PHENELFAMYCINS, A NOVEL COMPLEX OF ELFAMYCIN-TYPE ANTIBIOTICS

II. ISOLATION AND STRUCTURE DETERMINATION

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A novel complex of elfamycin-type antibiotics has been isolated from submerged fermentation of either *Streptomyces violaceoniger* AB 999F-80 or *Streptomyces violaceoniger* AB 1047T-33. Antibiotics were extracted from the fermentation broth with ethyl acetate and from the mycelia with acetone. Purification of individual components was achieved by a combination of solvent partitions, Sephadex LH-20 exclusion, C_{18} bonded-phase silica gel adsorption, diol partition and liquid-liquid countercurrent chromatographies. Seven closely related components were separated and assigned structures **4**, **11**, **12**, **13**, **14**, and **16** to phenelfamycins A to F respectively and structure **17** to unphenelfamycin. These structures were elucidated employing a variety of spectroscopic techniques, including extensive use of 1D and 2D NMR spectroscopy.

In the course of screening microorganisms for the production of antibiotics with activity against anaerobic bacteria, a *Streptomyces* species was discovered which produced a novel complex of elfamycin-type antibiotics. Companion papers[1,2] describe the taxonomy and fermentation of the producing organism and the biological properties of some of the individual antibiotics produced. This paper will describe the isolation of these antibiotics and the elucidation of their structures.

Results and Discussion

Isolation of Phenelfamycin A and Unphenelfamycin

Eighty liters of submerged fermentation broth of *Streptomyces violaceoniger* AB 999F-80 prepared as outlined in ref 1 was adjusted to pH 4.0 with H_2SO_4. Mycelial mass was then removed by centrifugation and filtration and retained. Filtered broth was extracted with EtOAc ($3\times1/3$ volumes), the EtOAc extracts were combined and concentrated under vacuum to leave 10 g of dark oil. This material was partitioned between *n*-hexane and MeOH (10 liters of each). The MeOH layer was concentrated under vacuum to an oil. This oil was applied to a diol-bonded silica gel column pre-equilibrated in the lower phase of toluene - EtOAc - MeOH - H_2O (1 : 1 : 1 : 1) and eluted with the upper phase of this solvent system. Active fractions from the diol column were pooled and concentrated to an oil. This oil was subjected to countercurrent chromatography on an Ito multilayered coil planet centrifuge (CPC) employing a solvent system of toluene - EtOAc - MeOH - H_2O (1 : 1 : 1 : 1) with the upper phase as mobile. This CPC separation yielded pure phenelfamycin A (3.0 mg/liter) and unphenelfamycin (0.15 mg/liter).

The mycelial mass from this same fermentation was extracted with acetone (5×1 liter). The combined acetone extracts were concentrated under reduced pressure to an oil which was applied to

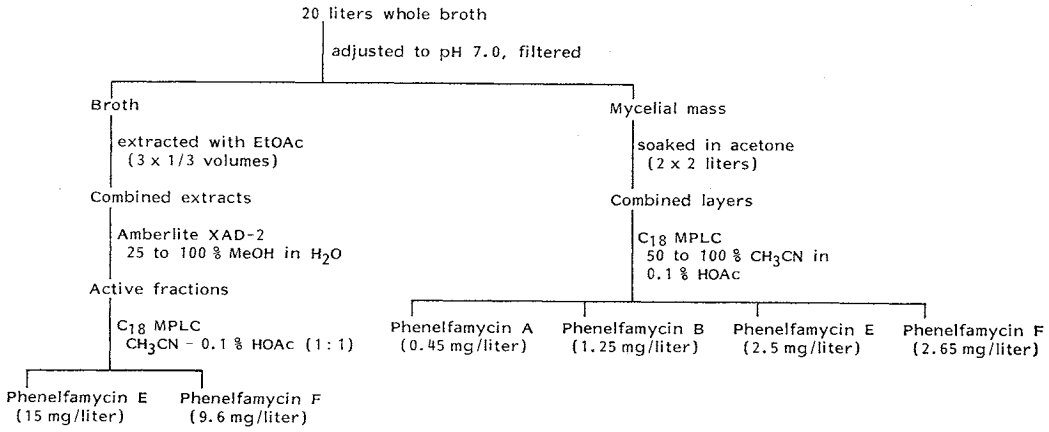
a Sephadex LH-20 column and eluted with MeOH. Active fractions from the Sephadex LH-20 column were combined, concentrated and applied to a Baker C_{18} bonded-phase column eluted with a gradient of 0 to 100% CH_3CN - MeOH (1 : 1) in water. This yielded pure phenelfamycin A at a level of 3.75 mg/liter of whole broth.

Isolation of Phenelfamycins B, C, D, E and F

The pH of a 5,100-liter fermentation of *S. violaceoniger* AB 999F-80 was adjusted to 4.3 with H_2SO_4. The whole broth was then passed through a desludging machine (DeLavol PX-207) to remove most of the mycelia. The clarified broth was extracted with EtOAc (3×1,500 liters), the EtOAc extracts were combined and concentrated under reduced pressure to leave *ca.* 4 liters of dark oil. This oil was partitioned between *n*-hexane and MeOH (60 liters of each). The MeOH layer was further extracted with *n*-hexane (2×60 liters) then concentrated under reduced pressure to a dark oil. This oil was partitioned between $CHCl_3$ - MeOH - aqueous 0.1% H_3PO_4 (20 liters of each). The lower layer of this partition was extracted with aqueous 0.05 M $NaHCO_3$, pH 9.5 (4×10 liters). Aqueous extracts were combined and concentrated under reduced pressure to leave a dark solid residue. This residue was chromatographed in ten equal portions on a diol-bonded silica gel column pre-equilibrated with the lower layer of $CHCl_3$ - EtOAc - MeOH - H_2O (3 : 3 : 2 : 4) and eluted with first the lower, then the upper layer of this solvent system. Active fractions from all ten chromatographies on this column were combined, concentrated and applied to a Baker C_{18} bonded-phase silica gel column eluted with a CH_3CN - 0.1% aqueous HOAc (1 : 1) to CH_3CN gradient. Active fractions from this column were combined and concentrated to a tan solid residue. A portion of this residue was applied to a Merck C_{18} bonded-phase Lobar column and eluted with CH_3CN - aqueous 0.1% H_3PO_4 (1 : 1) to yield pure phenelfamycins A, B, C, D, E and F. These components elute in the order A, C, E, B, D, F both here or on a Whatman analytical C_{18} bonded-phase HPLC column eluted with an aqueous acetonitrile gradient.

The mycelia initially removed by desludging from this large fermentation was soaked in acetone (2×200 liters) to lyse the cells and release antibiotic. The acetone layers were combined and concentrated to a dark viscous oil which was partitioned between *n*-hexane and MeOH (60 liters of each). The MeOH layer was further extracted with *n*-hexane (2×60 liters) then concentrated under reduced pressure to leave a dark oil. This oil was partitioned between $CHCl_3$ - MeOH - aqueous 0.1% H_3PO_4 (10 liters of each), the layers were separated and the lower layer was extracted with pH 9.5, 0.1 M bicarbonate buffer (20 liters). The lower layer from the bicarbonate extract was concentrated under reduced pressure to a dark solid. This solid was applied to a diol-bonded silica gel column and eluted with a 0 to 100% MeOH gradient in CH_2Cl_2. Active fractions from the diol column were pooled to yield pure unphenelfamycin (~20 mg/liter of whole beer). The bicarbonate layer from the above extract was concentrated under reduced pressure to yield a dark solid residue. This residue was triturated with $CHCl_3$ (20 liters) then filtered. $CHCl_3$ insoluble material was applied to an Amberlite XAD-2 column and eluted with a 1 : 1 water - MeOH to MeOH gradient. Active fractions from the Amberlite XAD-2 column were combined and concentrated under reduced pressure to a brown solid. To the $CHCl_3$ soluble portion of the above trituration was added 40 liters of *n*-heptane whereupon a flocculant precipitate formed. This precipitate was removed by filtration and combined with active fractions from the Amberlite XAD-2 column of the original $CHCl_3$ insoluble material. This combined material was adsorbed onto a diol-bonded silica gel column and eluted first with the upper then

Fig. 1. Isolation of phenelfamycins E and F from 20 liters of *Streptomyces violaceoniger* AB 1047T-33.



C_{18} MPLC: Merck C_{18} bonded-phase Lobar column chromatography.

the lower layer of the biphasic solvent system $CHCl_3$ - EtOAc - MeOH - H_2O (3 : 3 : 2 : 4). Two active bands were eluted from this diol column. The first was applied to a Sephadex LH-20 column and eluted with $CHCl_3$ - heptane - EtOH (10 : 10 : 1) to yield pure phenelfamycins A, B and C. The second was applied to a Baker C_{18} bonded-phase silica gel column eluted with a CH_3CN - 0.1% HOAc (1 : 1) to CH_3CN gradient to yield pure phenelfamycin A. Material which was soluble in the $CHCl_3$ - heptane soluble (triturated into $CHCl_3$ and did not precipitate with heptane) was concentrated under reduced pressure to leave a brown oil. This oil was chromatographed in five equal portions on a silica gel column eluted with 2-PrOH - CH_2Cl_2 (1 : 10). Active fractions from this column were pooled and concentrated under reduced pressure to a tan solid. Portions of this solid were applied to a Merck C_{18} bonded-phase Lobar column and eluted with CH_3CN - aqueous 0.1% H_3PO_4 (1 : 1). This column yielded pure phenelfamycins A, B, C, D, E and F.

Isolation of Phenelfamycins E and F from Culture B

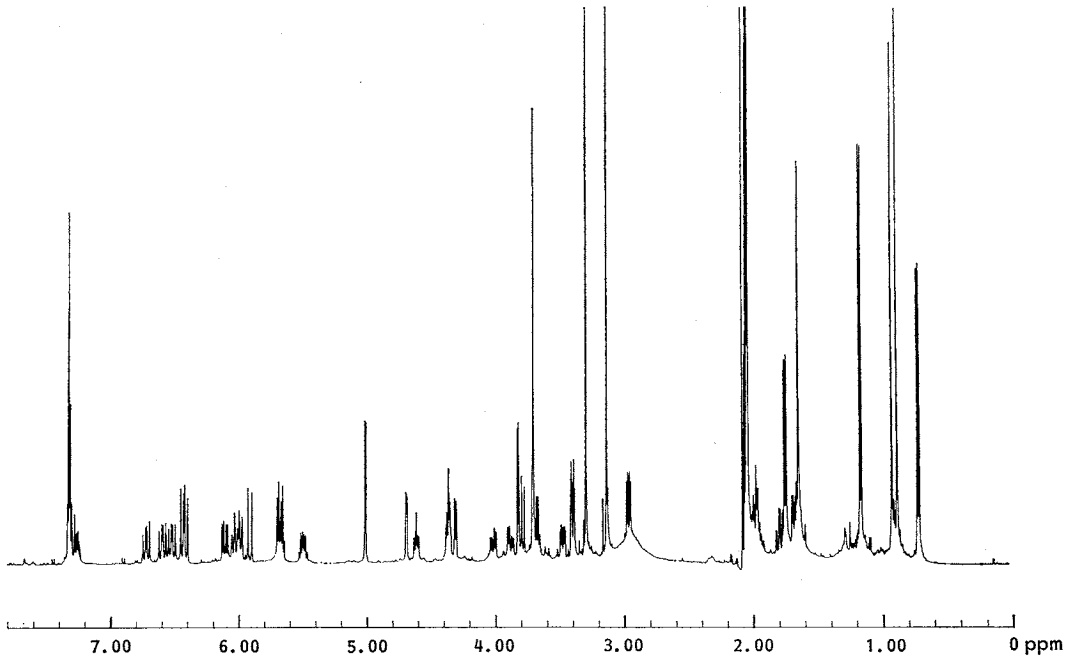
A second isolate, *S. violaceoniger* AB 1047T-33, was discovered which also produced the phenelfamycins. HPLC analysis monitored by diode array UV detection indicated that this culture was producing phenelfamycins E and F as the major members of the complex. Twenty liters of whole broth from 200×500 ml shaken flasks were harvested and phenelfamycins A (0.45 mg/liter), B (1.25 mg/liter), E (17.4 mg/liter), and F (12.25 mg/liter) were isolated as outlined in Fig. 1.

Structure Determination of Phenelfamycin A

The ^1H NMR spectrum of phenelfamycin A (Fig. 2) was complex in the region of δ 7.4 to 5.4 with 18 overlapping olefinic/aromatic protons. A 2D ^1H correlation spectroscopy (COSY)[8] spectrum and single frequency decoupling experiments helped to define four isolated spin systems. A diene system could be identified with protons at: δ 5.68 (1H, dd, J=15.3 and 6.0 Hz), 6.60 (1H, br dd, J=15.3 and 11.0 Hz), 6.03 (1H, tq, J=11.0 and 1.5 Hz), 5.50 (1H, dq, J=11.0 and 7.2 Hz) and 1.75 (3H, dd, J=7.2 and 1.5 Hz) indicating a methyl terminating fragment **1**. A second diene system was defined by protons at: δ 1.65 (3H, br s), 5.98 (1H, br d, J=10.9 Hz), 6.52 (1H, dddd, J=15.2, 10.9, 1.5 and 1.3 Hz), 5.67 (1H, dt, J=15.2 and 6.4 Hz) indicating a fragment **2**.

A cluster of signals at $\sim\delta$ 7.3 integrated to six protons. Examination of the COSY spectrum

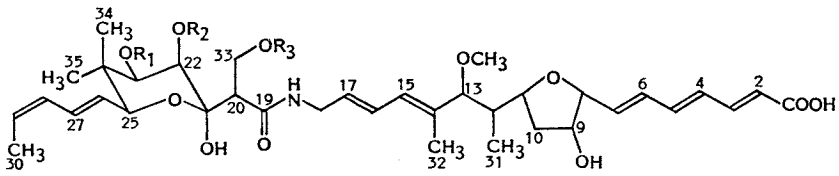
Fig. 2. Phenelfamycin A, ^1H NMR spectrum in acetone-d_6.



1 2 3

indicates that only a single proton in this region is coupled outside of the envelope. This signal can be followed through the COSY and single frequency decoupling experiments to define polarized triene fragment **3** with proton signals at δ 5.92 (1H, d, J=15.2 Hz), 7.30 (1H, dd, J=15.2 and 11.4 Hz), 6.43 (1H, dd, J=15.3 and 11.4 Hz), 6.72 (1H, dd, J=15.3 and 11.4 Hz), 6.43 (1H, dd, J=15.3 and 11.4 Hz) and 6.12 (1H, dd, J=15.3 and 6.3 Hz).

These three olefinic systems closely resembled those found in a class of antibiotics typified by aurodox[4,5], an antibiotic first discovered in 1973. The term "elfamycins" has been given to this class based upon the members common mode of action, the interference with protein synthesis at the step involving elongation factor Tu (or EF Tu). Most elfamycins of known structure, including efrotomycin[6], heneicomycin[7] and kirrothricin[8] contain an intact, or partially reduced, 4-hydroxy-α-pyridone moiety which does not appear to be present in the structure of phenelfamycin A. A heteronuclear multiple bond correlation (HMBC) experiment[9] indicated that a carbonyl carbon at δ 167.7 (quaternary (Q)) coupled into the triene **3** as evidenced by connectivity peaks to the olefinic proton at δ 5.92. This evidence, in addition to the extraction profile indicating the acidic nature of phenelfamycin A, led us to suspect a carboxylic acid triene moiety. Further substantiating this, a recently reported antibiotic,



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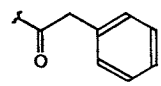

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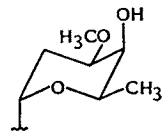

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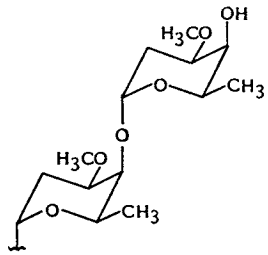
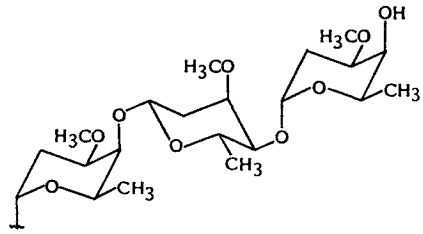


D =



Phenelfamycin A (**4**)	R_1=H	R_2=A	R_3=B	Phenelfamycin D (**13**)	R_1=A	R_2=H	R_3=C
10	R_1=B	R_2=A	R_3=H	Phenelfamycin E (**14**)	R_1=H	R_2=A	R_3=D
Phenelfamycin B (**11**)	R_1=A	R_2=H	R_3=B	Phenelfamycin F (**16**)	R_1=A	R_2=H	R_3=D
Phenelfamycin C (**12**)	R_1=H	R_2=A	R_3=C	Unphenelfamycin (**17**)	R_1=H	R_2=H	R_3=B

L-681,217[10], containing this same fragment served as a good UV spectral model.

Having established the class of antibiotics to which phenelfamycin A (**4**) belongs, we will here present the full structure for ease of presentation of the remaining data. Based upon a heteronuclear correlation map (HETCOR) experiment, proton signals were assigned to their respective carbon atoms. The results are outlined in Tables 1 and 2. The three partial structures **1**, **2** and **3** account for 13 of the 18 downfield protons.

The remaining five protons of phenelfamycin A with signals at *ca.* δ 7.3 were not coupled outside of that region and correlated to three carbon signals (degenerate) at δ 130.2, 129.1 and 127.6 indicative of an unsubstituted phenyl ring. An HMBC experiment indicated that this benzene ring was attached to a methylene with protons at δ 3.71 (2H, s) which was itself connected to an ester carbonyl at δ 171.4, hence defining a phenacetate moiety **5**.

The three olefinic fragments **1**~**3** previously described could each be extended *via* a series of homonuclear and heteronuclear correlation experiments as well as single frequency decoupling. The methyl terminating diene fragment **1** (carbons 26~30 in **4**) is coupled to an oxygenated methine with carbon and proton chemical shifts of δ 76.8 (CH) and 4.31 (1H, br d, J=6.0 Hz) respectively. This proton couples only into the olefinic system and hence is adjacent to a quaternary carbon atom. This carbon was identified as that at δ 39.5 (Q) by an HMBC correlation to the δ 4.31 proton. An isolated set of coupled methine protons with proton signals at δ 3.83 (1H, d, J=3.7 Hz) and 5.02 (1H, d, J=3.7 Hz) and attached to carbons with signals at δ 72.0 (CH) and 72.6 (CH) respectively were further

Table 1. ^{13}C NMR data (ppm) for phenelfamycins (in acetone-d_6).

Carbon No.	Phenelfamycin A	Phenelfamycin B	Phenelfamycin C	Phenelfamycin E	Phenelfamycin F	Unphenelfamycin
1	167.7 (Q)	168.1 (Q)	168.0 (Q)	167.7 (Q)	167.8 (Q)	167.2 (Q)
2	121.8 (CH)	121.8 (CH)	121.8 (CH)	121.2 (CH)	121.9 (CH)	121.2 (CH)
3	146.5 (CH)	145.4 (CH)	145.4 (CH)	146.5 (CH)	145.4 (CH)	144.4 (CH)
4	130.3 (CH)	130.3 (CH)	130.3 (CH)	130.2 (CH)	130.2 (CH)	129.4 (CH)
5	141.9 (CH)	141.2 (CH)	141.1 (CH)	141.9 (CH)	141.2 (CH)	140.2 (CH)
6	131.5 (CH)	131.5 (CH)	131.5 (CH)	131.4 (CH)	131.4 (CH)	130.6 (CH)
7	137.5 (CH)	137.4 (CH)	137.4 (CH)	137.1 (CH)	137.6 (CH)	136.5 (CH)
8	75.0 (CH)	75.0 (CH)	75.0 (CH)	75.1 (CH)	75.1 (CH)	74.0 (CH)
9	84.3 (CH)	84.2 (CH)	84.3 (CH)	84.3 (CH)	84.2 (CH)	83.4 (CH)
10	40.2 (CH_2)	40.1 (CH_2)	39.6 (CH_2)	40.2 (CH_2)	40.2 (CH_2)	39.2 (CH_2)
11	78.0 (CH)	78.1 (CH)	78.0 (CH)	78.0 (CH)	78.1 (CH)	77.2 (CH)
12	40.5 (CH)	40.6 (CH)	40.5 (CH)	40.5 (CH)	40.6 (CH)	39.7 (CH)
13	90.2 (CH)	90.2 (CH)	90.2 (CH)	90.2 (CH)	90.3 (CH)	89.3 (CH)
13-OCH_3	56.2 (CH_3)*	56.1 (CH_3)*	56.2 (CH_3)*	56.2 (CH_3)	56.2 (CH_3)	55.2 (CH_3)
14	136.0 (Q)	137.0 (Q)	137.5 (Q)	137.1 (Q)	137.1 (Q)	136.1 (Q)
15	129.5 (CH)	129.6 (CH)	129.5 (CH)	130.0 (CH)	129.5 (CH)	128.7 (CH)
16	128.5 (CH)	129.5 (CH)	128.3 (CH)	129.1 (CH)	128.1 (CH)	127.1 (CH)
17	130.0 (CH)	130.4 (CH)	130.3 (CH)	130.2 (CH)	129.7 (CH)	129.6 (CH)
18	41.7 (CH_2)	41.7 (CH_2)	41.6 (CH_2)	42.1 (CH_2)	42.0 (CH_2)	41.0 (CH_2)
19	174.8 (Q)	175.4 (Q)	174.6 (Q)	175.0 (Q)	175.5 (Q)	174.6 (Q)
20	49.6 (CH)	49.7 (CH)	49.6 (CH)	49.8 (CH)	49.8 (CH)	49.2 (CH)
21	98.5 (Q)	99.6 (Q)	98.4 (Q)	98.6 (Q)	99.7 (Q)	98.4 (Q)
22	72.6 (CH)	69.0 (CH)	72.6 (CH)	72.6 (CH)	69.3 (CH)	70.5 (CH)
23	72.0 (CH)	76.5 (CH)	71.9 (CH)	72.1 (CH)	76.6 (CH)	71.9 (CH)
24	39.5 (Q)	38.7 (Q)	40.1 (Q)	39.6 (Q)	38.8 (Q)	38.6 (Q)
25	76.8 (CH)	76.4 (CH)	76.8 (CH)	76.8 (CH)	76.4 (CH)	75.4 (CH)
26	130.3 (CH)	130.2 (CH)	129.9 (CH)	130.3 (CH)	130.5 (CH)	129.6 (CH)
27	127.4 (CH)	128.0 (CH)	127.4 (CH)	128.3 (CH)	127.6 (CH)	126.1 (CH)
28	130.1 (CH)	130.1 (CH)	130.1 (CH)	130.3 (CH)	130.1 (CH)	129.3 (CH)
29	126.3 (CH)	126.4 (CH)	126.4 (CH)	126.3 (CH)	126.4 (CH)	125.0 (CH)
30	13.6 (CH_3)	13.5 (CH_3)	13.5 (CH_3)	13.6 (CH_3)	13.6 (CH_3)	12.6 (CH_3)
31	10.5 (CH_3)	10.5 (CH_3)	10.4 (CH_3)	10.5 (CH_3)	10.6 (CH_3)	9.6 (CH_3)
32	11.0 (CH_3)	11.0 (CH_3)	11.0 (CH_3)	11.0 (CH_3)	11.0 (CH_3)	10.1 (CH_3)
33	64.0 (CH_2)	64.5 (CH_2)	64.0 (CH_2)	64.1 (CH_2)	64.7 (CH_2)	63.8 (CH_2)
34	15.3 (CH_3)	16.8 (CH_3)	15.4 (CH_3)	15.4 (CH_3)	16.8 (CH_3)	14.9 (CH_3)
35	24.2 (CH_3)	24.0 (CH_3)	24.2 (CH_3)	24 3 (CH_3)	24.0 (CH_3)	23.4 (CH_3)
1′	97.1 (CH)	97.5 (CH)	97.2 (CH)	97.3 (CH)	97.6 (CH)	96.6 (CH)
2′	30.8 (CH_2)	30.6 (CH_2)	31.7 (CH_2)	32.0 (CH_2)	32.1 (CH_2)	31.0 (CH_2)
3′	75.8 (CH)	75.8 (CH)	75.9 (CH)	75.1 (CH)	75.1 (CH)	75.0 (CH)
3′-OCH_3	55.3 (CH_3)*	55.3 (CH_3)*	55.1 (CH_3)*	55.5 (CH_3)	55.5 (CH_3)	55.2 (CH_3)
4′	67.5 (CH)	67.7 (CH)	74.6 (CH)	74.2 (CH)	74.3 (CH)	66.8 (CH)
5′	66.9 (CH)	66.9 (CH)	67.7 (CH)	67.3 (CH)	67.3 (CH)	66.0 (CH)
6′	17.2 (CH_3)	17.2 (CH_3)	17.4 (CH_3)	17.7 (CH_3)	17.8 (CH_3)	16.4 (CH_3)
1″			100.0 (CH)	99.4 (CH)	99.4 (CH)	
2″			30.2 (CH_2)	34.6 (CH_2)	34.6 (CH_2)	
3″			75.8 (CH)	77.8 (CH)	77.8 (CH)	
3″-OCH_3			56.4 (CH_3)*	57.2 (CH_3)	57.2 (CH_3)	
4″			67.8 (CH)	82.7 (CH)	82.7 (CH)	
5″			66.9 (CH)	69.6 (CH)	69.6 (CH)	
6″			17.6 (CH_3)	18.6 (CH_3)	18.6 (CH_3)	
1‴				101.1 (CH)	101.1 (CH)	
2‴				30.3 (CH_2)	30.9 (CH_2)	
3‴				75.7 (CH)	75.7 (CH)	
3‴-OCH_3				55.2 (CH_3)	54.4 (CH_3)	
4‴				67.8 (CH)	67.8 (CH)	
5‴				67.3 (CH)	67.4 (CH)	
6‴				17.4 (CH_3)	17.5 (CH_3)	
Phenacetyl CO	171.4 (Q)	171.5 (Q)	171.5 (Q)	171.4 (Q)	171.4 (Q)	
Phenacetyl CH_2	41.9 (CH_2)	42.0 (CH_2)	41.9 (CH_2)	41.7 (CH_2)	41.7 (CH_2)	
Aromatic	135.4 (Q)	135.6 (Q)	135.3 (Q)	135.4 (Q)	135.7 (Q)	
Aromatic	130.2 (CH)	130.2 (CH)	130.3 (CH)	130.2 (CH)	130.3 (CH)	
Aromatic	129.1 (CH)	129.1 (CH)	129.1 (CH)	129.5 (CH)	129.1 (CH)	
Aromatic	127.6 (CH)	127.6 (CH)	127.6 (CH)	127.5 (CH)	127.6 (CH)	

* These assignments may be interchanged within the column.
Q: Quaternary.

Table 2. ^{1}H NMR data (ppm) for the

Protons on carbon No.	Phenelfamycin A	Phenelfamycin B	Phenelfamycin C
2	5.92 (1H, d, J=15.2)	5.90 (1H, d, J=15.3)	5.90 (1H, d, J=15.3)
3	7.30 (1H, dd, J=15.2, 11.4)	7.32 (1H, dd, J=15.3, 11.4)	7.30 (1H, dd, J=15.3, 11.4)
4	6.43 (1H, dd, J=15.3, 11.4)	6.43 (1H, dd, J=15.3, 11.4)	6.43 (1H, dd, J=15.3, 11.4)
5	6.72 (1H, dd, J=15.3, 11.4)	6.71 (1H, dd, J=15.3, 11.4)	6.72 (1H, dd, J=15.3, 11.4)
6	6.43 (1H, dd, J=15.3, 11.4)	6.42 (1H, dd, J=15.3, 11.4)	6.39 (1H, dd, J=15.3, 11.4)
7	6.12 (1H, dd, J=15.3, 6.3)	6.10 (1H, dd, J=15.3, 6.1)	6.10 (1H, dd, J=15.3, 6.3)
8	4.37 (1H, m)	4.35 (1H, m)	4.35 (1H, m)
9	4.38 (1H, m)	4.35 (1H, m)	4.34 (1H, m)
10	2.00 (2H, m)	1.98 (2H, m)	1.95 (2H, m)
11	4.62 (1H, m)	4.61 (1H, m)	4.61 (1H, ddd, J=9.7, 6.6, 2.9)
12	1.70 (1H, m)	1.64 (1H, m)	1.63 (1H, obscured)
13	3.40 (1H, d, J=9.7)	3.38 (1H, d, J=9.7)	3.39 (1H, d, J=9.5)
13-OCH_3	3.30 (3H, s)*	3.29 (3H, s)	3.30 (3H, s)*
15	5.98 (1H, br d, J=10.9)	5.98 (1H, br d, J=11.1)	5.98 (1H, br d, J=10.9)
16	6.52 (1H, dddd, J=15.2, 10.9, 1.5, 1.3)	6.52 (1H, dddd, J=15.3, 11.1, 1.4, 1.3)	6.51 (1H, dddd, J=15.2, 10.9, 1.5, 1.3)
17	5.67 (1H, dt, J=15.2, 6.4)	5.69 (1H, m)	5.65 (1H, dt, J=15.2, 6.0)
18	4.02 (1H, br ddd, J=15.6, 6.4, 1.5)	4.02 (1H, br ddd, J=15.6, 6.5, 1.4)	4.00 (1H, br m)
	3.88 (1H, br ddd, J=15.6, 6.4, 1.3)	3.89 (1H, br ddd, J=15.6, 6.2, 1.3)	3.89 (1H, br m)
20	2.97 (1H, dd, J=10.7, 4.3)	3.24 (1H, dd, J=10.7, 4.5)	2.96 (1H, dd, J=10.7, 4.5)
22	5.02 (1H, d, J=3.7)	3.78 (1H, d, J=3.4)	5.00 (1H, d, J=3.6)
23	3.83 (1H, d, J=3.7)	4.93 (1H, d, J=3.4)	3.80 (1H, d, J=3.6)
25	4.31 (1H, br d, J=6.0)	4.32 (1H, br d, J=6.1)	4.29 (1H, d, J=5.6)
26	5.68 (1H, dd, J=15.3, 6.0)	5.66 (1H, dd, J=15.3, 6.1)	5.68 (1H, dd, J=15.0, 5.6)
27	6.60 (1H, br dd, J=15.3, 11.0)	6.58 (1H, br dd, J=15.3, 11.2)	6.58 (1H, br dd, J=15.0, 11.2)
28	6.03 (1H, tq, J=11.0, 1.5)	6.00 (1H, ddq, J=11.2, 10.8, 1.6)	6.00 (1H, ddq, J=11.2, 10.8, 1.3)
29	5.50 (1H, dq, J=11.0, 7.2)	5.48 (1H, dq, J=10.8, 7.1, 1.6)	5.48 (1H, dq, J=10.8, 7.0)
30	1.75 (3H, dd, J=7.2, 1.5)	1.73 (1H, dd, J=7.1, 1.6)	1.74 (3H, d, J=7.0)
31	0.72 (3H, d, J=6.9)	0.70 (3H, d, J=7.0)	0.70 (3H, d, J=6.9)
32	1.65 (3H, br s)	1.62 (3H, br s)	1.63 (3H, br s)
33	3.79 (1H, dd, J=10.7, 9.5)	3.85 (1H, dd, J=10.7, 9.1)	3.80 (1H, dd, J=10.7, 9.6)
	3.40 (1H, dd, J=9.5, 4.3)	3.62 (1H, dd, J=9.1, 4.5)	3.38 (1H, dd, J=9.6, 4.5)
34	0.94 (3H, s)	1.00 (3H, s)	0.90 (3H, s)
35	0.89 (3H, s)	0.67 (3H, s)	0.86 (3H, s)
Phenacetyl CH_2	3.71 (2H, s)	3.76 (1H, d, J=15.2) 3.69 (1H, d, J=15.2)	3.70 (2H, s)
Aromatic	7.30 (5H, m)	7.30 (5H, m)	7.30 (5H, m)

phenelfamycins (in acetone-d_6).

Phenelfamycin D	Phenelfamycin E	Phenelfamycin F	Unphenelfamycin
5.92 (1H, d, J=15.3)	5.85 (1H, d, J=15.3)	5.93 (1H, d, J=15.3)	5.91 (1H, d, J=15.4)
7.31 (1H, obscured)	7.28 (1H, dd, J=15.3, 11.4)	7.25 (1H, dd, J=15.3, 11.4)	7.29 (1H, dd, J=15.4, 11.6)
6.48 (1H, dd, J=15.3, 11.4)	6.44 (1H, dd, J=15.3, 11.4)	6.44 (1H, dd, J=15.0, 11.4)	6.43 (1H, dd, J=15.2, 11.6)
6.75 (1H, dd, J=15.3, 11.4)	6.71 (1H, dd, J=15.3, 11.4)	6.70 (1H, dd, J=15.0, 11.4)	6.71 (1H, dd, J=15.2, 11.4)
6.43 (1H, dd, J=15.3, 11.4)	6.41 (1H, dd, J=15.3, 11.4)	6.41 (1H, dd, J=15.0, 11.4)	6.39 (1H, dd, J=15.2, 11.4)
6.11 (1H, dd, J=15.3, 6.2)	6.10 (1H, dd, J=15.3, 6.3)	6.11 (1H, dd, J=15.0, 6.4)	6.10 (1H, dd, J=15.2, 6.5)
4.40 (1H, m)	4.38 (1H, m)	4.36 (1H, m)	4.37 (1H, m)
4.40 (1H, m)	4.42 (1H, m)	4.38 (1H, m)	4.37 (1H, m)
1.98 (2H, m)	2.00 (2H, m)	1.97 (2H, m)	1.96 (2H, m)
4.63 (1H, ddd, J=9.6, 6.6, 3.3)	4.64 (1H, ddd, J=9.7, 6.6, 2.9)	4.63 (1H, ddd, J=9.7, 6.6, 3.1)	4.62 (1H, ddd, J=9.5, 6.5, 3.0)
1.70 (1H, obscured)	1.72 (1H, m)	1.65 (1H, m)	1.70 (1H, m)
3.43 (1H, d, J=9.5)	3.40 (1H, obscured)	3.39 (1H, d, J=9.7)	3.38 (1H, d, J=8.5)
3.32 (3H, s)*	3.13 (3H, s)	3.12 (3H, s)	3.10 (3H, s)
6.00 (1H, br d, J=11.0)	5.98 (1H, br d, J=10.9)	5.98 (1H, br d, J=11.0)	5.99 (1H, br d, J=11.0)
6.55 (1H, br dd, J=15.3, 11.0)	6.54 (1H, br dd, J=15.1, 10.9)	6.53 (1H, br dd, J=15.2, 11.0)	6.53 (1H, br dd, J=15.2, 11.0)
5.64 (1H, m)	5.68 (1H, dt, J=15.1, 6.0)	5.64 (1H, dt, J=15.2, 5.8)	5.66 (1H, m)
4.02 (1H, br m)	4.02 (1H, br m)	4.04 (1H, br m)	4.04 (1H, br dd, J=15.8, 6.2)
3.92 (1H, br m)	3.90 (1H, br m)	3.90 (1H, br m)	3.89 (1H, obscured)
3.29 (1H, dd, J=10.7, 4.6)	2.98 (1H, dd, J=10.8, 4.1)	3.25 (1H, dd, J=10.5, 4.5)	3.19 (1H, obscured)
3.78 (1H, d, J=3.4)	5.01 (1H, d, J=3.4)	3.83 (1H, d, J=3.4)	3.59 (1H, m)
4.94 (1H, d, J=3.4)	3.81 (1H, d, J=3.4)	4.96 (1H, d, J=3.4)	3.59 (1H, m)
Unassigned	4.32 (1H, d, J=5.8)	4.33 (1H, d, J=5.8)	4.23 (1H, br d, J=6.0)
5.68 (1H, obscured)	5.70 (1H, dd, J=15.0, 5.8)	5.69 (1H, dd, J=15.1, 5.8)	5.70 (1H, m)
6.64 (1H, br dd, J=15.3, 11.2)	6.59 (1H, br dd, J=15.0, 11.4)	6.60 (1H, br dd, J=15.1, 10.8)	6.57 (1H, m)
6.00 (1H, m)	6.02 (1H, ddq, J=11.4, 10.6, 1.1)	6.00 (1H, tq, J=10.8, 1.4)	5.99 (1H, tq, J=11.0, 1.6)
5.48 (1H, dq, J=10.8, 7.3)	5.49 (1H, dq, J=10.6, 7.0)	5.47 (1H, dq, J=10.8, 7.0)	5.46 (1H, dq, J=11.0, 7.1)
1.71 (3H, dd, J=7.3, 1.6)	1.75 (3H, dd, J=7.0, 1.1)	1.75 (3H, dd, J=7.0, 1.4)	1.74 (3H, dd, J=7.1, 1.6)
0.73 (3H, d, J=6.9)	0.72 (3H, d, J=6.9)	0.72 (3H, d, J=6.9)	0.73 (3H, d, J=7.3)
1.69 (3H, br s)	1.66 (3H, br s)	1.66 (3H, br s)	1.66 (3H, br s)
3.81 (1H, dd, J=10.7, 9.1)	3.80 (1H, obscured)	3.87 (1H, dd, J=10.5, 9.2)	3.89 (1H, dd, J=11.9, 9.1)
3.61 (1H, obscured)	3.41 (1H, obscured)	3.60 (1H, dd, J=9.2, 4.5)	3.68 (dd, 1H, J=9.1, 4.5)
1.01 (3H, s)	0.95 (3H, s)	1.02 (3H, s)	0.92 (3H, s)
0.70 (3H, s)	0.89 (3H, s)	0.68 (3H, s)	0.89 (3H, s)
3.73 (1H, d, J=15.2)	3.70 (2H, s)	3.76 (1H, d, J=15.2)	
3.67 (1H, d, J=15.2)		3.68 (1H, d, J=15.2)	
7.36 (5H, m)	7.32 (5H, m)	7.30 (5H, m)	

Table 2.

Protons on carbon No.	Phenelfamycin A	Phenelfamycin B	Phenelfamycin C
1′	4.70 (1H, br d, J=3.0)	4.82 (1H, br d, J=2.9)	4.69 (1H, d, J=2.7)
2′	1.80 (2H, m)	1.83 (1H, dd, J=12.1, 3.4)	1.81 (1H, br dd, J=11.8, 2.9)
		1.69 (1H, m)	1.63 (1H, obscured)
3′	3.48 (1H, ddd, J=11.8, 4.9, 2.9)	3.50 (1H, ddd, J=11.9, 4.8, 3.4)	3.46 (1H, ddd, J=11.8, 4.9, 2.9)
3′-OCH_3	3.10 (3H, s)	3.12 (3H, s)	3.10 (3H, s)*
4′	3.70 (1H, obscured)	3.47 (1H, br d, J=4.8)	3.80 (1H, obscured)
5′	3.68 (1H, q, J=6.6)	3.73 (1H, q, J=6.5)	3.66 (1H, br q, J=6.4)
6′	1.17 (3H, d, J=6.6)	1.17 (3H, d, J=6.5)	1.13 (3H, d, J=6.4)
1″			4.90 (1H, t, J=1.0)
2″			1.86 (2H, m)
3″			3.59 (1H, m)
3″-OCH_3			3.34 (3H, s)*
4″			3.70 (1H, obscured)
5″			4.22 (1H, br q, J=6.5)
6″			1.16 (3H, d, J=6.5)
1‴			
2‴			
3‴			
3‴-OCH_3			
4‴			
5‴			
6‴			

* Assignments of these protons may be interchanged within the column.
Coupling constant: J in Hz.

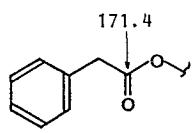
coupled into this quaternary carbon at δ 39.5. These observations fit well the oxygenation pattern common in elfamycins. In addition, the δ 3.83 proton (23-H) shows a strong nuclear Overhauser enhancement (NOE) to that at δ 4.31 (25-H) indicating a 1,3 diaxial relationship between the two. A coupling constant of 3.7 Hz between the 23- and 22-H's places the latter in an equatorial orientation. The chemical shift of δ 5.02 for 22-H suggests that this carbon carries an acyloxy group and as previously mentioned this hydrogen is coupled to the phenacetate ester carbonyl carbon thus setting the site of attachment for that group as C-22, and defining a partial structure **6**.

The second diene moiety **2** (carbons 14~17) is connected at C-17 to a methylene with proton and carbon chemical shifts of δ 4.02 (1H, br ddd, J=15.6, 6.4 and 1.5 Hz), 3.88 (1H, br ddd, J=15.2, 6.4 and 1.3 Hz) and 41.7 (CH_2) respectively. This is suggestive of an amide methylene thus correlating the fragment of carbons 14~19 in **7** to a similar entity in aurodox.

The triene carboxylic acid group of phenelfamycin A attaches to the structure at an oxygen-bearing methine with carbon (C-8) and proton chemical shifts of δ 75.0 (CH) and 4.37 (1H, m) respectively. A wide diagonal contour for the two proton envelope at ~δ 4.37~4.38 suggests that these two methine protons – the δ 4.38 correlating to a carbon at δ 84.3 – are coupled to one another. The

(Continued)

Phenelfamycin D	Phenelfamycin E	Phenelfamycin F	Unphenelfamycin
4.82 (1H, br d, J=2.9)	4.69 (1H, d, J=2.7)	4.79 (1H, br s)	4.84 (1H, br d, J=2.7)
Unassigned	1.75 (1H, obscured)	1.78 (1H, ddd, J=12.4, 12.1, 3.2)	1.90 (1H, m)
Unassigned	1.58 (1H, dd, J=12.2, 4.3)	1.60 (1H, br dd, J=12.4, 4.4)	1.69 (1H, m)
3.54 (1H, obscured)	3.43 (1H, m)	3.42 (1H, ddd, J=12.1, 4.4, 2.7)	3.54 (1H, ddd, J=12.4, 4.9, 2.8)
3.39 (3H, s)*	3.33 (3H, s)	3.34 (3H, s)	3.31 (3H, s)
3.81 (1H, obscured)	3.80 (1H, obscured)	3.91 (1H, br d, J=2.7)	3.74 (1H, br s)
3.62 (1H, obscured)	3.54 (1H, q, J=6.9)	3.69 (1H, br q, J=6.6)	3.77 (1H, br q, J=7.4)
1.16 (3H, d, J=6.5)	1.15 (3H, d, J=6.9)	1.16 (3H, d, J=6.6)	1.19 (3H, d, J=7.4)
4.91 (1H, t, J=1.0)	4.80 (1H, br d, J=9.0)	4.78 (1H, dd, J=9.6, 1.6)	
Unassigned	2.31 (1H, br d, J=12.3)	2.30 (1H, ddd, J=13.8, 3.4, 1.6)	
Unassigned	1.54 (1H, ddd, J=12.3, 9.0, 1.8)	1.53 (1H, ddd, J=13.8, 9.6, 2.2)	
Unassigned	3.68 (1H, obscured)	3.72 (1H, m)	
3.40 (3H, s)	3.41 (3H, s)	3.41 (3H, s)	
3.78 (1H, obscured)	3.20 (1H, dd, J=9.6, 2.6)	3.20 (1H, dd, J=9.5, 2.8)	
4.27 (1H, br q, J=6.5)	3.78 (1H, dq, J=9.6, 6.5)	3.84 (1H, dq, J=9.5, 6.3)	
1.17 (3H, d, J=6.5)	1.21 (1H, q, J=6.5)	1.19 (3H, d, J=6.3)	
	4.95 (1H, d, J=1.0)	4.94 (1H, br s)	
	1.80 (1H, m)	1.79 (2H, m)	
	1.78 (1H, m)		
	3.52 (1H, obscured)	3.52 (1H, ddd, J=11.9, 5.0, 2.9)	
	3.31 (3H, s)	3.31 (3H, s)	
	3.89 (1H, br d, J=1.3)	3.75 (1H, br s)	
	4.01 (1H, br q, J=6.6)	4.02 (1H, br q, J=6.7)	
	1.25 (3H, d, J=6.6)	1.23 (3H, d, J=6.7)	



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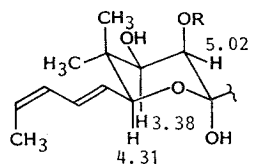

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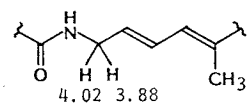

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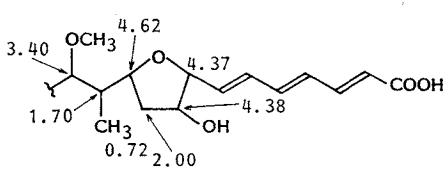

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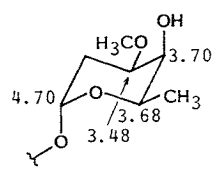


9

downfield proton of the two, δ 4.38, further couples into a methylene with proton and carbon signals at δ 2.00 (2H, m) and δ 40.2 (CH_2) respectively, this methylene is also coupled to a methine at δ 4.62, suggesting a 3-OH tetrahydrofuran moiety. Tracing the coupling pattern from the tetrahydrofuran, the δ 4.62 proton couples to a methine at δ 1.70 (1H, m), which is coupled to a methyl (δ 0.72 (3H, d, J=6.9 Hz)) and to another methine (δ 3.40 (1H, d, J=9.7 Hz)). This leads to definition of the fragment **8**.

Decoupling and COSY experiments also reveal a 2,6-dideoxy sugar moiety (**9**) present in phenelfamycin A. An anomeric proton at δ 4.70 (1H, br s) couples into a methylene signal at δ 1.80 (2H, m) by only two small coupling constants indicating that it must be in an equatorial orientation. The methylene protons then couple into a methine by two distinct coupling constants of J=11.8 and 4.9 Hz. The large coupling constant (11.8 Hz) between one methylene proton and the signal at δ 3.48 (3′-H) indicates that the 3′-H is axial. This axial 3′-H further couples into a signal at $\sim\delta$ 3.70 (4′-H) which is obscured by other protons in the region and hence whose multiplicity cannot be determined. However the small 3′,4′ coupling, as observed in the 3′-H splitting pattern, places the 4′-H in an equatorial orientation. A methyl (C-6′) whose protons appear at δ 1.17 (3H, d, J=6.6 Hz) is coupled to a methine (C-5′) at δ 3.68 (1H, q, J=6.61 Hz). This δ 3.68 methine experiences a strong NOE to the 3′ axial proton at δ 3.48. These observations define the relative stereochemistry of the sugar as shown in **9**. A second methoxy group in phenelfamycin A, as yet unplaced, can be assigned on C-3′ on the basis of chemical shift. Both 3′ and 4′ are oxygenated carbons with ^{13}C NMR chemical shifts of δ 75.8 and 67.5, respectively. The upfield δ 67.5 tends to imply an OH group here as 75.8 would imply an ether linkage.

The only signals in the ^1H NMR spectrum of phenelfamycin A not described above are 3 intercoupled signals at δ 2.97 (1H, dd, J=10.7 and 4.3 Hz), 3.79 (1H, dd, J=10.7 and 9.5 Hz) and 3.40 (1H, dd, J=9.5 and 4.3 Hz). The δ 3.79 and 3.40 signals correlate to a carbon at δ 64.0 (CH_2) and the δ 2.97 to a carbon at δ 49.6 (CH). Absent from the ^1H NMR spectrum of phenelfamycin A are signals which could be ascribed to an ethyl moiety present and attached at C-20 in previously described elfamycin antibiotics. It appears that phenelfamycin A contains instead an oxygenated methylene at this site.

Fragments **6** to **8** and this substituted C-20 moiety can be connected to describe an elfamycin-type antibiotic structure. It remains to determine the point of attachment of the sugar **9**. We originally assigned the site of sugar attachment based upon analogy to efrotomycin as being at C-23. Structure **10** was thus presented at the 1987 Intersci. Conf. on Antimicrob. Agents Chemother.[11]. Data presented at the 1988 International Conference on the Biotechnology of Microbial Products by Lederle authors[12], however, led us to re-investigate this assignment. These authors presented structures identical to our phenelfamycins E and F (described later in this paper) except for the site of trisaccharide attachment. ^{13}C NMR spectra run in acetone - H_2O and acetone - D_2O were therefore compared. In this experiment, carbons bearing an OH should differ somewhat in chemical shift between the two sets of conditions as an OD will exert different effects upon chemical shift of neighboring carbons than will an OH. In this case, the atoms C-23 and C-33 whose carbon signals appear at δ 72.0 and 64.0 respectively were examined. The δ 64.0 signal appeared at the same chemical shift in each solvent system whereas the δ 72.0 in acetone - H_2O appeared at δ 71.9 in acetone - D_2O. This shift was easily observed when spectral addition techniques were employed. Thus C-23 must bear

an OH group, leaving by default, the site of attachment of **9** at C-33 as in **4**. A mass spectrum run in the fast atom bombardment positive ion mode gave a parent peak at *m/z* 938, consistent with this structure whose molecule formula is $C_{51}H_{71}NO_{15}$.

Structure Determination of Phenelfamycin B

Phenelfamycin B (**11**) was isomeric with phenelfamycin A as evidenced by their mass spectra, each indicating a protonated molecular ion of *m/z* 938. The ^{13}C and ^{1}H NMR spectra of phenelfamycins A and B were quite similar (see Tables 1 and 2) with significant differences only in the region of carbon atoms 20~24 and groups attached to that portion of the backbone. In particular, carbons 22 and 23 differ most in shift values (2.6 and 4.5 ppm, respectively) between phenelfamycins A and B, and the axial methyl (C-34) at C-24 shows a smaller difference of 1.5 ppm. The ^{1}H NMR spectra of phenelfamycins A and B differ from one another at C-20 by 0.27 ppm and by 0.22 ppm at the attached C-33. Further, the proton chemical shifts of the methyls attached to C-24 differ by 0.06 and 0.22 for the axial and equatorial, respectively. Chemical shifts of the protons of sugar **9** differ at the anomeric and C-4′ by 0.12 and 0.23 ppm, respectively. In addition, differences occur in the signals assigned to the phenacetate methylene protons. These protons resonate as a 2H singlet at δ 3.71 in the spectrum of phenelfamycin A, but in that of phenelfamycin B they are resolved with ^{1}H NMR signals of δ 3.76 (1H, d, J=15.2 Hz) and 3.69 (1H, d, J=15.2 Hz). A CAMEL (NOE in rotating frame)[13] spectrum of phenelfamycin B demonstrates another significant variance from phenelfamycin A. 25-H appearing in the ^{1}H NMR spectrum of phenelfamycin A at δ 4.31 exhibited a strong NOE to its 1,3 diaxial partner at δ 3.83 (23-H). The corresponding 25-H at δ 4.32 in phenelfamycin B exhibits an NOE to an ester proton at δ 4.93. These data can be accounted for by a change in position of phenacetyl esterification from the hydroxyl at C-22 in phenelfamycin A to the hydroxyl at C-23 in phenelfamycin B (**11**).

Structure Determination of Phenelfamycins C and D

The mass spectrum of phenelfamycin C (**12**) indicated a molecular weight of 1,081. ^{1}H and ^{13}C NMR spectra and correlations (tabulated in Tables 2 and 3) indicate that phenelfamycin C contains all the structural entities found in phenelfamycin A. The site of attachment for the phenacetyl moiety is at C-22 as in phenelfamycin A as evidenced by an NOE between 25-H at δ 4.29 and an 23-H whose chemical shift of δ 3.80 indicates the lack of an ester linkage. Also of note is the fact that the phenacetyl methylene protons in phenelfamycin C appear as a singlet at δ 3.70 (3H, s) as in phenelfamycin A

Table 3. Selected ^{1}H NMR data for phenelfamycins A, B, C, D, E and F.

Protons on carbon No.	Phenelfamycin A	Phenelfamycin B	Phenelfamycin C	Phenelfamycin D	Phenelfamycin E	Phenelfamycin F
Phenacetyl methylene	3.71 (2H, s)	3.76 (1H, d, J=15.2) 3.69 (1H, d, J=15.2)	3.70 (2H, s)	3.73 (1H, d, J=15.2) 3.67 (1H, d, J=15.2)	3.70 (2H, s)	3.76 (1H, d, J=15.2) 3.68 (1H, d, J=15.2)
20	2.97	3.24	2.96	3.29	2.98	3.25
33	3.79	3.85	3.80	3.81	3.80	3.87
	3.40	3.62	3.38	3.61	3.41	3.60
34	0.94	1.00	0.90	1.01	0.95	1.02
35	0.89	0.67	0.86	0.70	0.89	0.68

Coupling constant: J in Hz.

(δ 3.71 (2H, s)) rather than differentiated as in phenelfamycin B (δ 3.76 (1H, d, J=15.2 Hz) and 3.69 (1H, d, J=15.2 Hz)). In addition, phenelfamycin C contained extra ^{13}C NMR signals at δ 100.0 (CH), 30.2 (CH_2), 75.8 (CH), 56.4 (CH_3), 67.8 (CH), 66.9 (CH) and 17.6 (CH_3) correlating to protons at δ 4.90 (1H, t, J=1.0 Hz), 1.86 (2H, m), 3.59 (1H, m), 3.34 (3H, s), 3.70 (1H, m), 4.22 (1H, br q, J=6.5 Hz) and 1.16 (3H, d, J=6.5 Hz) respectively. These data define a second sugar moiety in phenelfamycin C identical to the first. The site of attachment of the second sugar of phenelfamycin C is set at the 4′-OH of the directly attached sugar. This is evidenced by a difference in chemical shift from the C-4′ of phenelfamycin A at δ 67.5 to the same carbon in phenelfamycin C at δ 74.6 indicative of a difference between alcohol and ether carbons. NOE experiments also support the disaccharide structure of phenelfamycin C. The 1″ anomeric proton at δ 4.90 shows an NOE effect to both the 6′-H (δ 1.13) and 4′-H (δ 3.80) of the inner sugar. The 1′ anomeric, in contrast exhibits an NOE effect to protons of the aglycone at δ 2.96 (20-H) and δ 3.38 (33-H). This structure with a molecular formula of $C_{58}H_{83}NO_{18}$ is consistent with a protonated molecular ion of m/z 1,083†.

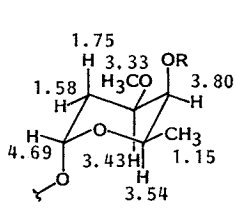
Phenelfamycin D (**13**) was isomeric with phenelfamycin C with a protonated molecular ion at m/z 1,083† consistent with a molecular formula of $C_{58}H_{83}NO_{18}$. Only a small quantity of this material was available and hence ^{13}C NMR data could not be acquired. ^1H NMR and COSY spectra suggested assignment (see Table 2) of a disaccharide structure, bearing the same relationship to phenelfamycin C as phenelfamycin B does to phenelfamycin A. A comparison of ^1H NMR data (see Table 3) between phenelfamycins A, B, C and D demonstrates this analogy. The same differences between phenelfamycins D and C can be noted as between phenelfamycins B and A in their ^1H NMR spectra, in particular: 1) Methylene protons on C-22 appear as a 2H singlet in both phenelfamycins A and C, and as an AB quartet in phenelfamycins B and D, 2) similar chemical shift changes are observed for the methylene protons on C-33 and the methine proton of C-20 between phenelfamycins C and D as between the analogous protons of phenelfamycins A and B and 3) singlet methyl signals for C-34 and C-35 exhibit similar differences between the spectra of phenelfamycins D and C or between those of phenelfamycins B and A. These data lead to the assignment of structure **13** to phenelfamycin D.

Structure Determination of Phenelfamycins E and F

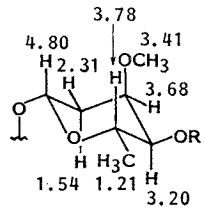
Phenelfamycin E (**14**) had a MW of 1,226 as determined by positive ion mode fast atom bombardment mass spectrometry (FAB-MS). ^{13}C and ^1H NMR data as compiled in Tables 1 and 2 define an aglycone portion for phenelfamycin E identical to that of phenelfamycins A and C. The site of attachment of the phenacetyl moiety is set at C-22 as in phenelfamycins A and C based upon an NOE between 25-H at δ 4.33 and an 23-H whose chemical shift of δ 3.81 indicates that this proton is not on a carbon bearing an acyloxy function. The phenacetyl methylene protons in phenelfamycin E appear as a singlet in the ^1H NMR spectrum (Table 3).

Remaining signals in the ^1H NMR spectrum of phenelfamycin E could be assigned to three sugar moieties **15a**, **15b** and **15c**. Two of these sugars, **15a** and **15c**, were identical to one another and to the sugars present in phenelfamycins A~D. (^1H NMR chemical shift assignments are recorded on the structure and multiplicities can be found within Table 2). A third sugar (**15b**), however, appeared

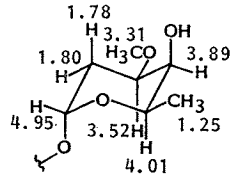
† Theoretical values for the molecular ion isotope cluster for $C_{58}H_{84}NO_{18}^+$ are 1,082.56 (49%), 1,083.57 (32%), 1,084.57 (12%), 1,085.57 (3.3%) and the average molecular mass for the parent molecule is 1,082.30. This and all other compounds reported here gave protonated molecular ions cluster at m/z values, and in relative intensities, correct for the formulae proposed.



15a



15b



15c

to be isomeric but not identical to **15a** and **15c**. This sugar was defined by proton signals at δ 4.80 (1H, br d, J=9.0 Hz, 1-H), 2.31 (1H, br d, J=12.3 Hz) and 1.54 (1H, ddd, J=12.3, 9.0 and 1.8 Hz, 2-H_2), *ca.* 3.68 (1H, 3-H), 3.20 (1H, dd, J=9.6 and 2.6 Hz, 4-H), 3.78 (1H, dq, J=9.6 and 6.5 Hz, 5-H), 1.21 (3H, d, J=6.5 Hz, 6-H). The large coupling constant of the anomeric proton requires that it be axial. Two large coupling constants for the δ 1.54 proton at carbon 2 suggests that it is an axial proton with one large (J=12.3 Hz) geminal coupling and a second axial-axial (9.0 Hz) coupling. The remaining small coupling constant (J=1.8 Hz) requires that the proton of carbon 3 be equatorial. Protons on carbons 4 and 5 are coupled to one another by a coupling constant of J=9.6 Hz indicating that each must be in an axial position. These data thus define a sugar of structure **15b**.

The order in which these sugars are attached to the aglycone was determined by ^{13}C NMR and heteronuclear multiple bond correlations with particular reference to the connectivities around C-4 of the three sugars. These carbons have ^{13}C NMR shifts of δ 82.7, 74.2 and 67.8 the last of which would be expected to be an alcohol bearing carbon and hence belongs to the terminal sugar. The sugar whose C-4 resonates at δ 67.8 has an anomeric proton at δ 4.95. A heteronuclear multiple bond correlation map reveals that this δ 4.95 proton is coupled to the C-4 at δ 82.7. This carbon is found in the sugar of structure **15b** and hence must be the centrally attached of the 3 sugars. Similarly, this central sugar has an anomeric proton at δ 4.80 and this shows a heteronuclear multiple bond correlation to the C-4 at δ 74.2 which appears in the third and therefore inner most sugar.

Phenelfamycin F (**16**) has a MW 1,226 consistent with a molecular formula of $C_{65}H_{95}NO_{21}$, isomeric to phenelfamycin E. That the aglycone portion of phenelfamycin F is equivalent to that of phenelfamycins A and C was determined by ^{13}C and ^{1}H NMR homo and heteronuclear experiments, the results of which are tabulated in Tables 1 and 2. The site of attachment for the phenacetyl group is determined to be C-23 based upon an NOE between a 25-H signal at δ 4.33 and a 23-H signal at δ 4.96 whose chemical shift indicates that C-23 is an ester bearing carbon. As illustrated in Table 3, ^{1}H NMR analogies for protons on carbon atoms 20, 33, 34, 35 and phenacetyl methylene hold between phenelfamycins E and F as between pairs phenelfamycins A, B and C, D. The trisaccharide portion of phenelfamycin F is identical to that of phenelfamycin E as determined by ^{1}H NMR decoupling and CAMEL techniques. ^{13}C and ^{1}H NMR signals for these sugars are tabulated in Tables 1 and 2.

Structure Determination of Unphenelfamycin

Unphenelfamycin (**17**) has a molecular weight of 819 as determined by positive ion FAB-MS of the sodium salt (m/z 842). This is consistent with a molecular formula of $C_{43}H_{65}NO_{14}$ for unphenelfamycin. Immediately obvious from the ^{1}H NMR spectrum of unphenelfamycin was the absence of the 5 proton signal of a phenacetyl moiety observed in the spectra of phenelfamycins A~F at ~δ 7.3. In addition, the unphenelfamycin ^{1}H NMR spectrum lacked an ester methine proton observed at

$\sim\delta$ 5.0 in the phenelfamycins spectrum and this was replaced by a proton signal at δ 3.59 indicative of a change from an ester to alcohol methine. The ^{13}C and ^{1}H NMR data (see Tables 1 and 2) for unphenelfamycin mapped very well, with the exception of the phenacetate ester noted, to that of phenelfamycin A and decoupling of the former led to identical structural assignments.

This spectroscopic analysis defines the phenelfamycins as a novel family of the elfamycin class of antibiotics. The family differs structurally from known members of this class by several interesting features. The lack of a terminal 3-hydroxypyridone moiety has only one previous precedent, namely L-681,217[10]. At C-20 all previously known elfamycins carry an ethyl group which, in the case of aurodox is butyrate derived[14]. Phenelfamycins have a substituted hydroxymethyl substituent at this position which is interesting biogenetically. The phenylacetate moiety is uncommon and without precedence in this class of antibiotics. All of the phenelfamycins are glycosides, whereas, the disaccharide, efrotomycin, is the only other known glycosylated elfamycin. The arrangement of sugars in the trisaccharide is particularly interesting in that the trisaccharides differ from the disaccharides by having a different sugar as the central moiety of the trisaccharide chain. This also raises interesting questions of biosynthesis.

Experimental

General Procedures

Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in 10 cm tubes. MP's were determined on a Hoover Unimelt and are reported uncorrected. IR spectra were recorded on a Perkin-Elmer 683 dual beam dispersive instrument and UV spectra on a Perkin-Elmer Lambda 3B UV-visible spectrophotometer. Mass spectra were measured on a Kratos MS-50 spectrometer. NMR spectra were acquired on either a General Electric GN300 or GN500 spectrometer with 5 mm probes. ^{13}C and ^{1}H NMR spectral data for the phenelfamycins are compiled in Tables 1 and 2.

Phenelfamycins elute from C_{18} bonded-phase silica gel in the order unphenelfamycin, phenelfamycins A, C, E, B, D, F in any aqueous MeOH or acetonitrile gradient.

Phenelfamycin A, $[\alpha]_D^{25}$ $-8.6°$ (*c* 0.1, MeOH) is a pale tan powder with decomposition point $\sim$140°C. A MW of 959 was established for the sodium salt of phenelfamycin A by positive ion FAB-MS. An IR spectrum measured in $CDCl_3$ contained bands at 3443, 3370, 3320, 2978, 2938, 1730, 1683, 1655, 1622, 1533, 1446, 1411, 1384, 1365, 1343, 1295, 1257, 1217, 1188, 1145, 1105, 1022, 1005, 985, 974 cm^{-1}.

Phenelfamycin B, $[\alpha]_D^{25}$ $-10.0°$ (*c* 1.12, MeOH) is a pale tan powder with a decomposition point of $\sim$118°C. A MW of 959 was established for the sodium salt of phenelfamycin B by positive ion FAB-MS. An IR spectrum measured in $CDCl_3$ contained bands at 3445, 3420, 2892, 2868, 1735, 1715, 1692, 1655, 1620, 1528, 1498, 1455, 1448, 1368, 1365, 1300, 1258, 1188, 1108, 1090, 1008 cm^{-1}.

Phenelfamycin C, $[\alpha]_D^{25}$ $-7.7°$ (*c* 0.51, MeOH) is a pale tan powder which decomposes at $\sim$88°C. A MW of 1,081 was established for phenelfamycin C by positive ion FAB-MS. An IR spectrum measured in $CDCl_3$ contained bands at 3445, 3420, 2892, 2868, 1735, 1715, 1692, 1655, 1620, 1528, 1448, 1368, 1365, 1258, 1222, 1188, 1108, 1090, 1008, 990, 987 cm^{-1}.

Phenelfamycin D, $[\alpha]_D^{25}$ $-37°$ (*c* 0.75, MeOH) is a light tan oil. A MW of 1,081 was established for phenelfamycin D by positive ion FAB-MS. An IR spectrum measured in $CDCl_3$ contained bands at 3445, 2985, 2925, 1725, 1685, 1650, 1615, 1525, 1455, 1378, 1302, 1290, 1256, 1182, 1104, 1024, 1002 cm^{-1}.

Phenelfamycin E, $[\alpha]_D^{25}$ $-16.5°$ (*c* 0.20, MeOH) is a pale tan powder with a decomposition point of $\sim$138°C. A MW of 1,225 was established for phenelfamycin E by positive ion FAB-MS. An IR spectrum of phenelfamycin E measured in $CDCl_3$ contained bands at 3342, 2984, 2935, 1728, 1682, 1648, 1616, 1524, 1448, 1444, 1376, 1364, 1252, 1216, 1191, 1095, 1087 cm^{-1}.

Phenelfamycin F, $[\alpha]_D^{25}$ $-17°$ (*c* 1.10, MeOH) is a pale tan powder with a decomposition point of

~121°C. A MW of 1,225 was established for phenelfamycin F by positive ion FAB-MS. An IR spectrum measured in $CDCl_3$ contained bands at 3440, 2988, 2936, 1718, 1685, 1653, 1618, 1446, 1367, 1260, 1217, 1192, 1168, 1147, 1099, 1022, 1007, 972 cm^{-1}.

Unphenelfamycin, $[\alpha]_D^{25}$ −17° (*c* 0.56, MeOH) is a light tan oil. A MW of 841 was established for the sodium salt of unphenelfamycin by positive ion FAB-MS. An IR spectrum measured in $CDCl_3$ contained bands at 3435, 2975, 2936, 1701, 1646, 1618, 1605, 1385, 1295, 1276, 1250, 1236, 1187, 1147, 1106, 1022, 1007, 985, 974 cm^{-1}.

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