure and absolute configuration of antibiotic X-537A have been determined by physical and chemical methods.

THE isolation of three crystalline antibiotics, X-206, X-464, and X-537A from three different Streptomyces species was reported from this laboratory[1] in 1951. The three antibiotics had similar biological activity as well as the unusual property that their alkali salts were soluble in such non-polar solvents as benzene and ether, but virtually insoluble in water.

Antibiotic X-464 has recently been shown[2] to be identical to nigericin[3] (polyetherin A[4]) using a combination of i.r., mass spectral, and polarimetric methods. Nigericin belongs to the same class of polyether antibiotics as the monensins.[5] These compounds have the common property of being monocarboxylic acids containing a number of cyclic ether moieties.

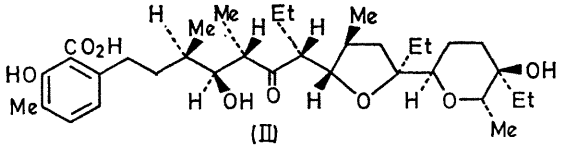
Antibiotic X-537A ($C_{34}H_{54}O_8$)[6] m.p. 110—114°, $[\alpha]_D^{25}$ −7·55° (*c* 1, MeOH) was unique in this class of antibiotics in possessing an aromatic chromophore with u.v. maxima at 248 (ϵ 6750) and 318 nm (ϵ 4200) in 50% aqueous isopropyl alcohol. The sodium salt ($C_{34}H_{53}O_8Na$) m.p. 168—171°, $[\alpha]_D^{25}$ −30° (*c* 1, MeOH) had an inflection in the u.v. at 245 and a maximum at 308 nm (ϵ 4100). The presence of a phenolic group was indicated by a positive iron(III) chloride reaction[7] in chloroform suggesting that the chromophore was a hydroxybenzoic acid. Both *m*- and *p*-hydroxybenzoic acids gave negative iron(III) chloride reactions whereas salicylic acid gave an identical reaction to the antibiotic. Potentiometric titration in 66% dimethylformamide gave a single pk_a = 5·13 (salicylic acid = 4·55, *m*-hydroxybenzoic acid = 6·8, 12·9). Further proof of a salicylic chromophore for the antibiotic was the hypsochromic shift in the u.v. on transformation of the free acid to its salt form. Salicylic acid behaves similarly, but *m*-hydroxybenzoic acid undergoes a bathochromic shift under these conditions. N.m.r. ($CDCl_3$) of the barium salt showed the presence of an aromatic methyl singlet at δ 2·14, and two aromatic protons at δ 6·38 and 6·91 (J_{ortho} 8 Hz). These results suggested a partial structure (I) for the antibiotic.



(I) (R = remainder of molecule)

Kuhn–Roth oxidation of antibiotic X-537A indicated at least 7 C-Me groups (Found: 17·52%; 7 C-Me, 17·9%; 8 C-Me, 20·3%). There was no evidence of methoxy-groups. The i.r. spectrum ($CHCl_3$) of the antibiotic showed the presence of a hydrogen-bonded carboxyl group (1650 cm^{-1}) and a saturated ketone (1700 cm^{-1}). In the salt form, these peaks were at 1600 (carboxylate) and 1710 cm^{-1} (ketone). There was also evidence for hydroxy-groups (3200—3500 cm^{-1}). Treatment of the i.r. sample with pyridine and phenyl isocyanate gave two urethane peaks at 1750 and 1783 cm^{-1} suggesting the presence of a secondary alcohol and phenol respectively.

From this evidence and the *X*-ray crystallographic analysis[8] of the barium salt of X-537A, the following structure (II) is suggested for the antibiotic:

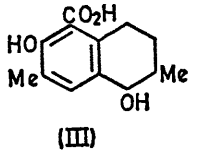


(II)

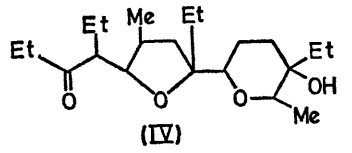
If a β-ketol system is present as in (II) the molecule should be cleaved by base. Treatment of the antibiotic with 10% aqueous sodium hydroxide in dioxan (1 : 1) yielded (III) and (IV) which were separated and identified.

Compounds (III) and (IV) arose from a retrograde aldol reaction. The initial products must be (IV) and (V) ($R^1 = R^2 = H$), but due to activation of the C-5 position of the salicylic acid chromophore towards electrophilic attack,

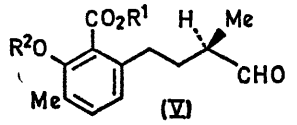
(V) immediately cyclises to (III). The latter was shown to be 2,5-dihydroxy-3,6-dimethyl-5,6,7,8-tetrahydro-1-naphthoic acid by n.m.r. (CD_3OD). There was no aldehydic



(III)



(IV)



(V)

proton in the spectrum, but a broad band at δ 3·92 showed the presence of an axial benzylic methine proton. There was an aromatic proton at δ 7·25 singlet and protons at δ 0·97 (d, 3, CH_3–CH, J 7 Hz), 1·0—2·0 (m, 3, CH_2–CH), 2·20 (s, 3, aromatic CH_3), and 3·0 (t, 2, benzylic CH_2). Mass spectrometry of (III) ($C_{13}H_{16}O_4$, M^+ 236) and (IV) ($C_{21}H_{38}O_4$, M^+ 354) supported a molecular formula of $C_{34}H_{54}O_8$ for the antibiotic rather than $C_{34}H_{52}O_8$ suggested earlier.[1] Reduction of the ketone group in (II) with sodium borohydride gave a compound which was resistant to base attack, confirming the retroaldol mechanism for the cleavage of the antibiotic β-ketol system.

Methylation of the antibiotic (MeI, Ag_2O) followed by pyrolysis at 170° (0·05 mm.) gave 6-(3-formylbutyl)-2-methoxy-3-methylbenzoic acid, methyl ester (V, $R^1 = R^2 = Me$) $C_{15}H_{20}O_4$, M^+ 264, $[\alpha]_D^{25}$ −1·07° (MeOH), ν_{max} ($CHCl_3$) 1720 (C=O), λ_{max} (MeOH) 275 nm (ε 1380), δ($CDCl_3$) 1·10 (d, 3, J 8 Hz, CH_3–CH), 1·5—2·3 (m, 3, CH_2–CH), 2·27 (s, 3, aromatic CH_3), 2·58 (m, 2, CH_2–CH_2), 3·75, 3·90 (2s, 6, aromatic CO_2CH_3 and OCH_3) 6·89, 7·15 (AB, 2, J_{ortho} 8 Hz, aromatic) 9·55 (d, 1, J 2Hz, –CHO). Compound (V; $R^1 = R^2 = Me$) exhibits a negative Cotton effect (in o.r.d. a trough at 312 nm, $[\phi] = +54°$; in c.d. a negative maximum at 303 nm $[\theta] = +68°$). By comparison with *S*-2-methylbutanal, which has been shown[9] to exhibit a positive Cotton effect, (V) must have the *R*-configuration.

From the *X*-ray analysis of the antibiotic salt, the relative configuration of the ten asymmetric centres was deduced and in combination with the polarimetric results on (V; $R^1 = R^2 = Me$), the complete structure of X-537A is 3-methyl-6-[7(*R*)-ethyl-4(*S*)-hydroxy-3(R),5(*S*)-dimethyl-6-oxo-7-{5(*S*)-ethyl-3(*S*)-methyl-5-[5(*R*)-ethyl-5-hydroxy-6(*S*)-methyl-2(*R*)-tetrahydropyranyl]-2(*S*)-tetrahydrofuryl}-heptyl]salicylic acid.

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