

FURTHER STUDY OF THE MATRIX EFFECT ON THE EXTENT OF FRAGMENTATION IN MOLECULAR IONS M^{++} PRODUCED UNDER FAST ATOM BOMBARDMENT (FAB) CONDITIONS

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ABSTRACT

The extents of fragmentation in the molecular ions M^{++} of prenylated flavonoids produced under FAB conditions using various liquid matrices are evaluated by comparison with the corresponding electron ionization (EI) mass spectral patterns at electron impact energies of 15–70 eV. The extents of fragmentation in the M^{++} ions produced with *m*-nitrobenzyl alcohol as a FAB matrix are always lower than those produced with glycerol and thioglycerol as the matrices. A few FAB spectral data obtained here indicate that the extents of FAB fragmentation in the M^{++} ions correspond to those of the EI fragmentation in the M^{++} ions produced at the electron impact energy of more than 70 eV. Consequently, it is concluded that FAB is an EI-like hard ionization process even when a liquid matrix is used.

INTRODUCTION

Positive ion FAB and liquid secondary ion (liquid SI) mass spectra of organic compounds generally show various molecular ion species $(M - H)^+$, M^{++} , $(M + H)^+$ and $(M + Na)^+$, and also show the corresponding electron ionization (EI)-like and/or chemical ionization (CI)-like fragment ions. According to recent studies on FAB fragmentation with a four-sector tandem mass spectrometer [1,2], such fragments are formed by an EI-like and a CI-like fragmentation from the molecular ion M^{++} and the protonated molecule $(M + H)^+$, respectively, produced under FAB conditions. Positive ion FAB and liquid SI mass spectra of volatile organic compounds such as *o*- and *m*-nitrophenols, anisol, tetramethylene sulphone, benzophenone and diphenylamine show the molecular ions M^{++} and the corresponding EI-like fragment ions, when a liquid matrix is not used [3–5]. In such a case, particle bombardment ionization methods such as FAB and liquid SI may not actually be referred to as a “soft ionization” [5].

We have recently reported [6] that the extents of fragmentation in the molecular ions $M^{+\cdot}$ of a prenylated flavonoid morusin (**1**) [7], produced under the FAB conditions with glycerol (G), thioglycerol (TG) and *m*-nitrobenzyl alcohol (m-NBA) as the matrices, correspond to those of the molecular ions $M^{+\cdot}$ produced at the electron impact energies of > 30, 30–70 and 21–22 eV, respectively. We thus proposed that FAB is an EI-like hard ionization process even when a liquid matrix is used. This paper describes further studies of the matrix effect on the extent of fragmentation in the molecular ions $M^{+\cdot}$ of a few prenylated flavonoids, produced under the FAB conditions with various liquid matrices.

EXPERIMENTAL

Mass spectra were obtained with a JEOL JMS-DX303 double-focusing mass spectrometer/JMA-DA5000 data system. The liquid matrices used in the FAB-MS were glycerol (G) (Kanto Chemical), thioglycerol (TG) (Fluka), *m*-nitrobenzyl alcohol (m-NBA) (Tokyo Kasei), m-NBA/DTDE (1:1), i.e., a 1:1 v/v mixture of m-NBA to 2,2'-dithiodiethanol (DTDE) (Aldrich Chemical) and a so-called Magic Bullet, a 1:3 v/v mixture of dithioerythritol (Tokyo Kasei) to dithiothreitol (Tokyo Kasei). The fast atom beam used was generated from xenon ions which were accelerated to 5 kV. Samples were usually dissolved in each matrix at a concentration of ca. $8 \mu\text{g} \mu\text{l}^{-1}$. Sufficient amounts of each solution (3–4 μl) were coated onto a sample holder tip. The electron impact energy in the EI-mode varied from 15 to 70 eV. The ionization current and the chamber temperature were 100 μA and 200°C, respectively. Tandem FAB mass spectra recorded by the linked-scanning technique at constant B/E were measured with a JEOL JMS-HX110/HX110 four-sector tandem mass spectrometer. Precursor ions were resolved at a resolution of 3000. The spectra were obtained by helium gas collision. The reproducibility was confirmed in all mass spectra, and the spectra were averaged over five or seven scans.

RESULTS AND DISCUSSION

EI-like fragmentation in FAB mass spectra

First of all, the EI-like and CI-like fragmentations from the molecular ions $M^{+\cdot}$ and the protonated molecules $(M + H)^+$ produced under FAB conditions were examined with four-sector tandem mass spectrometer. Figures 1–3 show the positive ion EI, FAB and tandem FAB mass spectra of prenylated flavonoids morusin (**1**), kuwanon C (**2**) [8] and 2'-hydroxy-3'-prenyl-2,4,4'-trimethoxychalcone (trimethoxychalcone, **3**) [9]. Major fragmentations in the EI-MS of **1** and **2** have already been reported [10], and the

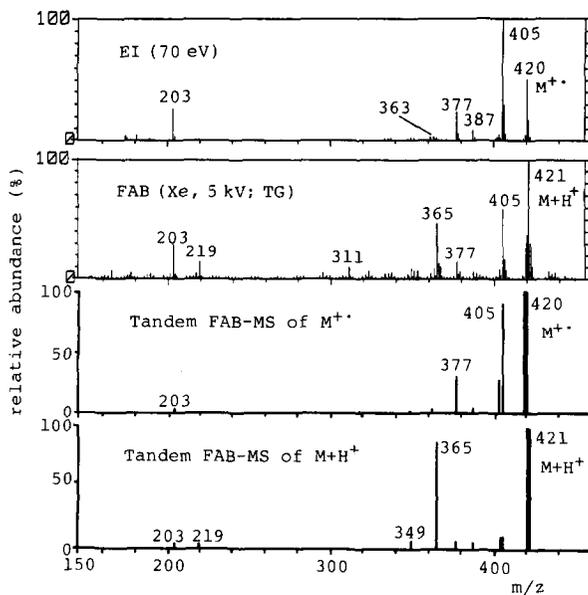


Fig. 1. EI, FAB and tandem FAB mass spectra of morusin (**1**).

EI-like and CI-like fragmentations in the FAB spectra have recently been reported [1,2].

The major fragmentations in the EI spectrum of morusin (**1**) can be illustrated as shown in Scheme 1. The EI-like fragment ions at m/z 405, 377

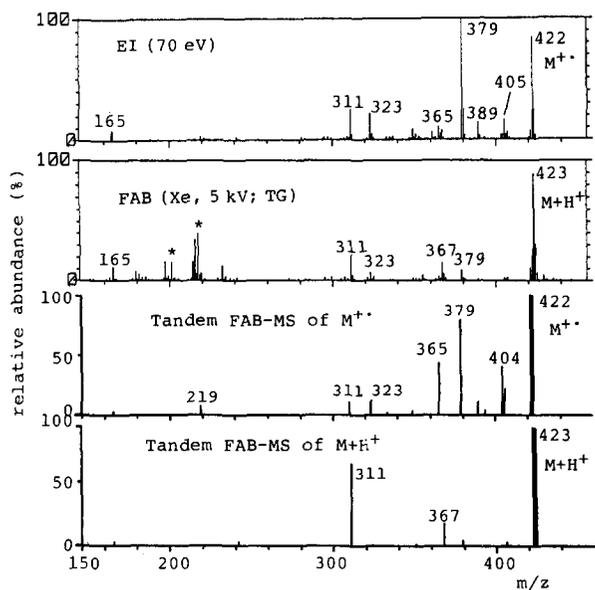


Fig. 2. EI, FAB and tandem FAB mass spectra of kuwanon C (**2**). Peaks originating from the TG matrix are marked with an asterisk.

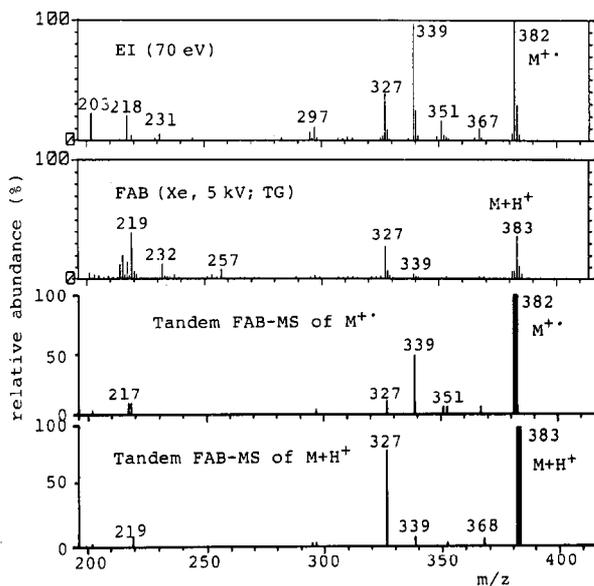
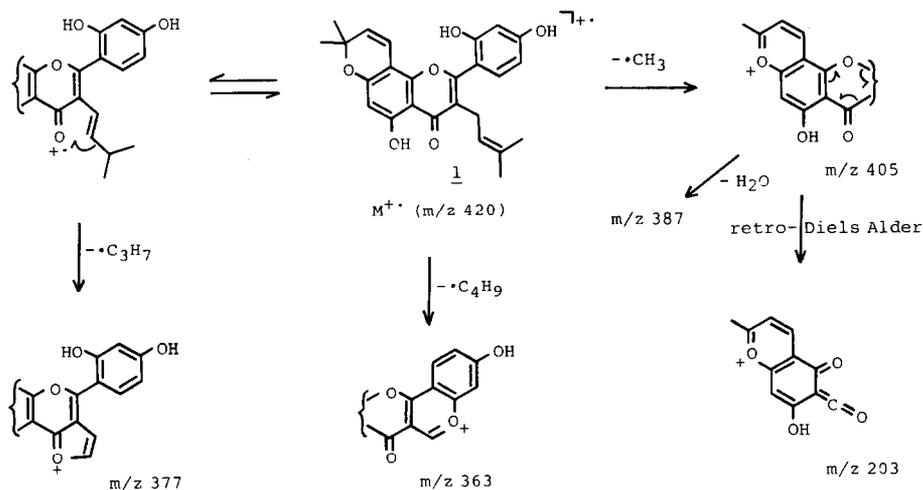


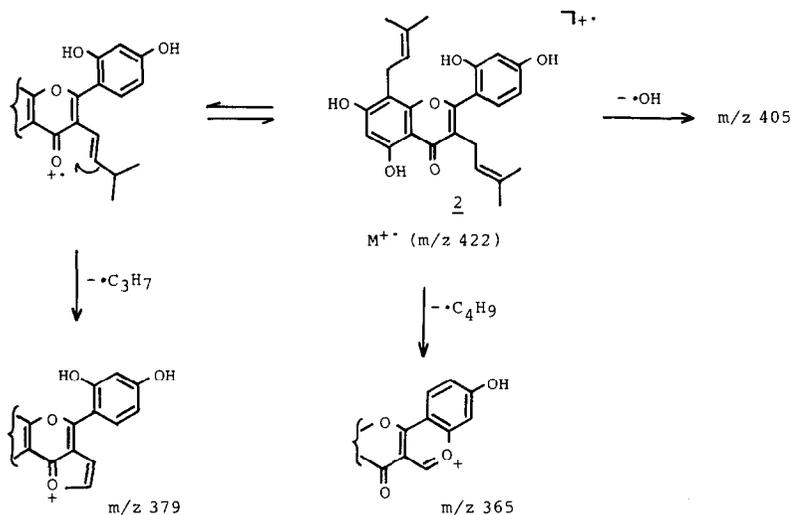
Fig. 3. EI, FAB and tandem FAB mass spectra of trimethoxychalcone (3).

and 363 in the FAB spectrum are possibly formed directly from the molecular ion $M^{+\bullet}$ (m/z 420, 38%) produced under FAB conditions, and the protonated molecule $(M + H)^+$, (m/z 421, 100%) results in the CI-like fragment ions at m/z 365 and 219. These fragmentations can be supported by the tandem FAB spectra in Fig. 1.

The EI-like fragment ions at m/z 405, 379 and 365 originating directly from



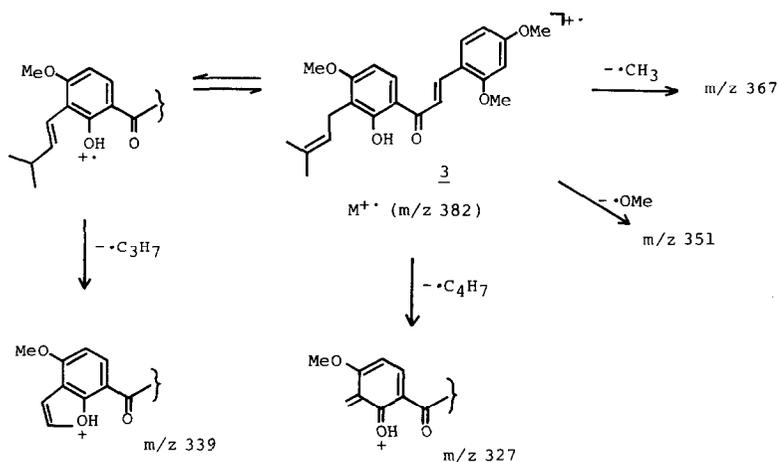
Scheme 1.



Scheme 2.

the molecular ion $\text{M}^{+\bullet}$ (m/z 422, 27%) in the FAB spectrum of kuwanon C (**2**) (Fig. 2), and the fragmentations can be illustrated as shown in Scheme 2. In the tandem FAB spectrum of the $(\text{M} + \text{H})^+$ ion as shown in Fig. 2, however, a small proportion of the ions m/z 405 and 379 originate from the $(\text{M} + \text{H})^+$ ion.

The major EI-like fragmentations in the FAB spectrum of trimethoxychalcone (**3**) in Fig. 3 may be illustrated as shown in Scheme 3. However, the fragment at m/z 327 is the EI-like and CI-like fragment ion which is formed by the loss of a neutral fragment C_4H_7 from the ion $\text{M}^{+\bullet}$ (m/z 382, 7%) and



Scheme 3.

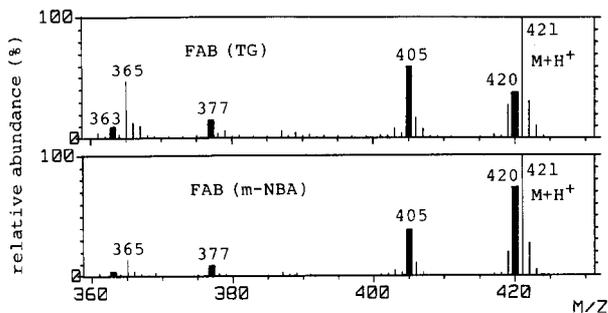


Fig. 4. EI-like fragmentation patterns in FAB mass spectra of morusin (**1**).

the loss of a neutral fragment C_4H_8 from the ion $(M + H)^+$, (m/z 383, 36%), respectively, produced under the FAB conditions.

The FAB mass spectra obtained here evidently show the fragmentation patterns with respect to the EI-like peaks.

EI-like spectral pattern in the FAB mass spectra

As described above, the FAB mass spectra here showed the EI-like spectral pattern which consists of the molecular ions $M^{+\cdot}$ and the EI-like fragment ions. As reported in the case of morusin (**1**) [6], the fragmentation pattern with respect to EI-like peaks at m/z 420, 405 and 377 varied significantly according to the liquid matrices used. The FAB spectra of **1** with TG and m-NBA as the matrices are again shown in Fig. 4. Furthermore, the partial FAB mass spectra of kuwanon C (**2**) and trimethoxychalcone (**3**) with TG and m-NBA at a common concentration of ca. $8 \mu g \mu l^{-1}$ are shown in Figs. 5 and 6, respectively. In all spectra, the fragment peaks drawn in bold lines represent the EI-like fragment ions originating directly from the molecular ions $M^{+\cdot}$. That is, the ions m/z 405, 377 and 363 in morusin (**1**), the ions m/z 379 and

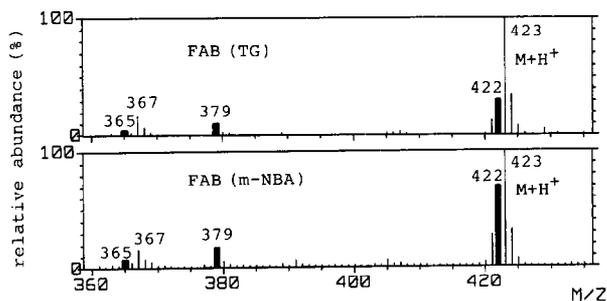


Fig. 5. EI-like fragmentation patterns in FAB mass spectra of kuwanon C (**2**).

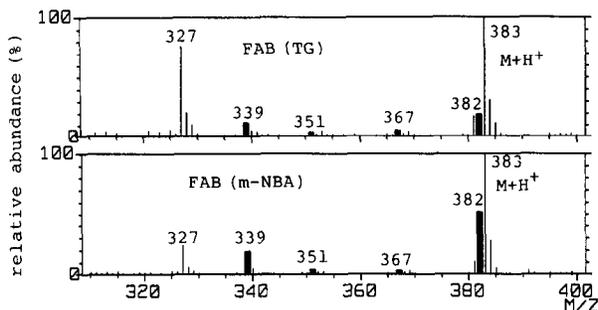


Fig. 6. EI-like fragmentation patterns in FAB mass spectra of trimethoxychalcone (3).

365 in kuwanon C (2) and the ions m/z 367, 351 and 339 in trimethoxychalcone (3) are formed mainly by EI-like fragmentation from the molecular ions $M^{+\cdot}$ (m/z 420, 422 and 382), respectively, produced under FAB conditions.

Figures 4–6 show that the respective FAB spectra with m-NBA decrease in the ratio $R(m_i)$ of the relative abundance of i th EI-like fragment $I(m_i)$ ($i = 1, 2, \dots$) to that of the molecular ion $I(M^{+\cdot})$ than those of the corresponding FAB spectra with TG. This implies that the extent of fragmentation (or the internal energy content) of the molecular ions $M^{+\cdot}$ produced under FAB conditions with m-NBA is lower than that of the $M^{+\cdot}$ ions produced with TG. For the purpose of quantitative comparison of the EI-like spectral pattern in the FAB spectrum with the corresponding EI-spectral pattern at the various electron impact energies, the ratio R is defined as

$$R = \sum_i I(m_i)/I(M^{+\cdot}) \quad (i = 1, 2, \dots)$$

For compounds 1, 2 and 3, the EI-like fragment ions m_i ($i = 1, 2, \dots$) have already been selected (Figs. 4–6). The R value obtained from the EI mass spectrum was plotted against the impact energies (IE) of 15–70 eV and the results are shown in Figs. 7–9.

The FAB mass spectra with various matrices G, TG, m-NBA, Magic Bullet and m-NBA/DTDE (1:1) were obtained for each compound, and the R values obtained from the FAB spectra were plotted so that the points (indicated as dots) were located on the corresponding R -IE curve in Figs. 7–9. The R values obtained are summarized in Table 1 with the exception that the FAB spectrum of trimethoxychalcone (3) with G as the matrix did not show the appropriate peaks for evaluation of the R values. The R value for morusin (1) indicates that the extent of fragmentation in the molecular ions $M^{+\cdot}$ produced under the FAB conditions with G and TG, Magic Bullet, and m-NBA and m-NBA/DTDE (1:1) corresponds to those of fragmentation in the $M^{+\cdot}$ ions produced at the electron impact energies of > 30, 23–24 and 21–22 eV, respectively. The R values for kuwanon C (2) with G, Magic Bullet and TG, and m-NBA and

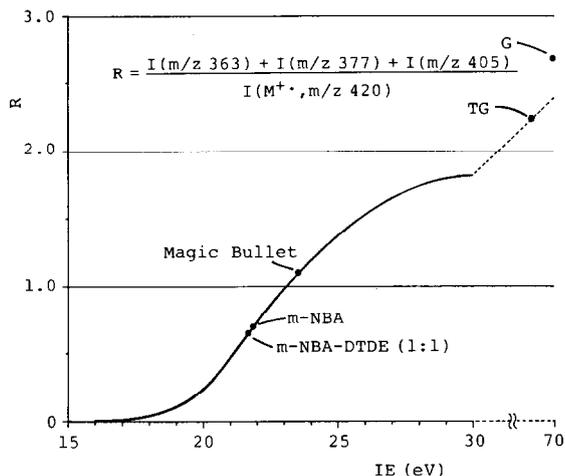


Fig. 7. R versus IE for morusin (1). Dots represent the R values obtained from the FAB mass spectra.

m-NBA/DTDE (1:1) correspond to the impact energies of 24–25, 21–22 and 20–21 eV, respectively. The R values for trimethoxychalcone (3) with Magic Bullet and TG, and m-NBA and m-NBA/DTDE (1:1) correspond to the impact energies of 18–19 and 16–17 eV, respectively. These results suggest that the matrices m-NBA and m-NBA/DTDE (1:1) provide milder ionization conditions than the matrices G, TG and Magic Bullet. The mildest matrix is the m-NBA/DTDE (1:1) which was prepared for low-polar organic compounds such as cholesterol and stearic acid methyl ester [11].

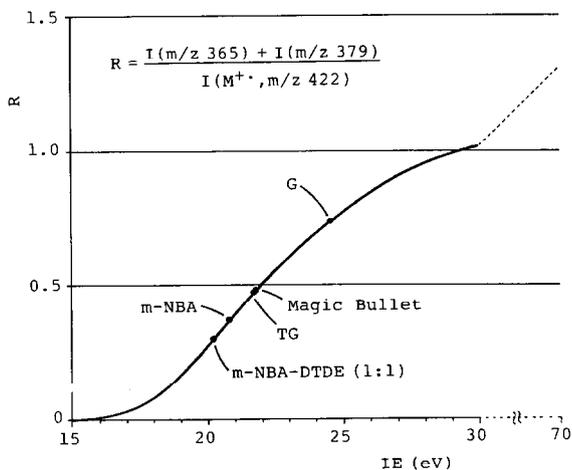


Fig. 8. R versus IE for kuwanon C (2). Dots represent the R values obtained from the FAB mass spectra.

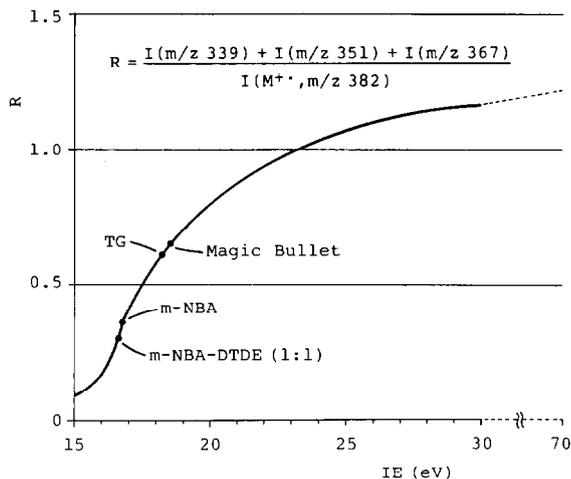


Fig. 9. R versus IE for trimethoxychalcone (3). Dots represent the R values obtained from the FAB mass spectra.

Effects of analyte concentration and the amount of matrix solution

In order to examine the effect of analyte concentration on the extent of fragmentation in the molecular ions $M^{+\cdot}$ produced under FAB conditions, the FAB mass spectra of morusin (1) were obtained with TG and m-NBA at analyte concentrations of ca. 0.4, 1.0, 2.0, 10 and $20 \mu\text{g} \mu\text{l}^{-1}$. A sufficient volume of each solution 3–4 μl was coated onto the sample holder tip to avoid an effect of the solution amount. The R values obtained from the FAB spectra of morusin (1) are summarized in Table 2. The R values obtained with TG indicate that increasing concentration tends to increase the extent of fragmentation in the molecular ions $M^{+\cdot}$ with a few exceptions. Such a tendency, however, was not observed in the case of m-NBA.

In addition, we examined an effect of the amount of matrix solution. The R values given in parentheses in Table 2 represent the values when the solution

TABLE 1

R values for prenylated flavonoids 1, 2 and 3 obtained from FAB mass spectra with various matrices

Analytes	Matrix				
	G	TG	m-NBA	Magic Bullet	m-NBA/DTDE (1:1)
Morusin (1)	2.69	2.24	0.70	1.10	0.66
Kuwanon C (2)	0.74	0.47	0.37	0.48	0.30
Trimethoxychalcone (3)		0.61	0.36	0.65	0.33

TABLE 2

R values for morusin (1) obtained from FAB mass spectra with TG and m-NBA matrices at different concentrations

Matrix	Analyte concentration ($\mu\text{g } \mu\text{l}^{-1}$)				
	0.4	1.0	2.0	10	20
TG	1.35 (1.88) ^a	2.01	2.30 (3.83) ^a	2.77 (4.96) ^a	2.71
m-NBA	0.93	0.83	0.86	0.90	0.93

^a Solution was thinly coated at sample holder tip.

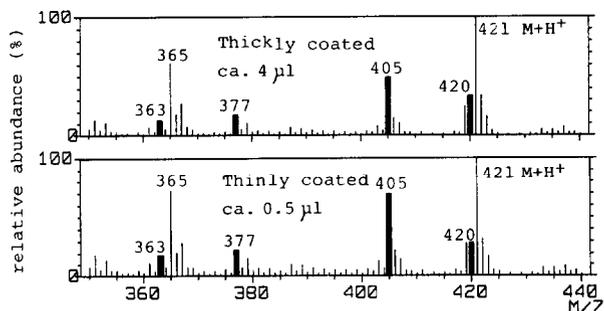


Fig. 10. Partial FAB mass spectra of morusin (1) with the matrix TG at a concentration of ca. $2 \mu\text{g } \mu\text{l}^{-1}$, where the matrix solution was either thickly or thinly coated on the sample holder tip.

at concentrations of ca. 0.4, 2.0 and $10 \mu\text{g } \mu\text{l}^{-1}$ was thinly coated on the holder tip (ca. $0.5 \mu\text{l}$). Such a decrease in the amount of matrix solution increased the *R* value at each concentration examined. The volume effect is no less important than the concentration effect. Figure 10 shows the partial FAB mass spectra of morusin (1) with TG at the concentration ca. $2.0 \mu\text{g } \mu\text{l}^{-1}$ when the solution was coated thickly and thinly on the holder tip. The *R* values obtained from the FAB spectra at 2.30 and 3.83 correspond to the electron impact energies of 30–70 and > 70 eV, respectively. This estimation is particularly surprising in view of the alternative analysis with respect to the internal energy distribution of the ions produced under FAB and liquid SI conditions, because the analysis suggests that the energy content is approximately in the range 0–3 eV [12,13].

CONCLUSION

The results obtained for the prenylated flavonoids indicate a marked tendency for the extent of fragmentation of the molecular ions $\text{M}^{+\cdot}$ produced under FAB conditions with m-NBA and m-NBA/DTDE (1:1) as matrices to be lower than those of the $\text{M}^{+\cdot}$ ions produced with G, TG and Magic Bullet

as the matrices. This suggests that the liquid matrices m-NBA and m-NBA/DTDE (1:1) provide milder ionization conditions with respect to the production of the molecular ions $M^{+\cdot}$ than other matrices used. Such a matrix effect has not previously been reported.

In addition, it is concluded that the extent of fragmentation in the $M^{+\cdot}$ ions produced under FAB conditions is affected not only by the amount of matrix solution at the sample holder tip, but also by the analyte concentration. For example, the R values for morusin (**1**) increased from 1.35, 2.30 and 2.77 to 1.88, 3.83 and 4.96, respectively, upon decreasing the amount of matrix solution (Table 2). It is striking that the relatively large R values of 2.77, 3.83 and 4.96 correspond to electron impact energies > 70 eV suggesting that sufficient energy is transferred to the $M^{+\cdot}$ ions produced under FAB conditions. Although the excess internal energy of the fragmenting molecular ions $M^{+\cdot}$ produced under FAB conditions cannot be estimated directly from the results obtained here, such internal energy seems to be equivalent to that of the fragmenting $M^{+\cdot}$ ions produced at electron impact energies of 16–70 eV or above. This supports a proposition that FAB is an EI-like hard ionization process even when a liquid matrix is used. It is, therefore, necessary to research the production mechanism and energetics (origin of the internal energy) of the molecular ions $M^{+\cdot}$ produced under FAB conditions in order to interpret the matrix effects obtained here.

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