THE STRUCTURE OF HENEICOMYCIN

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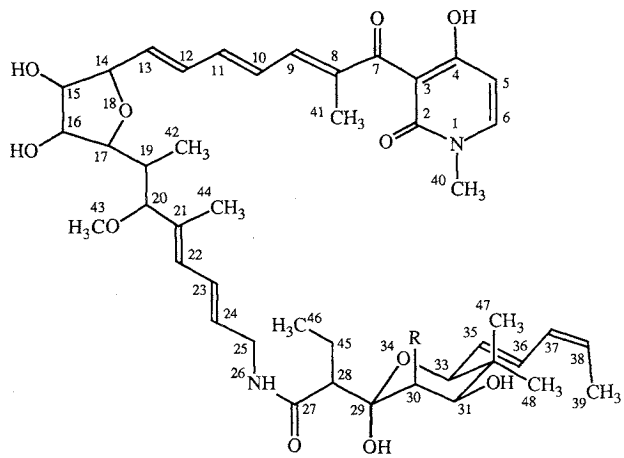
The antibiotic heneicomycin (**1**), $C_{44}H_{62}N_2O_{11}$, was isolated from cultures of *Streptomyces filipinensis* as an amorphous yellow powder. Mass spectral and NMR analysis showed the compound to be a deoxy modification of aurodox (**2**), a member of the elfamycin antibiotic family. A marked change in mass spectral fragmentation compared to aurodox and ^1H NMR couplings indicated the absence of the hydroxyl at position 30 of aurodox (position 3 of the tetrahydropyran).

Heneicomycin (A21A) was discovered in a culture of *Streptomyces filipinensis* (NRRL No. 11044)[1,2]. The following, physical chemical and spectral evidence indicated the structure as one of the elfamycin class of antibiotics.

Experimental

The low resolution mass spectra of heneicomycin were run on a LKB 7000 mass spectrometer as the underivatized compound or as its trimethylsilyl ether. The HR-MS data were acquired on a Varian MAT 731 mass spectrometer. All NMR spectra were recorded on a Varian SC300, XL300, or XL400 NMR spectrometer at ambient temperature. The ^1H NMR spectra were recorded in CD_3OD (buffered) at 300 MHz and in acetone-d_6 (spiked with CD_3COOD) at 400 MHz using the respective solvent peaks at δ 3.30 and 2.04 as internal standards. The ^{13}C NMR spectra were recorded in CD_3OD (buffered) at 75 MHz and acetone-d_6 (spiked with CD_3COOD) at 100 MHz using the solvent peaks at 49.0 and 29.8 ppm, respectively, as internal references. The ^1H NMR assignments in CD_3OD were made by spin-spin decoupling experiments at 300 MHz and COSY in acetone-d_6 at 400 MHz using the standard pulse sequence[3].



Heneicomycin (**1**) R=H
Aurodox (**2**) R=OH

Pure absorptive mode 2D NOE experiments in acetone-d_6 (4 mg/0.5 ml) were obtained for **1** and aurodox (**2**) by the hypercomplex method of STATES *et al.*[4]. Data sets (1 K × 1 K) were accumulated in 128 increments, using 96 transients for each value of t_1, a mix time of 0.5 second, and a delay of 2.5 seconds HETCOR were recorded in acetone-d_6 using the standard pulse sequence of BAX and MORRIS[5]. The experiment was optimized for $^1J_{CH}=135$ Hz using 64 increments and a delay of 1 second. The IR spectra were recorded on a Perkin Elmer Model 137; the optical rotation was taken on a Perkin Elmer 241 polarimeter, and the UV spectra on a Cary 15 recording UV spectrophotometer.

Heneicomycin (**1**) was purified by preparative TLC on silica gel in $CHCl_3$-CH_3OH-conc aq NH_3 (80:20:1). The product had an Rf of 0.3 and was isolated as an amorphous, slightly deliquescent, lemon yellow powder after lyophylization from benzene-methanol (20:1), $[\alpha]_D$ −74.3° (*c* 1, CH_3OH). IR (acetonitrile) cm^{-1} 3424, 3333, 1650, 1616, 1590, 1538; UV λ_{max} (0.1 M HCl in CH_3OH) nm (ε) 233 (64,300), 330 (30,800) and λ_{max} (0.1 M NaOH in CH_3OH) nm (ε) 224 (64,300), 318 (34,800).

Anal Calcd for $C_{44}H_{62}N_2O_{11}\cdot H_2O$: C 65.00, H 7.94, N 3.45.
Found: C 65.23, H 7.78, N 3.47.

Results and Discussion

The UV spectrum was typical of certain members of the elfamycin class of antibiotics such as aurodox[6], mocimycin[7] (kirromycin)[8] and efrotomycin[9]. Its antibacterial spectrum also showed similarities to these substances[1].

The MS data is summarized in Table 1. The FD-MS of **1** showed molecular ion peaks at *m/z* 795 $(M+H)^+$ and 817 $(M+Na)^+$. The mass spectrum of the trimethylsilyl derivative of **1** showed peaks up to *m/z* 1,226.7 for the hexatrimethylsilyl product. These were consistent with a deoxy modification of aurodox (**2**). The EI-MS of underivatized **1** showed low mass fragments at *m/z* 152, 138 and 123 characteristic of the 3-acyl-*N*-methyl pyridone portion found in this group of elfamycins. Heneicomycin showed a characteristic peak at *m/z* 245 ($C_{16}H_{21}O_2$) which could be ascribed to the ketene dihydropyran fragment, **3**. In contrast, **1** showed no peak at *m/z* 296, a characteristic of the spectra of mocimycin and aurodox.

Table 1. Mass spectral data for **1**.

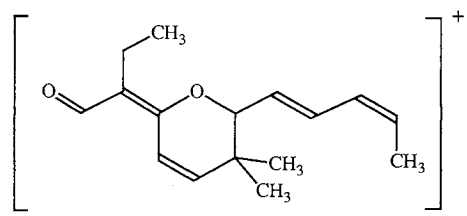
Method	Found (*m/z*)	Assignments
FD-MS	817	M+Na, $C_{44}H_{62}N_2O_{11}$+Na
	795.777, 759	M+H, M+H−H_2O, M+H−2H_2O
HREI-MS	758.4091	Calcd for $C_{44}H_{58}N_2O_9$: 758.4139
	648.3397	Calcd for $C_{37}H_{48}N_2O_8$: 648.3410, M−(H_2O, CH_3CH=$CHCH$=$CHCHO$, CH_3OH)
	384.2531	Calcd for $C_{24}H_{34}NO_3$: 384.2538
	338.1396	Calcd for $C_{20}H_{20}NO_4$: 338.1392
	245.1535	Calcd for $C_{16}H_{21}O_2$: 245.1541
EI-MS	758	M−2H_2O
	680	M−(H_2O, C_6H_8O)
	648	M−(H_2O, CH_3OH, C_6H_8O)
	620	M−(2H_2O, $C_9H_{14}O$)
	588	M−(2H_2O, CH_3OH, $C_9H_{14}O$)
	245	$C_{16}H_{21}O_2$
	152	$COC_5NH_2(OH)_2CH_3$, base peak
	123	$COC_3H_2CONCH_3$
	1,226.7[a]	M+6[$(CH_3)_3Si$−H]
	1,281.1[b]	M+6[$(CD_3)_3Si$−H]
	224[a]	$C_7H_6NO_3$+$(CH_3)_3Si$−H

[a] Trimethylsilyl derivative.
[b] Trimethylsilyl-d_9 derivative.

The *m/z* 296 fragment was assigned to a lactone pyran structure resulting from closure of the aurodox C-30 hydroxyl onto the amide carbonyl. In fact this product had been produced chemically from these antibiotics[7,10]. Similarly, in the case of efrotomycin a related fragment at *m/z* 630 had been observed and was attributed to a disaccharide derivative of this lactone[9].

The fragment of **1** at *m/z* 680 could be derived by loss of water at C-30 and C-31 in the tetrahydropyran ring followed by a reverse Diels-Alder to release the hexadienal derived from carbons at positions 33, 35~39. HR peak matching of *m/z* 648 supported this structure as *m/z* 680 with a loss of CH_3OH. Cleavage between 19 and 20 would lead to the fragments at *m/z* 338 ($C_{20}H_{20}NO_4$) containing the pyridone ring and at *m/z* 384 ($C_{24}H_{34}NO_4$) corresponding to the amide pyran fragment.

The ^1H NMR spectrum of **1** is shown in Fig. 1 and the assignments are compared with those for aurodox in Table 2. These may be compared with the recently published ^1H NMR data for kirromycin in phosphate buffered D_2O[11]. Decoupling studies suggested a $-CH_2-CHOH-$ moiety at positions 30, 31, where the vicinal couplings of $J=5.5$ and 11.5 Hz (Table 2) indicated an equatorial OH group at either C-30 or C-31. Comparison of the predicted chemical shift changes of the two methyl groups at C-32 for the two cases with those of aurodox allowed the OH group to be located at C-31 as shown in Fig. 2. Both methyl groups, C-47 and C-48, would be perturbed on one hand by the OH at C-30 whereas only the 47-CH_3 group at δ 0.74 and assigned to the axial position at C-32 is shifted upfield from δ 0.91 in aurodox. This is attributed to the absence of the 1,3-diaxial interaction between the OH at C-30 and the 47-CH_3 group in aurodox. A similar shift (0.25 ppm) was noted for 5α,14α-androstane and its 2β-hydroxy derivative[12] and noted in 1,3-diaxial methyl cyclohexanols[13]. Verification of the methyl assignments and the relative stereochemistry of the pyranose ring system was obtained *via* a pure absorptive mode 2D NOE experiment using a mix time of 0.5 second and an equilibration delay of 2.5 seconds. The correla-



3 *m/z* 245.1541 $C_{16}H_{21}O_2$

Fig. 1. ^1H NMR spectrum of heneicomycin (**1**) in buffered CD_3OD at 300 MHz.

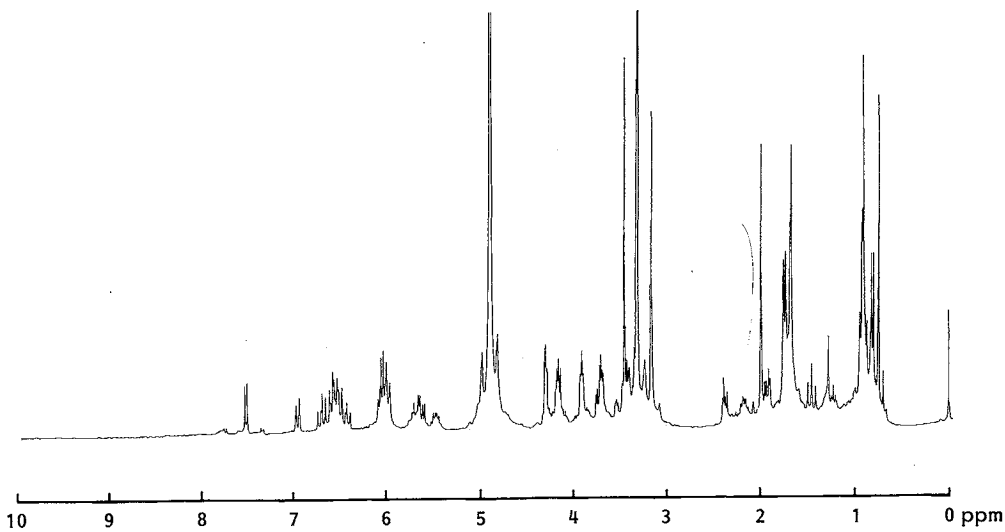
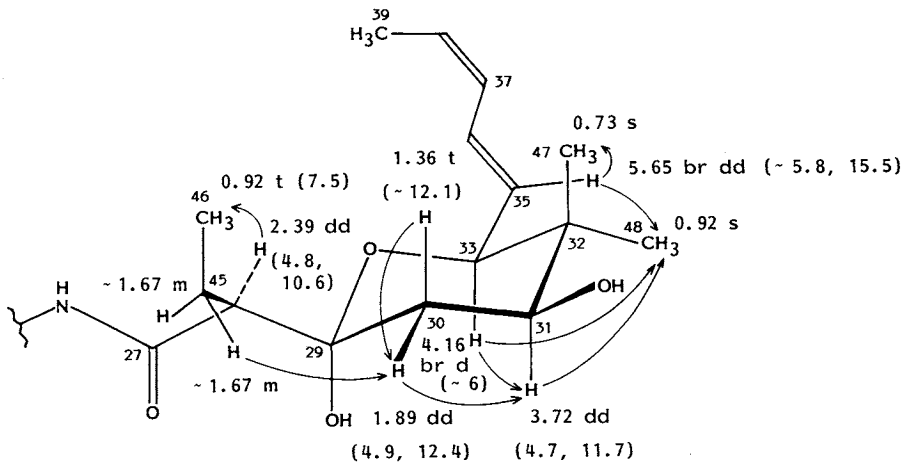


Table 2. Comparison of ^{1}H NMR data for heneicomycin (**1**) and aurodox (**2**)[a].

No.[b]	Heneicomycin[c]	Aurodox[c]	No.[b]	Heneicomycin[c]	Aurodox[c]
5-H	6.12 (1H, d, 7.7)	5.92 (1H, d, 7.6)	30-H	1.48 ($1H_{ax}$, dd, 12.5, 11.5), 1.94 ($1H_{eq}$, dd, 12.5, 5.5)	3.66 (1H, d, 3.6)
6-H	7.53 (1H, d, 7.7)	7.33 (1H, d, 7.5)			
9-H	6.97 (1H, br d, 11)	7.03 (1H, br d, 11.1)			
10-H	6.71 (1H, dd, 11, 14.7)	6.68 (1H, dd, 11.1, 14.0)	31-H	3.74 (1H, dd, 11.5, 4.8)	3.59 (1H, d, 3.5)
11-H	6.45 (1H, dd, 14.7, 10.6)	6.42 (1H, dd, 14.8, 10.8)	33-H	4.15 (1H, d, 6.7)	4.21 (1H, br d, 6.0)
			35-H	5.63 (1H, dd, 6.2, 15)	5.63 (1H, dd, 6, 15)
12-H	6.50 (1H, obscured)	6.00 (1H, obscured)	36-H	6.55 (1H, m, obscured)	6.56 (1H, dd, 15, 10)
13-H	6.25 (1H, dd, 14, 7.5)	5.98 (1H, obscured)	37-H	6.00 (1H, dd, 10, 11)	6.08 (1H, dd, 11, 10)
14-H	4.31 (1H, t)	4.27 (1H, ~t, obscured)	38-H	5.48 (1H, dq, 10.5, 7)	5.47 (1H, dd, 10.4, 6.8)
15-H	4.31 (1H, t)	4.28 (1H, ~t, obscured)	39-H	1.75 (3H, d, 7)	1.75 (3H, br d, 6.6)
16-H	4.21 (1H, t, 4)	4.18 (1H, ~t, 4.1)	40-H	3.45 (3H, s)	3.39 (3H, s)
17-H	3.70 (1H, dd, 3.5, 7)	3.69 (1H, dd, 4, 7)	41-H	2.01 (3H, br s)	1.98 (3H, s)
19-H	2.19 (1H, ddq, 2.2, 6.5, 6.5)	2.17 (1H, m)	42-H	0.82 (3H, d, 6.6)	0.82 (3H, d, 6.6)
			43-H	3.17 (3H, s)	3.14 (3H, s)
20-H	3.34 (1H, d, obscured)	3.36 (1H, d, 3)	44-H	1.67 (3H, s)	1.66 (3H, br s)
22-H	5.98 (1H, br d, 11)	6.65 (1H, d, 10.2)	45-H	1.66 (2H, obscured)	1.72 (2H, m, obscured)
23-H	6.55 (1H, m)	5.67 (1H, dd, 15, 10)	46-H	0.93 (3H, t, 7.5)	0.91 (3H, br t, 7.6)
24-H	5.69 (1H, m)	5.66 (1H, d~t, 15, 7)	47-H	0.74 (3H, s)	0.87 (3H, s)
25-H	3.92 (2H, dq)	3.90 (2H, m)	48-H	0.91 (3H, s)	0.87 (3H, s)
28-H	2.40 (1H, dd, 5.5, 10)	2.82 (1H, dd, 5.6, 9.5)			

[a] Chemical shifts are reported in ppm downfield of TMS. Number of protons, multiplicity and coupling constants (J=Hz) are shown in parentheses.
[b] Numbering system for aurodox.
[c] In CD_3OD-phosphate buffer.

Fig. 2. ^{1}H NMR assignments of the tetrahydropyran region of **1** in acetone-d_6 spiked with CD_3COOD and NOE correlations.



tions are shown in Fig. 2.

The proton decoupled ^{13}C NMR spectrum of **1** closely resembled that of aurodox and its des-*N*-methyl analog, kirromycin. A comparison of ^{13}C NMR spectra of heneicomycin in buffered methanol-d_4 and in acetone-d_6 spiked with acetic acid-d_4 and aurodox in acetone-d_6 spiked with acetic acid-d_4 in summarized in Table 3. Further comparison can be made with published data for aurodox in buffered methanol-d_4[14,15] and kirromycin in acetone-d_6[11], and assignments for protonated carbons in **1** and aurodox were

Table 3. ^{13}C NMR assignments of heneicomycin (**1**) and aurodox (**2**).

Assignment	Heneicomycin (**1**)[a]	**1**[b]	Aurodox[b]	Assignment	Heneicomycin (**1**)[a]	**1**[b]	Aurodox[b]
44-CH_3	11.2	11.2 q	11.2 q	29-HOCO	99.4	99.2 s	100.4 s
46-CH_3	12.4	12.3 q	12.3 q	5-CH	109.5	100.3 d	100.0 d
47-CH_3	11.9	12.4 q	15.9 q	C-3	113.0	109.2 s	108.8 s
41-CH_3	12.4	13.2 q	13.3 q	38-CH	126.4	125.8 d	125.7 d
39-CH_3	13.6	13.6 q	13.6 q	36-CH	127.9	127.0 d	126.8 d
42-CH_3	14.0	14.0 q	14.0 q	23-CH[d]	127.9	127.6 d	127.5 d
45-CH_2	21.5	21.4 t	21.0 t	10-CH	129.9	128.8 d	128.8 d
48-CH_3	23.0	23.0 q	24.5 q	37-CH	130.3	130.3 d	130.3 d
19-CH	36.8	36.6 d	36.6 d	22-CH	130.3	130.4 d	130.4 d
40-CH_3	36.5	36.9 q	36.9 q	35-CH	130.8	131.0 d	130.9 d
30	38.2	39.0 t	71.2 d	24-CH[d]	131.0	131.4	131.5 d
C-32	40.2	40.0 s	39.5 s	12-CH	133.6	132.7 d	132.8 d
25-CH_2	41.9	41.4 t	41.5 t	9-CH	135.7	135.9	135.4 d
43-CH_3O	56.2	56.1 q	56.1 q	C-21	135.7	135.7 s	135.6 s
28-CH	58.2	56.9 d	51.7 d	13-CH	135.7	135.9 d	135.8 d
31-CHOH	72.5	71.7 d	73.1 d	11-CH	139.0	138.8 d	138.6 d
15-CHOH	73.9	73.3 d[c]	73.3 d[c]	C-8	139.8	138.9 s	138.9 s
16-CHOH	74.6	74.7 d[c]	74.7 d[c]	6-CH	141.3	144.4 d	144.9 d
33-CH	77.5	76.7 d	76.3 d	4-CO	164.7	161.9 s	162.0 s
14-CHO	81.4	81.2 d	81.3 d	2-CO	176.6	173.1	174.0 s
17-CHO	85.0	84.5 d	84.5 d	27-CO	177.9	176.0 s	176.8 s
20-CHO	91.7	91.4 d	91.4 d	7-CO	202.5	201.2 s	201.6 s

[a] In buffered CD_3OD.
[b] In acetone-d_6 spiked with CD_3COOD.
[c,d] May be reversed.

corroborated using HETCOR experiments. Differences which can be ascribed to the loss of the hydroxyl in the pyran system are seen. The appearance of the peak at δ 39.0 is ascribed to the C-30 methylene carbon, and the loss of the resonance at δ 71.2 as seen in aurodox can be associated with the loss of the C-30 hydroxyl. Confirmation was also obtained by the effect on the chemical shifts of the two methyl groups at C-32. These could not be distinguished previously[11], but on the basis of the NOE and HETCOR experiments, the two methyls could be readily assigned in **1** and by analogy in aurodox (Table 3). The upfield resonance in this family of elfamycins is therefore assigned to the axial 47-CH_3. The appearance of a peak at δ 12.4 in **1** is therefore ascribed to an upfield shift of the axial methyl, C-32 at δ 15.9 in aurodox as a result of the loss of the C-30 axial hydroxyl. A similar 1,3-diaxial shift has been observed in 4β-hydroxy and 2β-hydroxy 10β-methyldecalins[16] and described in androstane and cholestane at the C-19 methyl for the 4β-,6β- or 2β-hydroxy derivatives[17]. A deshielding of 5 ppm for C-28 is consistent with a loss of a γ gauche effect of an axial OH in a six membered ring to the equatorial carbon. Little change is seen at the quaternary C-32. Essentially no shift was seen at C-10 in the steroid cases[17] by introduction of a β-hydroxyl at C-4 or C-6 or at C-10 of the 2 and 4β-hydroxy-10-methyl-*trans*-decalins[16].

Exposure of **1** to a trace of $NaOCD_3$ in CD_3OD resulted in the disappearance of the 28-H resonance at δ 2.40 in the ^1H NMR spectrum. This was attributed to the ring opening of the hemiketal at C-29, which would yield a β-ketoamide. Rapid deuterium substitution at C-28 would be expected from the enolization of this ring opened intermediate.

This pyran system is found in kirrothricin at the same level of hydroxylation[18]. The ^{13}C NMR spectrum of an aldehyde cleavage product containing the pyran system (structure **1**, ref 18, $CDCl_3$) showed

a peak at δ 39.2 corresponding to position 30 of heneicomycin and δ 71.8 corresponding to position 31 of heneicomycin. The antibiotic UK-69,753 has the same pyran oxygenation but as the disaccharide derivative analogous to efrotomycin[19]. Finally, a structurally related antibiotic complex, SB22484, was reported recently as having this C-30 deoxygenated pyran system and included components having C-31 equatorial and axial hydroxyl groups[20].

Heneicomycin represents another member of the class of elfamycins. The nonoxygenated C-30 position is consistent with the biosynthetic scheme proposed for aurodox[11] and efrotomycin (unpublished) were C-30 in **1** is derived from C-2 of acetate.

Acknowledgments

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