CHEMISTRY OF MADURAMICIN

II. DECARBOXYLATION, ABNORMAL KETALIZATION AND DEHYDRATION

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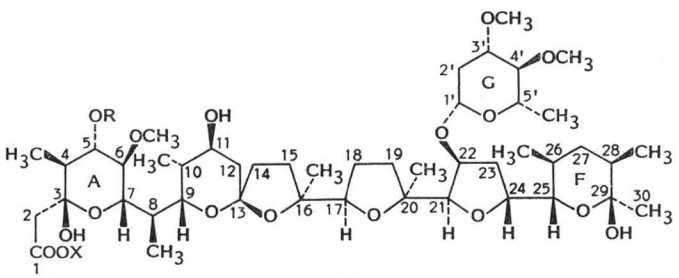
The behavior of the free acid and ammonium salt of maduramicin towards heat and alcohols is examined. In refluxing lower alcohols the free acid material is decarboxylated. In addition a bisketal decarboxylated compound as well as an A-ring monoketal decarboxylated derivative are formed. Heating the ammonium salt of the ionophores in suspension in water, or dissolved in inert solvents such as heptane or xylene can cause decarboxylation as well as concomitant dehydration of the F-ring. Reaction of dansyl chloride with the free acid of maduramicin can cause dehydration of the B-ring under very mild conditions.

Maduramicin† is a commercially important ionophore which is already in use in Europe and South America as an anticoccidial agent. In a previous paper we discussed the normal ketalization of the free acid of maduramicin, **I**, in lower alcohols.[1] By analysis of the ^{13}C NMR spectra of these ketals it was shown that normal ketalization occurs on the F-ring of the antibiotics. Our efforts were facilitated by the work of RAJAN *et al.* who had unequivocally assigned the ^{13}C chemical shifts of the backbone carbons of the sodium salt of the major component of maduramicin, **II**[2]. In this paper we discuss the results of further studies on the ketalization of maduramicin as well as its thermal degradation.

Since upon ketalization the pseudo-cyclic structure is undone and the molecule assumes an elongated conformation we felt that under forcing conditions we might obtain a bisketal derivative *via* an intermolecular catalytic effect.[3] Hence we refluxed maduramicin free acid in methanol over-

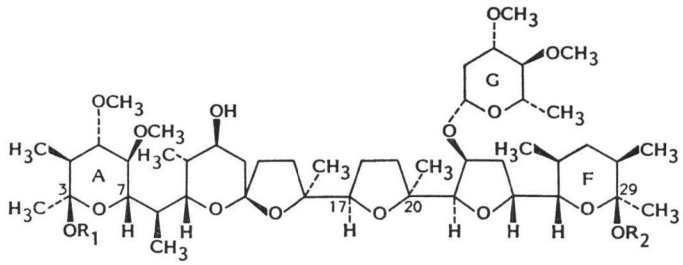
Fig. 1. Maduramicin consists of 2 to 4% of a minor component R=H and the remainder, a major component R=CH_3.



I R=X=H
II R=CH_3, X=Na

† The ammonium salt of maduramicin is marketed by American Cyanamid as an anticoccidial agent under the tradename CYGRO.

Fig. 2.



III $R_1=R_2=CH_3$
IV $R_1=CH_3$, $R_2=H$
V $R_1=R_2=H$
VI $R_1=R_2=C_2H_5$
VII $R_1=C_2H_5$, $R_2=H$

night. Upon examination of the reaction solution using TLC it was decided that there were at least three isolable products together with a number of minor components.

Careful silica gel chromatography on the reaction mixture yielded the decarboxylated methyl bisketal, **III**, the decarboxylated A-ring methyl ketal, **IV**, and the decarboxylated derivative, **V**. Because of the chromatography these compounds and those subsequently discussed in this paper are derivatives of the major component only. By refluxing the free acid of maduramicin in ethanol the corresponding decarboxylated ethyl ketals, **VI** and **VII**, as well as **V** were generated.

^{13}C NMR Studies on Compounds **III** through **VII**

The structures **III** through **VII** (Fig. 2) have been determined largely on the basis of ^{13}C NMR work. Table 1 gives the chemical shift assignments of all the carbons in these derivatives. For the purposes of this discussion the significant shift changes in the spectra of **III** through **VII** compared with those observed for **II** are concentrated in Table 2. It should be mentioned that we also studied the ^1H NMR spectra especially with respect to the doublet and singlet methyls and also methoxyls. Further, in the case of each of the new derivatives discussed in this paper we were able to obtain mass spectral confirmation of the molecular weights. The ^1H NMR data on methyl groups as well as the remainder of mass spectral information are given in the Experimental section.

All five compounds, **III** through **VII**, are decarboxylated since they lack the ^{13}C signal for C(1) observed at 179 ppm in **II** and between 171~173 ppm in free acid compounds of maduramicin. Because of decarboxylation, C(2) is now a methyl carbon observed at 21~22 ppm in **III**, **IV**, **VI** and **VII** and at 28 ppm in **V**. The 6 ppm upfield shift of the C(2) methyl carbon in the ketalized compounds, **III**, **IV**, **VI** and **VII**, is readily understood by analogy with the 4 ppm upfield shift of C(30) in the normal ketal compounds (ketalized at C(29)).[1]

Further evidence that **III**, **IV**, **VI** and **VII** are ketalized at C(3) is provided by the 2 ppm downfield displacement of the chemical shift of this carbon relative to the shift of the same carbon in the decarboxylated derivative, **V**. We never observed ketalization at this position in the intact ionophore.

The C(16) signal for the simple decarboxylated compound, **V**, is 2 ppm downfield from the same signal in **II**. A similar 2 ppm displacement of the same signal is observed for the free acids of the normal methyl and ethyl ketals indicating that this position of the ionophore is sensitive to conformational change. The C(16) methyl signal is moved 4 ppm upfield in the F-ring ketalized derivatives,

Table 1. ^{13}C NMR chemical shifts in ppm of decarboxylated maduramicin derivatives.

Carbon position	Compound					
	II	III	IV	V	VI	VII
1	179.1					
2	45.5	21.7	21.4	28.3	22.5	22.1
3	97.8	100.8	100.7	97.9	100.5	100.4
4	45.6	46.4	46.4	45.5	46.5	46.5
5	85.9	86.7	86.3	85.6	86.8	86.0
6	82.1	81.0	82.7	82.2	80.5	82.2
7	67.4	67.7	67.4	68.1	67.7	67.5
8	33.4	33.5	32.5	33.3	33.5	33.5
9	67.8	69.4	69.2	68.8	69.5	69.1
10	33.7	34.8	34.1	34.3	34.9	34.0
11	70.2	71.5	70.9	71.1	71.5	70.7
12	33.9	35.2	35.0	34.6	35.1	34.5
13	107.5	107.2	107.3	107.4	107.1	107.2
14	39.1	39.2	39.0	39.1	39.1	38.9
15	33.5	31.9	33.3	33.5	32.1	33.4
16	84.8	85.1	85.1	87.9	85.0	85.0
17	82.2	82.2	82.1	82.3	82.3	82.7
18	26.8	28.0	28.6	27.4	28.0	28.7
19	32.0	31.7	32.2	32.3	31.9	33.0
20	84.6	84.7	84.0	84.2	84.8	83.9
21	86.8	88.2	87.4	87.1	88.1	87.4
22	75.2	76.3	74.8	75.6	76.1	76.5
23	30.2	30.9	30.3	30.6	31.3	30.2
24	79.9	78.1	78.0	78.0	78.4	78.0
25	73.1	76.9	76.0	76.0	76.7	76.1
26	33.3	33.6	33.5	32.9	33.9	33.4
27	36.6	37.3	37.2	37.1	37.2	37.1
28	40.0	40.3	39.1	39.1	40.4	39.1
29	97.1	99.4	97.1	97.4	99.2	97.1
30	26.1	21.4	26.2	25.9	22.1	26.4
1′	95.9	95.8	95.7	95.7	95.8	95.7
2′	36.9	38.7	36.8	36.7	36.7	36.8
3′	80.9	80.9	80.9	80.9	81.0	80.9
4′	85.7	85.6	85.7	85.7	85.6	85.6
5′	71.3	71.5	71.2	71.2	71.2	71.2
Methyls						
4	12.0	11.6	11.8	12.2	11.8	11.8
8	11.0	10.6	10.4	10.6	10.6	10.4
10	10.5	10.2	10.1	10.3	10.2	10.2
16	27.7	23.6	27.2	27.8	23.0	27.2
20	22.3	22.8	23.9	23.6	22.9	23.9
26	17.6	17.5	17.6	17.5	17.7	17.6
28	17.0	16.4	16.7	16.7	16.4	16.7
51	18.0	18.0	17.9	17.9	18.0	17.9
Alkoxyls						
3-OCH_3		48.7	48.7			
3-OCH_2CH_3					55.8	55.9
3-OCH_2CH_3					15.5	15.2
5	60.4	60.6	60.7	60.6	60.6	60.6
6	59.4	60.3	59.2	60.0	60.3	60.2
29-OCH_3		47.4				
29-OCH_2CH_3					54.8	
29-OCH_2CH_3					15.4	
3′	57.0	56.8	56.8	56.8	56.8	56.9
4′	60.6	60.7	60.7	60.7	60.7	60.7

Table 2. Selected ^{13}C NMR chemical shifts in ppm of decarboxylated maduramicin derivatives.

Carbon position	Compound					
	II	III	IV	V	VI	VII
1	179.1					
2	45.5	21.7	21.4	28.3	22.5	22.1
3	97.8	100.8	100.7	97.9	100.5	100.4
16	84.8	85.1	85.1	87.9	85.0	85.0
16-CH_3	27.7	23.6	27.2	27.8	23.0	27.2
20-CH_3	22.3	22.8	23.9	23.6	22.9	23.9
21	86.8	88.2	87.4	87.1	88.1	87.4
25	73.1	76.9	76.0	76.0	76.7	76.1
29	97.1	99.4	97.1	97.4	99.2	97.1
30	26.1	21.4	26.2	25.9	22.1	26.4
3-OCH_3		48.7	48.7			
3-OCH_2CH_3					55.8	55.9
3-OCH_2CH_3					15.5	15.2
29-OCH_3		47.4				
29-OCH_2CH_3					54.8	
29-OCH_2CH_3					15.4	

III and **VI**. This of course is exactly analogous to the displacement of the same signal in normal F-ring ketals.[1]

Since the C(17) signal is unchanged in all of these derivatives and since X-ray data[3] indicate that much accommodation to normal ketalization occurs by rotation about the C(16)-C(17) bond it seems that in these decarboxylated compounds the pivoting occurs largely at C(16). Another signal, C(25), is displaced downfield by about 3 ppm in all the decarboxylated materials suggesting that here is another conformational adjustment point in these molecules.

The C(29) signal is quite significant since its location confirms F-ring ketalization in **III** and **VI** because of the 2 ppm downfield displacement. Similarly the pendant methyl, C(30), signal reflects F-ring ketalization in **III** and **VI** because of the approximate 4 ppm upfield displacement. All the alkoxyl signals in these decarboxylated, ketalized compounds are observed at the expected locations. They correspond approximately with those observed in the normal ketal derivatives.[1] To distinguish between methine, methylene, methyl and methoxy carbons distortionless enhancement by polarization transfer (DEPT) work was carried out on **III**, which substantiated these assignments made by comparison with those of **II**.

Fast atom bombardment mass spectroscopy (FAB-MS) on **III** using a thioglycerol-sodium chloride matrix showed intense peaks at *m/z* 1,075, 967 and 859. At first these were puzzling. However, if we assume that the molecule loses two molecules of methanol, then gains two molecules of thioglycerol before forming a sodium adduct, the *m/z* 1,075 is explained as M(900)$-2CH_3OH(64)+2$ thioglycerol (216)+Na(23) to give 1,075. The peaks at *m/z* 967 and 859 represent the loss of one and two molecules of thioglycerol respectively. Compound **IV** gives the same mass spectral pattern as **III**. To account for this we must consider the loss of one molecule of water and one molecule of methanol followed by the uptake of two thioglycerols and a sodium with the net result that this spectrum is identical to that of **I**. However, the use of field desorption mass spectroscopy (FD-MS) gave $(M+H)^+$ ions at *m/z* 901 and 887 for compounds **III** and **IV** respectively as expected. Since identical spectra are obtained from two different compounds (**III** and **IV**) under FAB-MS conditions it appears that methanol

Table 3. ^{13}C NMR chemical shifts in ppm of dehydrated maduramicin derivatives.

Carbon position	Compound				
	II	V	VIII	IX	X
1	179.1			177.7	176.0
2	45.5	28.3	28.9	44.8	43.0
3	97.8	97.9	98.0	97.8	97.6
4	45.6	45.5	45.5	45.5	46.5
5	85.9	85.6	85.7	86.5	86.2
6	82.1	82.2	82.3	82.0	82.9
7	67.4	68.1	68.3	67.7	69.2
8	33.4	33.3	33.2	33.3	33.1
9	67.8	68.8	69.0	68.8	72.1
10	33.7	34.3	34.5	33.4	35.5
11	70.2	71.1	71.7	70.4	125.3
12	33.9	34.6	34.6	35.1	136.1
13	107.5	107.4	107.4	107.6	105.4
14	39.1	39.1	39.6	39.6	38.2
15	33.5	33.5	33.5	31.6	33.3
16	84.8	87.9	85.6	85.6	83.4
17	82.2	82.3	81.9	81.8	82.1
18	26.8	27.4	27.4	29.2	27.4
19	32.0	32.3	31.6	31.6	28.9
20	84.6	84.2	84.0	84.6	83.9
21	86.8	87.1	87.3	86.3	87.2
22	75.2	75.6	76.0	75.7	75.9
23	30.2	30.6	29.6	29.7	29.6
24	79.9	78.0	81.6	79.9	78.4
25	73.1	76.0	88.6	78.5	73.8
26	33.3	32.9	32.8	32.4	33.3
27	36.6	37.1	34.5	34.4	37.0
28	40.0	39.1	100.4	100.2	39.9
29	97.1	97.4	143.9	143.4	97.3
30	26.1	25.9	17.9	17.7	28.1
1′	95.9	95.7	96.0	95.9	95.8
2′	36.9	36.7	36.8	36.7	36.9
3′	80.9	80.9	81.1	80.9	81.0
4′	85.7	85.7	85.7	85.7	85.8
5′	71.3	71.2	71.3	71.2	71.3
Methyls					
4	12.0	12.2	12.2	11.9	12.5
8	11.0	10.6	10.5	11.2	10.6
10	10.5	10.3	10.4	10.6	11.9
16	27.7	27.8	24.2	26.9	26.7
20	22.3	23.6	23.4	22.2	23.4
26	17.6	17.5	17.7	17.5	17.6
28	17.0	16.7	16.1	16.0	16.8
51	18.0	17.9	18.0	17.9	18.0
Methoxyls					
5	60.4	60.6	60.5	60.5	60.7
6	59.4	60.0	60.0	59.6	60.0
3′	57.0	56.8	56.9	56.9	57.0
4′	60.6	60.7	60.5	60.7	60.8

Table 4. Selected ^{13}C NMR chemical shifts in ppm of dehydrated maduramicin derivatives.

Carbon position	Compound				
	II	V	VIII	IX	X
1	179.1			177.7	176.0
7	67.4	68.1	68.3	67.7	69.2
9	67.8	68.8	69.0	68.8	72.1
10	33.7	34.3	34.5	33.4	35.5
11	70.2	71.1	71.1	70.4	125.3
12	33.9	34.6	34.6	35.1	136.1
13	107.5	107.4	107.4	107.6	105.4
14	39.1	39.1	39.6	39.6	38.2
16	84.8	87.9	85.6	85.6	83.4
16-CH_3	27.7	27.8	24.2	26.9	26.7
25	73.1	76.0	88.6	78.5	73.8
27	36.6	37.1	34.5	34.4	37.0
28	40.0	39.1	100.4	100.2	39.9
29	97.1	97.4	143.9	143.4	97.3
30	26.1	25.9	17.9	17.7	28.1

or water is readily lost at the hemiketal positions C(3) and C(29) giving rise to addition products with thioglycerol. Sodium then binds to these addition compounds and under FAB-MS conditions the resultant charged entities are observed as intense peaks.

Dehydration of Maduramicin with and without Decarboxylation

We were interested in the effect of heat on the ammonium salt of maduramicin. The behavior of the salt complex was examined under the following conditions:

(i) Heating as a suspension in water under autoclaving conditions,

(ii) In refluxing heptane (bp 98°C),

(iii) In refluxing xylene (bp 138.9°C).

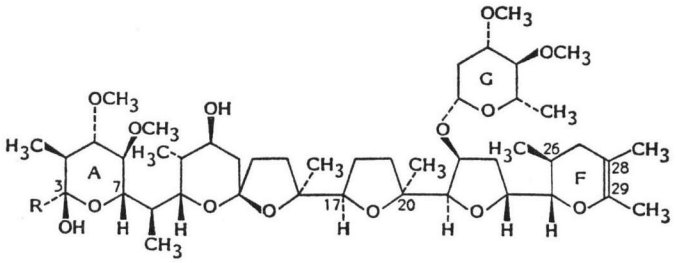
After heating the ammonium salt of the ionophore at 120°C overnight no starting material either in the form of ammonium salt or free acid survived. Of the degradation products which resulted we were able to isolate and identify two compounds, namely, the decarboxylated derivative, **V**, and **VIII** which was not only decarboxylated but also dehydrated in the F-ring.

Overnight refluxing in heptane also resulted in complete degradation of the intact ionophore. From the resultant reaction solution we isolated **V** and the F-ring dehydrated product, **IX**. As might be expected, refluxing in xylene had essentially the same effect as heating to 120°C in water. The two compounds isolated from the reaction mixture were **V** and **VIII**.

Clearly, decarboxylation of the ammonium salt of maduramicin can occur upon application of heat. For decarboxylation to occur ammonia is lost and the resultant free acid, being a potential β-keto acid, decarboxylates thermally through the well-known 6-membered transition state.[4] When the ammonium salt of maduramicin was refluxed overnight in methanol, HPLC analysis indicated a loss of 30% of starting material. Back titration of the entrapped gas from the reaction indicated that 25% of the ammonium ion had been discharged as ammonia gas. Refluxing of the sodium salt of the ionophore under similar conditions gave rise to 15% degradation of starting material as measured by HPLC.

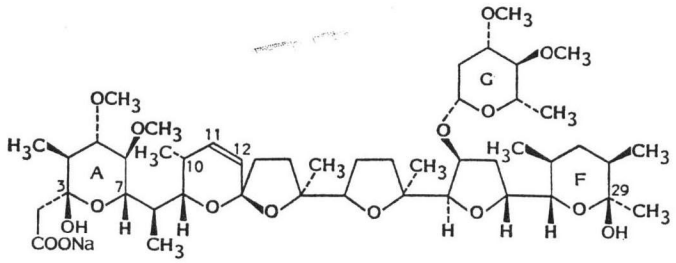
When the free acid of maduramicin was stirred at ambient temperature with an acetone solution

Fig. 3.



VIII R=CH_3
IX R=CH_2COONa

Fig. 4.



X

of dansyl chloride, still another kind of dehydration was observed. From this reaction a small quantity of **X** was isolated. This material differed from the parent material by being dehydrated in the B-ring. The complete assignments of the ^{13}C NMR chemical shifts of these decarboxylated and dehydrated derivatives are given in Table 3 and for convenience the significantly changed assignments are condensed in Table 4. The spectrum of **VIII** lacked the C(1) carboxylate signal and also contained unexpected signals at 143.9 and 100.4 ppm. It was quickly decided that they belonged to olefinic carbons in the more reactive F-ring. The signal at 143.9 ppm was assigned to C(29) and that at 100.4 ppm to C(28) in agreement with assignments in rickamycin which contains a similar enol-ether group.[5] Supporting this placement of the double bond is the observation that no olefinic protons are observed in the ^1H NMR spectrum of **VIII** and that the A-ring ^{13}C NMR signals are unchanged from those of starting material (Table 3). The presence of the double bond markedly affects the signal for C(25) which is now observed at 88.6 ppm, a downfield shift of 12 ppm from the related signal in **V**.

Compound **IX** retains the carboxylic group but again F-ring dehydration was indicated by olefinic signals at 143.4 and 100.2 ppm. The signal for C(25) in the distorted F-ring is observed in this compound at 78.5 ppm, not nearly as far downfield as the 88.6 ppm signal in **VIII**. The reason for the smaller displacement must somehow be connected to the presence of the carboxylate group although, since the pseudo-cyclic conformation is now eliminated with the disappearance of the C(29) hydroxyl group, the exact connection is not easily perceived. One other displacement which is further confirmation of the double bond at the position shown is that of C(30) from 26 ppm in **II** to 18 ppm in **VIII** and **IX** a chemical shift which is characteristic of vinyl methyls.[6]

In compound **X** the presence of a double bond was indicated by signals at 136.1 and 125.3 ppm which have been assigned to C(12) and C(11) respectively. Due to the presence of this double bond

the chemical shift of C(9) normally observed around 68 ppm is displaced to 72 ppm. In the spectra of all maduramicin derivatives examined so far the C(13) spirocarbon has been invariably observed around 107.5 ppm but due to the presence of unsaturation in the B-ring this carbon is now observed at 105.4 ppm. For the same reason the C(14) resonance, normally observed between 39 and 39.6 ppm, is shifted upfield to 38.2 ppm.

The placement of the double bond between C(11) and C(12) is due to the interpretation of the ^{1}H NMR spectrum. The olefinic proton at C(12) appears as a doublet at δ 5.55 (J=10 Hz), being split by the adjacent C(11) vinyl proton. The C(11) proton is also split by the single proton at C(10) (J=6 Hz) thus appearing as a doublet of doublets at δ 6.05.

Biological Activity of Decarboxylated Maduramicin Derivatives

The decarboxylated derivatives, **III**, **IV**, **V**, **VI**, **VII** and **VIII**, are virtually devoid of antibacterial or anticoccidial activity. In addition the acute toxicities of these compounds are dramatically lower than the parent compound. These results are perhaps not unexpected since the decarboxylation effectively destroys the ability of the derivatives to co-ordinate usefully with metal ions.

Experimental

^{1}H and ^{13}C NMR spectra were taken in $CDCl_3$ solutions of the ionophores using a Nicolet NT 300 WB instrument.

Mass spectral data were determined using a Kratos MS-50 equipped with an M-Scan FAB ion source. Melting points were taken on a Fisher-Johns hot plate and are uncorrected. TLC was carried out on silica gel F254 plates using the system EtOAc - $CHCl_3$ as 7 : 3 and detection was made possible by spraying the developed layers with 30% conc H_2SO_4 in MeOH followed by hot air treatment. HPLC conditions are described in reference number one.

Compounds III and IV

About 2 g of the free acid of maduramicin were refluxed overnight in 35 ml of MeOH. The solvent was evaporated and the resultant off-white solid was taken up in ether and 10 g of Silicar CC7 were added. The solvent was evaporated off and the loaded silica gel was added to a column of the same silica gel (43 cm bed depth and 1.9 cm i.d.) which had been slurried in heptane. The column was developed using 1-liter aliquots of 20, 30, 40 and 50% CH_2Cl_2 in heptane and then with 1-liter aliquots of 10, 20, 30, 40, 50, 60 and 80% EtOAc in CH_2Cl_2 - heptane (50 : 50). Fraction volumes were in the range 50~60 ml. Fractions 71 through 78 were combined based on TLC work and evaporated to dryness, taken up in ether, filtered and allowed to crystallize. A first crop of 200 mg of **III**, mp 172~175°C was obtained; $[\alpha]_D^{25}$ +30±4° (c 0.29, MeOH); FD-MS showed a weak $(M+H)^+$ at m/z 901 and a median peak at m/z 869 which represents $(M+H)^+-CH_3OH$. FAB-MS has been discussed in the body of the text. ^{1}H NMR ($CDCl_3$) δ CH_3 at 0.84 (d, J=6.5 Hz), 0.86 (d), 0.87 (d), 1.01 (d), 1.02 (d), 1.19 (s), 1.23 (s), 1.26 (s), 1.27 (s), 1.27 (d). Two extra OCH_3 at δ 3.18 and δ 3.20 over the normal $4\times OCH_3$ in the parent compound were observed.

Anal Calcd for $C_{48}H_{84}O_{15}\cdot\frac{1}{2}H_2O$ (909): C 63.37, H 9.35.
Found: C 63.59, H 9.28.

Fractions 85 through 90 off the column described in the previous entry were combined and evaporated to dryness. When taken up in ether, filtered and left to crystallize 200 mg of a powdery solid, **IV**, mp 105~110°C were obtained; $[\alpha]_D^{25}$ +23±2° (c 0.43, MeOH). By FD-MS there were weak signals at m/z 886 and 887 for M^+ and $(M+H)^+$. Upon addition of NaCl a medium signal at m/z 909 for $(M+Na)^+$ was observed. FAB-MS has been discussed in the text.

^{1}H NMR ($CDCl_3$) δ CH_3 at 0.84 (d, J=6.5 Hz), 0.86 (d), 0.90 (d), 1.01 (d), 1.03 (d); $2\times CH_3$ at 1.24 (s); CH_3 at 1.25 (s), 1.28 (d), 1.41 (s).

There is one extra OCH_3 at δ 3.19 over the $4\times OCH_3$ seen in the spectrum of the parent compound.

Anal Calcd for $C_{47}H_{82}O_{15}\cdot\frac{1}{2}H_2O$ (895): C 63.02, H 9.27.
Found: C 63.26, H 9.31.

Compound **V** was isolated from the above column but a purer preparation was obtained from heating the ionophore in aqueous suspension and is described later.

Compounds **VI** and **VII**

A 2-g aliquot of the free acid of maduramicin was refluxed overnight in 35 ml of abs EtOH. The solvent was evaporated off and the resultant solids charged to Silicar CC7 as described previously and developed using the same stepwise gradients as described for the isolation of **III** and **IV** except that fraction volumes were in the range 80 to 85 ml.

Fractions 26 and 27 were combined and evaporated to a solid which was allowed to crystallize from acetone to yield 234 mg, **VI**, mp 161~164°C; $[\alpha]_D^{25}$ +29±1° (*c* 0.88, MeOH). FAB-MS with **VI** in a tetramethylene sulfone and NaCl matrix $(M+Na)^+$ *m/z* 951.

^1H NMR ($CDCl_3$) δ 3×CH_3 at 0.87 (d); CH_3 at 1.01 (d), 1.02 (d); terminal CH_3's at 1.12 (t), 1.13 (t); CH_3 at 1.19 (s), 1.23 (s), 1.26 (s), 1.27 (s), 1.27 (d).

Anal Calcd for $C_{50}H_{88}O_{15}$ (928): C 64.66, H 9.48.
Found: C 65.23, H 9.32.

Fractions 31 through 34 from the above column were combined and evaporated to a solid which was taken up in ether. Evaporation of the ether gave rise to 260 mg, **VII**, mp 106~110°C; $[\alpha]_D^{25}$ +30±2° (*c* 0.06, MeOH). FAB-MS using tetramethylene sulfone and NaCl matrix $(M+Na)^+$ *m/z* 923.

^1H NMR ($CDCl_3$) δ CH_3 at 0.85 (d), 0.86 (d), 0.91 (d), 1.01 (d), 1.03 (d); terminal CH_3 at 1.14 (t); CH_3 at 1.25 (s), 1.26 (s), 1.27 (d), 1.41 (s).

Anal Calcd for $C_{48}H_{84}O_{15}$ (900): C 64.00, H 9.33.
Found: C 63.90, H 9.35.

Compounds **V** and **VIII**

About 5 g of the ammonium salt of maduramicin was slurried in 100 ml of H_2O and autoclaved overnight at 1.4 kg/cm² and 120°C. The solid was recovered by filtration and the partially dried cake was taken up in acetone and filtered again. The filtrate was evaporated to an off-white solid which was chromatographed over 400 g of Silicar CC7 using the stepwise gradients described previously. Fractions 235 to 251 were combined and concentrated to a white powder which resisted attempts at crystallization. Upon allowing a concentrated solution in ether to evaporate slowly approximately 1 g of white powder, **V**, was obtained mp 116~120°C; $[\alpha]_D^{25}$ +40±1° (*c* 0.73, MeOH).

FAB-MS using a thioglycerol, CH_2Cl_2 and NaCl matrix $(M+Na)^+$ *m/z* 895 (very strong). Using KCl instead of NaCl $(M+K)^+$ *m/z* 911 (medium).

^1H NMR ($CDCl_3$) δ 2×CH_3 0.86 (d); CH_3 at 0.91 (d), 1.02 (d), 1.05 (d), 1.22 (s), 1.27 (d), 1.32 (s), 1.35 (s), 1.41 (s).

Fractions 217 to 223 from the above column were combined to obtain 411 mg of **VIII**, mp 122~127°C; $[\alpha]_D^{25}$ +37±2°C (*c* 0.52, MeOH).

FAB-MS using thioglycerol, CH_2Cl_2 and NaCl matrix $(M+Na)^+$ *m/z* 877. Using KCl instead of NaCl $(M+K)^+$ *m/z* 893. ^1H NMR ($CDCl_3$) δ CH_3 at 0.86 (d), 0.98 (d), 1.03 (d), 1.06 (d), 1.21 (s), 1.25 (s), 1.28 (s), 1.36 (s), 1.55 (s), 1.69 (s).

Anal Calcd for $C_{46}H_{78}O_{14}\cdot H_2O$ (872): C 63.30, H 9.17.
Found: C 63.85, H 8.93.

Compound **IX**

About 2.5 g of the ammonium salt of maduramicin were refluxed in 100 ml of heptane (bp 98°C). The resultant products were chromatographed over Silicar CC7 as previously described using heptane/ CH_2Cl_2/ EtOAc stepwise gradients. Fractions 71 through 101 were combined and processed to yield 171 mg, **IX**, mp 120~123°C; $[\alpha]_D^{25}$ +29±2° (*c* 0.55, MeOH).

FAB-MS using a matrix of 1 part dithioerythritol and 5 parts of dithiothreitol and NaCl $(M+Na)^+$ *m/z* 921 (strong), $(M+Na)^+ - COO - H_2O$ *m/z* 859 (very strong).

^1H NMR ($CDCl_3$) δ 2×CH_3 at 0.85 (d); CH_3 at 0.95 (d), 1.02 (d), 1.04 (d), 1.21 (s), 1.27 (d), 1.40 (s), 1.53 (s), 1.69 (s).

The material was an estimated 50 : 50 mixture of Na^+ and NH_4^+ salts.

Anal Calcd for $C_{47}H_{77}O_{16}Na$ (920): C 61.30, H 8.37.
Calcd for $C_{47}H_{80}O_{16}N$: C 62.09, H 8.77.
Estimated average calcd values: C 61.50, H 8.56.
Found: C 62.09, H 8.77.

Compound X

A 2.5-g aliquot of the free acid of maduramicin in 20 ml of acetone was stirred with 7 ml of a commercial dansyl chloride in acetone reagent for 15 hours at ambient temperature. The solvent was evaporated and the resultant yellow solid was chromatographed over Silicar CC7 under the same conditions as mentioned above. Fractions 145 through 159 yielded 99 mg of white solid, **X**, mp 126~129°C. FAB-MS using the magic bullet matrix mentioned above with NaCl $(M+Na)^+$ *m/z* 921, $(M+Na)^+-COO-H_2O$ *m/z* 859. ^1H NMR ($CDCl_3$) δ CH_3 at 0.86 (d), 0.87 (d), 0.92 (d), 1.02 (d), 1.08 (d), 1.21 (s), 1.27 (s), 1.27 (d), 1.47 (s). Olefinic protons at δ 5.55 (d, J=10 Hz), 6.05 (dd, J=10 and 6 Hz). Evidently the hydroxyl group of the B-ring is a key co-ordinating group for metals. With this group missing the ionophore did not pick up the full complement of Na from the silica gel. This compound, **X**, is a 50 : 50 mixture of the free acid and Na salt of the dehydrated maduramicin. The C(1) signal appears at 176.0 ppm and when we prepared a 50 : 50 mixture of the free acid and Na salt of maduramicin C(1) appeared at 175.9 ppm in the ^{13}C spectrum.

Anal Calcd for $C_{47}H_{77}O_{16}Na\cdot\frac{1}{2}H_2O$: C 60.71, H 8.40.
Calcd for $C_{47}H_{78}O_{16}\cdot\frac{1}{2}H_2O$: C 62.18, H 8.60.
Average calcd values: C 61.44, H 8.55.
Found: C 61.09, H 8.53.

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References

1) ELLESTAD, G. A.; N. CANFIELD, R. A. LEESE, G. O. MORTON, J. C. JAMES, M. M. SIEGEL & W. J. MCGAHREN: Chemistry of maduramicin. I. Salt formation and normal ketalization. J. Antibiotics 39: 447~456, 1986
2) RAJAN, S.; H.-R. TSOU, P. C. MOWERY, M. W. BULLOCK & G. W. STOCKTON: Natural abundance two-dimensional double-quantum ^{13}C NMR spectroscopy of maduramicin, a polyether ionophore antibiotic and coccidiostat. J. Antibiotics 37: 1495~1500, 1984
3) WESTLEY, J. W.; C.-M. LIU, J. F. BLOUNT, R. H. EVANS, L. H. SELLO, N. TROUPE & P. A. MILLER: Novel polyether antibiotics X-14868A, B, C and D: Potent coccidiostats from *Nocardia*. *In* Trends in Antibiotic Research. Genetics, Biosyntheses, Actions & New Substances. *Ed.*, H. UMEZAWA *et al.*, pp. 125~134, Japan Antibiotics Res. Assoc., Tokyo, 1982
4) SOLOMONS, T. W. G.: Organic Chemistry. 3rd Ed., pp. 810~812, John Wiley & Sons, New York, 1984
5) JOHNSON, L. F. & W. C. JANKOWSKI: Carbon-13 NMR Spectra. A Collection of Assigned, Coded, and Indexed Spectra. Spectrum No. 484, John Wiley & Sons, New York, 1972
6) JOHNSON, L. F. & W. C. JANKOWSKI: Carbon-13 NMR Spectra. A Collection of Assigned, Coded, and Indexed Spectra. Spectrum No. 119, John Wiley & Sons, New York, 1972