Charles C. Clark,[1] *B.S.*

The Differentiation of Lysergic Acid Diethylamide (LSD) from *N*-Methyl-*N*-Propyl and *N*-Butyl Amides of Lysergic Acid

REFERENCE: Clark, C. C., **"The Differentiation of Lysergic Acid Diethylamide (LSD) from *N*-Methyl-*N*-Propyl and *N*-Butyl Amides of Lysergic Acid,"** *Journal of Forensic Sciences*, JFSCA, Vol. 34, No. 3, May 1989, pp. 532-546.

ABSTRACT: The *N*-methyl-*N*-propyl, *N*-methyl-*N*-isopropyl, *N*-butyl, *N*-isobutyl, *N-sec*-butyl and *N-tert*-butyl amides of lysergic acid were synthesized to determine the specificity of electron impact mass spectroscopy (EI/MS), when combined with other analytical techniques, for the identification of lysergic acid diethylamide (LSD). After separation of the C_8 axial and equatorial isomers by preparative thin-layer chromatography, the amides were subjected to gas-liquid chromatography (GLC), thin-layer chromatography (TLC), high pressure liquid chromatography (HPLC), and EI/MS. EI/MS, when combined with other analytical techniques, is shown to be capable of differentiating LSD from any of the other compounds included in this study.

KEYWORDS: toxicology, lysergic acid, spectroscopic analysis, chromatographic analysis

The dextrorotatory isomer of lysergic acid diethylamide (LSD) is probably the most potent hallucinogen known. It is a Schedule I substance under the Controlled Substance Act [*1*]. LSD is not naturally occurring but is made from lysergic acid. The lysergic acid is obtained by the alkaline hydrolysis of ergotamine or related alkaloids. Lysergic acid has two chiral carbon atoms, designated as C_5 and C_8. Thus, four stereoisomers are possible. However, the lysergic acid obtained by the alkaline hydrolysis of naturally occurring ergot alkaloids has exclusively the axial *(d)* hydrogen orientation at C_5, and the usual methods of synthesis used to produce LSD from lysergic acid preserve this orientation. Thus, LSD exhibits seen in forensic science laboratories contain only the *d*-LSD (C_8 amide group equatorial) and iso-LSD (C_8 amide group axial) isomers. Most of the difficulty encountered in obtaining an unequivocal identification of LSD is due to its low dosage. A single dose may contain as little as 50 μg of LSD. When soaked into paper, mixed and hardened with gelatin or mixed with excipients and binders and pressed into tablets, the LSD may constitute only 0.1% of the total weight. Early methods used for the identification of LSD include thin-layer chromatography (TLC) [*2-4*] and, later, high-pressure liquid chromatography (HPLC) [*5-7*]. These methods had adequate sensitivity for single-dose identifications, but chromatographic techniques alone are not generally regarded as specific. The use of infrared [*8, 9*] and mass spectroscopy (MS) [*10, 11*] for the identification of LSD has been suggested. A clean infrared spectrum of the LSD extracted from a specimen is difficult to obtain because of the small amount of LSD present. This necessitates a rigorous cleanup followed by the use of micro

Presented at the 40th Annual Meeting of the American Academy of Forensic Sciences, Philadelphia, PA, 15-20 Feb. 1988. Received for publication 28 June 1988; revised manuscript received 23 Aug. 1988; accepted for publication 25 Aug. 1988.

[1]Forensic chemist, U.S. Drug Enforcement Administration, Southeast Laboratory, Miami, FL.

pellets, beam condensors, and so on. Mass spectroscopy has the necessary sensitivity for single-dose identifications of LSD but, when the sample is introduced via a solid probe inlet system, a clean spectrum is difficult to obtain. The low mass end of the spectrum, in particular, is frequently contaminated with responses as a result of impurities in the specimen. Introduction of the specimen into the mass spectrometer through a gas-liquid chromatographic (GLC) system generally gives a cleaner mass spectrum. However, because LSD is thermally labile, the use of packed GLC columns for its chromatography has been generally unsuccessful without the use of derivative formation [*12, 13*] or lightly loaded packings [*14*]. The introduction to GLC of capillary columns with thermally stable cross-linked stationary phases on fused silica has made GLC of LSD possible. Many mass spectrometers now use a capillary column as an inlet system. Under electron impact (EI) conditions, LSD produces a prominent molecular ion. Thus, only compounds which have a molecular weight identical to that of LSD could be mistaken for LSD when EI/MS is used for the identification of LSD. Compounds expected to have an EI mass spectrum similar to *d*-LSD include its diastereomer iso-LSD and lysergic acid amides which have a rearrangement of the four carbon atoms of the diethyl group, for example, lysergic acid methylpropylamide (LAMPA). Levorotatory LSD, expected to have an EI mass spectrum identical to that of LSD, is not likely to be seen in forensic science laboratories for the reasons given above. In addition, although *dl*-lysergic acid has been synthesized [*15*], its synthesis is tedious and involves many steps and low yields. Since *l*-LSD is not believed to be a potent hallucinogen, there is little profit incentive to produce it. Because iso-LSD is a diastereomer of LSD, there may be differences between their EI mass spectra. It also is not a potent hallucinogen. Since iso-LSD can easily be converted to the much more potent LSD, most forensic science specimens do not contain large amounts of iso-LSD. Much work has been published showing that LAMPA can be separated from LSD by chromatographic means [*16–19*] and that their mass spectra can be distinguished [*17–19*]. However, analytical data for related compounds is scarce. This report presents the EI mass spectra and describes the TLC, HPLC, and GLC behaviors of lysergic acid methylisopropylamide and the *n*-butyl, isobutyl, *sec*-butyl, and *tert*-butyl amides of lysergic acid. Their structures are shown in Fig. 1 and Table 1. The corresponding information for iso-LSD and LAMPA is included for completeness.

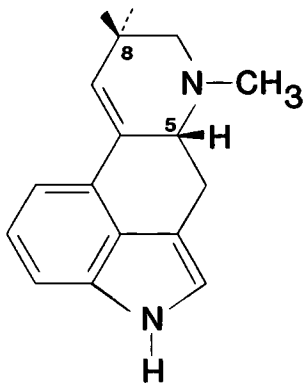

FIG. 1—*Structure of LSD and related compounds used in this study (see also Table 1).*

TABLE 1—*Structure of compounds studied.*

Compound	C_8 Amide
1A (LSD)	$CON(C_2H_5)_2$ equatorial
1B (iso-LSD)	$CON(C_2H_5)_2$ axial
IIA	$CON(CH_3)(n\text{-}C_3H_7)$ equatorial
IIB	$CON(CH_3)(n\text{-}C_3H_7)$ axial
IIIA	$CON(CH_3)(iso\text{-}C_3H_7)$
IIIB	$CON(CH_3)(iso\text{-}C_3H_7)$
IVA	$CONH(n\text{-}C_4H_9)$
IVB	$CONH(n\text{-}C_4H_9)$
VA	$CONH(iso\text{-}C_4H_9)$
VB	$CONH(iso\text{-}C_4H_9)$
VIA	$CONH(tert\text{-}C_4H_9)$
VIB	$CONH(tert\text{-}C_4H_9)$
VIIA-1	$CONH(sec\text{-}C_4H_9)$
VIIA-2	$CONH(sec\text{-}C_4H_9)$
VIIB-1	$CONH(sec\text{-}C_4H_9)$
VIIB-2	$CONH(sec\text{-}C_4H_9)$

Experimental Procedure

Apparatus

Mass spectra were obtained on a Finnigan 4530 quadrupole mass spectrometer. Ionizing voltage was 70 eV. Source temperature was 200°C. Scan rate was 0.5 s/scan, and the scan range was 40 to 400 mass units. Background subtraction was made. Specimen introductions were made via a 12-m by 0.32-mm inside diameter (ID) fused silica capillary column with a 0.52-μm film thickness of OV-1. Split ratio was approximately 25:1. Column temperature was 260°C and the injector temperature 270°C. Helium was used as a carrier gas at a flow of 1.5 mL/min. HPLC data was obtained on a Perkin-Elmer Series 4 Liquid Chromatograph equipped with a Rheodyne 7125-0785 injection valve fitted with a 20-μL loop. Detection was by ultraviolet absorption at 309 nm. A 25-cm by 4.6-mm stainless steel column packed with 5 μm of Supelcosil LC-18 was used. The eluent consisted of phosphate buffer: methanol (60:40). The phosphate buffer was prepared by the addition of 10 mL of phosphoric acid (H_3PO_4) to 1 L of water, followed by the addition of sufficient 2*N* sodium hydroxide (NaOH) solution to raise the pH to 6.5. Analytical gas-liquid chromatography was performed using a Hewlett-Packard 5880A Series Gas Chromatograph fitted with a capillary injector operated in the split mode at a split ratio of approximately 25:1. The column, column temperature, and injection temperature were the same as for mass spectral work. Hydrogen was used as a carrier gas at a flow of 1.5 mL/min. Flame ionization detection was used. TLC was carried out using commercially purchased glass plates having a 250-μm coating of silica gel G (Analtech, Newark, Delaware). Chloroform: methanol (9 + 1) and acetone were used as developing solvents. The plates were developed for 10 cm in an unlined tank, the solvent allowed to evaporate from the plate, and the amides located by their native fluorescence under long wavelength ultraviolet radiation.

Synthesis

The amides were prepared from commercially purchased *d*-lysergic acid by the mixed anhydride method of Pioch [*20*]. This procedure produces a mixture of C_8 equatorial and C_8 axial (iso) amides which were separated by preparative TLC. The plates, solvent (acetone), development, and visualization were the same as those described above. Following removal

of the separated isomers from the adsorbent, the amides were dissolved in sufficient methanol (CH_3OH) to obtain a concentration of approximately 1 mg/mL. These solutions were used for all analytical tests. No attempt was made to assign a C_8 amide orientation to a particular member of the separated set of isomers. Throughout the remainder of this paper, the member having the higher R_f under the preparative TLC conditions used is designated as Compound A, and the member having the lower R_f is designated as Compound B. Thus LSD, having the higher R_f, is called Compound IA, and iso-LSD, having the lower R_f, is called Compound IB.

Results and Discussion

Thin-Layer Chromatography

The results obtained using the two solvents are shown in Table 2. With both solvent systems, Compound IB (that is, iso-LSD), is easily differentiated from LSD. All of the di-substituted amides have lower R_f values and all mono-substituted amides have higher R_f values than their respective LSD counterparts. Two spots were noted for the *sec*-butylamide having the lower R_f. Because the *sec*-butyl group introduces a third chiral center into the molecule, four spots corresponding to the four dextrorotatory isomers are possible. The two isomers having the higher R_f values are apparently not resolved when using either TLC system. The resolution of the two isomers having the lower R_f value was not sufficient to allow the isolation of the individual isomers from the preparative plate. Thus, the two sections of the adsorbent removed from the preparative *sec*-butyl amide TLC plate each contain two diastereomers (Compounds VIIA-1, VIIA-2, VIIB-1, and VIIB-2). When only one system is used, some analogs could be misidentified as LSD, but this possibility can be reduced by using both TLC systems or by briefly heating the specimen in alcoholic KOH before chromatography. This procedure isomerizes the LSD, causing two spots corresponding to the two C_8 diastereomers to appear on the TLC plate. The R_f of the two spots can then be compared with those produced by both LSD and iso-LSD.

TABLE 2—*TLC data for LSD and related compounds.*[a]

Compound	Solvent	
	$CHCl_3 \cdot CH_3OH$ (9 + 1), R_R	Acetone, R_R
IA (LSD)	1.00 (R_f = 0.53)	1.00 (R_f = 0.58)
IB	0.53	0.29
IIA	0.85	0.84
IIB	0.51	0.22
IIIA	0.95	0.85
IIIB	0.41	0.24
IVA	1.26	1.50
IVB	0.83	0.92
VA	1.38	1.59
VB	1.02	0.89
VIA	1.42	1.57
VIB	0.85	1.16
VIIA-1	1.28	1.49
VIIA-2	1.28	1.49
VIIB-1	1.01	1.26
VIIB-2	0.96	1.13

[a]Plate: silica gel G, 5 by 20 cm.

High Pressure Liquid Chromatography

The results obtained using HPLC are shown in Table 3. No separation was obtained between LSD and Compound IIIA. Partial, but not complete separation, was achieved between LSD and iso-LSD and between LSD and Compound IIA (LAMPA). For all of the di-substituted amide diastereometric pairs studied, that member having the higher TLC R_f value has the shorter HPLC retention time. The reverse is true for all the mono-substituted diastereometric pairs studied. Compounds VIIA-1 and VIIA-2, unresolved and removed together from the preparative TLC plate, are partially resolved by HLPC. Compounds VIIB-1 and VIIB-2, partially resolved but removed together from the preparative plate, apparently coelute. With this HPLC system, LSD can be differentiated from, but not completely resolved from, all of the compounds studied except Compound IIIA.

Gas-Liquid Chromatography

The results obtained using GLC are shown in Table 4. Only a partial separation of LSD and iso-LSD was achieved. Similarly, only partial separations of the members of the C_8 diastereometric pairs of each of the studied di-substituted amides were achieved. For di-substituted amides, that pair member having the lower TLC R_f value has the shorter GLC retention time. Since both di-substituted amides studied have longer GLC retention times than LSD, the pair member having the lower TLC R_f elutes closer to LSD than does the member having the higher TLC R_f. Thus, iso-LAMPA (Compound IIB) elutes closer to LSD than does LAMPA (Compound IIA). Iso-lysergic acid methylisopropylamide elutes even closer. For all studied pairs of mono-substituted amides, that pair member having the lower TLC R_f value has the longer GLC retention time. Mono-substituted amide pair members were completely resolved except for the *sec*-butyl amides. Compounds VIIA-1 and VIIA-2, unresolved by TLC, were partially resolved by GLC. The opposite is true for Compounds VIIB-1 and VIIB-2.

TABLE 3—*HPLC data for LSD and related compounds.*[a]

Compound	R_t, min	R_R
IA (LSD)	5.34	1.00
IB	5.82	1.09
IIA	5.66	1.06
IIB	6.14	1.15
IIIA	5.34	1.00
IIIB	5.77	1.08
IVA	13.0	2.43
IVB	6.51	1.22
VA	12.9	2.42
VB	6.19	1.16
VIA	15.1	2.82
VIB	6.73	1.26
VIIA-1	11.7	2.20
VIIA-2	12.9	2.41
VIIB-1	5.71	1.07
VIIB-2	5.71	1.07

[a]Column: 25-cm by 4.6-mm ID packed with 5-μm Supelcosil LC-18. Mobile phase: pH 6.5 phosphate buffer, CH_3OH (60/40).

TABLE 4—*GLC data for LSD and related compounds.*[a]

Compound	R_t, min	R_R
IA(LSD)	7.28	1.00
IB	7.04	0.97
IIA	8.06	1.11
IIB	7.80	1.07
IIIA	7.54	1.05
IIIB	7.56	1.04
IVA	7.00	0.96
IVB	8.23	1.13
VA	6.45	0.88
VB	7.46	1.02
VIA	4.61	0.63
VIB	5.38	0.74
VIIA-1	5.60	0.77
VIIA-2	5.78	0.79
VIIB-1	6.71	0.92
VIIB-2	6.71	0.92

[a]Column: 12-m by 0.32-mm ID OV-1 260°C.

Electron Impact/Mass Spectrometry

Figures 2 to 16 show the EI mass spectra of the compounds included in this study. The combined mass spectra of Compounds VIIB-1 and VIIB-2, which could not be resolved by either TLC or GLC, are shown in Fig. 16. All the studied compounds have molecular weights of 323 and show prominent molecular ions. Di-substituted pair members having the higher TLC R_f and mono-substituted pair members having the lower TLC R_f show a m/z 221 base peak and all show prominent ions at m/z 207, 196, 181, 167, 154, 111, and 72. All except Compounds VIA and VIB (*tert*-butyl amides) have a prominent m/z 128. Thus, differentiation between LSD and the other studied compounds must depend upon the intensity ratios of the major fragments and the presence or absence of minor fragments. LSD (Compound IA) can be differentiated from iso-LSD (Compound IB) by the latter's more abundant m/z 207, 181, and especially 196 fragments. Similarly, for the other two pairs of di-substituted amides, the pair member having the lower TLC R_f can be distinguished from the other pair member by its more abundant m/z 207, 196, and 181 ions. The opposite is true for the mono-substituted amide pairs. For these pairs, except for Compound VIIA-2, that member having the higher TLC R_f value has a m/z 196 base peak. LSD can be differentiated from the members of the other two pairs of di-substituted amides by its m/z 44 fragment and the near absence of a m/z 43 fragment. This m/z 43 fragment is a prominent feature of the spectra of the members of the other two pairs of di-substituted amides but is nearly lacking in LSD and iso-LSD. The presence in the LSD spectrum of a m/z 100 fragment nearly as intense as the m/z 111 fragment response also serves to differentiate LSD from the other studied di-substituted amides. The LSD m/z 58 fragment intensity and the (m/z 58)/-(m/z 57) fragments intensity ratio differentiate LSD from all of the studied mono-substituted amides.

Conclusion

EI mass spectroscopy alone can differentiate between LSD and any of the studied compounds when standards are available for comparison and clean spectra can be obtained. However, since differentiation is based, in part, on low-mass fragments, a clean mass spec-

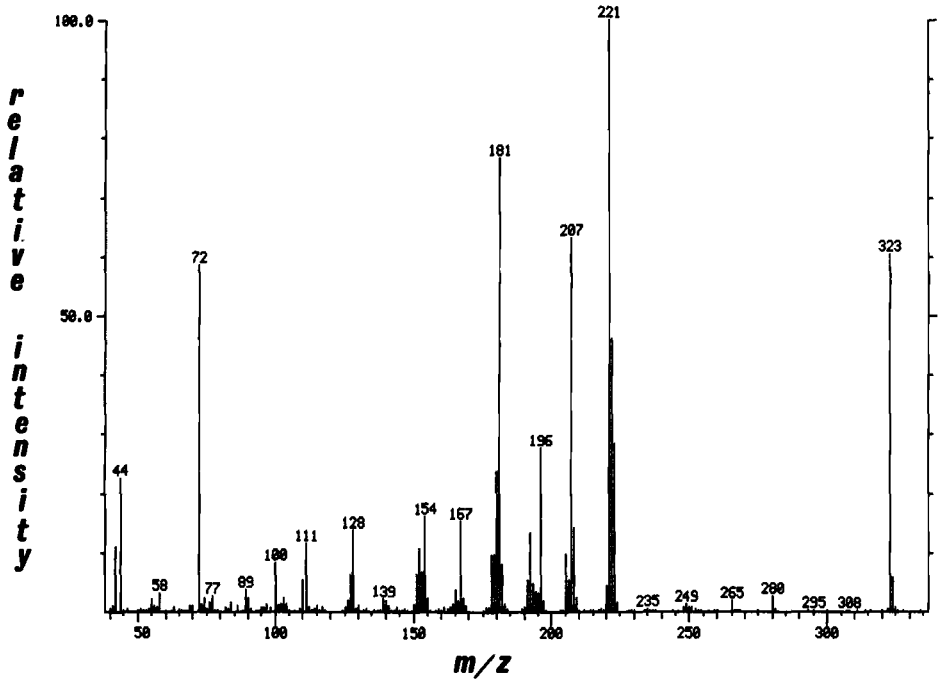

FIG. 2—*EI mass spectrum of Compound IA (LSD).*

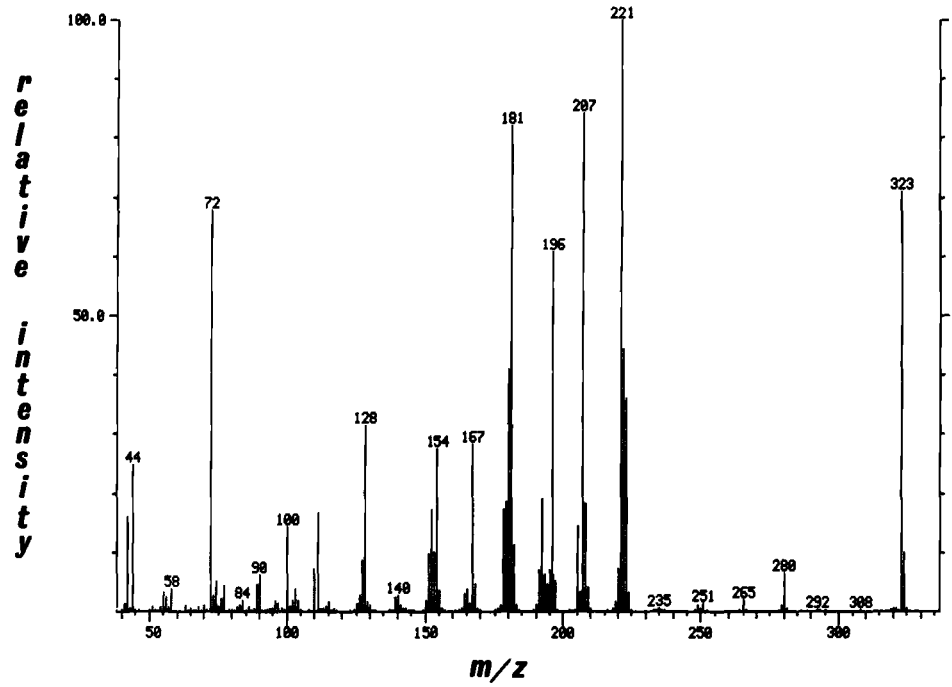

FIG. 3—*EI mass spectrum of Compound IB (iso-LSD).*

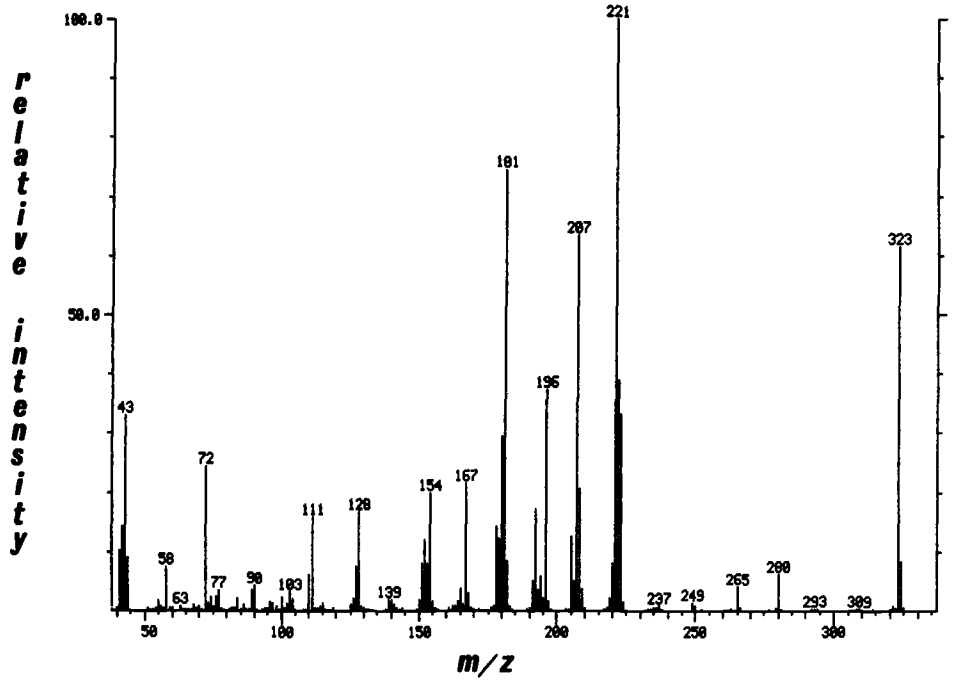

FIG. 4—*EI mass spectrum of Compound IIA (LAMPA).*

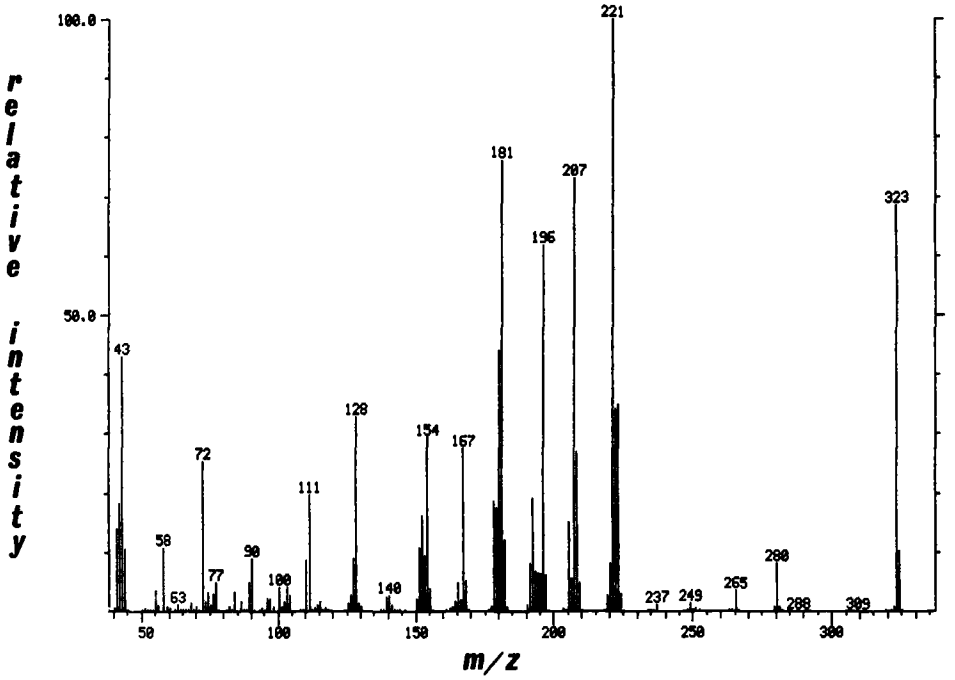

FIG. 5—*EI mass spectrum of Compound IIB (iso-LAMPA).*

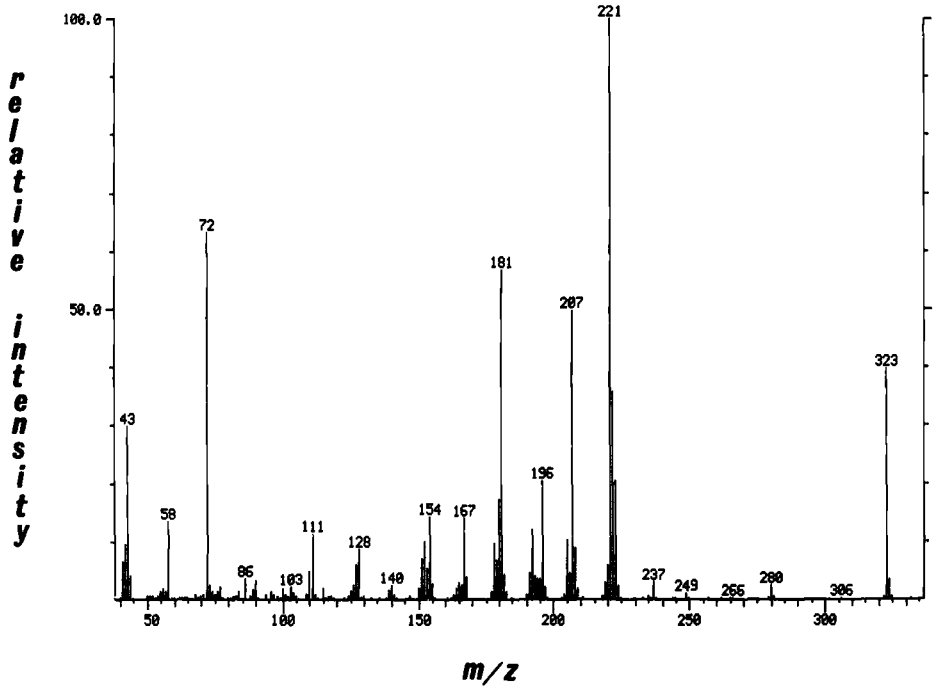

FIG. 6—*EI mass spectrum of Compound IIIA.*

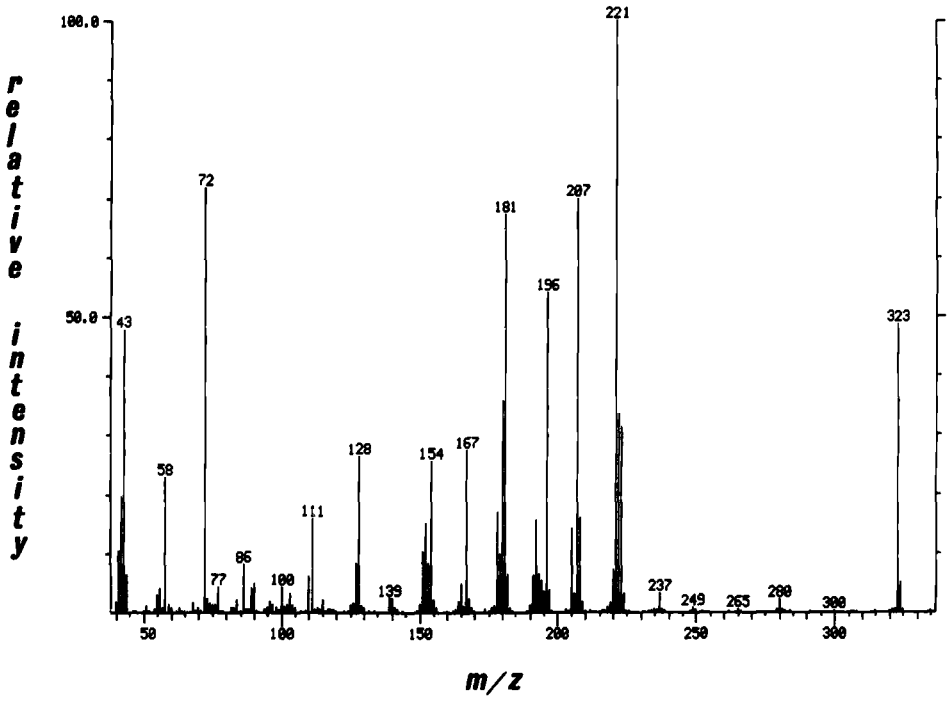

FIG. 7—*EI mass spectrum of Compound IIIB.*

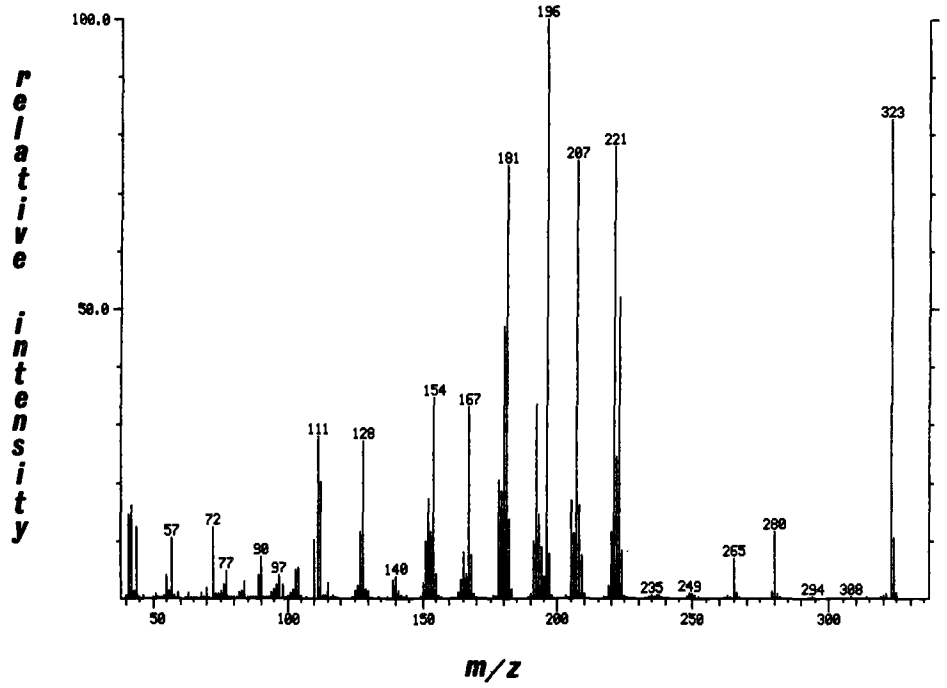

FIG. 8—*EI mass spectrum of Compound IVA.*

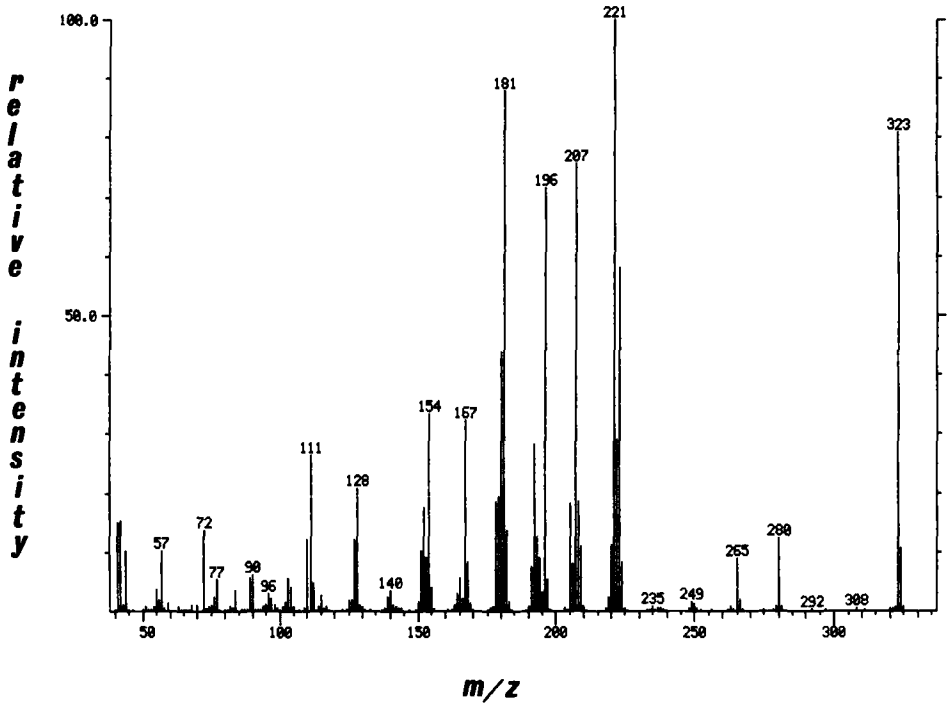

FIG. 9—*EI mass spectrum of Compound IVB.*

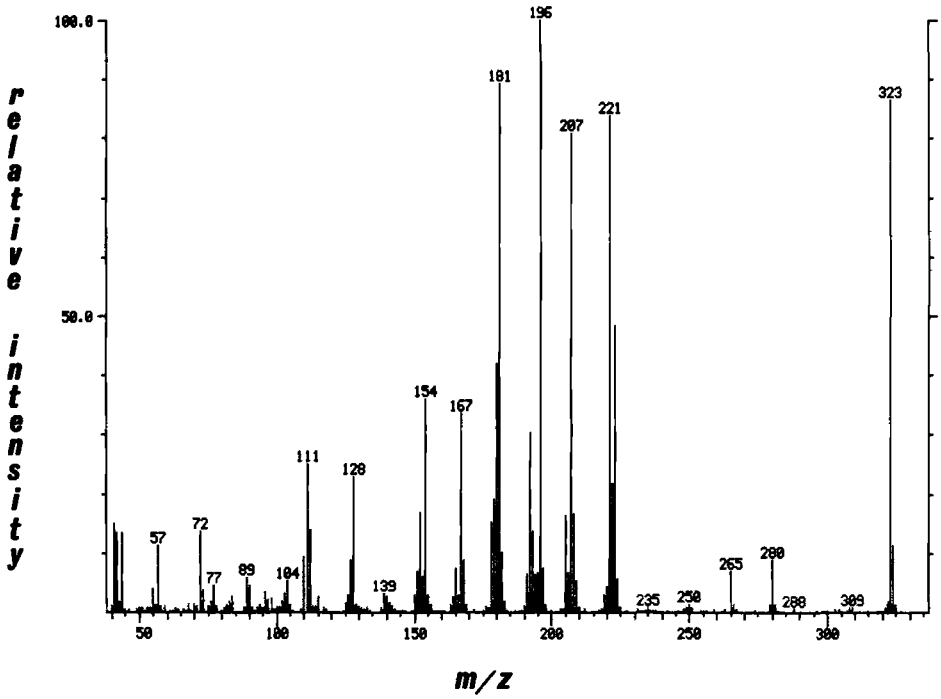

FIG. 10—*EI mass spectrum of Compound VA.*

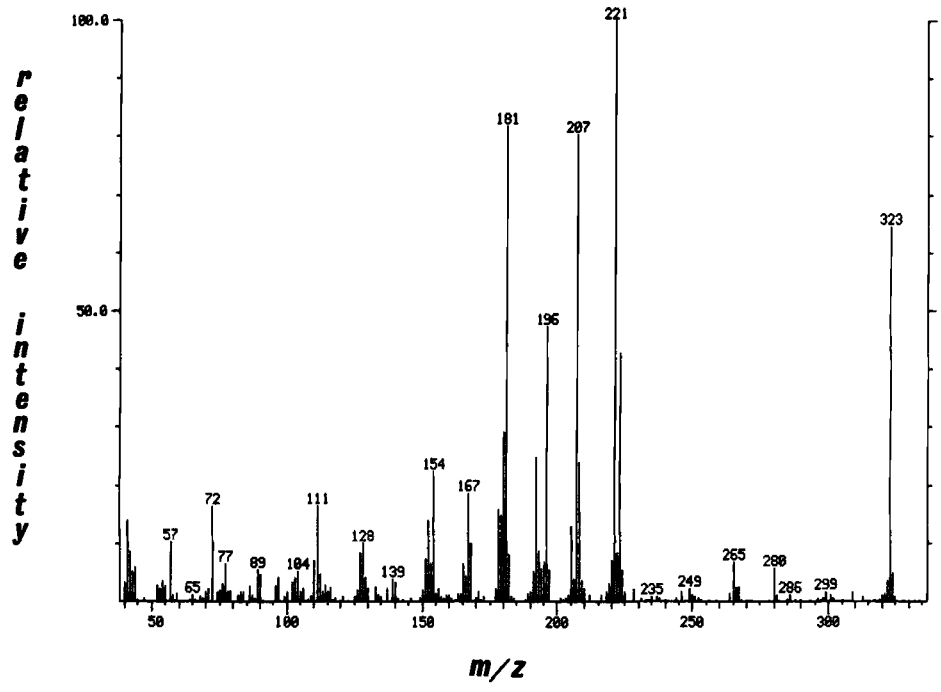

FIG. 11—*EI mass spectrum of Compound VB.*

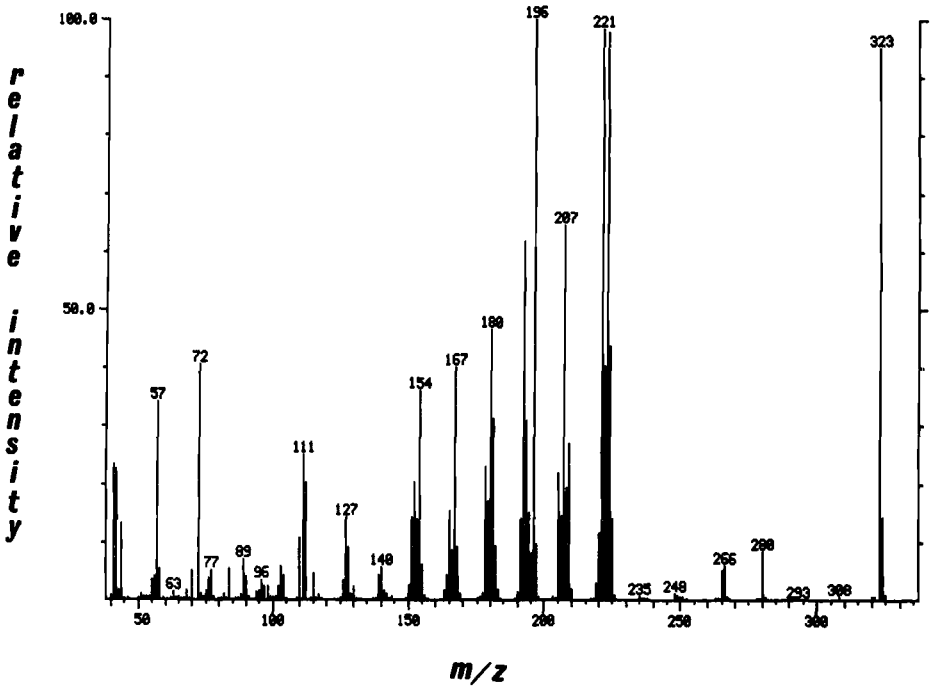

FIG. 12—*EI mass spectrum of Compound VIA.*

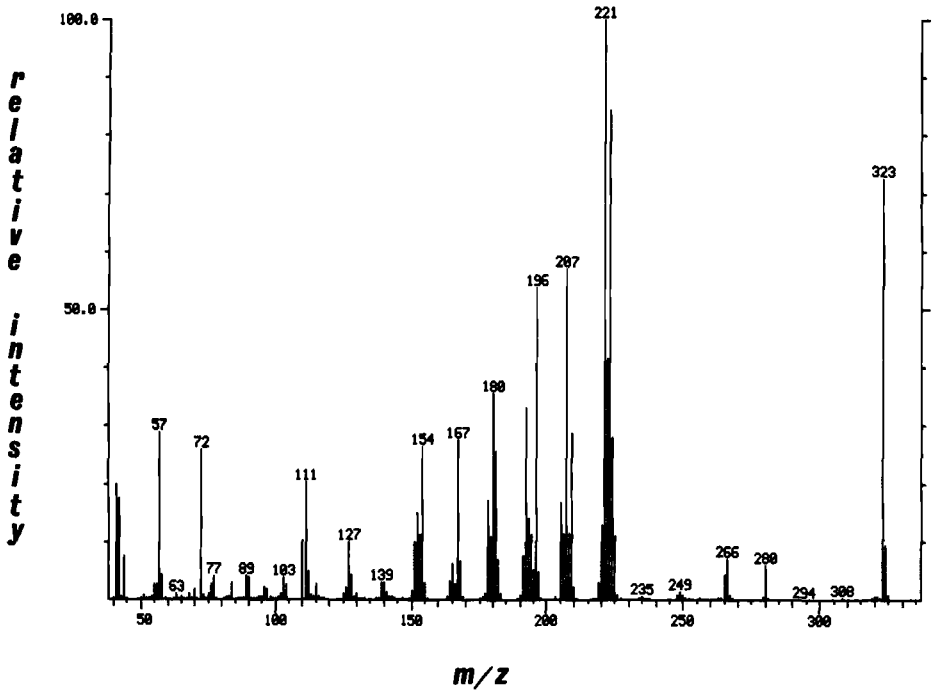

FIG. 13—*EI mass spectrum of Compound VIB.*

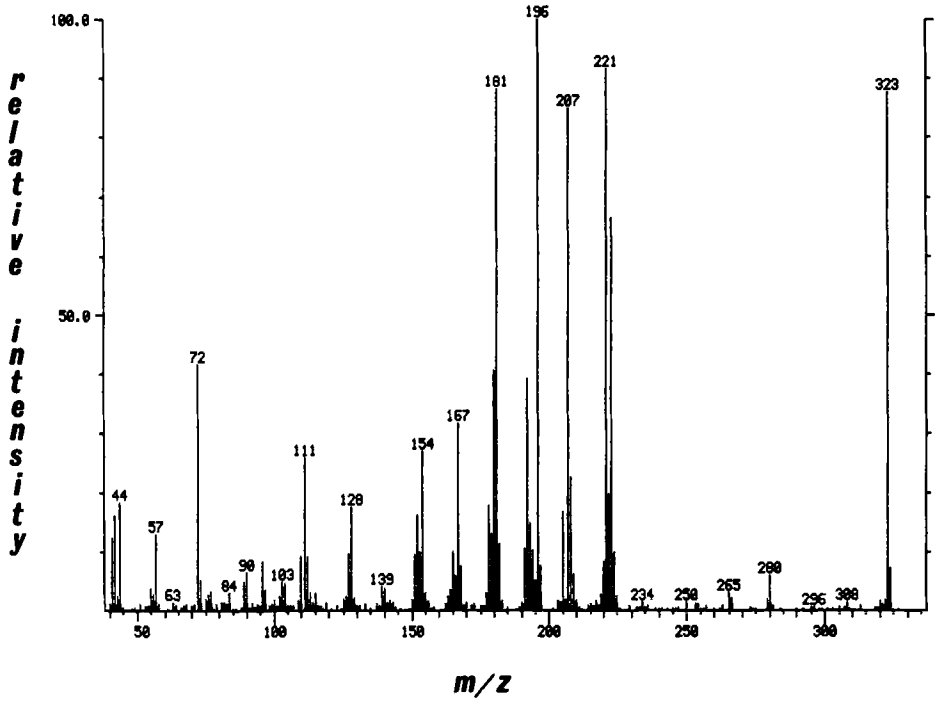

FIG. 14—*EI mass spectrum of Compound VIIA-1.*

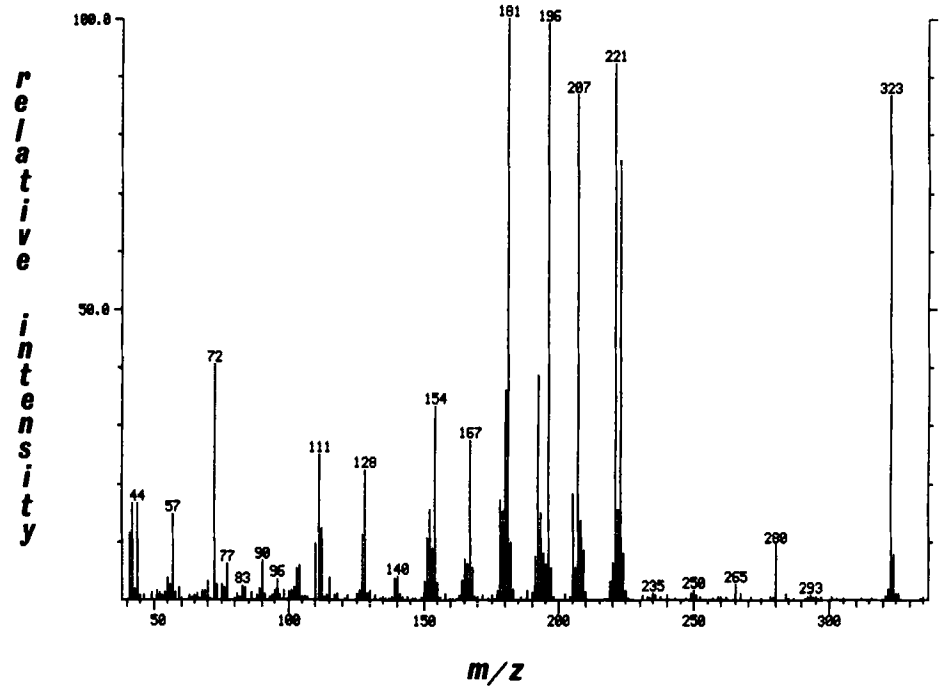

FIG. 15—*EI mass spectrum of Compound VIIA-2.*

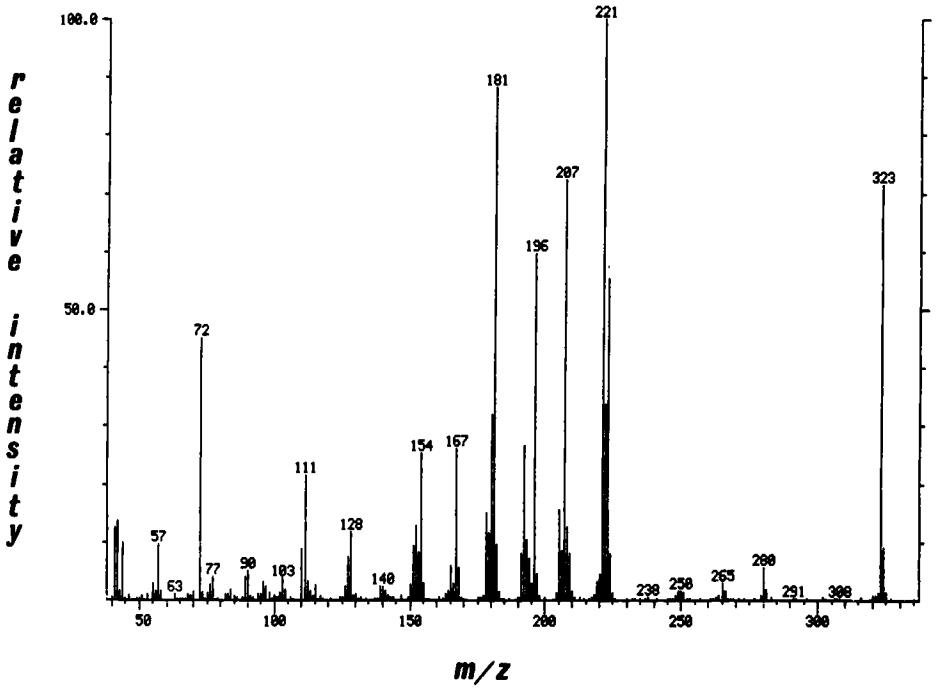

FIG. 16—*Combined mass spectra of Compounds VIIB-1 and VIIB-2.*

trum is necessary. Chromatographic techniques can be used to augment the differentiation. Mass spectroscopy combined with any of the chromatographic techniques covered in this paper can differentiate between LSD and any of the studied compounds.

References

[1] 21 CFR sec. 1308.11 d, *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC, 1970.

[2] French, W. N. and Wehrli, A. J., "Thin-Layer Chromatography of Ergot Alkaloids in Pharmaceutical Preparations," *Journal of Pharmaceutical Sciences*, Vol. 54, No. 10, Oct. 1965, pp. 1515-1516.

[3] Gough, T. A. and Baker, P. B., "Identification of Major Drugs of Abuse Using Chromatography," *Journal of Chromatographic Science*, Vol. 20, No. 7, July 1982, pp. 289-329.

[4] Fowler, R., Gomm, P. J., and Patterson, D. A., "Thin-Layer Chromatography of Lysergide and Other Ergot Alkaloids," *Journal of Chromatography*, Vol. 72, 1972, pp. 351-357.

[5] Heacock, R. A., Langille, K. R., MacNeil, J. D., and Frei, R. W., "A Preliminary Investigation of the High-Speed Liquid Chromatography of Some Ergot Alkaloids," *Journal of Chromatography*, Vol. 77, 1973, pp. 425-430.

[6] Jane, I. and Wheals, B. B., "The Characterization of LSD in Illicit Preparations by Pressure-Assisted Liquid Chromatography and Gas Chromatography," *Journal of Chromatography*, Vol. 84, 1973, pp. 181-186.

[7] Gill, R. and Key, J. A., "High-Performance Liquid Chromatography System for the Separation of Ergot Alkaloids with Applicability to the Analysis of Illicit Lysergide (LSD)," *Journal of Chromatography*, Vol. 346, 1985, pp. 423-427.

[8] Cromp, C. C. and Turney, F. G., "Infrared Identification of LSD and Related Compounds," *Journal of Forensic Sciences*, Vol. 12, Oct. 1967, pp. 538-546.

[9] Mesley, R. J. and Evans, W. H., "Infrared Identification of Lysergide (LSD)," *Journal of Pharmacy and Pharmacology*, Vol. 21, Nov. 1969, pp. 713-720.

[10] Bellman, S. W., "Mass Spectral Identification of Some Hallucinogenic Drugs," *Journal of the Association of Official Analytical Chemists*, Vol. 51, No. 1, Jan. 1968, pp. 164-175.
[11] Bellman, S. W., Turczan, J. W., and Kram, T. C., "Spectrometric Forensic Chemistry of Hallucinogenic Drugs," *Journal of Forensic Sciences*, Vol. 15, No. 2, April 1970, pp. 261-286.
[12] Lerner, M. and Kalsiaficas, M. D., "Analytical Separation of Mixtures of Hallucinogenics," *Bulletin on Narcotics*, Vol. 21, No. 1, 1969, pp. 47-51.
[13] Radecka, C. and Nigam, F. C., "Detection of Trace Amounts of Lysergic Acid Diethylamide in Sugar Cubes," *Journal of Pharmaceutical Sciences*, Vol. 55, No. 8, 1966, pp. 861-862.
[14] Katz, M. A., Tadjer, G., and Aufricht, W. A., "Direct Microanalysis of LSD by Gas Chromatography," *Journal of Chromatography*, Vol. 31, 1967, pp. 545-546.
[15] Kornfeld, C., Fornefeld, E. J., Kline, G. B., Mann, M. J., Jones, R. G., and Woodward, R. B., "The Total Synthesis of Lysergic Acid and Ergonovine," *Journal of the American Chemical Society*, Vol. 76, Oct. 1954, pp. 5256-5257.
[16] Japp, M., Gill, R., and Osselton, M. D., "The Separation of Lysergide (LSD) from Related Ergot Alkaloids and its Identification in Forensic Science Casework Samples," *Journal of Forensic Sciences*, Vol. 32, No. 4, July 1987, pp. 933-940.
[17] Stafford, D. T., Nicols, H. S., and Anderson, W. H., "Efficiency of Capillary Column Gas Chromatography in Separating Lysergic Acid Diethylamide (LSD) and Lysergic Acid Methylpropylamide (LAMPA)," *Journal of Forensic Sciences*, Vol. 29, No. 1, Jan. 1984, pp. 291-298.
[18] Bailey, K., Verner, D., and Legault, D., "Distinction of Some Dialkyl Amides of Lysergic and Iso-Lysergic Acids from LSD," *Journal of the Association of Official Analytical Chemists*, Vol. 56, No. 1, Jan. 1973, pp. 88-99.
[19] Nichols, H. S., Anderson, W. H., and Stafford, D. T., "Capillary G.C. Separation of LSD and LAMPA," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 6, Feb. 1983, pp. 101-103.
[20] U.S. Patent 2,736,728 to Eli Lilly and Co., 1956.

Address requests for reprints or additional information to
Charles C. Clark
Drug Enforcement Administration
Southeast Laboratory
5205 NW 84th Ave.
Miami, FL 33166