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THE STRUCTURE AND CONFIGURATION OF PSEUDOMONIC ACID C

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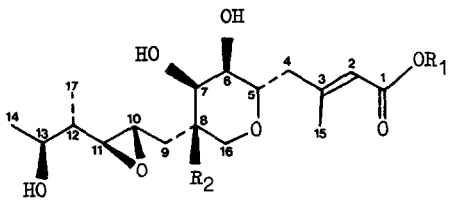
SUMMARY: A new antibiotic, pseudomonic acid C (2a), has been isolated from fermentations of the strain of Pseudomonas fluorescens which produces the known and structurally similar antibiotics pseudomonic acids A (1a) and B (1c).

The isolation and structural elucidation of pseudomonic acids A (1a) and B (1c), produced by fermentation of a strain of Pseudomonas fluorescens, have been reported.[1,2,3,4,5] We now wish to describe the isolation and structural characterization of the third member, which we have designated pseudomonic acid C (2a), of this novel class of antibiotics.

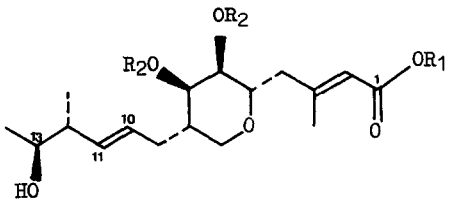
Close examination of the reverse phase high performance liquid chromatography of culture filtrates of Pseudomonas fluorescens revealed, in addition to the peaks corresponding to pseudomonic acids A and B, the presence of a third component of lower polarity which we have called pseudomonic acid C. The ratio of the metabolites was found to be pseudomonic acids A:B:C≡93:5:2. The fermentation brew was acidified and extracted with ethyl acetate, which after drying, was concentrated and diluted with diethyl ether. The major metabolite, pseudomonic acid A, was allowed to crystallize. The mother liquors now enriched in pseudomonic acid C were methylated and the resulting mixture of methyl esters chromatographed on silica gel (type 60) eluting with a gradient 0-4% methanol/chloroform. The fractions containing substantially pure methyl pseudomonate C (Rf 0.43 silica tlc, chloroform/methanol 9:1; methyl pseudomonate A Rf = 0.39 and methyl pseudomonate B Rf = 0.33) were combined and re-chromatographed to give pure methyl pseudomonate C (2b) m.p. 47-49°C, $[\alpha]_D^{20}$ + 13.7° (c, 1.0, $CHCl_3$), with the molecular formula $C_{27}H_{46}O_8$ (compared with $C_{27}H_{46}O_9$ and $C_{27}H_{46}O_{10}$ for methyl pseudomonates A and B respectively). The infra-red spectrum revealed an unsaturated ester at νmax 1710 (C=O) and 1650 (C=C) cm^{-1}, which was confirmed by the u.v. absorption at λmax 222nm (εm 14,900). These features were common to all three pseudomonic acid esters. One additional feature of the i.r. spectrum of methyl pseudomonate C was the

peak at 980cm^{-1}, indicative of a disubstituted trans carbon-carbon double bond. Comparisons of the ^{1}H and ^{13}C nmr spectra of methyl pseudomonates A, B and C clearly indicated structural similarities in the three molecules.

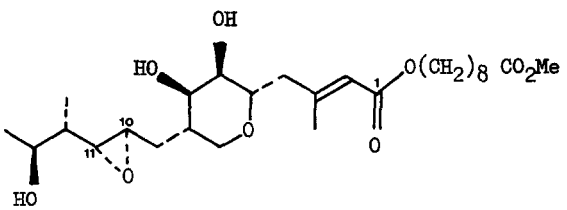


$9' \longleftarrow 1'$

1 a $R_1 = (CH_2)_8\ CO_2H$; $R_2 = H$
b $R_1 = (CH_2)_8\ CO_2Me$; $R_2 = H$
c $R_1 = (CH_2)_8\ CO_2H$; $R_2 = OH$
d $R_1 = (CH_2)_8\ CO_2Me$; $R_2 = OH$



2 a $R_1 = (CH_2)_8\ CO_2H$; $R_2 = H$
b $R_1 = (CH_2)_8\ CO_2Me$; $R_2 = H$
c $R_1 = (CH_2)_8\ CO_2Me$; $R_2 =$ 



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The 90 MHz ^{1}H nmr spectrum of methyl pseudomonate C revealed two secondary methyls at δ_H 0.98 and 1.13; a vinylic methyl at δ_H 2.18; a methylene envelope at δ_H 1.30 and a vinylic proton at δ_H 5.72. The most notable feature of the spectrum was the presence of a complex two-olefinic proton signal at δ_H 5.40 and the absence of epoxide protons. The observations clearly indicated that methyl pseudomonate C was structurally related to methyl pseudomonate A, the major difference being the replacement of the epoxide by an olefinic double bond. The structure was confirmed as (2b) by the off resonance ^{13}C nmr spectrum in which C_{10} and C_{11} appeared as low field doublets at δ_C 134.5 and 129.4. The noise-decoupled ^{13}C nmr spectra of methyl pseudomonates A, B and C are compared in the table.

The geometry of the 10,11-double bond could not be assigned from the ^{1}H nmr spectrum of (2b), since the similar chemical shifts of H-10 and H-11 did not permit measurement of the coupling constant, $J_{10,11}$. Treatment of (2b) with 2,2-dimethoxypropane and p-toluenesulphonic acid afforded, after chromatographic purification, the acetonide derivative (2c) in 64% yield, $C_{30}H_{50}O_8$, $[\alpha]_D^{20}$ -6.2° (c, 1.0, $CHCl_3$). It was anticipated that by blocking the glycol function in this way the remaining 13-hydroxyl group would be the major chelation site for a paramagnetic shift reagent such as Europium tris-[6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate] ($Eu(fod)_3$) and, thereby, influence a sufficient separation in the chemical shifts between H-10 and H-11 to enable measurement of the coupling constant, $J_{10,11}$.

This indeed proved to be the case. The ^1H nmr spectrum of the olefinic region of the acetonide (2c) in (i) the absence and (ii) the presence of 1.15 equivalents of Eu $(fod)_3$ can be seen in the figure. The coupling constant $J_{10,11}$ 15.1 Hz confirmed the trans geometry of the 10,11-double bond. The coupling constant $J_{11,12}$ 7.3 Hz and $J_{9,10}$ 6.5 Hz were confirmed by spin decoupling.

Table: Comparison of ^{13}C n.m.r. spectra

C*	(1b)†	(1d)†	(2b)†
1	166.7	166.8	166.8
2	117.5	117.8	117.6
3	156.6	156.3	156.8
4	42.7	42.7	43.1
5	74.8	70.2	74.8
6	68.8	72.9	68.9
7	70.1	74.6	70.4
8	39.4	72.1	42.0
9	31.6	37.1	32.4
10	55.5	51.8	{129.4
11	61.2	60.7	{134.5
12	42.7	41.7	44.7
13	71.0	71.5	71.2
14	20.7	21.2	20.4
15	19.1	19.2	19.1
16	65.4	68.6	64.8
17	12.7	12.1	16.6
1'	174.2	175.8	174.3
2'	34.1	34.1	34.1
3'	24.9	24.9	24.9
4'-6'	29.0	29.1	29.1
7'	25.9	26.0	26.0
8'	28.7	28.7	28.7
9'	63.8	63.9	63.8
MeO	51.4	51.4	51.4

* Assignments based on work of G. Mellows et al[9]

† P.p.m. to low field of Me_4Si; solvent $CDCl_3$

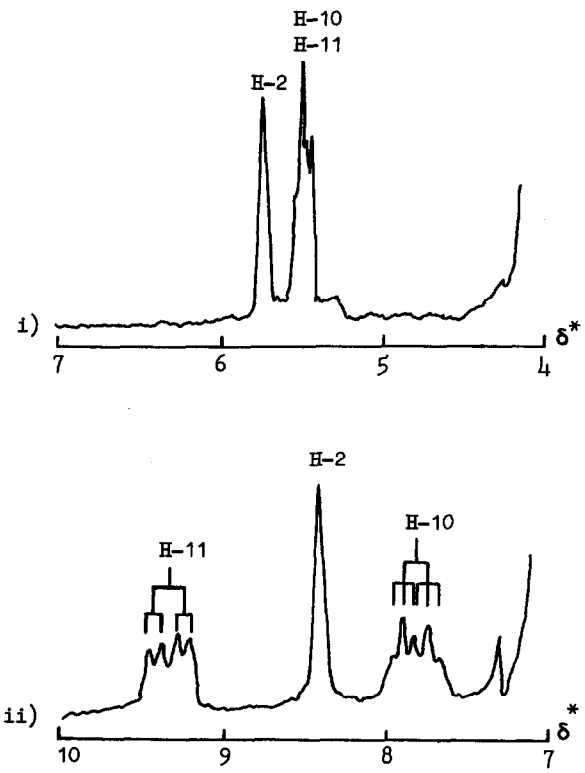

Figure: i) 90MHz ^1H-n.m.r. part spectrum in $CDCl_3$ of (2c)

ii) After addition of 1.15 equivalents of Eu$(fod)_3$ to (2c)

* ppm from $SiMe_4$ as internal standard

Unequivocal proof of the structure of pseudomonic acid C (2a) was afforded by the stereospecific conversion of methyl pseudomonate A (1b) into methyl pseudomonate C (2b) using potassium selenocyanate[7] in refluxing aqueous methanol. The methyl trans 10,11-deoxypseudomonate A obtained, albeit in poor yield (ca 10%), was chromatographically and spectroscopically identical in every respect to "natural" methyl pseudomonate C (2b). The poor yield and long reaction time (7 days) reflects the refractory nature of the epoxide function in pseudomonic acid A towards deoxygenation. Many of the published procedures for converting epoxides to olefins have been examined with little or no success.

In addition the conversion of methyl pseudomonate C to methyl pseudomonate A was investigated. After protection of the hydroxyl functions (2b) as trimethylsilyl ethers, treatment with m-chloroperbenzoic acid in dry methylene chloride afforded, after deprotection, a product which was chromatographically identical (t.l.c.) to methyl pseudomonate A (1b). However both ^{1}H and ^{13}C n.m.r. spectra of this product indicated as expected an epimeric mixture of epoxides (1b) and (3). The epoxide carbons of methyl pseudomonate A (1b) occurred at δ_C56.5 (C10) and 61.0 (C11), whilst those of the isomer (4) occurred at δ_C58.9 (C10) and 62.6 (C11) in CD_3OD. Additional features in the ^{13}C nmr spectrum were consistent with an epimeric mixture of (1b) and (3). The ratio of (1b) and (3) was estimated as 1:2 by the intensity of the ^{13}C epoxide signals and was confirmed by analytical h.p.l.c.

It is interesting to note that while pseudomonic acid A is unstable under both mild acidic and alkaline conditions with eventual total loss of antibiotic activity[8], pseudomonic acid C retains its antibiotic activity.

Acknowledgement

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