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Stereochemical Applications of Mass Spectrometry

Part 5—Fragmentation of Protonated and Methylated Maleic and Fumaric Acid and Derivatives

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The unimolecular metastable ion and collision-induced dissociation (CID) fragmentation reactions of protonated and methylated monoamides, monomethyl esters and methyl esters of the monoamides of maleic and fumaric acids were studied. In addition, some studies of the fragmentation of protonated and methylated maleic and fumaric acids were carried out. The $[MH - H_2O]^+$ ions derived from protonated maleic and fumaric acids show distinctly different CID mass spectra, that for the $[MH - H_2O]^+$ ion from the maleic acid being the same as that of protonated maleic anhydride; the results show that the stereochemistry about the double bond is retained in the $[MH - H_2O]^+$ ions. Fragmentation of specifically deuterium-labelled and protonated or deuterated maleic acids show that the added proton becomes scrambled with the carboxylic hydrogens prior to loss of H_2O. The fragmentation of similarly labelled fumaric acids show that a 1,3-H^+ migration followed by elimination of H_2O is not the only pathway to water elimination; the results implicate proton migration from one carboxyl group to the other as well as some involvement of the C-bonded hydrogens in the water-loss reaction. A major fragmentation reaction of protonated maleamic acid forms NH_4^+; this reaction is of only minor importance for protonated fumamic acid. Other primary fragmentation reactions involve elimination of NH_3 and H_2O from the protonated species. The protonated monomethyl esters fragment initially by loss of H_2O or loss of CH_3OH; the former is more prominent for the maleate whereas the latter dominates for the fumarate. Protonation of methyl maleamate and methyl fumamate results in loss of NH_3 or CH_3OH as primary fragmentation reactions; these primary fragment ions undergo less facile further fragmentation for the maleamate than for the fumamate. The CH_3^+ adducts of the monoamides fragment by loss of NH_3, H_2O and CH_3OH; the CID spectra of the adducts are distinctly different from those of the protonated methyl esters of the monoamides, indicating predominant addition of the methyl to the amide oxygen. The CH_3^+ adduct of monomethyl maleate fragments primarily by loss of methanol, the two methyl groups having become equivalent prior to fragmentation. A minor fragmentation route involves loss of dimethyl ether, a reaction not observed for protonated dimethyl maleate. Elimination of dimethyl ether is a major fragmentation channel for the CH_3^+ adduct of monomethyl fumarate. Since this reaction channel is not observed for protonated dimethyl fumarate, the results indicate predominant CH_3^+ addition to the carbomethoxy group of the monoester. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: maleic acid derivatives; fumaric acid derivatives; fragmentation; stereochemistry

INTRODUCTION

Mass spectrometry (MS) has been widely applied to stereochemical problems over the past 30 years and there now are many examples of stereochemical effects on the fragmentation of gaseous ions formed by electron ionization or chemical ionization (CI).[1–8] One class of compounds which has seen extensive study by chemical ionization methods are the *Z*/*E*-isomeric but-2-ene-1,4-dioic acids, their diesters and related compounds. Fales *et al.*[9] reported briefly on the isobutane CI mass spectra of diethyl maleate and diethyl fumarate; although MH^+ was the only product observed at a source temperature of 130 °C, above 250 °C some fragmentation was observed with the maleate ester showing a more facile elimination of ethanol. Harrison and Kallury[10] carried out a more thorough study of the Brønsted acid chemical ionization of the (*Z*)-dicarboxylic acids, maleic and citraconic acids and their *E*-

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isomers fumaric and mesaconic acids, as well as a number of esters of maleic and fumaric acids. These studies showed that there was a much more facile fragmentation of the protonated *Z*-isomers compared with the protonated *E*-isomers; the dominant fragmentation mode involved elimination of water from the protonated acids and an alcohol molecule from the protonated esters. This stereochemical effect has been interpreted[10] in terms of an interaction between the two carboxylate functions in the *Z*-isomers which permits a facile proton transfer from the carbonyl oxygen of one carboxyl group to the hydroxyl or alkoxyl oxygen of the other carboxyl group, a necessary transfer for elimination of water or alcohol. Since the carboxyl groups cannot interact in the *E*-isomers it was concluded that a 1,3-proton transfer was necessary prior to elimination of H_2O or ROH and that this transfer had a high energy barrier because it is symmetry forbidden. Support for the proton transfer reaction in protonated *Z*-isomers was provided by the observation that protonated methylethyl 2-*tert*-butylmaleates specifically eliminated an alcohol molecule incorporating the alkoxy group adjacent to the *tert*-butyl group both in fragmentation following Brønsted acid CI[11] and in low-energy collision-induced dissociation (CID) of the MH^+ ions.[12] Steric interaction of the bulky *tert*-butyl group leads to protonation at the carbonyl group remote from this group and, subsequently, elimination of ROH from the carboxyl function adjacent to the *tert*-butyl group.

The stereoisomeric but-2-ene-1,4-dioic acids and derivatives have also been popular substrates for study using less common reagent gas systems.[13] Das *et al.*,[14] in a study of the ammonia CI of the diethyl esters of maleic, fumaric, citraconic and mesaconic acid, observed that only the *Z*-isomers formed MH^+ ions whereas the *E*-isomers formed more abundant $[M + NH_4]^+$ ions and $[M + N_2H_7]^+$ ions; because of internal solvation of the added proton in the *Z*-isomers the proton affinity is higher than for the *E*-isomers. Using 1,2-dibromoethane as reagent gas, Vairamani *et al.*[15] observed a more abundant MH^+ ion for the *E*-isomers of the acids and esters studied, with more extensive fragmentation by loss of ROH (R = H or CH_3) for the protonated *Z*-isomers. Similar results were reported using acrylonitrile[16] and methylene chloride as the reagent gas.[17] With tetramethylsilane as reagent, the *E*-isomers of the acids showed an abundant $[M + (CH_3)_3Si - CH_4]^+$ ion signal whereas the *Z*-isomers exhibited a strong $[M + (CH_3)_3Si - H_2O]^+$ ion signal;[18] for the esters studied, the $[M + (CH_3)_3Si]^+$ adduct was more abundant for the *E*- than for the *Z*-isomers.

There have been relatively few studies of the collision-induced fragmentation of the protonated *Z*- and *E*-stereoisomers; in particular, there have been no studies to elucidate whether the stereochemistry is retained in the primary fragment ions. Mandelbaum and co-workers[19] studied the low-energy CID of the MH^+ ions of diethyl and dimethyl esters of maleic, fumaric, citraconic and mesaconic acid. The (*Z*)-ethyl esters, for example, showed abundant ion signals corresponding to $[MH - C_2H_5OH]^+$ and $[MH - C_2H_5OH - C_2H_4]^+$ whereas the *E*-isomers exhibited abundant $[MH - C_2H_4]^+$ and $[MH - 2C_2H_4]^+$ fragment ions. These highly stereospecific fragmentation processes indicate that the double bond configuration is retained under collisional activation conditions. Isbell and Brodbelt[20] reported on the CID in a quadrupole ion trap of both protonated and methyl-cationated *Z*- and *E*-isomeric acids and their dimethyl esters. The protonated acids fragmented by loss of H_2O whereas the methyl esters showed loss of CH_3OH. The methyl-cationated *Z*-isomers, maleic and citraconic acid, fragmented entirely by loss of H_2O whereas the methyl-cationated *E*-isomers, fumaric and mesaconic acid, fragmented primarily by loss of CH_3OH.

The studies to date of stereoisomeric but-2-ene-1,4-dioic acid derivatives have involved either the free acids or their diesters, where, with the exception of the work of Mandelbaum and co-workers,[11,12] the two ester functionalities have been the same. In the present work we concentrated largely on stereoisomers containing two different functionalities, the monoamides of maleic and fumaric acid, the monomethyl esters of the same acids and the methyl esters of the monoamides. Our studies involved metastable ion and CID studies of the fragmentation of the protonated and methyl-cationated species. Some experiments are also reported concerning the mechanism of fragmentation of the MH^+ ions of maleic and fumaric acid and on the fragmentation of the $[MH - H_2O]^+$ ions derived from these acids.

EXPERIMENTAL

Metastable ion studies and low-energy CID studies were carried out using a VG Analytical (Manchester, UK) ZAB-2FQ hybrid BEqQ mass spectrometer, which has been described in detail previously.[21] In the metastable ion studies, the appropriate ion beam was mass selected by the BE double-focusing mass spectrometer at 6 keV ion energy decelerated to *ca.* 20 eV kinetic energy and introduced into the r.f.-only quadrupole cell, q, in the absence of collision gas. The ionic products of unimolecular fragmentation in the cell were analysed by scanning the final mass-analysing quadrupole Q. Low-energy CID experiments were carried out in the same fashion but with the addition of N_2 collision gas to the collision cell at a pressure of $(1–2) \times 10^{-7}$ Torr (1 Torr = 133.3 Pa) as read by the ionization gauge attached to the pumping line for the quadrupole section. In the CID experiments the collision energy typically was varied from 5 to 45 eV (laboratory scale). The results of these energy-resolved experiments[22–24] are presented in the following in the form of breakdown graphs showing the fractional fragment ion abundances as a function of the collision energy. Alternatively, CID spectra of isomeric species obtained at the same collision energy are presented. In MS/MS/MS experiments the initial precursor ion was mass selected at 6 keV ion energy and underwent either unimolecular or collision-induced dissociation in the field-free region before the electric sector. The desired ionic product was mass selected according to its kinetic energy by the electric sector, decelerated to the desired low kinetic energy and introduced into the quadrupole collision cell where low-energy CID studies were carried out in the usual

fashion. In both the metastable ion and CID studies, 20–40 2 s scans were accumulated on a multi-channel analyser to improve the signal-to-noise ratio.

The ion source of the ZAB instrument was operated in the CI mode at 200 °C. Methane was used as the reagent gas to produce MH^+ ions. Where it was desired to produce MD^+ ions, CD_4 or $(CD_3)_2CO$ was used as the reagent gas. The use of CH_3OD as the reagent gas resulted in H–D exchange of the labile hydrogens of the analyte and production of the MD^+ ion of the exchanged species.[25] Interaction of the dicarboxylic acids with CH_3OD prior to introduction by the solids probe and the use of CH_4 as reagent gas allowed the preparation of the MH^+ ion of both the d_1- and d_2-dicarboxylic acids; these were mass selected for study. The methyl-cationated species were obtained using CH_3I–CH_4 as the CI reagent;[26] when CD_3^+ adducts were desired, CD_3I–CD_4 was used as the reagent.

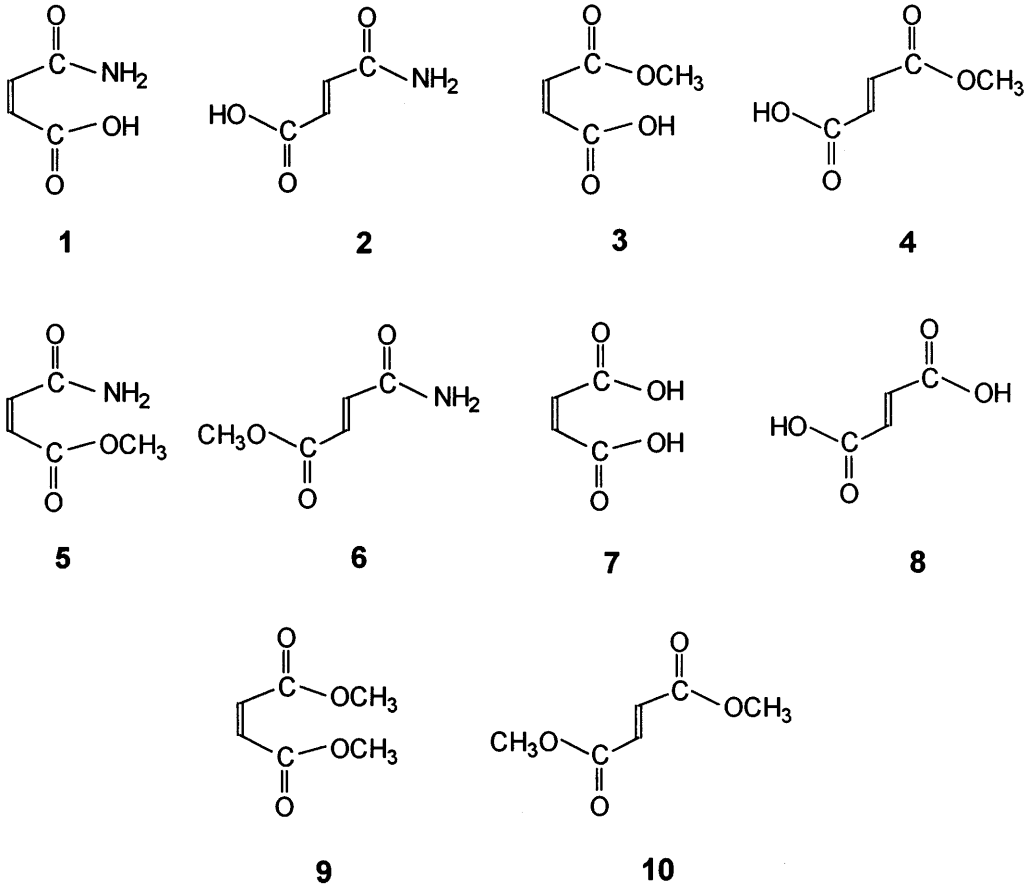
Maleic (**7**) and fumaric acid (**8**) were used as received commercially. Maleamic acid (**1**) and monomethyl maleate (**3**) were prepared by ammoniation and methanolysis, respectively, of maleic anhydride. Further reaction of **1** and **3** with acidic methanol afforded methyl maleamate (**5**) and dimethyl maleate (**9**). The preparation of all the *trans* isomers utilized fumaryl dichloride, which underwent controlled reaction with aqueous ammonia or methanol and subsequent hydrolysis to give fumamic acid (**2**) and monomethyl fumarate (**4**). Further esterification of **2** and **4** gave methyl fumamate (**6**) and dimethyl fumarate (**10**). After recrystallization, the purity of all compounds was established by MS and NMR spectrometry.

RESULTS AND DISCUSSION

Although the major focus of the present work is on the ion chemistry of the protonated and methylated monoamides, monomethyl esters and methyl esters of the monoamides of maleic and fumaric acid, compounds **1–6** in Scheme 1, some experiments were also carried out on the fragmentation of the protonated and methylated acids and dimethyl esters, **7–10** in Scheme 1.

Stereochemistry of fragment ions

As discussed in the Introduction, it is well established that the protonated *Z*/*E*-isomers of but-2-ene-1,4-dioic acids and their esters show strong stereochemical effects in that the *Z*-isomers show much more facile fragmentation that the *E*-isomers. Clearly, the configuration about the double bond is retained in the protonated species. In the present study we addressed the question of whether this stereochemistry is retained in the major fragment ions $[MH - H_2O]^+$, for the acids. Figure 1 compares the 35 eV CID mass spectra of the $[MH - H_2O]^+$ ions obtained on fragmentation of protonated fumaric and maleic acid. In these MS/MS/MS experiments, the MH^+ ion was mass selected by the magnetic sector and fragmentation in the field-free region between the magnetic and electric sectors gave rise to the $[MH - H_2O]^+$ (*m*/*z* 99) ions which were selected by the electric sector, decelerated and activated by collision with N_2 in the r.f.-only collision cell at 35



Scheme 1

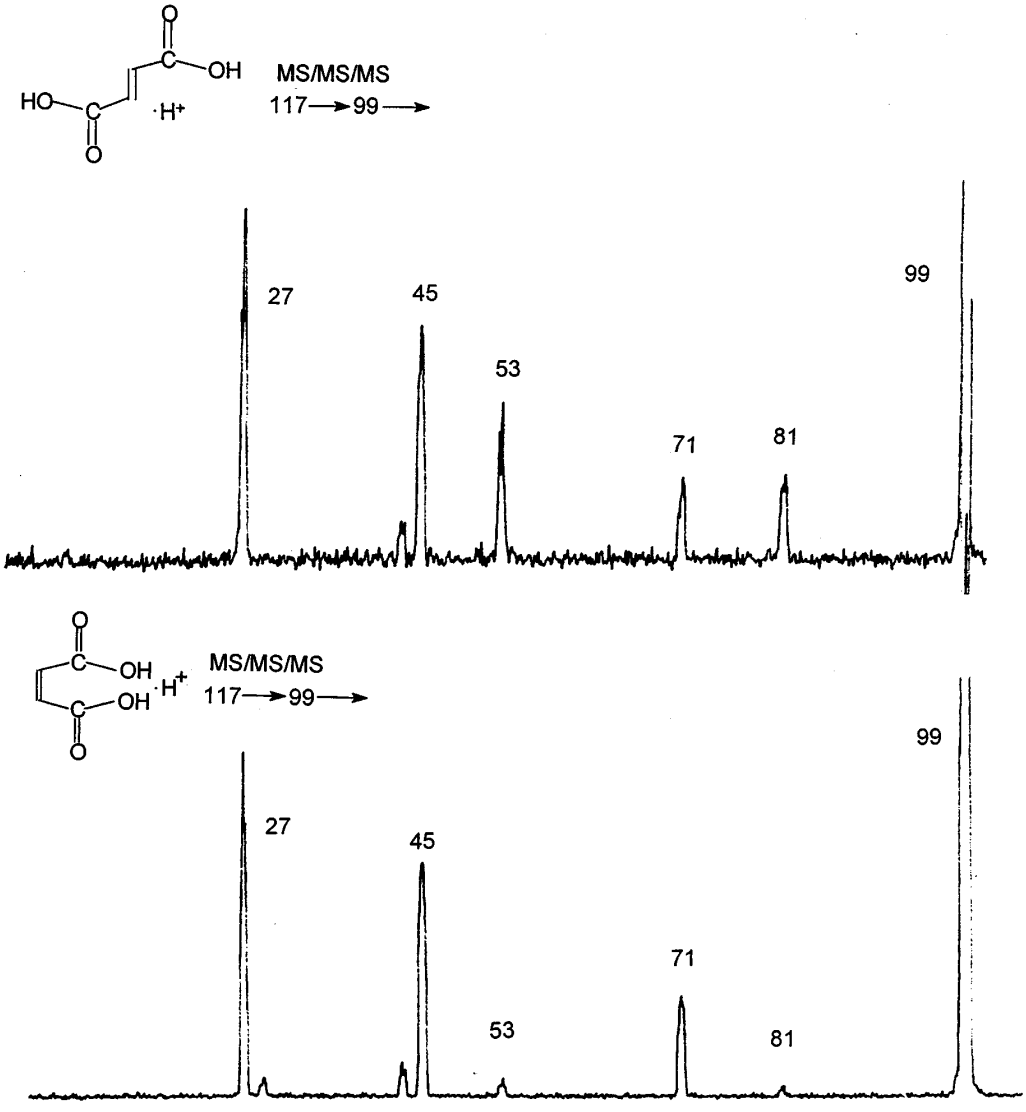


Figure 1. 35 eV CID mass spectra of $[MH - H_2O]^+$ ions derived by CID of MH^+ ions of fumaric and maleic acid.

eV collision energy. There are significant differences in the CID spectra obtained. Whereas that for the *m/z* 99 ion formed from protonated fumaric acid shows significant ion signals at *m/z* 81 and 53, these ion signals are barely detectable in the CID spectrum of the *m/z* 99 ion arising from protonated maleic acid. This difference is even more dramatic in the 10 eV CID spectra of source-generated $[MH - H_2O]^+$ ions shown in Fig. 2. At this collision energy, *m/z* 81 is the base peak in the spectrum of $[MH - H_2O]^+$ derived from fumaric acid but is of only minor importance in the CID spectrum of $[MH - H_2O]^+$ derived from maleic acid. In addition, the *m/z* 53 ion is much more abundant in the former spectrum. The bottom panel of Fig. 2 shows the 10 eV CID spectrum of protonated maleic anhydride, which is seen to be essentially identical with that of the $[MH - H_2O]^+$ ion derived from maleic acid, supporting the proposal that one of the driving forces for fragmentation of the protonated *Z*-isomers is cyclization to the stable cationated anhydride structure.

Obviously, the structures of at least the major portion of the $[MH - H_2O]^+$ ions derived from fumaric and maleic acid are different, since they show distinctly different CID spectra. This structural difference presumably reflects the fact that the stable structure for the ion derived from maleic acid has a cyclic protonated maleic anhydride structure, whereas that derived from fumaric acid is an acylium ion with a *trans* configuration about the double bond. A rationalization of the facile loss of water from the $[MH - H_2O]^+$ derived from fumaric acid is presented in Scheme 2. The orientation about the double bond of maleic acid precludes such a proton migration for its $[MH - H_2O]^+$ ion; the low-abundance *m/z* 81 ion observed in the spectrum of the $[MH - H_2O]^+$ ion derived from maleic acid presumably reflects a small extent of isomerization to the *E*-configuration or water elimination by a different, unknown, mechanism. The major fragmentation reactions of protonated maleic anhydride are rationalized in Scheme 3; a similar sequence is operative, in addition to the water-loss reaction, in the fragmentation of the $[MH - H_2O]^+$ ion derived from fumaric acid.

Mechanism of H_2O elimination from protonated acids

The low extent of fragmentation of protonated *E*-isomers of acids and esters has been attributed[10,27] to

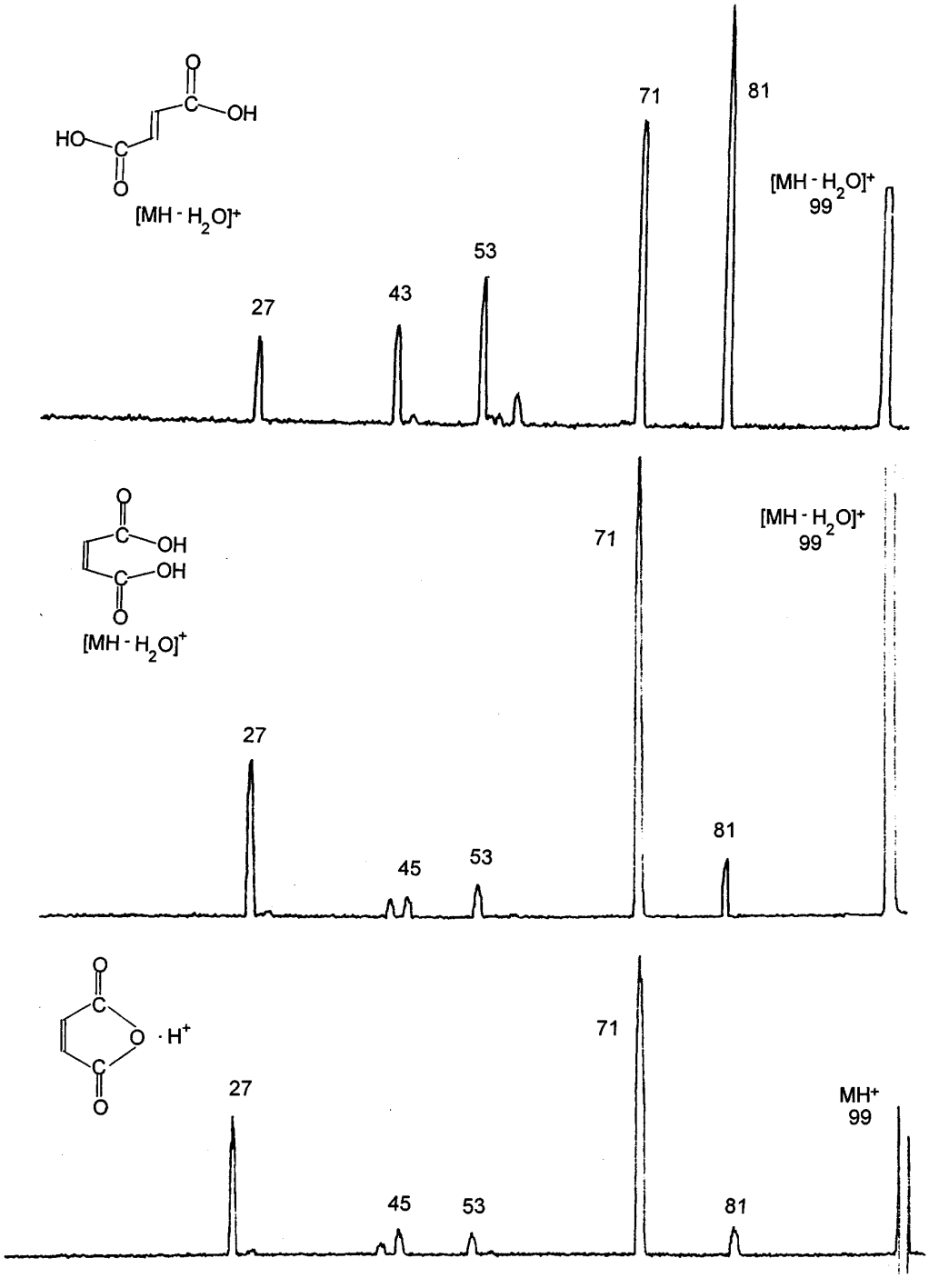
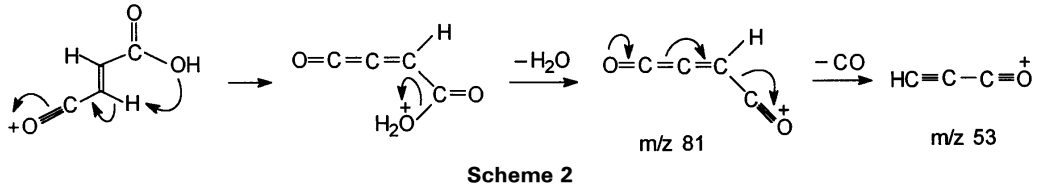
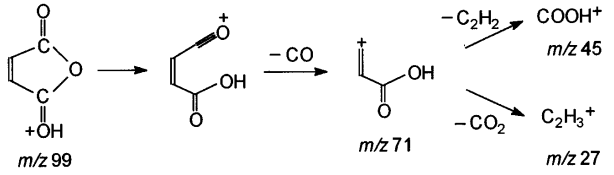


Figure 2. 10 eV CID mass spectra of $[MH - H_2O]^+$ ions formed in source from (a) fumaric acid and (b) maleic acid and (c) 10 eV CID mass spectrum of protonated maleic anhydride.

the necessity for a 1,3-H^+ migration in a carbonyl-protonated species before H_2O or ROH loss can occur (Scheme 4). This 1,3-migration is believed to be symmetry forbidden and, hence, to have a high energy barrier. By contrast, a 1,6-H^+ migration between two carbonyl groups can occur for protonated *Z*-isomers (Scheme 4); since this migration is not symmetry forbidden and has a low energy barrier, more facile fragmentation of protonated *Z*-isomers occurs in Brønsted acid chemical ionization mass spectra.

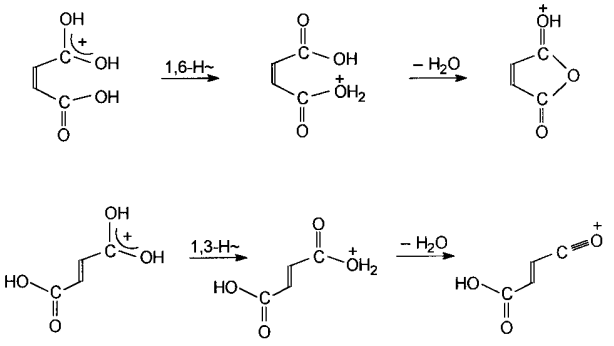


Scheme 2



Scheme 3

Further insight into the details of the fragmentation of protonated *Z*- and *E*-isomers was obtained from a study of the unimolecular metastable ion and collisionally activated dissociation of various protonated or deuterated fumaric and maleic acid species in which the carboxylic hydrogens had been replaced, partially or totally, by deuterium. The results of these studies are presented in Tables 1 and 2 in terms of the percentage elimination of H_2O, HDO or D_2O. The results for the maleic acid isotopomers (Table 1) are compared with distributions calculated on the assumption that the added H^+/D^+ undergoes complete exchange or scrambling with only the carboxylic hydrogens/deuteriums prior to fragmentation. The results for the fumaric acid isotopomers (Table 2) are compared with distributions calculated on the basis of the above model and also those calculated assuming that all H/D, including those bonded to carbon, become scrambled prior to fragmentation.



Scheme 4

The results obtained for the variously labelled maleic acid species (Table 1) are in reasonable agreement with the distribution calculated on the basis of scrambling of the added H^+/D^+ and the carboxylic hydrogens, particularly if one assumes that there is an isotope effect favouring elimination of water containing the fewest number of deuterium atoms. However, even in this apparently simple case there is some involvement of the C-bonded hydrogens in the water-loss reaction, particularly for long-lived metastable ions; this is evident from the minor ion signals corresponding to loss of H_2O from maleic acid-*O*-$d_2 \cdot H^+$ and maleic acid-*O*-$d_1 \cdot D^+$. The results for the labelled fumaric acids (Table 2) indicate that the water-loss reaction is much more complex

Table 1. Metastable ion (MI) and CID fragmentation of labelled maleic acids

		Neutral lost (% of total water loss signal)					
		Experimental			Calculated[a]		
Precursor	Fragmentation	H_2O	HDO	D_2O	H_2O	HDO	D_2O
7 $\cdot D^+$	MI	46.6	53.4		33.3	66.7	
	CID	40.6	59.4				
7-*O*-$d_1 \cdot H^+$	MI	44.2	55.8		33.3	66.7	
	CID	38.4	61.6				
7-*O*-$d_2 \cdot H^+$	MI	3.4	70.8	25.8		66.7	33.3
	CID	3.8	69.0	27.2			
7-*O*-$d_1 \cdot D^+$	MI	1.8	70.3	27.9		66.7	33.3
	CID		71.1	28.9			
7-*O*-$d_2 \cdot D^+$	MI			100			100
	CID			100			

[a] Assumes that added H/D scrambles with oxygen-bonded H/D only.

Table 2. Metastable ion (MI) and CID fragmentation of labelled fumaric acids

		Neutral lost (% of total water loss signal)					
		Experimental			Calculated[a]		
Precursor	Fragmentation	H_2O	HDO	D_2O	H_2O	HDO	D_2O
8 $\cdot D^+$	MI	54.3	45.7		33.3	66.7	
	CID	40.0	60.0		60.0	40.0	
8-*O*-$d_1 \cdot H^+$	MI	57.6	42.4		33.3	66.7	
	CID	50.3	49.7		60.0	40.0	
8-*O*-$d_1 \cdot D^+$	MI	23.4	66.0	10.6		66.7	33.3
	CID	12.9	65.5	21.6	30.0	60.0	10.0
8-*O*-$d_2 \cdot H^+$	MI	28.6	62.7	8.7		66.7	33.3
	CID	15.3	72.5	12.1	30.0	60.0	10.0
8-*O*-$d_2 \cdot D^+$	MI	8.4	51.4	40.2			100
	CID	3.6	29.0	67.4	10.0	60.0	30.0

[a] First entry for each compound assumes that added H/D scrambles with oxygen-bonded H/D. Second entry assumes that all H/D become scrambled, including those bonded to carbon, prior to fragmentation.

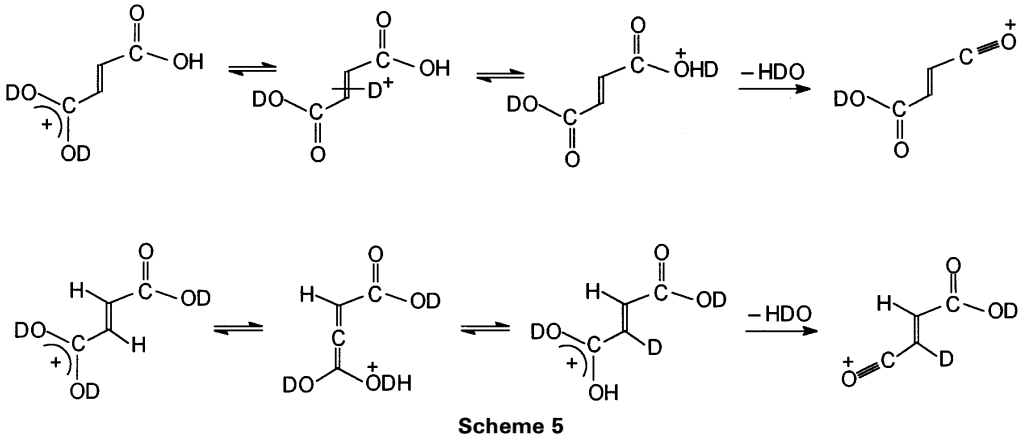
than that for the maleic acid system. The observed distributions are not in accord with the predictions of either scrambling model, nor are they in agreement with a model which assumes carbonyl oxygen protonation followed by a 1,3-H^+ migration leading to water elimination. Such a model would predict loss of only HDO from fumaric acid-*O*-$d_2 \cdot H^+$ and loss of only D_2O from fumaric acid-*O*-$d_2 \cdot D^+$. Two major points are evident. First, the H/D originally bonded to the two carboxylic acid groups are lost together as water to a significant extent; this is shown by the substantial loss of D_2O from fumaric acid-*O*-$d_2 \cdot H^+$. Second, the hydrogens originally bonded to carbon are involved to a substantial extent in the water-loss reaction. This is shown by the ion signal for loss of H_2O in the fragmentation of fumaric acid-*O*-$d_1 \cdot D^+$ and fumaric acid-*O*-$d_2 \cdot H^+$ and by the loss of both HDO and H_2O from fumaric acid-*O*-$d_2 \cdot D^+$. However, it does appear that a major portion of the fragmentation, particularly after collisional activation, can be accommodated by a 1,3-H^+ transfer reaction followed by water loss (Scheme 4). Such a pathway can lead, for example, to the prominent loss of D_2O from fumaric acid-*O*-$d_2 \cdot D^+$, loss of HDO and D_2O from fumaric acid-*O*-$d_1 \cdot D^+$, loss of HDO from fumaric acid-*O*-$d_2 \cdot D^+$ and loss of HDO from fumaric acid $\cdot D^+$. In Scheme 5 we propose that a proton may migrate from one carboxyl group to the other by way of a π-complex with the double bond. We also propose in Scheme 5 a possible mechanism for interchange of the C-bonded hydrogens with the O-bonded hydrogens which rationalizes the involvement of the former in the water-loss reaction. These migrations and interchanges undoubtedly have a significant energy barrier but become possible for the fumarate species because of the high energy barrier for the 1,3-H^+ migration. It should be noted that the results discussed above indicate that the $[MH - H_2O]^+$ ion from fumaric acid largely retains the original configuration about the double bond, although we cannot preclude some isomerization to the maleate configuration.

Fragmentation of protonated molecules

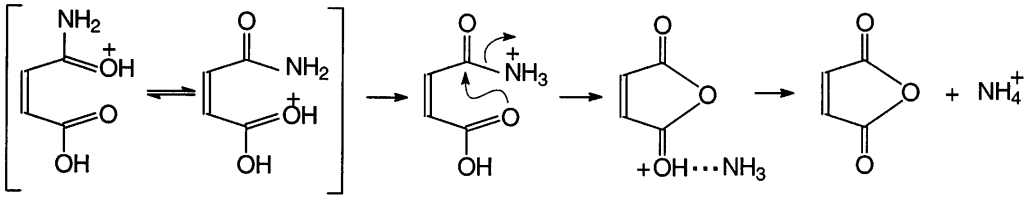
Table 3 records the metastable ion mass spectra resulting from unimolecular fragmentation of the MH^+ ions of **1**–**6**. The metastable ion mass spectra for the two protonated monoamides **1** and **2** show formation of NH_4^+, the fractional yield being much greater for $1H^+$. A similar formation of NH_4^+ in the fragmentation of protonated succinamic acid has been reported[28] while protonated aniline is a major ion in the Brønsted acid CI mass spectrum of maleanilic acid[10] and in the CID mass spectrum of protonated maleanilic acid.[29] The formation of the ammonium ion in the fragmentation of protonated maleamic acid ($\mathbf{1} \cdot H^+$) can be most readily rationalized (Scheme 6) in terms of formation of a protonated maleic anhydride–ammonia complex, which, at low internal energies, undergoes internal proton transfer to form the ammonium ion since the proton affinity of ammonia undoubtedly is greater than that of maleic anhydride. The low-intensity NH_4^+ ion signal for protonated fumamic acid ($\mathbf{2} \cdot H^+$) may arise directly from the *trans* configuration by an unknown mechanism or may reflect a small extent of isomerization to the maleate structure. In metastable ion fragmentation, protonated maleamic acid ($\mathbf{1} \cdot H^+$) shows a more pronounced loss of H_2O than loss of NH_3 plus formation

Table 3. Unimolecular fragmentation of MH^+ of compounds 1–6

	Product *m/z* (% of base peak)			
Precursor	$[MH - NH_3]^+$	$[MH - H_2O]^+$	$[MH - CH_3OH]^+$	NH_4^+
$\mathbf{1} \cdot H^+$	99 (14)	98 (100)		18 (51)
$\mathbf{2} \cdot H^+$	99 (100)	98 (65)		18 (8)
$\mathbf{3} \cdot H^+$		113 (100)	99 (12)	
$\mathbf{4} \cdot H^+$		113 (26)	99 (100)	
$\mathbf{5} \cdot H^+$	113 (34)		98 (100)	
$\mathbf{6} \cdot H^+$	113 (24)		98 (100)	



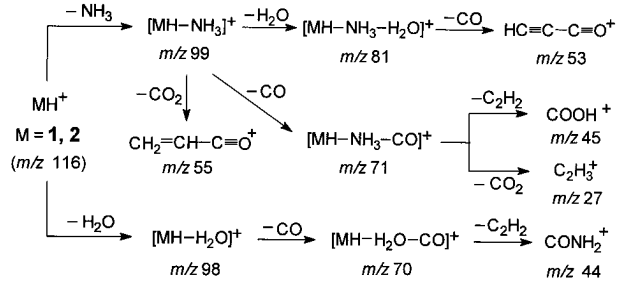
Scheme 5



Scheme 6

of NH_4^+; this is to be expected since H_2O loss should be more facile than NH_3 loss.[27] By contrast, protonated fumamic acid ($\mathbf{2} \cdot H^+$) shows more loss of NH_3 than loss of H_2O; this may reflect preferential protonation at the amide function followed by fragmentation without interfunctional proton migration.

The 35 eV CID mass spectra of the two protonated monoamides are shown in Fig. 3. Apart from the formation of NH_4^+ (*m/z* 18), the spectra can be interpreted by the fragmentation sequences shown in Scheme 7. Further fragmentation of the $[MH - NH_3]^+$ and $[MH - H_2O]^+$ primary fragment ions is more extensive for the fumamic acid species than for the maleamic acid. Thus, for the protonated fumamic acid *m/z* 27 ion



Scheme 7

($C_2H_3^+$) is more abundant than its precursor *m/z* 99 whereas the *m/z* 70 and 44 ions are more abundant than their precursor *m/z* 98 ion; the opposite is true for the protonated maleamic acid. This difference presumably reflects the greater stability of the $[MH - NH_3]^+$ and $[MH - H_2O]^+$ species for the maleamic acid system since cyclization to protonated maleic anhydride and protonated maleimide structures, respectively, undoubtedly occurs. The CID spectra (not shown) of the source-produced $[MH - NH_3]^+$ ions from both acids were each in good agreement with the corresponding spectra of the $[MH - H_2O]^+$ ions derived from the dicarboxylic acids (Fig. 1), providing support for the conclusion that cyclization has occurred on loss of NH_3 from protonated maleamic acid. Weisz *et al.*[19] noted that, in the CID of the protonated dimethyl esters, the $[MH - CH_3OH]^+$ ion from the fumarate ester underwent more extensive fragmentation than did the $[MH - CH_3OH]^+$ ion derived from the maleate ester.

The metastable ion mass spectra of the protonated monomethyl esters **3** and **4** are distinctly different (Table 3), with the maleate ester showing loss of H_2O as the dominant fragmentation reaction whereas the fumarate ester shows $[MH - CH_3OH]^+$ as the base peak. This differing behaviour carries over to the CID spectra.

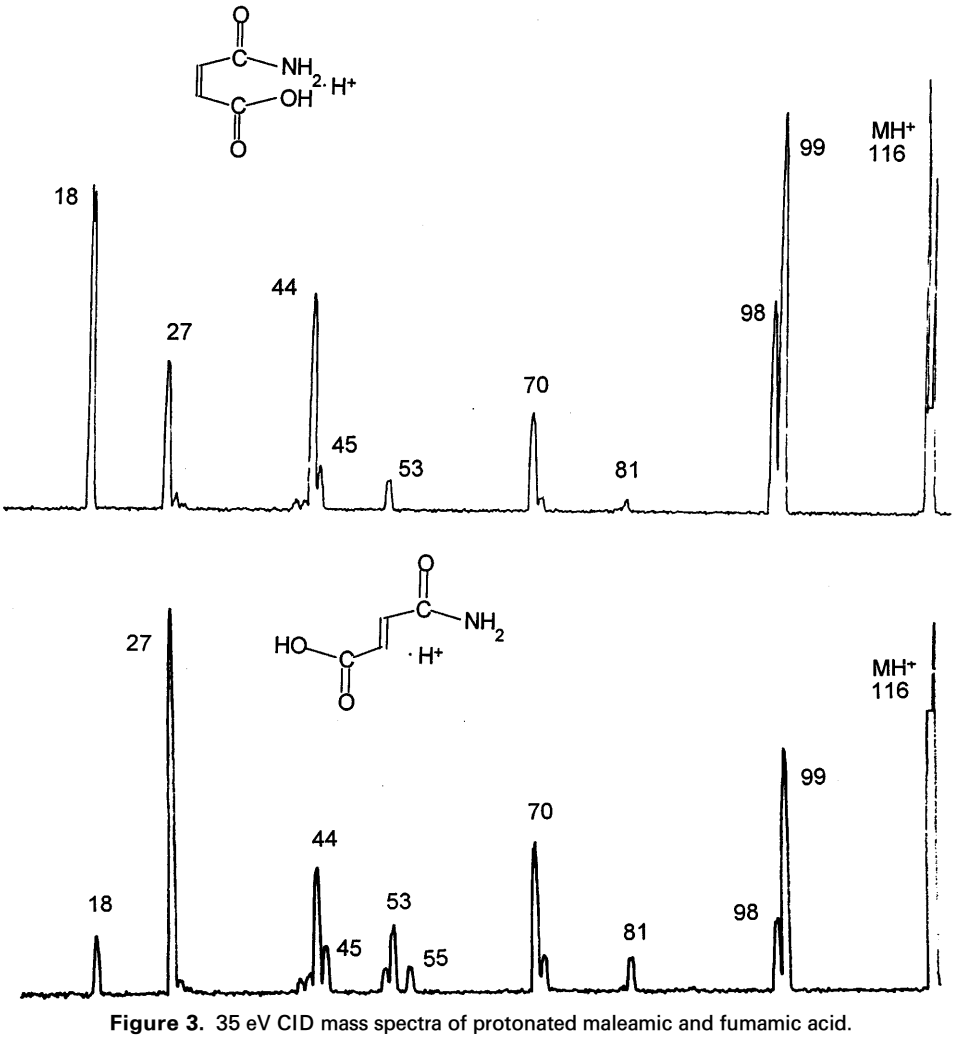


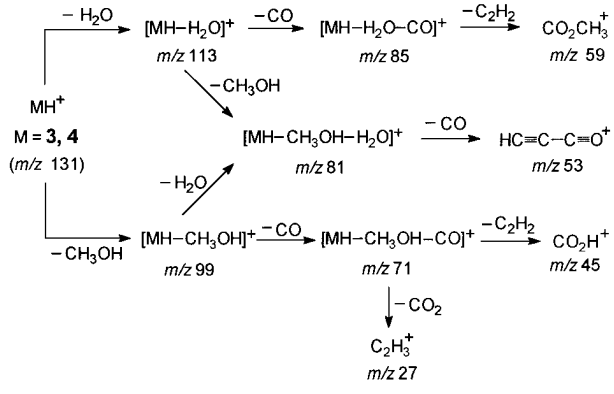
Figure 3. 35 eV CID mass spectra of protonated maleamic and fumamic acid.

Table 4. 40 eV CID mass spectra of protonated methyl maleate (3) and fumarate (4) and of methylated maleic (7) and fumaric (8) acid

		Intensity (% of base peak)			
m/z	Identity	**3**·H$^+$	**7**·CH$_3^+$	**4**·H$^+$	**8**·CH$_3^+$
113	$-H_2O$	100	100	7.6	10.3
99	$-CH_3OH$	61.6	62.9	100	100
85	$-[H_2O + CO]$	9.6	9.3	13.1	10.3
81	$-[CH_3OH + H_2O]$	2.9	2.7	35.0	34.3
71	$-[CH_3OH + CO]$	4.8	5.3	25.1	16.0
59	$COOCH_3^+$	67.3	66.7	28.0	18.8
53	$HCCCO^+$	6.7	5.3	53.1	52.6
45	$COOH^+$	8.4	9.3	23.0	20.6
33	$CH_3OH_2^+$	2.9	6.7		
27	$C_2H_3^+$	1.4	4.0	11.2	17.5

Table 4 records the 40 eV CID mass spectra of the MH$^+$ ions of the two monomethyl esters and, for comparison, the 40 eV CID mass spectra of the CH$_3^+$ adducts of the free maleic and fumaric acids. The spectra of the methyl adducts are in reasonable agreement with the CID spectra of the corresponding protonated monomethyl esters; this is to be expected given the mobility of the proton in these systems. The CID spectra can be rationalized by the fragmentation sequences in Scheme 8. Clearly, the maleates preferentially fragment by initial elimination of H_2O whereas the fumarates preferentially fragment by elimination of CH_3OH.

The preferential elimination of CH_3OH from protonated monomethyl fumarate might be taken to indicate that protonation occurs favourably at the carbonyl moiety of the ester function with subsequent 1,3-H$^+$ migration. In the light of the labelling results reported above for fragmentation of protonated fumaric acid, it is no surprise that the fragmentation of protonated monomethyl fumarate also is complex. The results for the metastable ion fragmentation of specifically labelled monomethyl fumarates are presented in Table 5. Fragmentation of **4**·D$^+$ and **4**-*O*-d_1·H$^+$ both show loss of CH_3OH: loss of $CH_3OD \approx 2:1$ whereas fragmentation of **4**-*O*-d_1·D$^+$ shows that loss of CH_3OH, involving a C-bonded hydrogen, accounts for *ca.* 10% of the fragmentation events. Under CID conditions (40 eV) **4**·D$^+$ showed $-CH_3OH/-CH_3OD \approx 1$ whereas **4**-*O*-d_1·H$^+$ continued to show $-CH_3OH/-CH_3OD \approx 2$. The



Scheme 8

Table 5. Unimolecular fragmentation of MH$^+$/MD$^+$ of monomethyl fumarates

	Neutral lost (% of base peak)			
Precursor	HDO	D_2O	CH_3OH	CH_3OD
4·D$^+$	12.3		100	47.7
4-*O*-d_1·H$^+$	11.5		100	45.0
4-*O*-d_1·D$^+$	2.0	8.1	10.5	100

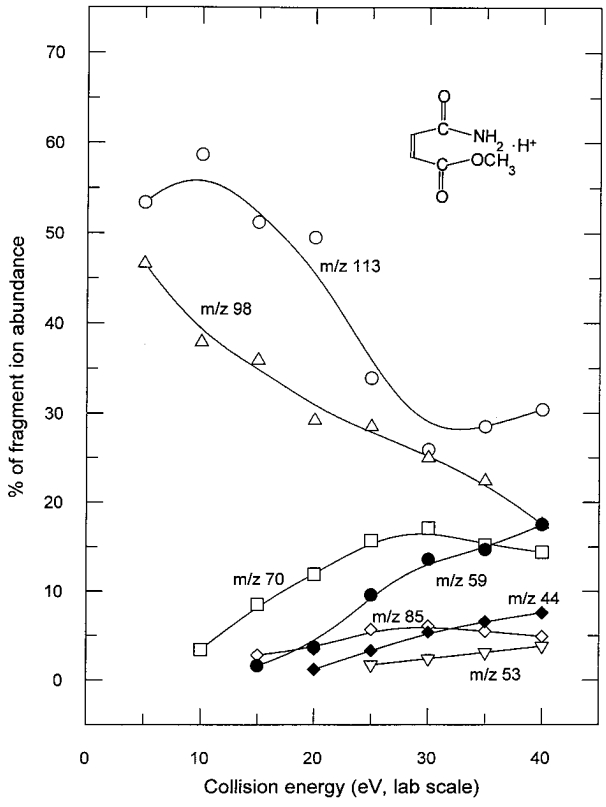


Figure 4. Breakdown graph for the MH$^+$ (*m/z* 130) ion of methyl maleamate.

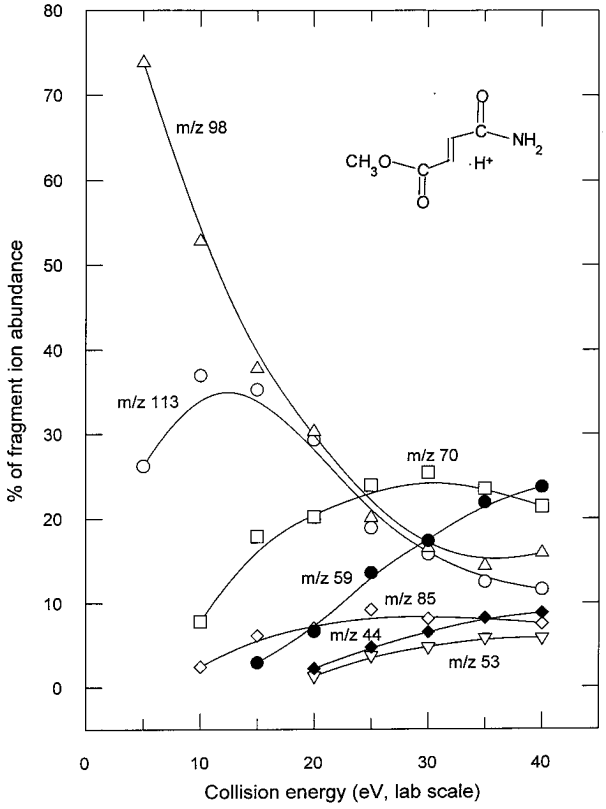
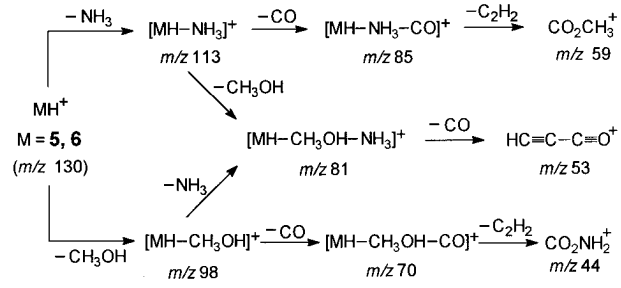


Figure 5. Breakdown graph for the MH^+ (*m/z* 130) ion of methyl fumamate.

metastable ion spectrum of **4**-*O*-$d_1 \cdot D^+$ shows that a C-bonded hydrogen is involved in about 20% of the water-loss reaction. Clearly, as for protonated fumaric acid, the detailed pathways of fragmentation are complex and cannot be rationalized solely on the basis of carbonyl protonation followed by a 1,3-H^+ shift and fragmentation.



Scheme 9

Table 6. Unimolecular fragmentation of CD_3^+ adducts of compounds 1–4

	Neutral lost (% of base peak)				
Compound	NH_3	H_2O	CH_3OH	CD_3OH	CD_3OCH_3
1	28	40		100	
2	38	38		100	
3			100	93	
4			99	100	70

The metastable ion fragmentation reactions for protonated methyl maleamate (**5**) and protonated methyl fumamate (**6**) are very similar (Table 3) with similar ratios for loss of CH_3OH and NH_3. However, fragmentations of the two protonated isomers under CID conditions show distinct differences. The breakdown graphs for $\mathbf{5} \cdot H^+$ and $\mathbf{6} \cdot H^+$ are shown in Figs 4 and 5 and the rationalization of the spectra observed is presented in Scheme 9. At collision energies greater than *ca.* 25 eV the fragment ions $[MH - CH_3OH - CO]^+$ and $COOCH_3^+$ become the most abundant ions for $\mathbf{6} \cdot H^+$ whereas the primary fragment ions $[MH - NH_3]^+$ and $[MH - CH_3OH]^+$ remain the most abundant ions for $\mathbf{5} \cdot H^+$. Clearly, as observed for the protonated monoamides, the primary fragment ions are more stable for the maleate species and undergo further fragmentation less readily.

Significant differences also are observed in the metastable ion fragmentation of $\mathbf{5} \cdot D^+$ and $\mathbf{6} \cdot D^+$. For both species the ammonia lost incorporated the added D; however, the maleate $\mathbf{5} \cdot D^+$ showed $-CH_3OH/-CH_3OD = 1.7$ whereas the fumarate $\mathbf{6} \cdot D^+$ showed $-CH_3OH/-CH_3OD = 0.15$. The former ratio is close to the ratio of 2.0 expected if the added D^+ scrambles with the amide hydrogens prior to methanol elimination. Clearly, for $\mathbf{6} \cdot D^+$ there is a decided preference for elimination of the added D^+ with the neutral methanol.

Fragmentation of methyl–cation adducts

The metastable ion spectra of the CD_3^+ adducts of the monoamides and monomethyl esters **1** to **4** are presented in Table 6. As can be seen, the CD_3^+ adducts of both monoamides **1** and **2** fragment on the metastable ion time-scale by elimination of CD_3OH, H_2O and NH_3 with very similar abundance ratios. Despite this similarity, the CID mass spectra of the adducts exhibit substantial differences, as shown by the 20 eV CID mass spectra of the CH_3^+ adducts (Fig. 6). The relative abundances for $[MCH_3 - NH_3]^+$ (*m/z* 113), $[MCH_3 - H_2O]^+$ (*m/z* 112) and $[MCH_3 - CH_3OH]^+$ (*m/z* 98) differ significantly for the two isomers. The *m/z* 84 and 80 fragment ions originate by loss of CO and CH_3OH, respectively, from the $[MCH_3 - H_2O]^+$ ion. The $[MCH_3 - H_2O]^+$ fragment ion from the fumamic acid adduct shows a distinctly different *m/z* 84/80 ratio to the maleamic acid adduct. In agreement with the latter spectrum, CID of the CH_3^+ adduct of maleimide (*m/z* 112) gave an *m/z* 80 ion, $[MCH_3 - CH_3OH]^+$, as the major fragment ion with minor formation of *m/z* 84. An unexpected observation is the formation of $CH_3OH_2^+$ (*m/z* 33, shifting to *m/z* 36 for the CD_3^+ adducts) in minor yield in both CID mass spectra. The mechanism by which this product is formed is not known.

The CID spectra of the CH_3^+ adducts of the monoamides differ substantially from the CID mass spectra of the protonated methyl esters of the monoamides (Figs 4 and 5). The latter show no signal for loss of H_2O (*m/z* 112) and no signal at *m/z* 84 and 80. Clearly, the major fraction of the CH_3^+ adducts has a structure different than that of the MH^+ ion of the methyl esters of

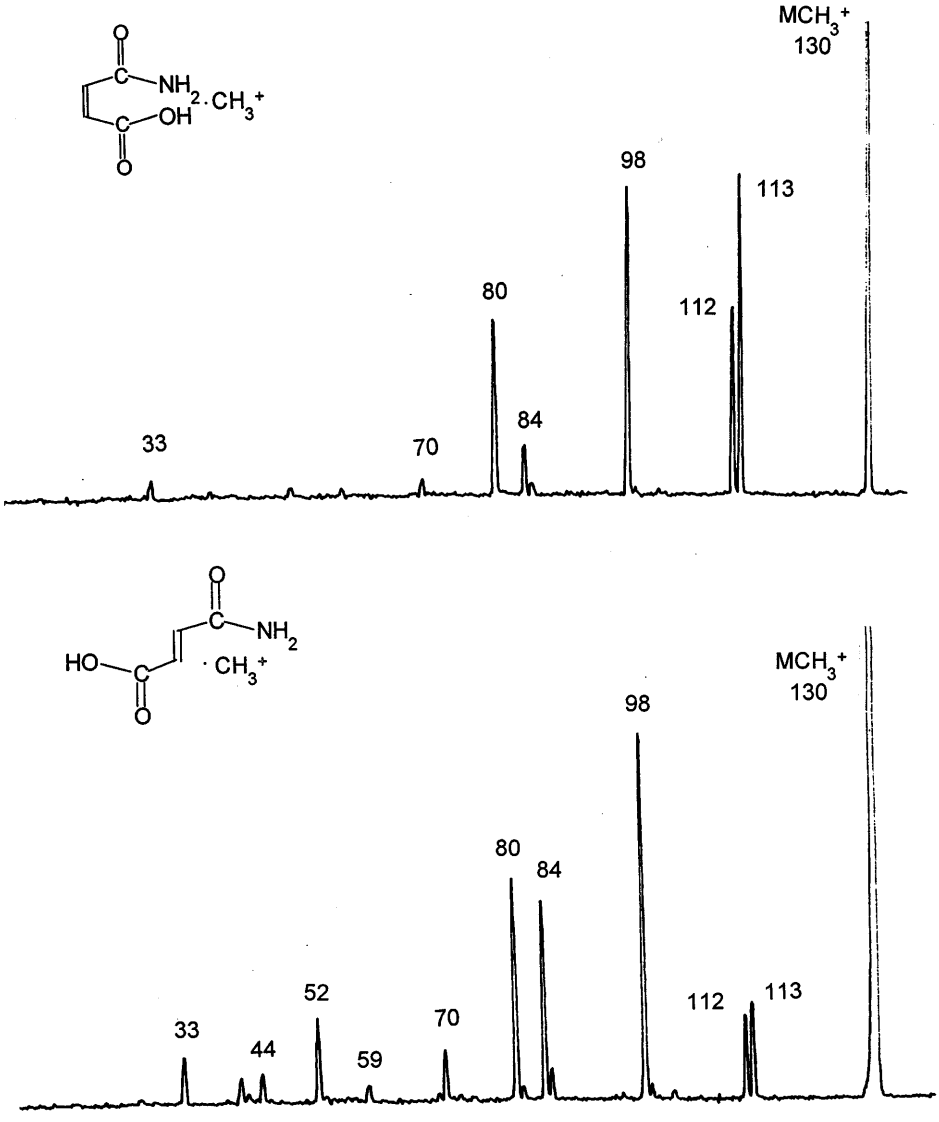


Figure 6. 20 eV CID mass spectra of CH_3^+ adducts of maleamic and fumamic acid.

the monoamides. This precludes significant methyl cation addition to either of the oxygens of the free carboxyl group of the monoamides. We conclude that the methyl cation adds primarily to the carbonyl oxygen of the amide function. CH_3^+ addition to the nitrogen would be expected to result in formation of either $[MCH_3 - CH_3NH_2]^+$ or $CH_3NH_3^+$, neither of which is observed in either CID mass spectrum.

The metastable ion mass spectra of the CD_3^+ adducts of the monomethyl esters **3** and **4** are substantially different (Table 6). Although both show loss of CD_3OH and CH_3OH in a *ca.* 1:1 ratio, the adduct with monomethyl fumarate also shows an intense metastable ion signal for elimination of CD_3OCH_3. Figure 7 compares the CID mass spectrum of the CH_3^+ adduct of the methyl-d_3 monoester of maleic acid-*O*-d_1 with the CID spectrum of protonated methyl-d_3 methylmaleate. The spectra are similar, the major primary fragmentation reaction involving loss of methanol before which the labelled and unlabelled methyl groups have become equivalent, as in the metastable ion spectrum. Loss of methanol is followed by loss of CO to give *m/z* 85 and 88 ions and further loss of C_2H_2 to give $COOCH_3^+$ and $COOCD_3^+$ (*m/z* 59 and 62). The methyl adduct of the monoester also shows a minor peak at *m/z* 100 in the CID spectrum, corresponding to loss of CH_3OCD_3 from the MCH_3^+ adduct; this product is not observed in the metastable ion spectrum, nor is it observed for the protonated diester. This fragmentation presumably arises from a species in which the methyl group has added to the carbomethoxy group of the monoester, at either the carbonyl or methoxy oxygen.

Elimination of dimethyl ether becomes a major fragmentation reaction in the CID spectrum of the CH_3^+ adduct of the monomethyl fumarate (**4**). This is evident from the intense ion signal at *m/z* 99 in the top spectrum of Fig. 8. This fragmentation reaction is not seen for protonated dimethyl fumarate (bottom spectrum, Fig. 8), indicating that at least a major part of the two ions of *m/z* 145 have different structures. Clearly, methyl cation addition to the monoester **4** must take place largely at the carbomethoxy group, but it is not certain whether this occurs at the carbonyl or methoxy oxygen. Isbell and Brodbelt[20] observed elimination of dimethyl

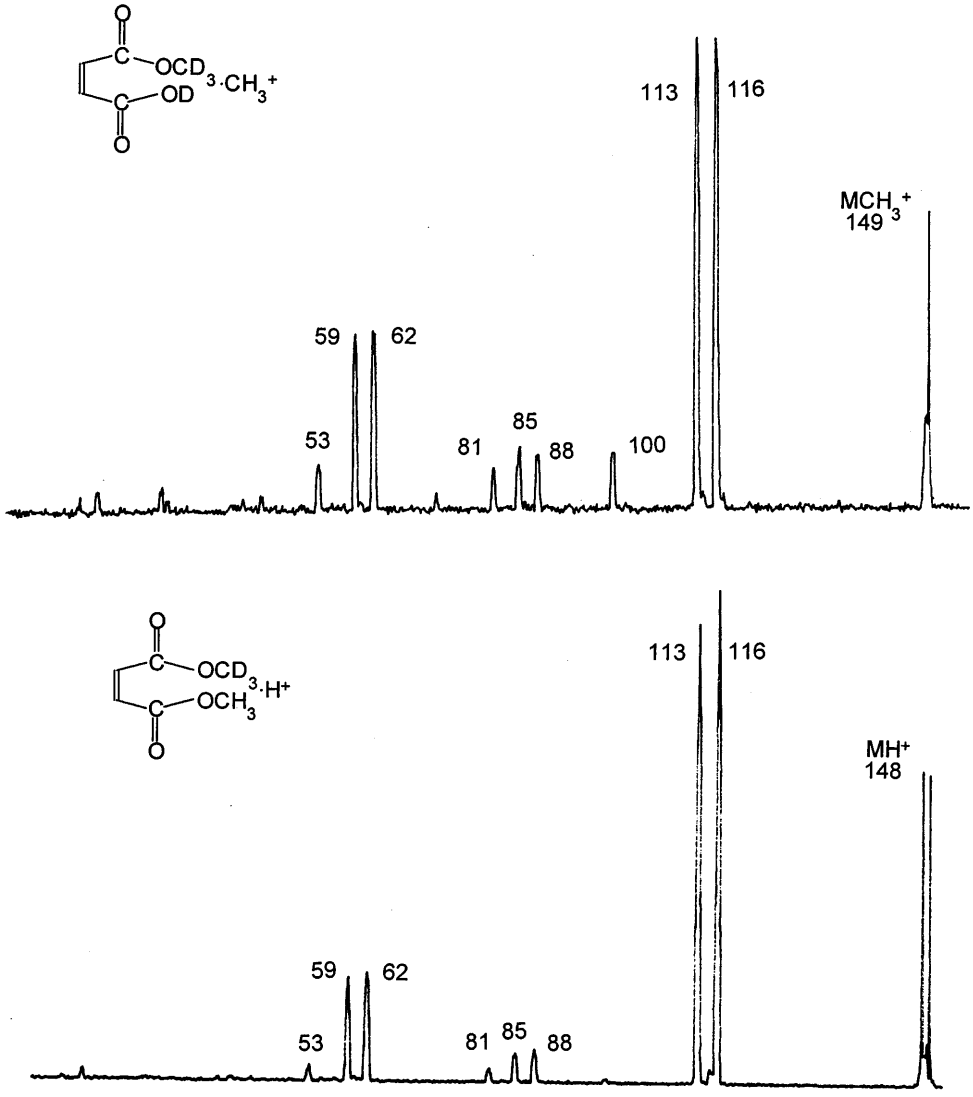


Figure 7. CID mass spectra (40 eV) of CH_3^+ adduct of methyl-d_3 maleate-O-d_1 and of protonated methyl-d_3 dimethylmaleate.

ether from methyl-cationated methyl propionate, methyl acrylate and methyl methacrylate.

CONCLUSIONS

A significant observation in the present work is that the fragmentation of protonated fumaric acid does not involve solely a 1,3-H^+ migration followed by H_2O elimination starting from a carbonyl oxygen protonated species. There is significant proton migration from one carboxyl group to the other as well as participation of the C-bonded hydrogens in the water-loss reaction. Despite the complexity of this water-loss reaction, the $[MH - H_2O]^+$ ion formed from protonated fumaric acid has a structure different from that of the $[MH - H_2O]^+$ ion derived from protonated maleic acid; this result indicates that the *cis/trans* configuration about the double bond is retained in the fragment ions.

The fragmentation of the protonated monoamides, monomethyl esters and the methyl esters of the monoamides show substantial stereochemical effects, making differentiation of the *cis* and *trans* isomers possible. Of particular note is the substantial formation of NH_4^+ in the fragmentation of protonated maleamic acid, which is best rationalized as occurring through the initial formation of a protonated maleic anhydride–ammonia ion–neutral complex.

The CH_3^+ adducts of maleamic and fumamic acids show a different fragmentation behaviour to the protonated methyl esters of the monoamides, indicating that the methyl cation adds mostly to the carbonyl oxygen of the amide function. Similarly, formation of the CH_3^+ adducts of the monomethyl esters appears to proceed primarily by addition to the carbomethoxy group rather than to the free carbohydroxy group.

Acknowledgement

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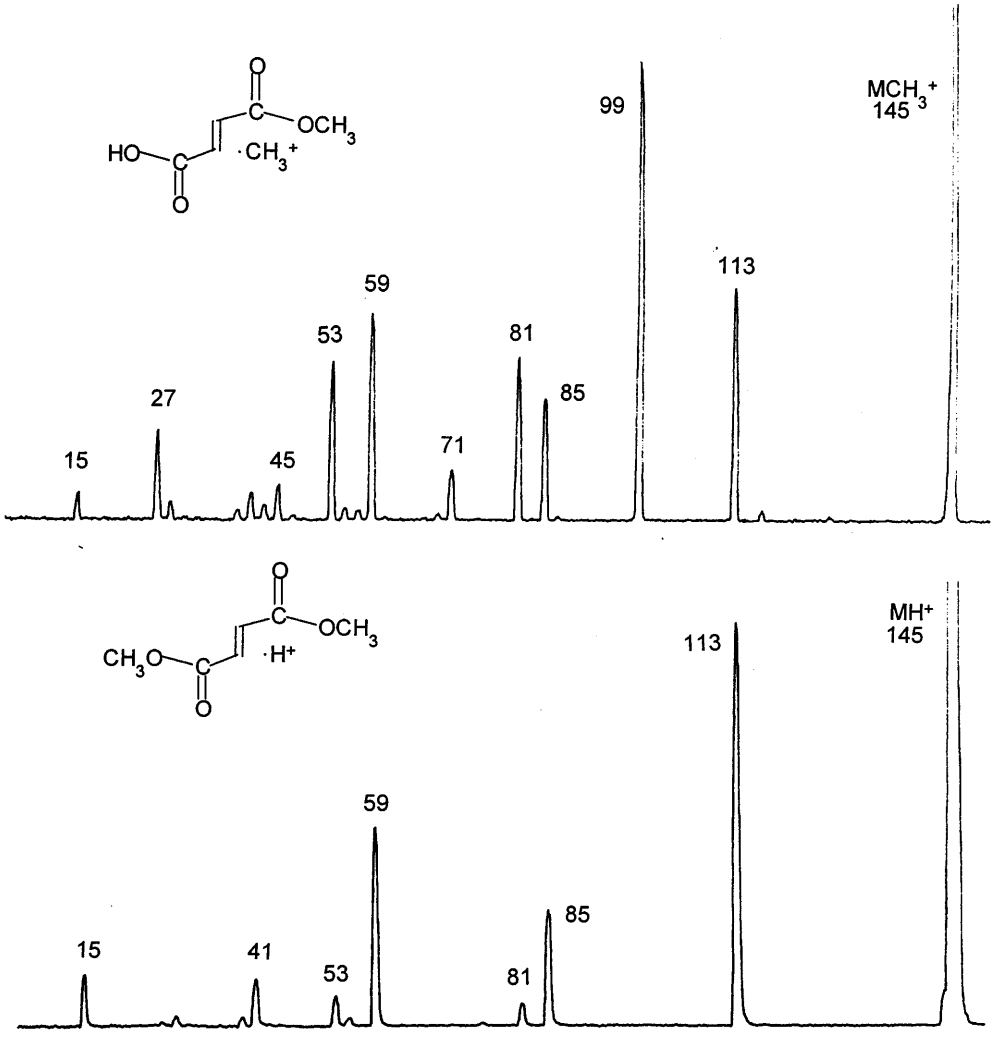

Figure 8. CID mass spectra (40 eV) of CH_3^+ adduct of monomethyl fumarate and of protonated dimethyl fumarate.

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