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THERMOSPRAY COLLISIONALLY INDUCED DISSOCIATION WITH SINGLE AND MULTIPLE MASS ANALYZERS

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SUMMARY

A thermospray discharge ionization source has been used to obtain a collisionally induced dissociation (CID) spectrum of nabilone (MW 372) using a single stage quadrupole mass spectrometer. The resulting CID-mass spectrometry (MS) data show only a few structural features. Since most of the important fragments are above mass 200, it is difficult to assign structural identities to the primary daughter fragments or the nabilone molecule.

Further information was obtained by using the thermospray discharge ionization source with a triple stage quadrupole mass spectrometer. Selected primary daughter ions were subjected to a second stage of CID fragmentation. Many of the resulting granddaughter ions are now in the mass range of 50–150 daltons and confident structure assignments can be made. The CID-MS–CID-MS data on nabilone showed the following features: a C_9 aliphatic chain with a branch giving a C_6 chain; losses of masses 58, 86, and 100 suggesting a ketonic structure that can break with up to 6 aliphatic carbons; losses of masses 60, 61, and 102 suggesting an ether or alcoholic oxygen; presence of one aromatic ring with one or two phenolic oxygens; low mass fragments of masses 55, 69, and 83 indicative of rings or unsaturation.

It is audacious to suggest that the information given here is sufficient to write the full structure of nabilone. Nevertheless, the granddaughter fragment information permits a reasonable reconstruction of possible structures that could not be made with the first-order collisional fragmentation.

INTRODUCTION

Although initially introduced as a liquid chromatography–mas spectrometry (LC–MS) interface method[1], thermospray has rapidly become recognized as a soft ionization technique in its own right. In many applications, thermospray has proven to be the preferred ionization method over established techniques such as fast atom bombardment, field desorption, or direct exposure probes. However, in common with most soft ionization processes, thermospray frequently gives only molecular weight

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information, usually as the molecular adduct ion, MH^+ or MNH_4^+. Such data are of tremendous value to the chemist, but lack specificity for target analysis and give essentially no information regarding the structure or functionality of unknowns.

Ionization in thermospray systems is achieved by one of three methods: buffer ionization, filament ionization, or discharge ionization. All three methods provide soft ionization and the molecular adduct ion is the anticipated ion product except for very thermally labile compounds. In favorable chemical systems, manipulation of the vaporizer and jet chamber temperatures can provide controlled fragmentation and a few examples have also been noted that give fragmentation with filament or discharge ionization but not with buffer ionization[2]. In general, however, temperature control and selection of ionization mode are not universal methods of obtaining fragmentation in thermospray analyses and can be used only for selected chemical systems.

The most popular process used to obtain fragmentation of molecular ions from soft ionization processes is collisionally induced dissociation (CID)[3]. Most commonly, a target mass is singled out in a primary mass analyzer, accelerated into a collision chamber at modestly elevated pressures, and the fragment ions formed by the collisions are mass analyzed in a second analyzer. Although commonly referred to as MS–MS, the process is in actuality MS–CID-MS. For mass spectrometer systems with multiple tandem mass analysis capability, the process can be further extended to give secondary daughter spectra via the process MS–CID-MS–CID-MS (usually designated MS–MS–MS).

Recently, it was observed that when operating in the discharge ionization mode, collisionally induced fragmentation can be produced by increasing the voltage on the repeller electrode of the Finnigan thermospray source[4]. The exact mechanism producing this fragmentation is uncertain since at the pressures involved (*ca.* 3–10 Torr), the ion mean free path is so small as to preclude significant accumulation of kinetic energy to provide a bond break. Nevertheless, it is observed that when the repeller voltage is increased to the range 70–100 V, fragmentation of the molecular adduct ion is induced. This process enables one to generate with a single mass analyzer a CID spectrum that is qualitatively equivalent to the spectrum obtained from multiple mass analyzers[2].

The actual voltage applied to the repeller for a desired degree of fragmentation may vary according to the surface condition of the repeller. Nevertheless, it is extremely easy to control the degree of fragmentation by monitoring the spectrum as the repeller voltage is increased. For most compounds, any desired degree of fragmentation can be obtained from mild dissociation of the molecular adduct ion to complete elimination of that ion so that only daughter ions are observed. This phenomenon thus greatly extends the value of a soft ionization thermospray system when used with a single mass analyzer and provides CID-MS data that is comparable to the MS–CID-MS data obtained from triple quadrupole systems or their equivalent.

It is similarly obvious that discharge ionization CID from the thermospray source can be used to generate an additional stage of fragmentation from any multiple mass analyzer. This can be particularly valuable when the information obtained from the MS–MS experiment gave insufficient data to determine an unknown structure. It is not uncommon that fragments in the 200–300 dalton range provide minimal information on the structure of a parent at mass 400. However, granddaughter fragments produced in a second collision from selected daughter ions will now reveal smaller ion fragments, usually in the mass range 40–150 daltons. Many of these ions can be immediately characterized and others may be assigned tentative structure from background information of the sample.

Application of the thermospray CID to structure elucidation is illustrated here for nabilone (MW 372). A single CID analysis shows considerable fragmentation, but since ions are formed from both sides of the molecule, structural interpretation is not obvious. When a second stage of collisional dissociation is performed on the primary daughter ions, the smaller mass ions thus formed are more easily related to the specific molecular characteristics of the primary daughter ions. The increased knowledge on the primary daughter now provides structural assignments that give better insight to the structure of the original molecular ion.

EXPERIMENTAL

The mass spectrometers used in this study consisted of a Finnigan Model 4600 single quadrupole and a triple stage TSQ™ Model 4600 equipped with a thermospray discharge ionization source. Liquid pumping was provided by a DuPont LC pump, Model 8800 or an Altech pump, Model 100A. Since the nabilone sample was known to be of high purity, it was injected directly through a loop at a flow-rate of 1 ml/min without use of an LC column. The LC-grade solvent was water–methanol (3:1, v/v) used without buffer. The nabilone sample was provided by Dr. H. R. Sullivan of Eli Lilly Research Laboratories.

Fig. 1 presents a schematic diagram of the Finnigan thermospray discharge ionization source. The discharge electrode is located 2 mm downstream from the tip of the vaporizer and protrudes into the jet chamber approximately 0.5 mm. Stable operation is achieved in the voltage range of 800–1000 V. To obtain spectra without CID fragmentation, the repeller was operated at a voltage of 60 V. To obtain CID fragmentation, the repeller was operated at 80–90 V. The degree of fragmentation achieved is easily controlled by monitoring the spectrum on the oscilloscope and varying the repeller voltage with successive loop injections of nabilone.

Preliminary MS and CID-MS experiments were performed using the Model 5600 single quadrupole system. The CID-MS–CID-MS experiments were performed on the TSQ Model 4600 by selecting appropriate primary CID daughter ions in Q1 and mass analyzing the secondary CID daughter ions in Q3.

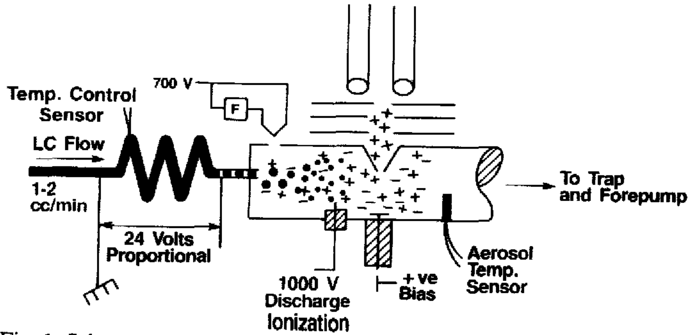

Fig. 1. Schematic diagram of thermospray LC–MS source.

RESULTS AND DISCUSSION

The mass spectrum of nabilone obtained at a repeller setting of 60 V is shown to consist of the molecular adduct ion MH$^+$ with essentially no useful fragmentation (Fig. 2). When the repeller voltage is increased to the range 80–90 V, the CID spectrum shown in Fig. 3 is obtained. This spectrum provides a large amount of additional information and for target compound analysis, would give almost complete specificity of identification. However, for an unknown sample with only modest background information, fragment ions in the mass range 200–300 daltons cannot be assigned an unequivocal structure, and interpretation of the spectrum is still very difficult.

Further information is obtained by selection of appropriate daughter ions and subjecting these ions to a second stage of collisional decomposition. For purposes of illustration, the ions selected for the second CID process were masses 315, 263, 249, 247, and 189. Granddaughter fragments are observed in the mass range 40–200 daltons and many can be assigned confident structural identities or reveal structural features not apparent from the first order daughter fragments.

Fig. 4 presents the granddaughter spectrum of the primary daughter ion of mass 315. This ion was formed from the parent ion by loss of mass 58 suggesting a component structure related to acetone. The further fragmentation of mass 315 clearly shows the existence of an aliphatic chain, presumably C_9H_{19} with a possible C_6 aliphatic chain at a branch point. The ion at mass 215, formed by loss of 100 units, may suggest another ketonic group, a cyclic ether oxygen moiety, or a possible concerted loss of C_6H_{13} and CH_3.

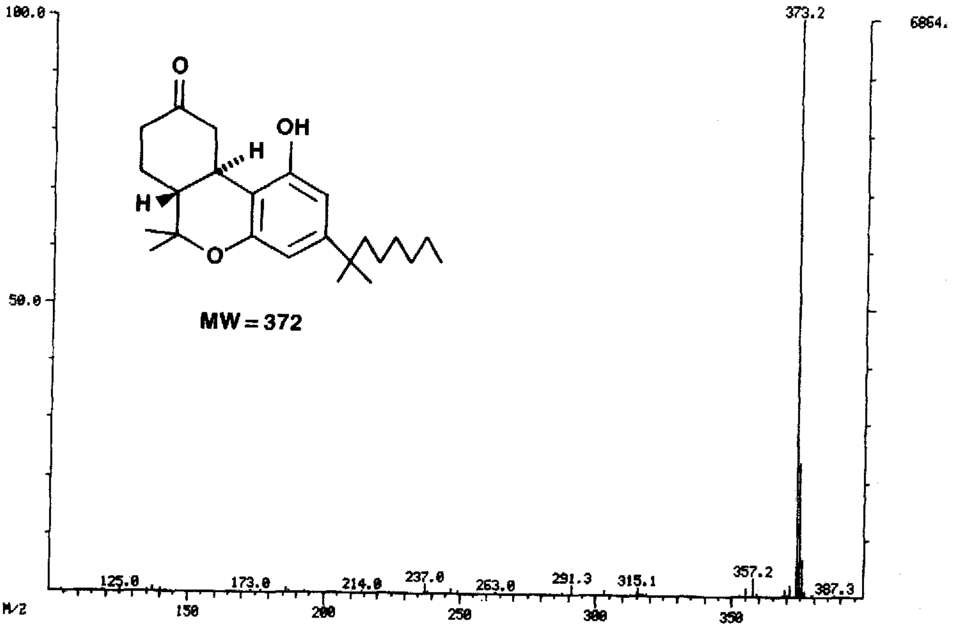

Fig. 2. Thermospray discharge ionization spectrum of nabilone at low repeller voltage (60 V).

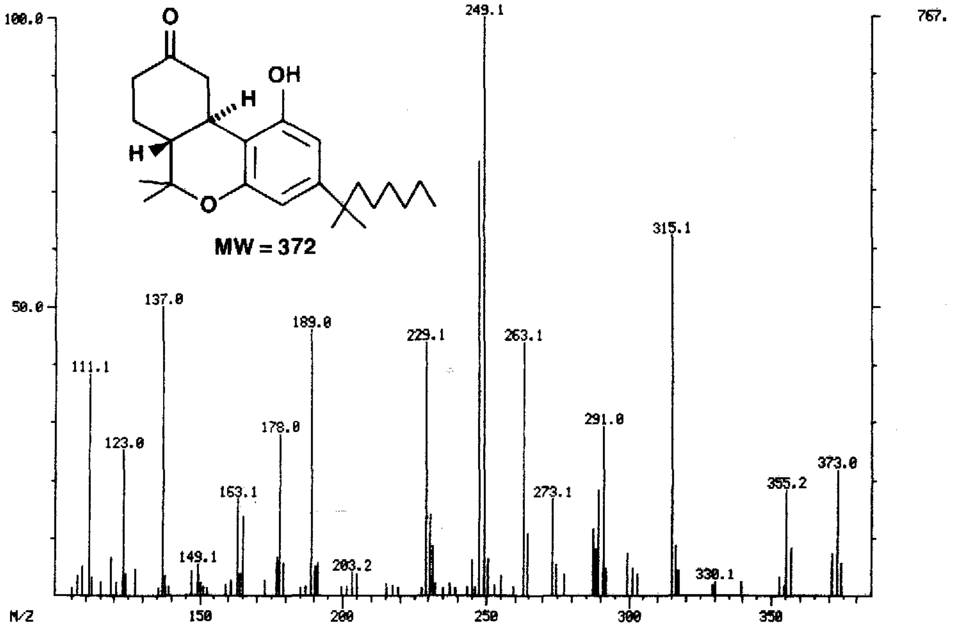

Fig. 3. Thermospray discharge ionization spectrum of nabilone at high repeller voltage (90 V) showing collisionally induced dissociation.

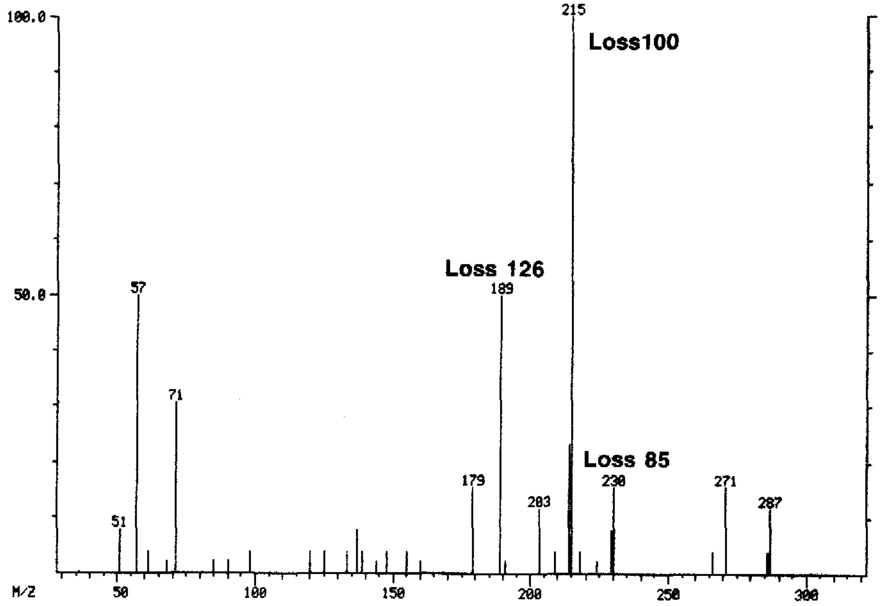

Fig. 4. Collisionally induced granddaughter spectrum from the primary daughter ion at mass 315.

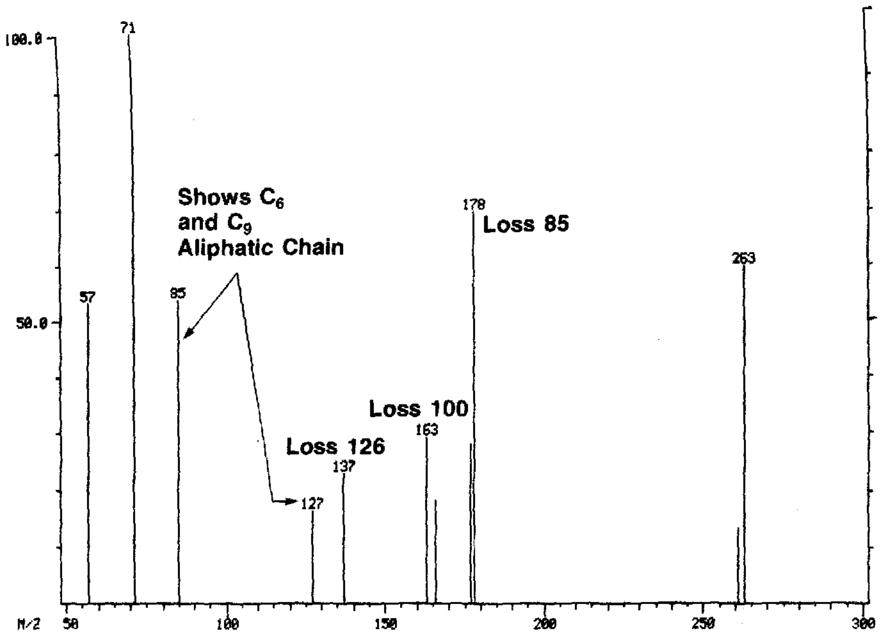

Fig. 5. Collisionally induced granddaughter spectrum from the primary daughter ion at mass 263.

Essentially the same information is obtained from the secondary daughter spectrum of the mass 263 ion (Fig. 5). This ion shows a loss of 100 units, thus further suggesting the presence of at least two ketonic or cyclic ether type oxygen atoms. The nature of the aliphatic chain is clearly shown by the ions at mass 178 (loss C_6H_{13}) and mass 137 (loss C_9H_{18}) and confirmed by the appearance of these moieties at

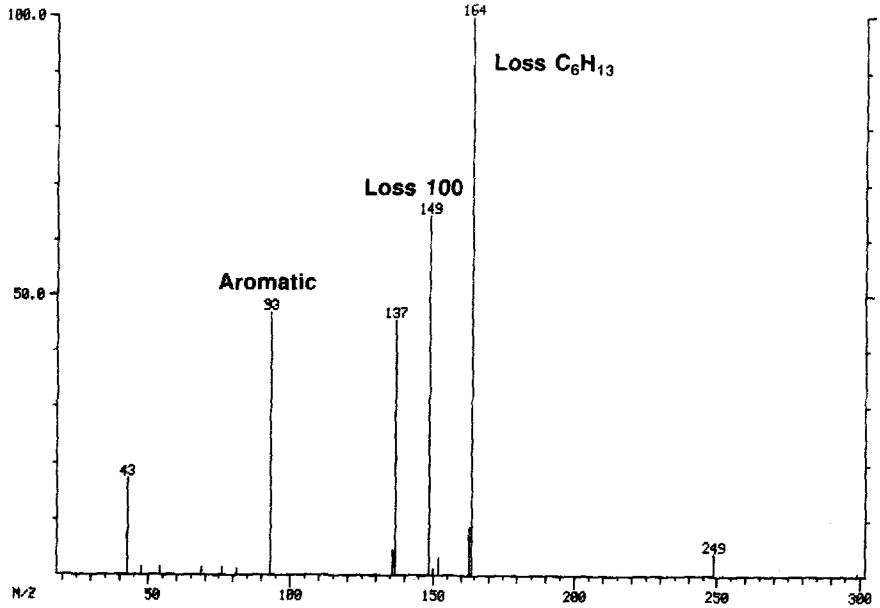

Fig. 6. Collisionally induced granddaughter spectrum from the primary daughter ion at mass 249.

masses 85 and 127. The absence of any C_7 or C_8 ions is in accord with the prior suggestion that the C_9 aliphatic chain has a C_6 chain at a branch point.

The primary daughter ions appearing at masses 249 and 247 provide an interesting comparison. It is often assumed that fragment ions which differ by two mass units might have a similar genesis but the secondary daughter spectra of these two ions show that this is not correct. The spectrum of the mass 249 daughter ion shown in Fig. 6 indicates from the loss of C_6H_{13} that the aliphatic chain was intact in the mass 249 parent. Absence of loss of C_9H_{18} is surprising, but may be explained if the first carbon of the aliphatic chain is incorporated into a tropylium structure. Such a rearrangement might also explain the appearance of the ions at masses 149 (concerted loss of C_6H_{13} and CH_3) and 137 (loss of C_8H_{16}). Aromaticity with a phenolic oxygen is shown by the presence of the mass 93 ion.

On the other hand, the daughter ions formed from the mass 247 ion show extensive fragmentation but virtually no indication of the now well established aliphatic chain (Fig. 7). This confirms the suggestion that the primary ion of mass 247 resulted from loss of C_9H_{18} and is not generically related to the mass 249 ion. Low mass ions at 55, 69, and 83 are indicative of cyclic or unsaturated structure. A ketonic structure is further confirmed by losses of 58 to give mass 189 and 86 to give 161. The ion observed at mass 187 is significant since the loss of 60 units strongly indicates an ether oxygen attached to 3 aliphatic type carbons that can fragment from the mass 247 ion as a rearrangement ion.

The final primary daughter chosen for this study was the ion at mass 189. The daughter spectrum of this moiety (Fig. 8) shows no indication of the C_9 aliphatic chain. Since 184 daltons were lost on formation of the 189 daughter, it appears that this primary daughter is formed in a two step process involving the loss of C_9H_{18}

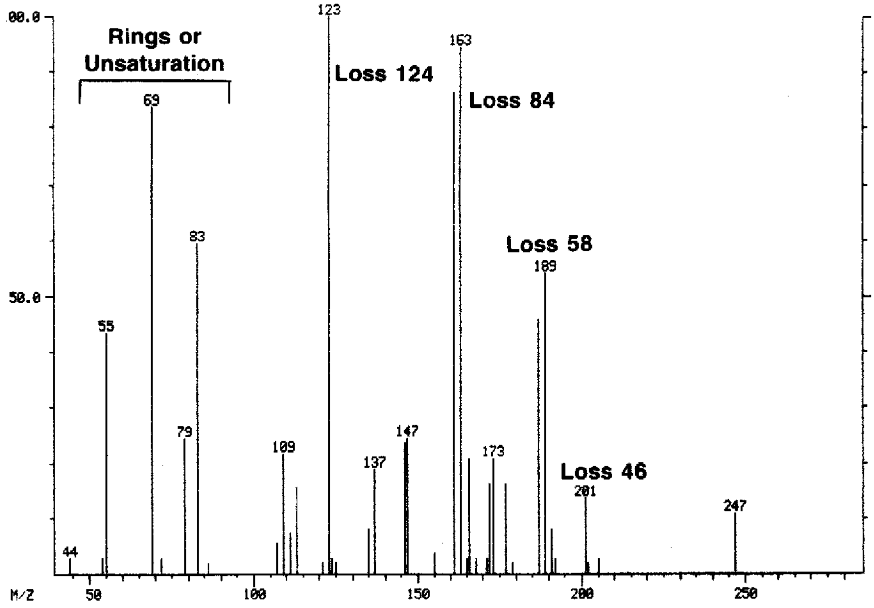

Fig. 7. Collisionally induced granddaughter spectrum from the primary daughter ion at mass 247.

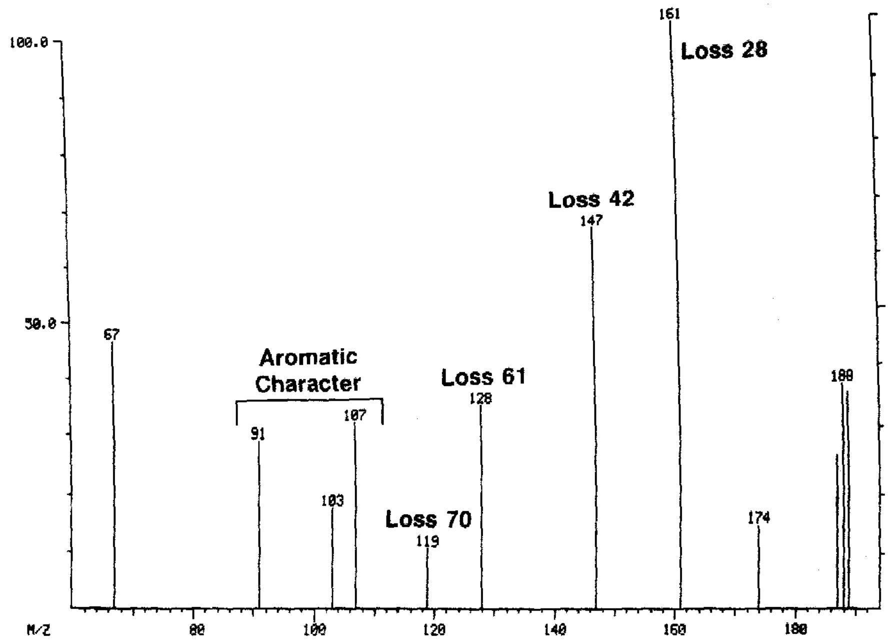

Fig. 8. Collisionally induced granddaughter spectrum from the primary daughter ion at mass 189.

(126 daltons) and C_3H_6O (58 daltons, the ketonic rearrangement loss previously noted). Other features of the granddaughter spectrum show the aromatic character at masses 91, 103, 107, a general loose cyclic structure that can lose C_2H_4, C_3H_6, C_5H_{10} (but not C_4H_8) to give ions at masses 161, 147, and 119, and a loss of mass 61 by extensive rearrangement to give the ion at mass 128.

Although these features were observed in some of the other granddaughter fragment ions, the information is important at this stage because this parent ion of mass 189 is considerably smaller than the others subjected to the second CID process. Thus, reconstruction of the indicated parts of the mass 189 ion, *i.e.*, an aromatic ring with two possible phenolic or ether oxygens and aliphatic or cyclic carbons that can be lost 2, 3, or 5 at a time will lead to a structure close to that expected for the mass 189 primary daughter. The other two features of the original molecule are the C_9 aliphatic chain and the C_3 ketonic group. If these are added to the proposed intermediate structure at this point, the result would be a tentative structure which would contain most of the features of the original nabilone molecule.

REFERENCES

1 C. R. Blakley, J. J. Carmody and M. L. Vestal, *J. Am. Chem. Soc.*, 102 (1980) 5931.
2 W. H. McFadden and S. A. Lammert, *J. Chromatogr.*, 385 (1987) 201.
3 J. V. Johnson and R. A. Yost, *Anal. Chem.*, 57 (1985) 758A.
4 D. Zackett, G. J. Kallos and P. J. Savickas, presented at the *34th Annual Conference on Mass Spectrometry and Allied Topics, June 1986, Cincinnati, OH,* paper MOB3.