ANTIMYCOPLASMAL ACTIVITIES OF THE PSEUDOMONIC ACIDS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF MONIC ACID A DERIVATIVES

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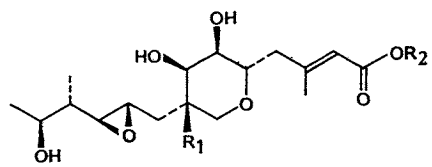
The antimycoplasmal activities of the pseudomonic acids isolated from *Pseudomonas fluorescens* NCIB 10586 are reported. Structure-activity relationships of a variety of ester, amide and thiol ester derivatives of the nucleus, monic acid A, are described. Enhanced antimycoplasmal activity is reported for a number of monic acid A esters and the most potent derivative, *m*-nitrobenzyl monate A, is a 100-fold more active against *Mycoplasma hyopneumoniae* than pseudomonic acid A.

The pseudomonic acids A (**1**),[1] B (**2**),[2] C (**3**)[3] and D (**4**)[4] (Figs. 1 and 2) are a family of naturally occurring antimicrobial agents produced by fermentation of *Pseudomonas fluorescens* NCIB 10586. Pseudomonic acid A,†† the major metabolite, has broad spectrum activity against Gram-positive bacteria[5] and is marketed as Bactroban††† for topical use in the treatment of skin infections. Pseudomonic acid A is unrelated to any other class of antibiotics and acts through the inhibition of bacterial protein synthesis by reversibly and specifically binding to bacterial isoleucyl tRNA synthetase.[6] The pseudomonic acids also possess antimycoplasmal activity which we report in this paper together with enhanced activities of a range of derivatives of the nucleus, monic acid A (**5**). We sought to improve the activity of the series against *Mycoplasma hyopneumoniae* by derivatization of monic acid A.

Fig. 1.



Pseudomonic acid A (**1**) $R_1 = H$ $R_2 = (CH_2)_8COOH$

Pseudomonic acid B (**2**) $R_1 = OH$ $R_2 = (CH_2)_8COOH$

Pseudomonic acid D (**4**) $R_1 = H$ $R_2 = (CH_2)_4\overset{H}{C}=\overset{H}{C}(CH_2)_2COOH$

Monic acid A (**5**) $R_1 = R_2 = H$

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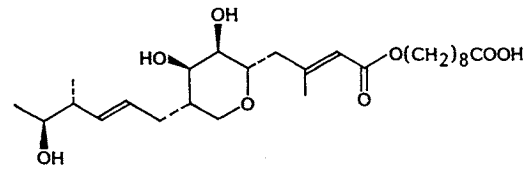
†† The approved generic name for pseudomonic acid A is mupirocin.

††† Trade mark of Beecham Group p.l.c.

Materials and Methods

Fig. 2.



Pseudomonic acid C (**3**)

The isolation and characterisation of the pseudomonic acids has already been described[1~4] and the preparation of monic acid A derivatives has been detailed in a series of Beecham patents.[7~13] In general terms the esters were prepared by reaction of the appropriate halide and sodium monate in *N,N*-dimethylformamide at temperatures ranging from ambient to 80°C. The amides and thiol esters of monic acid A were obtained by activation of monic acid A by mixed anhydride (isobutoxyformic) and addition of the appropriate amine or thiol. All derivatives used were substantially pure by various criteria. Purity was assessed by ^{1}H NMR, ^{13}C NMR and IR spectroscopic analysis, TLC and HPLC. Either elemental analysis or accurate mass measurement was obtained for the compounds described. Tylosin tartrate was obtained from the Sigma Chemical Company.

The mycoplasma strains used were either obtained from the National Collection of Type Cultures or from The American Type Culture Collection as indicated by the suffix code. MIC values for the mycoplasmas were determined by incorporating dilutions of the compounds from 0.01 μg/ml to 10 μg/ml in mycoplasma FRIIS medium,[14] solidified with 0.65% agarose (Miles Laboratories) and inoculating the surface with about 10^4 cells per 1 μl spot of the various mycoplasmas, using a Denley 400 multipoint inoculator. When dry, the plates were incubated aerobically in sealed moistened plastic bags at 37°C for 6 days. The MIC was the lowest concentration of drug which inhibited at least 50% of mycoplasmal growth.

Results and Discussion

The pseudomonic acids (**1**~**4**) display markedly different activities (Table 1). Pseudomonic acid A (**1**) is the most active metabolite against all five strains of mycoplasma. Pseudomonic acid D (**4**) differs only in the side chain in structure and has activity approaching that of pseudomonic acid A. Conversion of **1** to either an ester (**6**) or an amide (**7**) has little effect on activity (Table 2). Monic acid A (**5**), the major metabolic product of pseudomonic acid A in man and animals,[15] has no antimycoplasmal activity. Monic acids B and C are also inactive.

Substituted alkyl esters of monic acid A (Table 2) were all antimycoplasmally active. Although the activity varied widely it was concluded that compounds with a similar chain length to pseudomonic acid A displayed equal or better activity but polar functions when positioned close to the nucleus produced less active derivatives.

Pseudomonic acid A is highly bound to porcine serum (96%) and the alkyl esters (**23**~**25**) were prepared in a successful attempt to reduce this binding, *i.e.* **23** 18%; **24** 34%; and **25** 70%. In ad-

Table 1. Antimycoplasmal activity (MIC, μg/ml) of the pseudomonic acids and monic acid A.

Compound	*Mycoplasma hyopneumoniae* NCTC 10110	*M. hyorhinis* ATCC 23234	*M. bovis* NCTC 10131	*M. dispar* NCTC 10125	*M. pneumoniae* ATCC 15492
Pseudomonic acid A (**1**)	2.5	0.5	<0.01	1.0	5.0
Pseudomonic acid B (**2**)	25	10	0.05	2.5	50
Pseudomonic acid C (**3**)	10	2.5	<0.01	2.5	>10
Pseudomonic acid D (**4**)	5	1.0	0.05	2.5	5.0
Monic acid A (**5**)	>100	>100	>100	>100	>100
Tylosin	0.1	0.5	0.25	0.25	0.05

Table 2. Antimycoplasmal activity (MIC, μg/ml) of monic acid A esters.

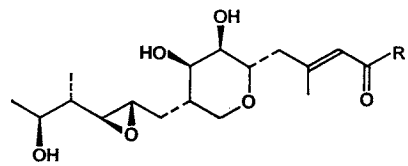
Compound	R (Fig. 3)	*Mycoplasma hyopneumoniae* NCTC 10110	*M. hyorhinis* ATCC 23234	*M. bovis* NCTC 10131	*M. dispar* NCTC 10125	*M. pneumoniae* ATCC 15492
6	$O(CH_2)_8COOCH_3$	2.5	0.5	<0.01	0.5	2.5
7	$O(CH_2)_8CONH_2$	1.0	0.25	<0.01	0.25	2.5
8	OCH_2COOH	>10	NR	10	NR	>10
9	$O(CH_2)_5COOH$	2.5	NR	<0.5	0.5	>10
10	$O(CH_2)_2OH$	2.5	1.0	0.05	0.5	5.0
11	$O(CH_2)_3OH$	1.0	0.5	0.025	0.25	5.0
12	$O(CH_2)_5OH$	0.5	0.25	<0.01	0.1	2.5
13	$O(CH_2)_6OH$	1.0	0.5	<0.01	0.05	2.5
14	$O(CH_2)_8OH$	0.5	0.25	<0.01	0.1	1.0
15	$O(CH_2)_8NH_2$	1.25	0.62	0.05	<0.02	0.62
16	$O(CH_2)_8NHCONH_2$	0.5	0.25	0.025	0.25	1.0
17	$O(CH_2)_8SO_3Na$	>10	10	0.25	5.0	>10
18	$O(CH_2)_3SO_2NH_2$	0.5	0.5	0.05	0.25	1.0
19	$O(CH_2)_6SO_2NH_2$	0.5	0.1	<0.01	0.05	2.5
20	$O(CH_2)_8SO_2NH_2$	0.5	0.25	<0.01	0.25	2.5
21	$O(CH_2)_2CONH_2$	5.0	2.5	0.25	0.5	10
22	$O(CH_2)_5CONH_2$	1.0	0.25	0.025	0.1	1.0
23	OCH_3	2.5	1.0	0.05	0.25	10
24	OCH_2CH_3	0.5	0.25	0.025	0.1	2.5
25	$O(CH_2)_3CH_3$	0.5	0.25	<0.01	0.1	2.5
26	OC_6H_5	0.5	NR	NR	0.5	1.25
Tylosin		0.1	0.5	0.25	0.25	0.05

NR: No result.

dition the ethyl and butyl esters were 2 to 5-fold more active than the parent compound.

A considerable improvement in activity was achieved against *M. hyopneumoniae*, *Mycoplasma hyorhinis* and *Mycoplasma dispar* when benzyl esters were studied (Table 3). Benzyl monate (**27**) proved to be 2 to 10-fold more active than the parent compound (**1**) and subsequent investigation of substitution of the benzene ring produced compounds up to 100-fold more active than pseudomonic acid A, *e.g.* the *meta*-nitrobenzyl ester (**29**) *M. hyopneumoniae* MIC 0.025 μg/ml. In general substitution with electron withdrawing groups produced more active compounds than electron donating substituents. Substitution in the *meta* or *para* positions was preferable to the *ortho* position. Substitution in the *meta* position for the cyano and nitro functions produced the most active derivatives but the methanesulfonyl function was better at the *para* position, perhaps reflecting a steric factor.

Fig. 3.



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Thiol esters of monic acid A (**38** and **39**) were slightly more active than the corresponding *O*-esters analogues (Table 4) but were found to be unstable *in vivo* and were not of further interest.

Amides of monic acid A (**40**~**44**) exhibited relatively poor antimycoplasmal activity. However, structure-activity relationship noted in the ester series were paralleled for the structurally analogous amides and the most active derivative was the *meta*-nitrobenzyl amide (**42**). The tertiary amide (**43**)

Table 3. Antimycoplasmal activity (MIC, μg/ml) of benzyl esters of monic acid A.

Compound	R (Fig. 3)	*Mycoplasma hyopneumoniae* NCTC 10110	*M. hyorhinis* ATCC 23234	*M. bovis* NCTC 10131	*M. dispar* NCTC 10125	*M. pneumoniae* ATCC 15492
27	$OCH_2C_6H_5$	0.25	0.25	<0.01	0.1	2.5
28	$OCH_2C_6H_4(o\text{-}NO_2)$	0.5	0.25	<0.01	0.1	5.0
29	$OCH_2C_6H_4(m\text{-}NO_2)$	0.025	<0.01	<0.01	<0.01	1.0
30	$OCH_2C_6H_4(p\text{-}NO_2)$	0.1	0.05	<0.01	0.025	0.25
31	$OCH_2C_6H_4(m\text{-}CN)$	0.1	0.05	<0.01	0.025	1.0
32	$OCH_2C_6H_4(p\text{-}CN)$	0.25	0.1	0.05	<0.01	0.25
33	$OCH_2C_6H_4(m\text{-}SO_2CH_3)$	1.0	0.5	0.025	0.25	2.5
34	$OCH_2C_6H_4(p\text{-}SO_2CH_3)$	0.25	0.1	<0.01	0.05	0.25
35	$OCH_2C_6H_4(o\text{-}OCH_3)$	2.5	1.0	0.025	0.5	NR
36	$OCH_2C_6H_4(m\text{-}OCH_3)$	2.5	0.5	0.025	0.25	10
37	$OCH_2C_6H_4(p\text{-}OCH_3)$	0.5	0.25	<0.01	0.025	NR
Tylosin		0.1	0.5	0.25	0.25	0.05

NR: No result.

Table 4. Antimycoplasmal activity (MIC, μg/ml) of thiol esters and amides of monic acid A.

Compound	R (Fig. 3)	*Mycoplasma hyopneumoniae* NCTC 10110	*M. hyorhinis* ATCC 23234	*M. bovis* NCTC 10131	*M. dispar* NCTC 10125	*M. pneumoniae* ATCC 15492
38	SCH_3	0.5	0.25	0.025	0.025	2.5
39	SCH_2CH_3	0.5	0.25	0.025	0.1	2.5
40	$NH(CH_2)_5COONa$	>10	5.0	0.25	10	>10
41	NH_2	>10	>10	1.0	5.0	>10
42	$NHCH_2C_6H_4(m\text{-}NO_2)$	0.25	0.1	0.05	0.1	10
43	$NHCH_2C_6H_4(m\text{-}CN)$	1.0	0.5	0.05	0.25	>10
44	$N(CH_3)_2$	>10	NR	1.0	2.5	>10
Tylosin		0.1	0.5	0.25	0.25	0.05

NR: No result.

for which there is no ester equivalent had poor antimycoplasmal activity.

In summary, of the pseudomonic acids (**1**~**4**), the most abundant metabolite, pseudomonic acid A, possesses the most useful activity against mycoplasma. Although the nucleus, monic acid A (**5**), is inactive, derivatives and particularly benzyl esters show enhanced activities against *M. hyopneumoniae*, *M. hyorhinis*, *M. dispar* and *Mycoplasma pneumoniae* but not *Mycoplasma bovis*. The most potent derivative tested was *m*-nitrobenzyl monate A (**29**).

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References

1) CHAIN, E. B. & G. MELLOWS: Pseudomonic acid. Part 1. The structure of pseudomonic acid A, a novel metabolite produced by *Pseudomonas fluorescens*. J. Chem. Soc. Perkin Trans. I 1977: 294~309, 1977 and refs sited therein
2) CHAIN, E. B. & G. MELLOWS: Pseudomonic acid. Part 3. Structure of pseudomonic acid B. J. Chem. Soc. Perkin Trans. I 1977: 318~322, 1977

3) CLAYTON, J. P.; P. J. O'HANLON & N. H. ROGERS: The structure and configuration of pseudomonic acid C. Tetrahedron Lett. 21: 881~884, 1980
4) O'HANLON, P. J.; N. H. ROGERS & J. W. TYLER: The chemistry of pseudomonic acid. Part 6. Structure and preparation of pseudomonic acid D. J. Chem. Soc. Perkin Trans. I 1983: 2655~2657, 1983
5) WHITE, A. R.; A. S. BEALE, R. J. BOON, K. E. GRIFFIN, P. J. MASTERS & R. SUTHERLAND: Antibacterial activity of mupirocin. *In* Bactroban (mupirocin). Proceedings of an International Symposium. Current Clinical Practice Series 16. *Ed.*, R. L. DOBSON *et al.*, pp. 19~36, Excerpta Medica, Amsterdam, 1985
6) HUGHES, J. & G. MELLOWS: On the mode of action of pseudomonic acid: Inhibition of protein synthesis in *Staphylococcus aureus*. J. Antibiotics 31: 330~335, 1978
7) ROGERS, N. H. (Beecham): Pseudomonic acid amides. Brit. UK Pat. Appl. 1,565,083, Feb. 20, 1976
8) CLAYTON, J. P.; K. LUK & N. H. ROGERS (Beecham): Monic acid esters. Brit. UK Pat. Appl. 1,587,059, Mar. 1, 1977
9) ROGERS, N. H. (Beecham): Monic acid amides. Eur. Pat. Appl. 0 001 914B, Nov. 5, 1977
10) CLAYTON, J. P.; S. COULTON & N. H. ROGERS (Beecham): Monic acid thiol esters. Eur. Pat. Appl. 0 002 371B, Dec. 12, 1977
11) O'HANLON, P. J. & N. H. ROGERS (Beecham): Monic acid sulphonamido esters. Eur. Pat. Appl. 0 026 611B, Sept. 28, 1979
12) O'HANLON, P. J. & G. WALKER (Beecham): Cyanobenzyl monate esters. Brit. UK Pat. Appl. 2,101,116B, Aug. 13, 1981
13) O'HANLON, P. J. & G. WALKER (Beecham): Nitrobenzyl monate esters. Brit. UK Pat. Appl. 2,090,256A, Sept. 9, 1981
14) FRIIS, N. F.: Some recommendations concerning *Mycoplasma suipneumoniae* and *Mycoplasma flocculare* — a survey. Nord. Veterinaermed. 27: 337~339, 1975
15) BASKER, M. J.; K. R. COMBER, J. P. CLAYTON, P. C. T. HANNAN, L. W. MIZEN, N. H. ROGERS, B. SLOCOMBE & R. SUTHERLAND: Ethyl monate A: a semisynthetic antibiotic derived from pseudomonic acid A. *In* Current Chemotherapy and Infectious Disease. Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. Vol. I. *Eds.*, J. D. NELSON & C. GRASSI, pp. 471~473, The American Society for Microbiology, Washington, DC, 1980