Base-catalyzed Isomerism in Elfamycin Antibiotics

Structures of Ganefromycins ε and ε_1

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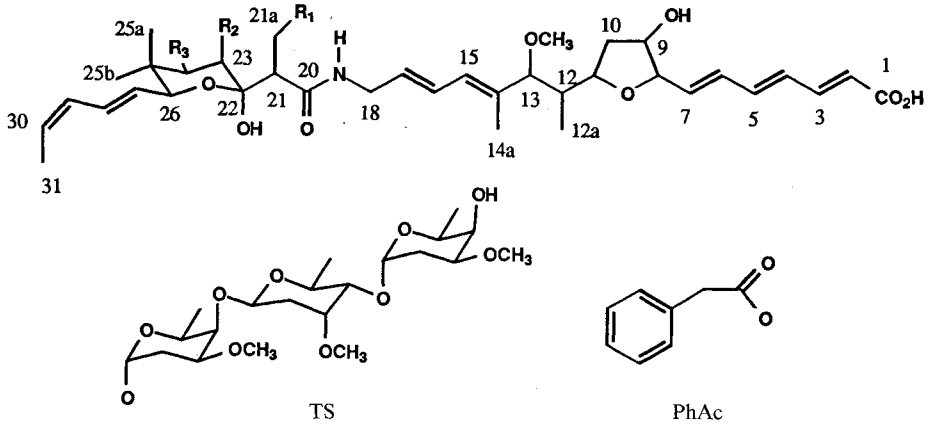
(Received for publication June 5, 1995)

The structures of ganefromycins ε (**2a**) and ε_1 (**2b**) have been determined by spectroscopic techniques. The compounds are isomeric deoxygenated precursors of the parent antibiotics ganefromycins α and β. The nature of the isomerism was determined by chemical interconversion experiments and spectroscopic analysis to be a change in configuration at C-21. Evidence is provided for other cases of this type of isomerism in the elfamycin class of antibiotics.

Ganefromycins are a family of elfamycin antibiotics produced by *Streptomyces lydicus* spp. *tanzanius* [1,2], currently under development for use as performance enhancement agents in livestock[3]. The chemistry of the ganefromycins revealed during structure elucidation experiments was described previously[4]. Of particular interest was the behavior of the compounds under basic conditions. It was found that sodium methoxide treatment promoted the elimination of the trisaccharide and decomposition of the aglycone, whereas sodium bicarbonate effected the equilibration of the α and β forms *via* acyl migration. Treatment of either ganefromycin α or β with concentrated ammonia in acetonitrile resulted in a product which had the trisaccharide replaced by a phenylacetamide group. Thus it was determined that neutral or slightly acidic conditions were necessary to maintain optimum stability of the antibiotics[5].

In the course of isolation and identification of ganefromycin congeners, two isomeric compounds of molecular formula $C_{36}H_{53}NO_9$, designated ganefromycins ε and ε_1, were encountered††. Although these compounds were readily separable by reverse phase HPLC, they differed only slightly in physical properties and their interrelationship was not immediately obvious. Isomerism in the elfamycin family has been noted several times previously. The nature of the isomerism for kirromycin (mocimycin) was attributed to keto-enol tautomerism



	R_1	R_2	R_3
Ganefromycin α	TS	PhAc	OH
Ganefromycin β	TS	OH	PhAc
β aglycone (**1**)	OH	OH	PhAc
Ganefromycin ε/ε_1 (**2**)	H	H	OH

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†† The isolation of these compounds will be described elsewhere.

involving the terminal keto-pyridone moiety[7]. In the cases of ganefromycin α and β[4], δ_3 and δ_4[8] and phenelfamycins A~F[9], pairs of regioisomers exist, varying in the position of the phenylacetate ester. More recently the structures of the SB22484 antibiotics were reported[10] as homologous pairs, epimeric at the hemiacetal carbon.

In this paper are presented the structure determinations of ganefromycins ε and ε_1, which are deoxygenated precursors of the parent compounds ganefromycins α and β. The epimeric nature of the compounds is unprecedented in the elfamycin family of antibiotics. On the basis of chemical interconversion experiments and spectroscopic analysis, a general theory is advanced regarding analogous isomerism in other elfamycins.

Results and Discussion

The structures of these isomeric compounds were determined following completely parallel arguments and only the data for ε will be discussed in detail. Ganefromycin ε was determined to have a molecular formula of $C_{36}H_{53}NO_9$ on the basis of high resolution FAB-MS measurements. Signals for 36 carbons were observed in the ^{13}C NMR spectrum (Table 1). Carbon-hydrogen correlation experiments (HMQC) established the presence of 7 methyl, 3 methylene and 21 methine groups, accounting for 48 protons, leaving 5 protons bonded to heteroatoms. As shown in Table 1, the ^1H and ^{13}C chemical shift values for ε correlate very closely with those for the backbone portion of the aglycone of ganefromycin β (**1**) in the fatty amino acid portion (C-1 through 18). Thus it was quite clear that ε had the identical structure as the β aglycone in this region. Comparison of the two sets of data for the portion defined by C-20 through 31 showed numerous differences, although key similarities were recognized. One obvious feature lacking in ε was the phenylacetate ester group (C-32~37). Two other quite substantial differences were observed for signals attributed to C-21a and C-23. In **1**, these carbons bear oxygen atoms and give rise to signals at δ 60.63 and 69.39, respectively. The corresponding signals for ε are at δ 13.68 and 38.53, indicating that these carbons are not oxygenated. ^1H-^1H NMR coupling data for ε fully support this assignment as the signal for H-21 (δ 2.54) appears as an isolated quartet with a 7.2 Hz coupling constant to the doublet for CH_3-21a at δ 1.17, as revealed by COSY measurements. Similarly, the signal for H-24 appears at δ 3.73 as a dd (J=4.9, 11.7 Hz) indicative of coupling to the signals for two protons, axial H-23a at δ 1.32 (dd ($J_{23(\alpha),24}$=11.7, $J_{23(\alpha),23(\beta)}$= 12.4 Hz)) and equatorial H(β)-23 at δ 1.87 (dd ($J_{23(\beta),24}$= 4.9, $J_{23(\alpha),23(\beta)}$=12.4 Hz)). The magnitude of the coupling constant between H-23a and H-24 provides evidence that H-24 is axial, as it is in all other ganefromycin congeners. The difference in chemical shift observed for H-24 in ε versus **1**, is readily accounted for by removal of the ester group†. The structure of the remaining portion of the compound was determined through detailed analysis of HMQC, ^1H-^1H COSY, HMBC and ROESY 2-D NMR experimental data, which are summarized in Table 1. Figure 1 shows the key correlations used to establish this part of the structure. Combination of the defined moieties yields **2**, the 21a, 23-dideoxy-desphenacyl aglycone of the parent compounds. The gross structure of ε_1 was determined analogously and the pertinent NMR data are summarized in Table 2.

Although the gross structure for $\varepsilon/\varepsilon_1$ was readily deduced as described above, their structural distinction was less clear. As indicated previously, the two isomers were readily separable by reverse phase HPLC, but their physicochemical properties were nearly identical. Significantly different ($\Delta\delta$ >0.5 ppm) ^{13}C chemical shift values observed for carbons 20, 21, 21a, 22, 23 and 26, located the isomeric region of the molecules.

The conformation of the tetrahydropyran rings of ε and ε_1 are essentially the same as that of the corresponding moieties found in **1** and other elfamycins[11], as determined by ROESY measurements and the coupling constants between H(α)-23, H(β)-23, and H-24 (Tables 1 and 2). Thus in both isomers, the 1,3-diaxial orientations of H-24 and H-26 result in ROESY crosspeaks, and both of these protons show interactions with the equatorial methyl group C-25b indicative of their *cis* relationship.

In an attempt to understand the nature of the relationship between ε and ε_1, chemical interconversion experiments were conducted. Basic treatment of ε, employing 1% Na_2CO_3 in aqueous dioxane, resulted in equilibration of the two forms. Approximately a 1:1 mixture of the two isomers was obtained, as determined

† In the course of this work it became apparent that the original NMR assignments[3] for the 25a (axial) and b (equatorial) methyl groups in ganefromycin β should be reversed. ROESY and HMQC analyses of **1** and other β series compounds (*i.e.* those substituted with phenylacetate at position 24) clearly show the equatorial 25-methyl group resonates at higher-field than the axial methyl group in both ^1H and ^{13}C spectra.

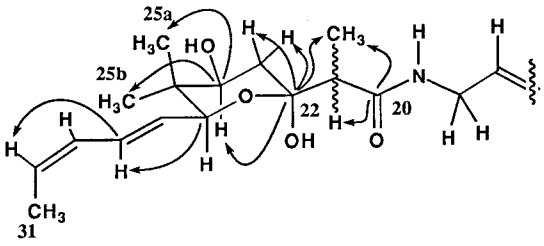
Table 1. NMR data for aglycone (**1**) and ganefromycin ε (**2a**).

C	1		2a				
	^{13}C	^{1}H	^{13}C	HMQC	COSY	HMBC	ROESY
OCH_3	56.10	3.11	56.11	3.21	—		
1	167.81	—	167.75	—	—	H3	
2	121.71	5.90	121.71	5.90	H3		
3	145.53	7.30	145.49	7.30, dd, J=11.3, 15.1 Hz	H2, H4	H5	
4	130.12	6.41	130.28*	6.41	H3, H5	H2	
5	141.25	6.71	141.22	6.71	H4, H6	H4, H6, H7	
6	131.50	6.41	131.51*	6.41	H5, H7	H8	
7	137.53	6.09	137.52	6.09	H6, H8		H8
8	84.28	4.36	84.30	4.35	H7, H9	H6, H7	H7
9	75.10	4.35	75.10	4.34	H10	H10	H10
10	40.27	1.95	40.28	1.96	H9, H11		H9, H11
11	78.17	4.68	78.15	4.62	H10, H12	H13, H14a	H8/H9#, H10, H14a
12	40.59	1.64	40.59	1.64	H12a, H11, H13, H14a	H13, H14a	
12a	10.54	0.70, d, J=7.2 Hz	10.52	0.70, d, J=6.8 Hz	H12, H13, H14a		
13	90.29	3.37	90.27	3.38, d, J=9.8 Hz	H12, H13, H14a	H12a, H14a, H15, OMe	H15
14	136.57	—	136.78	—	—		
14a	10.96	1.61	10.95	1.62	H12, H13, H15	H13	H11, H15
15	129.13*	5.96	129.11	5.96	H14a, H16, H17	H13	H14a
16	127.52	6.55	127.71	6.47	H15, H17, H18		
17	130.25	5.67	130.82	5.65	H16, H18	H16	H18, H19
18	41.58	3.98	41.62	3.91	H16, H17, H19, H21a	H16	H17, H19
19-NH	—	—	—	7.58	—	—	H17, H18, H21
20	175.42	—	177.25	—	—	H21, H21a	
21	52.52	3.14	48.70	2.54, q, J=7.2 Hz	H21a		H21a
21a	60.63	3.83	13.68	1.17, d, J=7.2 Hz	H21		H19, H21, H(α)23, H(β)23
22	100.03	—	99.39	—	—	H21, H21a, H(α)23, H(β)23	
23	69.39	—	38.53	(α) 1.32, dd, J=11.7, 12.4 Hz	H(β)23, H24		H(β)23, H21a
		(β) 3.82		(β) 1.87, dd, J=4.9, 12.4 Hz	H(α)23, H24		H21a, H(α)23
24	76.51#	4.96, d, J=3.4 Hz	71.70	3.73, dd, J=4.9, 11.7 Hz	H(α)23, H(β)23	H(α)23, H(β)23, H25a, H25b	H25b, H26
25	38.75	—	39.87	—	—	H25a, H25b	
25a	23.98	1.00	12.38	0.73	H25b	H24, H25b	
25b	16.78	0.68	22.99	0.92	H25a	H24, H25a	H24, H26
26	76.51#	4.31	77.03	4.16, d, J=6.3 Hz	H27, H28	H25a, H25b, H27, H28	H25b, H24
27	130.24	5.62	130.69	5.62	H26, H28, H29	H29	
28	127.61	6.59	127.22	6.53	H26, H27, H29	H26, H30	
29	130.67	6.00	129.62	5.99	H27, H28, H31		H31
30	126.41	5.47	125.93	5.45	H29, H31		H31
31	13.56	1.73, dd, J=1.1, 6.8 Hz	13.53	1.72, dd, J=1.5, 7.2 Hz	H29, H30	H29, H30	H29, H30
32	171.36	—					
33	41.78	3.63					
34	135.67	—					
35, 39	130.24	7.28					
36, 38	129.12*	7.28					
37	127.67	7.25					

* Carbon assignments interchangable # Signals overlapped.

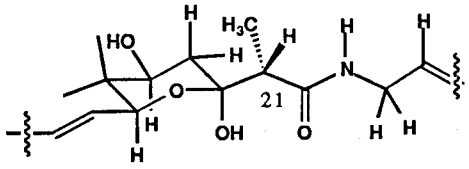
by HPLC analysis of the reaction mixture following workup. When this experiment was repeated using deuterated solvents and the reaction mixture was monitored by ^{1}H NMR analysis, the major change in the spectrum was the disappearance of the signal for H-21. Thus it was apparent that H-21 was abstracted in this process, most likely resulting in an enolate intermediate. Reprotonation of the enolate during workup presumably yields the two isomers. Since both isomers were shown by ROESY experiments to have the

Fig. 1. Key HMBC correlations for C-20 to C-31.

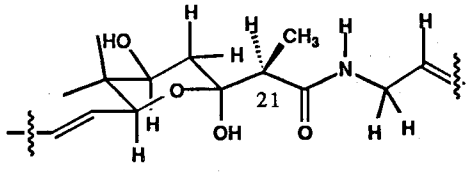


same tetrahydropyran conformation, this effectively precludes epimerization at the hemiacetal center C-22 as being responsible for the isomerism, nor would significant hydrolysis of the hemiacetal be expected under basic conditions. Comparison of the ROESY data in Tables 1 and 2 reveals telling differences for the C-21 substituents between ε and ε_1. In the case of ε, methyl group 21a showed through-space interactions with both H(α)-23 and H(β)-23 and the NH. Conversely, for ε_1 it was methine proton H-21 which showed the ROESY cross

Fig. 2. Structures of ganefromycins ε and ε_1.



epsilon (2a)



epsilon$_1$ (2b)

Table 2. Ganefromycin ε_1 (**2b**) NMR data

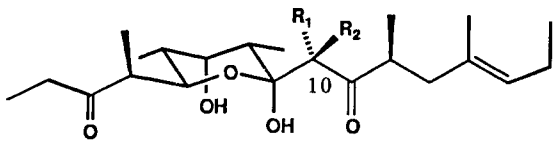
Carbon	δ ppm ^{13}C	HMQC	COSY	HMBC	ROESY
OCH_3	56.12	3.12			
1	167.81	—		H3	
2	121.71	5.90	H3		
3	145.54	7.30	H2, H4	H5	H5
4	130.27	6.41	H3, H5, H7	H2, H3, H6	
5	141.25	6.71	H4, H6	H3, H4, H6, H7	H3
6	131.51	6.41	H5, H7	H8	H8
7	137.54	6.10	H6, H8	H5, H13	
8	84.28	4.38	H7	H6, H7, H9	H6
9	75.13	4.37	H10	H10	H10
10	40.31	1.95	H9, H11		H8/H9, H11, H12a
11	78.18	4.59	H10	H8, H12a, H13	H10, H12
12	40.65	1.64	H12a, H13	H12a, H13	H11, H8/H9
12a	10.58	0.69, d, J=6.8 Hz	H12	H11, H13	H10, H13
13	90.32	3.37, d, J=9.8 Hz	H12, H12a, H15	H12a, H14a, OMe	H12a, H15
14	136.72	—		H14a	
14a	11.01	1.62			H16
15	129.65	5.97	H13, H16	H13, H14a	H13, H17
16	127.76	6.47	H15, H17		H18
17	130.97	5.69	H16, H18		H15, H19
18	41.75	3.90, dd, J=6.4, 12.8 Hz	H17, H19		H16, H19
20	174.41	—		H21, H21a	
21	51.23	2.58, q, J=7.2 Hz	H21a	H21a	H21a, H23a/b, H19
21a	12.39	1.20, d, J=7.2 Hz	H21	H21	H21, H19
22	98.55	—		H21, H21a	
23	37.11	(α) 1.70	H24		H21
		(β) 1.84	H24		H21, H24, H26
24	71.98	3.76, dd, J=4.9, 11.3 Hz	H(α)23, H(β)23	H(α)23, H25b, H26	H(β)23, H25b, H26
25	39.90	—		H25b, H26	
25a	12.35	0.73	H25b	H24, H25b, H26	
25b	23.02	0.90	H25a	H26	H24, H26, H27
26	77.77	4.18, d, J=6.4 Hz	H27, H28	H25a, H28	H24, H25b
27	130.79	5.64	H26, H28	H26	H25b
28	127.54	6.54	H26, H27, H29	H26	H26, H31
29	130.15	5.99	H28, H31	H31	
30	126.13	5.45	H31	H31	
31	13.48	1.72	H29, H30		H28
19-NH		7.26			H17, H18, H21, H21a

peaks to both H-23's. These observations suggest the two isomers have the relative configurations and adopt the conformations depicted in Fig. 2.

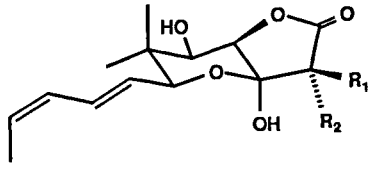
An analogous case of isomerism was described several years ago for two polypropionate metabolites, denticulatins A(**3**) and B(**4**), isolated from the marine mollusc *Siphonaria denticulata*[12]. These compounds, which contain a similarly substituted tetrahydropyran, showed a similar pattern of NMR chemical shift differences as described for ε and ε_1. Denticulatins A and B were also interconverted by basic treatment. After identical relative stereochemistry at the hemiacetal carbon was established by observation of W coupling between the hemiacetal OH and the vicinal methine proton in each isomer, it was determined that the compounds differed only in the configuration of the C-10 methyl group.

Another example of this type of isomerism is found in a key step in the total synthesis of the elfamycins[13]. In the synthesis of intermediate IV (**5**), a degradation product of aurodox, the initial product mixture obtained by condensation of ethyl butyrate with a lactone precursor of the tetrahydropyran system resulted in predominantly isomer **6**. This was remedied by equilibration in base, which provided a mixture of the two epimers.

Although this particular type of isomeric relationship has not been reported previously, reports have appeared in the literature regarding isomerism in the elfamycin series. In the case of the SB 22484 antibiotics[10], two pairs of homologous products were described, where the difference between the pairs was attributed to opposite configurations at the hemiacetal carbon, as indicated in Fig. 3. The evidence for the structure assignments was that there were significant ^{1}H NMR chemical shift differences between the NH, 28-CH_3 and 30-CH_2 groups, and that the isomers were observed to equilibrate in aqueous solution; no ^{13}C NMR data were given. What was ignored in this analysis is that the conformation of the tetrahydropyran ring was apparently unchanged in the interconversion of isomeric forms as indicated by the

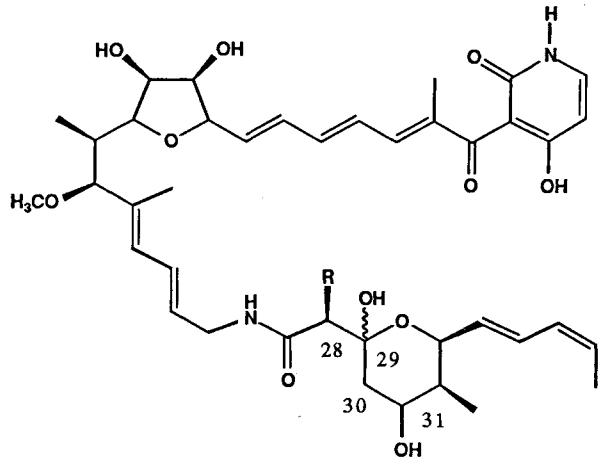


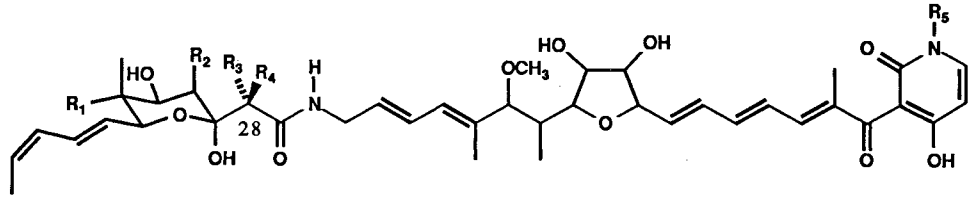
3 R_1=H, R_2=CH_3
4 R_1=CH_3, R_2=H



5 R_1=CH_2CH_3, R_2=H
6 R_1=H, R_2=CH_2CH_3

Fig. 3. Reported structures of SB22484 antibiotics[10] (R = CH_3 or CH_2CH_3).





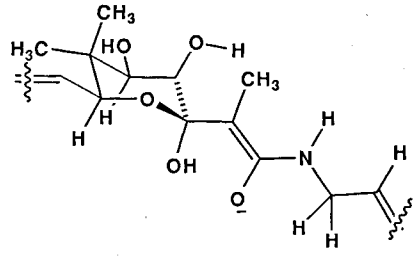
	Compound	R_1	R_2	R_3	R_4	R_5
7, 8	SB 22484A-1 & 3	H	H	H/Me	H/Me	H
9, 10	SB 22484A-2 & 4	H	H	H/Et	H/Et	H
11	Aurodox	Me	OH	Et	H	Me
12	28-*epi*-aurodox	Me	OH	H	Et	Me
13	Heneicomycin	Me	H	Et	H	Me
14	28-*epi*-heneicomycin	Me	H	H	Et	Me

nearly identical set of chemical shift values observed for H-31, 32 and 33. Inversion of configuration at C-29 would certainly require inversion of the tetrahydropyran conformation to position the long chain substituent at C-29 in the equatorial orientation. Although no coupling constants were provided for the ring protons, it is very unlikely that the chemical shift values of these protons would remain so nearly the same in both axial and equatorial positions. On the basis of these data alone, it is more reasonable to postulate that these pairs of compounds also differ in configuration at the carbon adjacent to the amide carbonyl (C-28 in Fig. 3), and have the structures **7**~**10**.

To further investigate the generality of this type of isomerism in the elfamycin series, aurodox (**11**) and heneicomycin (**13**) were subjected to equilibration with 1% Na_2CO_3 in CD_3OD/D_2O. The reactions were monitored by HPLC analysis and ^1H NMR spectroscopy. In both instances the proton adjacent to the amide carbonyl (H-28) was removed and/or exchanged with deuterium as evidenced by disappearance of the dd signal for that proton in the ^1H NMR spectrum (δ 2.81 aurodox, 2.40 heneicomycin). HPLC analysis of the reaction mixtures, which was carried out in an acidic medium, showed two components with identical UV spectra in approximately a 1 : 1 ratio by area for heneicomycin, one of which corresponded to the starting antibiotic, while aurodox remained as a single peak with identical retention time. By analogy with $\varepsilon/\varepsilon_1$ and the SB22484 compounds, the new component formed in the heneicomycin reaction must be epimer **14**. Apparently very little or no epimer **12** was formed in the aurodox reaction. Exchange of this proton in heneicomycin with $NaOCD_3$ in CD_3OD was observed previously[14], although the formation of epimeric products was not described.

It appears that C-30 substitution (C-23 in the ganefromycin series) determines the extent to which epimerization occurs at the carbon α to the amide carbonyl in the elfamycins. For ganefromycin ε, the SB 22484 antibiotics, and heneicomycin, which are unsubstituted at this position, epimerization occurs readily, as substantial amounts of both isomers are observed by HPLC. In the case of aurodox, which has an axial hydroxyl group at C-23, no epimerization was detected by HPLC nor was there evidence of epimerization by ^1H or ^{13}C NMR analysis of the final reaction product. An enolate intermediate of the type shown in Fig. 4 can be invoked to explain the product compositions. The axial hydroxyl group is shown positioned behind the plane of the enolate, thus blocking the approach of a proton from that side, while approach from the front would be much less hindered. Since protonation must take place from a direction perpendicular to the plane of the enolate[15], it should occur primarily from the front side resulting predominantly in the original configuration. In the compounds lacking hydroxyl substitution, approach from either direction should be about equally favored, resulting in a mixture of epimers.

Fig. 4. Proposed enolate intermediate for aurodox.



Experimental

NMR spectra were recorded on a Bruker-AMX-300 instrument in acetone-d_6. Mass spectra were measured on a VG-ZAB SE instrument. HPLC analyses were done with Hewlett Packard 1090 instruments equipped with diode-array UV-Vis detectors. Preparative reverse phase separations were accomplished with a Rainin Dynamax system. Samples of aurodox and heneicomycin were obtained from Lederle Laboratories Antibiotic Collection.

Preparation of Ganefromycin β Aglycone (**1**)

Ganefromycin β (10 g) was dissolved in MeOH (50 ml) and was then cooled in an ice water bath for 30 minutes before adding 12 N HCl (0.5 ml) dropwise while stirring. The reaction was monitored by analytical HPLC (C18, Rainin Microsorb-MV, 10 cm × 4.6 mm, 1 : 1 acetonitrile - 0.1 M NH_4OAc pH 4, 1.0 ml/minute, UV 290 nm) indicating the decrease in the amount of starting material (retention time 16.8 minutes) and an increase over time of the desired aglycone product (retention time 7.3 minutes). After the observed completion of the reaction by analytical HPLC, H_2O (100 ml) was added and the reaction mixture was extracted twice with equal volumes of ethyl acetate. The ethyl acetate layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to a yellow brown oil containing the aglycone. The aglycone in the reaction mixture was purified by preparative HPLC (C18, 4.1 cm × 30 cm, 2 : 3 acetonitrile - 0.1 M NH_4OAc pH 4, 30 ml/minute, UV 290 nm, collection in 10 minutes fractions, retention time 170 minutes) followed by back extraction with CH_2Cl_2 and evaporation of the solvents, yielding the desired aglycone (1.3 g).

Molecular weight: 793 (FAB-MS $=m/z$ 816 corresponding to $(M+Na)^+$)

Molecular formula: $C_{44}H_{59}NO_{12}$

HRFAB-MS $(M+Na)^+ = m/z$ 816.3946 (calc. 816.3935)

Specific rotation: $[\alpha]_D^{25} = +33.3°$ (0.54, MeOH)

UV absorption (MeOH) λ_{max} (ε): 232 nm (39,600), 290 nm (22,500)

Characterization data for ganefromycin ε (**2a**):

Molecular weight: 643 (FAB-MS $=m/z$ 666 corresponding to $(M+Na)^+$)

Molecular formula: $C_{36}H_{53}NO_9$

HRFAB-MS $(M+Na)^+ = m/z$ 666.3650 (calc. 666.3618)

Specific rotation: $[\alpha]_D^{25} = +23.7°$ (0.54, MeOH)

UV absorption (MeOH) λ_{max} (ε): 232 nm (52,000), 290 nm (38,000)

IR absorption spectrum (KBr) ν_{max}: 3384, 2973, 2935, 1690, 1639, 1619, 1548, 1451, 1385, 1298, 1151, 1007 cm^{-1}.

Characterization data for ganefromycin ε_1 (**2b**):

Molecular weight: 643 (FAB-MS $=m/z$ 666 corresponding to $(M+Na)^+$)

Molecular formula: $C_{36}H_{53}NO_9$

HRFAB-MS $(M+Na)^+ = m/z$ 666.3625 (calc. 666.3618)

Specific rotation: $[\alpha]_D^{25} = +14.3°$ (0.3, MeOH)

UV absorption (MeOH) λ_{max}: 232 nm (41,700), 289 nm (26,400).

IR absorption spectrum (KBr) ν_{max}: 3400, 2973, 1690, 1642, 1618, 1248, 1178, 1149, 1083, 1007 cm^{-1}.

Chemical Interconversion Experiment $\varepsilon/\varepsilon_1$

Ganefromycin ε (57 mg) was dissolved in CD_3OD (0.5 ml) and 0.5 ml of a solution of Na_2CO_3 in D_2O (200 mg/10 ml) was added resulting in a somewhat cloudy pale yellow solution. A sample (10 μl) was immediately removed and was diluted ten-fold with 1:1 methanol-water for HPLC analysis. The remaining material was held at rt in a capped vial for 30 minutes, then heated to 50° for 60 minutes. HPLC samples were taken (as described above) at 15 minutes and 90 minutes. The reaction mixture was then filtered through a cotton plug into an NMR tube and a ^1H NMR spectrum was recorded. HPLC analysis was performed with a C18 column (2.1 × 100 mm) eluted with a mixture of 35% acetonitrile/65% aqueous acetic acid (0.5% v/v) at a flow rate of 0.5 ml/minute. Detection was by UV absorbance at 235 nm. In this system ganefromycins ε and ε_1 have retention times of 5.6 and 3.3 minutes, respectively. At time zero, HPLC showed only ε to be present. After 15 minutes at rt a 95/5 mixture of ε to ε_1 existed, and at the end of the experiment the ratio was approximately 50/50. The ^1H NMR spectrum of the reaction mixture after 90 minutes lacked the quartet for H-21 at 2.5 ppm, indicating deuterium exchange at this position. In addition, several of the signals were doubled owing to the presence of nearly equal amounts of ε and ε_1.

Treatment of Heneicomycin with Na_2CO_3/D_2O/MeOD

Heneicomycin (1.6 mg) was dissolved in CD_3OD (0.5 ml) and a 2% solution of Na_2CO_3 in D_2O (0.25 ml) was added. After 30 minutes at rt the reaction mixture was analyzed by HPLC: C18 column, 5 micron particle size, 4.6 × 150 mm, mobile phase acetonitrile/0.5% HOAc (40/60), flow rate 1.0 ml/minute, detection UV 300 nm and by ^1H NMR. Two major peaks were observed in the chromatogram of approximately equal area, one at the original retention time of heneicomycin (9.0 minutes) and the other at 5.3 minutes.

Treatment of Aurodox with Na_2CO_3/D_2O/MeOD

Aurodox (23.2 mg) was dissolved in CD_3OD (0.5 ml) and a 2% solution of Na_2CO_3 in D_2O (0.25 ml) was added. The solution was held at rt for 105 minutes, then heated at 50° for 10 minutes. After cooling to rt (20 minutes) the sample was analyzed by ^1H and ^{13}C NMR spectroscopy. HPLC was used to monitor the course of the reaction: C18, 3 micron particle size, 4.6 × 100 mm, mobile phase acetonitrile/0.5% HOAc (35/65) flow rate 1.0 ml/minute, detection UV 290 nm. Aurodox retention time 13 minutes.

Acknowledgments

We gratefully acknowledge the helpful discussions held with Professors D. JOHN FAULKNER, RAYMOND ANDERSEN and our colleagues in the Natural Products Chemistry Department. We thank Dr. FRANK KOEHN for his assistance in obtaining some of the NMR data and Dr. RUSS TSAO for the FAB-MS data.

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