PACIDAMYCINS, A NOVEL SERIES OF ANTIBIOTICS WITH ANTI-*PSEUDOMONAS AERUGINOSA* ACTIVITY

II. ISOLATION AND STRUCTURAL ELUCIDATION

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A novel class of antibiotics was isolated from cultures of *Streptomyces coeruleorubidus* strain AB 1183F-64. The antimicrobial activity of the pacidamycins is selective against *Pseudomonas aeruginosa*. The various congeners are nucleoside peptides which differ in the terminal amino acid residues. The structures were determined using MS-MS and 2D NMR techniques.

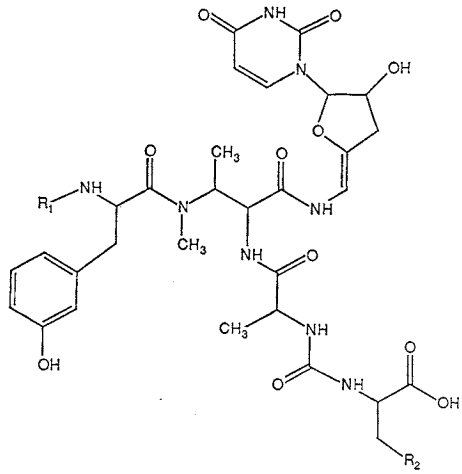
In the course of screening soil microorganisms for the production of novel antibiotics, a new class of compounds, the pacidamycins, with highly selective activity against *Pseudomonas aeruginosa*, was discovered. Several unique structural features are found in this nucleoside-peptide hybrid. The nucleoside part of the molecule consists of a uridine analogue with an unusual amino sugar. The peptide portion, which contains the rare amino acid *m*-tyrosine, changes direction twice. The first is through a diamino butyrate residue, and the second occurs, one residue later, by the incorporation of a urea residue. The pacidamycins are produced as a series of compounds which differ in their terminal residues. The distribution of congeners can be controlled by media supplementation with the appropriate amino acids. Antibiotic production and microbiological data are covered in companion papers[1,2]. The isolation and structural characterization of these compounds are described in this paper.

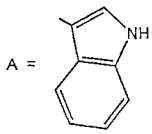
Characterization and Structural Determination

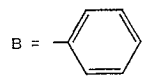
The pacidamycins are soluble in aqueous solutions from pH 2 to 10, but greater solubilities are achieved at alkaline pH. All dissolve in dimethyl sulfoxide and pyridine, and have only marginal solubilities in aqueous alcohols (methanol, ethanol, and butanol). The phenylalanine derivatives were the only congeners soluble in dry methanol. This article will only present a detailed discussion for the structural elucidation of pacidamycin 1 (structure **1** in Fig. 1). The data for pacidamycins 2 and 3 (structures **2** and **3**, respectively) are listed (Tables 2 and 3), and the basis for their assignments are identical to those given for **1**.

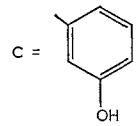
The high resolution (HR) mass measurement (positive ion fast atom bombardment (FAB)) of the protonated molecular ion from **1** was recorded as *m/z* 875.3687, and corresponds to a formula of $C_{41}H_{50}N_{10}O_{12}+H^+$ (calcd 875.3691). The UV spectrum of **1** in aqueous solution is characterized by a single maximum at 259 nm (ε 17,200). The optical rotation was measured as $[\alpha]_D^{24}$ +0.6°, where *c* 0.66. Table 1 presents a comprehensive list of NMR data for compound **1**. In the first column, the 41 carbon atoms are listed in the order of chemical shift. The attached protons (heteronuclear correlation, HETCOR[3]) are given in the second column and the number of attached protons, as

Fig. 1. Structures of pacidamycins.









Pacidamycin 1	(1)	R_1=Alanyl	R_2=A
Pacidamycin 2	(2)	R_1=Alanyl	R_2=B
Pacidamycin 3	(3)	R_1=Alanyl	R_2=C
Pacidamycin 4	(4)	R_1=H	R_2=A
Pacidamycin 5	(5)	R_1=H	R_2=B
Pacidamycin 6	(6)	R_1=Glycyl	R_2=A
Pacidamycin 7	(7)	R_1=Glycyl	R_2=B

determined by distortionless enhancement by polarization transfer (DEPT), are in the third column. In the fourth column, the results of a ^{1}H correlation spectroscopy (COSY) experiment are given for the protons listed in column two. In the last column the assignment of the carbon is given.

The NMR data were recorded in slightly alkaline D_2O (pD 8) to avoid the spectral complexities caused by rotational conformers about the amide bonds. This problem, typical for peptides, was more severe in dimethyl sulfoxide at 25°C, but upon heating the sample to 120°C (Fig. 2) the olefinic signals of the rotamers were observed to coalesce. At this temperature however, the sample degraded in less than 30 minutes and the spectra became uninterpretable.

From inspection of the proton and carbon NMR data, it was determined that **1** was in part comprised of two alanines, a *m*-tyrosine, and a tryptophan residue. The assignments are shown in Table 1 and agree with literature values[4]. The presence of alanine and *m*-tyrosine was corroborated by an amino acid analysis. The tryptophan residue was not detected in the standard analysis, because under acidic conditions, a prevalent side reaction might result in tryptophan-alanine hydantoin formation (Fig. 3)[5]. This product was resistant to further hydrolysis.

The 2-amino-3-methylaminobutyrate region of the molecule was characterized from the NMR data. The proton resonances at 1.18, 4.93, and 4.57 ppm are coupled sequentially and correspond to positions 4, 3, and 2 of the butyrate, respectively. The carbon shifts of the two methines (51.1 and 55.7 ppm) were indicative that neither was bonded to an oxygen, but rather to acylated nitrogens.

Table 1. NMR data for pacidamycin 1.

C shift	H shift	LR-HETCOR	DEPT	COSY	Assignment
13.5	1.18	4.57	CH_3	4.93	Butyrate-4
17.1	1.26		CH_3		Ala-1
19.5	1.22	3.54	CH_3	3.54	Ala-2
28.6	3.13, 3.27	4.39	CH_2	4.39	Trp
30.3	3.09		NCH_3		Butyrate
33.4	2.62, 2.88		CH_2	4.35, 5.94	Sugar
37.0	2.67, 2.95	4.96	CH_2	4.96	Tyr
50.0	3.54	1.22	CH	1.22	Ala-2
50.3	4.14	1.26	CH		Ala-1
51.1	4.93	1.18, 3.09, 4.57	CH	1.18, 4.57	Butyrate-3
51.4	4.96	2.67, 2.95	CH	2.67, 2.95	Tyr
55.7	4.57	1.18	CH	4.93	Butyrate-2
56.5	4.39	3.13	CH	3.13, 3.27	Trp
72.8	4.35	2.62	O-CH	2.62, 2.88, 6.01	Sugar
93.6	6.01	2.62	O-CH-N	4.35	Sugar
97.2	5.94	2.88	CH=	2.88	Sugar
102.7	5.47		CH=	6.96	Urea
110.7		3.13, 4.39, 7.22	Ar C		Trp-3
112.0	7.49	7.16	Ar CH	7.22	Trp-7
114.7	6.78	6.73	Ar CH	7.22	Tyr-4
116.2	6.73	2.67, 6.78	Ar CH		Tyr-2
119.1	7.69	7.22	Ar CH	7.16	Trp-4
119.5	7.16	7.49	Ar CH	7.22, 7.69	Trp-5
121.1	6.78	2.69, 6.73, 6.78	Ar CH	7.22	Tyr-6
122.0	7.22	7.69	Ar CH	7.16, 7.49	Trp-6
124.5	7.22	3.13	Ar CH		Trp-2
127.6		3.13, 7.16, 7.22, 7.49	Ar C		Trp-9
130.6	7.22		Ar CH	6.78, 6.78	Tyr-5
136.4		7.22, 7.69	Ar C		Trp-8
138.4		2.67, 7.22	Ar C		Tyr-1
139.9	6.96	5.47, 6.01	CH=	5.47	Urea
145.1		2.62, 2.88, 4.35, 5.94, 6.01	>C=		Sugar
151.7		6.96	N-CO-N		Urea
156.4		6.73, 7.22	Ar C-O		Tyr-3
159.0		4.39	N-CO-N		Urea
168.1		6.96	CO-N		Urea
173.0		2.67, 2.95, 3.09	CO-N		Tyr
173.4		3.09, 4.93	CO-N		Butyrate-1
176.6		1.26	CO-N		Ala-1
177.0		1.22, 3.54	CO-N		Ala-2
180.0		4.39	COOH		Trp

The carbonyl which resonated at 173.4 ppm and coupled to the protons (long range heteronuclear correlation, LR-HETCOR[6]) (Fig. 4) at positions 3 and 2 (in the cases of **2** and **3**) was assigned as the butyryl carbonyl. The *N*-methyl protons, 3.09 ppm, coupled to carbon 3, and thus indicated the location of the methylated amine. Also coupled to the *N*-methyl protons is the *m*-tyrosine carbonyl, 173.0 ppm; this was indicative of an amide bond from *m*-tyrosine to the 3-methylamine of the butyrate.

The heteronuclear coupling of the α proton of tryptophan, 4.39 ppm, to the relatively high field carbonyl at 159.0 ppm indicated that the α-amine of the tryptophan residue was not acylated to a

Table 2. NMR data for pacidamycin 2.

C shift	H shift	LR-HETCOR	DEPT	COSY	Assignment
13.5	1.22		CH_3	4.91	Butyrate-4
16.8	1.48	4.00	CH_3	4.00	Ala-1
17.2	1.27	4.12	CH_3	4.12	Ala-2
30.2	3.12		NCH_3		
33.4	2.63, 2.88		CH_2	4.42, 5.93	Sugar
36.6	2.69, 2.93	4.97, 6.73, 6.81	CH_2	4.97	Tyr
38.6	2.91, 3.07	4.29, 7.24	CH_2	4.29	Phe
49.0	4.00	1.48	CH	1.48	Ala-1
50.3	4.12	1.27	CH	1.27	Ala-2
51.2	4.91	1.22, 3.12, 4.58	CH	1.22, 4.58	Butyrate-3
51.9	4.97	2.69	CH	2.69, 2.93	Tyr
55.5	4.58	1.22	CH	4.91	Butyrate-2
56.8	4.29	2.91, 3.05	CH	2.91, 3.07	Phe
72.7	4.42	2.63	O-CH	2.63, 2.88, 6.01	Sugar
93.6	6.01	2.63	O-CH-N	4.42	Sugar
97.2	5.93		CH=	2.63, 2.88	Sugar
102.5	5.47		CH=	7.01	Urea
114.6	6.78	6.73, 6.81	Ar CH	7.21	Tyr-4
115.9	6.73	2.69, 6.78, 6.81	Ar CH		Tyr-2
121.0	6.81	2.69, 6.73, 6.78	Ar CH	7.21	Tyr-6
127.0	7.29	7.24	Ar CH	7.35	Phe
128.8	7.35	7.35	Ar CH	7.24, 7.29	Phe-3,5
128.8	7.35	7.35	Ar CH	7.24, 7.29	Phe-3,5
129.7	7.24	2.91, 3.07, 7.24, 7.29	Ar CH	7.35	Phe-2,6
129.7	7.24	2.91, 3.07, 7.24, 7.29	Ar CH	7.35	Phe-2,6
130.6	7.21		Ar CH	6.78, 6.81	Tyr-5
138.0		2.91, 3.07, 4.29, 7.35	Ar C		Phe-1
138.4		2.69, 7.21	Ar C		Tyr-1
140.3	7.01	5.47	CH=	5.47	Urea
144.6		2.63, 2.88, 4.42, 5.93, 6.01	>C=		Sugar
151.2		7.01	N-CO-N		Urea
156.1		6.73, 7.21	Ar C-O		Tyr-3
158.8		4.29	N-CO-N		Urea
166.0		7.01	CO-N		Urea
168.1		4.58, 5.93	CO-N		Butyrate-1
170.6		1.48, 4.00	CO-N		Ala-1
173.0		2.69, 3.12, 4.97	CO-N		Tyr
176.6		1.27	CO-N		Ala-2
179.0		2.91, 3.07, 4.29	COOH		Phe

peptide carbonyl, but possibly a carbamoyl or urea group. Mass spectral evidence (Fig. 5) confirmed that the acylating functionality was a urea group. Tryptophan was assigned as the *C*-terminus based on the relatively low field resonance of its carbonyl (180.0 ppm), which was suggestive of a free acid.

Another proton spin system which was characterized by NMR was that of the unusual nucleoside moiety. The spectral data for the uracil base were easily recognized and are in good agreement with literature values. The pentose, however, is novel. The COSY and LR-HETCOR data established the skeletal features of this sugar. The anomeric proton, 6.01 ppm, couples to the methine at 4.35

Table 3. NMR data for pacidamycin 3.

C shift	H shift	LR-HETCOR	DEPT	COSY	Assignment
13.6	1.25	4.64	CH_3	4.96	Butyrate-4
17.3	1.32		CH_3	4.19	Ala-1
18.8	1.30	3.70	CH_3	3.70	Ala-2
30.3	3.17		NCH_3		Butyrate
33.5	2.71, 2.98		CH_2	4.46, 5.98	Sugar
36.9	2.75, 2.99	5.02	CH_2	5.02	Tyr-1
38.6	2.92, 3.07	4.33	CH_2	4.33	Tyr-2
49.7	3.70	1.30	CH	1.30	Ala-2
50.4	4.19		CH	1.32	Ala-1
51.1	4.96	1.25, 3.17, 4.64	CH	1.25, 4.64	Butyrate-3
51.6	5.02	2.75	CH	2.75, 2.99	Tyr-1
55.5	4.64	1.25	CH	4.96	Butyrate-2
56.9	4.33	2.92	CH	2.92, 3.07	Tyr-2
72.8	4.46	2.71	CH-O	2.71, 2.98, 6.07	Sugar
93.6	6.07		O-CH-N	4.46	Sugar
97.3	5.98		CH=	2.71, 2.98	Sugar
102.7	5.53		CH=	7.05	Urea
114.0	6.83	6.86	Ar CH	7.28	Tyr-4
114.6	6.83	6.86	Ar CH	7.28	Tyr-4
116.1	6.76	2.75, 6.86	Ar CH		Tyr-1-2
116.5	6.78	2.92, 6.86	Ar CH		Tyr-2-2
121.1	6.86	2.75, 6.76, 6.83	Ar CH	7.28	Tyr-1-6
121.9	6.86	2.92, 6.78, 6.83	Ar CH	7.28	Tyr-2-6
130.2	7.28		Ar CH	6.83, 6.86	Tyr-5
130.6	7.28		Ar CH	6.83, 6.86	Tyr-5
138.4		2.75, 7.28	Ar C		Tyr-1-1
140.0		2.92, 4.33, 7.28	Ar C		Tyr-2-1
140.2	7.05	5.53	CH=	5.53	Urea
144.6		5.98, 6.07	>C=		Sugar
152.0		7.05	N-CO-N		Urea
155.8		6.78, 7.28	Ar C-O		Tyr-2
156.3		6.76, 7.28	Ar C-O		Tyr-1
158.9		4.33	N-CO-N		Urea
166.9		7.05	CO-N		Urea
168.2		4.64, 5.98	CO-N		Butyrate-1
173.3		2.75, 3.17	CO-N		Tyr-1
175.2		1.30, 3.70	CO-N		Ala-2
176.7		1.32	CO-N		Ala-1
179.3		2.92, 4.33	COOH		Tyr-2

ppm. The methine couples to the allylic methylene protons, 2.62 and 2.88 ppm, which in turn show weak coupling to the vinyl proton at 5.94 ppm. The furanose ring was indicated by the coupling (LR-HETCOR) across the oxygen bridge by the anomeric proton, 6.01 ppm, to the enol ether carbon at 145.1 ppm. Heteronuclear coupling of the anomeric proton to the uracil carbon at 139.9 ppm indicates that the base is *N*-glycosidically attached to the pentofuranose at the anomeric position. In a nuclear Overhauser effect (NOE) study, exchange between the 6.96 ppm proton of uracil and the methine at 4.35 ppm indicated that the relative orientation of the base to the hydroxyl is *anti*. The carbon shifts of the β-(acylamino) enol ether were consistent with a model compound, 3-(acetylamino)-2-methoxy-2-propenamide[7], with allowance for the appropriate substitution of the amide by an alkyl substituent. The *cis* orientation of the heteroatoms about the olefin was evident by the NOE from

the vinyl proton to the methylene protons of the sugar. The same vinyl proton couples to the butyryl carbonyl (LR-HETCOR) (observed in **2** and **3**), and establishes that the ene-amine nitrogen is acylated by the butyrate.

The total structure was identified utilizing the MS-MS fragmentation data of the protonated molecular ion, *m/z* 875, (Fig. 5) to assemble the substructures that had been generated by NMR. Starting from the *N*-terminus of the peptide chain, the loss of alanine (*m/z* 802) and alanyl-*m*-tyrosine (*m/z* 641) were detected. Therefore, an alanine residue is at the *N*-terminus position and linked *via* a peptide bond to *m*-tyrosine. As was already concluded (NMR coupling) that the *m*-tyrosine was connected through an amide bond to the 3-methylamine of the butyrate, this was confirmed by the daughter ions *m/z* 611 and 266. Both fragments arose from the scission of the nitrogen-carbon bond at C-3 of the butyrate.

From the *C*-terminus, loss of indole (*m/z*

Fig. 2. Variable temperature ^{1}H NMR of pacidamycin 1.

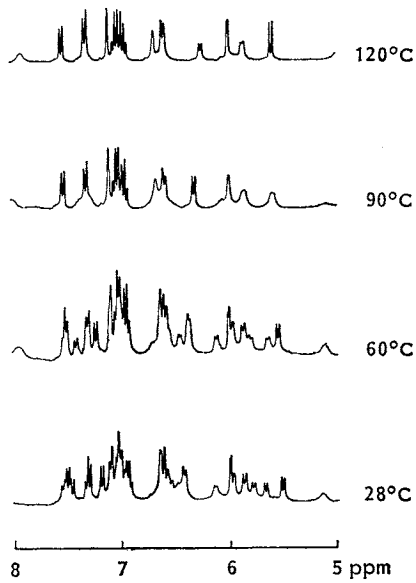


Fig. 3. Tryptophan-alanine hydantoin.

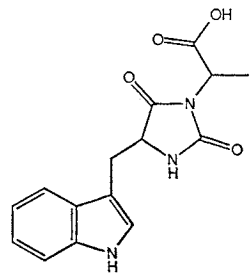


Fig. 4. LR-HETCOR coupling connectivities of pacidamycin 1.

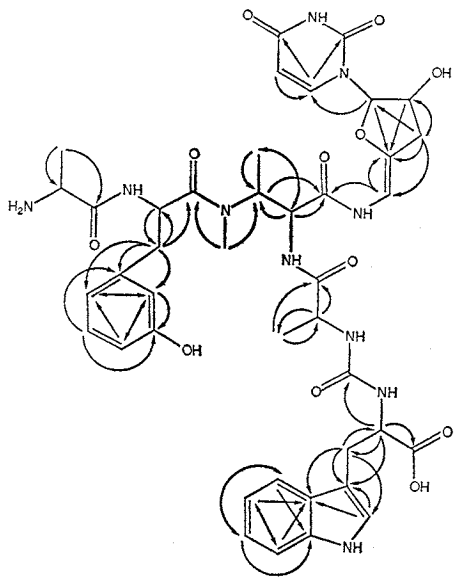
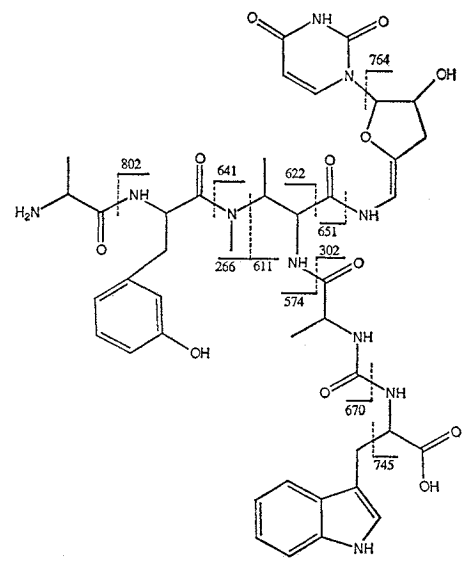


Fig. 5. MS-MS fragmentation of pacidamycin 1.



745) and loss of tryptophan (*m/z* 670) reaffirmed the presence and location of this residue. The NMR data had indicated that the α-amine of tryptophan was derivatized as the urea. Extending the molecule on the opposite side of the urea carbonyl is the second alanine residue, with its α-amine also part of the urea. The daughter ions which verify this assignment are *m/z* 302 (alanylurea-tryptophan) and *m/z* 574. Both intense signals were derived from the highly favorable cleavage of the acyl alanyl bond. The location of the alanyl acylation was at the 2-amino of the butyrate. This assignment was based on an intense daughter ion at *m/z* 622 which was derived from the α cleavage of the amine toward the carbonyl. In addition, cleavage of the amide bond between the butyrate and the nucleoside resulted in an ion, *m/z* 651, consisting of the "peptide". These two daughter ions along with *m/z* 611, from the loss of the *N*-terminal dipeptide, support the assignment and the proposed structure. The daughter ion *m/z* 764, from a loss of uracil from the molecular ion, confirms the nucleoside base.

The two other congeners with significant bioactivity were pacidamycin 2 (**2**) and pacidamycin 3 (**3**). Their structures varied from **1** in the nature of the *C*-terminal amino acid. Instead of tryptophan, **2** contains phenylalanine and **3** contains a second *m*-tyrosine residue. In water, **2** had a UV maximum at 256 nm (ε 12,700) and an optical rotation of $[\alpha]_D^{24}$ $-4.5°$ (*c* 0.63). The UV spectrum of **3** in aqueous solution gave a maximum at 257 nm (ε 13,000) and its optical rotation was measured as $[\alpha]_D^{24}$ $-1.7°$ (*c* 0.65). Inspection of the NMR data (Tables 2 and 3) shows minor chemical shift differences, except in the substituted residue. All the analogous coupled spin systems seen in **1** were observed in **2** and **3**. In both **2** and **3**, the α-proton of the *C*-terminal amino acid was coupled to the urea carbonyl (LR-HETCOR), as it was in **1**. HR-MS supports the composition for **2**, *m/z* 836.3579 as $C_{39}H_{49}N_9O_{12}+H$ (calcd 836.3582), and **3**, *m/z* 852.3518 as $C_{39}H_{49}N_9O_{13}+H$ (calcd 852.3531). The MS-MS daughter ions of **2** were indicative of the *C*-terminus substitution. These ions, which were 39 amu less than analogous ions produced by **1**, were: *m/z* 611, 602, 263, and 724. The fragment *m/z* 670 was seen in both spectra as it is unaffected by the different substitutions. Also the phenylalanine fragment appeared at *m/z* 166.

Characterization of Minors

The two minor active compounds, pacidamycin 4 (**4**) and pacidamycin 5 (**5**), were isolated and contributed less than 10% to the total activity of the complex. NMR data indicated **4** to be similar to **1** except for the lack of signals from one of the alanine residues. A similar relationship was seen for **5** and **2**. The alanine deletions were verified by HR-MS, **5** gave *m/z* 765.3208 corresponding to $C_{36}H_{44}N_8O_{11}+H$ (calcd 765.3211) and **4** gave *m/z* 804 corresponding to $C_{38}H_{45}N_9O_{11}+H$. The MS-MS spectrum of **5** also corroborates the loss of the *N*-terminal alanine. The fragment ions at *m/z* 263 and 601 (these were seen in the related compound **2** and are analogous to *m/z* 302 and 641 in **1**) indicate the region of the deletion can not be at the *C*-terminus or the nucleoside region. The fragment which contains the *N*-terminus and the nucleoside, produced a signal at *m/z* 574 in congeners **1** and **2**, but in **5** this fragment was at *m/z* 500, due to the alanine deletion.

Two species, **6** and **7**, are minors related to **1** and **2**, respectively, and were found as contaminants of the purified parent compounds. In both cases the *N*-terminal alanine was replaced with a glycine residue. Neither was separable from the parent congener, and were only observed and identified by their mass spectra. Both **6** and **7** produced a protonated molecular ions 14 amu less and at 10 to 20% the intensity of **1** and **2**. The HR-MS of **6** was recorded as *m/z* 861.3539 which corresponds to $C_{40}H_{48}N_{10}O_{12}+H$ (calcd 861.3535). This "loss of methylene" was localized to the *N*-terminal alanine

by MS-MS. The daughter ion at *m/z* 641 arose from the loss of glycine-*m*-tyrosyl. The identical fragment is seen in **1** and indicates that **6** does not lack the *N*-methylation. Also, the other alanine is intact and is observed in the ion fragment at *m/z* 302. Amino acid analysis detects the glycine at a concentration relative to alanine at 0.15 residue.

A series of structurally undetermined compounds[5] (LL-BO2964α, β, and γ) with comparable bioactivity, possess similar structural elements and are likely of the same class. Components of the LL-BO2964 mixtures are uracil, alanine, β-alanine, glycine, *m*-tyrosine, and 2-amino-3-*N*-methyl-aminobutyric acid. They are also able to form hydantoins from alanine and another amino acid, which in the description was either phenylalanine, tyrosine, or *m*-tyrosine. The main differences between the two sets of compounds are that the pacidamycins do not contain β-alanine, and neither a carbohydrate moiety or a tryptophan residue have been identified in the LL-BO2964 compounds.

Experimental

Isolation

Sixty six liters of broth (pH 7.0, unadjusted) from a fermentation of *Streptomyces coeruleorubidus* strain AB 1183F-64 were filtered through glass wool to remove the mycelial mass. The filtrate was batch extracted by addition of 6 liters of Amberlite XAD-2. The suspension was stirred for 12 hours at 8°C. The extracted broth was decanted and the resin was washed with 6 liters of water. This was then washed, in batch, with a step gradient begining with water and ending with methanol in 25% increments, at 2×3 liters per wash. Active fractions, 25 to 75% methanol, were pooled to give 18 liters of solution. The methanol was removed and the volume was reduced to 800 ml on a vertical evaporator under reduced pressure. The aqueous solution was extracted twice with equivalent volumes of butanol. The aqueous layer was adjusted to pH 3 with 6 N HCl and reextracted three times with equal volumes of butanol. The organic extracts were pooled to give 4 liters of solution. Reduction of the volume to 1 liter resulted in the precipitation of the active components. The suspension was centrifuged and the pellet was washed twice with 200 ml of ethyl acetate. Reduction of the mother liquor to 200 ml yielded a second crop of precipitate which was collected and washed. The combined yield of the two crops was 16.7 g. Aliquots of 1 g were chromatographed on Fractogel TSK HW-40 (S) (2.5×100 cm) in 50 mM ammonium acetate, adjusted to pH 8 with NH_4OH. The flow rate was 1.5 ml/minute (0.18 kg/cm^2) and the effluent was monitored by UV at 254 nm. Three major bioactive compounds were collected, **1** (170 mg), **2** (77 mg), and **3** (149 mg), along with two related minor peaks, **4** and **5**. The capacity factors for the compounds were: **1** k′=4.50, **2** k′=2.13, **3** k′=2.38, **4** k′=5.13, and **5** k′=2.63. Capacity factor k′ is defined as $(V_1-V_0)/V_0$, where V_1 is the elution volume and V_0 is the void volume of the column. The activity was monitored by the disk diffusion bioassay on agar plates which had been inoculated with *P. aeruginosa* BMH 1.

General Methods

NMR spectra were recorded with either a General Electric GN300 or GN500 spectrometer. ^{1}H NMR COSY data were acquired at 500.1 MHz. ^{1}H NMR CAMEL (NOE) data was measured at 300.1 MHz. ^{13}C NMR and DEPT experiments were recorded at 125.8 and 75.5 MHz. HETCOR and LR-HETCOR data were acquired at 500.1 MHz. Mass spectra and high resolution peak match values were recorded on a Kratos MS-50. MS-MS data were acquired on a Nermag 3010, a triple quadrapole spectrometer. UV spectra were measured on a Perkin-Elmer Lambda 3B UV/Vis spectrophotometer. Amino acid analyses were performed on a Beckman 6300. Optical rotations were measured in a 10-cm tube on a Perkin-Elmer model 241 polarimeter.

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