R. Martin Smith,[1] *Ph.D.*

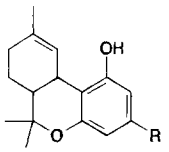
Identification of Butyl Cannabinoids in Marijuana

REFERENCE: Smith RM. Identification of butyl cannabinoids in marijuana. J Forensic Sci 1997;42(4):610–618.

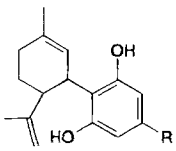
ABSTRACT: Homologs of Δ^9-tetrahydrocannabinol (THC; Ic), cannabidiol (CBD; IIc) and cannabinol (CBN, IIIc) having butyl, rather than pentyl, side chains on the aromatic ring were identified by gas chromatography/mass spectrometry as minor constituents of marijuana samples that also contained propyl cannabinoids. The mass spectra of butyl-THC (Ib), butyl-CBD (IIb), and butyl-CBN (IIIb) are analogous to those of the corresponding propyl and pentyl homologs and are consistent with the proposed structures.

KEYWORDS: forensic science, marijuana, cannabis sativa, cannabinoids, tetrahydrocannabinol, analogs, butyltetrahydrocannabinol, butylcannabinol, butylcannabidiol, gas chromatography/mass spectrometry, biosynthesis

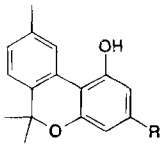
Although the major cannabinoids from marijuana (*Cannabis sativa* L.) all have an *n*-pentyl group attached to a dioxygenated aromatic ring, homologs of Δ^9-*trans*-tetrahydrocannabinol (THC; Ic), cannabidiol (CBD; IIc) and cannabinol (IIIc) having methyl and *n*-propyl (Ia–IIIa) side chains were identified in some marijuana varieties in the late 1960s and early 1970s (1–5). The corresponding butyl homologs (Ib–IIIb), however, have never been reported either in marijuana or as a result of synthesis. The relative absence of these compounds in nature is not entirely unexpected because, like naturally occurring straight chain fatty acids containing odd numbers of carbons, the olivetol ring of cannabinoids is an acetogenin whose normal biosynthesis proceeds via the accretion of two-carbon units (6).



Ia: Δ^9-Tetrahydrocannabivarin (propyl-THC; R = n-C_3H_7)
Ib: Butyl-THC (R = C_4H_9)
Ic: Δ^9-Tetrahydrocannabinol (THC; R = n-C_5H_{11})



IIa: Cannabidivarin (propyl-CBD; R = n-C_3H_7)
IIb: Butyl-CBD (R = C_4H_9)
IIc: Cannabidiol (CBD; R = n-C_5H_{11})



IIIa: Cannabivarin (propyl-CBN; R = n-C_3H_7)
IIIb: Butyl-CBN (R = C_4H_9)
IIIc: Cannabinol (CBN; R = n-C_5H_{11})

In contrast to the methyl homologs, which apparently occur only in trace amounts in some samples (1), our laboratory routinely identifies *n*-propyl cannabinoids in many marijuana samples, with concentrations varying from 0 to about 20% those of the corresponding *n*-pentyl compounds. Careful analysis of the cannabinoids in these samples by gas chromatography/mass spectrometry (GC/MS) often indicated small quantities of Ib or IIb at retention times midway between those of the propyl and pentyl homologs. Although the presence of propyl cannabinoids appears to be necessary for finding butyl homologs in marijuana, a survey of numerous samples showed no relationship between the relative concentrations of propyl and butyl cannabinoids. However, in no case was the concentration of butyl cannabinoids greater than about 1% that of the pentyl homolog. Thin-layer chromatography (TLC) of two exhibits led to spectra of Ib, IIb, and IIIb that were nearly free of contamination.

Experimental

All exhibits were received as routine illicit samples and physically were unremarkable. After preliminary screening tests, a sample from each exhibit (0.5–1.2 g) was extracted with either hexane, methanol, or a combination thereof, and injected without further purification into a Hewlett-Packard 5890 Gas Chromatograph/5970 Mass Spectrometer equipped with a 12-m HP-1 capillary column. The column temperature for Exhibit 1 was programed to run from 200 to 280°C at a rate of 20°C/min, then hold at 280°C for 3 min. The temperature program for Exhibit 2 was from 200 to 230°C at 10°C/min, from 230 to 250°C at 5°C/min, from 250 to 280°C at 20°C/min, then hold at 280°C for 2 min. Mass spectral data were collected and processed with a Hewlett-Packard 59940 Unix ChemStation (Exhibit 1) and HP G1034C software for the MS ChemStation (DOS Series; Exhibit 2).

Exhibit 1 was from phenotype 3 plants (7) and contained predominantly CBD with virtually no THC. Our normal analysis scheme involved increasing the relative concentration of THC in this sample by subjecting it to preparative TLC on silica gel (250-μ plates) using benzene as the developing solvent. Standards of CBD and THC were spotted for reference along one edge of the plate. After development and evaporation of the solvent, the plate was covered with a clean glass plate so as to expose only the standards and a narrow vertical band of the sample. The exposed area was sprayed with aqueous Fast Blue BB to locate the standards in relation to the components of the sample. A horizontal band of the unsprayed silica gel at the approximate R_f of THC was scraped from the plate, extracted with 1–2 mL of chloroform, filtered and concentrated under air. This solution was used to generate the data reported here.

[1]Quality control chemist, Wisconsin Department of Justice, Madison Crime Lab, Madison, WI.

Received 3 July 1996; and in revised form 30 Sept. 1996; accepted 21 Oct. 1996.

Exhibit 2 was from phenotype 1 plants (7), showing a preponderance of THC over other cannabinoids. Preparative TLC of this sample in the manner described above led to two colored bands—a red band corresponding to THC at $R_f \sim 0.75$ and an orange band at $R_f \sim 0.55$ containing several cannabinoids in moderate abundance. Neither colored band contained significant amounts of Ib. However, scraping and extracting the area on the plate between the two colored bands gave primarily Ia–Ic and IIIa–IIIc.

Results and Discussion

The total ion chromatogram for Exhibit 1 is shown in Fig. 1. Even after isolation by preparative TLC, the presence of IIa, IIc, and IIIc dominate the chromatogram. Almost as interesting as the presence of IIb in this exhibit are the relative concentrations of *cis*- and *trans*-THC. Although *cis*-THC is identified routinely in extracts of phenotype 3 samples (8), its concentration rarely approaches that of the *trans*-isomer. Approximately midway between the peaks for IIa and IIc is a small peak (R_t3.333 min) whose mass spectrum is shown in Fig. 2b. This spectrum, when compared with those for IIa and IIc (Figs. 2a and c), is consistent with that expected for structure IIb.

Both the low and high mass ion patterns in Fig. 2b are very similar to those shown by propyl- and pentyl-CBD, although major high mass ions for IIb (m/z 217, 232, 257, 285, and 300) are found at m/z values consistent with methylene insertion or deletion from IIa and IIc respectively. Whereas the ions at m/z 300 and 285 are due to the molecular ion and methyl loss respectively, those at m/z 232 and 217 result from retro Diels-Alder elimination from the limonene ring, with subsequent ring closure and methyl loss to produce the highly aromatic benzopyranyl ion (Fig. 3) (9,10). Formation of the small ion at m/z 257 may be analogous to the 43 Dalton loss from the molecular ion observed in homologs of THC itself (see below). In each of these fragmentations, the aliphatic side chain on the catechol ring remains intact.

Three ions between m/z 160 and 200 are consistent with fragmentation of the benzopyranyl ion (Fig. 4). Those at m/z 165, 179, and 193 in the spectra of IIa, IIb, and IIc respectively can result from loss of CO (11), although simple cleavage of an alkyl radical from the side chain of the catechol ring could produce the common ion at m/z 174. γ-Hydrogen rearrangement involving the side chain leads to the less intense ion at m/z 175, and is expected to be more prominent in the spectra of butyl- and pentyl-CBD than in that of the propyl homolog (12).

The total ion chromatogram for Exhibit 2 (Fig. 5a) shows the presence of Ib at a retention time (R_t 4.10) midway between those of Ia and Ic. Analysis of the sample after removal of major cannabinoids by TLC (Fig. 5b) not only improved the quality of the spectrum of Ib; it also uncovered the presence of IIIb at a retention time (R_t 4.56) midway between those of propyl- and pentyl-CBN.

The spectrum of Ib, when compared with those of Ia and Ic (Fig. 6), is consistent with its proposed structure. The low mass ion pattern is very similar to those of Ia and Ic. Most of the major ions above m/z 150 have counterparts in the spectra of either Ia, Ic or both, with those in Fig. 6b occurring either at the same m/z values as or at m/z values midway between those for the propyl and pentyl homologs. For example, all three compounds show losses of 15 (methyl), 43 (C_3H_7) and 83 Daltons (m/z 285, 257, and 217 for Ib) that do not involve cleavage of the alkyl side chain (Figs. 7 and 8). In addition, the spectrum of Ib has ions at m/z 258 and 243 in common with Ic (m/z 243 in the spectrum of Ia is analogous to m/z 271 in the spectrum of Ic; Fig. 8) (9,10). The ion at m/z 258 results from loss of the alkyl side chain by γ-hydrogen rearrangement, with subsequent methyl loss producing the ion at m/z 243. γ-Hydrogen rearrangements in *n*-propylbenzene

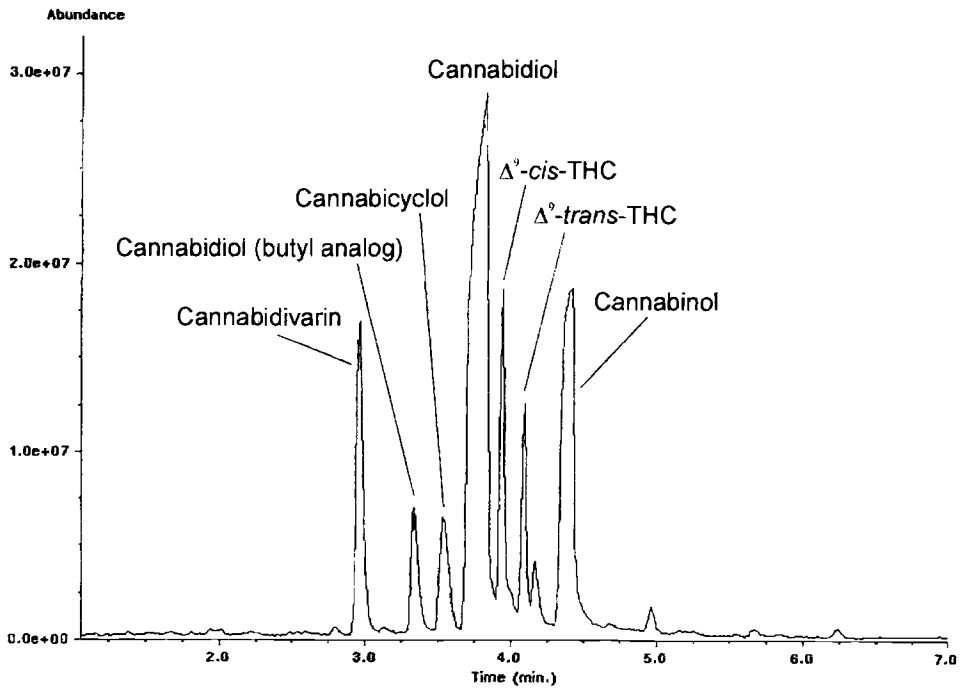


FIG. 1—*Total ion chromatogram for Exhibit 1 after TLC. The most intense peaks are distorted due to sample overloading in order to enhance the mass spectra of minor constituents.*

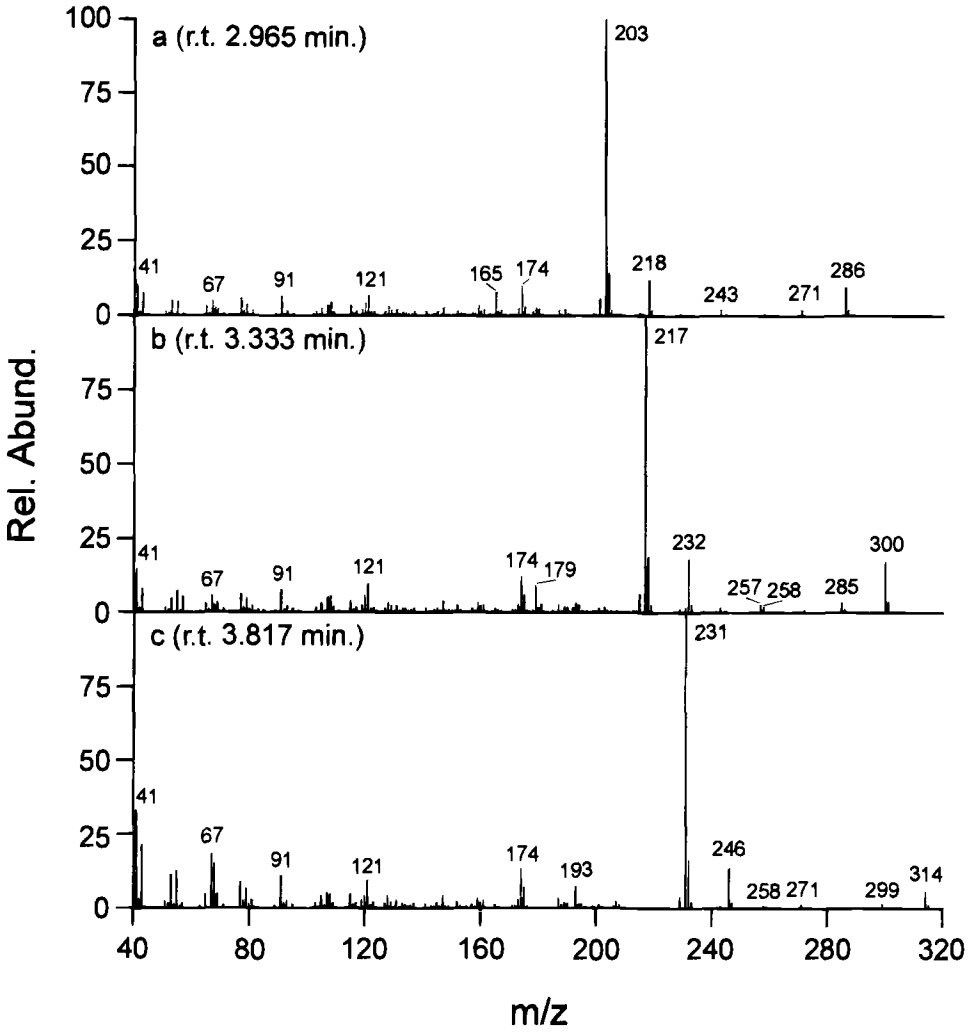


FIG. 2—*Mass spectra for homologs of cannabidiol obtained from Exhibit 1: a) cannabidivarin (IIa); b) butyl-CBD (IIb); c) cannabidiol (IIc). Spectra are not corrected for background ions.*

derivatives are usually overshadowed by other fragmentations (12), so that the intensity of this ion for Ia is very weak.

The spectrum in Fig. 6b also has ions at m/z 174 and 175 in common with those of Ia and Ic, and an ion at m/z 179 midway in mass between the m/z 165 and 193 ions for Ia and Ic respectively. This behavior is analogous to that observed for IIb above (Figs. 2 and 4).

The spectrum of IIIb shares nearly all of the important ions below m/z 260 with those of IIIa and IIIc (Fig. 9). Indeed only the ions at m/z 296 and 281, corresponding to the molecular ion and methyl loss respectively, are found at the expected m/z values midway between those of IIIa and IIIc. A rationalization of this behavior is given in Fig. 10. Note that the ions at m/z 223 probably do not result from methyl loss from m/z 238. A more likely explanation involves loss of CO from m/z 251, in a manner analogous to that shown for the benzopyranyl ion in Fig. 4 (11).

Although these data do not unambiguously specify an *n*-butyl group for these structures, chain branching appears less likely, if only by analogy with the pentyl and propyl homologs. It seems reasonable to assume that the biosyntheses of all three groups of cannabinoids proceed through common early intermediates, with side chain length being determined during a fairly late step (6). In addition, a *tert*-butyl sidechain on Ib or IIb could not undergo γ-hydrogen rearrangement, and an *sec*-butyl group should show enhanced loss of ethyl by α-cleavage from the carbon next to the aromatic ring. It is unclear what effect an isobutyl side chain would have on the mass spectra of these compounds.

Synthesis of Ic can be achieved by combining readily available starting materials. In contrast, the butyl catechol needed for synthesis of Ib cannot be easily prepared. Thus no attempt was made to synthesize these compounds.

Acknowledgments

The author wishes to thank Martin G. Koch, Stephanie Ross, and Robert Block for providing samples and performing preliminary analyses.

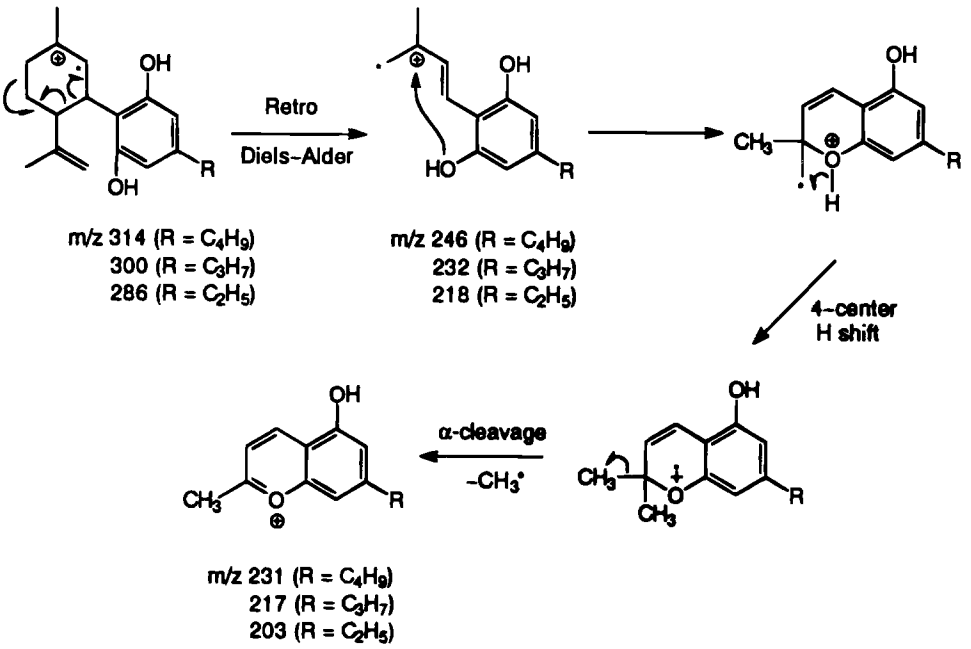


FIG. 3—*Proposed fragmentation pathway for formation of benzopyranyl ions in homologs of cannabidiol.*

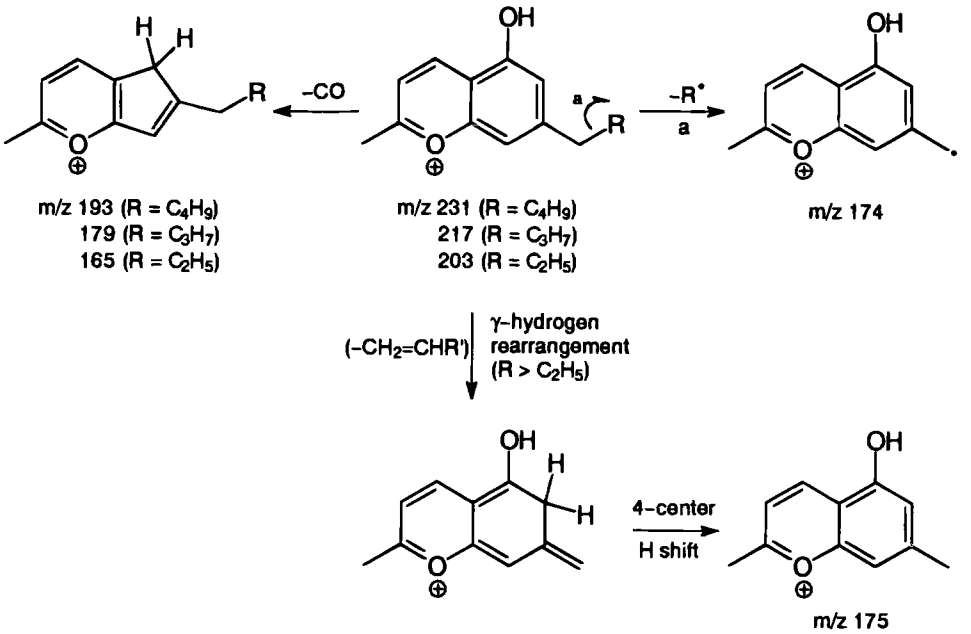


FIG. 4—*Proposed fragmentations of major benzopyranyl ions found in the spectra of cannabinoids.*

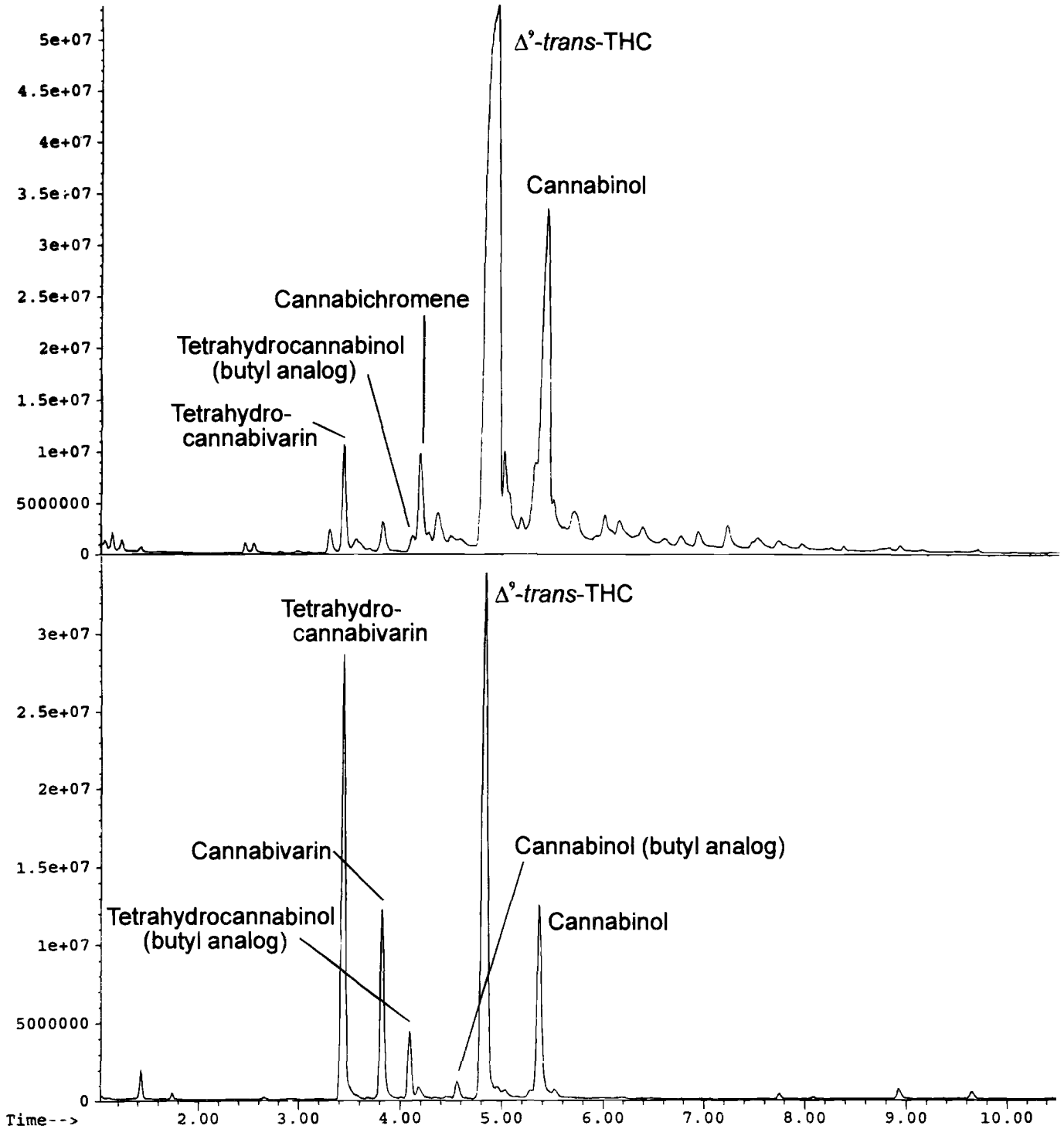


FIG. 5—*a) Total ion chromatogram for Exhibit 2. The THC peak is distorted due to sample overloading in order to enhance the mass spectra of minor constituents. b) Total ion chromatogram for Exhibit 2 after removal of most of the major cannabinoids by TLC.*

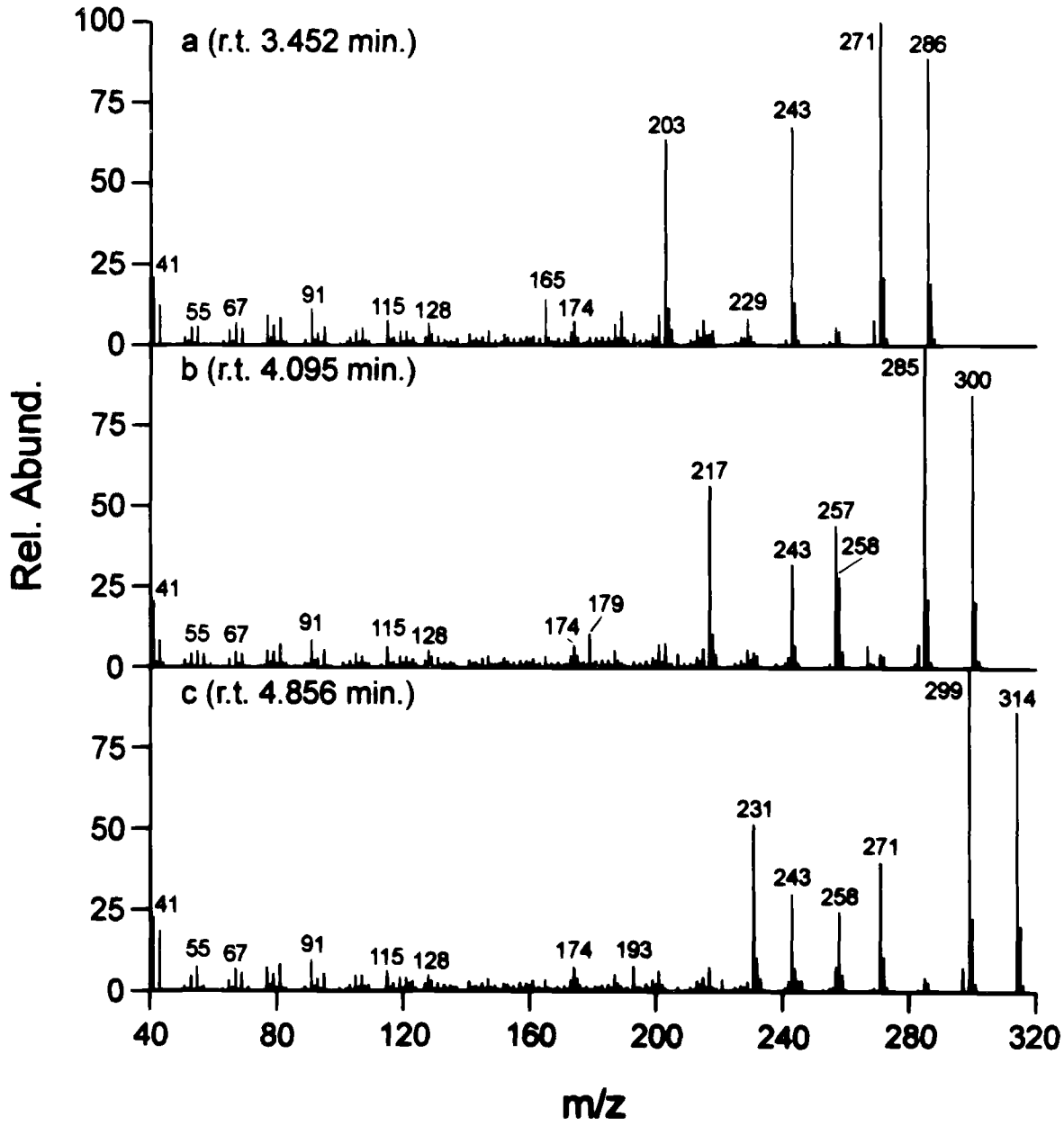


FIG. 6—*Mass spectra for homologs of Δ^9-tetrahydrocannabinol from Exhibit 2: a) tetrahydrocannabivarin (Ia); b) butyl-THC (Ib); and c) THC (Ic). Spectra are not corrected for background ions.*

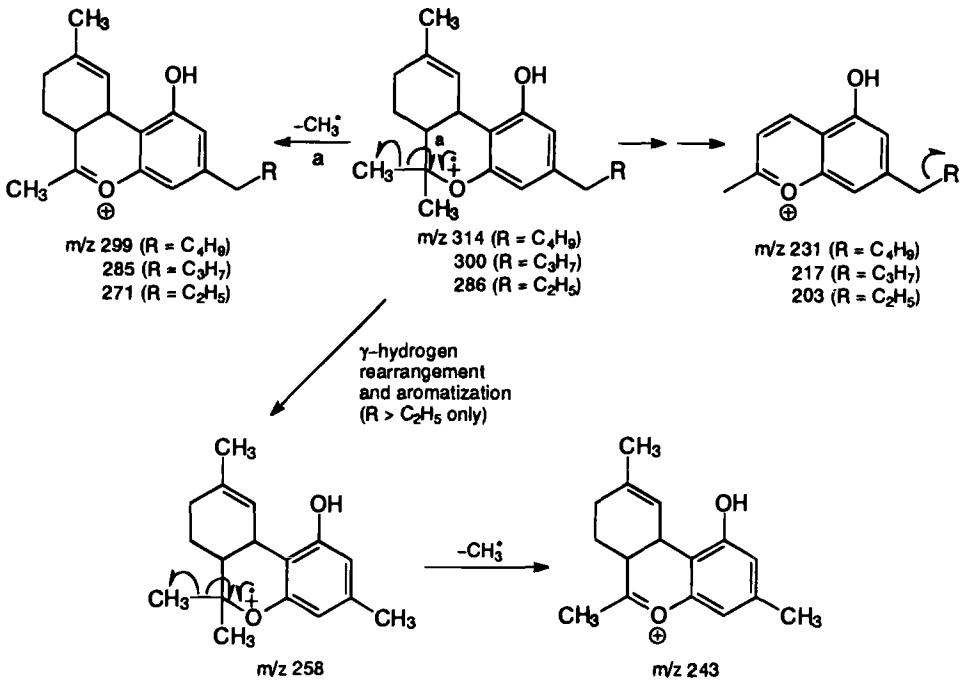


FIG. 7—*Proposed fragmentation pathways for production of intense high mass ions in homologs of tetrahydrocannabinol.*

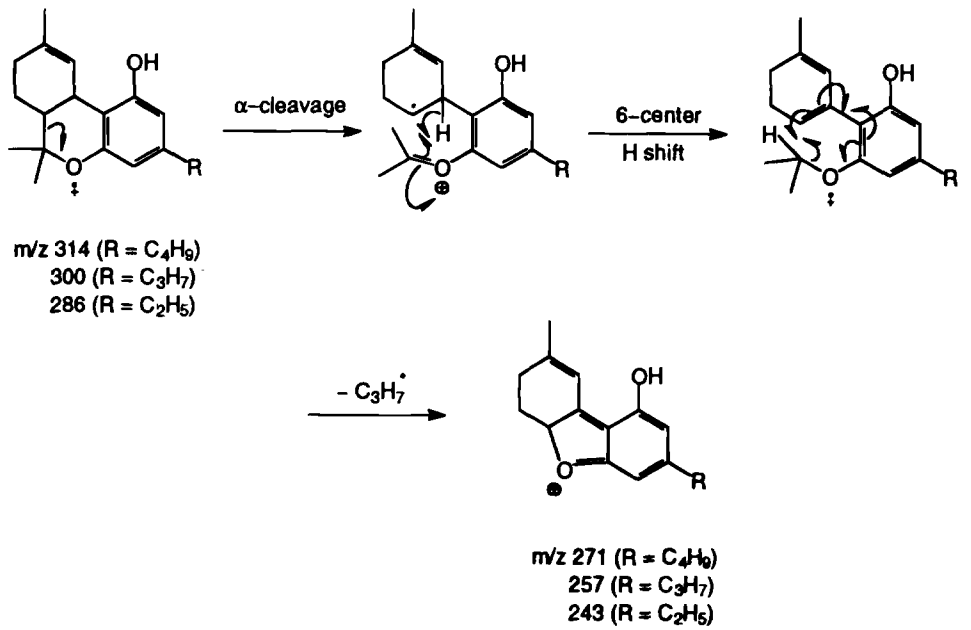


FIG. 8—*Proposed fragmentation mechanism for loss of C_3H_7 from the molecular ion in homologs of tetrahydrocannabinol.*

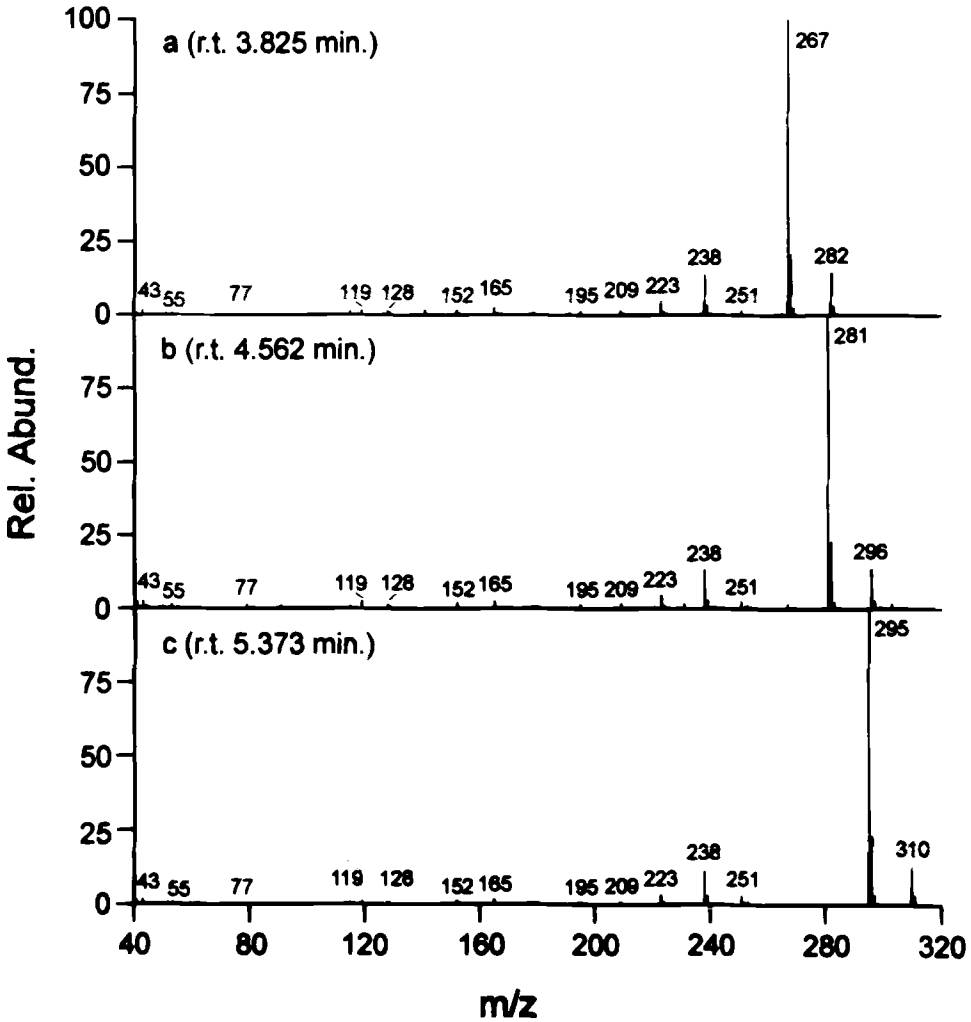


FIG. 9—*Mass spectra for homologs of cannabinol from Exhibit 2: a) cannabivarin (IIIa); b) butyl-CBN (IIIb); and c) cannabinol (IIIc). Spectra are not corrected for background ions.*

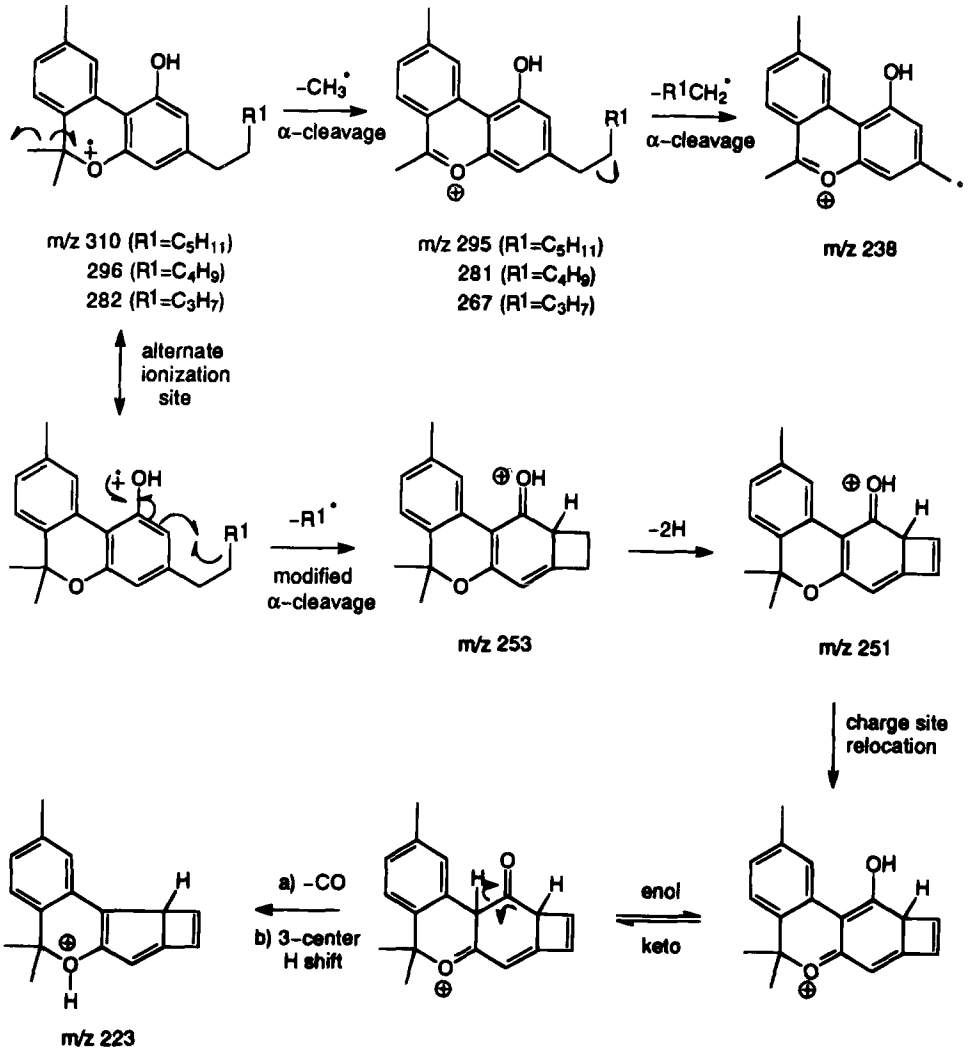


FIG. 10—*Proposed fragmentation pathways for major high mass ions in the spectra of homologs of cannabinol.*

References

1. Vree TB, Breimer DD, van Ginneken CAM, van Rossum JM. Identification in hashish of tetrahydrocannabinol, cannabidiol and cannabinol analogues with a methyl side chain. J Pharm Pharmacology 1972;24:7–12.
2. Vollner L, Bieniek D, Korte F. Haschisch. XX. Cannabidivarin—ein neuer haschischinhaltsstoff. Tetr Letters 1969;145–7.
3. Gill EW, Patton GWDM, Pertwee RG. Preliminary experiments on the chemistry and pharmacology of Cannabis. Nature 1970; 228:134–6. Gill EW. Propyl homologue of tetrahydrocannabinol; its isolation from Cannabis, properties and synthesis. J Chem Soc—C 1971;579–82.
4. Merkus FWHM. Cannabivarin and tetrahydrocannabivarin: Two new constituents of hashish. Nature 1971;232:579–80.
5. de Zeeuw RA, Wijsbeek J, Breimer DD, Vree TB, van Ginneken CAM, van Rossum JM. Cannabinoids with a propyl side chain in Cannabis: Occurrence and chromatographic behavior. Science 1972;175:778–9.
6. Hendrickson JB. The molecules of nature. WA Benjamin Inc., New York, NY, 1965:22–33.
7. Small E, Beckstead HD. Cannabinoid phenotypes in Cannabis sativa. Nature 1973;245:147–8.
8. Smith RM, Kempfert KD. Δ^1-3,4-cis-Tetrahydrocannabinol in Cannabis sativa. Phytochem 1977;16:1088–9.
9. Budzikiewicz H, Alpin RT, Lightner DA, Djerassi C, Mechoulam R, Gaoni Y. Massenspektroskopie und ihre anwendung auf strukturelle und stereochemische probleme—LXVIII: Massenspektrokopische untersuchung der inhaltstoffe von haschisch. Tetrahedron 1965;21:1881–8.
10. Vree TB. Mass spectrometry of cannabinoids. J Pharm Sci 1977;66:1444–50.
11. McLafferty FW, Tureček F. Interpretation of mass spectra. 4th ed., University Science Books, Mill Valley, CA, 1993:245.
12. Beynon JH, Saunders RA, Williams AE. The mass spectra of organic molecules. Elsevier publishing company, Amsterdam/New York, 1968:124–6.

Additional information and reprint requests:
R. Martin Smith, Ph.D.
Wisconsin Department of Justice
Madison Crime Laboratory
4706 University Avenue
Madison, WI 53705