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Article in *European Mass Spectrometry* · January 1995

DOI: 10.1255/ejms.123

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## Fast atom bombardment (FAB) mass spectrometry of diterpene resin acid derivatives

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The behaviour of methyl dehydroabietate and its ring C-substituted derivatives has been investigated by fast atom bombardment (FAB) mass spectrometry conditions. Similar FAB mass spectra were obtained in a 3-nitrobenzyl alcohol matrix using argon or xenon as the target gas, which has been attributed to the hydrophobic character of these compounds. Significant  $[M - H]^+$ ,  $M^{+\bullet}$  and  $[M + H]^+$  peaks and minor abundant  $[M - 3H]^+$ ,  $[M - 2H]^{+\bullet}$  and  $[M + 2H]^{+\bullet}$  ions have been observed to occur in the spectra. The intense  $M^{+\bullet}$  peaks generally observed have been rationalised in terms of the relatively aprotic and hydrophobic character of the matrix 3-nitrobenzyl alcohol and the hydrophobic nature of the compounds studied. The molecular ion represents the base peak in the molecular mass region of the amino abietates when 3-nitrobenzyl alcohol or triethyltetramine are used as matrices, presumably reflecting the stability of these radical cations. The relatively aprotic character of the matrix has been suggested to favour the ionization to  $M^{+\bullet}$  ions of the amino derivatives. The electrophilic nitro groups and/or a proton acceptor carbonyl group, have been found to play an important role in the formation of  $[M + H]^+$  with respect to  $M^{+\bullet}$  ions. The enhanced abundance of  $[M + H]^+$  with respect to  $M^{+\bullet}$  ions, when HCl was added to the matrix, as well as in a thioglycolic matrix, has been suggested to derive from a contribution of solution chemistry for the compounds bearing one to two unsubstituted amino groups. An important role played by the common hydrocarbon skeleton has been suggested to account for the significantly abundant  $[M - H]^+$  ions observed.

### Introduction

Resin acids generally occurring in nature are of wide use in the chemical industry and some of their derivatives have been prepared as surfactants.<sup>1</sup> Dehydroabietic acid, the main component of disproportionated rosin, is a readily available hydrophenanthrene derivative and a useful starting material for the synthesis of industrial and/or physiologically important products, through the introduction in the aromatic ring of suitable substituents, such as nitro or amino groups. Methyl dehydroabietate and other similar diterpene resin acid derivatives have been extensively studied by EI mass spectrometry.<sup>2,3</sup> Methyl dehydroabietate, often preferred to the acid as it provides a protected group which inhibits the carboxyl group from taking part in the derivatisation reactions of the aromatic ring, exhibits a 70 eV EI mass spectrum with only two fragment ions of significant abundance and relatively low abundant molecular ions. We decided therefore to investigate the

behaviour of this compound and its substituted ring C derivatives under fast atom bombardment (FAB) mass spectrometry conditions, a technique which has proved to be a useful tool for structural elucidation since its appearance in the last decade.<sup>4</sup>

### Experimental

Methyl dehydroabietate and its substituted derivatives were directly solubilised in 4  $\mu$ l 3-nitrobenzyl alcohol (NBA).

The FAB mass spectra obtained from  $\sim 10^{-1}$  mg compound added to the matrix on the target were recorded with a Kratos MS 25 RF mass spectrometer. An argon or xenon beam with an energy of 5 keV was used at a source housing pressure of  $\sim 5 \times 10^{-5}$  Torr (1 Torr = 133.3 Pa) and a gun current of 1 mA. Where applicable the matrix was acidified with concentrated HCl ( $10^{-2}$  ml HCl added to 1 ml matrix) before application to the probe tip.

Table 1. Ion abundances in the molecular mass region of compounds 1–8 in a neutral NBA matrix and using xenon as target gas [relative abundance (%) with  $m/z$  in parentheses].<sup>a-c</sup>

Compound	Ions					
	$[M - 3H]^+$	$[M - 2H]^{2+}$	$[M - H]^+$	$M^{2+}$	$[M + H]^+$	$[M + 2H]^{2+}$
1	2.9 (311)	7.7 (312)	61.1 (313)	51.9 (314)	100.0 (315)	0.9 (316)
2	3.2 (326)	5.5 (327)	25.2 (328)	100.0 (329)	40.2 (330)	1.1 (331)
3	2.6 (341)	6.9 (342)	25.3 (343)	100.0 (344)	45.3 (345)	1.1 (346)
4	1.6 (371)	2.6 (372)	15.4 (373)	100.0 (374)	64.5 (375)	3.0 (376)
5	0.7 (356)	1.5 (357)	19.7 (358)	13.0 (359)	100.0 (360)	1.7 (361)
6	3.5 (401)	2.7 (402)	19.2 (403)	5.8 (404)	100.0 (405)	2.5 (406)
7	2.9 (413)	3.1 (414)	14.9 (415)	19.1 (416)	100.0 (417)	0.5 (418)
8	1.1 (425)	2.5 (426)	14.0 (427)	55.2 (428)	100.0 (429)	2.0 (430)

<sup>a</sup>Relative abundances have been normalised to a value of 100 units for the most intense peak in the molecular mass region of the spectrum.

<sup>b</sup>Five averaged mass spectra were used to obtain these data.

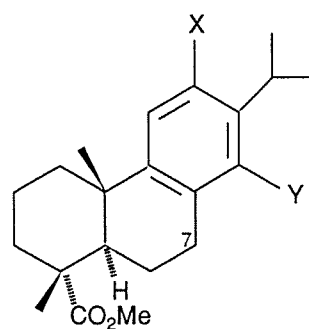
<sup>c</sup>Abundances have been corrected for isotopic contribution.

The spectra presented are an average of five successive scans unless stated otherwise. The contribution of the matrix has been eliminated in the FAB mass spectra of all compounds, with the exception of methyl dehydroabietate, compound 1.

Compound synthesis is described elsewhere.<sup>5</sup>

## Results and discussion

Methyl dehydroabietate and its ring C substituted derivatives, 1–8 are presented in Scheme 1. Owing to the high solubility of these compounds in 3-nitrobenzyl alcohol (NBA)



Compound	X	Y
1	H	H
2	NH <sub>2</sub>	H
3	NH <sub>2</sub>	NH <sub>2</sub>
4	NH <sub>2</sub>	NO <sub>2</sub>
5	H	NO <sub>2</sub>
6	NO <sub>2</sub>	NO <sub>2</sub>
7	NHCOCH <sub>3</sub>	NO <sub>2</sub>
8	NHCOCH <sub>3</sub>	NHCOCH <sub>3</sub>

Scheme 1.

and to the low solubility in glycerol, the former has been selected as a preferential matrix. Since the X or Y substituted derivatives give identical FAB mass spectra, just one isomer of each compound has been investigated. The FAB mass spectrum of methyl dehydroabietate, 1, using argon as target gas, Figure 1(a), typically shows abundant peaks in the molecular mass region, and in general rather abundant fragment ions. The FAB mass spectra of methyl dehydroabietate and its derivatives, 1–8, using argon or xenon as target gas appear not to be significantly different, as may be observed, for example, when comparing the FAB mass spectra of 1, Figures 1(a) and (b). This observation may be attributed to the tensioactive character in general exhibited by these compounds, as has been suggested for compounds with a hydrophobic nature.<sup>6</sup> Compound 1, in particular, effectively suppresses the matrix peaks as may be observed in Figures 1(a) and (b).

The most striking and characteristic common feature of the FAB mass spectra of methyl dehydroabietate and its derivatives 1–8, using NBA as matrix, is the occurrence of significant  $[M - H]^+$ ,  $M^{2+}$  and  $[M + H]^+$  peaks and minor abundant  $[M - 3H]^+$ ,  $[M - 2H]^{2+}$  and  $[M + 2H]^{2+}$  ions (Table 1).

Considering the relatively aprotic and hydrophobic character of the matrix NBA and the hydrophobic nature of these compounds, the appearance of significantly abundant molecular ions is not surprising and is in agreement with previous findings.<sup>7–11</sup> The fact that  $M^{2+}$  ions correspond to the base peak in the molecular mass region of derivatives bearing one or two primary amino groups, 2–4, seemed improbable in view of the high basicity of these compounds. FAB MS/MS experiments performed on the protonated molecule of these compounds showed that  $M^{2+}$  ions were not produced by dissociation of the  $[M + H]^+$  ions. CI experiments using methane as reagent gas, on 3, a compound with two amino substituents which has been used as a model compound, showed the presence of  $[M - H]^+$ ,

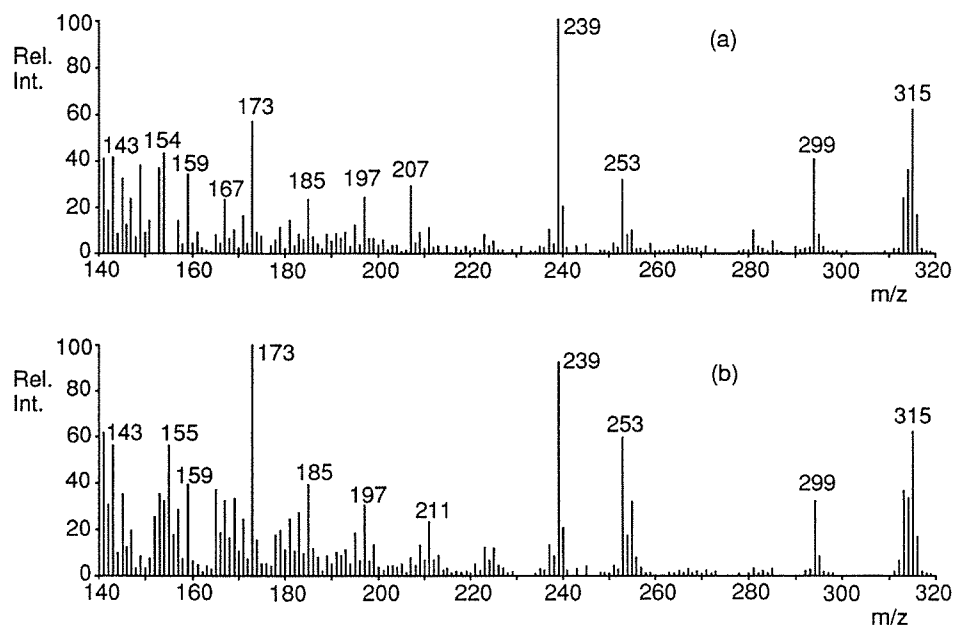


Figure 1. Partial positive-ion FAB mass spectrum of methyl dehydroabietate **1**, obtained in a NBA matrix using (a) argon and (b) xenon as the target gas.

$M^{+•}$  and  $[M + H]^+$  ions with 13.0, 41.1 and 100% relative abundances, respectively.<sup>12</sup> These results suggest that besides other mechanisms, including possibly collision interactions between analytes *M* and recoiling matrix molecular ions,<sup>11</sup> CI-like charge transfer processes may partially contribute to  $M^{+•}$  ion formation. Further experiments on **3**, in triethyltetraamine (TTA), a liquid matrix which cannot provide a good source of hydrogens, but which can on the other hand accept protons, showed that in this case the protonation of the molecule ( $[M + H]^+$  rel. int. = 15.1, whereas  $M^{+•}$  rel. int. = 100%) is even less effective than in NBA (Table 1). In the protic, polar matrix glycerol, however,  $[M + H]^+$  ions correspond to the base peak while  $M^{+•}$  ions account for 75.2% rel. int. These findings seem to suggest an important role played by the matrix in the  $M^{+•}$  ion formation of the amino derivatives.

Predominant molecular ions have been related to several properties including very low ionisation potentials<sup>13,14</sup> and/or high volatility<sup>15–17</sup> of compounds, formation of charge-transfer complexes with the help of solvation energy of the ions<sup>18</sup> and ease of radical cation formation.<sup>19</sup> At present, the lack of

appropriate data does not enable us to draw conclusions regarding the ionisation potential of the amino abietates with respect to the other derivatives, although we assume it to be rather low. This would be consistent with the occurrence of charge-transfer reactions leading to the significant  $M^{+•}$  ions observed in the CI mass spectra of these compounds. High volatility does not appear to be the case. The possibility that the amino abietates and the electron scavenging matrices undergo charge transfer complexation does not appear to be a determining factor, since undissociated complexes were detected in very low concentrations <1% for these derivatives. Although agreement on many aspects of FAB has gradually been reached, in a few cases consensus has not yet been attained, as noted by Sunner.<sup>20</sup> The mechanism(s) for the formation of molecular ions in a liquid matrix seem to be a good example of the latter. For the aminoabietates we tend to suggest an easy radical formation at the allylic 7-position, with the consequent stabilisation of the molecular cation, due to the delocalised  $\pi$ -system. This process which will be favoured in a liquid matrix easily accepting protons such as TTA, may also account for the observed effect. When the nitrogen atoms in

Table 2. Relative intensity<sup>a-b</sup> ratio of peaks in the molecular mass region of compounds **1–8** in an NBA matrix acidified with conc. HCl and using xenon as the target gas (values in the neutral NBA matrix in parentheses).

Species	Compounds							
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
$[M + H]^+/M^{+•}$	1.9 (1.9)	1.0 (0.4)	1.7 (0.4)	1.1 (0.6)	8.0 (7.7)	18.9 (17.2)	5.0 (5.2)	1.8 (1.8)

<sup>a</sup>Five averaged mass spectra were used to obtain these data.

<sup>b</sup>Abundances have been corrected for isotopic contribution.

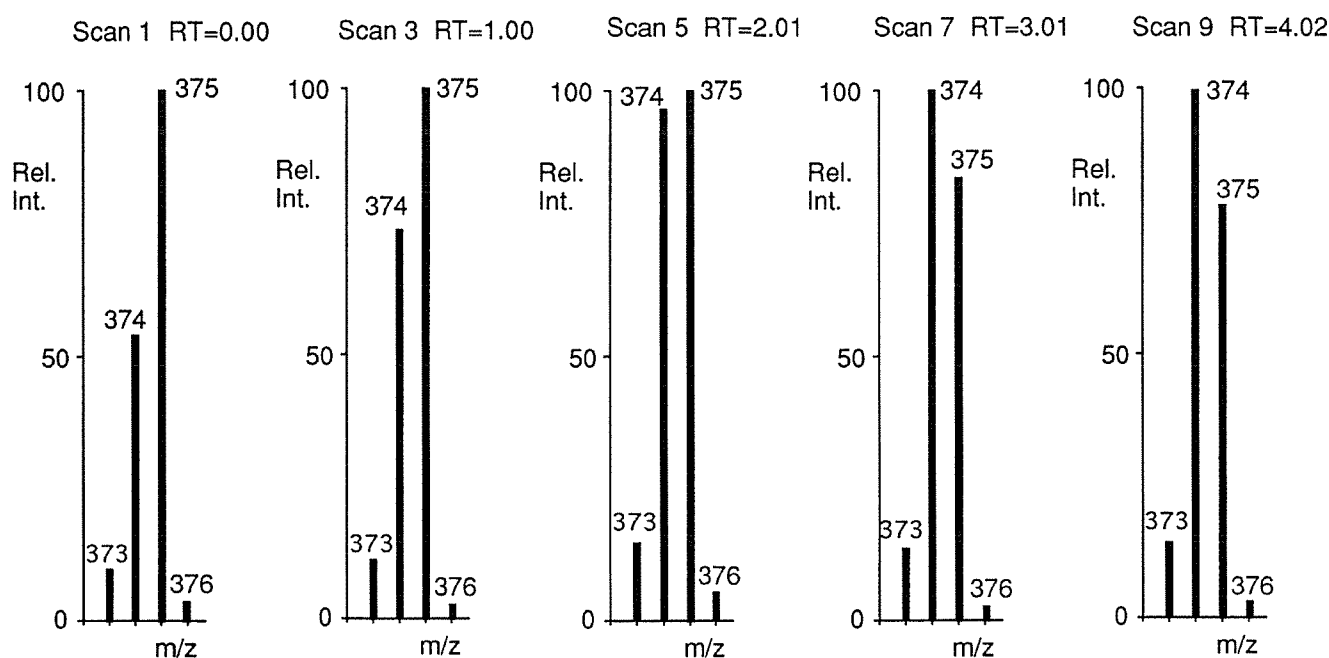


Figure 2. Ion peak intensities versus time (RT) in minutes, in the molecular mass region of the positive-ion FAB mass spectra of compound 4, in a NBA matrix acidified with concentrated HCl. All relative intensities have been corrected for isotopic contribution.

the aromatic substituent lack basic character as in the amides, **7** and **8**, the behaviour approaches that of **1**, the unsubstituted compound.

A similar phenomenon to the one we observed for the unsubstituted amino derivatives has been reported for *o*- and *m*-nitroanilines in NBA as well as in a 2-nitrophenyl octyl ether (NPOE) matrix<sup>13</sup> and attributed to a significant decrease in basicity of the *o*- and *m*- isomers upon excitation. Such an hypothesis might be worth investigating since the excited state acid–base properties of the amino derivatives could also possibly contribute for the observed results.

On the other hand, the acetylated derivatives **7** and **8** already show a preference for  $[M + H]^+$  formation over  $M^{+•}$ , which could be attributed to the presence of a proton acceptor carbonyl group, consistent with previous reports in the literature.<sup>7</sup> This observation may be attributed to the presence of the proton acceptor carbonyl group and/or the hydrogen-bonding interaction between this group and the alcoholic hydroxyl group of the matrix NBA, as proposed by Takayama *et al.*<sup>7</sup> for compounds bearing significant acceptor groups. Moreover, the relatively small value of the ratio  $[M + H]^+/M^{+•}$  for compound **4** with respect to the acetylated derivative **7** (Table 2) seems to provide further experimental evidence for the important influence of acetylation on  $[M + H]^+$  formation.

In addition, the ratio  $[M + H]^+/M^{+•}$  appears to be significantly enhanced for the mononitro and dinitro derivatives **5** and **6**, with a higher value for the latter. The mechanisms for  $M^{+•}$  and  $[M + H]^+$  ion formation may be competitive and of a complex nature. Nevertheless, these results seem to suggest that the electrophilic nitro groups play an important role in the

formation of  $[M + H]^+$  with respect to  $M^{+•}$  ions when NBA, a matrix with known electron scavenging properties is used. No significant  $[M + 2H]^{+•}$  ions are observed in the FAB spectra of this type of compound.

When HCl is added to the matrix, the ratio  $[M + H]^+/M^{+•}$  increases two to four times for compounds **2–4** with respect to the neutral matrix, otherwise this ratio is fairly constant (Table 2). Moreover, with the addition of HCl to the matrix,  $[M + H]^+$  ions correspond in general to one of the most intense peaks in the molecular mass region of the spectra for **2–4**. Nevertheless, with the depletion of HCl,  $M^{+•}$  ions may regain their former value in the neutral matrix, as illustrated for compound **4** (Figure 2). The protonation of the amino substituents in the acidified matrix may be responsible for the observed effect. Further experiments performed on compounds **2–4** using thioglycolic acid (TGA) as matrix, a compound which combines both acid and reducing agent characteristics<sup>21</sup> resulted in the enhancement of  $[M + H]^+$  ions which would represent the base peak in the molecular mass region of these compounds. Moreover, relatively large values of the ratio  $[M + H]^+/M^{+•}$ , i.e. 2.9, 2.3 and 3.6 for compounds **2**, **3** and **4**, respectively, when compared to the ones in the acidified NBA matrix (Table 2), were found. The results we obtained seem to argue for some contribution to  $[M + H]^+$  ion formation, from solution chemistry mechanisms, including possibly acid–base and oxidation–reduction reactions.

The significantly abundant  $[M - H]^+$  ions observed in both neutral and acidified matrices are noteworthy, since hydride abstraction has very seldom been observed under FAB conditions.<sup>16, 22, 23</sup> The enhanced abundance of  $[M - H]^+$  ions for **1**

when compared to its substituted derivatives 2–8, argues for an important role played by the common hydrocarbon skeleton. The ease of proton abstraction from the allylic 7-position may account, at least partially, for the observed effect. The contribution from the large hydrocarbon skeleton, which may provide several reaction sites as has been suggested for long chain ethers<sup>23</sup> may also not be excluded. Likewise, the occurrence of  $[M - 3H]^+$  and  $[M - 2H]^{2+}$  ions albeit small, is somewhat surprising. These aspects are currently under investigation.

### Conclusions

Similar FAB mass spectra are obtained using argon or xenon as the target gas which may be attributed to the hydrophobic character of these compounds. The significant  $M^{2+}$  peaks occurring in the FAB mass spectra may be rationalised in terms of the relatively aprotic and hydrophobic character of the matrix NBA and the hydrophobic nature of the compounds studied. For the derivatives bearing at least one amino group, the molecular ion corresponds to the base peak in the molecular mass region when NBA or TTA are used as matrices. The ease of radical ion formation may very well account for the observed effect. These processes appear to be favoured in a matrix with a relatively aprotic character. The formation of  $[M + H]^+$  with respect to  $M^{2+}$  ions seems to be enhanced by the electrophilic nitro groups. The addition of HCl to the matrix NBA, as well as a thioglycolic acid matrix, which increases the abundance of  $[M + H]^+$  with respect to  $M^{2+}$  ions for the compounds bearing one to two unsubstituted amino groups, seems to indicate a contribution from solution chemistry to the formation of  $[M + H]^+$  ions. The significantly abundant  $[M - H]^+$  ions observed seem to argue for an important role played by the common hydrocarbon skeleton. Moreover, the ease of proton abstraction from the allylic 7-position may also account for the observed effect.

### Acknowledgement

Stimulating discussions with Professor Ana Lobo are gratefully acknowledged.

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Received: 15 November 1994

Revised: 18 July 1995

Accepted: 1 August 1995

