A NOVEL SERIES OF MILBEMYCIN† ANTIBIOTICS FROM *STREPTOMYCES* STRAIN E225

II. ISOLATION, CHARACTERIZATION, STRUCTURE ELUCIDATION AND SOLUTION CONFORMATIONS

G. H. BAKER, R. J. J. DORGAN*, J. R. EVERETT††
J. D. HOOD††† and M. E. POULTON†††

Beecham Pharmaceuticals Research Division,
Great Burgh, Yew Tree Bottom Road,
Epsom, Surrey KT18 5XQ, UK
††B.P.R.D.,
Brockham Park, Betchworth, Surrey RH3 7AJ, UK
†††B.P.R.D.,
Walton Oaks, Tadworth, Surrey KT20 7NT, UK

(Received for publication February 9, 1990)

A novel series of milbemycin antibiotics were isolated from the fermentation broth of a *Streptomyces* species designated E225. The structures of the four main metabolites VM 44857 (**1**), VM 44864 (**2**), VM 44865 (**3**) and VM 44866 (**4**) were determined by NMR techniques. In addition we describe the solution conformations of the major metabolite VM 44857 (**1**).

In a previous paper[1] a novel *Streptomyces* species designated E225 and the anthelmintic activity of four of the milbemycin metabolites produced by this organism were described. In this paper we describe the structure elucidation of these metabolites which are related to other known milbemycins but are unusual in possessing an unsaturated C-25 side chain and being either unsubstituted at C-22 and C-23 or possessing a single C-22-hydroxyl group instead of the more common C-23-hydroxyl substitution.

Materials and Methods

Fermentation

The seed medium consisted of (all concentrations in g or ml/litre): Special peptone (Oxoid) 2.5, beef extract (Oxoid Lab. Lemco) 2.5, Tryptone (Oxoid) 2.5, Neutralised soya peptone (Oxoid) 2.5, soluble starch (BDH) 2.5, glucose 2.5, malt extract (Oxoid) 2.5, glycerol 2.5 and trace elements solution 5.

The composition of the trace elements solution was (all concentrations in g/litre): $CaCl_2 \cdot 2H_2O$ 10, $MgCl_2 \cdot 6H_2O$ 10, NaCl 10, $FeCl_3$ 3, $ZnCl_2$ 0.5, $CuCl_2 \cdot 2H_2O$ 0.5, $MnSO_4 \cdot 4H_2O$ 0.5 and $CoCl_2 \cdot 6H_2O$ 1.

Seed medium (50 ml) in 250-ml Erlenmeyer flasks was inoculated with *Streptomyces* species E225 by addition of small areas of growth cut from an agar plate. Flasks were incubated at 27°C for 48 hours on a gyratory shaker at 240 rpm.

The production medium consisted of (all concentrations in g or ml/litre): Soluble starch (BDH) 20, casein (Sigma) 2, soyabean flour (Arkasoy 50) 10, glucose 20, calcium carbonate 5, magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 1, casein hydrolysate 2, K_2HPO_4 0.5 and trace element solution 10.

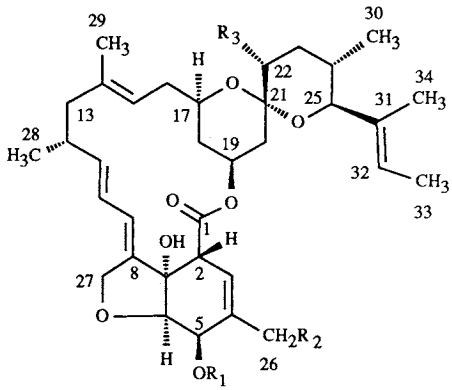
Production medium (50 ml) contained in 250-ml Erlenmeyer flasks was inoculated with 2 ml of the seed, and incubated at 27°C on a gyratory shaker at 240 rpm.

During the fermentation, samples were removed, treated with an equal volume of acetone, filtered and the filtrate assayed by HPLC (Ultrasphere ODS 5 μm column 25 cm × 4.6 mm, eluted with 90 : 10 methanol-water at 1 ml/minute monitored by UV absorption at 246 nm) to determine optimum time for harvest. After 13 days, the whole broth from 200 flasks was combined and centrifuged.

† These milbemycin metabolites were reported in Eur. Pat. Appl. 254, 583 published Jan. 27, 1988.

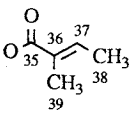
Instrumental

^{1}H and ^{13}C NMR spectra were recorded at ambient temperature, in a 5-mm $^{1}H/^{13}C$ dual probe on a Brüker AM400 spectrometer using standard software. The 2D ^{1}H COSY-45 NMR spectra[2] for **1** to **4** were acquired using 16 or 32 scans for each of 512×2K FID's. The FID matrix was zero-filled to 2K×1K prior to double FT. The 2D $^{1}H,^{13}C$ COSY NMR spectra for **1** and **2** were acquired with ^{1}H decoupling in both dimensions[2] and were tuned for $^{1}J_{CH}=140$ Hz, with 64 scans for each of 256×4K FID's. The 2D $^{1}H,^{13}C$ COLOC NMR spectrum[2] of **3** was tuned for $^{n}J_{CH}=9.5$ Hz and acquired with 800 scans for each of 240×4K FID's. The compounds were dissolved in $CDCl_3$. HR-MS data were obtained using a VG Analytical ZAB-IF instrument.



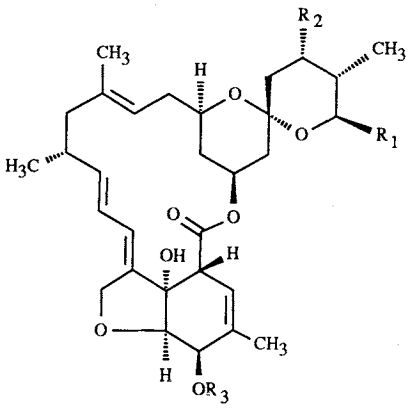
VM 44857 (**1**) $R_1=H$ $R_2=H$ $R_3=H$

VM 44864 (**2**) $R_1=CH_3$ $R_2=H$ $R_3=OH$



VM 44865 (**3**) $R_1=CH_3$ $R_2=$ $R_3=OH$

VM 44866 (**4**) $R_1=H$ $R_2=H$ $R_3=OH$



Milbemycin D (**5**) $R_1=CH(CH_3)_2$ $R_2=H$ $R_3=H$

Factor C or LL-F28249 β (**6**) $R_1=$  $R_2=OH$ $R_3=H$

7 $R_1=$  $R_2=OCOCH_3$ $R_3=COCH_3$

Results and Discussion

The metabolites were detected using an *in vitro* assay measuring activity against the nematode *Haemonchus contortus*[1], and the HPLC method described above.

Isolation

The cell mass was slurried in water (0.75 litre), stirred with acetone (1.0 litre) for 30 minutes and filtered through Hyflo supercel (BDH Chemicals Limited). The residue was washed with acetone (3×1 litre) and the combined filtrates were evaporated to remove the acetone. The aqueous residue (600 ml) was extracted with chloroform (4×500 ml), the combined chloroform extracts dried ($MgSO_4$) and evaporated to yield a brown oil (6 g). The oil was applied to a short silica column (100 g) and the column eluted sequentially with 200 ml volumes of hexane - ether mixtures of the following compositions (1 : 0, 3 : 1, 1 : 1, 1 : 3 and 0 : 1). The bulk of the milbemycin metabolites were contained in the last two fractions which were evaporated to yield white solids (356 and 522 mg, respectively). These two solids were separately chromatographed on silica (75 g) eluted with 0～100% ether in hexane and the fractions monitored by HPLC. The fractions containing VM 44857 (**1**), VM 44864 (**2**) and VM 44865 (**3**) were evaporated to yield the pure metabolites (280, 107 and 11 mg, respectively) as white amorphous solids. The fractions containing VM 44866 (**4**) were further purified by preparative TLC on silica gel taper plates (Analtech) eluted with 5% methanol in dichloromethane to yield pure VM 44866 (**4**) (6 mg) as a white amorphous solid.

Structure and Solution Conformations of VM 44857 (1)

The metabolite VM 44857 (**1**), possessed a UV λ_{max}^{MeOH} nm (ε) 224 (31,300) suggesting a conjugated diene, $[\alpha]_D^{25}$ +111.2° (*c* 0.25, acetone) and the mass spectrum gave a molecular ion (*m/z* 568.3412) corresponding to a molecular formula $C_{34}H_{48}O_7$. The ^{13}C NMR spectrum exhibited 34 resonances and was very similar to that reported[3] for milbemycin D (**5**), with the signals for the macrocyclic ring system C-1 to C-30 all within 0.8 ppm with the exception of C-25. This suggested that VM 44857 was a milbemycin related to milbemycin D (**5**), with a different substituent at C-25.

The ^1H and ^{13}C NMR spectra (Table 1) were assigned using 2D ^1H COSY-45 and 2D ^1H,^{13}C COSY experiments and the structure for VM 44857 shown to be **1**, which is unsubstituted at both C-22 and C-23, similar to milbemycin D (**5**), and possessing an unsaturated C-25 side chain. The same C-25 side chain has recently been reported[4] to occur in another natural milbemycin (**6**) although this milbemycin, unlike VM 44857 (**1**), possesses an axial C-23-hydroxyl group.

The observation of an NOE between 17-H and 19-H, (*i.e.* NOE[17-H]19-H) as well as NOE[18-H_{ax}]20-H_{ax} confirms the 1,3-diaxial relationship of these protons and consequently the conformation of the O-17～C-21 tetrahydropyran ring (Fig. 1). The stereochemistry of the spiro ring fusion was confirmed by the observation of NOE[17-H]25-H. The large value of $^3J_{24,25}$ (9.3 Hz) indicates that the O-21 to C-25 tetrahydropyran ring also exists in a chair conformation with C-30 and C-31 in equatorial positions. This was confirmed by the observation of NOE[25-H]23-H_{ax}.

The configuration of the C-25 side chain was confirmed by the observation of only a small NOE[32-H]34-CH_3 indicative of their *trans* orientation, and a large NOE[25-H]32-H.

The assignment of the ^{13}C NMR spectrum for VM 44857 (**1**) is in complete agreement with that reported[3] for milbemycin D (**5**) for C-1 to C-30 and contrary to a recent suggestion[5] concerning the spectral

Table 1. ^{1}H and ^{13}C chemical shifts δ(ppm)[a], ^{1}H NOE's and $^{1}H,^{1}H$ coupling constants ^{n}J (Hz)[b] for VM 44857 (**1**).

Atom	δ_H	δ_C	$^{2}J_{HH}$	$^{3}J_{(HaHb)}$ Hz	^{1}H NOE[c]
1	—	173.6			
2	3.26	45.7		$J(2,3)\sim2.4$	3(S),5(S),6(S),9(S),27a(S)
3	5.4	118.1			
4	—	137.7			
5	4.28	67.7		$J(5,6)\sim5.6$	3(S),6(L),26(S)
6	3.95	79.2		$J(6,5)\sim6.1$	2(S),5(L),10(S),27b(S)
7	—	80.1			
8	—	139.4			
9	5.77	120.2		$J(9,10)\sim11$	
10	5.72	123.3		$J(10,9)\sim11$, $J(10,11)\sim13$	12(S),27a(S),27b(S)
11	5.33	142.8		$J(11,10)\sim12.8$, $J(11,12)\sim10.1$	
12	2.42	35.9		$J(12,11)\sim10.0$, $J(12,13_{eq})\sim4.0$, $J(12,13_{ax})\sim11.8$	10(M),11(S),13_{eq}(S),28(S),29(S)
13_{eq}	2.20	48.5			
13_{ax}	1.85		12.5	$J(13,12)\sim12.1$	11(M),13_{eq}(L),15(M),28(S)
14	—	136.8			
15	4.98	121.0		$J(15,16)\sim9$, $J(15,16)\sim7.5$	11(S),13_{ax}(M),16(S),17(S)
16	2.3~2.2	34.7			
17	3.56	67.4		$J(17,18_{eq})\sim2.2$, $J(17,18_{ax})\sim11.3$, $J(17,16)\sim9.4$, $J(17,16)\sim6.0$	15(S),16(S),18_{eq}(S),19(M),25(S)
18_{eq}	1.80	36.5	12.6	$J(18,17)\sim2.2$, $J(18,19)\sim4.9$	9(S),10(S),17(S),18_{ax}(M),19(M)
18_{ax}	0.85		12.6	$J(18,17)\sim11.6$, $J(18,19)\sim11.6$	
19	5.35	68.7		$J(19,18_{ax})\sim11.5$, $J(19,18_{eq})\sim5.4$, $J(19,20_{ax})\sim11.5$, $J(19,20_{eq})\sim5.4$	
20_{eq}	2.02	41.1	12.0	$J(20,19)\sim5.0$	19(S),20_{ax}(L)
20_{ax}	1.34		11.7	$J(20,19)\sim11.7$	18_{ax}(S),19(S),20_{eq}(L),22(S)
21	—	97.6			
22	1.7~1.5	35.6			
23	1.7~1.5	27.6			
24	1.55	31.5			30(S)
25	3.45	82.4		$J(25,24)\sim9.3$	17(M),23_{ax}(M),30(S),32(L)
26	1.86	19.9			3(L),5(M)
27a	4.69	68.4	14.0		
27b	4.65		14.0		
28	0.99	22.3		$J(28,12)\sim6.7$	11(S),12(M),13_{eq}(S),13_{ax}(S)
29	1.53	15.5			10(S),12(M),13_{eq}(S),16(S)
30	0.68	17.7		$J(30,24)\sim5.8$	24(M),25(M),32(S), 34(S)
31	—	134.9			
32	5.4	122.9		$J(32,33)\sim7.1$	
33	1.64	13.1		$J(33,22)\sim6.7$	32(M)
34	1.59	11.0			25(S),30(S),32(S)

[a] For δ_H, δ TMS=0 for δ_C, δ $CDCl_3$=77.0.

[b] In addition the following long range coupling constants were recorded: $^{5}J_{2,26}\sim2.3$ Hz, $^{4}J_{9,27a}\sim^{4}J_{9,27b}\sim2.3$ Hz, $^{4}J_{15,17}\sim1.2$ Hz, $^{4}J_{15,29}\sim1.2$ Hz, $^{4}J_{18_{eq},20_{eq}}\sim2.1$ Hz, $^{5}J_{33,34}\sim1.0$ Hz and $^{4}J_{34,32}\sim1.0$ Hz.

[c] S=small (0~2%), M=medium (2~5%), L=large ($\geq$5%).

assignments for milbemycin D (**5**), the 2D $^{1}H,^{13}C$ COSY and 2D ^{1}H COSY-45 NMR spectra, Figs. 2 and 3, respectively, clearly show that the signal for C-17 is at higher field than that for C-19. The apparent reversal[5] of the relative positions of the C-17 and C-19 signals in the related milbemycin (**6**) is possibly caused by the effect of hydrogen bonding between the axial 23-hydroxyl group, present in this particular metabolite, and O-17 affecting the shielding at C-17. The presence of hydrogen bonding between the 23_{ax}-hydroxyl group and O-17 has been observed[6] in the X-ray structure of a closely related metabolite.

Using ^{1}H NOE difference spectroscopy experiments it was also possible to compare the solution state

conformation of VM 44857 (**1**) with the solid state conformation of a related milbemycin (**7**) for which X-ray crystallographic data are available[6]. Hydrogen atoms were added to the X-ray structure using standard bond lengths and angles for each atom type and the distances between adjacent protons measured. Overall 67 NOE's (Table 1) were observed in the NOE difference spectroscopy experiments and of these 57 corresponded to protons less than 3.1Å apart in the modified crystal structure of **7**. A further 9 NOE's were very small (<0.6% enhancement) and were attributed to longer range interactions with relatively isolated protons. The remaining NOE[34-CH_3]25-H was attributed to some degree of conformational freedom around the C-25~C-31 bond, although the large NOE[25-H]32-H indicates that the solution conformation of the C-25 side chain is predominantly the same as in the crystal structure of **7**. The solution conformation of VM 44857 (**1**) was consequently concluded to be essentially the same as that in the crystal structure of **7**. A number of NOE's merit particular mention. The observation of NOE[2-H]27-Ha, NOE[6-H]27-Hb and NOE[6-H]5-H confirmed the relative stereochemistry of the C-2 to C-27 hexahydrobenzofuran part of the molecule. The NOE's observed between 29-CH_3 and 10-H, 12-H, 13-H_{eq} and 16-H are consistent with the crystal structure for **7**. The cross-ring NOE[18-H_{eq}]9-H is further confirmation of the macrocyclic ring conformation, although the NOE[18-H_{eq}]11-H could not be observed due to interference from NOE[18-H_{eq}]19-H.

Fig. 1. Stereochemistry of spiroketal portion of VM 44857.

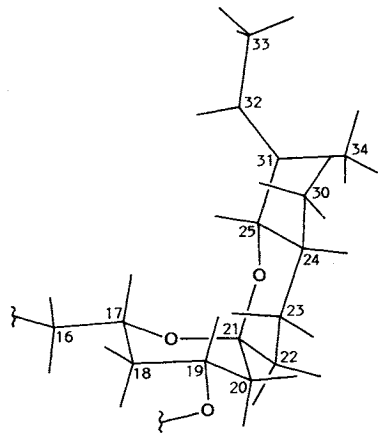


Fig. 2. Portion of 2D ^1H,^{13}C COSY NMR spectrum of VM 44857 (**1**).

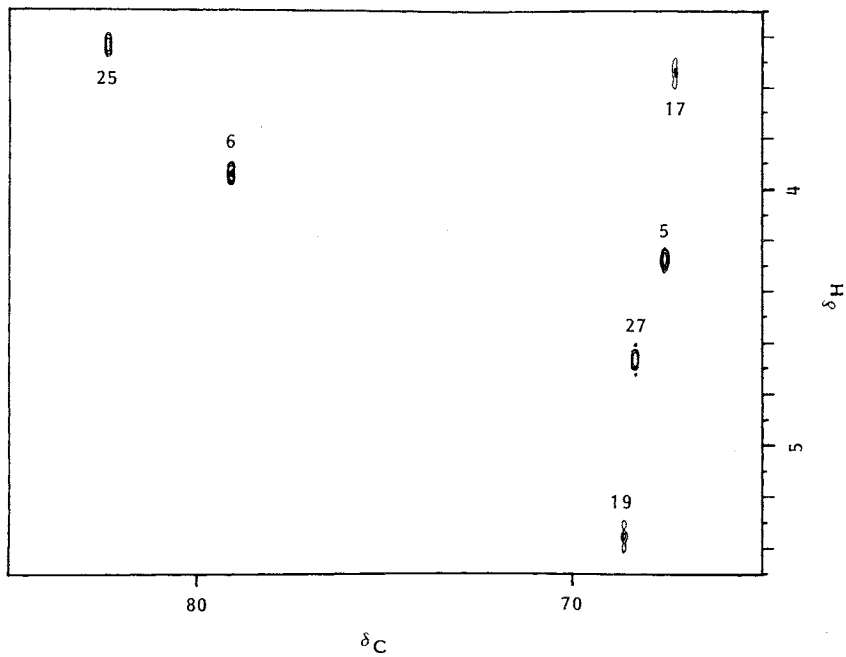
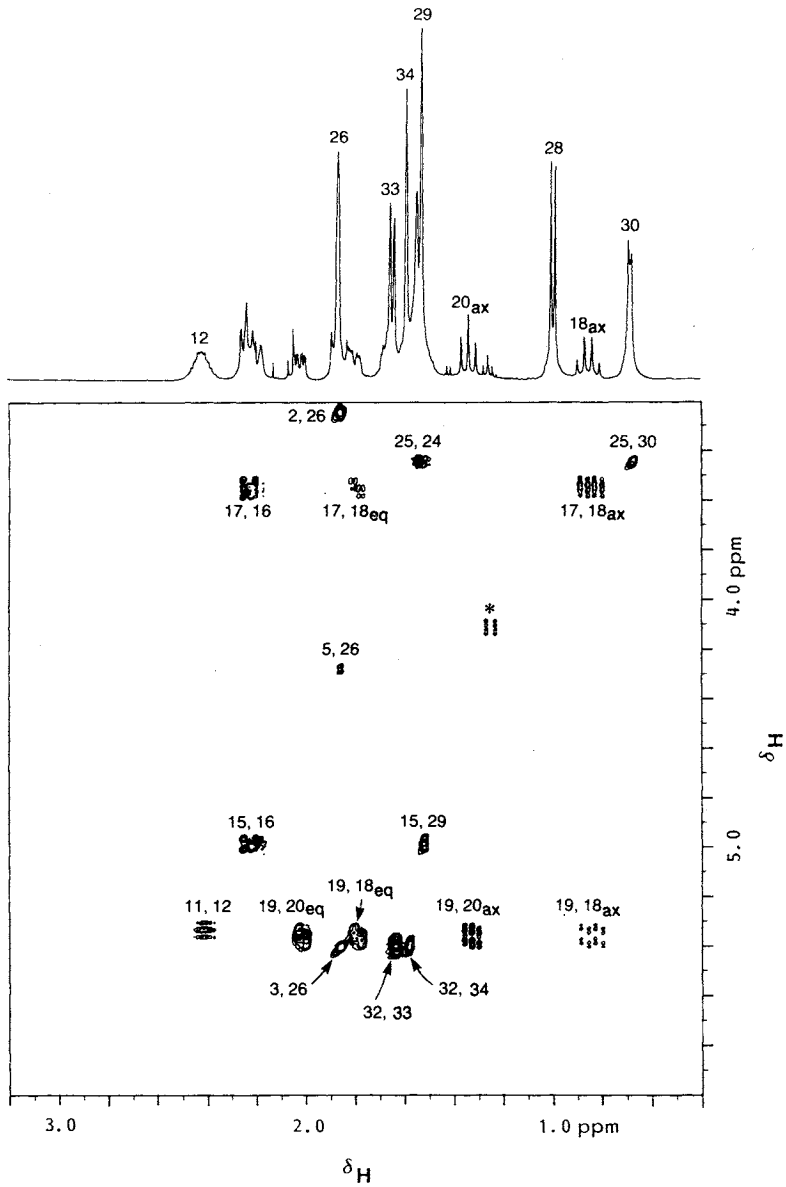


Fig. 3. Portion of 2D ^{1}H COSY-45 NMR spectrum of VM 44857 (**1**).



Structures of VM 44864 (**2**), VM 44865 (**3**) and VM 44866 (**4**)

The structures of the other three milbemycin metabolites VM 44864 (**2**), VM 44865 (**3**) and VM 44866 (**4**) were deduced by analysis of their respective 1D and 2D ^{1}H and ^{13}C NMR spectra (Table 2 and 3) in the light of the unambiguous assignments obtained for VM 44857.

VM 44864 (**2**) and VM 44866 (**4**) were obviously structurally related on the basis of their ^{13}C NMR spectra. Analysis of the ^{1}H-^{1}H connectivities in the 2D ^{1}H COSY-45 NMR spectrum of VM 44866 (**4**) gave a similar connectivity map to VM 44857 (**1**), except that the spectrum indicated that C-22 was hydroxylated. This was consistent with the mass spectral data which gave a molecular ion (*m/z* 584.3335) corresponding to a molecular formula of $C_{34}H_{48}O_8$. The observation of 23-H_{ax} as a highfield quartet ($^{3}J_{23,22}\sim^{3}J_{23,24}\sim^{2}J_{23,23}$

Table 2. Assignment of ^1H NMR data for metabolites **1** to **4**.

Atom	δ_H in ppm[a]			
	VM 44857 (1)	VM 44864 (2)	VM 44865 (3)	VM 44866 (4)
1	—	—	—	—
2	3.26	3.31	3.37	3.27
3	5.4	5.39	5.74	5.41
4	—	—	—	—
5	4.28	3.96	4.19	4.29
6	3.95	4.02	4.06	3.97
7	—	—	—	—
8	—	—	—	—
9	5.77	5.74	5.75	5.75
10	5.72	5.74	5.75	5.75
11	5.33	5.32	5.35	5.33
12	2.42	2.42	2.43	2.42
13_{eq}	2.20	2.20	2.21	2.20
13_{ax}	1.85	1.85	1.85	1.85
14	—	—	—	—
15	4.98	4.98	4.99	4.99
16	2.3~2.2	2.23	2.24	2.23
17	3.56	3.62	3.62	3.62
18_{eq}	1.80	1.80	1.80	1.80
18_{ax}	0.85	0.89	0.90	0.87
19	5.35	5.32	5.35	5.35
20_{eq}	2.02	1.88	1.9	1.9
20_{ax}	1.34	1.92	1.9	1.9
21	—	—	—	—
22	1.7~1.5	3.34(ax)	3.35(ax)	3.33(ax)
23	1.7~1.5	1.87(eq), 1.41(ax)	1.87(eq), 1.41(ax)	1.87(eq), 1.41(ax)
24	1.55	1.70	1.70	1.70
25	3.45	3.40	3.40	3.40
26	1.86	1.81	4.78(a), 4.69(b)	1.85
27a	4.69	4.69	4.70	4.69
27b	4.65	4.62	4.64	4.65
28	0.99	1.00	1.00	1.00
29	1.53	1.54	1.54	1.54
30	0.68	0.70	0.70	0.70
31	—	—	—	—
32	5.4	5.40	5.41	5.40
33	1.64	1.64	1.65	1.65
34	1.59	1.57	1.58	1.57
OCH_3	—	3.50	3.49	—
35	—	—	—	—
36	—	—	—	—
37	—	—	6.89	—
38	—	—	1.80	—
39	—	—	1.85	—

[a] δ TMS = 0.

Table 3. Assignment of ^{13}C NMR data for metabolites **1** to **4**.

Atom	δ_C in ppm[a]			
	VM 44857 (1)	VM 44864 (2)	VM 44865 (3)	VM 44866 (4)
1	173.6	173.8	173.3	173.7
2	45.7	45.6	45.5	45.7
3	118.1	118.4	121.0	118.0
4	137.7	135.8	134.9	137.8
5	67.7	76.9	74.2	67.7
6	79.2	77.5	77.3	79.1
7	80.1	80.3	80.4	80.1
8	139.4	139.7	139.4	139.5
9	120.2	119.4	119.8	120.2
10	123.3	123.47	123.4	123.4
11	142.8	142.3	142.6	142.7
12	35.9	35.9	35.9	36.0
13	48.5	48.5	48.5	48.5
14	136.8	137.2	137.2	137.2
15	121.0	120.5	120.5	120.6
16	34.7	34.6	34.6	34.6
17	67.4	67.9	68.0	68.0
18	36.5	36.3	36.3[b]	36.4[b]
19	68.7	68.6	68.9	68.6
20	41.1	36.4	36.4[b]	36.5[b]
21	97.6	98.8	98.8	98.8
22	35.6	71.5	71.5	71.6
23	27.6	36.8	36.9	36.9
24	31.5	32.0	32.1	32.1
25	82.4	81.8	81.9	81.9
26	19.9	19.8	64.3	19.9
27	68.4	68.2	68.3	68.4
28	22.3	22.3	22.3	22.3
29	15.5	15.5	15.6	15.5
30	17.7	17.4	17.5	17.5
31	134.9	134.0	134.1	134.1
32	122.9	123.53	123.6	123.6
33	13.1	13.1	13.1	13.1
34	11.0	10.9	11.0	10.9
OCH_5	—	57.7	57.8	—
35	—	—	167.5	—
36	—	—	128.4	—
37	—	—	137.6	—
38	—	—	14.4	—
39	—	—	12.1	—

[a] δ $CDCl_3$ = 77.0 ppm.
[b] Assignment uncertain.

~12.0 Hz) indicated that the C-22-hydroxyl group was equatorial. The observation of a methoxyl group in the ^1H spectrum of VM 44864 (**2**) and the close similarity between the ^1H and ^{13}C spectra of VM 44864 (**2**) and VM 44866 (**4**), with the exceptions of 5-H and 6-H and C-5 and C-6, lead to the conclusion that VM 44864 (**2**) was the 5-methoxy derivative of VM 44866 (**4**). Additional physico-chemical data was recorded as follows:

VM 44866 (**4**) UV λ_{max}^{MeOH} nm (ε) 244 (30,500), $[\alpha]_D^{25}$ +81.9° (*c* 0.16, acetone).

VM 44864 (**2**) UV λ_{max}^{MeOH} nm (ε) 244 (28,300), $[\alpha]_D^{25}$ +96.8° (*c* 0.25, acetone), HR-MS *m/z* 598.3504 (M^+, $C_{35}H_{50}O_8$).

The mass spectrum of VM 44865 (**3**) gave a molecular ion (*m/z* 696) corresponding to a molecular formula of $C_{40}H_{56}O_{10}$. Both the ^1H and ^{13}C spectra indicated that 26-CH_3 was absent and the ^{13}C spectrum showed a new CH_2 signal at 64.3 ppm. In addition, the ^1H NMR spectrum showed a new set of resonances corresponding to a second dimethylvinyl residue and the ^{13}C spectrum showed the presence of a new ester carbonyl group. Comparison of the ^1H and ^{13}C spectra with those for the other metabolites indicated that this was a 22-hydroxy-5-methoxy derivative and it was therefore deduced that it was oxidatively functionalised on 26-CH_3 with a 2-methylbut-2-enoate moiety. The structure of VM 44865 (**3**) was conclusively confirmed by the results of a 2D ^1H,^{13}C COLOC experiment which showed connectivities from C-26 to 3-H and from C-35 to 39-H and 26-Hb. The stereochemistry of the 2-methylbut-2-enoate moiety was confirmed as *E* on the basis of the absence of an NOE between 37-H and 39-H.

VM 44864 (**2**), VM 44865 (**3**) and VM 44866 (**4**) are particularly interesting in that they all possess an equatorial hydroxyl group at C-22, unlike the more common C-23 axial hydroxy compounds. Only one previous milbemycin has been reported[7] which possesses a 22 hydroxy-23-dihydro functionality although a number of 22,23-dioxygenated milbemycins are known[8], and, as we have previously shown[9], at least some of these have diequatorial stereochemistry at C-22 and C-23.

Acknowledgements

The authors are indebted to Mr. G. RISBRIDGER for mass spectral data and to Mrs. MARY DRISCOLL for typing this manuscript.

References

1) HOOD, J. D.; R. M. BANKS, M. D. BREWER, J. P. FISH, B. R. MANGER & M. E. POULTON: A novel series of milbemycin antibiotics from *Streptomyces* strain E225. I. Discovery, fermentation and anthelmintic activity. J. Antibiotics 42: 1593～1598, 1989
2) DEROME, A. E. (*Ed.*): Modern NMR Techniques for Chemistry Research. Pergamon Press, 1987
3) ONO, M.; H. MISHIMA, Y. TAKIGUCHI, M. TERAO, H. KOBAYASHI, S. IWASAKI & S. OKUDA: Milbemycins, a new family of macrolide antibiotics. Studies on the biosynthesis of milbemycins α_2, α_4 and D using ^{13}C labeled precursors. J. Antibiotics 36: 991～1000, 1983
4) CARTER, G. T.; J. A. NIETSCHE & D. B. BORDERS: Structure determination of LL-F 28249 α, β, γ, and λ, potent antiparasitic macrolides from *Streptomyces cyaneogriseus* spp. *noncyanogenus*. J. Chem. Soc. Chem. Commun. 1987: 402～404, 1987
5) CARTER, G. T.; J. A. NIETSCHE, M. R. HERTZ, D. R. WILLIAMS, M. M. SIEGEL, G. O. MORTON, J. C. JAMES & D. B. BORDERS: LL-F28249 antibiotic complex: A new family of antiparasitic macrocyclic lactones. Isolation, characterization and structures of LL-F28249 α, β, γ, λ. J. Antibiotics 41: 519～529, 1988
6) RAMSAY, M. V. J.; S. M. ROBERTS, J. C. RUSSELL, A. H. SHINGLER, A. M. Z. SLAWIN, D. R. SUTHERLAND, E. P. TILEY & D. J. WILLIAMS: Novel antiparasitic agents derived by modification of a new natural product series. Tetrahedron Lett. 28: 5353～5356, 1987
7) MCCORMICK, P. A. & R. T. GOEGELMAN (Merck): Anthelmintic macrocyclic lactones and their production by fermentation. Eur. Pat. Appl. 204, 421, Dec. 10, 1986
8) MISHIMA, H.; J. IDE, S. MURAMATSU & M. ONO: Milbemycins, a new family of macrolide antibiotics. Structure determination of milbemycins D, E, F, G, H, J and K. J. Antibiotics 36: 980～990, 1983 and references cited therein
9) BLANCHFLOWER, S. E.; R. J. J. DORGAN, J. R. EVERETT & S. A. READSHAW: An investigation of the stereochemistry of a 22,23-dioxygenated milbemycin. Tetrahedron Lett. 29: 6645～6648, 1988