Structure of Pseudomonic Acid, an Antibiotic from *Pseudomonas fluorescens*

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Summary The antibiotic, pseudomonic acid, produced by a strain of *Pseudomonas fluorescens*, is shown to have structure (**1a**).

In a previous publication,[1] we reported the isolation and purification of a group of antimicrobially active substances produced by submerged fermentations of a strain of *Pseudomonas fluorescens*. We ascribed the name pseudomonic acid to the parent member of this family isolated as its methyl ester. The presence of a 9-hydroxy-nonanoic acid residue, the hydroxyl group of which is joined to the rest of the molecule through an α,β-unsaturated ester linkage was also shown. We now present evidence which is in accord with structure (Ia) for pseudomonic acid.

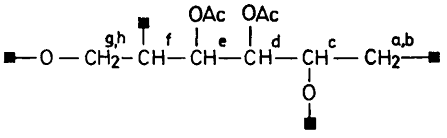


Figure 1. Part structure (a) of (IIIa)

Methyl pseudomonate, (Ib), m.p. 77—8°, $[\alpha]_D -9°$† has a molecular formula $C_{27}H_{46}O_9$ (m.s.) and hence the molecular formula of the parent acid, (Ia), is $C_{26}H_{44}O_9$. The ^{13}C n.m.r. spectrum of (Ib)‡ showed the presence of the following groupings: 2-COR, $-\underset{|}{C}=CH-$, 6 $-\underset{|}{C}H{\cdot}O-$, 2–$CH_2{\cdot}O-$, Me·O–, 2 $-\underset{|}{C}H-$, 9 $-CH_2-$ and 3 Me–. The ^1H n.m.r. spectrum of (Ib) confirmed that two of the methyls were secondary whilst the other was vinylic and showed a methylene envelope and a vinyl proton. Of the nine oxygens in (Ib), four can be accounted for in the ester and α,β-unsaturated ester groupings. Methyl pseudomonate, (Ib), formed the following oily derivatives: a triacetate, (Ic) $[\alpha]_D -1°$ and a tribenzoate, (Id) $[\alpha]_D -11°$, which showed no hydroxyl absorbance in the i.r., an acetonide (Ie), $[\alpha]_D -25°$ and the C-13 keto-derivative of (Ie) $[\alpha]_D -49°$. Thus, (Ib) contains three hydroxyl groups. Treatment of (Ib) with $NaIO_4$ (1 mole uptake) in aqueous EtOH gave an unstable dialdehyde as the sole product. Two of the hydroxyl groups are therefore present in a glycol system, which must be present in a ring. Of the remaining two oxygens in (Ia), it was inferred from the n.m.r. spectra of (Ib) and its derivatives, that one was present in an oxiran ring. The other oxygen must therefore be in an ethereal linkage. The correctness of this assumption becomes apparent in the following discussion.

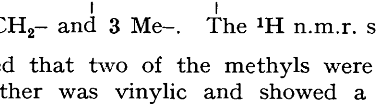


Figure 2. Part structure (b) of (IIIa).

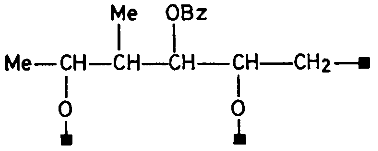


Figure 3. Part structure (c) of (VI).

Treatment of (Ic) with OsO_4–py followed by aqueous sodium metabisulphite gave a diastereomeric mixture of diols, (II), which was cleaved with $NaIO_4$ in aqueous EtOH to give (IIIa), oil $[\alpha]_D -17°$ and (IV), oil (semicarbazone m.p. 162·5—164°); (IIIb) and (IIIc) were similarly obtained from (Id) and (Ie). The formation of (IV) and the methyl ketones (IIIa, b, and c) provided additional proof of the α,β-unsaturated ester linkage of the 9-hydroxynonanoic acid unit and established that the vinylic methyl group is attached to the β-carbon of the double bond. The chemical shift of the vinylic methyl of (Ib) was also in accord with a *trans*-(*E*)-orientation of the double bond.[3]§

Spin decoupling experiments on the methyl ketone triacetate, (IIIa), demonstrated the spin systems shown in Figures 1 and 2. The chemical shifts of H^a,H^b, and the

† Optical rotations were taken in $CHCl_3$; satisfactory analytical and spectral data was obtained for all new compounds.

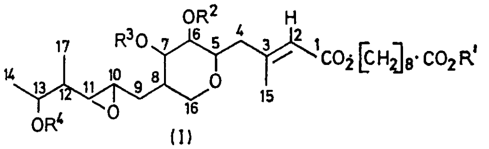
‡ The full assignment of the ^{13}C n.m.r. spectrum of (Ib) will be reported elsewhere.[6]

§ This has been further substantiated by comparison of the ^{13}C n.m.r. spectrum of (Ib) with that of synthetic 8-methoxycarbonyloctanyl-3,3-dimethyl acrylate and literature citations.[5]

absence of further coupling into these two protons, other than from H^c, was in accord with the attachment of Me.CO at this point. The spin–spin coupling of H^k to H^l was proven by the INDOR method on (Id) and the vicinal coupling was consistent with a *trans*-orientation of the epoxide group.[4]

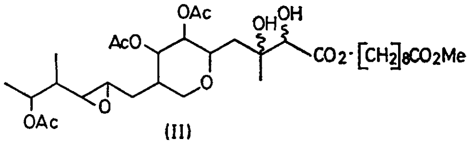
Although it was not possible to demonstrate the coupling of H^f to H^l and H^j, the part structures (a) and (b) (Figures 1 and 2) can only be accommodated in structure (IIIa) for the methyl ketone triacetate. Hence, the structure of pseudomonic acid must be (Ia).

Attempts to confirm the presence of the epoxide by ring opening of (Ic) or (Id) to the corresponding diol failed. On treatment of either (Ic) or (Id) with 60% $HClO_4$ in EtAc, a facile rearrangement to the oily triacetate, (Va), $[\alpha]_D$ +17°, or the tribenzoate (Vb), m.p. 122—123°, $[\alpha]_D$ −2°, occurred. The ^1H n.m.r. spectrum of (Vb) indicated that modification had taken place involving C-10, C-11 and C-13, which was clearly seen in the spectrum of (VI), m.p. 138—139°, $[\alpha]_D$ −4° obtained from (Vb) along with (IV), by the OsO_4 route. The ^1H n.m.r. spectrum (220 MHz) of (VI) confirmed that the substituted perhydropyran ring had remained intact and decoupling experiments (100 MHz) were in accord with ring opening of the epoxide to the part structure (c) (Figure 3) which could only be accommodated in structure (VI).

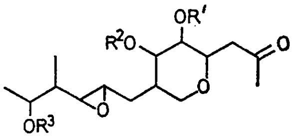


(I)

a; $R^1=R^2=R^3=R^4=H$
b; $R^1=Me$; $R^2=R^3=R^4=H$
c; $R^1=Me$; $R^2=R^3=R^4=Ac$
d; $R^1=Me$; $R^2=R^3=R^4=COPh$
e; $R^1=Me$; $R^2=R^3=Me_2C<$; $R^4=H$
f; $R^1=R^2=R^3=R^4=Me$

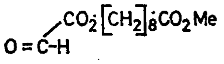


(II)

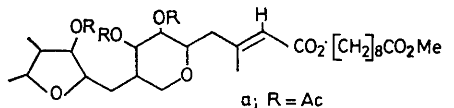


(III)

a; $R^1=R^2=R^3=Ac$
b; $R^1=R^2=R^3=COPh$
c; $R^1=R^2=Me_2C<$; $R^3=H$

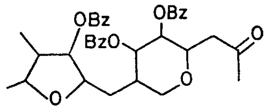


(IV)

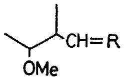


(V)

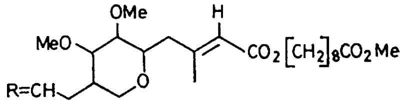
a; R = Ac
b; R = COPh



(VI)



(VII)



(VIII)

a; R=O
b; $R=N \cdot NH \cdot C_6H_3(NO_2)_2$

To overcome the above rearrangements, the oily trimethyl ether, (If), $[\alpha]_D$ −15°, was prepared by treatment of (Ib) with MeI and NaH in dimethyl acetamide. Reaction of (If) with HIO_4 (1·1 equiv.) in ether afforded 3-methoxy-2-methylbutyraldehyde (VIIa) and the aldehyde (VIIIa), isolated as their 2,4-dinitrophenylhydrazone derivatives: (VIIb), m.p. 76—78° $[\alpha]_D$ +6°: (VIIIb), oil, $[\alpha]_D$ −11°. The assigned structure for pseudomonic acid is also supported by mass spectral data of (Ib) and its derivatives and by biosynthetic labelling studies.[6]

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