The Chemistry of Pseudomonic Acid. Part 6.[1] Structure and Preparation of Pseudomonic Acid D

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Pseudomonic acid D (1c), a minor antibiotic produced by *Pseudomonas fluorescens*, has been isolated and identified. The preparation of pseudomonic acid D from monic acid A (1e) is described.

Fermentation of *Pseudomonas fluorescens* NCIB 10586 produces a group of novel antibiotics collectively known as the pseudomonic acids. In addition to the previously reported pseudomonic acids A (1a),[2] B (1b),[3] and C (2)[1,4] a new minor metabolite, designated pseudomonic acid D (1c), has recently been isolated and characterised. Extraction of culture filtrates of *P. fluorescens* followed by purification provided material containing over 90% pseudomonic acid A and *ca.* 2% of pseudomonic acid D. Because of its ability to co-crystallise and co-chromatograph with pseudomonic acid A (1a) it was difficult to isolate the metabolite in pure form. However, the more polar pseudomonic acid D (1c) was isolated and purified by high performance liquid chromatography (h.p.l.c.) using a semi-preparative, reverse phase, Altex ultrasphere-octyl column.

U.v., i.r., and n.m.r. spectra indicated structural features similar to those of pseudomonic acid A (1a). ^1H and ^{13}C N.m.r. data confirmed that pseudomonic acid D (1c) and A (1a) possessed the same nucleus, monic acid A (1e),[5] but differed only in respect to the ester side chain. The presence of a *trans* carbon–carbon double bond at C-4′ in the 9-hydroxynonenoic acid side chain was shown by spin–spin decoupling experiments with the coupling constant $J_{4',5'} = 15$ Hz confirming the geometry.

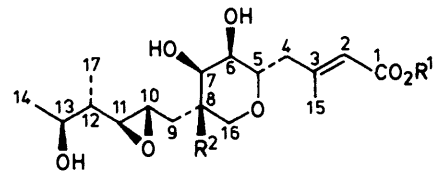
Proof of the structure was obtained by partial synthesis involving esterification of sodium monate A (1f) with (*E*)-9-chlorononon-4-enoic acid methyl ester (3a) followed by hydrolysis. However it will become apparent from the ensuing reactions that this synthetic route unavoidably produces a mixture of geometrical isomers (1c) and (1i) of which pseudomonic acid D (1c) was the major. (*Z*)-9-Hydroxynon-4-enoic acid (3b) was prepared in 20% yield by treating 2-hydroxypyran with phosphorus ylide derived from the phosphonium salt (4) and dimsyl-lithium in dimethyl sulphoxide and tetrahydrofuran. The *cis*-geometry as expected from the Wittig reaction was confirmed by the coupling constant $J_{4',5'} = 11$ Hz in the ^1H n.m.r. spectrum. Gas chromatographic analysis of the product as its methyl ester indicated the presence of 8% of the *trans*-isomer.

Irradiation of a benzene solution of the *cis*-acid (3b) and 1 equiv. of diphenyl disulphide under argon with a 6-W low-pressure mercury lamp afforded an isomeric mixture of acids (3b) enriched in the *trans*-isomer. The optimum period for this photoisomerisation was 42 h. Treatment of this mixture of acids (3b) with thionyl chloride followed by reaction in methanol gave the chloro-ester (3a) which consisted of a mixture of 86% *trans*- and 14% *cis*-isomers in a combined yield of 70%.

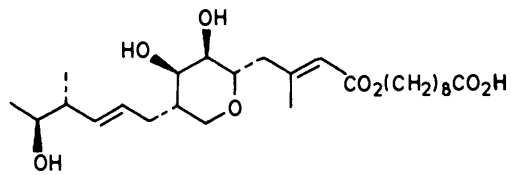
Reaction of the chloride (3a) with sodium monate A (1f) in the presence of sodium iodide in *N*,*N*-dimethylformamide at 80 °C afforded 32% of a mixture of methyl pseudomonate D (1g) and its $\Delta^{4'}$ *cis*-isomer (1h) in the ratio 4 : 1 as shown by the ^1H and ^{13}C n.m.r. spectra. Enzymatic hydrolysis of the methyl esters with bakers' yeast gave a similar 4 : 1 isomeric mixture of sodium pseudomonate (1d) and isomer (1j) which were isolated in a combined yield of 50%.

The geometrical isomers of the methyl esters (1g) and (1h) and of the free acids (1c) and (1i) were not separable by either h.p.l.c. or t.l.c.

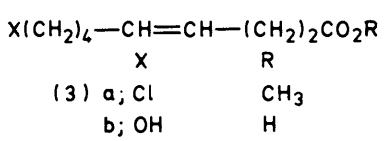
Pseudomonic acid D (1c) and its methyl ester (1g) displayed spectra of antimicrobial activity similar to that of pseudomonic acid A (1a).[6]



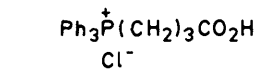
	R¹	R²
	9′ ← 1′	
(1) a;	$(CH_2)_8CO_2H$	H
b;	$(CH_2)_8CO_2H$	OH
c;	$(CH_2)_4CH{=}CH(CH_2)_2CO_2H$	H
d;	$(CH_2)_4CH{=}CH(CH_2)_2CO_2Na$	H
e;	H	H
f;	Na	H
g;	$(CH_2)_4CH{=}CH(CH_2)_2CO_2Me$	H
h;	$(CH_2)_4CH{=}CH(CH_2)_2CO_2Me$	H
i;	$(CH_2)_4CH{=}CH(CH_2)_2CO_2H$	H
j;	$(CH_2)_4CH{=}CH(CH_2)_2CO_2Na$	H



(2)



	X	R
(3) a;	Cl	CH_3
b;	OH	H



(4)

Experimental

^{1}H N.m.r. data were recorded at either 60 MHz on a Perkin-Elmer R24A or 250 MHz on a Bruker WM250 instrument and ^{13}C measurements were obtained using a Bruker WM250 spectrometer; all n.m.r. data were recorded at ambient temperatures with tetramethylsilane as internal standard. The numbering system used for assigning the chemical shifts is that shown in formula (1). Mass spectra were obtained at 70 eV using a VG 70—70F instrument operating at 8 eV. Column chromatography was carried out on Merck Kieselgel H (type 60). G.c. analyses were performed on a Pye model 104, using a 0.3 mm × 25 m glass WCOT SP1000 column. Tetrahydrofuran, *N*,*N*-dimethylformamide, and dimethyl sulphoxide were dried over calcium hydride and distilled before use.

Fermentation of Pseudomonas fluorescens (*NCIB* 10586) *and Isolation of Pseudomonic Acid D* (1c).—The bacterium was cultured on an agar slope and the culture was flooded with sterile water. A sample was added to a seed-stage medium containing Oxoid yeast (2.0%, w/v), glucose (0.11), Na_2HPO_4 (0.26), and KH_2PO_4 (0.24). The culture was grown at 28 °C overnight and was used to inoculate a production-stage medium containing corn steep liquor (0.3% w/v), glucose (2.0), glycerol (0.5), $(NH_4)_2SO_4$ (0.2), $CaCO_3$ (0.4), KH_2PO_4 (0.04), Na_2HPO_4 (0.065), $MnCl_2{\cdot}4H_2O$ (0.0003), KCl (0.05), $MgSO_4{\cdot}7H_2O$ (0.0375), and P2000 silicone antifoam. The fermentation was carried out at 25 °C for 48 h after which time production was essentially complete. After removal of the cells by centrifugation, the supernatant liquid was partitioned into methyl isobutyl ketone at pH 4.5. The organic layer was extracted with aqueous sodium hydrogen carbonate. The aqueous extract was acidified to pH 4.5 and re-extracted with methyl isobutyl ketone. The organic extract was washed with deionised water, dried ($MgSO_4$), and evaporated to low volume. n-Heptane was added and the mixture allowed to crystallise. The product was filtered off and dried *in vacuo*. This material was shown by h.p.l.c. analysis to contain *ca.* 92% of pseudomonic acid A (1a) and *ca.* 2% of pseudomonic acid D (1c). Crude product (1.5 g) so produced was dissolved in dry THF (1.5 ml) and chromatographed in portions (200—250 μl) by h.p.l.c. using an Altex ultrasphere-octyl (250 × 10 mm) reverse phase column and eluting at a flow rate of 3 ml/min of 0.1M-ammonium acetate buffer (pH 5.7)–THF (3 : 1). Pseudomonic acid D had a retention time of 30 min (pseudomonic acid A R_t = 41 min). The combined eluants containing the pseudomonic acid D peaks (detected at $\lambda_{max.}$ 240 nm) were evaporated to dryness after which the residue was dissolved in water and adjusted to pH 4 using 1M-hydrochloric acid, under a layer of ethyl acetate (20 ml). The organic phase was separated and the aqueous phase further extracted with ethyl acetate (3 × 20 ml); the combined extracts were washed with brine, dried ($MgSO_4$), and evaporated to dryness. The pseudomonic acid D obtained was rechromatographed by h.p.l.c. as above and the combined eluants evaporated to remove the THF; the resulting aqueous solution was adjusted to pH 7 and washed with ethyl acetate. The aqueous layer was acidified to pH 4 using 1M-hydrochloric acid under a layer of ethyl acetate (25 ml) and the aqueous layer further extracted with ethyl acetate (3 × 25 ml). The combined extracts were dried ($MgSO_4$) and evaporated to yield pseudomonic acid D (30 mg) as an oil; $\nu_{max.}$ ($CHCl_3$) 3 400, 1 720, and 1 650 cm^{-1}; $\lambda_{max.}$ (EtOH) 220 nm (ε 15 499); δ_H ($CDCl_3$) 5.76 (1 H, m, 2-H), 5.45 (2 H, m, 4′- and 5′-H, *J* 15 Hz), 4.08 (3 H, t, 9′-H_2), 3.91 (1 H, dd, 7-H), 3.87 and 3.54 (2 H, m, 16-H_2), 3.80 (1 H, m, 13-H), 3.76 (1 H, m, 5-H), 3.47 (1 H, dd, 6-H), 2.83 (1 H, dt, 10-H), 2.73 (1 H, dd, 11-H), 2.59 and 2.30 (2 H, m, 4-H_2), 2.40 (2 H, t, 2′-H_2), 2.32 (2 H, m, 3′-H_2), 2.21 (3 H, d, 15-H_3), 2.02 (2 H, m, 6′-H_2), 2.00 (1 H, m, 8-H), 1.73 (2 H, m, 9-H_2), 1.63 (2 H, m, 8′-H_2), 1.44 (2 H, m, 7′-H_2), 1.38 (1 H, m, 12-H), 1.22 (3 H, d, 14-H_3), and 0.94 (3 H, d, 17-H_3); δ_C ($CDCl_3$) 178.0 (C-1′), 166.9 (C-1), 156.6 (C-3), 131.4 (C-5′), 128.5 (C-4′), 117.8 (C-2), 75.2 (C-5), 71.4 (C-13), 70.6 (C-7), 69.2 (C-6), 65.5 (C-16), 63.8 (C-9′), 61.3 (C-11), 55.7 (C-10), 42.9 (C-4 and C-12), 39.6 (C-8), 34.2 (C-2′), 32.0, 31.8, 28.1, 27.8 and 25.9 (C-6′, -9, -8′, -7′, and -3′), 20.8 (C-14), 19.3 (C-15), and 12.7 p.p.m. (C-17); *m*/*z* (relative intensity) 498 (M^+, 3%), 396(4), 327(10), 309(16), 291(5), 254(23), 227(92), 209(30), 141(49), 137(39), 129(22), and 111(100) (Found: *m*/*z* 498.2860. $C_{26}H_{42}O_9$ requires *m*/*z* 498.2892).

(Z)-9-*Hydroxynon*-4-*enoic acid* (3b).—(3-Carboxypropyl)-triphenylphosphonium chloride [7] (5) (3.84 g) was added to a solution of butyl-lithium (1.18M in toluene, 17 ml) in dimethyl sulphoxide (20 ml) at 0 °C. The resulting deep red solution was diluted with tetrahydrofuran (20 ml) and stirred at room temperature for 2 h after which it was treated with 2-hydroxytetrahydropyran (1.1 g) and stirred overnight. After being poured into water the solution was acidified to pH 2 and extracted with diethyl ether. The combined organic layers were extracted with 1M-sodium hydroxide and the aqueous extract finally acidified to pH 2 and extracted with diethyl ether. The extracts were dried ($MgSO_4$) and evaporated to an oil which was chromatographed on silica (10 g) with 2—6% methanol in dichloromethane as eluant to yield the product as an oil (0.346 g, 20%); g.c. analysis after on-column methylation with trimethylanilinium hydroxide (MethElute) in methanol revealed 8% of the *trans*-isomer; $\nu_{max.}$ (film) 2 300—3 700br and 1 715br cm^{-1}; δ_H ($CDCl_3$) 1.43 (2 H, m, 7-H_2), 1.57 (2 H, m, 8-H_2), 2.09 (2 H, q, 6-H_2), 2.38 (4 H, m, 2-H_2 and 3-H_2), 3.64 (2 H, t, 9-H_2), and 5.40 (2 H, m, 4-H and 5-H, *J* 11 Hz); δ_C ($CDCl_3$) 176.7 (C-1), 131.1 (C-5), 127.8 (C-4), 62.4 (C-9), 34.2 (C-2), 32.1 (C-8), 26.9 (C-7), 25.7 (C-6), and 22.8 (C-3) (Found: C, 62.5; H, 9.6. $C_9H_{16}O_3$ requires C, 62.8; H, 9.4%).

(E)-*Methyl* 9-*Chloronon*-4-*enoate* (3a).—A solution of (*Z*)-9-hydroxynon-4-enoic acid (0.5 g) and diphenyl disulphide (0.634 g) in benzene (150 ml) was photolysed (6-W low-pressure mercury lamp) for 42 h under argon. The solution was evaporated to an oil which was redissolved in diethyl ether and extracted with 1M-sodium hydroxide. After acidification to pH 2, the aqueous phase was re-extracted with diethyl ether and the extracts dried ($MgSO_4$), evaporated to an oil, and taken up in benzene (20 ml). Thionyl chloride (2 ml) was added and the solution refluxed for 4 h. After evaporation of the solvent and excess of reagent, methanol (25 ml) was added and the solution stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue dissolved in diethyl ether and washed with aqueous sodium hydrogen carbonate and brine and then dried ($MgSO_4$). Evaporation of the solvent under reduced pressure afforded the product as an oil (0.415 g, 70%) which consisted of 86% *trans* and 14% *cis* as determined by g.c. analysis; $\nu_{max.}$ (film) 1 738 cm^{-1}; δ_H ($CDCl_3$) 1.49 (2 H, m, 7-H_2), 1.63 (2 H, m, 8-H_2), 1.98 (2 H, m, 6-H_2), 2.32 (4 H, m, 2-H_2 and 3-H_2), 3.48 (2 H, t, 9-H_2), 3.62 (3 H, s, OCH_3), and 5.42 (2 H, m, 4-H and 5-H, *J* 15 Hz) (Found: C, 56.4; H, 9.1. $C_9H_{17}ClO_2$ requires C, 56.1; H, 8.9%).

Methyl Pseudomonate D (1g).—(*E*)-Methyl 9-chlorononon-4-enoate (0.415 g), sodium iodide (0.3 g), and sodium monate A (0.817 g) in *N*,*N*-dimethylformamide (25 ml) were heated at 80 °C for 18 h. After removal of the solvent under reduced pressure, the residue was extracted with diethyl ether–brine and the organic phase washed with aqueous sodium hydrogen carbonate and brine and then dried ($MgSO_4$). The solvent was

evaporated under reduced pressure and the residual oil chromatographed on silica (6 g) with 0—4% methanol in dichloromethane as eluant. Fractions containing methyl pseudomonate D (1g) and its $\Delta^{4'}$ *cis*-isomer (1h) were combined and evaporated to an oil (0.333 g, 32% as isomeric mixture); $\nu_{max.}$ ($CHCl_3$) 3 430br, 1 722, 1 708, and 1 645 cm^{-1}; $\lambda_{max.}$ (EtOH) 219 nm (ε 18 579); δ_H and δ_C indicated the product consisted of 80% *trans*- and 20% *cis*-$\Delta^{4'}$ isomers. *trans*-Isomer δ_H ($CDCl_3$) 0.95 (3 H, d, 17-H_3), 1.22 (3 H, d, 14-H_3), 1.42 (2 H, m, 7′-H_2), 1.63 (2 H, m, 8′-H_2), 2.01 (2 H, m, 6′-H_2), 2.21 (3 H, d, 15-H_3), 2.32 (4 H, m, 2′-H_2 and 3′-H_2), 3.67 (3 H, s, OCH_3), 4.07 (2 H, t, 9′-H_2), 5.43 (2 H, m, 4′-H and 5′-H, *J* 14 Hz), and 5.75 (1 H, m, 2-H); *trans*-isomer δ_C ($CDCl_3$) 173.8 (C-1′), 166.9 (C-1), 156.9 (C-3), 131.5 (C-5′), 128.5 (C-4′), 117.5 (C-2), 75.0 (C-5), 71.0 (C-13), 70.3 (C-7), 69.0 (C-6), 65.4 (C-16), 63.6 (C-9′), 61.2 (C-11), 55.6 (C-10), 51.5 (OCH_3), 42.9 (C-12), 42.8 (C-4), 39.6 (C-8), 34.1 (C-2′), 32.0 (C-6′), 31.7 (C-9′), 28.1 (C-8′), 27.9 (C-3′), 25.8 (C-7′), 20.7 (C-14), 19.1 (C-15), and 12.6 p.p.m. (C-17); *m/z* (rel. int.) 512 (M^+, 1%), 410(1), 364(2), 327(6), 309(8), 268(10), 227(37), 111(72), 95(73), 67(87), 55(85), and 41(100) (Found: *m/z*, 512.3026. $C_{27}H_{44}O_9$ requires *m/z*, 512.3070).

Sodium Pseudomonate D (1d).—The 4 : 1 mixture of methyl pseudomonate D (1g) and $\Delta^{4'}$ *cis*-isomer (1h) (0.136 g) was dissolved in *N*,*N*-dimethylformamide (4.5 ml) and diluted with 0.05M-phosphate buffer (pH 7) (60 ml). Bakers' yeast (5 g) was added and the reaction stirred vigorously for 18 h at room temperature. The yeast was removed by centrifugation and the aqueous layer acidified to pH 4 and extracted with ethyl acetate (4 × 25 ml). The combined extracts were dried ($MgSO_4$), evaporated under reduced pressure and the residual oil dissolved in methanol (1 ml) and treated with sodium hydrogen carbonate (0.013 g) in water (1 ml). Evaporation to dryness gave a mixture of sodium pseudomonate D (1d) and its geometrical isomer (1j) (0.076 g, 50%); $\nu_{max.}$ (Nujol) 3 400br, 1 710, 1 647, and 1 558 cm^{-1}; $\lambda_{max.}$ (EtOH) 223 nm (ε 14 404); δ_H and δ_C indicated the product consisted of 80% *trans* and 20% *cis* $\Delta^{4'}$ isomers. *trans*-Isomer δ_H CD_3OD) 0.95 (3 H, d, 17-H_3), 1.20 (3 H, d, 14-H_3), 1.42 (2 H, m, 7′-H_2), 1.65 (2 H, m, 8′-H_2), 2.01 (2 H, m, 6′-H_2), 2.18 (3 H, d, 15-H_3), 2.23 (4 H, m, 2′-H_2 and 3′-H_2), 4.06 (2 H, t, 9′-H_2), 5.46 (2 H, m, 4′-H and 5′-H, *J* 15 Hz), and 5.74 (1 H, s, 2-H); *trans*-isomer δ_C (CD_3OD) 181.8 (C-1′), 168.4 (C-1), 158.8 (C-3), 131.5 (C-5′), 130.9 (C-4′), 118.3 (C-2), 76.3 (C-5), 71.6 (C-13), 70.8 (C-7), 70.0 (C-6), 66.3 (C-16), 64.7 (C-9′), 61.5 (C-11), 56.8 (C-10), 43.9 (C-4), 41.5 (C-8), 38.8 (C-2′), 33.1 and 33.0 (C-6′ and -9), 30.5 (C-7′), 29.3 (C-8′), 27.0 (C-3′), 20.5 (C-14), 19.4 (C-15), and 12.3 p.p.m. (C-17) (Found: C, 59.7; H, 7.8. $C_{26}H_{41}NaO_9$ requires C, 60.0; H, 7.9%).

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References

1 Part 5, J. P. Clayton, P. J. O'Hanlon, N. H. Rogers, and T. J. King, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2827.
2 A. T. Fuller, G. Mellows, M. Woolford, G. T. Banks, K. D. Barrow, and E. B. Chain, *Nature (London)*, 1971, **234**, 415; E. B. Chain and G. Mellows, *J. Chem. Soc., Chem. Commun.*, 1974, 847; *J. Chem. Soc., Perkin Trans. 1*, 1977, 294 and R. G. Alexander, J. P. Clayton, K. Luk, and N. H. Rogers, *ibid.*, 1978, 561.
3 E. B. Chain and G. Mellows, *J. Chem. Soc., Perkin Trans. 1*, 1977, 318.
4 J. P. Clayton, P. J. O'Hanlon, and N. H. Rogers, *Tetrahedron Lett.*, 1980, **21**, 881.
5 J. P. Clayton, K. Luk, and N. H. Rogers, *J. Chem. Soc., Perkin Trans. 1*, 1979, 308.
6 R. Sutherland, K. R. Comber, L. W. Mizen, B. Slocombe, and J. P. Clayton, ' Pseudomonic Acid, an Antibiotic Produced by *Pseudomonas fluorescens*,' presented at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, U.S.A., 1976; M. J. Basker, K. R. Comber, J. P. Clayton, P. C. T. Hannan, L. W. Mizen, N. H. Rogers, B. Slocombe, and R. Sutherland, ' Current Chemotherapy and Infectious Disease,' ' Proceedings of the 11th I.C.C. and the 19th I.C.A.A.C.,' *Am. Soc. Microbiol.*, 1980, 471.
7 D. B. Denney and L. C. Smith, *J. Org. Chem.*, 1962, **27**, 3404.

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