Taylor, R. L.; Conrad, H. E. *Biochemistry* **1972**, *11*, 1383.
Theander, O.; Åman, P. "Dietary Fibers: Chemistry and Nutrition"; Academic Press: New York, San Francisco, and London, 1979; pp 215-245.
Utille, J. P. Thèse Doctorat Etat, Université de Grenoble, Grenoble, France, 1979a.
Utille, J. P., C.E.R.M.A.V., Grenoble, France, unpublished data, 1979b.
Wilkie, K. C. B. *Adv. Carbohydr. Chem. Biochem.* **1979**, *36*, 266.
Wilkie, K. C. B.; Woo, S. L. *Carbohydr. Res.* **1977**, *57*, 145.

Received for review June 1, 1981. Revised manuscript received November 3, 1981. Accepted January 6, 1982.

Direct Characterization of Nutmeg Constituents by Mass Spectrometry-Mass Spectrometry

Dean V. Davis and R. Graham Cooks*

Constituents of *Myristica fragrans* (nutmeg) in the molecular weight range 100–700 amu are characterized via MS–MS (mass spectrometry–mass spectrometry). The nutmeg sample is analyzed directly; no extraction, derivatization, or other sample preparation is necessary. The analysis is facilitated by temperature profiling and by utilizing different reagent gases in the chemical ionization (CI) source of the mass spectrometer. Data were taken with both a sector type and a triple quadrupole MS–MS instrument. Compounds were characterized by recording the mass spectrum of fragments formed by collision-induced dissociation of particular ions, often the protonated molecules. Groups of compounds with particular structural units were characterized by scans of all precursor ions which yield mass-selected fragment ions. This also was used to pinpoint instances where MS–MS spectra contained contributions from more than one structural isomer. In this study, one new compound, a dehydrodiphenylpropanoid derivative of myristicin, was identified. Correlation of the results with literature data demonstrates that analysis of foodstuffs by MS–MS is rapid and provides detailed structural information.

Mass spectrometry–mass spectrometry (MS–MS) is a developing technique for the identification and quantitation of organic constituents present in complex mixtures (Hunt et al., 1980; Kondrat and Cooks, 1978; Unger and Cooks, 1979; Shushan and Boyd, 1980). Due to the large variety of samples which have been analyzed by MS–MS in the last several years, the methodology is fast becoming established as an alternative and/or supplement to gas chromatography–mass spectrometry (GC–MS). This study on the identification of constituents in the spice *Myristica fragrans* (nutmeg) was undertaken to explore some recent refinements in the MS–MS technique, including comparisons between data taken on a high energy sector instrument and a triple quadrupole device.

Up until now most MS–MS work on complex mixtures has been targeted at one or just a few prespecified components; however, the need exists for more complete characterization of such mixtures. The capability of MS–MS for such detailed characterization has not yet been demonstrated, with the possible exception of recent work on coal liquids (Zakett et al., 1981, 1979b; Wilson et al.; 1981; Hunt and Shabanowitz, 1981). This particular aim is here facilitated by using temperature profiling in conjunction with MS–MS. The complexity of the mixture actually present in the ion source at any time is reduced by repetitively recording mass spectra as a function of probe temperature. In other words, selective volatilization allows more complex mixtures to be studied without increasing the spectral complexity.

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907.

Most of the data in this paper consist of daughter ion spectra as shown in eq 1. In addition, most of these data

$$m_1^+ \rightarrow m_2^+ + m_3 \quad (1)$$

fix m_1^+, scan m_2^+: daughter spectrum

fix m_2^+, scan m_1^+: parent spectrum

are recorded at high collision energy. Using a triple quadrupole instrument which utilizes much lower energy collisions, one can compare the effects of collision energy on MS–MS spectra and also access two other types of scans (Yost and Enke, 1979). Referring to reaction 1, one can fix m_2^+ and scan m_1^+, the so-called parent scan (Gallegos, 1976), or fix m_3 and scan m_1^+, termed a neutral loss scan (Lacey and MacDonald, 1979; Zakett et al., 1979a; Haddon, 1980). Both of these latter spectra have potential in identification of all individual compounds which contain a particular functional group. Investigation of the practical utility of these newer types of scans was one of the objectives of this study.

A further motivation behind this work was to test the potential of MS–MS in the area of foods analysis. Background on current techniques in this growing area of analysis is provided is recent reviews by Self (1979) and Kolor (1979). Analysis of foods by MS–MS is expected to be particularly attractive not only because it has the necessary speed and sensitivity but also because it should require little derivatization or other sample workup prior to analysis.

The complex mixture of organic species which make up nutmeg has been studied previously by both standard extraction techniques and by gas chromatography–mass spectrometry. Extraction and characterization of the

0021-8561/82/1430-0495$01.25/0

Table I. Some Constituents of *M. fragrans*[a]

monoterpenes	esters
α-pinene	bornyl acetate
β-pinene	linalyl acetate
sabinene	α-terpenyl acetate
β-phellandrene	citronellyl acetate
α-terpinene	
limonene	aliphatics
sesquiterpenes	2-methyl-4-decen-1-ol
β-caryophylene	3-methyl-4-decernyl acetate
α-copanene	fatty acids
α-humulene	myristic
α-farnesene	lauric
	palmitic
aromatics	steric
safrol	oleic
eugenol	tridecanoic
cis-isoeugenol	
trans-isoeugenol	diphenylpropanoids
methyl eugenol	dehydroisoeugenol
myristicin	5-methoxydehydroisoeugenol
elemicin	
cis-isoelemicin	
trans-isoelemicin	
monoterpene alcohols	
linalool	
terpinene-4-ol	
α-terpineol	

[a] Data from Schenk and Lamparsky (1981), Forrest et al. (1973, 1974), and Harvey (1975).

Table II. Temperature Profiled CIMS of Nutmeg Using Nitrogen as the Reagent Gas

temp, °C	mass	intensity	temp, °C	mass	intensity
125	136	33.2	250	228	18.1
	137	5.1		236	4.0
	146	13.2		238	10.2
	148	6.8		256	5.6
	150	8.9		262	7.1
	160	10		263	5.3
	161	3.7		264	41.4
	162	25.4		265	5.8
	163	11.0		278	3.3
	164	26.6		280	3.5
	176	8.0		282	18.3
	177	34.7		283	3.8
	178	80.2		284	8.8
	179	8.3		300	7.5
	190	7.0		323	5.7
	191	29.6		324	12.3
	192	100		325	14.1
	193	17.1		326	24.6
	194	3.7		327	6.9
	200	3.9		340	3.5
	204	8.1		344	3.3
	207	14.7		354	7.1
	208	42.3		355	3.7
	209	4.0		356	12.3
	210	11.7		357	4.5
				370	13.8
				371	3.9
				400	5.7
				401	3.4

separated constituents by NMR, IR, and UV spectroscopy and electron impact (EI) mass spectrometry followed by synthesis of authentics have been reported by Forrest et al. in a series of papers on diphenylpropanoids in nutmeg (Forrest et al., 1973, 1974). GC–MS work on nutmeg oil was initiated by Sammy and Nawar (1968), who identified a number of volatile components, and further expanded by Harvey (1975) through the use of trimethylsilyl derivatives. Harvey's work confirmed the presence of the diphenylpropanoids seen in Forrest's studies. Very recent work by Schenk and Lamparsky (1981) has further clarified the information available on nutmeg oil constituents by separating the isobaric ions of the monoterpenes, sesquiterpenes, and monoterpenols, confirming those already suspected to be present and showing the presence of a number of new constituents. Table I gives the structures of a representative selection of the compounds known to be present in nutmeg.

EXPERIMENTAL SECTION

The ground nutmeg sample (Durkee's brand) was acquired commerically. The spice was placed in a capillary tube and then inserted via a solids probe into the source of the mass spectrometer. The sample was ionized by chemical ionization (CI) (Richter and Schwarz, 1978); when protonated molecular ions $(M + H)^+$ were desired, isobutane was used as the reagent gas. When radical ions $(M^{+\cdot})$ of the type commonly seen in EI mass spectrometry were required, the reagent gas used was N_2, which gives radical ions by charge exchange (Lindholm, 1966) with $N_2^{+\cdot}$.

During ionization, the temperature of the sample was varied in order to selectively vaporize various constituents. The lowest molecular weight species yield intense ions at ambient probe temperature. The higher mass species (ca. 280–400 amu), however, required increased probe temperatures to be desorbed and subsequently ionized. A temperature of approximately 300 °C was necessary to generate good intensities for the highest mass constituents studied.

All authentic compounds except trimyristin were obtained from Aldrich Chemical Co. and were used without further purification. Trimyristin was obtained by extraction of nutmeg itself (Reimer and Will, 1885; Helmkamp and Johnson, 1968).

The high-energy mass and MS–MS spectra were obtained on a reverse geometry mass-analyzed ion kinetic energy (MIKE) spectrometer described in detail elsewhere (Beynon et al., 1973; Kondrat and Cooks, 1978). The procedures used consisted of manually tuning a magnetic sector to transmit ions of a given mass/charge ratio, under conditions of better than unit mass resolution. This preselected ion was then excited in a collision cell (collision energy 7000 eV), and its dissociation products were mass resolved by using an electric sector scan. This scan, data acquisition, and plotting were all done under computer control (Schoen et al., 1981). The collision gas used was air at a nominal pressure of $(2–3) \times 10^{-5}$ torr, as read on a Bayard–Alpert type ion gauge (estimated actual cell pressure 0.5 to 1.0×10^{-3} torr). The low-energy MS–MS spectra were obtained on a commercial Finnigan triple quadrupole system (Slayback and Story, 1981). The collision energy was approximately 15 eV, and the pressure in the collision quadrupole approximately 7×10^{-4} torr of argon. The reagent gas was isobutane. All of these data were again acquired under computer control. Exact mass measurements were made by using electron ionization on a CEC 110B mass spetrometer and are accurate to ±0.003 amu.

RESULTS AND DISCUSSION

Temperature-profiled isobutane CI mass spectra of ground nutmeg (Figures 1 and 2) show a large number of peaks covering a mass range of approximately 130–410 amu while at still higher temperatures higher mass ions are also observed and some are discussed below. The choice of isobutane as reagent gas ensures that most compounds appear as protonated molecules (MH^+) in the mass spectrum and that little fragmentation occurs. In fact, as will be evident from the MS–MS results, most of the ions seen

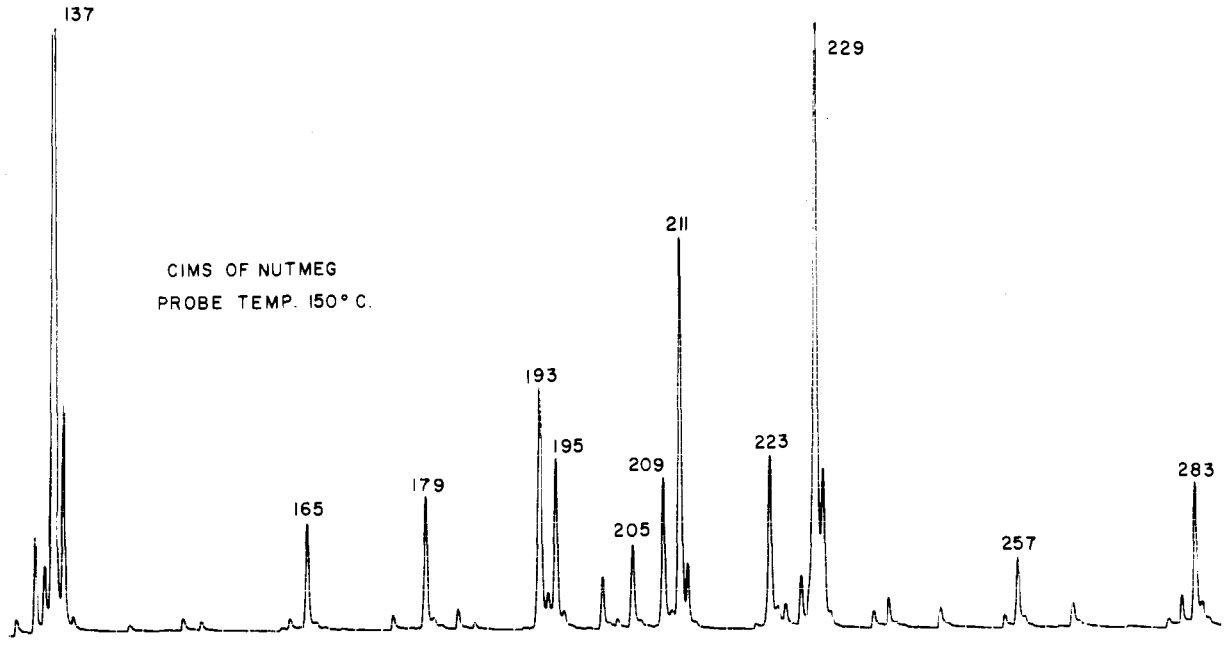


Figure 1. Isobutane chemical ionization mass spectrum of nutmeg at a probe temperature of 150 °C (low-mass region).

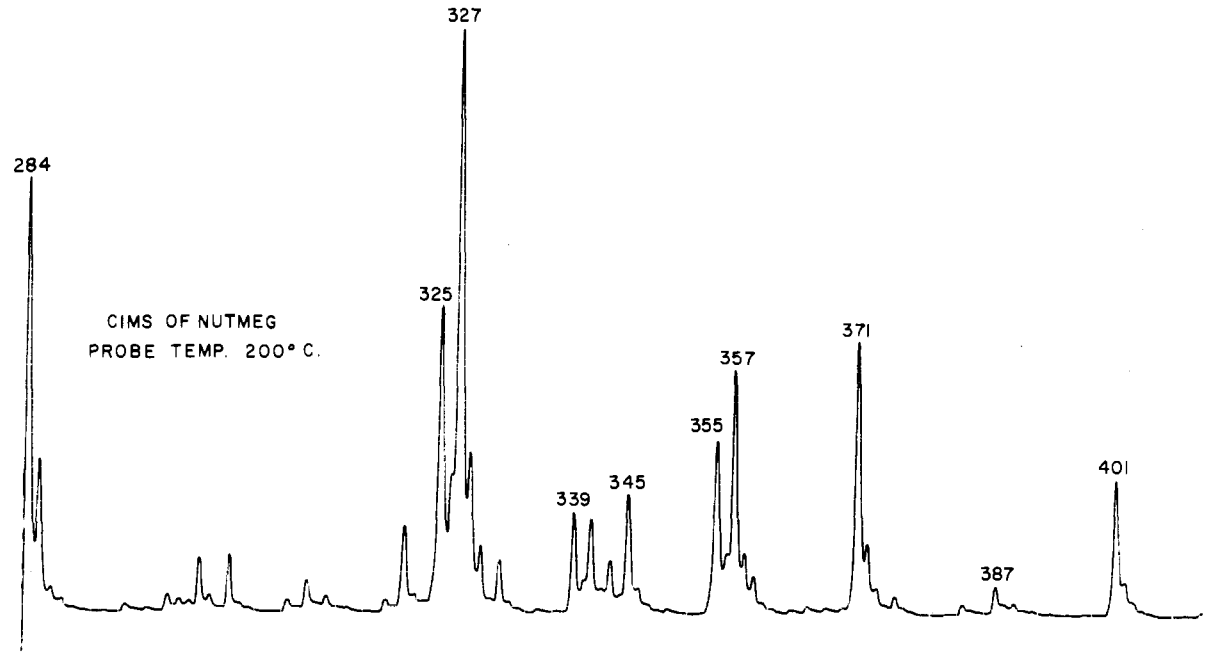


Figure 2. Isobutane chemical ionization mass spectrum of nutmeg at 200 °C (high-mass region). Note that 327^+ in this figure is approximately 20 times less intense than the 229^+ ion in Figure 1.

in Figures 1 and 2 do correspond to intact, protonated molecules. An alternative and complementary form of mass spectrum is that obtained by using nitrogen as a charge-exchange reagent. Table II gives charge-exchange mass spectra of nutmeg at two selected probe temperatures. Many prominent ions are radical cations of the type $M^{+\cdot}$, and the molecular weights of dozens of nutmeg constituents are evident from a comparison of the results of Table II and Figures 1 and 2.

Further identification of these individual compounds is achieved by obtaining mass spectra of the corresponding ions, viz., MH^+ and $M^{+\cdot}$, for a compound of molecular weight M. Note that the ion serves as a surrogate for the neutral compound of interest. This procedure is illustrated in Figure 3 which shows a comparison of the MS–MS spectrum (daughter scan) of 137^+ from nutmeg with that from 137^+ generated by protonation of β-pinene, molecular weight 136. It can be seen that the MS–MS spectrum of protonated β-pinene is very similar to that of the 137^+ ion from nutmeg, the comparison being suggested initially by the fact that nutmeg oil is known to be rich in terpenes, including the pinenes. This comparison does not establish the presence of β-pinene in nutmeg, since protonated limonene, for example, gives a very similar MS–MS spectrum. It does confirm that the 137^+ peak arises from a protonated monoterpene. No effort was made to determine isomers individually, although such information can sometimes be obtained in MS–MS (Zakett et al., 1979a). The peak at mass 205^+ corresponds to the next member of the terpene series, the sesquiterpene ($C_{15}H_{24}$). Figure 4 shows the MS–MS spectrum of 205^+ from nutmeg compared with that of 205^+ generated by chemical ionization of β-caryophylene. The match here is not as good as that shown in Figure 3, indicating that nutmeg is likely to contain a mixture of sesquiterpenes, and possibly other compounds, in addition to β-caryophylene. A third ion,

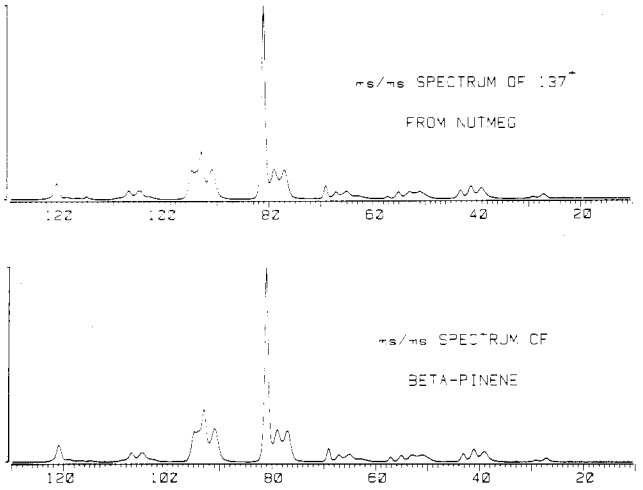


Figure 3. Comparison of MS–MS spectra generated from the 137^+ ion of nutmeg and protonated authentic β-pinene.

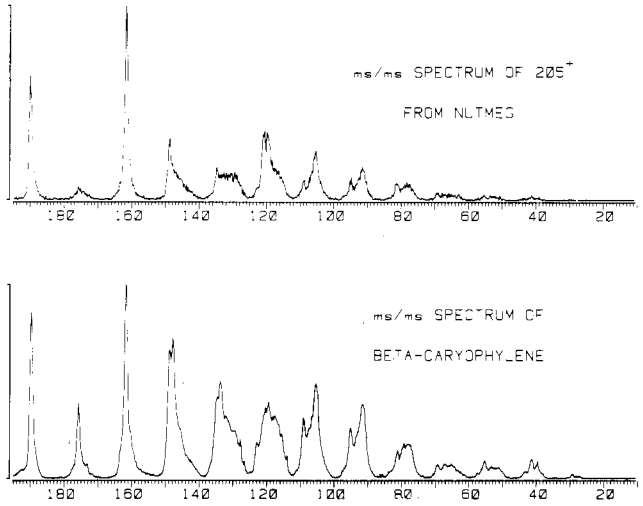
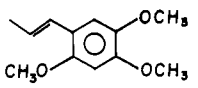


Figure 4. Comparison of the MS–MS spectra generated from the 205^+ ion of nutmeg and protonated authentic β-caryophylene.

mass 221^+, was chosen for study as it corresponds to molecular weight 220 and could be a sesquiterpene alcohol ($C_{15}H_{24}O$). It was established by comparison with an authentic MS–MS spectrum that this peak was not due to protonated caryophylene oxide, but in the absence of a library of MS–MS spectra which might be searched for evidence on the structure, no further work was done to identify it.

A second class of compounds (m/z 165, 179, 193, and 209 in Figure 1) were distinguished on the basis of the overall appearance of their isobutane MS–MS spectra and previous literature data (Sammy and Nawar, 1968), both factors indicating them to be multiply substituted aromatic compounds. Two members of this series were studied in greater depth. The compound which occurred as a protonated species at 209^+ using isobutane CI also appeared at mass 208 in the charge-exchange mass spectrum and was found to have an exact mass of 208.111 amu, establishing its molecular formula as $C_{12}H_{16}O_3$. A comparison was made (Figure 5) between the MS–MS spectrum of 209^+ and the protonated molecular ion of authentic 2,4,5-trimethoxypropenylbenzene (I), and this showed that the



I

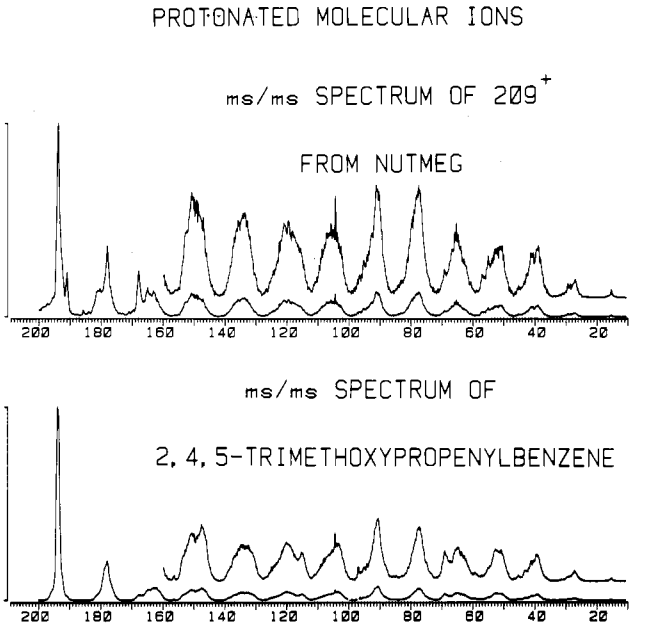


Figure 5. Comparison of the MS–MS spectra generated from the 209^+ ion of nutmeg and authentic 2,4,5-trimethoxypropenylbenzene (isobutane used as the CI reagent gas).

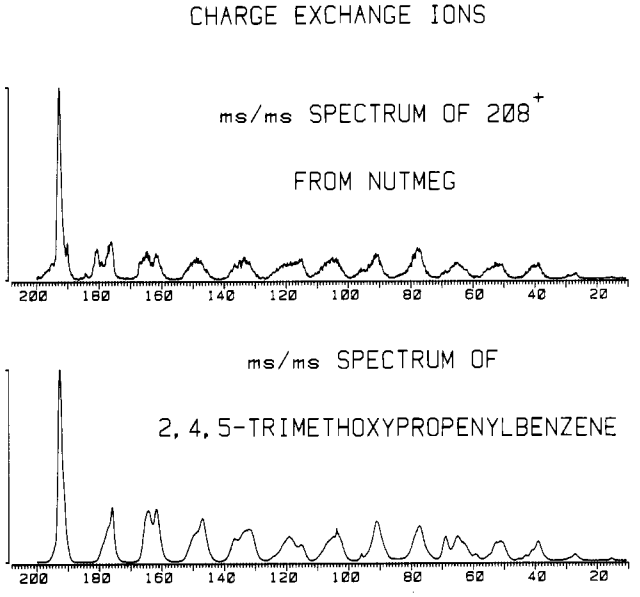
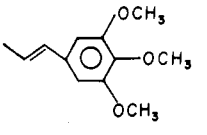


Figure 6. Comparison of the MS–MS spectra generated from the 208^+ ion of nutmeg with authentic 2,4,5-trimethoxypropenylbenzene (nitrogen used as the CI reagent gas).

compound responsible for m/z 209 was very closely related to compound I. The exact position of the three methoxy groups was not determined by MS–MS as all of the necessary authentic compounds were not available. However, other work (Self, 1979) has demonstrated that the actual isomer is likely to be the protonated form of *trans*-isoelmecin (II). To further test the conclusion that a tri-



II

methoxypropenylbenzene is present, an MS–MS spectrum was taken by using the alternative ionization method, N_2 charge exchange. A comparison of MS–MS spectra generated from radical ions of compound I and of nutmeg

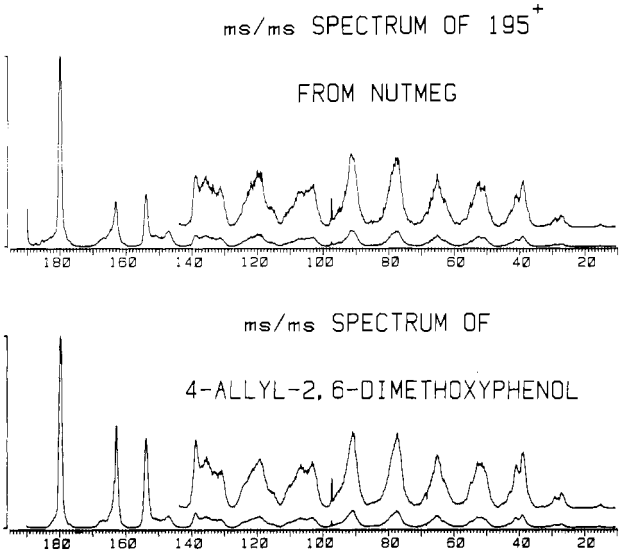
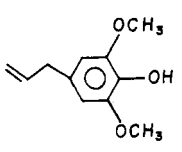


Figure 7. Comparison of MS–MS spectra generated from the 195^+ ion of nutmeg and authentic 4-allyl-2,6-dimethoxyphenol (isobutane used as the CI reagent gas).

(208^+) is shown in Figure 6. This comparison corroborates the conclusion drawn from the isobutane chemical ionization results. (If only one ionization reaction is to be selected, protonation is preferable because the mass spectrum is simpler, resulting in higher molecular ion abundances and more assurance of structural identity between the neutral molecule and the ion which is analyzed as its proxy.)

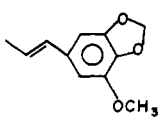
The presence of a homologous series of compounds of which II is a member is indicated by the several mass spectra of nutmeg (Figures 1 and 2; Table II). For confirmation of this, the ion 14 mass units below 209^+, at mass 195^+, was selected for study. A comparison of its MS–MS spectrum with that of 209^+ (compare Figures 5 and 7) confirmed the homologous relationship. The corresponding ions generated by charge exchange (208^+ and 194^+) also show MS–MS spectra which appear to be those of homologous ions (Figures 6 and 8). The structure of the lower homologue is confirmed as the dimethoxyphenol [4-allyl-2,6-dimethoxyphenol (III) or a ring-substituent



III

isomer] by the comparison made in Figure 7 and cross-checked by using radical cations in Figure 8. In both comparisons agreement is excellent, but the exact substitution pattern on the ring is not confirmed.

A third member of this set of compounds occurs at 193^+ [$(M + H)^+$] and is tentatively associated with myristicin (IV) even though the authentic compound was not avail-



IV

able. This conclusion is based on the chemical similarity

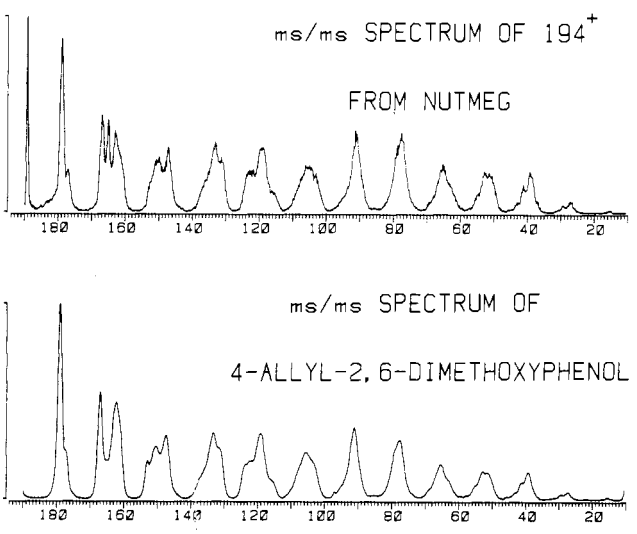
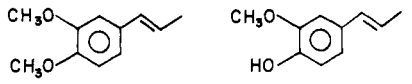


Figure 8. Comparison of MS–MS spectra generated from the 194^+ ion of nutmeg and authentic 4-allyl-2,6-dimethoxyphenol (nitrogen used as the CI reagent gas).

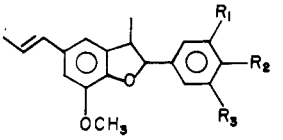
between myristicin and other compounds already confirmed as being present. It was corroborated by an exact mass measurement of 192.070 which corresponds to a molecular formula of $C_{11}H_{12}O_3$ and by interpretation of the MS–MS spectra of 192^+ and 193^+ corresponding to the molecular ion and the ionized molecule, respectively. Both MS–MS spectra showed characteristic aromatic ions centered on 27, 29, 39, 51, 63, 65, 76, 77, 91, and 105. In addition, the molecular ion spectrum was dominated by loss of a methyl radical as expected for an arylmethoxy structure like compound IV. The other two abundant members of this class (165^+ and 179^+ in the CI mass spectrum), while not studied further, are likely to correspond to protonated methylengenol (V) and eugenol (VI)



V VI

which have been reported to be present in nutmeg (see Table I).

A third class of compounds was delineated in analogous fashion to the two just discussed. It consists of one of the two types of diphenylpropanoids which can be formed from the aromatic compounds just discussed. The ions 325^+, 327^+, and 355^+ were assigned to this class because they show great similarities in their daughter MS–MS spectra (see Figure 9) and have appropriate molecular weights. The slight differences in the overall appearance of the MS–MS spectrum of 355^+ compared to that of the other two compounds (Figure 9) led to a more detailed study which uncovered a spectral overlap at nominal mass 355^+. This will be discussed in greater detail below when dealing with the triple quadrupole results. The structures of these three ions, as well as additional ions of the same basic type but of lower intensity, are given below (VII). The common structural backbond accounts for their very similar MS–MS spectra. The positions of the ring substituents in VII are not confirmed by MS–MS although they are known from organic extraction studies and biosynthetic considerations. Such determinations are often only possible in MS–MS if authentic compounds are available.

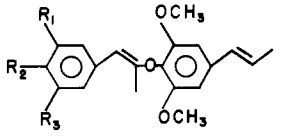


VII

mass $(M + H)^+$ ion	R_1	R_2	R_3
325	$O-CH_2-O$		H
327	OCH_3	OH	H
341	$O-CH_2-O$		OH
341	OCH_3	OCH_3	H
343	OCH_3	OH	OH
355	$O-CH_2-O$		OCH_3
357	OCH_3	OH	OCH_3
371	OCH_3	OCH_3	OCH_3

Diphenylpropanoids are also known which differ from those of type VII in that they contain acyclic rather than cyclic ether linkages. These ions do not generate molecular ions in the CI mass spectra; instead, they generate intense ions 18 mass units below the expected $(MH)^+$ species. These ions are created by protonation of neutrals which have undergone dehydration (see Scheme I), probably before volatilization. This unavoidable dehydration is the result of the high temperatures necessary to vaporize these fragile species and points out what may be a problem in MS–MS analysis: transformations which occur in the sample in the course of analysis.

While this problem is significant, the dehydrated molecular ions contain most of the structural information present in the original neutral molecules as shown by the MS–MS spectra of several $(M + H - H_2O)^+$ ions (Figure 10). The spectra of these three compounds are almost identical with one another for two reasons. First, they all contain a common framework (VIII), and second, within



VIII

mass of $(M + H^+ - H_2O)$ ions	R_1	R_2	R_3
355	$O-CH_2-O$		H
357	OCH_3	OH	H
371	$O-CH_2-O$		OH
371	OCH_3	OCH_3	H
387	OCH_3	OH	OCH_3
401	OCH_3	OCH_3	OCH_3

this framework there occurs an easily cleaved bond which leads to a particularly stable aryloxy fragment ion (193^+) which dominates each of the MS–MS spectra.

Positions of the ring substituents are not established by MS–MS due to lack of appropriate authentics but are again known from organic extraction work and biosynthetic considerations. Once again some subtle differences observed in the spectra were found to be due to contributions from more than one species at the mass of the selected ion, features which were further elucidated in the triple quadrupole studies (below).

All of the diphenylpropanoids (VII and VIII) are likely reaction products of the simple aromatic compounds already discussed. However, it seems that the diphenylpropanoids are present in the sample itself and are not artifacts formed in the ion source, for three reasons. First, ions corresponding to these constituents are present when

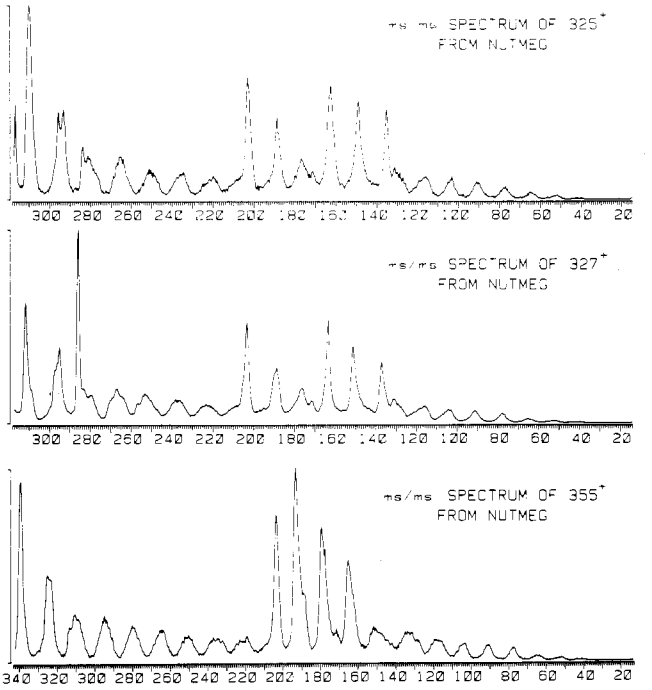


Figure 9. MS–MS spectra of the protonated diphenylpropanoids containing cyclic ether linkages which are present in nutmeg.

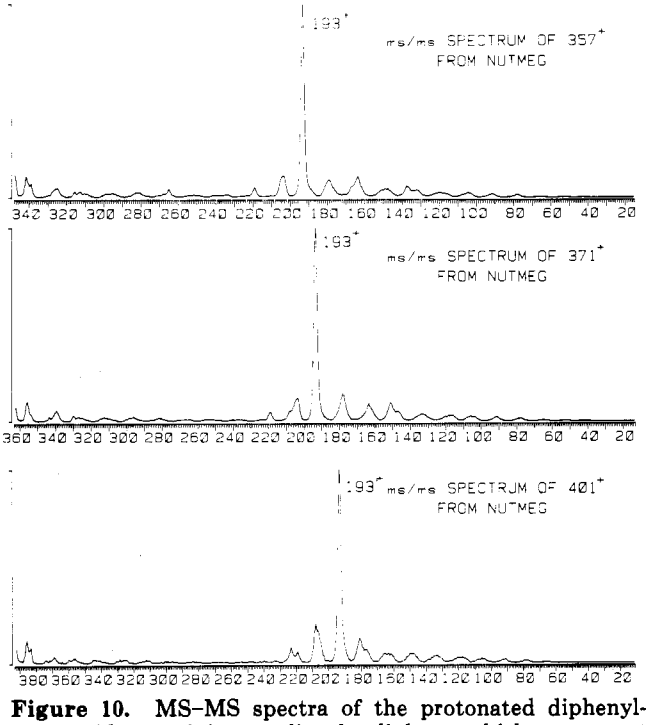
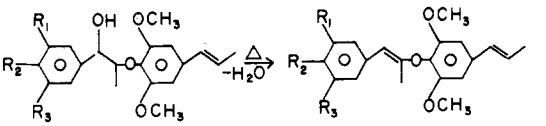


Figure 10. MS–MS spectra of the protonated diphenylpropanoids containing acyclic ether linkages which are present in nutmeg.

Scheme I



different ionizing techniques are used. They are seen in three distinct ionization procedures; EI, CI, and charge exchange which employ different source conditions. Second, the temperatures at which the compounds are desorbed are not high, and hence only extremely facile thermal reactions are likely. Third, such species have been

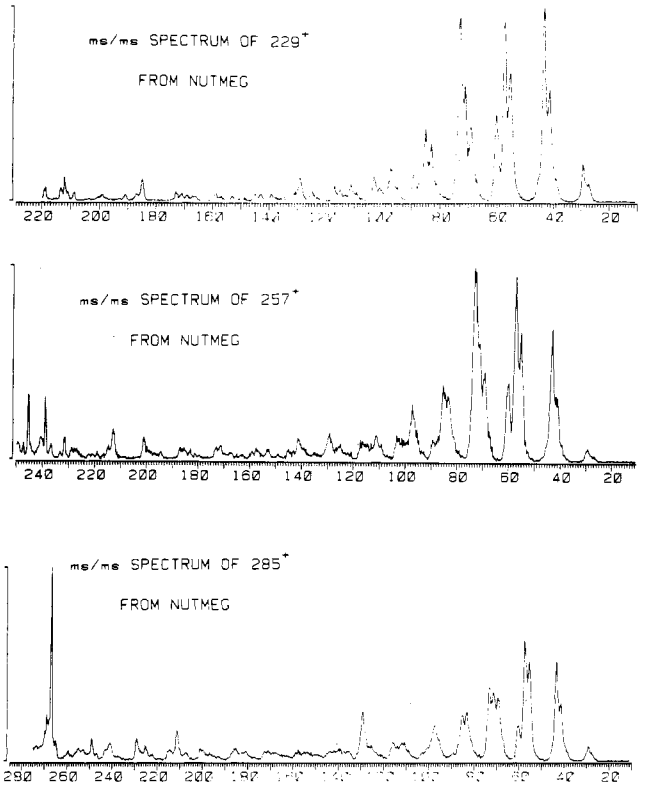


Figure 11. MS–MS spectra of ions corresponding to protonated forms of some of the fatty acids (myristic, palmitic, and stearic) present in nutmeg.

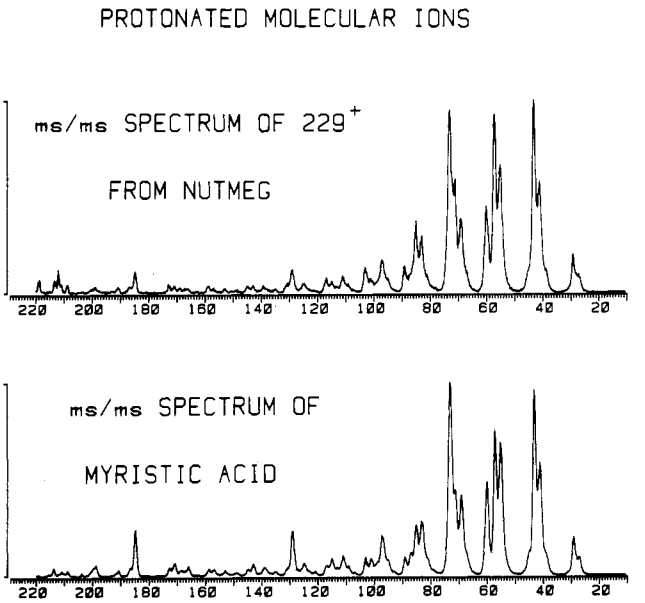


Figure 12. Comparison of MS–MS spectra generated from the 229^+ ion of nutmeg and of authentic myristic acid (isobutane used as the CI reagent gas).

observed previously in GC–MS and by conventional isolation techniques.

The final class of compounds encountered in this MS–MS study is again defined by a highly diagnostic set of fragment ions. This is a series of peaks in the low-mass range with highly characteristic relative intensities (see Figure 11). This group of compounds appears to be made up of fatty acids and their various glycerides. The lowest mass peak of the series is observed at m/z 229 in the CI mass spectrum. This was confirmed by exact mass measurement to have a molecular weight of 228.208 and a

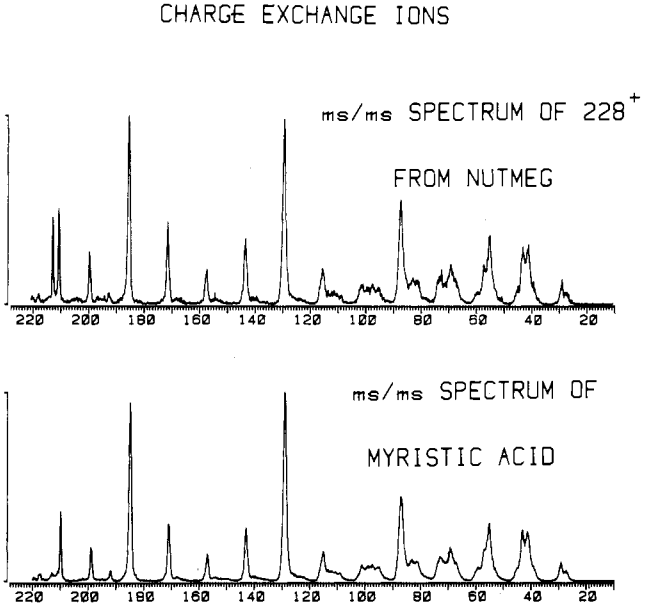


Figure 13. Comparison of MS–MS spectra generated from the 228^+ ion of nutmeg and of authentic myristic acid (nitrogen used as the CI reagent gas).

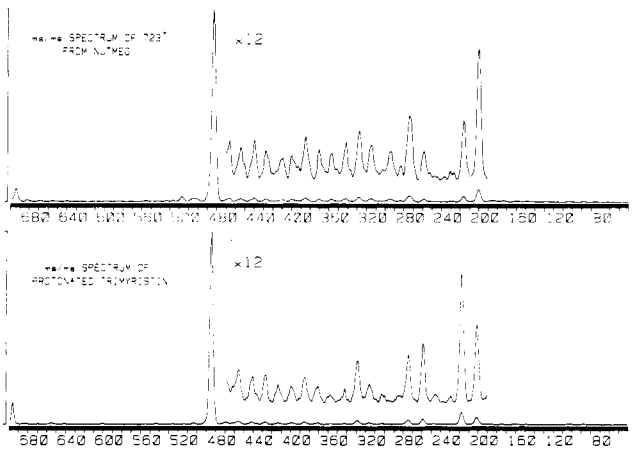


Figure 14. Comparison of MS–MS spectra generated from the 723^+ ion of nutmeg and of authentic trimyristin (isobutane used as the CI reagent gas).

molecular formula of $C_{14}H_{28}O_2$, strongly suggesting that it is myristic acid. Also seen in the nutmeg mass spectrum (Figures 1 and 2) are ions 257^+ corresponding to palmitic acid ($C_{16}H_{32}O_2$) and 285^+ corresponding to stearic acid ($C_{18}H_{36}O_2$). A comparison of the MS–MS spectrum of 229^+ derived from nutmeg with that obtained from authentic myristic acid is shown in Figure 12, and a comparison of radical ions generated by charge exchange from the same two sources is shown in Figure 13. Similarly good agreement with authentics is also seen for palmitic, stearic, and oleic acids. In the higher mass range, a peak at 513^+ is observed and found by MS–MS to correspond to the protonated diglyceride of myristic acid. An ion which gives a similar MS–MS spectrum to that of 513^+ is observed at mass 511^+; this apparently corresponds to the diglyceride with an additional double bond in one of the long-chain fatty acids. The ion occurring at mass 723^+ is due to the protonated intact trimyrisitin (the triglyceride of myristic acid). An MS–MS spectrum of authentic trimyrisitin was taken, and it shows fair agreement with that of the 723^+ peak from nutmeg (Figure 14). This MS–MS spectrum is distinguished by an abundant McLafferty rearrangement ion at 495^+ due to loss of myristic acid from the intact

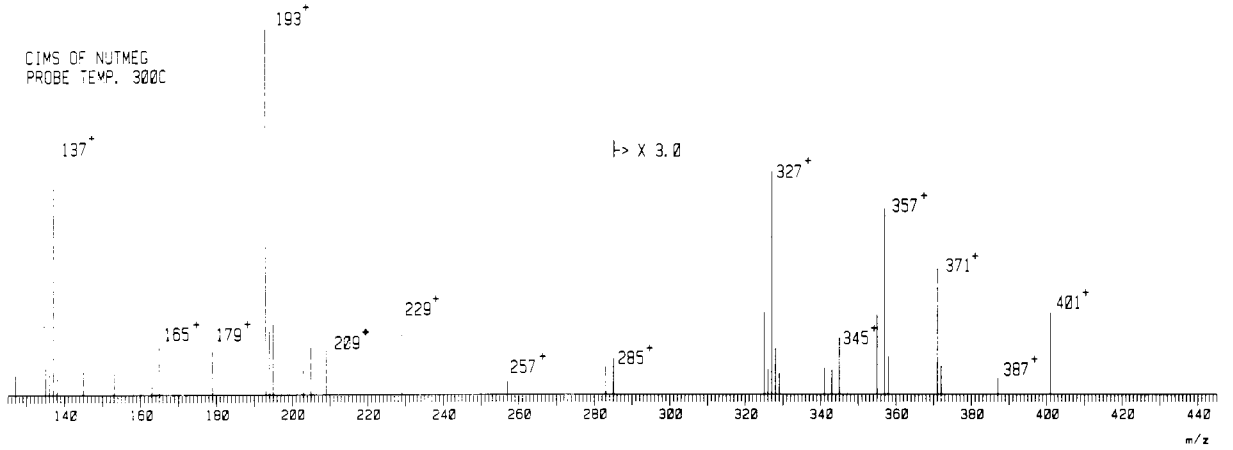


Figure 15. Isobutane chemical ionization mass spectrum of nutmeg taken on a triple quadrupole instrument.

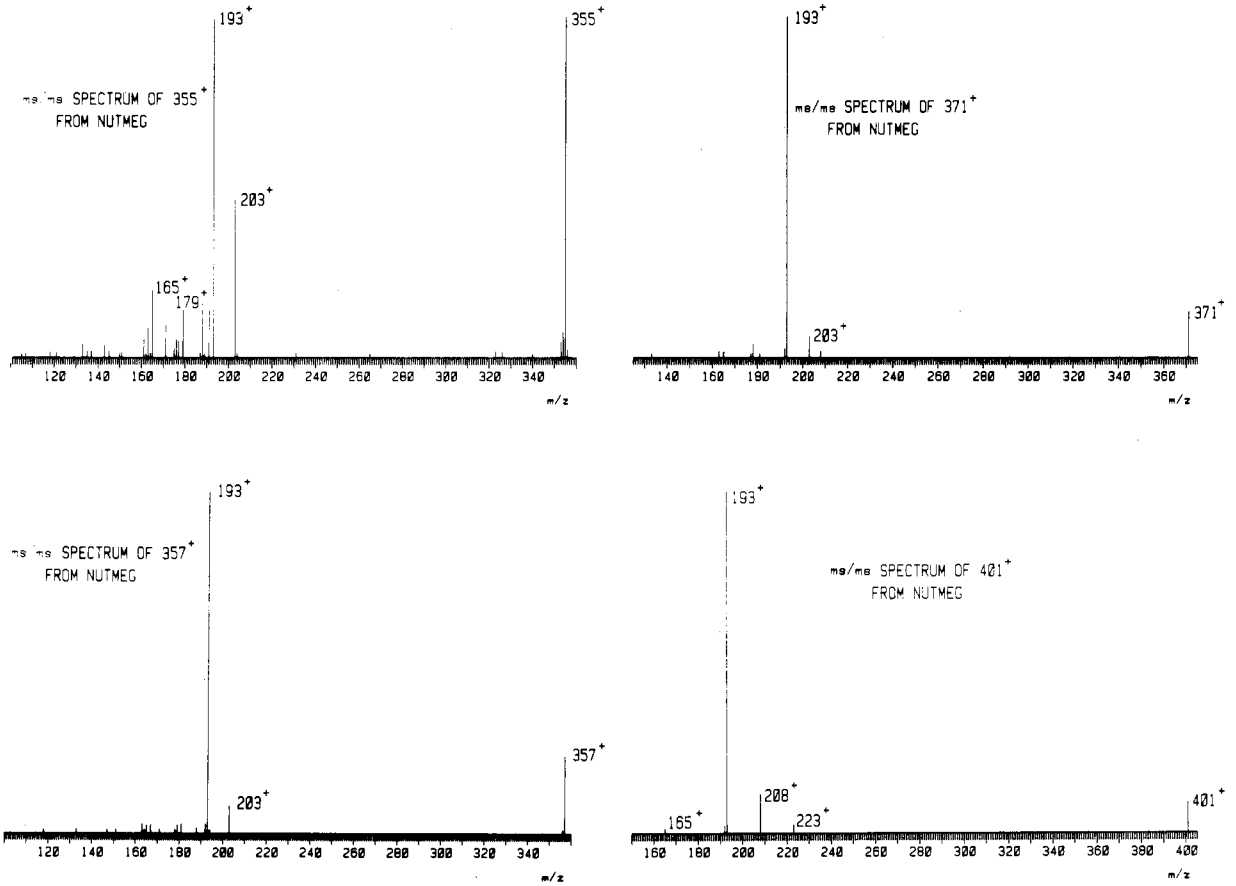
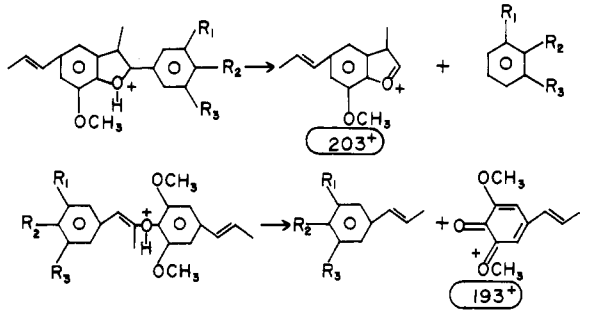


Figure 16. Low-energy MS–MS spectra of 355^+, 357^+, 371^+, and 401^+ taken by using a triple quadrupole instrument.

species. It also shows an ion at 707^+ due to loss of methane and an ion at 211^+ which corresponds to the acylium ion generated from myristic acid. While these esters have long been known to be present in nutmeg by means of the extraction or by GC–MS after derivatization, this appears to be the first time they have been determined directly without prior sample workup. This result demonstrates that MS–MS analysis is certainly not limited to low molecular weight compounds.

The diphenylpropanoids of both types (cyclic and acyclic) were studied further with the use of a triple quadrupole MS–MS instrument. These data served two purposes: first, comparisons between low-energy and high-energy MS–MS data can be made and, second, some of the spectral overlap problems referred to above can be solved by using alternate scanning modes which are not available on our high-energy MIKES instrument. An isobutane CI mass spectrum taken on the triple quadrupole instrument is shown (Figure 15) for comparison with that from the MIKES (Figures 1 and 2). The same ions occur in both sets of spectra, but quantitative comparisons would require that the probe temperature and heating rate be fixed. A direct comparison of low-energy MS–MS data and high-energy MS–MS data on the same components of the sample can be made by comparing the spectra of Figure 16 with the appropriate spectra of Figures 9 and 10. While the spectra shown are in fact very similar, this is so only because of careful selection of the triple quadrupole collision energy and collision cell pressure. The low-energy MS–MS spectra are in fact a strong function of both of these variables. High-energy MS–MS spectra are much less sensitive to changes in the collision param-

Scheme II



eters (McLuckey et al., 1981); hence, these parameters do not have to be as strictly controlled in a high-energy experiment.

Close examination of the high-energy MS–MS data revealed that each of the two groups of diphenylpropanoids gave unique fragment ions; for compounds of type VII, 203^+, and for type VIII, 193^+. Rationalization of these fragmentations is shown in Scheme II. For confirmation that these ions actually are diagnostic of a particular structural type, parent spectra were obtained on the triple quadrupole instrument. The results of these experiments are shown in Figure 17. These data clearly show two things: first, that these two ions are indeed specific to these two different types of compounds and, second, that some of the CIMS–MS daughter spectra are due to fragmentations of more than one component. For example, in the cases of 387^+ and 401^+, spectral overlap is not possible as none of the type VII ions are of appropriate molecular weight to overlap the type VIII ions which occur at these masses; correspondingly we find that these masses give only 193^+ fragment ions. On the other hand, 325^+, 327^+, and 341^+, which have masses lower than the limit for type VIII ions, must be of type VII, and indeed they give exclusively the 203^+ fragment. The parent ions 355^+, 357^+, and 371^+, which can be due to either type of diphenylpropanoid, occur in parent spectra when either 193^+ or 203^+ is specified as the fragment ion. This shows that they consist of some type VII and some type VIII species. This result is particularly important because the spectral overlap which occurs is due to isomeric species, and thus it is not possible to separate the contributing structures by high-resolution mass spectrometry. Moreover, parent spectra represent a particularly rapid and direct method of seeking compounds with specific structural characteristics. A significant consequence is the confirmation of a type VII compound at mass 355^+; the compound responsible is the dehydrodiphenylpropanoid derivative of myristicin (a major component of nutmeg) and has not to our knowledge been discovered before although it has been specifically sought (Harvey, 1975). Using MS–MS, we also sought a type VIII myristicin derivative at mass 385^+ but found none.

CONCLUSION

The results show that MS–MS is an analytical method which allows extensive (but not necessarily a priori) characterization of a highly complicated mixture of natural products. This can be accomplished by using either low or high energy sector or quadrupole instrumentation and is facilitated by temperature profiling which simplifies the complexity of the mixture being studied at any one time. Daughter spectra taken by using both protonation and charge exchange, along with exact mass measurements and supplementary information on the types of structures present, were sufficient to identify the diverse species

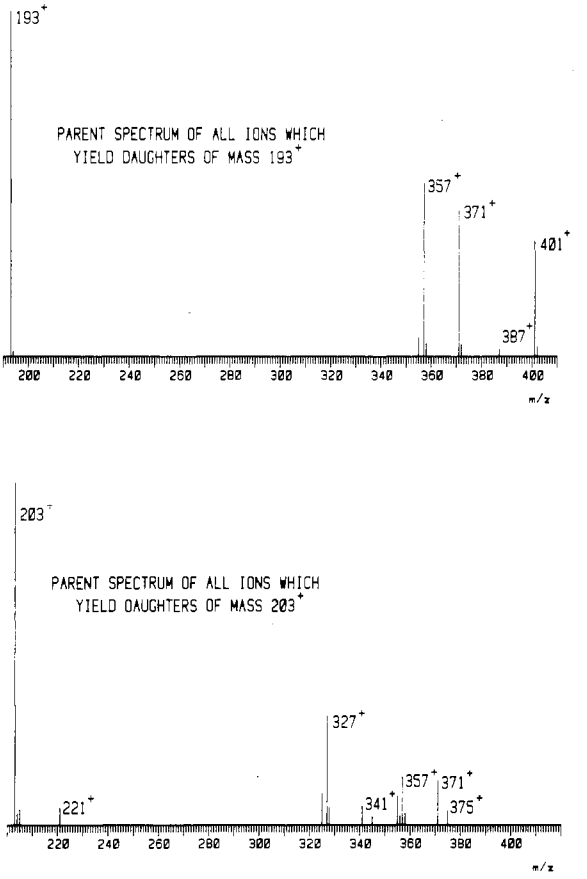


Figure 17. Parent spectra taken on a triple quadrupole instrument. Masses correspond to the mass of the parents which generate the 193^+ and 203^+ daughter ions.

present in the nutmeg sample. Access to a variety of scan types is a very valuable feature. Parent spectra are shown to be particularly useful in increasing analysis speed and the quality of the information which can be derived from the basic MS–MS experiment.

The ability to identify organic compounds in complex matrices without any sample preparation remains the greatest asset of MS–MS. In this study, many compounds which could previously be identified only after extraction and/or derivatization were identified without sample workup. The discovery of a new component of nutmeg illustrates the power of MS–MS as an analytical tool in mixture analysis. Shortcomings of MS–MS include poor isomer specificity and the possibility of changes in the sample in the course of analysis. Processes such as thermal reactions and rearrangements must be carefully considered when interpreting MS–MS data taken at elevated probe temperatures. The success of this study, especially the agreement with previous data, suggests further exploration of the considerable potential of MS–MS for food analysis.

ACKNOWLEDGMENT

We acknowledge valuable discussions with Dr. John Slayback and thank Dr. Karl Wood for assistance with some experiments.

LITERATURE CITED

Beynon, J. H.; Cooks, R. G.; Amy, J. W.; Baitinger, W. E.; Ridley, R. Y. *Anal. Chem.* **1973**, *45*, 1023A.

Forrest, J. E.; Heacock, R. A.; Forrest, T. P. *Experientia* **1973**, *29*, 139.

Forrest, J. E.; Heacock, R. A.; Forrest, T. P. *J. Chem. Soc., Perkin Trans. 1* **1974**, 205–209.

Gallegos, E. J. *Anal. Chem.* **1976**, *48*, 1348.

Haddon, William F. *Org. Mass Spectrom.* **1980**, *15*, 539.

Harvey, D. J. *J. Chromatogr.* **1975**, *110*, 91-102.
Helmkamp, G. K.; Johnson, H. W., Jr. "Selected Experiments in Organic Chemistry 2nd Edition"; W. H. Freeman: San Francisco, CA, 1968.
Hunt, D. F.; Shabanowitz, J., Proceedings of the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN, May 24-29, 1981.
Hunt, D. F.; Shabanowitz, J.; Giordani, A. B. *Anal. Chem.* **1980**, *52*, 386-390.
Kolor, M. G. "Mass Spectrometry Part A"; Merrit, C., Jr.; McEwen, C. N., Eds.; Marcel Dekker: New York, 1979.
Kondrat, R. W.; Cooks, R. G. *Anal. Chem.* **1978**, *50*, 81A-92A.
Lacey, M. J.; MacDonald, G. G. *Anal. Chem.* **1979**, *51*, 691.
Lindholm, E. "Ion Molecule Reactions in the Gas Phase"; American Chemical Society: Washington, DC, 1966; Adv. Chem. Ser. No. 58, p 1.
McLuckey, S. A.; Glish, G. L.; Cooks, R. G. *Int. J. Ion Mass Spectrom. Ion Phys.* **1981**, *34*, 219-230.
Reimer, C. L.; Will, W. *Ber. Dtsch. Chem. Ges.* **1885**, *18*, 2011.
Richter, W. J.; Schwarz, H. *Angew Chem., Int. Ed. Engl.* **1978**, *17*, 424-439.
Sammy, G. M.; Nawar, W. W. *Chem. Ind. (London)* **1968**, 1279-1280.
Schenk, H. P.; Lamparsky, D. *J. Chromatogr.* **1981**, *204*, 391-395.
Schoen, A. E.; Zakett, D.; Korenzeniowski, R., Proceedings of the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN, May 24-29, 1981.
Self, R. *Biomed. Mass Spectrom.* **1979**, *6*, 361-373.
Shushan, B.; Boyd, R. K. *Org. Mass Spectrom.* **1980**, *15*, 445-453.
Slayback, J.; Story, M. *Ind. Res./Dev.* **1981**, *23*, 129-134.
Unger, S. E.; Cooks, R. G. *Anal. Lett.* **1979**, *12* (B11), 1157-1167.
Wilson, B. W.; Pelroy, R. A.; Lee, M.; Later, D., Proceedings of the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, MN, May 24-29, 1981.
Yost, R. A.; Enke, C. G. *Anal. Chem.* **1979**, *51*, 1251A-1264A.
Zakett, D.; Ciupek, J. D.; Cooks, R. G. *Anal. Chem.* **1981**, *53*, 723-726.
Zakett, D.; Schoen, A. E.; Kondrat, R. W.; Cooks, R. G. *J. Am. Chem. Soc.* **1979a**, *101*, 6782.
Zakett, D.; Shaddock, V. M.; Cooks, R. G. *Anal. Chem.* **1979b**, *51*, 1849-1852.

Received for review September 21, 1981. Accepted December 23, 1981. This work was supported by National Science Foundation Grant CHE 80-11425.

Nomilin, a New Bitter Component in Grapefruit Juice

Russell L. Rouseff

A new bitter component in grapefruit juice has been separated and conclusively identified as nomilin from chromatographic, chemical, and mass spectral evidence. Nomilin was shown to be a natural component of grapefruit juice vesicles. In commercial grapefruit juice, produced during the 1978-1979 season, nomilin concentrations ranged from 1.6 to less than 0.1 ppm. Nomilin concentrations were found to be greatest in early season juices and decreased rapidly with increasing fruit maturity. Nomilin concentrations fell more rapidly than that of limonin during the 1978-1979 season. In November the nomilin/limonin ratio was 0.125 and by May it was 0.04. Nomilin concentrations increase with increasing extractor pressure up to a point but unlike limonin increase very little under very heavy squeeze conditions. For fruit harvested on the same day, juice nomilin and limonin contents were lower in the Duncan cultivar than the Marsh seedless cultivar.

Nomilin is a limonoid first isolated from the seeds of oranges and lemons (Emerson, 1948). It is bitter (Emerson, 1948, 1951, Dreyer, 1965) and is reported to be about twice as bitter as limonin (Hashinaga et al., 1977). Emerson (1949) reported that limonin was the sole bitter principle isolated from the juice of navel oranges. In some unpublished data, Bennett (1972) reported finding minor amounts of deacetylnomilin, nomilin, obacunone, deacetylnomilinic acid, and nomilinic acid in the peel of navel oranges. Limonoids such as limonin and nomilin are actively synthesized in orange and lemon leaves, particularly in young, immature leaves according to Hasegawa and Hoagland (1977). Hashinaga et al. (1977) found both limonin and nomilin in the seeds, segment membrane, peel, and flesh in ponkan mandarins. Hasegawa et al. (1980) found the ratio of nomilin to limonin was considerably lower in mature compared to immature leaves and fruit tissue of Eureka lemons.

Florida Department of Citrus, Scientific Research Department, Lake Alfred, Florida 33850.

While limonin was thought to be the sole bitter component in orange juice, the bitterness of grapefruit juice was ascribed to flavanone neohesperidosides, primarily naringin. Later Maier and Dreyer (1965) found the bitter limonin present in grapefruit juice as well.

In a recently developed high-pressure liquid chromatographic separation for citrus limonoids (Rouseff and Fisher, 1980), a peak with the same retention time as nomilin was observed in grapefruit juice samples. Therefore, the primary purpose of this study was to determine if yet another bitter limonoid (nomilin) is present in grapefruit juice. Since grapefruit seeds are known to contain nomilin in concentrations greater than 800 ppm (Hasegawa et al., 1980; Rouseff and Nagy, 1982), experiments were conducted to determine if nomilin is a natural component of grapefruit juice vesicels or is found in juice as a result of seeds ruptured during juice manufacture. Another goal was to determine how juice nomilin concentrations change with increasing fruit maturity and what concentration ranges are likely to be found in commercial juices from a given processing season. A final goal was to determine how juice nomilin concentrations are affected

0021-8561/82/1430-0504$01.25/0