Notes

ESPERAMICINS, A NOVEL CLASS OF POTENT ANTITUMOR ANTIBIOTICS I. PHYSICO-CHEMICAL DATA AND PARTIAL STRUCTURE

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Recently we have isolated several members of a family of exquisitely potent antitumor antibiotics from *Actinomadura verrucosospora*, strain H964-62 (BBM-1675, ATCC 39334).[1] The producing organism was collected at Pto Esperanza, Misiones, Argentina, consequently we have named these compounds esperamicins. The recent appearance of several patents and papers describing what appear to be related compounds prompts us to report the physicochemical properties of esperamicins A_1 (**1**), A_2 (**2**), and A_{1b} (**3**) as well as the partial structure of esperamicins A_1 and A_2.[2,3]

The esperamicins have been isolated from the fermentation beers *via* broth extraction with butanol, concentration, and precipitation by *n*-hexane.† The solids thus obtained were chromatographed on Sephadex LH-20, silica gel and finally on reverse phase HPLC supports. To date we have isolated esperamicins A_1, A_{1b}, A_2, A_3, A_4, B_1 and B_2, compound A_{1b} and the latter four components being minor ones.[1]††

Esperamicin A_1 was isolated as white to pale yellow crystals, mp 156~158°C (dec), $[\alpha]_D^{24}$ −207° (*c* 0.0351, $CHCl_3$) and $[\alpha]_D^{27}$ −191° (*c* 0.5, $CHCl_3$). The IR spectrum of **1** had bands characteristic of hydroxyl groups, amide, ester and α,β-unsaturated ketone (IR bands at 3440, 3360, 2960, 2920, 1715, 1668, 1608, 1592, 1520, 1446, 1405, 1380, 1308, 1250, 1210, 1150, 1110, 1070, 1015, 985 cm^{-1}). The UV spectrum of **1** in MeOH showed bands at λ_{max} (nm) 320 (*a* 12.4), 280 (sh), 253 (*a* 25.1), 210 (*a* 25.5). No significant shifts were observed upon addition of either acid or base. The elemental formula of esperamicin A_1 was determined to be $C_{55}H_{84}N_4O_{22}S_3$ using elemental analysis and FAB high resolution mass spectroscopy. The elemental analysis gave values of C 52.17%, H 6.15%, N 4.63%, S 9.09% and O 27.96% (by difference). The ^1H NMR spectrum of **1** at 360 MHz in $CDCl_3$ exhibited resonances at δ 11.75 (1H, s); 8.55 (1H, s); 7.45 (1H, s); 6.61 (1H, m); 6.23 (1H, br s); 6.17 (1H, br s); 5.93 (1H, d, J=9.3 Hz); 5.82 (1H, d, J=9.3 Hz); 5.70 (1H, br s); 5.49 (1H, m); 5.45 (1H, d, J=2.3 Hz); 5.38 (1H, br s); 4.95 (1H, d, J=10.2 Hz); 4.64 (2H, m); 4.54 (1H, d, J=2.3 Hz); 4.20 (1H, s); 4.15~3.35 (26~28H including 4.10 (1H, m), 4.02 (1H, br s), 3.95 (3H, s), 3.85 (3H, s), 3.79 (3H, s), 3.46 (1H, m), 3.40 (3H, s)); 2.82~2.70 (3H, br m); 2.50 (3H, s); 2.47 (1H, m); 2.38~2.22 (5H); 2.12 (1H, m); 2.11 (3H, s); 1.60~1.05 (22H including 1.39 (3H, d, J=6.3 Hz), 1.31 (3H, d, J=6.3 Hz), 1.29 (3H, d, J=6.3 Hz) 1.08 (6H)). The ^{13}C NMR spectrum of **1** is recorded in Table 1 together with those of **2**, **4**, and **5** for comparison purposes.

Esperamicin A_2 (**2**) was isolated as white crystals, mp 147~149°C, $[\alpha]_D^{27}$ −179.4° (*c* 0.5, $CHCl_3$). The IR and UV spectra of **2** were very similar to those of **1**. The elemental formula and molecular weight of **2** were shown to be identical to that of **1** by FAB high resolution mass spectroscopy. The elemental analysis gave values of C 52.71%, H 5.94%, N 3.94%, S 9.39%, O 28.02% (by difference). The ^1H NMR spectrum of **2** at 360 MHz in $CDCl_3$ exhibited some differences from that of **1**. The resonances were observed at δ 11.91 (1H, s); 8.62 (1H, s); 7.58 (1H, s); 6.56 (1H, m); 6.22

† A manuscript detailing the taxonomy, fermentation, and biological activities of the esperamicins is in preparation, T. MIYAKI, K. TOMITA, H. KAMEI *et al.*

†† Full details of the isolation procedures will be forthcoming, M. KONISHI, H. OHKUMA, J. A. MATSON & D. E. NETTLETON.

Table 1. ^{13}C NMR spectra of compounds **1**, **2**, **4** and **5**.[a]

Carbon	[b]	1	2	4	5	Carbon	[b]	1	2	4	5
1	q	13.7	13.7			29	d	76.6	76.9		
2	q	16.6	16.9	16.6	16.9	30	u	77.1	77.7		
3	q	17.5	17.5			31	d	77.3	78.1		
4	q	19.8	19.8			32	s	83.4	83.3		
5	q	22.2	22.3			33	d	86.6	86.2		
6	q	22.6	22.6			34	s	88.4	88.4		
7	q	23.4	23.4			35	t	90.5	90.4	90.6	90.4
8	t	29.0	33.1	29.0	33.3	36	d	97.2	97.2		
9	t	34.0	34.0			37	s	98.3	98.3		
10	t	35.1	35.1			38	d	99.0	99.1	99.0	98.8
11	t	39.5	39.3			39	d	99.5	99.1		
12	d	47.2	47.6			40	d	99.5	99.5		
13	q	52.5	52.6			41	d	103.7	103.8	103.8	103.9
14	u	55.6	55.7			42	s	107.1	107.6	107.1	107.1
15	q	56.0	56.0	56.0	56.1	43	d	112.5	112.4	112.6	112.7
16	q	56.0	56.1	56.0	56.1	44	d	123.1	123.2		
17	q	56.0	56.1	56.0	56.1	45	d	124.9	124.8		
18	d	57.1	57.6			46	d	130.1	129.9		
19	t	62.3	62.4			47	s	131[c]	131[c]		
20	d	64.5	64.5			48	s	136.7	137.3	136.8	137.5
21	d	66.7	65.9	66.6	65.2	49	s	144.0	144.1	144.1	144.2
22	t	68.2	68.2			50	s	147[c]	147[c]		
23	d	68.8	73.6	68.8	73.4	51	s	153.8	154.2	153.9	154.4
24	d	69.2	69.2			52	s	154.4	154.5	154.4	154.5
25	t	69.6	69.7			53	s	160.7	160.9	160.9	160.9
26	d	70.2	64.9	70.2	64.7	54	s	166.7	167.9	166.5	168.0
27	d	71.8	71.9			55	s	191.8	192.0		
28	d	76.0	75.8								

[a] Recorded at 90 MHz in $CDCl_3$ on a Bruker WM360.
[b] Multiplicity q=quartet, t=triplet, d=doublet, s=singlet, u=uncertain.
[c] Broad diffuse signals.

(1H, s); 6.15 (1H, br s); 5.91 (1H, d, J=9.6 Hz); 5.83 (1H, d, J=9.6 Hz); 5.70 (1H, m); 5.45 (1H, d, J=2.2 Hz); 5.44 (1H, s); 5.34 (1H, br s); 4.95 (1H, d, J=10.2 Hz); 4.75 (1H, m); 4.65 (1H, d, J=6.8 Hz); 4.54 (1H, d, J=2.2 Hz); 4.47 (1H, m); 4.18 (1H, s); 4.10 (1H, br s); 4.05~3.50 (20~24H, including 3.96 (3H, s), 3.87 (3H, s), 3.77 (3H, s)); 3.46 (1H, m); 3.39 (3H, s); 2.79 (1H, m); 2.73 (2H, m); 2.50 (3H, s); 2.50 (1H, m); 2.38~2.22 (3H, m); 2.14 (1H, m); 2.10 (3H, s); 1.98 (2H, m); 1.65~1.45 (6~8H); 1.38 (3H, d, J=6.0 Hz); 1.34 (3H, d, J=6.0 Hz); 1.22 (3H, d, J=6.8 Hz); 1.10 (6H).

Esperamicin A_{1b} (**3**) was isolated as a minor congener of the isolation of **1**. Examination of its physico-chemical properties led to the conclusion that it is identical with WS 6049A discovered earlier by KIYOTO *et al.*[2~4] The ^{13}C NMR spectrum recorded for **3** was identical with that reported for WS 6049A within experimental error. One discrepancy between our data and that of KIYOTO's group is the molecular weight. We have determined the molecular weight of **3**, using FAB high resolution mass spectroscopy, to be 1,235.469 corresponding to an [M+H] ion $C_{54}H_{83}N_4O_{22}S_3$. This represents a difference between compounds **1** and **2** and compound **3** of a single methyl function which is in complete accord with the ^{13}C evidence. The Fujisawa group reports an [M+H] ion at 1,311 determined by FABQ-MS. There are several possible reasons for the discrepancy one of which may be a matrix effect described in further detail below.

Mass Spectroscopy

The molecular weights of esperamicins A_1 and A_2 (**1** and **2**) were determined to be 1,248.456

using high resolution FAB-MS. This value corresponds to an elemental composition of $C_{55}H_{84}N_4O_{22}S_3$ which was determined by an analysis of all the data. Initially the molecular weight determinations were complicated by unexpected matrix effects which lead to incorrect assignments of the [M+H] ion. In order to unambiguously assign the molecular formulas of the esperamicins we have studied the FAB-MS using three matrices, thioglycerol, dithiothreitol/dithioerythritol "magic bullet"* (MB), and glycerol.

The thiol containing matrices yield abundant high mass ions at [M+H] and [M+H+matrix]. The FAB experiments with glycerol did not contain abundant high mass ions. Addition of DMSO to the glycerol matrix facilitated dissolution of **1** while at the same time accelerating its decomposition leading to very complex spectra which changed over time greatly diminishing the value of these experiments.**

In the thiol containing matrices, **1** and **2** yield a protonated molecular ion [M+H] cluster at *m/z* 1,249 daltons and more intense [M+H+matrix] ion clusters at *m/z* 1,357 and *m/z* 1,403 (when thioglycerol and MB matrices are used respectively). The [M+H] ion clusters were confirmed by adding NaCl to the mixtures. This yielded more intense ions at [M+Na] *m/z* 1,271 and [M+Na+matrix]. For spectra taken in thioglycerol with and without addition of NaCl the ratio of the [M+H] to the [M+H+matrix] was 0.20. A single matrix could lead to mistaking the [M+H+matrix] ion cluster for the [M+H] ion.[2~6] However a comparison of the thioglycerol and MB matrix spectra leads to proper assignment of the [M+H] ion. The matrix effect observed for **1** and **2** was also observed for **3** thus implying that this may be a general effect for molecules of this class.

* "Magic bullet" (MB) is a mixture of dithiothreitol: dithioerythritol (3:1) developed by J. CARTER COOK & K. RINEHART.

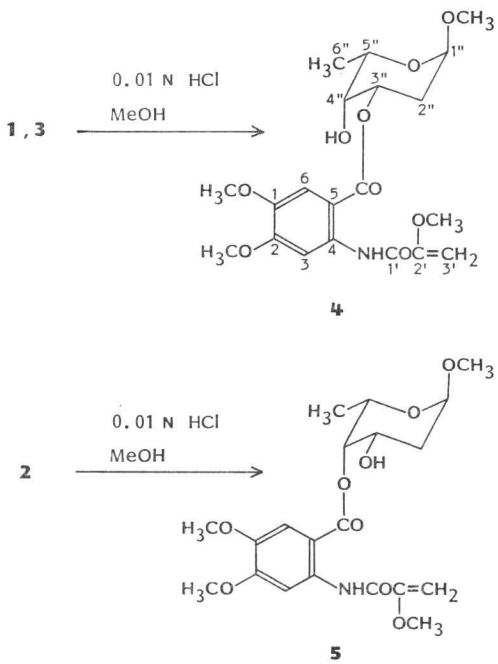
** The esperamicins are extremely reactive molecules. Consequently it is necessary that samples for FAB-MS be run immediately following preparation. The isotope ratios of prominent ion clusters change within five minutes of sample preparation. A matrix effect was observed for DMSO solutions of compound **1**, *i.e.* there was a strong [M+H+DMSO] ion at *m/z* 1,325.

Partial Structure Determination

Examination of the physico-chemical data for the esperamicins lead to the conclusion that these molecules consist of a chromophore conjugated to a number of sugars as shown by the anomeric carbons in the ^{13}C together with the corresponding protons in the ^1H NMR spectra. There is also evidence that there is an α,β-unsaturated ketone present in the molecule (^{13}C NMR signal at 192 ppm). Unfortunately the complexity of the esperamicin structure(s) precluded a complete NMR analysis. Consequently we have carried out a number of degradations of **1**, **2**, and **3** with a view to providing simpler fragments.

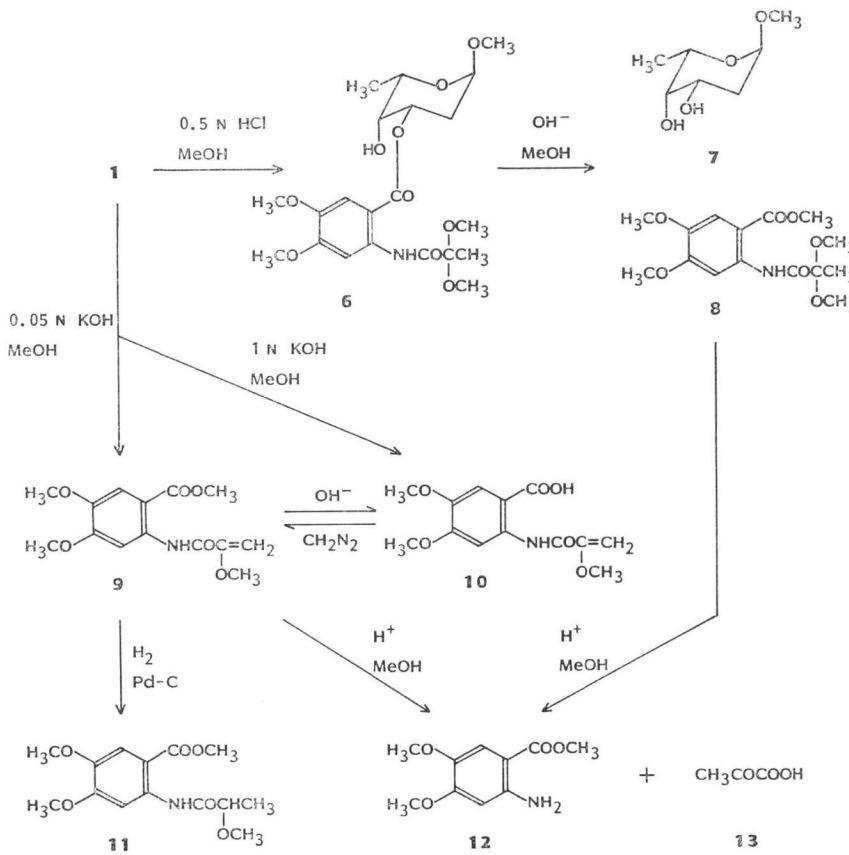
From an analysis of the ^1H and ^{13}C NMR spectra of **1** and **2** together with the mass spectra evidence it was apparent that **1** and **2** were close structural analogs if not isomers of the same basic structure. Methanolysis of **1** using 0.01 N hydrogen chloride (Scheme 1) yielded fragment **4** together with several other products. Compound **4** was also obtained upon methanolysis of **3**. When compound **2** was subjected to these conditions, **5**, a compound closely related to but different from **4**, was obtained. The IR and UV spectra of **4** and **5** were essentially superimpossible. The IR spectrum had absorptions

Scheme 1.



which corresponded to hydroxyl groups, aromatic ester, amide, and enol ethers; 3470, 3250, 2940, 2840, 1690, 1610, 1600, 1530, 1450, 1410, 1370, 1350, 1310, 1255, 1215, 1160, 1125, 1080, 1050, 1005, 990, 945, 920, 880, 860, 780, 750 cm^{-1}. The UV spectrum in MeOH indicated that the chromophore from the esperamicins was present in the fragments, λ_{max} nm, 323 (*a* 27.7), 252 (*a* 63.7), 210 (*a* 19.1) (*c* 0.0204 g/liter). The ^1H NMR spectrum of **4** in $CDCl_3$ at 360 MHz showed resonances at δ 11.75 (1H, bs, NH); 8.49 (1H, s, C3-H); 7.42 (1H, s, C6-H); 5.48 (1H, d, J=2.5 Hz, C3′-H); 4.50 (1H, d, J= 2.5 Hz, C3′-H); 5.38 (1H, m, C3″-H); 4.85 (1H, m, C1″-H); 4.03 (1H, dq, C5″-H); 3.93 (3H, s, C1-OCH_3); 3.93 (1H, m, C4″-H); 3.83 (3H, s, C2-OCH_3); 3.74 (3H, s, C2′-OCH_3); 3.34 (3H, s, C1″-OCH_3); 2.21, 1.97 (2H, m, C2″-H_2); 1.29 (3H, d, C6″-CH_3). The ^1H NMR spectrum of **5** in $CDCl_3$ at 360 MHz was similar to that of **4** with the exception of the resonances for C3″-H (at δ 4.31), C4″-H (at δ 5.28), and C5″-H (at δ 4.90) indicating that **4** and **5** differ in the substitution at the C3″ and C4″ hydroxyl groups of a 2-deoxyfucose unit. This was further supported by the ^{13}C NMR spectra (listed in Table 1) of compounds **4** and **5**. The resonances for all 20 carbons in **4** and **5** have been unequivocally assigned* as follows — compound **4**: 16.6 (C6″), 29.0 (C2″), 54.7 (C1″-OCH_3), 56.0 (C1-OCH_3, C2-OCH_3, C2′-OCH_3), 66.6 (C5″), 68.8 (C4″), 70.2 (C3″), 90.6 (C3′), 99.0 (C1″), 103.8 (C3), 107.6 (C5), 112.6 (C6), 137.5 (C4), 144.1 (C1), 153.9 (C2), 154.4 (C2′), 160.9 (C1′), 166.5 (C5-COOR). Compound **5**: 16.9 (C6″), 33.3 (C2″), 54.9 (C1″-OCH_3), 56.1 (C1-OCH_3, C2-OCH_3, C2′-OCH_3), 65.2 (C5″), 73.4 (C4″), 64.7 (C3″), 90.4 (C3′), 98.8 (C1″), 103.9 (C3), 107.1 (C5), 112.7 (C6), 137.5 (C4), 144.2 (C1), 154.4 (C2), 154.5 (C2′), 160.9 (C1′), 168.0 (C5-

Scheme 2.



* The proton and carbon assignments were made by homonuclear and heteronuclear correlation spectroscopy.

COOR). From an analysis of the NMR data it is evident that in compound **4** the 2-deoxyfucose is substituted at C3″ while in **5** it is substituted at C4″. Examination of the ^{13}C NMR spectra of **1** and **2** in comparison with those of **4** and **5** indicates that **1** and **2** are isomers about C3″ and C4″ of the 2 deoxyfucose unit as well.

Further evidence for the structures of **4** and **5** was provided by additional degradative work on compound **1**. (Scheme 2). Methanolysis of **1** under more vigorous conditions (0.5 N HCl) yielded **6**. The NMR spectrum of **6** was similar to that of **4** with the exception that the vinylogous methylene unit of **4** had disappeared to be replaced by a singlet methyl group. In addition there was an additional methoxyl group in **6**. Treatment of **6** with dilute base in MeOH gave the α-methylglycoside of 2-deoxyfucose **7** plus compound **8**. Further methanolysis of **8** (1.5 N HCl) gave **12** identified as methyl 4,5-dimethoxyanthranilate by comparison with an authentic sample. Treatment of the hydrolysate residues from this experiment with 2,4-dinitrophenylhydrazine gave the hydrazone of pyruvic acid. The absolute configuration of methyl α-2-deoxyfucopyranoside (**7**) was determined by application of the CD method to the 3,4-di-*p*-bromobenzoate of **7**. The CD spectrum of this compound exhibited a strong negative first Cotton effect and split CD curve ($\Delta\varepsilon_{253\,nm}$ −20.2, $\Delta\varepsilon_{244\,nm}$ 0, $\Delta\varepsilon_{237\,nm}$ +11.2). This is consistent with 2-deoxy-L-fucose.[7]

Base treatment of **1** with dilute hydroxide (0.05 N KOH) in MeOH gave **9**. When more vigorous treatment was applied (1 N KOH-MeOH) the carboxylic acid **10** corresponding to **9** was obtained. Compound **9** could be converted to **10** *via* base hydrolysis and **10** to **9** *via* treatment with diazomethane. Hydrogenation of **9** gave **11** in which the exomethylene unit of **9** had disappeared. Compound **11** exhibited a new doublet methyl signal and one of the methoxyl groups in **9** had shifted to higher field (δ 3.50) thus confirming in **9** the presence of a $C{=}CH_2$ unit. Hydrolysis of **9** gave **12**.
$\overset{|}{O}CH_3$

Discussion

The above data establishes partial structures for esperamicins A_1, A_2 and A_{1b} (**1**~**3**) consisting of an aromatic chromophore ester of 2-deoxy-L-fucose as an α-glycoside of the remaining portion of the molecule. In compounds **1** and **3** the C3 hydroxyl function of the sugar is esterified while in **2** the C4 hydroxyl group is substituted. Structural studies on the esperamicins are in progress and their full structures will be the subject of future manuscripts.

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