STRUCTURE OF THE QUINONE ANTIBIOTIC EM5519 AND THE BEHAVIOR OF QUINONES IN FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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Fast atom bombardment (FAB) mass and MS/MS spectra of a novel quinone antibiotic are presented. Their interpretation is based upon the examination of reductive behavior of model quinones in FAB solvents.

Saframycins[1], a new family of antibiotics isolated from *Streptomyces*[2] and marine sponges[3] have attracted considerable interest due to their antitumor activities[4]. They possess a unique skeleton containing a 1,3′-dimeric structure of two isoquinoline quinones[5], and a stereo-controlled total synthesis has now been reported[6]. Recently we and another group have independently isolated a new isoquinoline quinone antibiotic **1** from a bacterial source[7,8]. This compound is clearly similar to the saframycins and possesses broad-spectrum antibacterial activity. Structure **1** is given to EM5519 on the basis of chemical and spectroscopic studies*, including fast atom bombardment (FAB) mass spectrometry (MS), and by analogy to the closely related compound safracin A (**3**) and to safracin B[8,9]. In connection with these studies, we also examined the properties of model quinone compounds by FAB MS**. We observed $(M+H)^+$ ions, together with abundant $(M+2H)^+$ and $(M+3H)^+$ ions corresponding to the molecular ion and the protonated molecule of the hydroquinone, respectively. Similar observations have been recently reported for field desorption mass spectra of other quinone antibiotics although no details were given[10]. The apparent reduction of saframycin R under FAB conditions has also been noted[11]. We studied the conditions of quinone to hydroquinone reduction as evident from the positive and negative FAB MS of these quinones. Details of these results in combination with high resolution and mass spectrometry/mass spectrometry (MS/MS) data aided in the assignment of structure **1**, the details of which are presented herein.

The quinone antibiotic **1** has a molecular formula $C_{28}H_{36}N_4O_7$. The IR spectrum contained bands at 3350 cm^{-1} (NH/OH) and 1650 cm^{-1} (quinone). The UV spectrum at 270 nm shifted to 292 nm with the addition of base, indicating the presence of a phenolic group. The ^1H NMR spectrum contained signals at δ 0.80 (d, 3H, J=7 Hz) due to the alanine-methyl, 1.86 (s, 3H), 2.23 (s, 3H), 3.76 (s, 3H) and 4.03 (s, 3H) due to the methyl and methoxyl groups on the quinone and aromatic rings, and at 2.31 (s, 3H; shifting to 2.67 in acid) due to the *N*-methyl group. The signal at δ 6.73 was assigned to the single aromatic proton, while the signal at δ 3.38 (q, 1H, J=7 Hz) was due to the alanine-methyne.

The assignment of the remaining ring protons is shown in Table 1, and was made on the basis of further double irradiation and D_2O exchange experiments. Irradiation of the ddd signal at δ 1.50 (H_a)

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* EM5519 is identical to safracin B[7~9].

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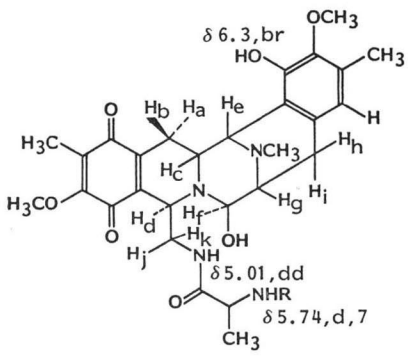
Table 1. ^{1}H NMR assignments of quinones **1** and **2**.



1 R = H

2 R = $COCH_3$

Proton δ (ppm)	J (Hz)
H_a, 1.50	ddd, 2.8, 11, 17
H_b, 2.90	dd, 4, 17
H_c, 3.25	ddd, 1.6, 4, 11
H_d, 4.20	m, $J_{a,d}$=2.8
H_e, 4.00	d, 1.6
H_f, 4.40	d, 2
H_g, 3.15	m, 2, 7.7
H_h, 2.28	d, 19
H_i, 2.95	dd, 19, 7.7
H_j, 2.90	d, 16
H_k, 3.70	dd 16, 4

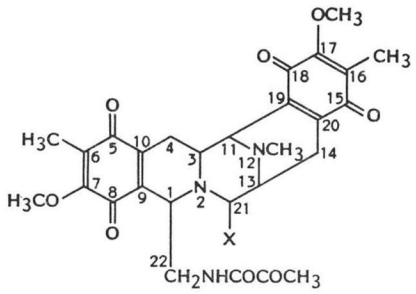
led to the collapse of the signal at δ 2.90 (H_b) from a dd to a d, and also caused collapse of the H_c absorption at δ 3.25 from a ddd to a dd. H_a and H_b show a coupling constant $J_{a,b}$=17 Hz characteristic of geminal protons. The coupling constants among H_a, H_b, H_c, H_d were determined as $J_{a,d}$=2.8, $J_{b,d}$=0.0, $J_{a,b}$=17, $J_{a,c}$=11.0, $J_{b,c}$=4 Hz. As in the cases of reported saframycins[12], the magnitude of the coupling between H_a and H_d over five bonds is consistent with such long range coupling for a homoallylic system[13]. This requires that H_a and H_d be orthogonal to the quinone ring and thus permits stereo-chemical assignment of H_a and H_d.

^{13}C NMR spectral measurements were taken on the *N*-acetyl derivative of **1**, namely compound **2**. The assignments of the carbons were determined using the INEPT[14] technique. Assignments were made by comparison to ^{13}C NMR data of saframycin A (**4**)[2] and saframycin S (**5**)[15]. These data are presented in Table 2, and it is apparent that a close similarity exists. It should be pointed out that both saframycin S (**5**) and compound **2** exhibit a carbon resonance at *ca.* 82 ppm that is assigned to the C-21 carbinolamine carbon. When the C-21 is substituted with a cyano group as in **4**, the C-21 resonance is shifted to 58.3 ppm. Only two quinone carbonyls appear in the spectrum of **2** at δ 187.5 and 182.3 and are attributable to the C_5 and C_8 carbonyls respectively. One of the rings is reduced and the ^{13}C resonances indicate a tetra-substituted phenol.

The positive FAB MS (8 KeV Xe°; thioglycerol matrix) of compound **1** (Fig. 1) and compound **2** (Fig. 2) showed ions at *m/z* 525 and 567, respectively. High resolution analysis of the 525^+ ion yielded 525.2703, requiring a molecular formula of $C_{28}H_{37}N_4O_6$ (calcd 525.2713). Ions at *m/z* 523 (50% relative intensity to 525^+) and at *m/z* 565 (25% relative intensity to 567^+) were initially attributed to H_2 loss from their respective parent ions. These ions, however, varied with changes in solvent (thioglycerol, glycerol, tetramethylene sulfone), the total fast atom flux, and the analysis time. These factors, along with other spectroscopic data, led us to consider the origin of these ions.

In order to accomodate the above results with the proposed structure we suggest that **1** is partially converted to the hydroquinone form under FAB conditions. The hydroquinone then fragments according to the scheme given in Fig. 3. The protonated molecule of either the quinone or hydroquinone form of **1** was not observed (expected 541^+ and 543^+, respectively). Loss of H_2O from these parents yield ions at 523^+ and 525^+. The $(M+2H)^+$ ion (expected *m/z* 542) was also not seen but contributes by OH· loss to the 525^+ fragment. High resolution analysis of the daughter ions supports the fragmentation scheme presented. The daughter ions at *m/z* 204 and 425 are analogous to those proposed by IKEDA[9], while the 218^+ ion is assigned a different structure based upon high resolution analysis.

Table 2. ^{13}C NMR chemical shifts for saframycin A, S, and quinone **2***.



Saframycin A (**4**) X = CN
Saframycin S (**5**) X = OH

C-No.	4	5	2	C-No.	4	5	2
5	186.5	188.6	187.5	21	58.3	81.6	82.0
8	183.4	182.9	182.3	OCH_3(7)	61.1	61.1	61.4
15	185.2	185.6	121.9	OCH_3(17)	61.0	61.1	60.5
18	180.8	180.9	144.7	1,	56.3,	55.0,	53.8,
				11	54.6	54.1	50.4
7	155.6	156.0	156.3	3,	54.3,	52.9,	50.1,
				13	54.0	49.7	58.2
17	155.9	156.0	137.2	NCH_3	41.6	42.5	40.8
10	141.6	141.1	142.2	22	40.7	41.1	42.8
20	141.2	133.9	118.0	4	25.1	25.2	25.4
9	135.6	137.3	134.1	14	21.6	21.0	24.8
19	135.6	135.1	112.1	CH_3(6)	8.7	8.8	9.6
6	129.2	129.5	128.9	CH_3(16)	8.7	8.8	16.4
16	128.3	127.8	130.9				

*_N_-Acetyl alanyl chemical shifts: 17.8 (CH_3), 22.8 (CH_3), 50.3 (CH) and 173.1, 175.2 ppm (C=O).

These fragmentations confirm the locations of the quinone ring and the tetra-substituted phenol ring with respect to each other and eliminate the possibility of reversal.

The closely related compound[8] safracin A (**3**) (see Fig. 3) was distinguished from **1** by the presence of parent ions at m/z 525 and 527. High resolution of the 525^+ ion of **1** (525.2703) and of **3** (525.2737) failed to distinguish the two structural forms. This is expected in light of their common molecular formula ($C_{28}H_{37}N_4O_6$, calcd 525.2713). A distinction of the two related compounds from their mass spectra alone is difficult due to their extreme similarity, the presence of both oxidized and reduced forms of each, and chemical noise common to FAB spectra.

These difficulties were overcome using two stages of mass spectrometry (*i.e.*, MS/MS)[16,17]. Mass-analysis of the 525^+ ion, collisional activation, and subsequent kinetic energy analysis of the resultant daughter ions yield MS/MS spectra of **1** and of **3**. These spectra are shown in Fig. 4 and illustrate the distinguishing features of these isomeric forms. The relative daughter ion intensities of $218^+/204^+$ differentiate **1** (ratio=0.5) from **3** (ratio=2.0). The resultant ion formed by direct protonation of the quinone form of **3** differs from that generated by water loss from the hydroquinone form of **1**. These differences are reflected in their rates of dissociation, and hence, in their relative daughter ion intensities. Such a distinction of the $218^+/204^+$ daughter ion ratio is extremely difficult from their conventional mass spectra due to the presence of solvent background.

Fig. 1. Positive FAB MS of compound **1** in a *p*-toluenesulfonic acid and thioglycerol matrix.

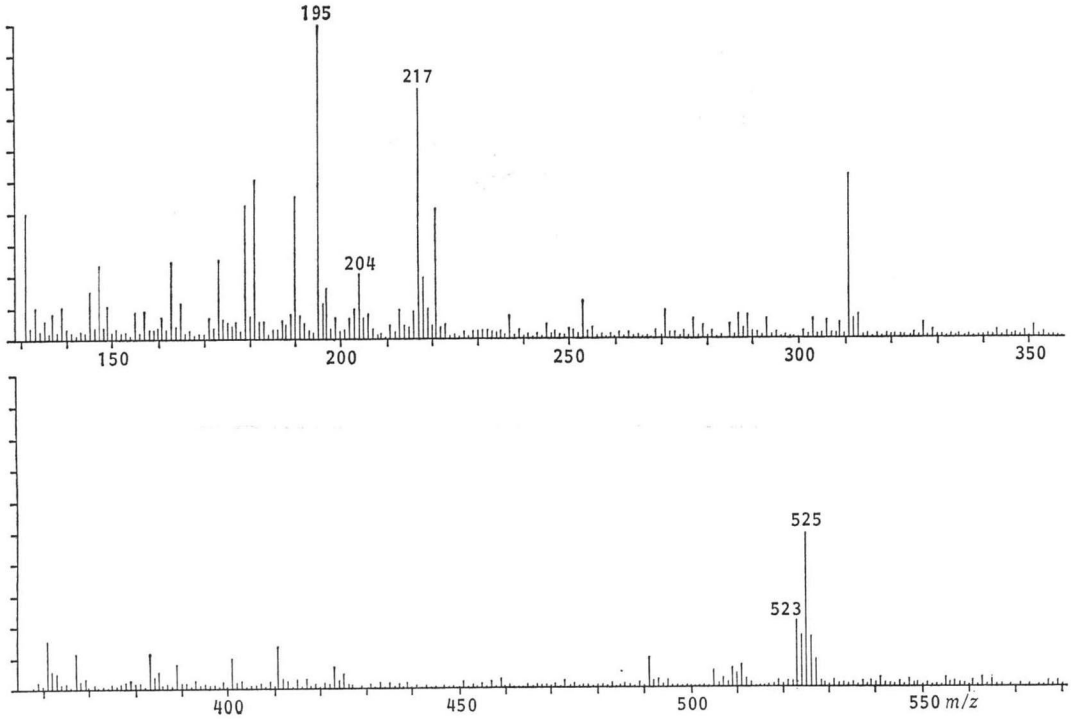


Fig. 2. Positive FAB MS of compound **2**, the *N*-acetyl derivative of **1**, in a thioglycerol matrix.

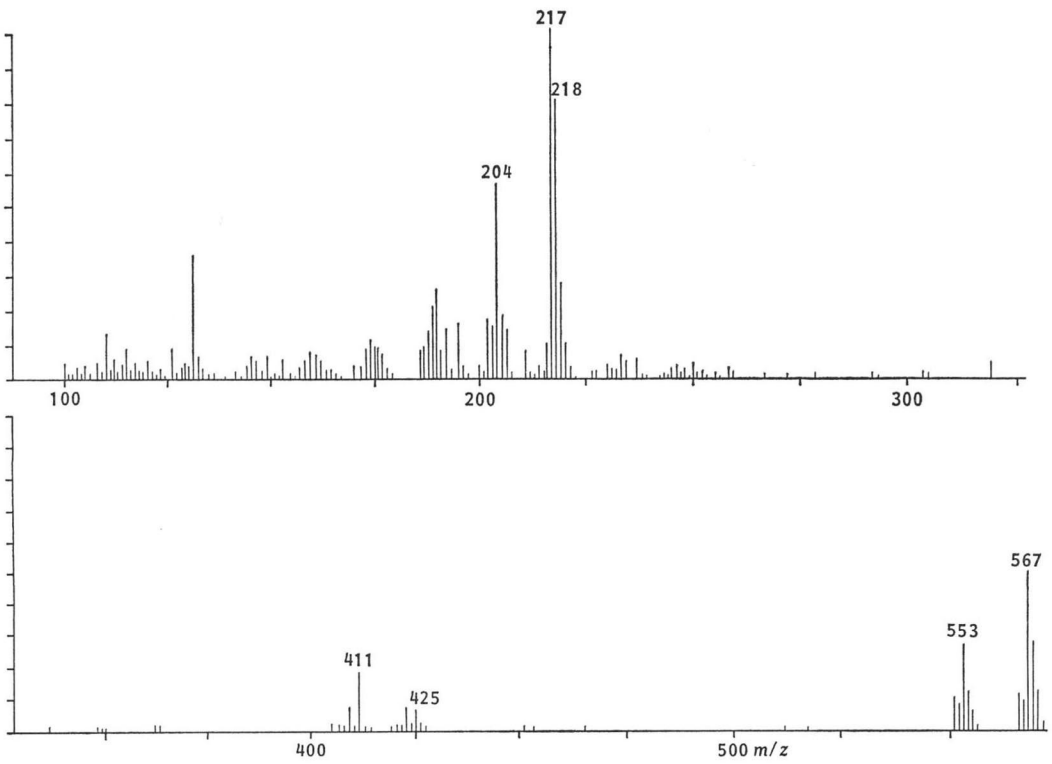
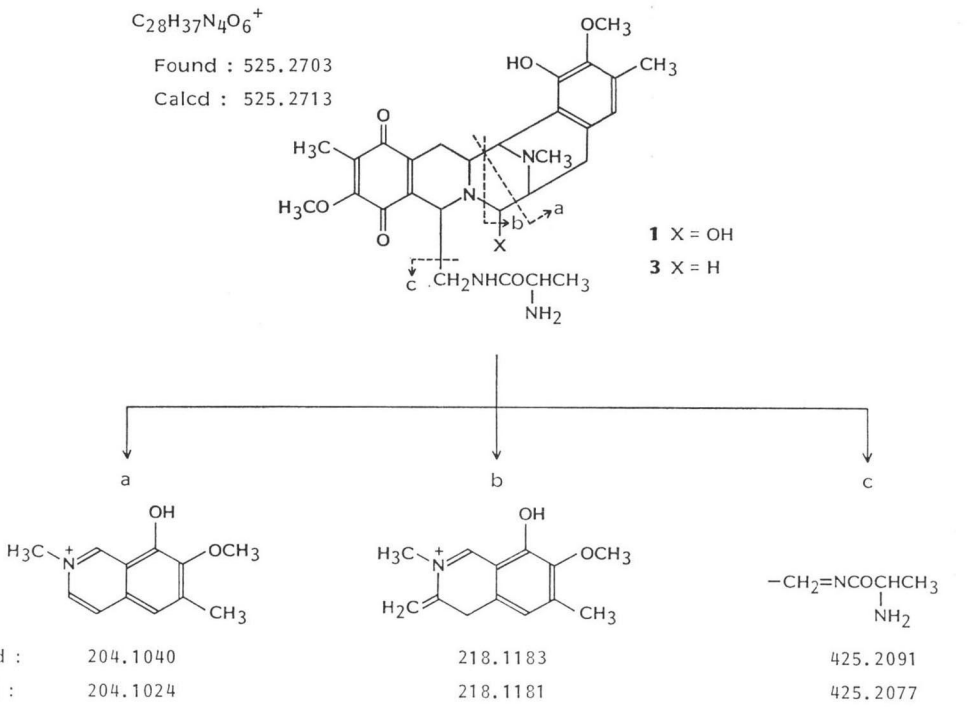


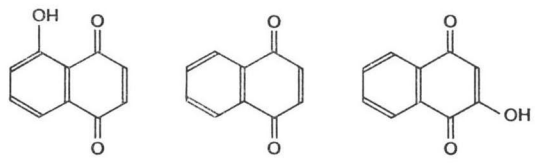
Fig. 3. Details of fragmentation observed from the 525^+ ion of quinone **1**.



The reduction and dehydroxylation behavior of quinone **1** seen in FAB is also supported by the observation of $(M+2H-H_2O)^{\cdot+}$ ions in electron impact mass spectra of the tetra trimethylsilyl (TMS) derivative, *m/z* 812, and of the *N*-acetyl, trimethylsilyl derivative, *m/z* 782. Incorporation of the single TMS or acetyl group, into the amide side chain of **1** is indicated by the presence of a common daughter ion, 639^+, for both derivatives.

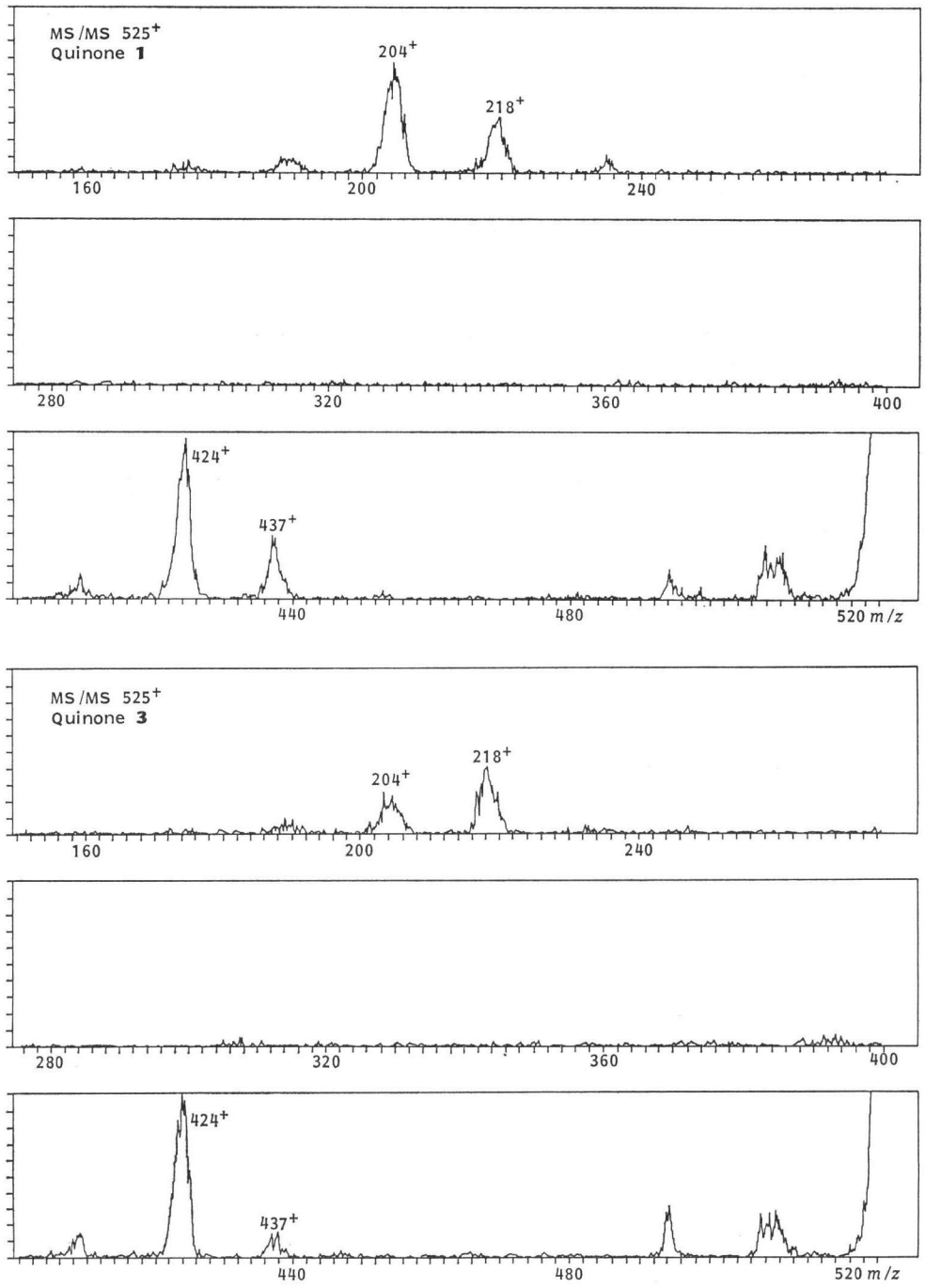
Closer examination of model quinones by FAB MS provided an understanding of the previous results. Quinones **6** (5-hydroxy-1,4-naphthaquinone), **7** (1,4-naphthaquinone), and **8** (2-hydroxy-1,4-naphthaquinone) showed significant $(M+2H)^{\cdot+}$ and $(M+3H)^+$ ions. The ratio of reduced to unreduced forms as given by the $[(M+2H)^{\cdot+}+(M+3H)^+]/(M+H)^+$ ion intensity varied approximately $20\sim50\%$ during the $10\sim15$ minutes analysis time. The average value was structure and solvent specific. For example, quinone **6** yielded a ratio of 2.0 in thioglycerol while **7** gave 0.70 and **8** yielded 1.0. The ratios for **6**~**8** were slightly higher in glycerol indicating that the -SH group of monothioglycerol was not responsible for the observed reduction.

The ratio of negative ion intensities, $[(M+H)^-+M^{\cdot-}]/(M-H)^-$, also paralleled those seen in the positive FAB MS. Quinone **6** gave 2.2, **7** produced a 1.1 ratio, and **8** yielded 1.2. The reduction potentials as measured by polarography[18] compare favorably with the measured positive and negative ion ratios. Quinone **6** showed one and two electron reduction potentials



6 **7** **8**

Fig. 4. MS/MS comparison of the 525^+ ion from quinone **1** with that of quinone **3**.



of -0.51 volt and -1.17 volt, **7** gave -0.72 volt and -1.63 volt, while **8** yielded -0.64 volt and -1.20 volt. These reduction potentials were measured using dropping mercury and saturated calomel electrodes in dimethylformamide at 25°C.

While it is difficult to quantify these relationships, the trend is evident. Quinones with low reduc-

tion potentials such as 5-hydroxy-1,4-naphthaquinone exhibit large $(M+2H)^{\cdot}$ and $(M+3H)^{+}$ ion intensities. Negative ion intensities also reflect this behavior as intense $(M+H)^{-}$ and $M^{\cdot-}$ ions are observed. The ratio of reduced to unreduced forms is influenced by (1) choice of solvent, (2) length of analysis, and (3) quinone structure. Recent experiments have indicated the reduction of two quinones in a single molecule (SINGH, P. & S. E. UNGER, unpublished results) and demonstrate the complexity of ions which may be formed under FAB conditions. However, this phenomenon, if recognized, can provide useful structural information regarding quinone antibiotics.

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