MARTINOMYCIN, A NEW POLYETHER ANTIBIOTIC PRODUCED BY *Streptomyces salvialis*

II. ISOLATION AND STRUCTURE DETERMINATION

GUY. T. CARTER*, GERHARD SCHLINGMANN, GRANT B. KENION, LISA MILNE, MAHENDER R. ALLURI, JOSEPH D. KORSHALLA, DAVID R. WILLIAMS, FERNANDO PINHO and DONALD B. BORDERS

American Cyanamid Company, Medical Research Division, Natural Products Chemistry, Lederle Laboratories, Pearl River, N.Y. U.S.A. 10965,

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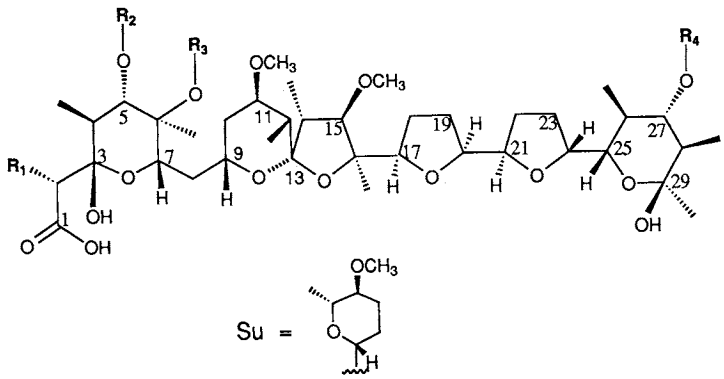
The antibacterial and insecticidal activities exhibited by fermentation broths of *Streptomyces salvialis* (culture LL-D37187), have been attributed to the new polyether antibiotic martinomycin (**1**)[1]. This compound is structurally related to K-41A (**2**)[2], which has the familiar monosaccharide moiety, 4-*O*-methylamicetose, attached to the terminal (F) ring. This substitution pattern is quite rare and was previously known only for K-41A and K-41B[2], and most recently CP-96,797[3]. In this report, the isolation and structure determination of martinomycin are presented.

Streptomyces salvialis was maintained as frozen vegetative material in a liquid medium containing 10% glycerol. The frozen vegetative material was then used as the source of inoculum for a seed medium consisting of: 1.0% glucose, 2.0% dextrin, 0.5% yeast extract, 0.5% N-Z Amine type A, and 0.1% $CaCO_3$. After cultivation for 3 days at 28°C at 200 rpm, this primary seed stage was used as a 10% inoculum for a secondary seed stage which was then used as inoculum (10%) for a 30-liter fermentation tank. The production medium consisted of 2.0% molasses, 1.0% dextrin, 1.5% nutrisoy, and 0.1% Mississippi Lime. Aeration was provided at 1 v/v/m while stirring at 550 rpm, and silicone antifoam agent FD 82 was added as required. The fermentation broth was harvested after 96 hours when the pH had risen to 8.0.

The antibiotic was recovered by extraction of the whole mash with an equal volume (29 liters) of ethyl acetate. The oily residue obtained upon evaporation of the ethyl acetate extract was partitioned between hexane and wet methanol (5% H_2O). The hexane layer containing the bulk of the polyether was evaporated to an oil. Trituration of this oil with methanol (twice 250 ml) provided a solution of material suitable for chromatography. The residue obtained upon concentration of the methanol was redissolved in ethyl acetate - hexane (1 : 3), and was charged onto a column (2.5 × 35 cm) packed with silica gel (100 g, 63 ~ 200 microns, 60 Å) in the same solvent mixture. The column was developed with this solvent mixture at a flow rate



Compound	Name	R_1	R_2	R_3	R_4
1	Martinomycin	CH_3	CH_2	CH_3	Su
2	K-41A	OH	CH_3	CH_3	Su
3	CP-82,009	CH_3	CH_3	Su	CH_3
4	A-204A	CH_3	Su	CH_3	CH_3

of 4.0 ml per minute and fractions were collected at 6.5 minute intervals. Fractions were assayed by TLC on silica gel (silica gel GF, ethyl acetate mobile phase, detected with vanillin-H_2SO_4 spray). Fractions 21~30 yielded a colorless film upon concentration, which was redissolved in *t*-butanol and freeze-dried to give martinomycin (180 mg) as a fluffy white solid.

Martinomycin was recognized as a polyether on the basis of its hexane extractability, red color reaction on TLC plates with vanillin-sulfuric acid spray and its propensity to give larger zones of inhibition against Gram-positive bacteria on agar media at pH 6.0 versus pH 7.8. The compound is optically active, $[\alpha]_D^{26}$ +21° (*c* 1.018%, $CHCl_3$) and exhibits the following significant IR bands (KBr): 3443, 2971, 2934, 2830, 1700, 1459, 1377, 1287, 1165, 1119, 1101, 1069, 1020, 1002, 983, 957 cm^{-1}. Initially the crude extract was analyzed by thermospray mass spectrometry where the characteristic pattern observed in the positive ion mode of $(M+NH_4)^+$ and $(M+Na)^+$ ions gave the first indication of the 944 molecular weight. FAB-MS experiments confirmed the molecular weight and in the high resolution mode afforded a measured *m/z* value of 967.5675 for the $(M+Na)^+$ ion, corresponding to a molecular formula of $C_{49}H_{84}O_{17}$ (Calcd. *m/z* 967.5606).

Two previously reported polyether antibiotics with the same molecular formula as martinomycin are A204A (**3**)[4], and the recently described *Actinomadura* product CP-82,009 (**4**)[5]. These structures conform to the type 3 polyether class established by SETO and OTAKE[6]. ^{13}C NMR data for **1**~**4** are presented in Table 1. As noted in the table, several of the values for **2** and **3** have been reassigned in accordance with our own observations and especially with regard to the assignments for **4**, which were obtained by current two-dimensional correlation experiments[5]. Comparison of ^1H and ^{13}C NMR data assigned for **4** with those of **1** reveals localized differences in chemical shift values at both ends of the carbon chains, while values for C-8 through C-24 are essentially the same. Careful comparison of the chemical shifts for **1** and **2** shows significant differences occur only at the carboxy terminus through the A-ring. Polyether **2** bears a hydroxy substituent at C-2 instead of a methyl group common to **3** and **4**, while **1** retains the 2-methyl substituent on the basis of the observed ^{13}C and ^1H NMR signals (see below). Thus, the hypothesis was advanced that the structure of martinomycin was the 2-deoxy-2-methyl analog of **2**.

Table 1. ^{13}C NMR chemical shifts ($CDCl_3$) for the Na-salts of **3**, **4**, **1**, and **2**.

Carbon	3[a]	4[b]	1	2[c]
1	180.3	180.8	180.8	178.4
2	45.5	45.2	45.6	71.8
3	99.3	99.6	99.6	98.2
4	41.1	40.7	40.5	38.7
5	78.7[d]	88.8	84.6	85.5
6	77.3	80.3	78.4	78.5
7	64.1	67.6	65.8	66.7
8	32.7	32.5	32.6	32.5
9	61.2	61.6	61.3	61.3
10	31.2	31.1	31.2	31.2
11	79.5[d]	79.8	79.6	79.7
12	36.8	36.9	36.9	36.9
13	106.5	106.7	106.7	106.8
14	46.0	46.1	46.0	46.2
15	94.4	94.7	94.7	94.5
16	83.1	83.3	83.2	83.3
17	83.2[d,e]	83.5[e]	83.4	83.6
18	25.5[d]	23.0	23.0	23.2
20	79.1[d,e]	79.1[e]	79.1	79.3
21	79.3[d,e]	79.3[e]	79.2	79.3
22	29.1	29.2	29.1	29.2
23	24.1	24.2	24.1	24.3
24	80.0	80.3	80.4	80.3
25	73.7	73.9	74.4	74.3
26	39.2	39.4	39.0	39.1
27	84.4	84.7	83.2	82.8
28	46.2	46.3	47.1	47.0
29	98.1	98.4	98.3	98.2
2-Me	11.5	11.5	11.5	—
4-Me	13.5[d]	12.0	11.9	12.3
5-OMe	—	61.7	60.9	60.9
6-Me	12.6	9.9	11.4	11.1
6-OMe	49.5	—	50.5	50.8
11-OMe	58.8	58.9	59.0	59.2
12-Me	12.5	12.5	12.6	12.7
14-Me	11.5	11.5	11.5	11.7
15-OMe	60.0	60.2	60.1	60.2
16-Me	28.4	28.4	28.4	28.5
26-Me	13.1[d]	13.1	13.3	13.6
27-OMe	59.7	59.9	—	—
28-Me	12.5	12.7	12.7	12.7
29-Me	26.5	26.6	26.6	27.0
1′	98.3	96.6	102.6	102.5
2′	29.8	31.9	30.5	30.6
3′	23.3	27.7	27.3	25.7
4′	81.2	80.3	80.3	80.7
5′	68.2	74.3	74.4	74.3
4′-OMe	56.2	56.8	56.7	56.7
5′-Me	18.5	18.5	18.3	18.4

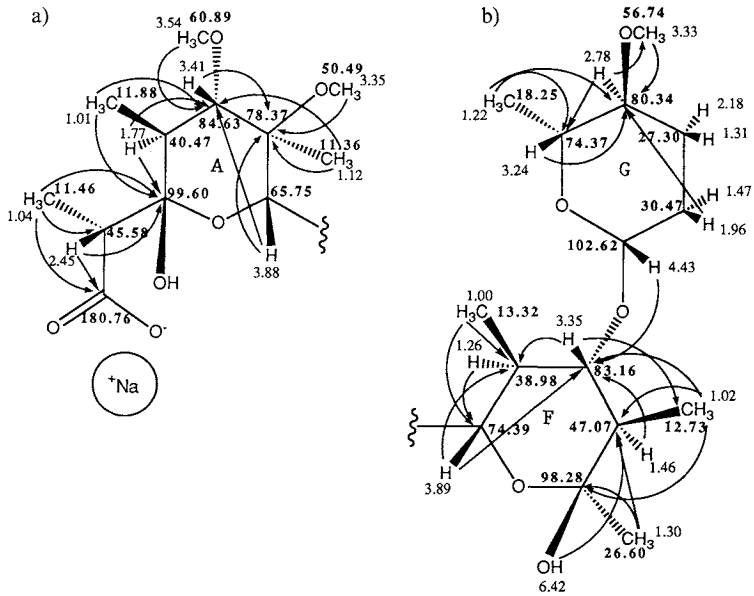
[a] From ref 4.
[b] From ref 5.
[c] From ref 7, assignments by analogy with those for **1**, **3**, and **4**.
[d] Reassigned by analogy with **1**, **2**, and **4**.
[e] Reassigned by analogy with **1**.

Table 2. ^{13}C and ^{1}H NMR data for the Na-salt of martinomycin ($CDCl_3$ 100 mg/ml).

Assignment	Function	Mult	^{13}C Shift	^{1}H Shift	^{1}H-^{1}H COSY
1	COONa	s	180.76	—	
13	O–C–O	s	106.74	—	
1′	O–CH–O	d	102.62	4.43	1.47, 1.96
3	O–C–O	s	99.60	—	
29	O–C–O	s	98.28	—	
15	HC–O	d	94.70	3.51	2.13
5	HC–O	d	84.63	3.41	1.77
17	HC–O	d	83.38	3.69	1.77, 1.91
16	C–O	s	83.24	—	
27	HC–O	d	83.16	3.35	1.26, 1.46
24	HC–O	d	80.37	4.33	2.13, 1.81, 3.89
4′	HC–O	d	80.34	2.78	1.31, 2.18, 3.24
11	HC–O	d	79.63	3.36	1.13, 2.15
21	HC–O	d	79.23	4.53	3.90, 1.95, 1.39
20	HC–O	d	79.07	3.9	4.51, 1.73
6	C–O	s	78.37	—	
25	HC–O	d	74.39	3.89	4.33, 1.26
5′	HC–O	d	74.37	3.24	1.22, 2.78
7	HC–O	d	65.75	3.88	1.51
9	HC–O	d	61.32	4.00	1.13, 1.51, 3.88
5-OMe	O–CH_3	q	60.89	3.54	—
15-OMe	O–CH_3	q	60.14	3.39	—
11-OMe	O–CH_3	q	59.02	3.45	—
4′-OMe	O–CH_3	q	56.74	3.33	—
6-OMe	O–CH_3	q	50.49	3.35	—
28	–CH–	d	47.07	1.46	3.35, 1.02
14	–CH–	d	46.03	2.12	3.51, 1.01
2	–CH–	d	45.58	2.45	1.04
4	–CH–	d	40.47	1.77	3.41, 1.01
26	–CH–	d	38.98	1.26	3.35, 1.00
12	–CH–	d	36.88	1.79	0.98
8	–CH_2–	t	32.56	1.51	4.00, 3.88
10	–CH_2–	t	31.24	2.15, 1.13	4.00, 3.36, 2.15, 1.13
2′	–CH_2–	t	30.47	1.96, 1.47	4.43, 1.47, 1.31
22	–CH_2–	t	29.13	1.95, 1.39	4.53, 2.13, 1.81
16a	CH_3	q	28.38	1.61	—
3′	–CH_2–	t	27.30	2.18, 1.31	2.78, 2.18, 1.96, 1.31, 1.47
29a	CH_3	q	26.60	1.30	—
18	–CH_2–	t	25.57	1.91, 1.77	3.67
23	–CH_2–	t	24.12	2.13, 1.81	1.81, 1.95, 1.39
19	–CH_2–	t	22.95	1.73	3.89
6′	CH_3	q	18.25	1.22	3.24
26a	CH_3	q	13.32	1.00	1.26
28a	CH_3	q	12.73	1.02	1.47
12a	CH_3	q	12.58	0.98	1.79
4a	CH_3	q	11.88	1.01	1.77
2a	CH_3	q	11.46	1.04	2.45
14a	CH_3	q	11.46	1.01	2.12
6a	CH_3	q	11.36	1.12	—

Evidence in support of this hypothesis was obtained by ^{1}H-^{1}H COSY, DEPT, HETCOR and HMBC experiments, the results of which are presented in Table 2. Regarding the proposed substitution pattern at the carboxy terminus of martinomycin, the HMBC data confirm the methyl substituent at C-2 as illustrated in Fig. 1a. The signal for the carboxylate carbon C-1 (180.76 ppm) correlates with a methine proton signal at 2.45 ppm (H-2) and a methyl resonance at 1.04 ppm. Linkage of the latter proton signal (doublet) to the carbon signal of the same methyl group (δ_c 11.46) was

Fig. 1. Heteronuclear multiple bond correlations observed for martinomycin a) ring A and b) rings F and G.



established *via* HETCOR analysis. Both COSY (δ_H 1.04 to δ_H 2.43) and HMBC (δ_H 1.04 to δ_C 45.58) experiments affirm this assignment. Connectivity between methyl group 2a, H-2, and C-3 (99.60 ppm) is also revealed by HMBC data, which show the expected 2- and 3-bond correlations. The additional correlations indicated in Fig. 1a confirm the structure of the A-ring.

Twenty-two of the ^{13}C NMR signals and their corresponding proton signals, (Table 2), are readily assigned to the core portion of **1**, from the methylene group at position 8, through rings B, C, D, and E. Although we suggest the reassignment of signals of carbons 17, 20 and 21, the structure of this core element is identical to those of **2**~**4** which were established independently, and is fully supported by the 2D-NMR data, the details of which will not be discussed herein.

The observed HMBC and COSY correlations depicted in Fig. 1b and Table 2, respectively, suffice to establish the structure for the F-ring. Definitive evidence for the C-27 sugar linkage is found in the observed correlation between the C-27 signal (83.16 ppm) and the anomeric proton resonance (4.43 ppm).

The identification of the attached deoxysugar moiety as a 2,3,6-trideoxy-4-*O*-methylpyranose was also established by COSY and HMBC correlations. The proton whose signal appears at 4.43 ppm is bonded to C-1′ (102.62 ppm) as evidenced by HETCOR. This anomeric proton signal shows strong coupling in the COSY experiment to two protons (δ_H 1.96 and 1.47) which are bonded to C-2′. A three-bond HMBC between one of the H-2′ proton signals (1.96 ppm) and the signal (80.34 ppm) for the carbon bearing the methoxy group (C-4′) was observed. This carbon in turn can be tied to the methyl signal at δ_H 1.22 ppm (Me-6′) and a proton resonating at 3.24 ppm (H-5′) by HMBC. These connectivities are fully supported by the COSY results showing all expected couplings including those between the rather high-field proton signal at 2.78 ppm (H-4′) and those for two protons at C-3′ (δ_H 2.18 and 1.31).

Verification of the monosaccharide structure was obtained by GC/MS comparison of the methyl glycosides obtained from martinomycin and septamycin[8] by methanolysis. Both reaction mixtures gave identical GC/MS profiles showing two isomeric components with molecular ions at *m/z* 160 due to the formation of α and β forms of methyl 4-*O*-methylamicetoside.

Combining the structural components defined by the NMR data leads to the complete structure **1** for martinomycin. Owing to the congruence of chemical shift values for **1**~**4** as they pertain to the core structure (C-8 through C-24), the relative stereochemistry in that region was taken to be identical. Comparison of chemical shift values for **1** and **2** in the region from C-25 to C-30 where their

gross structures are the same, also shows a close match indicative of the same relative stereochemistry. Similarly, the relative stereochemistry of C-1 through C-7 appears to be the same as for **3** and **4**, however, in this case a direct comparison is not possible, so the stereochemistry of **1** in this region cannot be assigned with certainty.

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