TECHNICAL NOTE

Robert J. Lewis,[1] Ph.D.; David Reed,[2] Ph.D.; Adrian G. Service,[1] B.S.; and Alan M. Langford,[1] Ph.D.

The Identification of 2-Chloro-4,5-methylenedioxymethylamphetamine in an Illicit Drug Seizure

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ABSTRACT: This work outlines the unequivocal identification of the "ecstasy" analog, 2-chloro-4,5-methylenedioxymethylamphetamine, using combined gas chromatography/mass spectroscopy (GC/MS) and proton magnetic resonance spectroscopy (^{1}H-NMR).

This compound was identified along with 3,4-methylenedioxymethylamphetamine (MDMA) in an illicit tablet seizure, which included 26 off-white tablets.

KEYWORDS: forensic science, 2-chloro-4,5-methylenedioxymethylamphetamine, GC/MS, ^{1}H-NMR

Ecstasy, the popular name for 3,4-methylenedioxymethylamphetamine (MDMA), has become a generic term for "designer amphetamines." The analysis of this class of compounds by GC/MS, using electron ionization conditions, is well documented (1–4). Nitrogen-driven α-cleavage is the predominate fragmentation process of this class of compound. As a result, the higher mass ranges are often generally weak and molecular ions can be difficult to detect. Heptafluorobutyryl derivatives of the 3,4-methylenedioxyamphetamine (MDA) analogs, however, yield characteristic mass spectra which allow greater structural elucidation (5).

Proton and carbon-13 assignments of MDA and some related analogs have been detailed in the literature (6–8). An extensive review (9) of NMR spectroscopy in forensic science has been published with particular emphasis on drug analysis.

A routine analysis of an ecstasy tablet by gas chromatography/mass spectroscopy (GC/MS) using electron ionization after derivatization with heptafluorobutyric anhydride (HFBA) identified the major component as MDMA. A second peak of approximately two-thirds the intensity of the MDMA peak was also evident from the total ion current chromatogram (TIC). Closer scrutiny of the mass spectral data for this peak indicated a structure similar to MDMA to which is substituted a single chlorine atom.

[1] Lothian and Borders Police Forensic Laboratory 11, Edinburgh, Scotland, UK.

[2] Department of Chemistry, Joseph Black Building, University of Edinburgh, Edinburgh, Scotland, UK.

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This substance was isolated by thin layer chromatography (TLC) and subsequently identified as 2-chloro-4,5-methylenedioxymethlamphetamine using data obtained from proton magnetic resonance spectroscopy (^{1}H-NMR), and GC/MS using electron impact and positive ion chemical ionization methods.

Methods

Thin Layer Chromatography

TLC plates: Silica stationary phase; layer thickness, 0.25 mm; plate size, 20 cm $\times$ 20 cm. Eluent: toluene diethylamine (95:5).

A sample of a tablet (approximately 50 mg) was placed into a glass vial containing methanol (approximately 0.5 mL), from which 16 extracts were spotted at regular intervals, using a capillary tube, 1 cm from the base of the TLC plate. This procedure was repeated six times on separate TLC plates. A standard MDMA solution, as a reference, and a methanol blank were also spotted once on each plate. The samples were eluted in toluene and diethylamine (95:5 v/v). The bands were visualized under an ultraviolet (UV) light.

Gas Chromatography/Mass Spectroscopy

Positive Ion Chemical Ionization—Analyses were performed using a Hewlett Packard 5890 Series II gas chromatograph with a Hewlett Packard 7673 GC-STC autosampler, coupled to a Hewlett Packard 5989A quadrapole mass spectrometer. The compounds of interest were detected using the following GC/MS conditions.

One microliter of sample was injected into the instrument operating in the splitless mode; the carrier gas was helium 13.5 psi (93 kPa); the reagent gas, methane (purity 99.95%).

Initial temperature, 100°C for 1 min., 1st ramp rate, 10°C/min to 150°C, 2nd ramp rate, 20°C/min to 300°C held for 2 min., injection temperature 250°C; transfer line temperature, 250°C; source temperature, 200°C; quadrapole temperature, 100°C; source manifold pressure, 2.7×10^{-4} torr; analyzer manifold pressure, 4.3×10^{-6} torr; foreline pressure, 1.4×10^{-1} torr; methane pressure, 1.8 torr.

GC column, HP5; length, 30 m $\times$ 0.25 mm, film thickness 0.25 μm.

The mass spectrometric scanning control parameters were as follows: start mass 40 amu; end mass 500 amu; scan time 0.9 s; interscan time 0.10 s.

Electron Ionization—Analyses were performed using a Hewlett Packard 5890 Series II gas chromatograph with a Hewlett Packard 7673 GC-STC autosampler, coupled to a Hewlett Packard 5972 quadrapole mass spectrometer.

The compounds of interest were detected using the GC conditions and the MS setup applicable to EI as detailed above.

^{1}H-NMR Spectroscopy

The NMR spectra were measured in deuteriochloroform solutions using a Bruker DPX 360MHz spectrometer operating at 360.13 MHz for protons. Spectra were recorded at 303 K, and were referenced to internal TMS at 0 ppm.

Derivatization

A sample of the tablet (10 mg) was placed into a screwtop glass vial (2 mL) to which was added an aqueous solution of 12.5 mM diphenylpyraline (DPP) as an internal standard (250 μL). An aqueous solution of 1 M Tris buffer solution (250 μL) followed by toluene (500 μL) was added to the vial which was then vortexed for approximately 20 s. An aliquot of the supernatant (25 μL) was removed and diluted 1:10 in toluene. Heptafluorobutyric anhydride (5 μL) together with a 10% solution of sodium hydrogen carbonate (250 μL) was added to the dilution and vortexed for approximately 20 s.

The supernatant layer was removed and dried over anhydrous sodium sulfate prior to GC/MS analysis.

A sample of the unknown component, isolated by TLC, was also derivatized as above.

Materials

TLC plates produced by Macherey-Nagel Polygram®.

(±) 3,4-Methylenedioxymethylamphetamine hydrochloride (MDMA-HCl), heptafluorobutyric anhydride solution (HFBA) (>98%) and diphenylpyraline (DPP) were obtained from Sigma. Toluene was obtained from Rathburn Chemicals Ltd. Diethylamine was obtained from BDH. Anhydrous sodium sulfate and tris(hydroxymethyl) methylamine (Tris buffer) were obtained from Fisons. Sodium hydrogen carbonate was obtained from May and Baker. Deuteriochloroform was obtained from Apollo Scientific. All chemicals were of analytical grade unless otherwise stated.

Results and Discussion

TLC

Each TLC plate was viewed under UV light, which visualized two clearly defined and resolved bands. The calculated relative front (R_f) value for the MDMA standard (R_f = 30.3) corresponded to one of the separated bands of the extracted sample. The R_f value of the remaining band was calculated as R_f = 34.9. This band (R_f = 34.9) was scraped from each plate, divided in two and placed into separate vials. One portion was extracted with toluene (1 mL) and the other with deuteriochloroform (1 mL) for subsequent derivatization prior to GC/MS and ^{1}H-NMR studies, respectively, as previously described.

GC/MS

Electron Ionization (EI/MS)—The heptafluorobutyryl derivatives of MDMA (retention time = 9.29 min) and the unknown compound, (retention time = 10.02 min) were clearly defined and separated according to their retention times (Fig. 1). The internal standard (DPP) was also noted (retention time = 11.49). The EI/MS spectra of both MDMA and the unknown compound (Figs. 2*a* and 2*b*) indicate structural similarities. Both spectra display a base peak at *m/z* 254 which has been previously assigned to *N*-methylamphetamines $[CH(NCH_3COC_3F_7)CH_3]^+$ (5). Further significant ions associated with the MDMA spectrum include *m/z* 135 $[CH_2O_2C_6H_3CH_2]^+$, *m/z* 162 $[CH_2O_2C_6H_3CH_2CHCH_2]^+$, and *m/z* 389 $[M]^+$.

The spectrum produced by the unknown compound is similar to

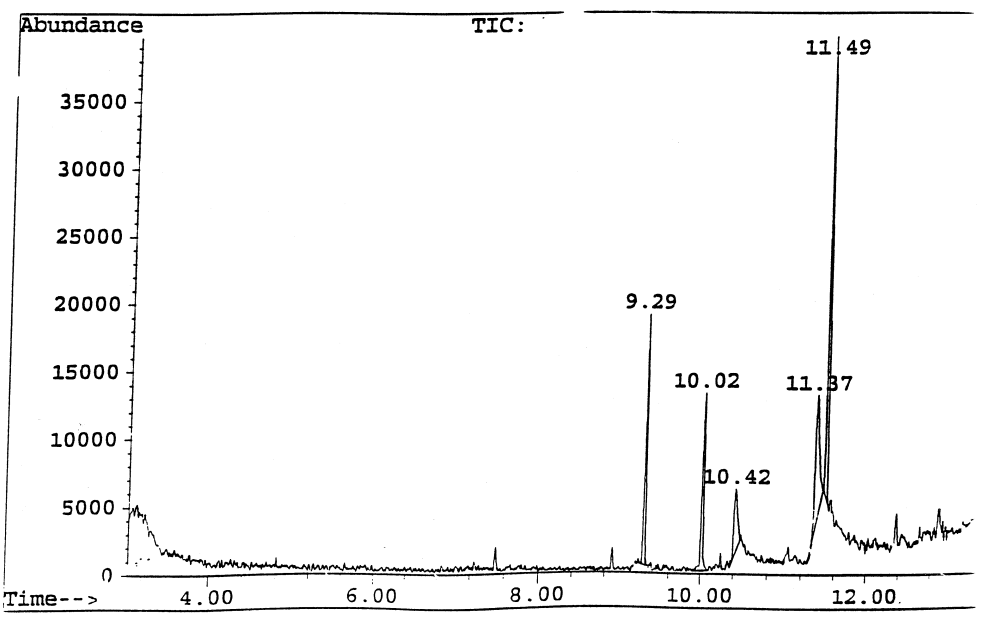

FIG. 1—*TIC of derivatized extract from tablet.*

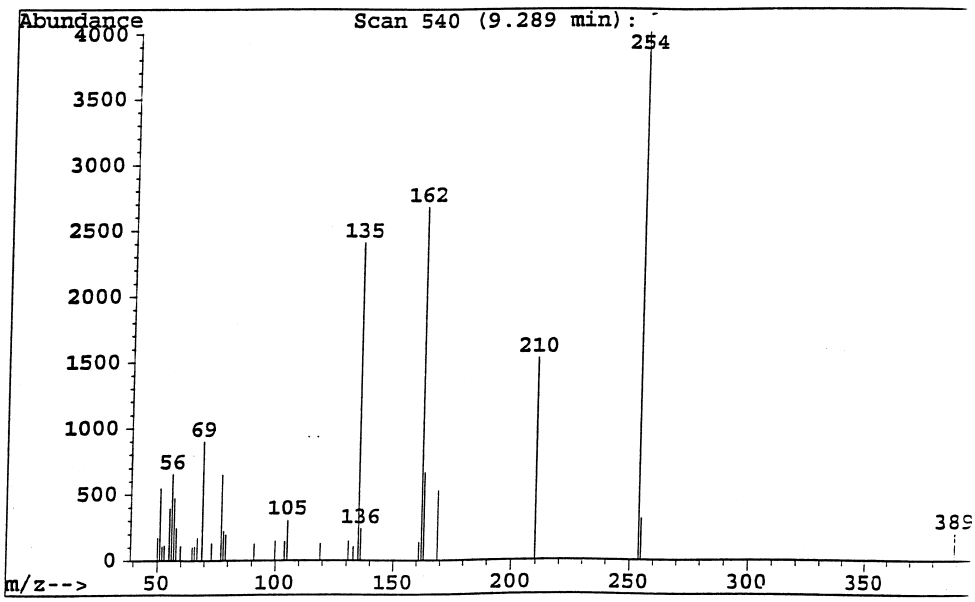

FIG. 2*a*—*EI/MS of MDMA.*

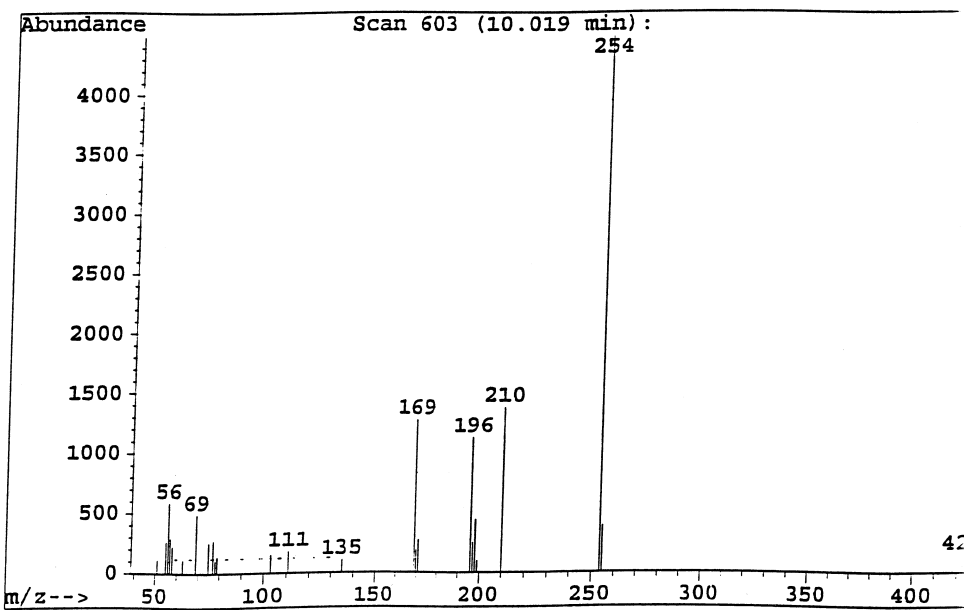

FIG. 2*b*—*EI/MS of CI-MDMA.*

that yielded by the MDMA. The two ions at *m/z* 169 and 196 (i.e., *m/z* 135 − 1 + 35 and 162 − 1 + 35), both of which exhibit characteristic A + 2 isotopic clusters, indicate a chlorine atom substituted to the MDMA structure. The weak ion at *m/z* 423 (389 − 1 + 35) is tentatively assigned as the molecular ion of a chlorinated MDMA (Cl-MDMA) structure.

The HFBA derivative of the unknown band (R_f = 34.9) recovered from the TLC experiments produced a mass spectrum identical to the proposed Cl-MDMA analogue (results not shown).

Positive Ion Chemical Ionization (PCI/MS)—The unknown compound isolated by TLC was derivatized (with HFBA) prior to analysis. The base peak m/e 424 $[M + 1]^+$ exhibits a characteristic A + 2 isotopic cluster indicative of a mono-chloro substituted compound (Fig. 3).

^{1}*H-NMR Spectroscopy*

3,4-MDMA Standard—MDMA was studied, as its hydrochloride salt, using ^{1}H-NMR in deuteriochloroform solution. The spectrum comprised 10 signals (Fig. 4, the signals being indicated by the labels A-K).

Signal A, a doublet with J = 6.4 Hz and of relative integral 3, arises from the H(3) (methyl) protons, the coupling being due to the interaction with the adjacent CH, i.e., H(2).

Signal B, a triplet with J = 5.1 Hz and relative integral 3, arises

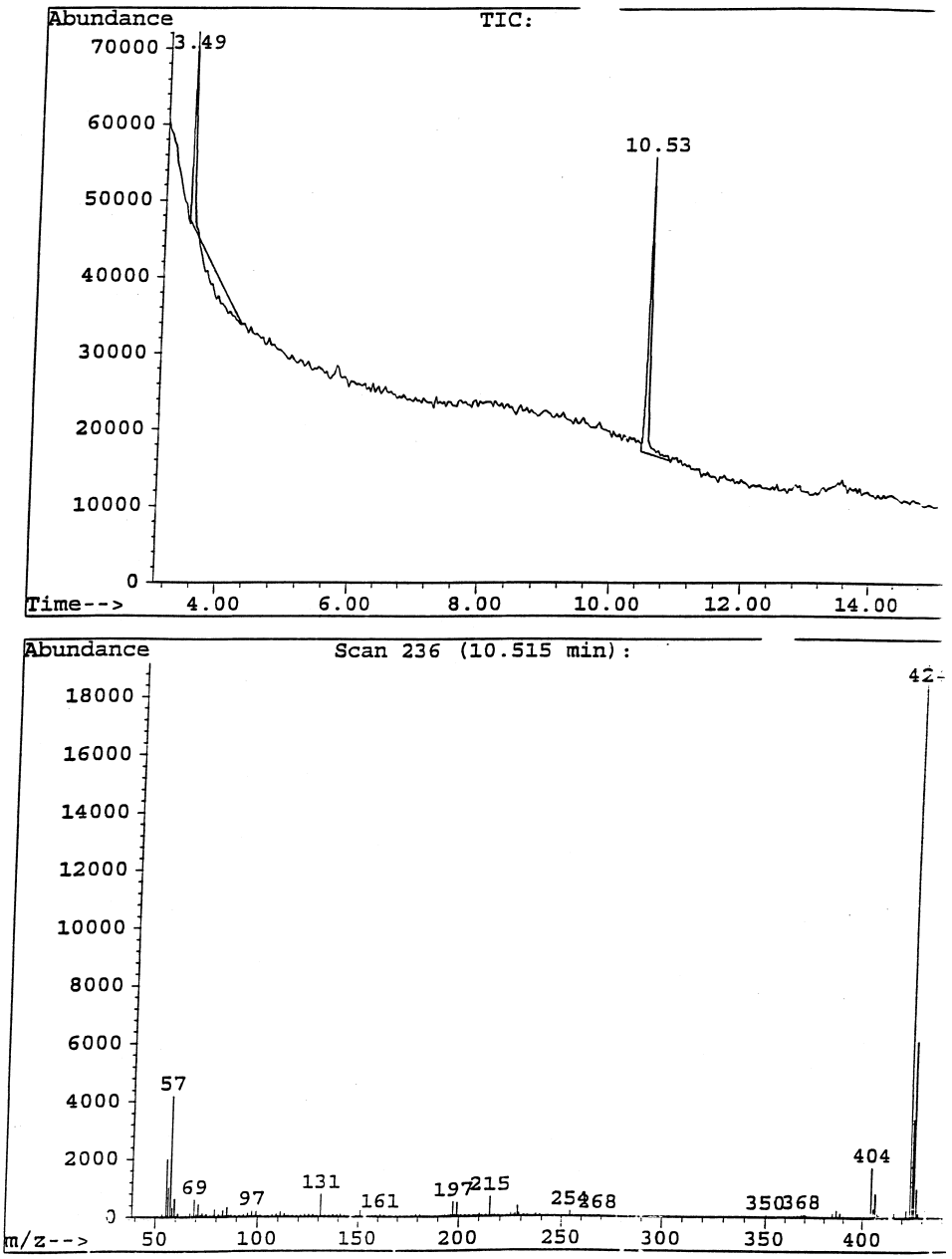

FIG. 3—*TIC and +ve CI/MS of CI-MDMA.*

from the methyl protons of the {$-NH_2$ -Me} group, the triplet structure due to coupling of these H's with the NH_2 protons. Signals C and E are due to the diastereotopic H's of C(1). Each is a doublet of doublets of relative integral 1, each showing coupling to its diastereotopic partner (13.2 Hz) and to the adjacent CH, H(2), (10.3 Hz for C and 4.2 Hz for E).

Signal D is a broad complex multiplet of relative integral 1, and is due to H(2). The complexity of the signal is due to it being coupled to the protons on carbon atoms 1 and 3, and to the NH_2 protons.

Signal F, of relative integral 2, is due to the methylenedioxy protons.

Signals G, H, and J are due to the aromatic protons H(2′), H(5′), and H(6′). Signal J is a doublet J = 7.8 Hz, and hence arises from H-5′, coupling with H-6′. Signal G is a doublet of doublets J = 7.8, 1.7 Hz, from H-6′ coupling to H-5′ and H-2′, respectively. With signal H as a narrow doublet, J = 1.7 Hz, due to H-2′.

Signal K, of relative integral 2, is hence assigned as the {$-NH_2$-Me} protons.

These data are entirely consistent with the known structure (Fig. 5).

Chlorinated MDMA Analog—The ^{1}H-NMR spectrum of the chlorinated analogue (in its base form), isolated by TLC, was also acquired, again in deuteriochloroform solution (Fig. 6). The spectrum was made up of seven distinct groups of signals, A-G (see Table 1 for chemical shift values). The remaining signals arise from impurities either in the solvent, or carried over from the iso-

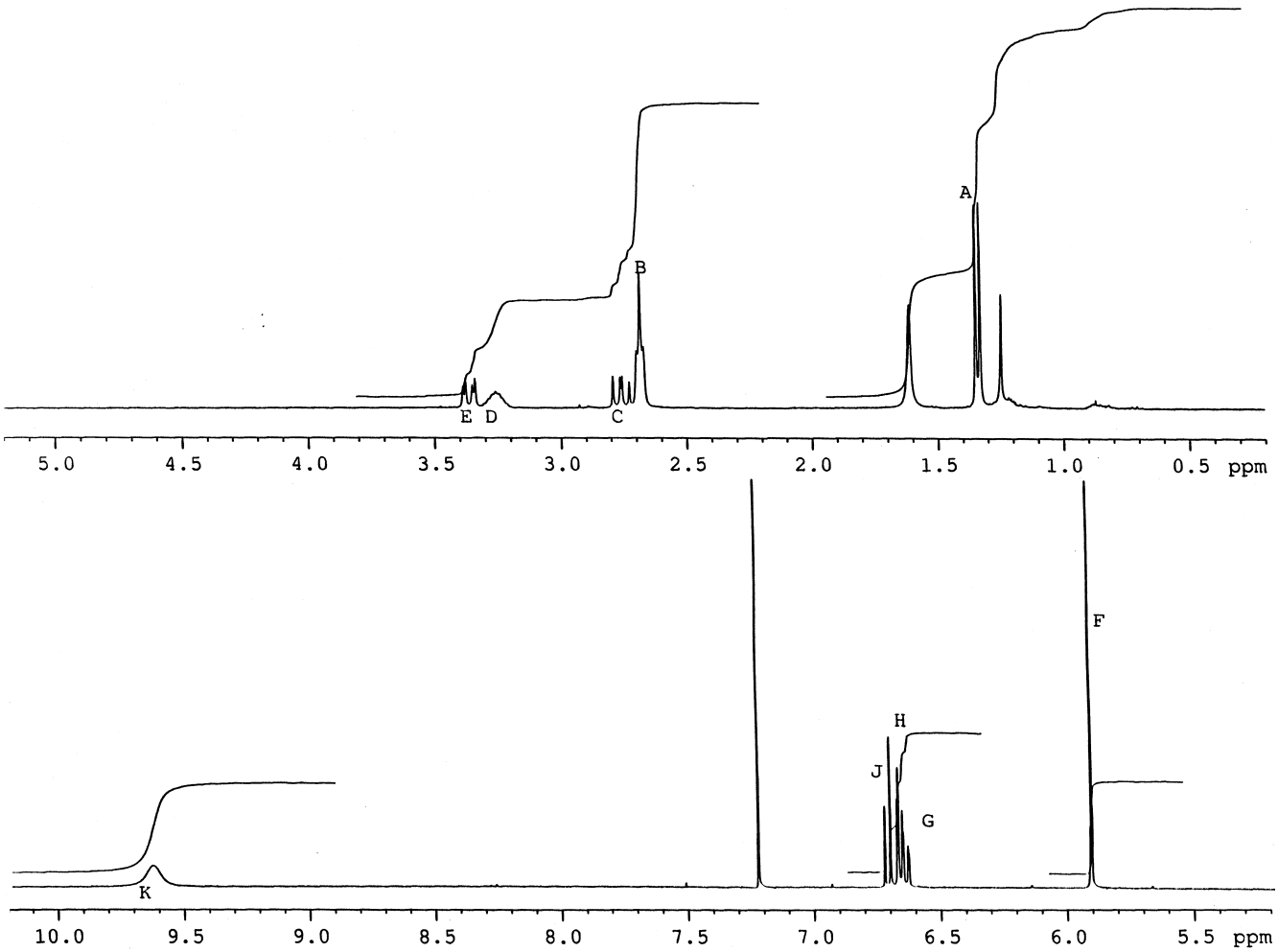

FIG. 4—*The 360.13 MHz ^1H NMR spectrum of MDMA (HCI form) in deuteriochloroform.*

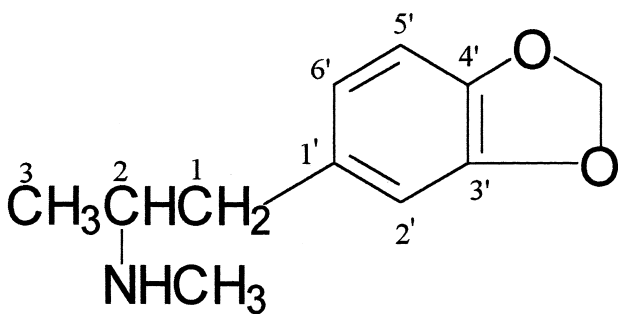

FIG. 5—*3,4-Methylenedioxymethylamphetamine.*

lation procedure. Signals F and G (each relative integral 1) are due to the aromatic protons 3′ and 6′. These two aromatic H's with their singlet appearance confirm the *para* arrangement of the protons on the aromatic ring, i.e., in the 3′ and 6′ sites.

Signal E (relative integral 2) arises from the methylenedioxy protons. Signal A, a doublet (relative integral 3), derives from the H-3 (methyl) protons and B (also relative integral 3) arises from the methyl protons of the {−NH-Me} group. In this case no coupling is observed to this signal. Signal C (integral 1), a doublet of doublets, J = 15.5, 8.9 Hz, is due to one of the diastereotopic H-1 pair, with the peak labeled D (integral 2) resulting from the overlapping of the other H-1 and the H-2 signal. The trigonal N-H is not observed.

Once again, this spectrum is entirely consistent with the proposed structure (Fig. 7).

In accordance with IUPAC rules, the numbering scheme differs to that of the parent around the aromatic ring, thus substitution is thought to have taken place at the 2′ position, as indicated. (Insufficient sample was available to convert the base form of the Cl-MDMA to its hydrochloride salt for further analysis.)

Tablet Sample—The ^1H-NMR spectrum of a deuteriochloroform extract from a tablet sample was acquired, with the result as shown in Fig. 8.

The spectrum consists of 18 signals, labeled A-T. Some of these signals are assignable to MDMA on the basis of either signal intensity or analogy with the spectrum of pure MDMA, the clearest of these being A,C,E,K,M,N, and P. Although there is some overlap of some of the remaining signals, others can be as-

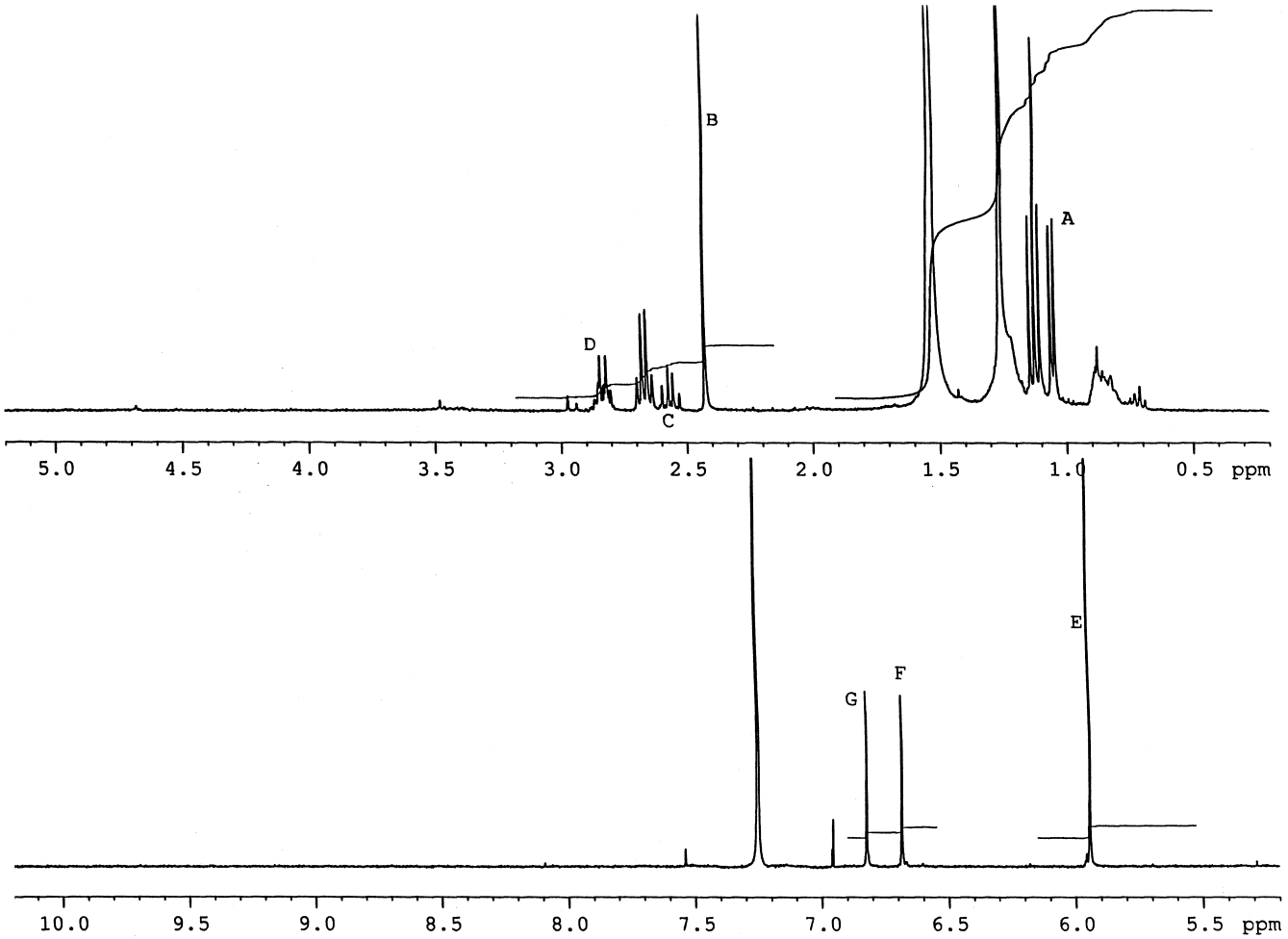

FIG. 6—*The 360.13 MHz ^1H NMR spectrum of CI-MDMA (base form) in deuteriochloroform.*

TABLE 1—*Chemical shifts of chlorinated MDMA analog.*

Assigned Signal	Chemical Shift (*d*) Value (ppm)
A	1.05
B	2.42
C	2.56
D	2.83
E	5.94
F	6.68
G	6.82

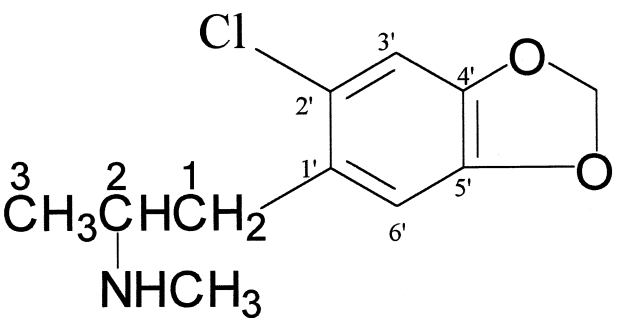

FIG. 7—*2-Chloro-4,5-methylenedioxymethylamphetamine.*

signed directly to the Cl-MDMA analog. For example, signals Q and R, both singlets of relative integral 1, are ascribable to the *para* protons of Cl-MDMA. The aliphatic region of this spectrum is very congested, particularly in the area between signals C and J. Nonetheless, some distinct signals due to Cl-MDMA can be seen. Signal D is analogous to signal C, and arises from the methyl protons of the $\{-NH_2\ -Me\}$ group, the triplet structure due to coupling of these H's with the NH_2 protons S/T. Hence, confirming both analogs to be present in the salt form. Signal F, like signal E, is a doublet of doublets, J = 14.1, 11.1 Hz, and arises from one of the diastereotopic H-1 protons. Overall therefore, the main difference between the ^1H-NMR spectra of the two analogs (as the HCl salts) lies in the appearance of the aromatic region and, as described above, it is this region of the spectrum that is critical in determining the position of the Cl atom on the aromatic ring.

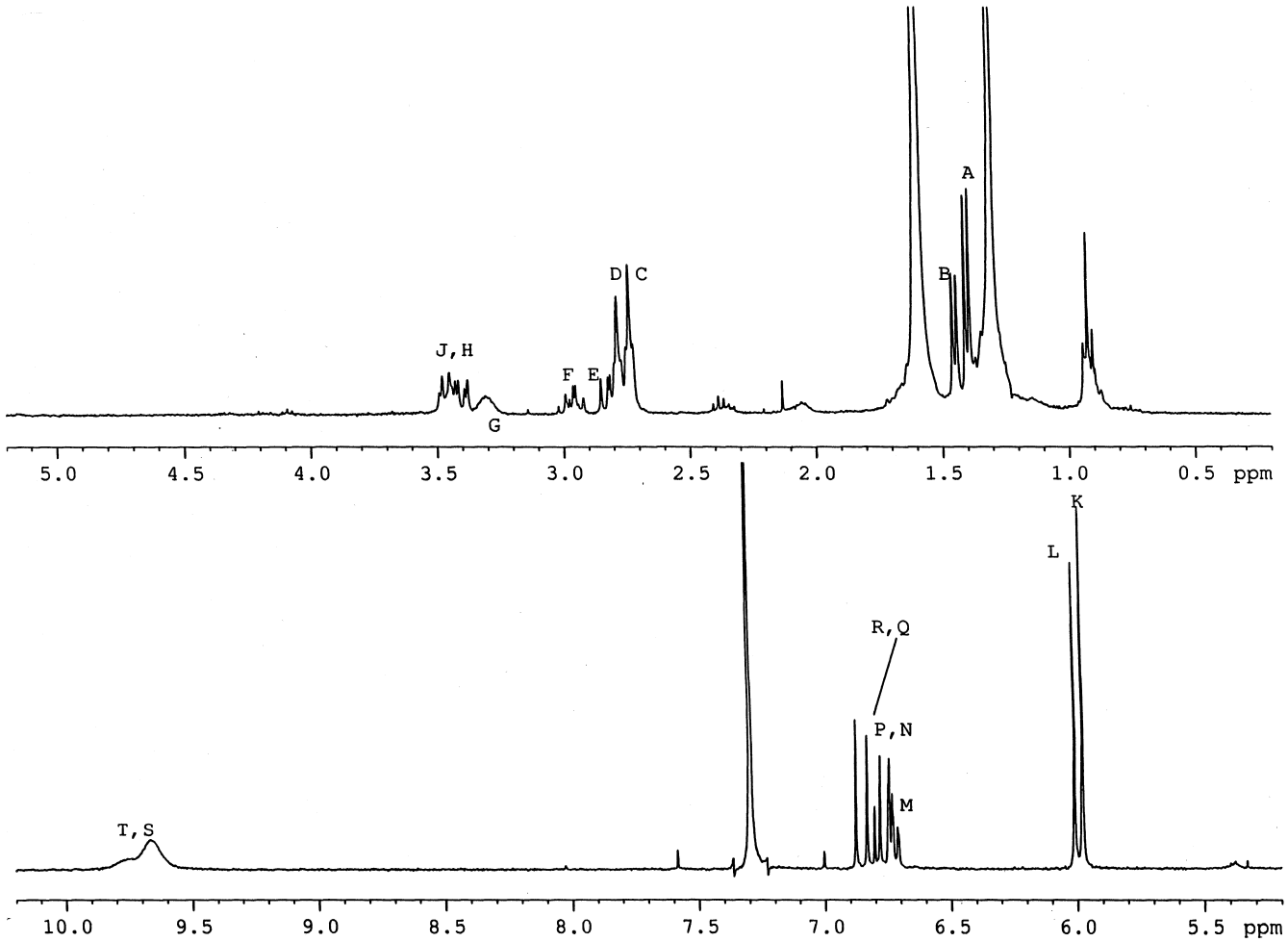

FIG. 8—*The 360.13 MHz ^1H NMR spectrum of the mixture of MDMA and CI-MDMA (HCI forms) in deuteriochloroform.*

Conclusions

The analysis of "designer" drugs such as ecstasy by chromatographic methods is complicated by the unavailability of reference materials. However, by using a systematic approach involving techniques such as GC/MS and ^{1}H-NMR, it is relatively simple to achieve an unambiguous identification of these drug types. In this instance we have identified a chlorinated analog of MDMA by mass spectrometry, and have confirmed its isomeric form as 2-chloro-4,5-methylenedioxymethylamphetamine by ^{1}H-NMR.

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Additional information and reprint requests:
Robert J Lewis, Ph.D.
Lothian and Borders Police Forensic Science Laboratory
11, Howden Hall Road
Edinburgh
Scotland, UK. EH16 6TF