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SEMDURAMICIN AND TWO DESMETHYL ANALOGS: ^{13}C NMR AND FAB MASS SPECTRAL CORRELATIONS

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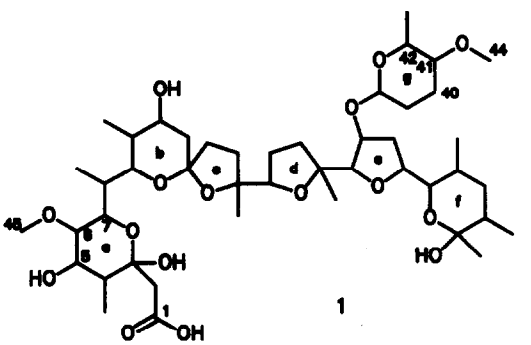
ABSTRACT: ^{13}C NMR and FAB mass spectral study of semduramicin and two desmethyl structural analogs reveals the sites of the missing methyl groups, and further clarifies the mass spectral fragmentation of this molecule.

INTRODUCTION: Semduramicin (1) is a polyether antibiotic currently being developed as an animal feed supplement for the prevention of coccidial diseases in fowl[1] and as a nutrient utilization enhancer in ruminants. Two analogs of semduramicin have been isolated and studied by both FAB mass spectrometry and ^{13}C NMR spectroscopy. Comparison of the spectral details of these three compounds with information on related polyether ionophores further clarifies the FAB mass spectral behavior of semduramicin.



EXPERIMENTAL: Semduramicin is produced directly by fermentation or by chemical modification of another polyether compound from *Actinomadura roseorufa*[2,3]. The two desmethyl analogs were observed as minor components in different lots of semduramicin, and isolated by preparative HPLC. FAB mass spectral observations were made from a 3:1 dithiothreitol-dithioerythritol matrix[4] using a VG Analytical 70S magnetic sector instrument. ^{13}C DEPT NMR measurements[5] were done on a Brüker AM-500 500 MHz instrument.

RESULTS AND DISCUSSION: The ring designations and carbon numbering used here for semduramicin is shown in 1. Carbon numbering starts with the carboxyl carbon as 1 and proceeds around the rings[6,7,8]. Carbons 44 and 45 are the methoxyl carbons on, respectively, the g and a rings. Stereochemistry is not shown here because of its minimal relevance to the mass spectrometry. The ^{13}C NMR line assignments for semduramicin have been determined[5,9,10].

Figure 1 shows the FAB mass spectrum of semduramicin. The peak at m/z 895 is the $[M+Na]^+$. Neutral losses of CO_2, H_2O and CH_3OH account for the peaks between m/z 833 and m/z 895. The m/z 833 peak corresponds to loss of both CO_2 and H_2O from $[M+Na]^+$, mechanisms for which are proposed in Scheme 1. Siegel *et al.*[11] reported similar fragmentations of maduramicin, proposing that their tandem mass spectrometric results suggested a concerted mechanism for CO_2 and H_2O loss, rather than the sequential mechanism indicated in Scheme 1. Maduramicin differs from semduramicin stereochemically and in having additional methoxyl groups attached to rings a and g.

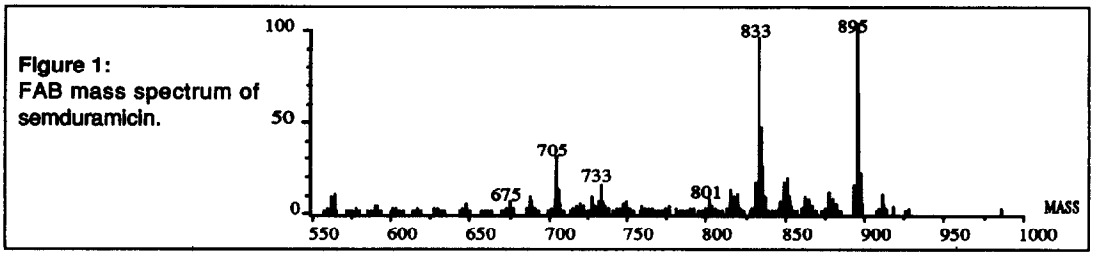
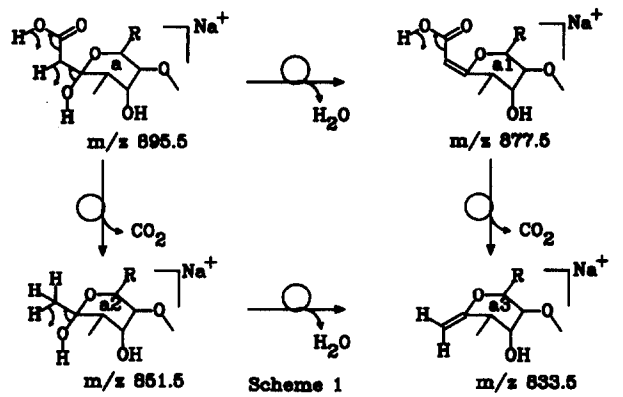


Figure 1:
FAB mass spectrum of semduramicin.



Scheme 1

All of the ion structures proposed in Scheme 1 include an Na^+, its presence being supported by accurate mass measurements[12]. The Na^+, however, is not associated with the carboxylic acid functional group. The m/z 833 ion has lost the carboxylate group, but still contains the Na^+. This behavior is consistent with the fact that semduramicin and related compounds show high monovalent cation affinities. The absence of any significant $[M+H]^+$ for semduramicin is consistent with the observations on maduramicin by Siegel *et al.*[11]. They expended considerable effort to prepare salt-free maduramicin, only to be rewarded with very weak $[M+H]^+$. Our attempts to produce samples of "free acid" showed a similar tenacity to retain Na^+. This tight cation binding is made even more remarkable by the electron ionization observations reported on other polyether ionophores by Chamberlin and Agtarap[6] and by Occolowitz *et al.*[13,14]. They report that, regardless of their efforts to eliminate sources of Na^+ contamination, their EI probe distillation experiments still indicated sodium complexes of molecular ions and fragments. They concluded that the Na^+ could have come only from their compounds' scavenging Na^+ from unusual places.

The FAB mass spectra of the two structural analogs are shown in Figures 2 and 3. The spectra clearly show $[M+Na]^+$ peaks at m/z 881, 14 daltons less than that of semduramicin. The peak at m/z 819 corresponds to $[M+Na-CO_2-H_2O]^+$, and the small peaks between it and $[M+Na]^+$ correspond to H_2O, CO_2 and CH_3OH neutral losses. These clearly indicate that the carboxyl group and immediately adjacent structures remain intact in both analogs.

^{13}C DEPT NMR examination clearly shows the two methoxyl carbons of semduramicin -- carbons 44 and 45. Their ^{13}C chemical shifts are given in Table 1. Similar study of the two desmethyl analogs clearly identified which methoxyl carbon is missing in each. Effects on the chemical shifts of carbons 5, 6 and 7 of the a-desmethyl analog, and of carbons 40, 41 and 42 in the g-desmethyl analog, are noted.

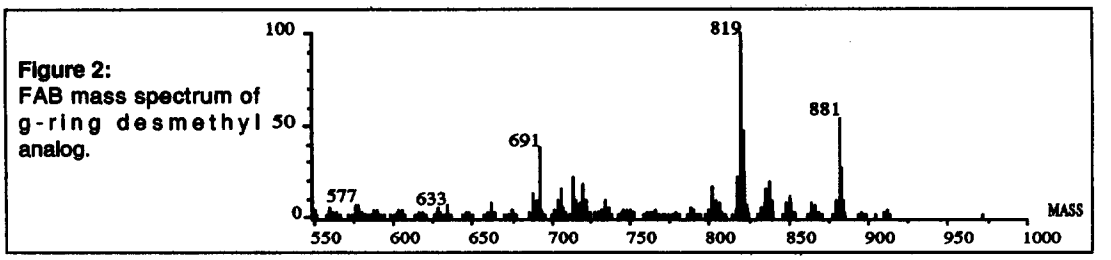


Figure 2:
FAB mass spectrum of g-ring desmethyl analog.

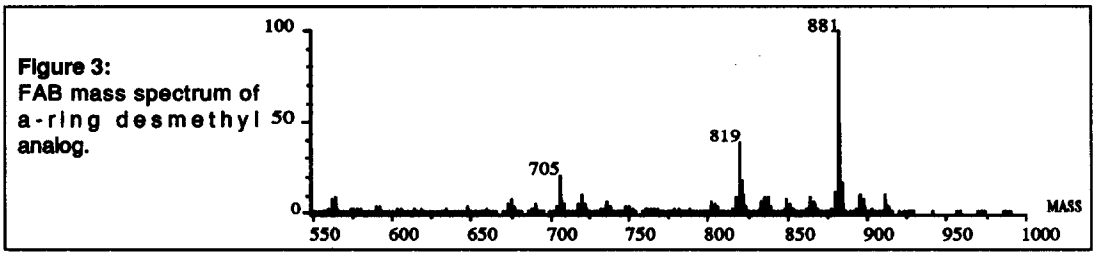


Figure 3:
FAB mass spectrum of a-ring desmethyl analog.

Table 1: ^{13}C NMR Chemical Shifts

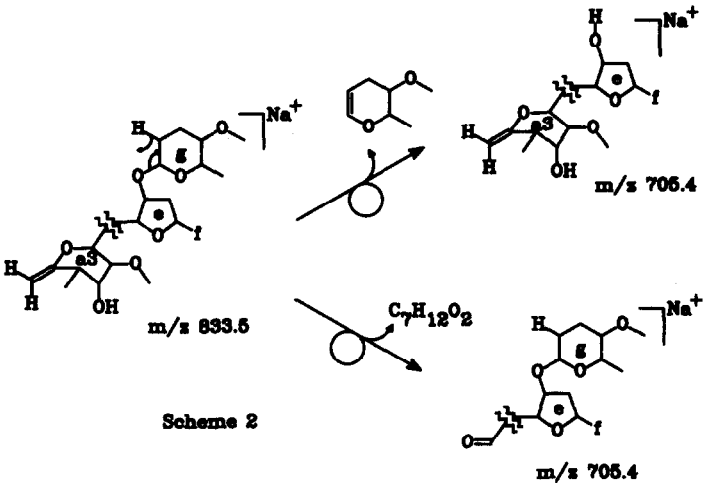
Carbon[a]	Semduramicin	g-desmethyl	a-desmethyl
5	74.9	74.9	75.9[b]
6	82.1	82.0	72.9[b]
7	66.9	66.8	67.8[b]
40	27.0	31.0	26.9[b]
41	80.0	75.8	79.9[b]
42	74.7	73.0	74.6[b]
44	56.9	--.-	56.8
45	59.1	59.0	--.-

a. Refer to structure 1 for the carbon numbering scheme of semduramicin
b. These ^{13}C chemical shifts were provided by Dirlam *et al.*[15].

Knowing the identity of the missing methoxyl carbon constrained our interpretation of the FAB mass spectral fragmentation of these two desmethyl analogs. Initially, the peak at m/z 705 in the spectra of both semduramicin and the analog of Figure 3 was interpreted according to the upper reaction of Scheme 2. (The m/z values given in Scheme 2 are for 1.) This led to our initial mis-assignment of this compound as a g-desmethyl analog. Both semduramicin and a g-desmethyl compound would fragment by this reaction to give the m/z 705 fragment. ^{13}C NMR clearly indicated, however, that methoxyl carbon 44 was still present in the compound of Figure 3, and carbon 45 was missing. The compound of Figure 3 was therefore an a-desmethyl analog, and another rationalization of the m/z 705 peak was needed.

The lower reaction of Scheme 2, however, offers a reaction across the a ring, which loses a fragment of the same elemental composition as the upper reaction -- a constraint required by accurate mass measurements. Fragmentation of the analog demonstrated by ^{13}C NMR to be the a-desmethyl analog -- the compound of Figure 3 -- would produce the required m/z 705 fragment. The analog in Figure 2, identified by ^{13}C NMR to be the g-desmethyl analog, would fragment according to the lower reaction of Scheme 2 to give a fragment at

m/z 691. The data are consistent with fragmentation across the a ring of semduramicin and the two desmethyl analogs, and for the FAB mass spectrum of maduramicin shown by Siegel *et al.*[11].



Scheme 2

Crystallography [16] shows that Na^+, and also Ag^+ in the silver complex[10], is bound by a coordination "sphere" formed by the oxygens of rings b through f. Such a complex would stabilize the involved portions of the molecule toward mass spectral fragmentation. Interaction of the a ring with the cation is through the carboxylate group. Once the carboxylate is gone, both g and a rings would be "loose" and available for further reactions. Fragmentation chemistry, however, appears to show preference for the a ring.

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