Structural Studies of MM46115, A Novel Tetronic Acid Containing Macrolide with Antiviral and Antibacterial Activity Isolated from *Actinomadura pelletieri*

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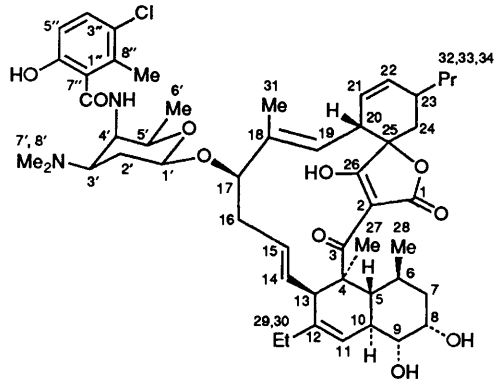
MM46115, a novel antiviral antibiotic produced by the fermentation of *Actinomadura pelletieri* IP/729.63, has been shown by spectroscopic studies to have a tetronic acid containing macrolide structure and be a new member of the tetrocarcin and kijanimicin family of antibiotics. The molecule also contains a novel diaminohexopyranose linked to a substituted salicylic acid moiety *via* an amide bond. Evidence for the proposed structure is presented.

In the course of screening microbial culture filtrates for compounds with antiviral activity, a novel macrolide, MM46115 **1**, containing an acyl tetronic acid moiety and exhibiting antiviral and antibacterial activity was isolated from the fermentation of *Actinomadura pelletieri* IP/729.63.[1] Herein, we report the structural studies of this natural product by spectroscopic methods.

Results and Discussion

MM46115 **1** was obtained from dichloromethane–methanol as



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elongated pentagonal plates; m.p. 196–201 °C; $[\alpha]_D^{25}$ − 122.1° (*c* 0.84, $CHCl_3$). The UV spectrum [λ_{max} 235sh and 280 (ε 11 000) nm in methanol] was very complex and sensitive to both acid and base, indicating the presence of more than one chromophore with an ionisable function. The IR spectrum ($CHCl_3$) indicated the presence of hydroxy groups (*v* 3480br cm^{-1}), a five-membered ring ketone or lactone (*v* 1745 cm^{-1}), a secondary amide (*v* 3412, 1662 and 1505 cm^{-1}), and a number of alcohol or ether groups (*v* 1000–1100 cm^{-1}).

The molecular formula of **1**, $C_{50}H_{67}ClN_2O_{10}$, was established by NMR spectroscopy, MS, XES and microanalysis studies. Heavy atom analysis by X-ray emission spectroscopy (XES)[2] indicated the presence of chlorine only. From the ^{13}C NMR spectra, it was concluded that **1** had 13 quaternary, 21 methine and 7 methylene carbons in addition to the 9 methyl groups indicated in the ^1H NMR spectrum (Table 1). Therefore, it followed that 50 carbons and 62 non-exchangeable protons (directly attached to carbon atoms) were present. The number of exchangeable protons could not be determined unambiguously from the ^1H NMR spectrum. Both FABMS and EIMS showed a highest mass ion at *m/z* 891 and 890 respectively. When sodium ions were present in the glycerol or

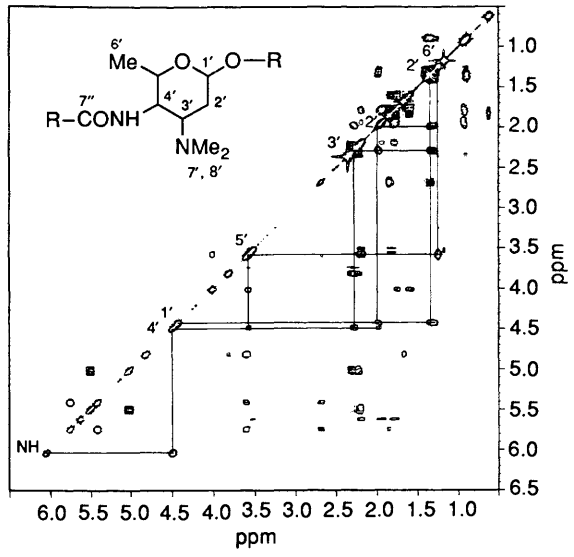


Fig. 1 Contour plot of ^1H COSY–45 NMR spectrum of **1** detailing the correlation of the partial structure shown. The numbers refer to the carbon numbering system.

nitrobenzyl alcohol matrix, the FAB mass ions *m/z* 913, 935 and 957 were observed in the ratio of 1:2:1. This result confirmed that *m/z* 891 was the [*M* + H] ion. In addition, the facile exchange of two further sodium ions indicated the presence of two acidic protons. High resolution measurement of the molecular ion in the EIMS gave 890.4487 and 890.4507 (calculated for $C_{50}H_{67}ClN_2O_{10}$ 890.4484) establishing the molecular formula. Elemental analysis (C, H, N) was satisfactory for the monohydrate. The molecule, therefore, had a double-bond equivalent of 18.

The NMR spectroscopic data for **1** are summarised in Table 1. Assignment of the ^1H NMR spectrum was achieved with the aid of a ^1H COSY–45 NMR experiment and additional homonuclear decoupling. Analysis of the proton–proton connectivities derived from the COSY plot yielded several structural fragments. These structural fragments are illustrated in the appropriately labelled COSY-45 plots of Figs. 1–3. Correlation between the protons and their directly bonded carbon nuclei was established using a ^1H, ^{13}C COSY NMR experiment. Differently tuned ^1H, ^{13}C COLOC NMR experiments were used to confirm the nature of the various structural fragments. In addition, the proton–carbon connectivities derived from these latter experiments enabled the structural fragments to be pieced together with the incorporation of the quaternary carbons. This method of structure determination led to the macrolide structure of **1** from C-3 to C-26 as well as the nature of the C-17 functionality.

Table 1 NMR Spectral data for MM46115

C no.	δ_C	Mult.[a]	δ_H	J_H (Hz)	COLOC[b]	NOEs[c]
1	166.8	C				
2	105.6	C				
3	208.7	C			5, 27	
4	53.7	C			5, 7eq, 10/13, 27	
5	38.0	CH	3.54	$^3J_{5,6}$ $^3J_{5,10}$ 10.0	7eq, 10/13, 11, 27, 28	7 ax, 14, 10
6	30.5	CH	1.84		7eq, 7ax, 8, 10, 28	
7	39.9	CH_2	ax 1.61	^2J 14.7; $^3J_{7ax,6}$ 12.1; $^3J_{7ax,8}$ 3.0	28	5, 7 eq, 8, 9
			eq 1.77			
8	69.9	CH	4.02	$^3J_{7ax,8}$ 3.0; $^3J_{7eq,8}$ $^3J_{8,9}$ 2.7	7eq	7ax, 7eq, 9
9	75.1	CH	3.58	$^3J_{8,9}$ 3.4; $^3J_{9,10}$ 10.8	7eq, 8, 10, 11	
10	40.9	CH	2.20		5, 8, 11	
11	119.1	CH	5.63		10/13	5, 9, 10, 29, 30
12	138.5	C			10/13, 30	
13	57.2	CH	2.22	$^3J_{13,14}$ 10.0	11, 15, 27	
14	132.8	CH	5.51	$^3J_{14,15}$ 15.0; $^3J_{14,13}$ 10.3; $^4J_{14,16}$ 1.8	13, 16	5, 13, 15
15	127.1	CH	5.02	$^3J_{14,15}$ 15.0; $^3J_{15,16}$ 10.5; $^3J_{15,16}$ 4.5	13	13, 14, 16, 17
16	36.4	CH_2	2.24	$^3J_{15,16}$ 4.5; $^3J_{16,17}$ $^4J_{14,16}$ 2.3	14	
			2.32	^2J 14.2; $^3J_{15,16}$ $^3J_{16,17}$ 10.4		
17	84.6	CH	3.82	$^3J_{16,17}$ 10.4; $^3J_{16,17}$ 2.5	16, 19, 31, 1′	19, 1′
18	141.6	C			16, 20, 31	
19	123.2	CH	4.82	$^3J_{19,20}$ 10.2; $^4J_{19,31}$ 1.5	17, 20, 21, 31	17, 21, 20
20	42.1	CH	3.60		19, 21, 22, 24	
21	124.8	CH	5.42	$^3J_{21,22}$ 10.0; $^3J_{20,21}$ $^4J_{21,23}$ 2.2	19, 20, 31	19, 20, 22
22	132.4	CH	5.75	$^3J_{21,22}$ 10.1; $^3J_{22,23}$ $^4J_{22,20}$ 2.5	20	20, 21, 23, 32/33
23	34.0	CH	2.70		21, 22, 24	22, 24, 32/33
24	36.9	CH_2	1.87		22	
25	84.7	C			20, 21, 24	
26	199.9	C			20, 24	
27	16.7	CH_3	1.19		5, 13	6, 10, 13, 11, 28
28	19.9	CH_3	0.64	$^3J_{6,28}$ 7.0		5, 6, 7, 7ax, 7eq, 27
29	27.5	CH_2	1.80, 1.96	^2J 14.8; $^3J_{29,30}$ 7.4	10/13, 11, 30	11, 29, 30
30	12.5	CH_3	0.94	$^3J_{29,30}$ 7.5		
31	11.5	CH_3	1.69	$^4J_{19,31}$ 1.4	19, 17	16, 20
32	37.8	CH_2	1.3–1.4			
33	19.7	CH_2	1.3–1.4		34	
34	14.2	CH_3	0.91	$^3J_{33,34}$ 7.0		
1′	98.8	CH	4.43	$^3J_{1',2'ax}$ 9.8; $^3J_{1',2'eq}$ 2.3	17, 2′eq, 2′ax	17, 2′eq, 3′, 5′, 2′ax
2′	31.7	CH_2	ax 1.32		1′, 4′	
			eq 2.00			1′, 2′ ax, 3′
3′	64.0	CH	2.30		2′eq, 2′ax, 4′, 7′, 8′	
4′	48.5	CH	4.49	$^3J_{4',NH}$ 11.0; $^3J_{3',4'}$ 3.4; $^3J_{4',5'}$ 1.0	2′eq, 6′	3′, 5′, 6′, 4′-NH
5′	70.4	CH	3.58	$^3J_{4',5'}$ 1.6; $^3J_{5',6'}$ 6.4	6′	
6′	17.3	CH_3	1.26	$^3J_{5',6'}$ 6.3		4′-NH, 4′, 5′
7′, 8′	43.1	CH_3	2.37		3′	
4′-NH			6.04	$^3J_{4',NH}$ 11.0		2′ax, 4′, 6′
1″	125.2	C			5″, 8″	
2″	133.7	C			4″, 8″	
3″	125.0	C			5″, 8″	
4″	131.2	CH	7.21	$^3J_{4'',5''}$ 8.8	8″	
5″	116.2	CH	6.67	$^3J_{4'',5''}$ 8.9; $^6J_{4'',8''}$ 0.4	8″	
6″	153.1	C			4″, 5″	
7″	167.5	C			4′, 4′-NH	
8″	17.2	CH_3	2.34			

[a] From ^{13}C Dept 135 experiment. [b] Protons showing ^nJ couplings (where $n > 1$, usually 2 or 3) to the carbon in column 1. [c] Protons showing positive NOE to those attached to the carbon in column 1.

Assignment of the nature of substitution for the C(11)–C(12) double-bond was hindered by the almost coincident chemical shifts of 10-H and 13-H. The fact that the olefinic proton 11-H showed long-range carbon–proton correlations with C-5 and C-9 rather than C-4 or C-14, indicated that the ethyl substituent was at C-12. Further evidence that the olefinic proton was at C-11 was provided by the NOE enhancements observed for 5-H, 9-H and 10-H upon irradiation of 11-H. The nature of the amino-sugar was readily established from the pattern of proton–proton connectivities shown in Fig. 1. The position of the glycosidic linkage was confirmed by the COLOC correlations of the anomeric carbon C-1′ and its attached proton with nuclei of the macrolide ring [*i.e.* C(17)–(1′)H and C(1′)–(17)H]. Further evidence supporting the (17)O–C(1′) glycosidic linkage was provided by the mutual NOE observed between 17-H and the anomeric proton 1′-H.

The high resolution EIMS gave ions at *m/z* 705, 566 ($C_{34}H_{46}O_7$), 548 ($C_{34}H_{44}O_6$, 566 − H_2O), 530 ($C_{34}H_{42}O_5$, 548 − H_2O) and 169 ($C_8H_6ClO_2$) consistent with the fragmentation shown in **2**. This evidence identified the chlorophenol ring, its attachment to the amino-sugar *via* an amide bond, and the two hydroxy-groups in the aglycone. The position of the amide bond was also evident from the secondary isotope effects[3] observed in the ^{13}C NMR spectrum of partially deuteriated **1** as reported in Table 2. In addition to confirming the position of the amide functionality, the secondary isotope

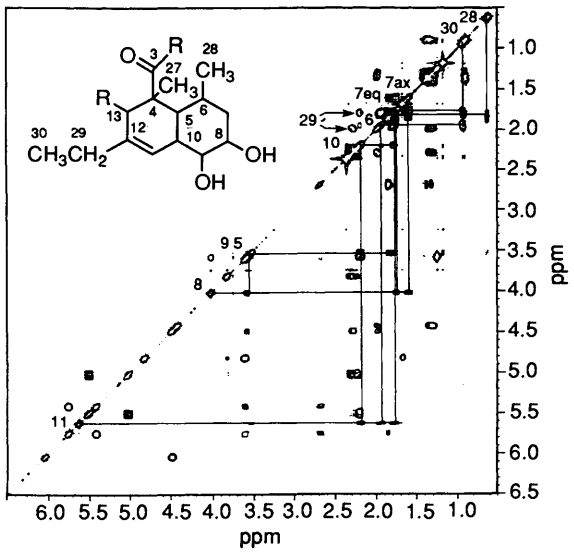


Fig. 2 Contour plot of ^{1}H COSY–45 NMR spectrum of **1** detailing the correlation of the partial structure shown. The numbers refer to the carbon numbering system.

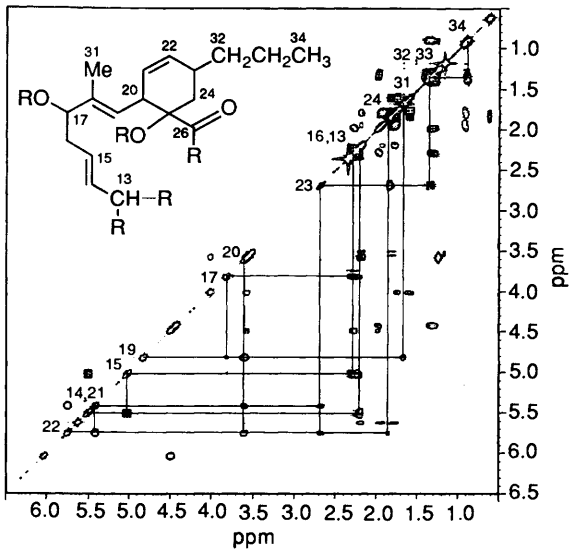
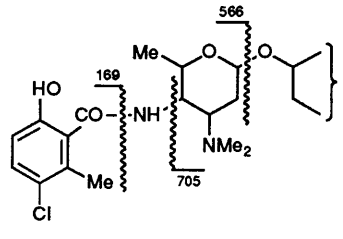


Fig. 3 Contour plot of ^{1}H COSY–45 NMR spectrum of **1** detailing the correlation of the partial structure shown. The numbers refer to the carbon numbering system.



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effects also enabled the chlorophenol carbon C-1″ to be assigned. The upfield secondary isotope observed for C-2″ resonating at 133.7 ppm was interpreted as a 4-bond effect.

The value of 8.9 Hz for the scalar coupling between the aromatic protons dictated that they had to be *ortho* to each other. Furthermore, the ^{1}H COSY–45 NMR spectrum showed correlations indicative of scalar coupling between the C-8″ methyl and both aromatic protons. However, only the scalar coupling (J 0.4 Hz) to the higher field proton (5″-H) could be resolved in the 1D ^{1}H NMR spectrum. The lack of any NOE between the aromatic protons and the C-8″ methyl protons implied that the methyl group could not be *ortho* to these protons and thus precluded any 4-bond coupling. Since the magnitude of benzylic coupling[4] is generally $^6J \geq {}^5J$, the C-8″ methyl group was assigned *para* to the 5″-H. Further evidence for the above assignment was provided by the ^{1}H, ^{13}C connectivities observed in the COLOC spectra. In addition, these ^{1}H, ^{13}C COLOC correlations together with ^{13}C NMR chemical shifts were used to deduce the remaining substitution pattern of the chlorophenol ring. The substituents in C-1″ and C-3″ could have been interchanged but the lack of coupling between C-7″ and 4″-H tentatively led to the assignment of C-7″ *ortho* to the phenolic hydroxy-group.

Table 2 Isotope effect[a] ($^n\Delta$) observed over n bonds upon partial deuteriation of **1**

Atom	$^{13}C^n\Delta$ (ppb)
C-26	—[b]
C-4′	+75.3(2)
C-1″	+42.2(3)
C-2″	−15.5(4)
C-7″	+74.2(2)

[a] A negative sign implies an upfield shift; the number of bonds n over which the effect operates is given in parentheses. [b] Broadening observed.

A reasonable connection between C-3 and C-26 incorporating the remaining quaternary carbons through a tetronic acid led to the full structure of MM46115 **1**. Comparison with related tetronic acid containing macrolides, kijanimicin[5] and the tetrocarcins[6] (antlermicin A[7]), shows good agreement in the IR carbonyl stretching frequency and the ^{13}C NMR spectroscopic chemical shifts[8,9] exhibited by the tetronic acid.

The stereochemical assignments in 13 of the 15 chiral centres and the two macrolide double-bonds in **1** were determined by the analysis of ^{1}H NMR coupling constants and NOE observations. The macrocyclic ring is *cis*-fused to a cyclohexene ring which, in turn, is *trans*-fused to a functionalised cyclohexane ring. The C(5)–C(10) *trans* ring fusion was revealed by the *trans* diaxial coupling of 5-H and 10-H ($^3J_{5,10}$ 10.0 Hz). The large $^3J_{5,6}$ (10.0 Hz) and $^3J_{6,7ax}$ (12.1 Hz) indicated that 6-H had to be *trans*-diaxial relative to both 5-H and 7ax-H and thus the C-28 methyl substituent had to be equatorial. The values of $^3J_{9,10}$ (10.8 Hz), $^3J_{7ax,8}$ (3.0 Hz) and $^3J_{8,9} \sim {}^3J_{7eq,8}$ (2.7 Hz) were consistent with an axial 9-H proton and an equatorial 8-H proton and therefore the *cis* C-8 and C-9 diol. The mutual NOEs observed between 5-H and 7ax-H, and the NOE [7ax-H]9-H, supported the *syn*-1,3-diaxial relationship for these protons. The *cis*-ring fusion at C-4 and C-13 was supported by the assignment of the C-27 methyl group, 10-H, and 13-H as being *syn*/*cis* to one another as evident from the NOEs [27-H]10-H and [27-H]13-H. The stereochemistry at C-13 was further defined by the mutual NOEs observed between 5-H and 14-H thus indicating their close spatial proximity. Therefore, it could be concluded that **1** had a cyclohexane chair and a cyclohexene half chair conformation similar to those defined in the crystal structures of tetronolide[10] and kijanolide.[8,9]

The macrocyclic ring contains two *trans* (*E*) double-bonds. The values of $^3J_{14,15}$ (15.0 Hz) and $^3J_{13,14}$ (10.3 Hz) were consistent with a C(14)–C(15) *trans* (*E*) double-bond and an antiperiplanar conformation for 13-H and 14-H, respectively. The NOE [15-H]13-H, and the mutual NOEs between 5-H and 14-H, confirmed the double-bond configuration and the C(13)–C(14) conformation. The values of $^3J_{15,16ax}$ (10.5 Hz) and $^3J_{15,16eq}$ (4.5 Hz) were consistent with 15-H being antiperiplanar relative to one of the 16-H methylene protons and *gauche* relative to the other. The stereochemistry of the C(18)–C(19) double-bond was assigned as *trans* (*E*) on the basis of the NOE [31-H]20-H as well as the mutual NOEs observed

between 17-H and 19-H. The value of $^3J_{19,20}$ (10.2 Hz) indicated that 20-H was antiperiplanar to 19-H. Further evidence for the (19)H–(20)H antiperiplanar arrangement, and subsequently the assigned C-20 stereochemistry, arose from the observed NOE [31-H]20-H. The large mutual NOEs observed between 17-H and 19-H indicated that 17-H was orientated *trans* or antiperiplanar to 31-H and hence spatially close to 19-H. The values of $^3J_{16ax,17}$ (10.4 Hz) and $^3J_{16eq,17}$ (2.5 Hz) established that 17-H was antiperiplanar to one of the 16-H methylene protons and *gauche* to the other. This is different from the solution state behaviour found for kijanimicin derivatives which had 17-H *gauche* to both the 16-H methylene protons.[8] It therefore was concluded that the C-17 relative stereochemistry of **1** was inverted relative to that of kijanimicin and the tetrocarcins. The relative chirality at C-23 and C-25 could not be ascertained from our present study though the consistency of the IR and ^{13}C NMR spectral characteristics of **1** with those of the known tetronic acid macrolides implied the same C-25 stereochemistry existed in **1**.

The equatorial linkage of the amino-sugar to the aglycone was revealed by the axial 1′-H ($^3J_{1',2'ax}$ 9.8 Hz, $^3J_{1',2'eq}$ 2.3 Hz). The values of $^3J_{3',4'}$ (3.4 Hz) and $^3J_{4',5'}$ (1.0 Hz) were consistent with the assigned sugar stereochemistry. The 4-bond proton–proton connectivity observed between 2′eq-H and 4′-H was characteristic of a W-type arrangement of these protons, and therefore, indicated that 4′-H was orientated equatorial to the sugar ring. The NOE [4′-NH]2′ax-H confirmed that the benzamide substituent was axial and indicated that the NH proton was orientated above the sugar ring towards 2′ax-H. The NOEs [1′-H]3′-H and [1′-H]5′-H supported the *syn* 1,3-diaxial inter-relationships for these protons and hence the stereochemical assignments. The relative stereochemistry between the amino-sugar and the aglycone remains undetermined.

Due to the small quantity of MM46115 available for structural studies, it has yet to be degraded chemically to solve the remaining unknown stereochemistry. The crystals obtained were found to be unsuitable for X-ray crystallography studies.

Conclusion

MM46115 is therefore a novel member of a class of tetronic acid containing macrolide antibiotics of which the tetrocarcins[6,7,11] and kijanimicin[5] are the only other known members. They differ in the aglycone, particularly the stereochemistry at C-17, as well as the sugar components. Neither the amino-sugar nor the chlorosalicylic acid in **1** have been reported in the literature though the latter has been previously isolated as the phenolic methyl ether in chlorothricin.[12] It is of interest to note that the other tetronic acid containing macrolides, chlorothricin[12] and PA-46101 A and B,[13] have an extra oxygen in the macrolide ring giving an acyloxy instead of an acyl tetronic acid function. MM46115 has shown inhibitory activity against parainfluenza virus and good activity *in vitro* against a range of gram-positive bacteria.[1]

Experimental

The m.p. was recorded in a Reichert Kofler apparatus and was uncorrected. The following instruments were used: Perkin-Elmer 241 polarimeter, Kontron 810 UV–VIS spectrometer and Perkin-Elmer 580 IR spectrometer with a model 3600 Data Station.

^1H and ^{13}C NMR spectroscopy experiments were conducted on a Bruker AM400 spectrometer in a 5 mm $^1H/^{13}C$ dual probe, using standard Bruker software and a 0.05 mol dm^{-3} $CDCl_3$/TMS solution. The 2D ^1H COSY-45 NMR spectrum[14] was acquired using 64 scans for each of 512 × 2K FIDs. The FID matrix was zero-filled to 2K × 1K prior to double Fourier transformation. The 2D ^1H, ^{13}C COSY NMR spectrum[14] was acquired with ^1H decoupling in both dimensions and was tuned for $^1J_{CH}$ = 140 Hz with 64 scans for each of 256 × 4K FIDS. The 2D ^1H, ^{13}C COLOC experiments[14,15] were tuned for $^nJ_{CH}$ = 7 Hz and $^nJ_{CH}$ = 8.5 Hz and acquired with 700 scans for each of 256 × 8*K* FIDs. The sweep widths for all the 2D NMR experiments were optimised prior to acquisition. The NOE difference experiments were conducted using a modification of the method of Hall and Saunders[16] as described previously.[17]

Mass spectra were acquired using either a V.G. ZAB or JEOL SX-102 mass spectrometer operating under fast atom bombardment conditions, using a glycerol, thioglycerol, or nitrobenzyl alcohol matrix or electron impact (EI) conditions. Accurate mass measurements were carried out using EI ionization at 10 000 resolving power by manual peak matching using perfluorokerosene reference ions.

The isolation and purification of **1** (Found: C, 65.75; H, 8.0; N, 3.45. $C_{50}H_{67}ClN_2O_{10}\cdot H_2O$ requires C, 66.05; H, 7.65; N, 3.1%) has been reported.[1]

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References

1 R. J. Ashton, M. D. Kenig, K. Luk, D. N. Planterose and G. Scott-Wood, *J. Antibiot.*, 1990, **43**, 1387.
2 D. Vaughan, *Energy-Dispersive X-ray Microanalysis*, Kevex Corp., Foster City, 1983.
3 J. C. Christofides and D. B. Davies, *J. Am. Chem. Soc.*, 1983, **105**, 5099; J. Reuben, *J. Am. Chem. Soc.*, 1984, **106**, 6160; 1985, **107**, 1747, 1756; 1986, **108**, 1735; 1989, **109**, 316; M. S. Morales-Rios, R. E. del Rio and P. Joseph-Nathan, *Magn. Reson. Chem.*, 1988, **26**, 552; M. S. Morales-Rios and P. Joseph-Nathan, *Magn. Reson. Chem.*, 1989, **27**, 75; J. R. Everett, G. H. Baker and R. J. J. Dorgan, *J. Chem. Soc., Perkin Trans. 2*, 1990, 717.
4 T. Schaefer and R. Laatikainen, *Can. J. Chem.*, 1983, **61**, 2785; T. Schaefer, R. Sebastian and G. H. Penner, *Can. J. Chem.*, 1985, **63**, 2597; G. H. Penner, *Can. J. Chem.*, 1987, **65**, 538.
5 J. A. Waitz, A. C. Horan, M. Kalyanpur, B. K. Lee, D. Loebenberg, J. A. Marquez, G. Miller and M. G. Patel, *J. Antibiot.*, 1981, **34**, 1101.
6 F. Tomita, T. Tamaoki, K. Shirahata, M. Kasai, M. Morimoto, S. Ohkubo, K. Mineura and S. Ishii, *J. Antibiot.*, 1980, **33**, 668.
7 K. Kobinata, M. Uramoto, T. Mizuno and K. Isono, *J. Antibiot.*, 1980, **33**, 244.
8 A. K. Mallams, M. S. Puar, R. R. Rossman, A. T. McPhail, R. D. Macfarlane and R. L. Stephens, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1497.
9 T. Tamaoki and F. Tomita, *J. Antibiot.*, 1983, **36**, 595.
10 N. Hirayama, M. Kasai, K. Shirahata, Y. Ohashi and Y. Sasada, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2984.
11 T. Tamaoki, M. Kasai, K. Shirahata and F. Tomita, *J. Antibiot.*, 1982, **35**, 979.
12 W. Keller-Schierlein, R. Muntwyler, W. Pache and H. Zahner, *Helv. Chim. Acta*, 1969, **52**, 127.
13 M. Matsumoto, Y. Kawamura, Y. Yoshimura, Y. Terui, H. Nakai, T. Yoshida and J. Shoji, *J. Antibiot.*, 1990, **43**, 739.
14 A. Derome, *Modern NMR Techniques for Chemistry Research*, Pergamon Press, Oxford, 1987.
15 H. Kessler, C. Griesinger and J. Lautz, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 444.
16 J. K. M. Saunders and J. D. Mersch, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1982, **15**, 353.
17 J. R. Everett and J. W. Tyler, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1659.

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