A83016F, A NEW MEMBER OF THE AURODOX FAMILY†

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A new member of the aurodox family of antibiotics, A83016F, has been isolated from an unidentified actionmycete designated A83016. The structure and relative stereochemistry of A83016F were elucidated by NMR examination of the parent compound and its diacetate derivative. A83016F exhibits only weak antimicrobial activity.

As part of a screening program for antibiotics, a new member of the aurodox[1] family, A83016F, has been isolated from the culture broth of an unidentified actinomycete designated A83016. Several members of the aurodox family, such as kirromycin (mocimycin)[2], efrotomycin[3], heneicomycin[4], and kirrothricin[5], have been previously reported, and are known for antibacterial activity and growth-promotant activity in chicks[6]. As in the related compounds L-681,217[7] and the phenelfamycins[8], A83016F lacks the pyridone moiety common in most members of this class. The strain A83016 also produces several kinamycin-type antibiotics including the novel compound A83016A[9]. This paper describes the isolation, structural elucidation, and antimicrobial activity of A83016F.

Strain A83016 Fermentation

Fermenter Inoculum

Stock cultures of the producing organism were preserved in glycerol-lactose (10%:5%) solutions stored in the vapor phase of liquid nitrogen. Fermenter inoculum was prepared by transferring the culture into wide-mouth 250 ml flasks containing 50 ml of a medium composed of glucose 1%, soluble starch 2%, yeast extract 0.5%, NZ-Amine A (Sheffield Products) 0.5% and $CaCO_3$ 0.1% in deionized water (pH 7.0 prior to sterilization). These flasks were incubated for 48 hours at 30°C on a shaker rotating in a 5.07 cm circle at 250 rpm. The resulting mycelial suspension was transferred to wide-mouth 2-liter flasks containing 400 ml of the same medium and incubated an additional 30 hours, then used to inoculate stirred reactors (0.8%).

Fermenters

Fermentations were conducted for three days at 30°C in fully baffled reactors of conventional design with two 6-bladed turbine impellors, a total capacity of 165 liters and a height-diameter ratio of approximately 1:1 for the post-inoculation medium volume of 115 liters. The fermentation medium (composed of glucose 2.5%, yeast extract 0.5%, NZ-Amine A 0.3%, blackstrap molasses 1.5%, $MgSO_4$ 0.1%, and $CaCO_3$ 0.2% in tap water) was sterilized through the application of 22~25 heating units by the F_0 method[10]. Dissolved oxygen was maintained at 45% of air saturation with 0.34 atmospheres of

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internal vessel pressure. The major portion of the A83016F produced was elaborated into the aqueous fermentation broth but additional amounts could be obtained by methanolic extraction of the biomass. Antibiotic production was monitored by a conventional disc-plate agar diffusion technique employing *Bacillus subtilis* and/or *Escherichia coli* as the test organism.

Isolation

A 83016 whole broth (212 liters) was extracted with an equal volume of ethyl acetate, forming a heavy emulsion. The emulsion was passed through a continuous-flow type centrifuge, and the resulting solids extracted with 25 liters of methanol. The methanolic extract was concentrated *in vacuo* to 2 liters of aqueous solution, which was extracted twice with one liter of chloroform. The combined chloroform extracts were concentrated *in vacuo* to 200 ml, and 2.5 liters of hexane were added. The resulting precipitate was recovered by filtration and dried *in vacuo* to yield 4.4 g of tan powder. A portion (450 mg) of this powder was then chromatographed over ~450 ml of Sephadex LH-20 in methanol. Fractions which were active vs. *E. coli* were combined and evaporated. The residue (91 mg) was chromatographed over a 2.5 cm i.d. × 30 cm column containing Chromegabond C18 (ES Industries) using methanol - 0.2% aqueous acetic acid buffered to pH 4.75 with NaOH (67 : 33) as the solvent. Fractions containing pure A83016F were combined, concentrated *in vacuo* to aqueous, and extracted with ethyl acetate. The extracts were evaporated *in vacuo* to yield 45 mg of A83016F (**1**).

Preparation of A83016F Diacetate

A83016F (80 mg) was dissolved in 6 ml of pyridine and 3 ml of acetic anhydride, and stirred at room temperature for 18 hours. Methanol (6 ml) was added, and the solution was evaporated *in vacuo*. The residue (121 mg) was chromatographed on three preparative TLC plates (EM Silica gel 60, 0.5 mm thickness) using toluene - EtOH (4 : 1). The major UV band was scraped off and eluted with 9 : 1 methylene chloride - methanol. The eluate was evaporated, and the residue (55 mg) was chromatographed over a 2.5 cm i.d. × 30 cm column containing Chromegabond C18 (ES Industries) using MeOH - 0.2% aqueous AcOH buffered to pH 4.75 with NaOH (74 : 26) as the mobile phase. Fractions containing pure A83016F diacetate were combined, concentrated *in vacuo* to aqueous, and extracted with ethyl acetate. The extracts were evaporated *in vacuo* to yield 10 mg of A83016F diacetate (**2**).

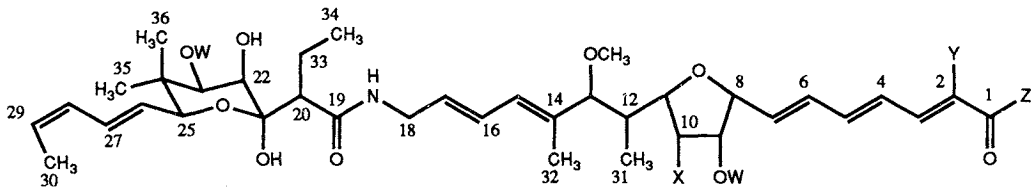
Structure

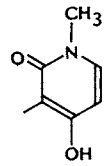
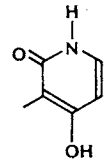
Aurodox (**3**) and mocimycin (=kirromycin) (**4**) were the first members of the elfamycin group of antibiotics, and their complete structures (including absolute stereochemistry) have been determined by chemical degradation and crystallographic studies[1,11]. Complete ^{1}H and ^{13}C NMR assignments have been reported for kirromycin[12] and for related compounds such as UK-69,753[13] and the phenelfamycins (including unphenelfamycin (**5**))[8]. Similar spectroscopic studies described in this paper have led to the structure elucidation of **1** and have allowed the evaluation of the relative stereochemistry of **1** and **2**.

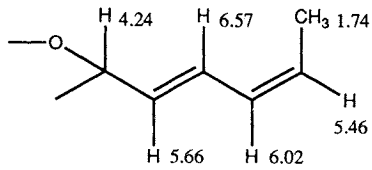
Elucidation of the structures of **1** and **2** was based on a variety of NMR spectral methods, including COSY, NOESY, DEPT, ^{1}H-^{13}C COSY, and selected proton-proton decoupling experiments; spectra were recorded in acetone-d_6 at 27°C on a Bruker AM500 spectrometer.

Compound 1

The molecular formula was found to be $C_{37}H_{55}NO_{10}$ by fast atom bombardment mass spectrometry



	W	X	Y	Z
A83016F (**1**)	H	H	H	OH
A83016F diacetate (**2**)	Ac	H	H	OH
Aurodox (**3**)	H	OH	CH_3	
Kirromycin (**4**)	H	OH	CH_3	
Unphenelfamyin (33~34 replaced by $-CH_2O-(C_7H_{13}O_3)$) (**5**)	H	H	H	OH



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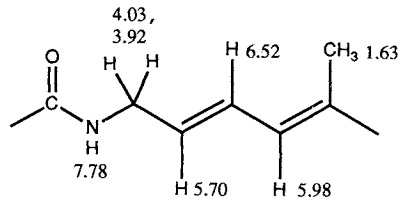

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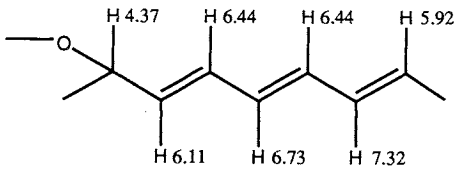

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Table 1. Physico-chemical properties for **1**.

Molecular formula	$C_{37}H_{55}NO_{10}$
MS (*m/z*) Found:	696.3746 $(M+Na)^+$
Calcd:	696.3724
UV λ_{max}^{MeOH} nm (ε)	234 (36,400), 298 (21,300)
IR ν_{max} (KBr) cm^{-1}	3425, 2970, 1697, 1260, 1095
$[\alpha]_D^{25}$	+7.95° (*c* 0.01, MeOH)

(see Table 1). Comparison of the DEPT and ^1H-^{13}C correlation spectra indicated the presence of 7 CH_3's, 3 CH_2's, and 22 CH's ($C_{32}H_{49}$); the remaining six protons are exchangeable. Examination of the COSY spectrum and the ^1H-^{13}C correlation results for **1** allowed the carbon-bound protons to be sorted into four major substructures and several smaller fragments, as follows:

1) Two diene-containing systems, **6** and **7**.
2) A triene-containing substructure, **8**.

The proton at 6.11 ppm is coupled into a two-proton resonance at 4.37 ppm; examination of the ^{1}H-^{13}C correlation map shows that there are two separate CH–O groups with protons at 4.37 and carbon resonances at 75.2 and 84.3 ppm.

3) A six-carbon aliphatic fragment, **9**.

The COSY spectrum provides no evidence to decide whether the 4.37 ppm resonance in **9** is the same as the one in **8**, so that **8** and **9** would actually be a single 12-carbon fragment, or whether the two substructures are separate but connected by adjacent 4.37 ppm CH–O groups.

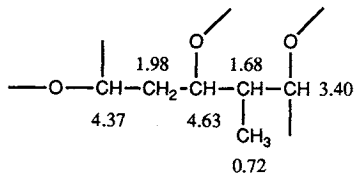
4) Smaller fragments and methyls.

The two isolated protons at 3.68 and 3.61 ppm are attached to separate carbons at 71.2 and 73.2 ppm, respectively, leading to substructure **10**; the coupling constant between the two protons is 3.6 Hz. The final fragment derived from the COSY map is the three-carbon piece **11**; examination of the ^{1}H-^{13}C correlation spectrum leads to three additional methyl groups which show no correlations in the COSY results: an $-OCH_3$ (3.13/56.1 ppm) and two singlet CH_3's (0.91/24.5 ppm and 0.91/15.9 ppm).

The carbon spectrum of **1** contains five quaternary resonances: two carbonyls (176.8 and 167.9), one olefinic (136.7), one anomeric (100.4), and one aliphatic (39.5). The olefinic carbon and one of the carbonyls have been accounted for in substructure **7**. All of the carbon and proton resonances for **1** have been collected in Table 2, and the proton resonances are compared there with the corresponding values for the related compound unphenelfamycin, **5**. The two proton listings correspond very closely with the exception of resonances in fragment **11**; the ethyl group in **11** (positions 33 and 34) is replaced by $-CH_2O-(C_7H_{13}O_3)$ in unphenelfamycin[8]. This comparison indicates that A83016F has the structure **1**. The carbonyl resonance at 167.9 ppm is assigned to position 1 by analogy to the phenelfamycins, where a heteronuclear multiple bond correlation experiment for phenelfamycin A indicated a long-range connectivity between a carbonyl resonance at 167.7 ppm and an olefinic proton at 5.92 ppm (see substructure **8**)[8]. The six exchangeable protons are the amide NH, the acidic COOH, and four hydroxyl protons (positions 9, 21, 22, and 23).

The $-OCH_3$ is placed at position 13 by analogy to all other elfamycins, but this placement is confirmed by NOESY correlations from the $-OCH_3$ (3.13 ppm) to the 13-H proton (3.40) and to the methyl singlet (1.63 ppm) attached to C-14. Other correlations involving the protons at positions 15, 16, and 17 confirm the *trans* double bond geometry in the central diene fragment; these correlations are listed in Table 2.

The double bond geometries shown in fragments **6** and **8** are also confirmed by NOESY results listed in Table 2; correlations in parentheses appear to connect remote segments of the molecule and may indicate molecular folding or intermolecular aggregation. Experiments to investigate these



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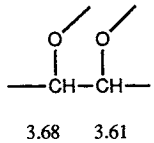

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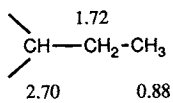

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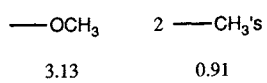


Table 2. Proton and carbon NMR data for **1** in acetone-d_6.

Position	Type	δ_C (ppm)	δ_H (ppm)	J_{H-H} (Hz)	NOESY correlations[a]	δ_H, **5**[b]
1	COOH	167.9	—			—
2	CH	121.7	5.92 d	15	6.40, (5.70)	5.91
3	CH	145.6	7.32 dd	11, 15	6.73	7.29
4	CH	131.6[c]	6.44 dd	11, 15	5.92	6.43
5	CH	141.3	6.73 dd	11, 15	7.32, (6.57), 6.11	6.71
6	CH	130.3[c]	6.44 dd	11, 15		6.39
7	CH	137.5	6.11 dd	6, 15	6.73	6.10
8	CH	84.3	4.37			4.37
9	CH	75.2	4.37			4.37
10	CH_2	40.3	1.98			1.96
11	CH	78.2	4.63 td			4.62
12	CH	40.6	1.68			1.70
13	CH	90.3	3.40 d		5.98, 3.13, 0.72	3.38
13′	OCH_3	56.1	3.13		3.40, 1.63, (0.91)	3.10
14	C	136.7	—			—
15	CH	129.7	5.98 d	11	5.70, 3.40	5.99
16	CH	127.7	6.52 dd	11, 15	1.63	6.53
17	CH	130.9	5.70 td	7, 15	5.98, (5.92)	5.66
18	CH_2	41.6	4.03, 3.92			4.04, 3.89
19	CO(NH)	176.8	—			—
20	CH	51.7	2.79 dd			3.19
21	C	100.4	—			—
22	CH	71.2	3.68 d		1.72	3.59
23	CH	73.2	3.61 d		4.24, 0.91	3.59
24	C	39.5	—			—
25	CH	76.4	4.24 d		3.61, 0.91	4.23
26	CH	130.8	5.66 dd	6, 15	6.02, 0.91	5.70
27	CH	126.9	6.57 dd	11, 15	(6.73), 1.74	6.57
28	CH	130.4	6.02 t	11	5.66	5.99
29	CH	125.8	5.46 dq	5.5, 11		5.46
30	CH_3	13.6	1.74 d		6.57	1.74
31	CH_3	10.5	0.72 d		3.40, 1.63	0.73
32	CH_3	11.0	1.63		6.52, 3.13, 0.72	1.66
33	CH_2	20.9	1.72		3.68	[d]
34	CH_3	12.3	0.88 t			[d]
35	CH_3	24.5	0.91		4.24, 3.61, (3.13)	0.89
36	CH_3	15.9	0.91		5.66	0.92
	NH		7.78 t			

[a] Cross peaks representing protons on adjacent carbons are not listed.
[b] Corresponding unphenelfamycin proton resonances in acetone-d_6; ref 8.
[c] These assignments may be interchanged.
[d] Not comparable.

possibilities were not attempted.

25-H at 4.24 ppm (see **6**) shows a strong NOE to 23-H at 3.61 ppm (see **10**), indicating their 1,3 diaxial relationship, and both 23-H and 25-H have strong NOEs to one of the equivalent *gem*-dimethyls at 0.91 ppm. The six-proton resonance at 0.91 also has a strong NOE to the olefinic peak at 5.66 ppm (26-H). Since 23-H (3.61) is axial, the 3.6 Hz coupling constant with the resonance at 3.68 ppm requires 22-H to be equatorial. A NOESY correlation between 22-H and the methylene resonance at 1.72 ppm confirms the attachment of fragment **11** to the substituted sugar moiety.

The major remaining ambiguity in the structure of **1** is the relative stereochemistry in the vicinity of

the tetrahydrofuran ring. The degenerate chemical shifts for most of the ring protons in **1** prevents investigation of this question by either coupling constant values or by NOE observations. More useful results were obtained for **2**, however.

Compound **2**

The product of the acetylation reaction of **1** had a molecular weight of 780 ($(M+Na)^+$, FAB-MS);

Table 3. Proton and carbon NMR data for **2** in acetone-d_6.

Position	Type	δ_C (ppm)	Δ_C[a]	δ_H (ppm)	Δ_H[b]	NOESY correlations
1	COOH	167.7	−0.2	—	—	
2	CH	122.1	0.4	5.94	0.02	6.46
3	CH	145.4	−0.2	7.30	−0.02	6.72
4	CH	132.0[c]	0.4	6.46	0.02	5.94
5	CH	140.7	−0.6	6.72	−0.01	7.30, 5.95
6	CH	131.0[c]	0.7	6.46	0.02	4.59
7	CH	135.7	−1.8	5.95	−0.16	6.72
8	CH	82.2	−2.1	4.59	0.22	6.46, 5.39
9	CH	77.3	2.1	5.39	1.02	4.59, 2.17
10	CH_2	37.6	−2.7	2.17, 2.07	0.19, 0.09	5.39, 0.74, 4.55
11	CH	78.7	−0.5	4.55	−0.08	3.41, 2.07
12	CH	40.6	0.0	1.71	0.03	
13	CH	90.3	0.0	3.41	0.01	5.96, 4.55, 3.14
13′	OCH_3	56.1	0.0	3.14	0.01	3.41
14	C	136.5	−0.2	—	—	
15	CH	130.0	0.3	5.96	−0.02	5.72, 3.41
16	CH	127.8	0.0	6.52	0.0	1.63
17	CH	131.1	0.2	5.72	0.02	5.96
18	CH_2	41.5	−0.1	4.01, 3.93	−0.02, 0.01	
19	CO(NH)	176.8	0.0	—	—	
20	CH	51.3	−0.4	2.81	0.02	0.90
21	C	100.7	0.3	—	—	
22	CH	68.7	−2.5	3.85	0.17	4.96, 1.66
23	CH	76.6[d]	1.4	4.96	1.35	4.36, 3.85, 0.81
24	C	38.7	−0.8	—	—	
25	CH	76.5[d]	0.1	4.36	0.12	6.61, 4.96, 0.81
26	CH	130.2	−0.6	5.66	0.0	6.02, 1.04, 0.81
27	CH	127.6	0.7	6.61	0.04	4.36, 1.75
28	CH	130.3	−0.1	6.02	0.0	5.66, 5.49
29	CH	126.3	0.5	5.49	−0.03	6.02, 1.75
30	CH_3	13.6	0.0	1.75	0.01	6.61, 5.49
31	CH_3	10.7	0.2	0.74	0.02	2.17, 1.63
32	CH_3	11.0	0.0	1.63	0.0	6.52, 0.74
33	CH_2	20.9	0.0	1.66	−0.06	3.85
34	CH_3	12.2	−0.1	0.90	0.02	2.81
35	CH_3	24.2	−0.3	0.81	−0.1	5.66, 4.96, 4.36, 1.04
36	CH_3	16.9	1.0	1.04	0.13	5.66, 0.81
	NH	—		7.78	0.0	
	$COCH_3$	170.7, 170.5				
	$COCH_3$	20.9, 20.9		2.06, 1.99		

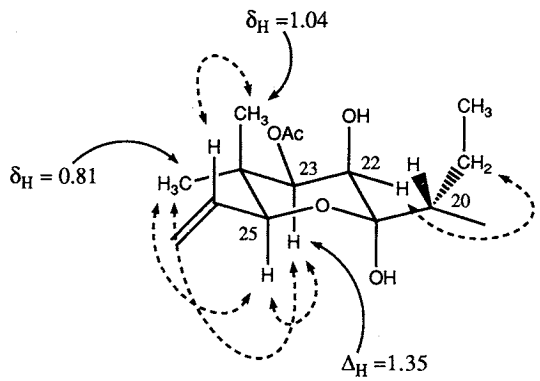
[a] $\Delta_H = \delta_C$(diacetate) − δ_C(A83016F).
[b] $\Delta_H = \delta_H$(diacetate) − δ_H(A83016F).
[c,d] These assignments may be reversed within each pair.

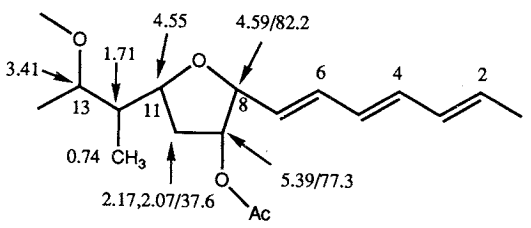
the gain of 84 units indicated the addition of acetate groups to two of the four available hydroxyls in the parent antibiotic. Proton and carbon NMR assignments for the diacetate were derived from COSY, NOESY, and ^{1}H-^{13}C correlation spectra and are listed in Table 3, where the shifts in resonance positions going from the parent compound to the diacetate are also listed: Δ_H and Δ_C represent the proton and carbon resonance changes, respectively. In the substituted sugar ring the pair of CH resonances corresponding to substructure **10** now occur at 3.85 and 4.96 ppm, and the 4.96 resonance shows an intense NOESY crosspeak to 25-H at 4.36 ppm, marking the 4.96 resonance as 23-H. The large downfield shift for 23-H in the diacetate ($\Delta_H = 1.35$ ppm) indicates that the 23-hydroxyl is the site of one of the acetate groups. The NOESY correlation between 22-H (3.85 ppm) and the methylene resonance at 1.66 ppm is consistent with the same configuration at C-20 as found for aurodox by X-ray crystallography[14]. Neither the spectra of A83016F nor those of the diacetate show evidence of the epimerization of the anomeric center as reported for the antibiotic SB22484[15]. The chemical shift of the anomeric carbon C-21 is essentially the same in **1** and **2** (100.4 and 100.7 ppm, respectively) as in aurodox and kirromycin (101.0 and 100.4 ppm, respectively)[1,12] indicating the same axial orientation at C-21 in both A83016F and aurodox[1].

A consequence of the esterification of the 23-OH is the nonequivalence of the *gem*-dimethyl proton resonances attached to carbon 24; the axial CH_3 shifts to 1.04 ppm and the equatorial CH_3 to 0.81. These changes are similar to results for the phenelfamycins, where factors which are glycosylated at the 23-OH have non-equivalent *gem*-dimethyl resonances differing by more than 0.3 ppm (phenelfamycins B, D, and E), while the methyl resonances differ by 0.06 ppm or less for factors which have a free 23-OH (phenelfamycins A, C, E, and unphenelfamycin)[8]. The two methyl peaks show the same NOEs in the diacetate as observed in the parent antibiotic.

The second acetate group is attached to the lone remaining hydroxyl site, the 9-OH. This modification disrupts the degeneracy of proton chemical shifts in the tetrahydrofuran ring of the parent antibiotic, allowing fragment **12** to be defined from the COSY spectrum.

9-H at 5.39 ppm shows a Δ_H of 1.02 ppm downfield from its position in **1**, leaving it well-separated from 8-H at 4.59 ppm. The ^{1}H-^{13}C correlation map for the diacetate shows clearly that C-8 has a chemical shift of 82.2 ppm and C-9 a shift of 77.3 ppm; these assignments suggest that in the parent antibiotic, where the proton resonances for positions 8 and 9 coincide, the carbon resonance for C-8 is 84.3 ppm and that for C-9 is 75.2 ppm. This set of assignments leads to $\Delta_C = 2.1$ ppm at the site of the acetate attachment (C-9) and $\Delta_C = -2.1$ and -2.7 ppm for the adjacent sites 8 and 10, respectively; similar trends around the acetate site





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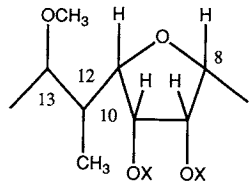


13 (X=H)	**14** (X=Ac)
$J_{12,13}=9.6$ Hz	$J_{12,13}=10.5$ Hz
$J_{11,12}=7.9$ Hz	$J_{11,12}=7.5$ Hz
$J_{10,11}=4.2$ Hz	$J_{10,11}=3.5$ Hz
$J_{9,10}=5.2$ Hz	$J_{9,10}=5.0$ Hz
$J_{8,9}=6.8$ Hz	$J_{8,9}=7.5$ Hz

at carbon 23 are listed in Table 3. This set of carbon assignments for positions 8 and 9 for the parent antibiotic, however, is reversed from results found for the analogous positions in unphenelfamycin[8].

The non-equivalence of the resonances for protons 8 and 9 and for the two resonances from position 10 in the diacetate make it possible to examine each site around the tetrahydrofuran ring individually by decoupling studies and NOE measurements.

Approximate coupling constants measured for partial structure **12**, based on multiplet patterns produced during selective decoupling, include the following: $J_{12,13}=9.7$ Hz, $J_{11,12}=3.4$ Hz, $J_{10A,11}=6.1$ Hz, $J_{10B,11}=10.4$ Hz, $J_{9,10A}=\sim 1.2$ Hz, $J_{9,10B}=4.9$ Hz, and $J_{8,9}=4.0$ Hz, where 10A-H is the proton at 2.07 ppm and 10B-H is at 2.17 ppm. Coupling constants may be obtained from the literature for mocimycin[12] (**13**), and for an acetylated derivative of mocimycin[16], as in **14**.

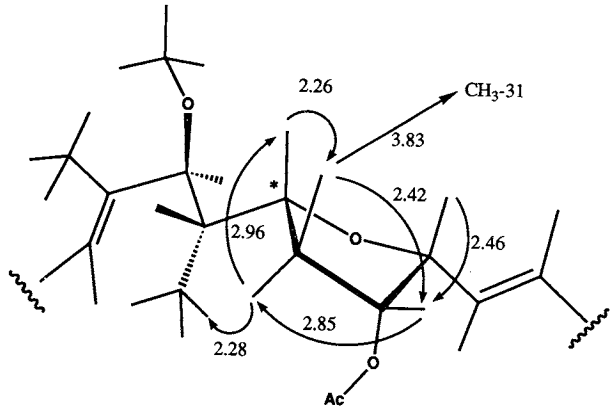
The all-*cis* configuration for the tetrahydrofuran moiety in aurodox (and kirromycin) was based initially on coupling constants for the tetrahydrofuran ring on which the two hydroxyls are tied together in an *O*-isopropylidene derivative[17]; in models of this type *J* values for vicinal *cis* hydrogens ranged from 3~6 Hz, while *trans* protons had *J* values of <1 Hz. Applying this relationship and comparing the coupling constants for **12**, **13**, and **14** suggests that in **2** 10A-H (2.07 ppm) is the hydrogen *trans* to proton 9, replacing 10-OX in **13** and **14**. This comparison is less helpful when relating the protons at site 10 to 11-H; $J_{10,11}(cis)$ is about 4 Hz for both **13** and **14**, but in **12** both values for $J_{10,11}$ are large (6.1 and 10.4 Hz). Another difference between **12** and **13**/**14** involves $J_{11,12}$; the coupling in **2** is much smaller (3.4 Hz) than in kirromycin and its acetylated derivative (7.9 and 7.5 Hz, respectively). The possibility that **1** might differ from aurodox and kirromycin by inversion of the configuration at site 11 was supported by difference NOE results and the NOESY correlations listed in Table 3, and by molecular modeling, as described below.

A strong NOE is observed between 8-H and 9-H in both the 1D and 2D experiments; irradiation of 10B-H produces difference NOE peaks at 9-H and 31-CH_3. Irradiation at 2.07 ppm (10A-H and one of the acetates) produces no NOE at 31-CH_3, an NOE at 11-H, and a smaller NOE at 9-H. These results suggest that protons 8, 9, and 10B are on one face of the tetrahydrofuran ring and protons 10A and 11 are on the opposite face, *cis* to each other. The conclusion that 10A-H is *trans* to 9-H is supported by the coupling constants; *i.e.*, a value of $J_{10A,11}(cis)=6.1$ Hz is consistent with previous observations. However, the value of $J_{10B,11}(trans)=10.4$ Hz is difficult to reconcile with the previous models. The computational package Macromodel[18] (MM2) was used to explore this difficulty.

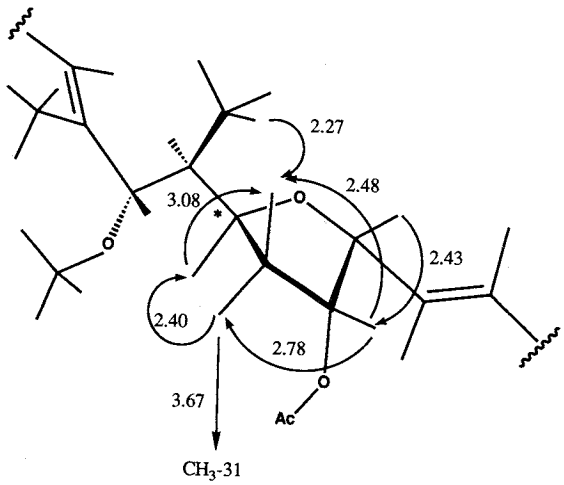
The first model was constructed using the diacetate structure as in **2**, but terminated at site 20 with a methyl group and having the aurodox stereochemistry at all sites[1]. After obtaining a minimum energy conformation (model 1) using MM2, a second model was obtained by inverting the stereochemistry at position 11 and repeating the MM2 minimization, producing model 2. The central portions of both models are shown in Fig. 1.

Both models predict very small values of $J_{9,10A}(trans)$, based on dihedral angles of approximately

Fig. 1. Two models of the A83016F diacetate tetrahydrofuran region; the two structures differ only by inversion at one site (*).



Model 1

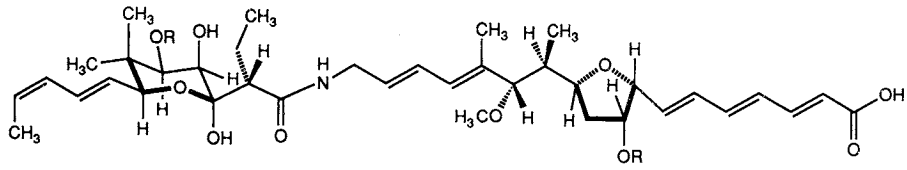


Model 2

Each structure was minimized separately using MM2 in the Macromodel computational package. Interproton distances are in Å.

90° and 80°, respectively[19]. However, both also predict large values of $J_{10B,11}$, regardless of whether the protons are *cis* or *trans*. In model 1, the aurodox-like structure, 10B-H and 11-H are almost eclipsed ($J \cong 9.6$ Hz) while 10A-H and 11-H are at an angle of about 120° ($J \cong 3.6$ Hz). Such an arrangement would lead to a maximum NOE between 10B-H and 11-H, in contrast to actual observations. Model 1 also predicts that 10A-H should be quite close to 31-CH_3, while 10B-H and that methyl would be remote. In model 2, the dihedral angles from 10A-H and 10B-H are approximately 40° and 165°, respectively, which would lead to $J_{10A,11} \cong 5.4$ Hz and $J_{10B,11} \cong 11.1$ Hz. Although these coupling constants fit the observed results slightly better than do those from model 1, the differences are not substantial enough to lead to a conclusion about the configuration at site 11. The interproton distances predicted by model 2, however, are more helpful. The distances between 8-H and 9-H, 9-H and 10A-H, and between 9-H and 10B-H are about the same in the two models and are consistent with the NOEs observed for **2**.

Fig. 2. Structure of A83016F (**1**) and A83016F diacetate (**2**).



1 R=H
2 R=$COCH_3$

However, model 2 predicts that 10B-H (but not 10A) will be in proximity to 31-CH_3, in agreement with the observed NOE and in contrast to model 1 results. Model 2 also predicts a greater NOE between 10A-H and 11-H (distance=2.40 Å) than between 10B-H and 11-H (3.08 Å), as observed. These results and the others listed in Tables 2 and 3 suggest that **1** and **2** have the structures shown in Fig. 2.

Table 4. Antimicrobial activity of **1**.

Test organism	MIC (μg/ml)
Staphylococcus aureus	>128
S. epidermidis	>128
Streptococcus pyogenes	32
S. pneumoniae PARK	16
Enterococcus faecium	>128
Salmonella sp. 514	>128
Pseudomonas sp. 258	>128
Serratia marcescens	>128
Haemophilus influenzae	64

Biological Activity

1 showed weak activity against a few microorganisms (see Table 4) in a broth dilution test.

Addendum in Proof

A83016F appears to be identical to the compound described in:

MARGRAFF, R.; T. KIENER & A. KIES (Rhone-Poulenc): New non-antibiotic elfamycin derivative — from *Streptomyces* CBS 473.89, for stimulating growth of monogastric animals. European Patent Appl., EP 442783-A, Aug. 21, 1991

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