CHEMISTRY OF MADURAMICIN

I. SALT FORMATION AND NORMAL KETALIZATION

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The formation of monovalent and divalent salt complexes of maduramicin is described and the ^{13}C NMR chemical shift assignments of these materials are tabulated and discussed. In the spectrum of the thallium salt, 20 of the 47 signals are split due to the thallium-carbon coupling. Similarly the preparation of both the free acid and the sodium salt complexes of the normal methyl and ethyl ketal derivatives of maduramicin are outlined. Their ^{13}C NMR spectra are fully assigned together with discussion of displacements observed due to this derivatization.

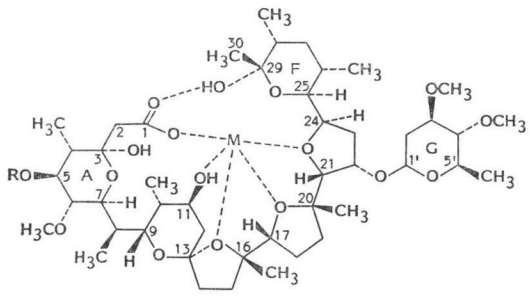
Actinomadura yumaensis (Lederle culture C23024) elaborates major and minor metabolites in the ratio of 20 or 30 to 1 which together are referred to as maduramicin. These metabolites belong to the lipophilic class of antibiotics known as ionophores which can complex monovalent and divalent cations and transport them across membranes in living systems.[1] Coccidiosis is a wide-spread poultry disease caused by protozoan parasites of the genus *Eimeria* which if left untreated, gives rise to poor weight gain, lower rate of egg laying and increased mortality. Monensin, lasalocid, nigericin and salinomycin are ionophores which are already in use as anticoccidial agents.[1] Maduramicin is about an order of magnitude more potent than monensin as an anticoccidial agent.[2~4]

During development efforts on maduramicin a number of different salt forms were prepared. At this time it became evident that the preparation of these salts is not necessarily straightforward and so we describe here the methods used in preparing these complexes, their physical constants and ^{13}C NMR chemical shift assignments. In addition, we describe very mild conditions for ketalization in the presence of simple alcohols and their stability in the free acid and salt form which caused some confusion during initial isolation work.†

Maduramicin forms salts with both monovalent and divalent cations. In the fermentation media it is elaborated as the free acid (II) and being a superb cation scavenger, it picks up monovalent cations immediately. It shows the most affinity for ammonium, potassium, and sodium ions but the predominance of any of these ions is likely to depend on mass action effect due to abundance of the cation in the fermentation medium. Isolation procedures for ionophores frequently involve silica gel adsorption chromatography and during this process the cation content of the ionophore may be changed due to sodium exchange with the silica gel. Because of this the purified ionophores are often converted to the free acid and then the desired salt is prepared from the free acid. In our hands the most practical

† The structures of the major and minor components of maduramicin have been determined by X-ray crystallography.[5] The NMR spectrum of the sodium salt of the major component (I) has been completely assigned by two-dimensional double-quantum ^{13}C NMR spectroscopy on the natural abundance material.[6] All of the ^{13}C NMR chemical shifts discussed in this paper are based on those assignments.

Fig. 1.



Major maduramicin component (**I**)
M=Na, R=CH_3
Minor maduramicin component (**Ia**)
M=Na, R=H
II M=H, R=CH_3

way to prepare these salts involved treating the acetone solution of the free acid of the ionophore with either a solution or suspension of the metal oxide in water.

X-Ray crystallography of the salts of several ionophores has shown that they form pseudocyclic structures with the lipophilic methyl groups on the external periphery and the ether oxygens directed inward.[7] Fig. 1 is our attempt to convey the information from X-ray work on maduramicin in terms of chemical formalism. The numbering system used is largely that of WESTLEY with the modifications proposed by SETO and ŌTAKE where methyl and alkoxyl groups are simply related to the backbone carbon to which they are attached.[8,9] The sugar carbons of the G-ring are numbered one through five and superscripted.

Fig. 1 illustrates how the pseudocyclic structure is held together by hydrogen bonding between the starting carboxylic acid oxygen and the hemiketal hydroxyl group at the end of the backbone chain. The cation fits into this cyclic site in a fashion that neutralizes the charge and provides maximum metal-oxygen contact. The site displays significant flexibility since ions varying in size from lithium to thallium can be accommodated there.

We have studied the ^{13}C NMR spectra of several monovalent (Table 1) and divalent salt complexes (Table 2) of maduramicin. Table 1 gives the complete ^{13}C NMR assignments of a number of monovalent complexes including those of the free acid preparation. All the spectra mentioned in this paper were taken on deuterated chloroform solutions. The monovalent salts, all gave sharp, clean spectra at room temperature. Attached proton tests (APT) were carried out on the lithium and thallium salt solutions and on the free acid compound and the data was used to confirm some of the comparison assignments.

Examination of the chemical shifts in Table 1 indicate certain expected and unexpected observations. The signals for the carbonyl carbons are observed near 174 ppm in the free acid compound and about 4.5 to 5.0 ppm further downfied in the metal complexes. The chemical shifts of the sugar carbons of the G-ring are unaffected by the presence or absence of different cations. This is to be expected since the X-ray work indicated no involvement of this moiety in complexation. The four methoxy carbons are also virtually unchanged from compound to compound as are the pendant methyl signals. It was anticipated that there might be some correlation between the chemical shifts of those carbons adjacent to coordinated oxygens and the various cations based on cation size. However, as seen from the tabulations in Tables 1 and 2, there are no significant chemical shift changes between the various salts. Thus, one concludes that this pseudocyclic ionophore can accommodate a variety of cation sizes ranging from lithium to thallium without sizeable conformational changes at least as reflected by the ^{13}C NMR resonances. A number of minor shift changes are observed in the spectrum of the lithium salt. It appears that this cation in the pseudocyclic site gives rise to some small conformational change since the specific rotation of this material is out of line with all the others (Table 3).

The spectrum of the silver salt was particularly sharp and clean exhibiting a total of 45 signals,

Table 1. ^{13}C NMR chemical shifts in ppm of monovalent maduramicin complexes.

Carbon position	Compound							
	H^+	Na^+	K^+	NH_4^+	Ag^+	Li^+	Tl^+	(J in Hz)
1	173.7	179.1	178.6	178.4	179.1	178.1	178.6	(26.4)
2	43.7	45.5	45.2	45.2	45.2	45.0	44.2	(36.8)
3	97.8	97.8	98.0	98.0	97.5	98.1	98.4	(9.1)
4	45.6	45.5	45.7	45.6	45.2	45.5	45.6	
5	85.1	85.7	85.8	85.7	85.6	85.8	85.6	
6	81.9	82.0	82.2	82.0	81.9	81.4	81.9	
7	67.2	67.5	67.8	67.9	67.4	67.7	67.7	
8	33.4	33.4	33.3	33.5	33.4	33.0	33.2	
9	68.7	67.6	68.5	68.4	67.5	69.7	69.7	(45.9)
10	34.2	33.8	34.2	34.1	34.1	33.4	33.2	
11	70.5	70.3	70.1	70.3	71.2	69.8	70.7	(42.5)
12	34.6	33.9	34.1	34.2	33.9	33.6	34.1	(7.6)
13	107.5	107.5	107.5	107.2	108.0	106.7	107.7	(38.4)
14	38.7	39.0	39.4	39.2	38.9	39.0	39.3	
15	34.2	33.6	33.8	33.1	33.6	33.0	33.6	(10.3)
16	85.0	84.7	84.7	84.5	84.6	84.9	83.8	(6.0)
17	83.2	82.3	82.8	83.2	82.5	82.1	82.4	(6.0)
18	27.1	26.9	27.6	27.7	27.0	27.0	28.0	(9.3)
19	33.0	32.1	32.3	32.4	32.2	31.7	32.6	(18.5)
20	84.5	84.5	83.9	84.4	84.4	85.6	85.7	(22.3)
21	86.2	86.9	86.9	86.3	89.3	86.4	86.0	(47.4)
22	75.6	75.3	75.7	75.6	75.1	74.4	75.1	(12.3)
23	30.1	30.2	29.8	29.6	30.1	30.5	29.8	(8.4)
24	78.0	79.9	78.7	78.7	79.9	80.4	80.2	(11.3)
25	73.6	73.0	74.4	74.4	73.4	73.5	73.9	(6.1)
26	33.2	33.3	33.1	32.9	33.3	32.9	32.7	
27	36.7	36.5	36.4	36.1	36.5	36.3	35.9	
28	39.7	40.0	40.5	40.3	39.9	40.0	40.7	(28.1)
29	97.3	97.0	97.5	98.0	96.9	98.0	97.6	(11.0)
30	26.4	26.1	26.7	26.6	26.1	26.1	27.0	
1′	95.8	95.9	95.8	95.6	95.8	95.9	95.6	
2′	36.8	36.9	36.9	36.7	36.8	36.9	36.7	
3′	80.8	80.9	80.9	80.8	80.8	80.9	80.8	
4′	85.6	85.7	85.7	85.7	85.6	85.7	85.6	
5′	71.2	71.3	71.4	71.2	71.3	71.2	71.2	
Methyls								
4	11.9	12.0	12.2	12.2	12.0	12.0	12.1	
8	10.8	11.0	11.2	11.1	11.0	11.0	11.7	
10	10.3	10.5	10.3	10.3	10.7	10.5	10.2	
16	27.9	27.6	27.7	27.7	27.5	28.0	27.3	
20	23.2	22.4	22.9	23.2	22.6	22.4	23.2	
26	17.5	17.6	17.5	17.6	17.6	17.6	17.7	
28	16.9	17.0	17.0	17.0	17.0	17.0	16.9	
5′	17.9	17.9	17.9	17.9	17.9	17.9	17.9	
Methoxyls								
5	60.7	60.5	60.5	60.6	60.6	60.5	60.6	
6	60.0	59.5	59.4	59.7	59.6	59.6	59.5	
3′	56.9	57.0	57.0	57.0	57.0	57.0	57.0	
4′	60.8	60.7	60.6	60.7	60.7	60.7	60.7	

Table 2. ^{13}C NMR chemical shifts in ppm of divalent maduramicin complexes.

Carbon position	Compound						
	Na^+	Ca^{++}	Mg^{++}	Zn^{++}	Ba^{++}	Sr^{++}	Pb^{++}
1	179.1	180.4	178.8	179.5	179.8	179.0	181.3
2	45.5	43.5	44.5	43.8	44.5	43.5	45.6
3	97.8	98.0	97.6	97.8	97.9	97.9	97.8
4	45.5	45.5	45.5	45.5	45.5	45.4	45.6
5	85.7	85.9	85.6	85.6	85.6	85.6	85.5
6	82.0	82.1	82.2	82.0	82.0	82.0	81.9
7	67.5	69.0	67.8	68.1	68.0	68.0	68.5
8	33.4	33.3	33.3	33.4	33.4	33.3	33.3
9	67.6	69.1	68.2	68.1	68.2	68.5	68.1
10	33.8	34.1	34.0	33.8	33.6	33.8	33.7
11	70.3	71.4	71.2	71.2	71.2	71.2	71.2
12	33.9	33.9	33.9	34.0	33.9	33.8	33.8
13	107.5	107.1	107.4	107.2	107.7	107.1	107.9
14	39.0	39.5	39.3	39.0	39.6	39.5	39.2
15	33.6	33.7	33.7	33.4	33.3	33.6	33.3
16	84.7	84.6	84.1	85.2	85.4	86.7	86.4
17	82.3	82.8	82.2	82.6	82.5	82.5	82.5
18	26.9	27.6	27.6	26.1	28.0	27.3	28.2
19	32.1	33.1	32.2	32.9	32.1	33.2	31.0
20	84.5	85.9	86.8	84.6	84.7	86.0	84.2
21	86.9	87.3	87.7	86.5	86.3	86.7	86.8
22	75.3	75.9	75.8	75.8	75.3	76.0	75.5
23	30.2	31.1	31.0	30.9	30.9	30.7	30.4
24	79.9	81.1	81.0	78.2	79.4	77.5	79.6
25	73.0	72.5	72.4	72.2	75.0	75.7	74.5
26	33.3	33.7	33.1	33.4	33.2	33.3	33.2
27	36.5	37.0	36.8	36.8	36.2	36.8	36.4
28	40.0	39.7	39.3	39.3	39.7	39.5	39.7
29	97.0	97.9	97.6	97.4	97.9	97.7	97.8
30	26.1	26.1	25.6	26.1	27.1	27.3	27.1
1′	95.9	96.1	95.9	95.9	95.9	95.7	95.8
2′	36.9	37.0	36.8	36.8	36.7	36.8	36.7
3′	80.9	81.1	81.0	80.9	80.7	80.9	80.8
4′	85.7	85.5	85.6	85.6	85.6	85.6	85.6
5′	71.3	71.4	71.2	71.2	71.2	71.2	71.2
Methyls							
4	12.0	12.1	11.9	11.9	12.1	12.2	11.9
8	11.0	11.4	10.8	10.7	10.8	10.6	11.2
10	10.5	10.9	10.7	10.7	10.8	10.6	10.3
16	27.6	27.3	27.6	27.2	27.1	27.3	27.3
20	22.4	23.5	23.0	23.3	22.9	22.9	22.6
26	17.6	17.5	17.5	17.5	17.4	17.6	17.5
28	17.0	16.6	16.7	16.6	16.8	16.8	16.9
5′	17.9	18.0	18.0	17.9	17.9	17.9	17.9
Methoxyls							
5	60.5	60.2	60.7	60.6	60.4	60.6	60.7
6	59.5	59.3	59.7	59.7	59.5	59.4	59.7
3′	57.0	57.0	56.8	56.8	56.9	56.8	57.0
4′	60.7	60.5	60.7	60.6	60.6	60.6	60.7

Table 3. Physico-chemical data on maduramicin salt complexes.

Cation	Major maduramicin component by HPLC (%)	$[\alpha]_D^{25}$ in MeOH	Theoretical percents by atomic absorption	Ash (%)	H_2O by K.F. (%)
H^+	96.4	+34.0°	—	0.02	3.61
Na^+	99.2	28.1°	98.5	4.93	—
K^+	98.1	32.1°	100	4.60	4.19
NH_4^+	90.0	38.1°	93.0*	0.10	2.19
Ag^+	87.9	41.2°	89.5	10.72	2.03
Li^+	103.0	15.0°	ND	ND	3.54
Tl^+	ND	42.0°	ND	ND	0.83
Ca^{++}	98.2	32.1°	90.5	2.96	3.28
Mg^{++}	90.0	42.1°	91.0	2.27	4.84
Ba^{++}	96.0	33.1°	98.3	9.01	3.21
Sr^{++}	91.0	34.1°	87.2	7.37	2.60
Zn^{++}	98.3	39.1°	76.0	3.42	2.96
Pb^{++}	ND	47.0°	ND	15.19	1.90

* Nitrogen determination by Kjeldahl.
ND; Not determined.

those at 45.2 and 85.6 ppm being doubled. One signal in this spectrum, that of C21 was downfield by about 2 ppm when compared to those of the other salts or the free acid material. At this stage, we have no explanation of this shift change. The thallium salt spectrum was also sharp and clean showing a total of 45 signals with doubling at 33.2 and 85.6 ppm. The distinctive feature of this spectrum taken in $CDCl_3$ is the splitting of certain ^{13}C signals by spin coupling with the Tl^+ nucleus. Since the gyromagnetic radii of the ^{203}Tl and ^{205}Tl nuclei differ by a percent or so, the splittings observed are simple doublets.[10] The couplings are observed as would be expected with those signals adjacent to oxygens which are co-ordinated to the metal. In all, 20 signals are split and among those are the signals for C25, C28 and C29 hence, it is clear that the thallium is complexed to the ether oxygen of the F-ring in solution. In Fig. 1 the diagram represents what is known from X-ray work on I (sodium salt). Both X-ray work and our solution studies indicate that when M equals Tl co-ordination occurs between the metal and the F-ring ether oxygen.[5] An interesting feature of the Tl-^{13}C splitting (Table 1) is the wide range of values (6~47 Hz). It is assumed that the variation is largely dependent on distance, angle and probably basicity of the co-ordinated oxygen. However, the four-bond coupling of 45.9 Hz for C9 is hard to explain by the above criteria as is the zero coupling for C10.

The ^1H NMR spectrum of the same material shows no hydrogen-thallium splitting. According to BYSTROV *et al.* the existence of spin-spin coupling is evidence of slow Tl^+ exchange between complexes. When the solvent polarity is increased by the addition of about 20% by volume of methanol, the exchange rate is increased and the coupling is decreased. Coupling is eliminated entirely when the spectrum is taken in 100% CD_3OD. Coupling is also eliminated when the number of thallium ions in solution is less than the number of maduramicin molecules. We prepared the Tl^+ salt of the normal methyl ketal (see below for normal ketalization studies). X-Ray data on the Na^+ salt of the methyl ketal maduramicin showed the presence of one Na^+ and one H_2O molecule for every two molecules of maduramicin methyl ketal. One can assume that the same arrangement obtains in the case of the thallium salt hence the number of metal ions will be in short supply relative to molecules of ionophore and consequently no splitting should occur. In fact none was observed in this spectrum.

Table 2 shows a comparison of the signals of six divalent metal complexes including those of Ca^{++}, Mg^{++}, Zn^{++}, Ba^{++}, Sr^{++}, and Pb^{++} with those of the sodium salt. In general the spectra of the divalent complexes were not as well resolved or as readily interpretable as those of the monovalent salts. The spectra of Ca^{++}, Sr^{++}, Zn^{++}, and Pb^{++} salts were very much improved by taking them at 60°C. The spectrum of the barium salt was only slightly improved at the higher temperature.

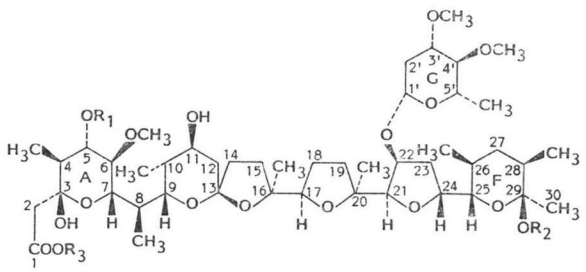
The remarks with regard to C1, the sugar carbons, the pendant methoxyls and methyls which were made in reference to Table 1 essentially carry over to Table 2. The largest changes observed in Table 2 are connected with C16 and C25 and they probably reflect the changes necessary to accommodate various divalent cation sizes in the pseudocyclic site.

Normal Ketalization of Maduramicin

During the course of analytical HPLC studies on maduramicin, it was observed that under slightly acidic conditions a new component developed in the presence of methanol. Replacing the methanol by ethanol caused the development of still another component. When the alcohol used was 2-propanol, the appearance of another component was much slower and with *tert*-butanol no reaction product was observed. The free acid of maduramicin was stirred overnight in methanol at ambient temperature and when the reaction solution was allowed to stand unagitated for a few hours, crystals appeared. By TLC on silica gel using an ethyl acetate - chloroform developing system these crystals had an Rf of 0.03 compared with maduramicin Rf 0.44. Attempts to recrystallize this material frequently resulted in the regeneration of small amounts of starting material as indicated by TLC on the recrystallized product. Spectroscopic data showed the crystalline material to be the normal ketal (**III**) of the free acid of maduramicin. A solution of this material could readily be converted to the sodium salt (**IV**) which could be recovered and recrystallized with impunity from acetone or ether. The corresponding normal ethyl ketals (**V** and **VI**) were obtained by replacing the methanol solvent with ethanol. We also prepared the thallium salt of the methyl ketal of maduramicin (**VII**) to show that coupling between Tl and ^{13}C is eliminated by exchange when the number of Tl ions in solution is less than the ionophore molecules. As expected, no doublets were observed. Structures **III** through **VII** are shown in Fig. 2.

The ^{13}C NMR spectra of compounds **III** through **VII** were most informative and Table 4 gives the complete chemical shift assignments of each compound. All four ketal derivatives contain the appropriate extra alkoxyl signals consistent with methyl or ethyl ketalization. The C29 signal in each

Fig. 2.



III	$R_1=CH_3$,	$R_2=CH_3$,	$R_3=H$
IV	$R_1=CH_3$,	$R_2=CH_3$,	$R_3=Na$
V	$R_1=CH_3$,	$R_2=C_2H_5$,	$R_3=H$
VI	$R_1=CH_3$,	$R_2=C_2H_5$,	$R_3=Na$
VII	$R_1=CH_3$,	$R_2=CH_3$,	$R_3=Tl$

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

Carbon position	Free acid of maduramicin	III	IV	V	VI	VII
1	173.7	171.0	178.4	171.7	179.5	178.7
2	43.7	44.7	45.6	44.9	45.7	45.6
3	97.8	97.3	97.8	97.5	98.1	98.2
4	45.6	45.5	45.3	45.6	45.3	46.2
5	85.1	85.2	85.4	85.4	85.6	85.6
6	81.9	81.7	81.6	81.8	81.7	82.1
7	67.2	68.3	67.3	68.4	67.5	67.9
8	33.4	33.3	33.3	33.5	33.3	33.3
9	68.7	68.9	69.1	69.1	69.2	69.1
10	34.2	33.6	33.0	34.2	33.4	33.3
11	70.5	72.4	70.0	72.3	70.1	71.2
12	34.6	34.1	33.5	34.4	33.6	34.2
13	107.5	107.2	108.0	107.3	108.2	107.3
14	38.7	39.2	39.4	39.4	39.4	39.7
15	34.2	31.5	34.5	31.8	34.6	32.7
16	85.0	86.7	85.3	87.0	85.5	87.1
17	83.2	81.2	82.0	81.5	82.1	81.9
18	27.1	28.1	27.6	28.2	27.6	28.5
19	33.0	32.1	32.3	32.6	32.6	32.4
20	84.5	84.7	84.4	84.8	84.5	84.6
21	86.2	88.5	86.0	88.3	86.0	86.8
22	75.6	76.1	75.5	76.1	75.6	75.2
23	30.1	31.1	31.4	31.7	31.5	32.7
24	78.0	78.3	80.0	78.9	80.4	80.7
25	73.6	76.8	77.2	76.8	77.5	76.7
26	33.2	33.5	33.1	33.8	33.0	33.3
27	36.7	37.2	36.4	37.3	36.6	36.7
28	39.7	40.2	40.0	40.6	40.5	40.3
29	97.3	99.5	99.5	99.4	99.6	99.7
30	26.4	21.7	21.6	22.4	22.0	21.7
1′	95.8	95.5	96.4	96.0	96.6	95.7
2′	36.8	36.6	36.7	36.9	36.9	36.8
3′	80.8	80.9	80.8	81.2	81.0	80.9
4′	85.6	85.5	85.6	85.8	85.8	85.7
5′	71.2	71.2	71.2	71.6	71.5	71.2
Methyls						
4	11.9	11.6	12.0	11.7	12.0	12.1
8	10.8	10.9	11.3	10.8	11.3	11.4
10	10.3	10.6	10.5	10.6	10.5	10.3
16	27.9	23.9	25.9	23.9	26.0	26.8
20	23.2	22.9	22.1	22.9	22.4	23.0
26	17.5	17.6	17.5	17.8	17.5	17.9
28	16.9	16.4	16.3	16.5	16.4	16.4
5′	17.9	17.9	18.0	18.0	18.0	18.2
Alkoxyls						
5	60.7	60.7	60.5	60.6	60.4	60.7
6	60.0	59.9	59.4	59.8	59.3	59.6
29-OCH_3	—	47.5	49.9	—	—	48.0
29-OCH_2CH_3	—	—	—	56.9	55.2	—
29-OCH_2CH_3	—	—	—	15.5	15.4	—
3′	56.9	57.0	56.9	56.8	57.0	56.9
4′	60.8	60.7	60.7	60.7	60.6	60.6

is shifted downfield by about 2 ppm from the values observed for the same signal in either the sodium salt or free acid of maduramicin. Also the C30 signal in each of the ketals is shifted upfield about 4 ppm or more from the same signal in the parent compound. These observations are consistent with ketalization of the F-ring. The greater reactivity of the F-ring hemiketal over that of the A-ring has already been observed for polyether A204.[1] It is this process which we describe as normal. In another paper we describe conditions where ketalization occurs in the A-ring and we refer to that process as abnormal.

X-Ray work on the methyl ketal of the major ionophore shows that the pseudocyclic structure of the parent compound is disrupted since the terminal hydroxyl group is covered by alkylation. This gives rise to a large rotation about the bond joining the C- and D-rings. The sodium cation is coordinated only with a carboxylate oxygen, the hydroxyl of the B-ring and the ether oxygen of the C-ring in effect, giving the molecule and extended conformation. These X-ray determinations explain some of the chemical shift displacements observed in the spectra of **III** through **VI**. As an example the signal for the methyl carbon attached to C16 is shifted 4 ppm upfield in the spectra of **III** and **V** and 2 ppm in the same direction in the spectra of **IV**, **VI** and **VII** as compared to its location in the spectrum of the parent compound. In addition, the C17 signal exhibits approximately half those relative displacements for the corresponding compounds. These shifts are clearly indicative of rotation about the C16–C17 bond. They further indicate that the conformations of both free acid ketals are similar and in turn are different from the conformations of the corresponding sodium salt ketals. Similarly the chemical shifts of C24 and C25 are moved downfield in the ketal compounds which is further evidence of the extended as opposed to the pseudocyclic conformation of these molecules.

Experimental

Most of the work described in this paper was carried out on CYGRO* which contained amounts of the minor component varying from 1 to 4%. TLC was carried out on silica gel plates using the system EtOAc - $CHCl_3$ as 7: 3. Detection was made by spraying with 30% conc H_2SO_4 in MeOH followed by slight heating. The ionophore spots turned yellow in a minute or so and browned on the application of heat. All salt complexes as well as the free acid appeared at Rf 0.44. Normal ketal derivatives appeared at Rf 0.03. HPLC was carried out on a Waters μBondapak C18 column using the system CH_3CN - H_2O, 70: 30, 0.01 M H_3PO_4 with the pH adjusted to 7.0 using dil NaOH solution. Detection was by end absorption at 190 nm or by refractive index. Under these conditions the relative retention time of the minor component was 0.55 relative to the major component 1.0. ^1H and ^{13}C NMR spectra ($CDCl_3$) were taken on a NICOLET NT 300 WB instrument. Mass spectra were recorded using a KRATOS MS-50 high-performance mass spectrometer equipped with an M-Scan FAB ion source.

Preparation of Free Acid of Maduramicin

About 20 g of a pilot plant preparation of maduramicin was dissolved in 100 ml of CH_2Cl_2 and filtered. The filtrate was stirred with 1,000 ml of H_2O at pH 2.5 for 30 minutes. The pH was maintained if necessary by adding 0.1 N HCl solution. The solvent phase was separated and extracted twice with deionized, distilled H_2O and then evaporated to a powder; $[\alpha]_D^{25}$ + 34 ± 1° (*c* 1.2, MeOH).

Anal Calcd for $C_{47}H_{80}O_{17}\cdot H_2O$ (934): C 60.38, H 8.78.
Found: C 60.53, H 8.57.

FAB-MS on this material did not give a molecular ion but the addition of NaCl in a tetramethylene sulfone matrix gave $(M+Na)^+$ *m/z* 939.

* Maduramicin is being marketed by American Cyanamid as an anti-coccidial agent in Europe and South America under the tradename CYGRO.

In order to compare data with those reported in ref 5, we prepared the free acid of the major component of maduramicin mp 155~156°C. From this we prepared the sodium salt to get crystals mp 171~173°C in agreement with the published value of 172.5~174°C.[5] However, the sodium content as determined by atomic absorption was 1.69% which is approximately 40% higher than the expected theoretical value of 1.21%.

General Method for Salt Formation

Approximately 2 g of the free acid of maduramicin was stirred in 50 ml of acetone. A 2.5% solution or suspension of a metal oxide was added dropwise until the pH of the combined solution or suspension was 9.0 and stirring was continued for 2 hours at room temperature. In the case of ZnO, the pH never did rise above 7.0 so stirring was continued overnight. The maximum incorporation of this metal which we observed was 76% of the theoretical value. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The separated solvent phase was back extracted with H_2O and the salt powder was recovered by evaporation. Salts were best crystallized from acetone or ether. One exception to this procedure was the preparation of the Pb^{++} salt where we used lead acetate rather than lead oxide. The physico-chemical data collected on the various preparations made are given in Table 3.

Preparation of III

Approximately 2 g of the free acid of maduramicin was stirred at ambient temperature overnight in 35 ml of absolute MeOH. The following morning upon allowing the resultant solution to stand unagitated for a few hours, crystals appeared. They were recovered to yield 1.2 g of III, mp 158~160°C; $[\alpha]_D^{25}$ +39±1° (*c* 0.96, MeOH). FAB-MS in a tetramethylene sulfone matrix with NaCl added showed $(M+Na)^+$ *m/z* 953, other peaks at *m/z* 921 for $(M+Na)^+-CH_3OH$ and *m/z* 891 for $(M+Na)^+-CO_2-H_2O$. ^1H NMR ($CDCl_3$) δ $3CH_3$ at 0.87 (d, J=6.5 Hz), CH_3 at each of the following: 1.04 (d), 1.05 (d), 1.20 (s), 1.23 (s), 1.27 (d) and 1.28 (s). An extra OCH_3 signal at δ 3.17 was observed over and above the four methoxy signals in the parent compound.

Anal Calcd for $C_{48}H_{82}O_{17}\cdot 1H_2O$ (948): C 60.76, H 8.86.
Found: C 60.58, H 8.60.

Preparation of IV

The filtrate from which III was separated was made alkaline to pH 9.0 using 1 N NaOH solution and stirred for 30 minutes. The solvent was then evaporated to dryness and the resultant solid was taken up in ether, treated with a little Darco G60 and after filtration, the clear ether solution was concentrated and left standing to yield 600 mg of white crystals, mp 163~165°C; $[\alpha]_D^{25}$ +32±1° (*c* 0.92, MeOH). ^1H NMR ($CDCl_3$) δ $2CH_3$ at 0.86 (d, J=6.3 Hz), CH_3 at each of the following: 0.98 (d), 1.02 (d) and 1.04 (d), $2CH_3$ at 1.22 (s), CH_3 at 1.28 (d) and 1.46 (s). An extra OCH_3 was observed at δ 3.18 compared to the spectrum of the parent compound.

Anal Calcd for $C_{96}H_{163}O_{34}Na\cdot 1H_2O$ (1,900): C 60.63, H 8.68.
Found: C 60.43, H 8.62.

Preparation of V

About 2 g of maduramicin free acid was stirred overnight in 35 ml of abs EtOH. Upon being allowed to stand, crystallization occurred to yield 600 mg of V, mp 173~174°C; $[\alpha]_D^{25}$ +38±2° (*c* 0.55, MeOH); ash 0.07% FAB-MS in tetramethylene sulfone matrix with NaCl added $(M+Na)^+$ *m/z* 967. ^1H NMR ($CDCl_3$) δ $3CH_3$ at 0.83 (d, J=6.5 Hz), CH_3 at 1.04 (d), 1.05 (d), 1.13 (t, J=~7 Hz), 1.21 (s), 1.23 (s), 1.27 (d) and 1.29 (s).

Anal Calcd for $C_{49}H_{84}O_{17}\cdot 1H_2O$ (962): C 61.12, H 8.94.
Found: C 61.20, H 8.75.

Preparation of VI

The filtrate from which V was recovered was made alkaline to pH 9.0 using 1 N NaOH and stirred for 1 hour. The solvent was evaporated to dryness and the resultant powder taken up in ether, filtered, concentrated and allowed to stand. About 700 mg of crystals of VI were recovered, mp 170~172°C; $[\alpha]_D^{25}$ +30±1° (*c* 0.62, MeOH).

FAB-MS on a tetramethylene sulfone matrix indicated $(M+Na)^+$ m/z 967. ^1H NMR ($CDCl_3$) δ CH_3 observed at 0.86 (d), 0.87 (d), 0.89 (d), 1.02 (d), 1.04 (d), 1.18 (t), $2CH_3$ at 1.22 (s), one at 1.28 (d) and 1.47 (s).

Preparation of **VII**

About 2 g of **II** were stirred in 35 ml of MeOH overnight. Then dil TlOH was added until the pH was 8.5 and stirring was continued for 30 minutes. The reaction solution was diluted with H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract was back extracted with H_2O and then evaporated to dryness. The resultant off-white solid was taken up in ether, treated with a small amount of Darco G60, filtered and left at room temperature for solvent to evaporate.

Approximately 1.7 g of white crystals (**VII**) were obtained, mp 145~148°C; $[\alpha]_D^{25}$ $+41\pm1°$ (c 0.95, MeOH).

Anal Calcd for $C_{48}H_{81}O_{17}Tl_{1/2}\cdot 4H_2O$: C 52.22, H 8.07.
Found: C 51.90, H 7.92.

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