TECHNICAL NOTE

CRIMINALISTICS

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Stereochemical Analysis of Methorphan Using (−)-Menthyl Chloroformate

ABSTRACT: Forensic laboratories around the world have seen an increase in the number of drug seizures containing methorphan. Levomethorphan is a narcotic and a controlled substance, and its enantiomer dextromethorphan is an antitussive agent used in over-the-counter medications. For the forensic analysis of seized drugs containing methorphan, it is important to report the stereochemical composition of the drug. Ideally, a method based on common forensic laboratory instrumentation is desirable. The use of the chiral derivatizing agent (−)-menthyl chloroformate followed by routine gas chromatography–mass spectrometry analysis of the derivative was shown to successfully determine the stereochemical composition of methorphan. This approach was applied to a street seizure containing methorphan proving that it was pure *dextro*methorphan. The derivatives of *dextro*- and *levo*methorphan were subjected to mass spectroscopic and nuclear magnetic resonance analysis, which confirmed that the structure of the derivatives remained unchanged as a result of the derivatization process.

KEYWORDS: forensic science, forensic chemistry, levomethorphan, dextromethorphan, stereochemical analysis, gas chromatography–mass spectrometry

Levomethorphan is similar in structure to morphine and is also a potent opiate narcotic analgesic. Listed in the *Controlled Substances (General) Regulations 2000* under the *Controlled Substances Act 1984*, levomethorphan is a Schedule 1 controlled drug. On the other hand, dextromethorphan (structure 1 in Fig. 1) shows no analgesic activity, but is an effective antitussive agent that is used in many over-the-counter (OTC) cough and cold medications (1–3). At therapeutic doses, dextromethorphan is considered a safe drug, but at high doses, it acts as a dissociative hallucinogen with effects similar to those produced by phencyclidine and ketamine (4–6). These effects are thought to be caused by the major active metabolite of dextromethorphan, dextrorphan (structure 2 in Fig. 1), that has been shown to have a high binding affinity for the *N*-methyl-D-aspartate (NMDA) receptor (4,7).

Recreational abuse of dextromethorphan has been on the rise as it is seen as a cheap and readily available alternative to ecstasy (3,4-methylenedioxymethampetamine) (4,6). Despite the reported abuse of dextromethorphan around the world, it has not been listed as an internationally controlled substance (6,8). For legal purposes, it is important to be able to determine the stereochemical composition of methorphan found in seized drugs (9). The ability to discriminate between such enantiomers is also important for toxicological studies (9).

As *dextro*- and *levo*methorphan are enantiomers of each other, they cannot be differentiated using conventional gas chromatography–mass spectrometry (GC–MS), the method of choice for the analysis of such illicit drugs in forensic laboratories. Differentiation

may be achieved using a chiral column, but these are expensive and tend to be very specific for a particular class of chiral compounds and so are not always successful (10). Kim et al. (11) developed a method for the separation of *dextro*- and *levo*methorphan by high performance liquid chromatography using a chiral column, while Lurie and Cox (3) achieved separation of *dextro*- and *levo*methorphan using capillary electrophoresis with dynamically coated capillaries. Alternatively, the traditional approach is to convert enantiomers to diastereoisomers using commercial chiral derivatizing reagents that can then be separated by conventional GC using achiral columns. Recently, we have used this method to successfully determine the stereochemical composition of methamphetamine obtained by the biotransformation of benzaldehyde (12).

Most alkaloid derivatization procedures involve the production of amides or carbamates, but methorphan is a tertiary amine, and consequently, it is not amenable to direct derivatization as this would lead to a quaternary ammonium salt that is not volatile and so not suitable for GC analysis. In such cases, chloroformate reagents can be used to achieve derivatization as they involve the dealkylation of the intermediate quarternary ammonium salt, resulting in the
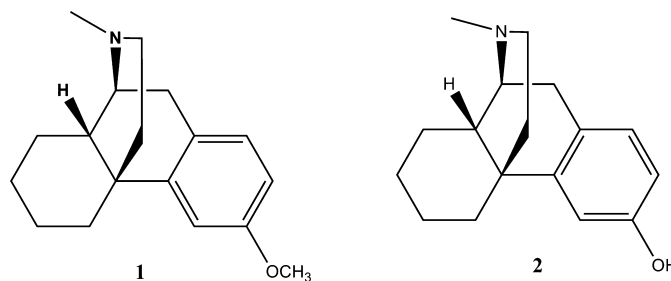
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FIG. 1—*Structure of dextromethorphan (1) and its metabolite, dextrorphan (2).*
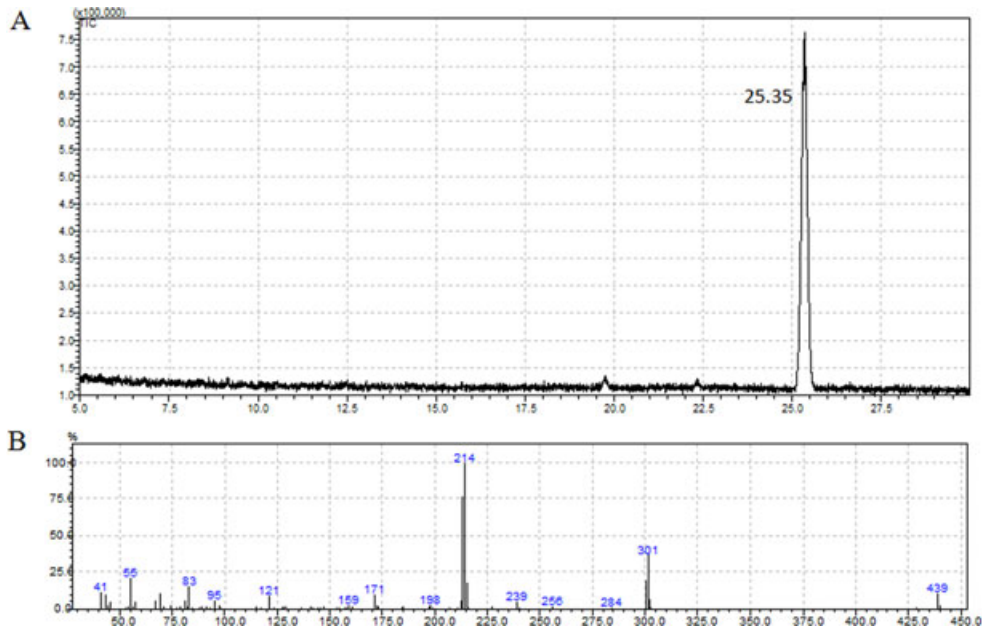
FIG. 2—(A) Total ion chromatogram and (B) mass spectrum of the dextromethorphan derivative.
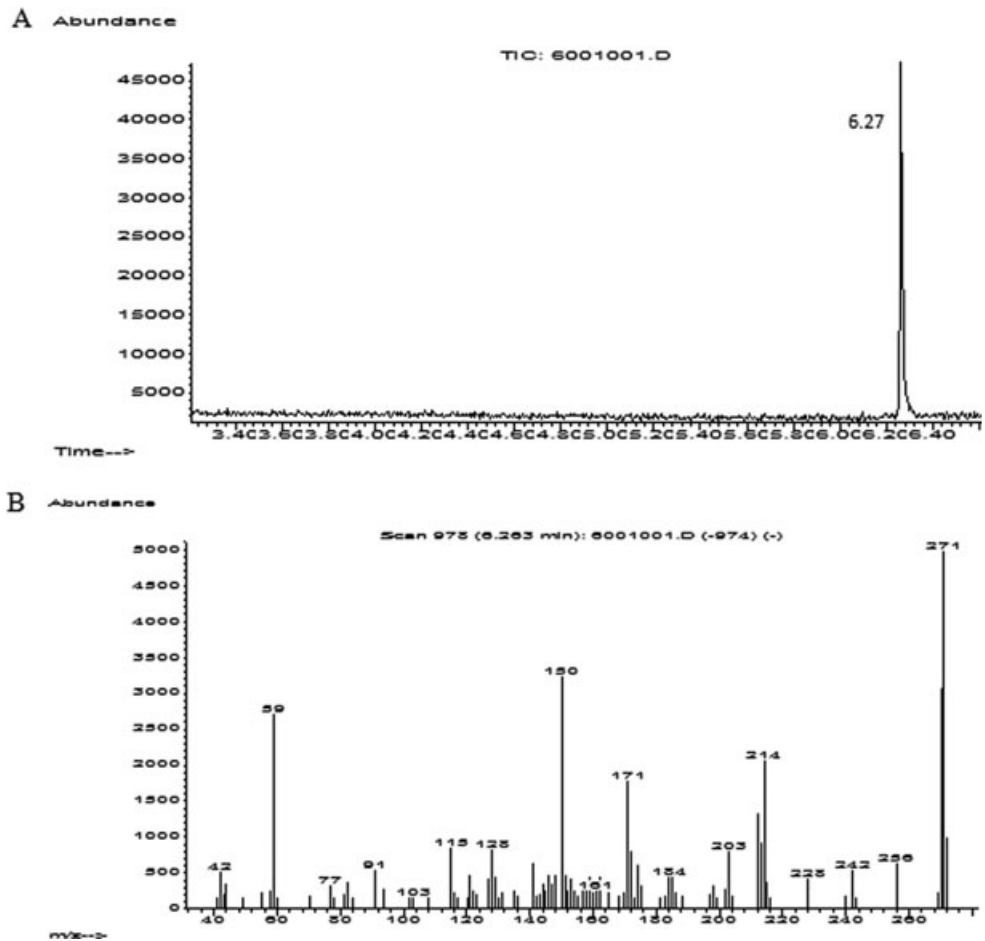
FIG. 3—(A) Total ion chromatogram and (B) mass spectrum of underivatized dextromethorphan (HP 6890 plus GC [15 m × 0.25 mm × 0.25 µm], HP-1MS column, HP5973 MSD, helium carrier gas, injector temperature 300°C, oven temperature 60°C, then ramped at 45°C/min to 300°C, then held for 4 min).

formation of acylated or carbamate derivatives (13). Recently, chloroformates have emerged as the preferred reagents for achieving *N*-demethylation of tertiary *N*-methyl amines (14).

Kristensen and Angelo (10) successfully derivatized methadone using (−)-menthyl chloroformate resulting in the formation of diastereomeric carbamate derivatives, which were separated on an achiral GC column. The reaction also was found to preserve the structure and not affect any of the chiral centers present in methadone, a crucial requirement for such a reagent. This led to our investigation into the use of (−)-menthyl chloroformate as a possible derivatizing agent for methorphan, and we report herein the results of these studies.

Materials and Methods

Chemicals and Reagents

(−)-Menthyl chloroformate (99% ee/GC) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI) *Robitussin Dry Cough Forte* (Wyeth Consumer Healthcare Pty Ltd, Baulkham Hills, New South Wales, Australia) was purchased from a local pharmacy. Levomethorphan (99%) was purchased from Cerilliant Analytical Reference Standards (Round Rock, TX). All other solvents and reagents used were of analytical reagent grade and were purchased from Merck Pty Ltd (Kilsyth, Victoria, Australia).
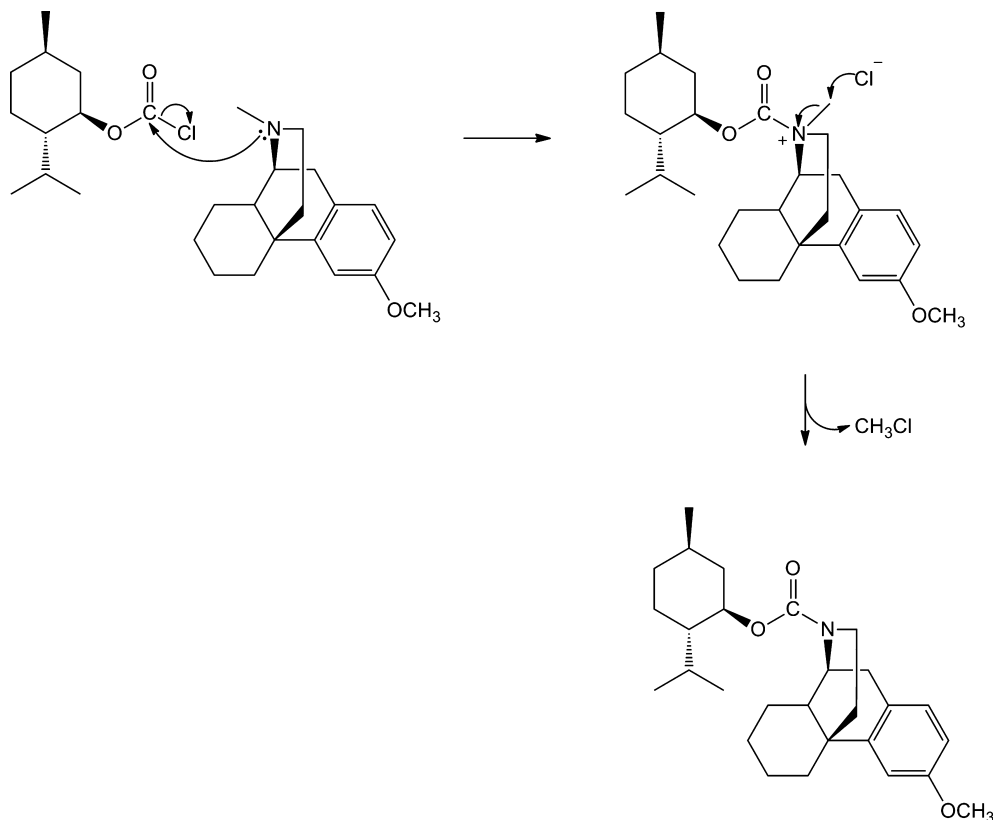

FIG. 4—*Proposed mechanism for the derivatization of dextromethorphan using (−)-menthyl chloroformate.*
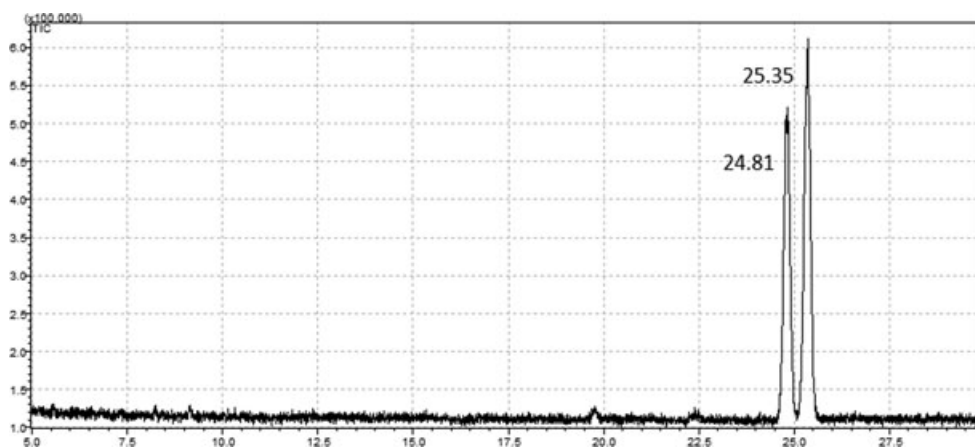

FIG. 5—*Gas chromatograph of an approximately equimolar mixture of the pure levomethorphan derivative (24.81 min) and the pure dextromethorphan derivative (25.35 min).*

Instrumentation

GC–MS analyses were performed on a Shimadzu GCMS-QP2010 (Rydalmere, New South Wales, Australia) using an Agilent HP-5MS capillary column (30 m × 0.25 mm ID, 0.25-μm film thickness; Mulgrave, Victoria, Australia). Helium was used as the carrier gas at a linear flow rate of 35 cm/s. The injector temperature was set to 250°C, with an isothermal oven temperature of 260°C. The mass selective detector operated between 40 and 500 m/z in electron impact mode with an ionization energy of 70 eV.

Nuclear magnetic resonance (NMR) spectra were run on a Bruker Avance III 600 (Alexandrina, New South Wales, Australia). ^{1}H, ^{13}C, COSY, heteronuclear multiple bond coherence (HMBC), and heteronuclear multiple quantum coherence (HMQC) spectra were acquired using standard Bruker pulse programs. Spectra were recorded at 90°C in deuterated dimethyl sulfoxide-d$_6$ (DMSO) and referenced to a residual solvent signal. Chemical shifts (δ) are reported as parts per million (ppm).

Purification of the dextromethorphan derivative was achieved using a Chromatotron Model 7924T (T-Squared Technologies, Inc., San Bruno, CA). Silica gel (Merck Kieselgel GF$_{254}$ Type 60) and calcium sulfate hemihydrate were the stationary phase used to coat the rotors. The mobile phase consisted of 5% ethyl acetate/hexane.

Purification of the levomethorphan derivative was achieved using normal-phase thin-layer chromatography (TLC) purchased from Merck with 10% ethyl acetate/hexane as the mobile phase.

Extraction of Dextromethorphan from Cough Syrup

Robitussin Dry Cough Forte (100 mL) was diluted with a solution of 10% NaOH (100 mL) and extracted with dichloromethane (DCM) (3 × 100 mL). The DCM extracts were combined, dried (Na$_2$SO$_4$), filtered, and evaporated to give an oil (226 mg).

Derivatization of Dextromethorphan

(−)-Menthyl chloroformate (182.5 μL) was added to the crude extract of dextromethorphan (226 mg) dissolved in toluene (2.26 mL). The reaction mixture was refluxed for 1 h. After this time, the reaction mixture was cooled, and ammonia solution (1 mL) was added. The resultant mixture was then extracted with ether (30 mL). The ethereal layer was washed with a solution of 10% HCl (3 × 10 mL), dried (Na$_2$SO$_4$), filtered, and evaporated to give a pale-yellow sticky oil (193 mg). Purification of the dextromethorphan derivative by way of centrifugal chromatography resulted in a colorless gum (103 mg, 28% purified yield).

Derivatization of Levomethorphan

(−)-Menthyl chloroformate (4.4 μL) was added to levomethorphan (5 mg) dissolved in toluene (50 μL). The reaction was heated in a sealed GC vial at 110°C for 1 h. After this time, the reaction mixture was cooled, and a few drops of ammonia solution were added to the reaction mixture, which was then diluted with DCM. Water (2 mL) was added, and the reaction mixture was extracted with DCM (4 mL). The DCM layer was dried (Na$_2$SO$_4$), filtered,
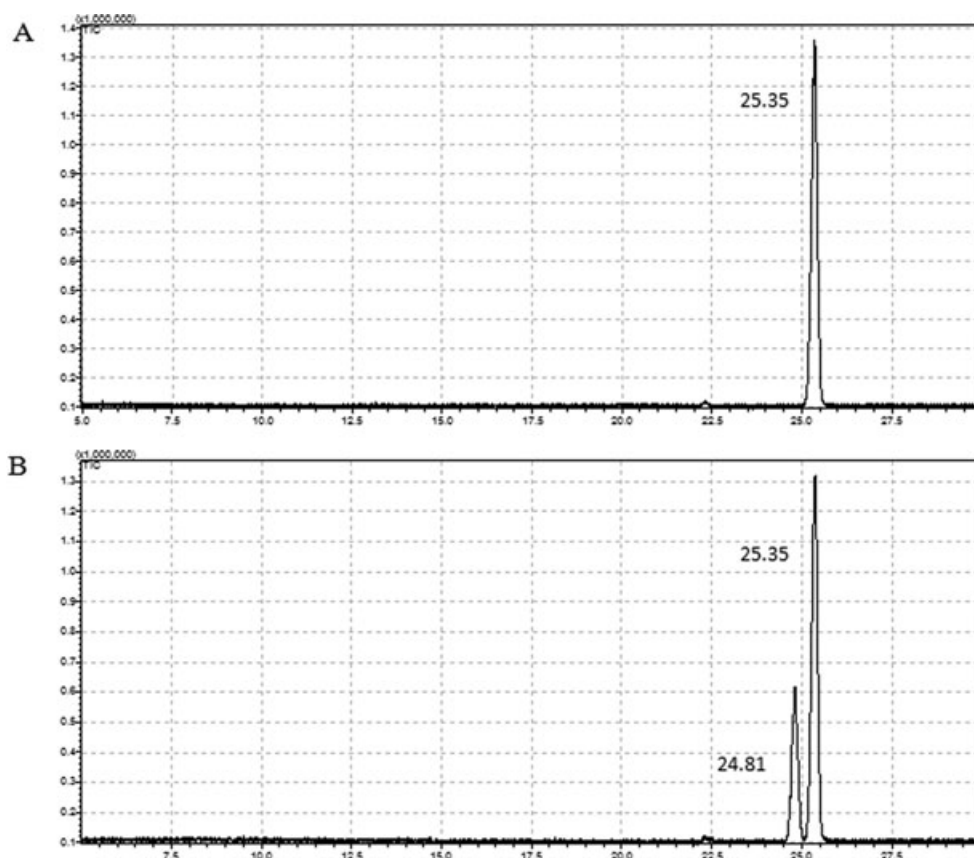

FIG. 6—*(A) Total ion chromatogram of a derivatized tablet extract (known to contain methorphan) showing one pure enantiomer, dextromethorphan (25.35 min), and (B) the derivatized tablet extract spiked with the levomethorphan derivative (24.81 min).*

and evaporated to give a white powder (9.1 mg). Purification of the levomethorphan derivative by way of TLC resulted in a white powder (1.3 mg, 16% purified yield).

Derivatization of a Case Sample Containing Methorphan

A solution of 20% sodium hydroxide (5 mL) was added to 100 mg of a crushed tablet known to contain methorphan. The mixture was then extracted with DCM (3 × 2 mL). The combined DCM extract was dried (Na$_2$SO$_4$), filtered, and evaporated. Toluene (100 μL) was added to the residue, and (−)-menthyl chloroformate (8.3 μL) was added, and the reaction mixture was heated at 110°C for 1 h. After this time, the reaction mixture was cooled, and a solution of 10% potassium hydroxide (100 μL) was added. The basified reaction mixture was then extracted with DCM (1 mL), and 1 μL was injected onto the GC–MS for analysis.

Results and Discussion

Derivatization of Methorphan Using (−)-Menthyl Chloroformate

Initially, the derivatization of dextromethorphan using (−)-menthyl chloroformate was investigated as it was readily available from OTC medicines. The reaction involved heating a mixture of (−)-menthyl chloroformate and dextromethorphan dissolved in toluene, as described in Materials and Methods. GC–MS analysis of the reaction mixture after 1 h showed that the starting material had been consumed and that the major product (retention

time = 25.35 min) gave a molecular ion of 439 m/z, consistent with the molar mass of the derivative. Figure 2 shows the resulting total ion chromatogram and mass spectrum of a purified sample of the dextromethorphan derivative. For comparison, the GC–MS of dextromethorphan (retention time = 6.27 min) is shown in Fig. 3. The mass spectrum of the peak as a result of dextromethorphan shown in Fig. 3B gives a molecular ion at 271 m/z consistent with the molar mass of methorphan. For the derivatization of levomethorphan, the same procedure was applied. Interpretation and structure confirmation of methorphan derivatives by MS and NMR will be discussed later.

A proposed mechanism for the derivatization of dextromethorphan is shown in Fig. 4. The mechanism involves initial nucleophilic attack of the tertiary amine at the chloroformate carbonyl carbon, with subsequent displacement of the chloride ion producing a quaternary ammonium salt. Demethylation is achieved by nucleophilic attack (in this case by the chloride ion) on the N-methyl group to yield the dextromethorphan derivative.

Chromatography of Levomethorphan and Dextromethorphan Derivatives

Injection of an approximately equimolar mixture of the levomethorphan and dextromethorphan derivatives onto a 30-m HP-5 capillary column, using an isothermal GC oven temperature of 260°C resulted in baseline separation, as can be seen in Fig. 5. Comparison with the retention times of the pure diastereoisomers indicates that the levomethorphan derivative elutes at 24.81 min and, the dextromethorphan derivative elutes at 25.35 min.
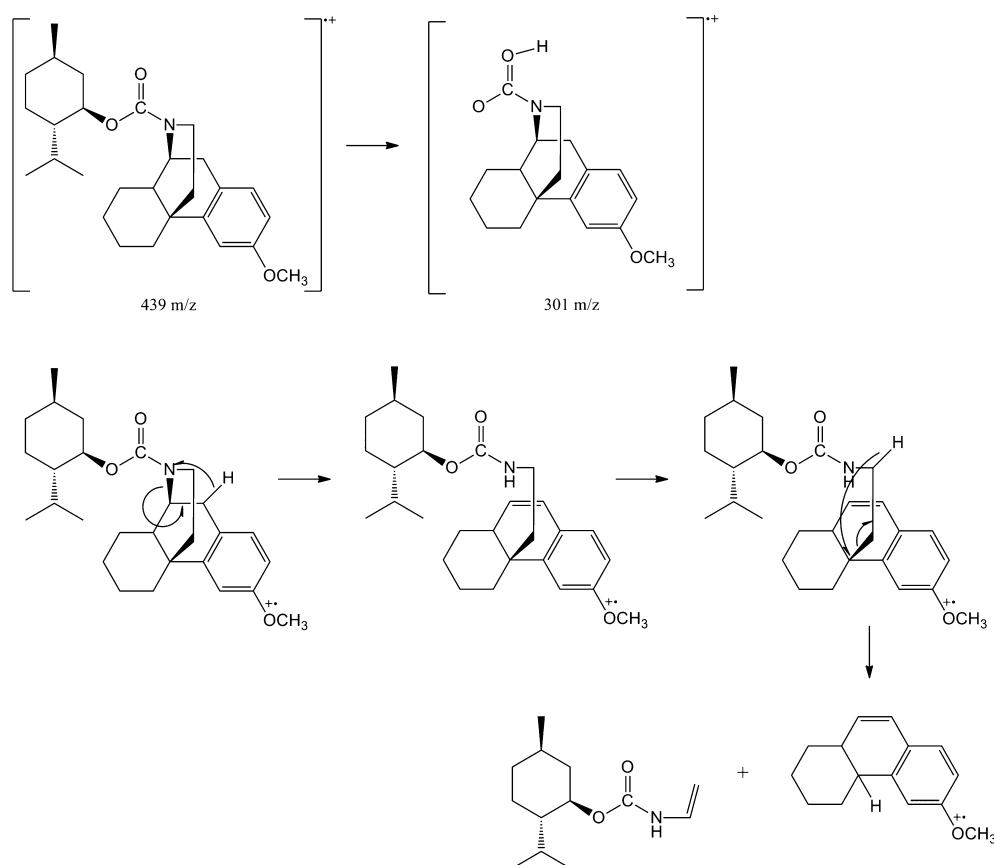

FIG. 7—Rationalization of the mass spectral fragmentation of the dextromethorphan derivative.

Stereochemical Analysis of an Unknown Methorphan Sample

Analysis was performed on a tablet taken from a case sample where previous analysis indicated that it contained methorphan. The tablet was crushed, extracted, and derivatized as described in Materials and Methods. GC–MS analysis of this derivatized sample resulted in the total ion chromatogram shown in Fig. 6A. Based on the retention time and comparison to our standards, the stereochemistry was determined to be pure *dextro*. To further support our stereochemical assignment, the sample was then spiked with the pure levomethorphan derivative, which on analysis resulted in the total ion chromatogram shown in Fig. 6B.

These results demonstrate that (−)-menthyl chloroformate can be used to derivatize *dextro-* and *levo*methorphan, yielding products that can be separated by conventional GC, thus allowing unequivocal determination of which enantiomer is present in street seizures containing methorphan. The next task was to confirm that the derivatization process did not alter the methorphan structure in any way and so retains the stereochemical integrity of the compounds under investigation.

Characterization of the Dextromethorphan Derivative Based on Mass Spectral Fragmentation

The mass spectrum of the dextromethorphan derivative appears to be consistent with its proposed structure. The 439 m/z molecular ion is consistent with the molar mass of the expected product. Hydrogen abstraction from the menthol moiety followed by electron rearrangement is believed to lead to the 301 m/z ion, as shown in Fig. 7. While loss of the ethanamine bridge from the molecular ion, typical of opiate alkaloids (15), is believed to lead to the 214 m/z base peak (Fig. 7). This loss is also evident in the mass spectrum of dextromethorphan shown in Fig. 3 also resulting in an ion at 214 m/z. Further analysis of the proposed derivative structure was performed by detailed NMR spectroscopy to affirm that the structure of the methorphan part of the derivative had not been altered in any way by the derivatization process.

Characterization of Methorphan Derivatives Using NMR

A sample of the purified dextromethorphan derivative was analyzed by proton NMR under standard conditions. Compared with the NMR spectrum of dextromethorphan, obtained under identical conditions, a significant increase in spectral complexity was observed. This made comparison of the two spectra and interpretation of the dextromethorphan derivative spectrum impossible. Experience with other such amides (16) and with reference to the NMR literature (17) indicated that the increase in spectral complexity was the result of restricted rotation around the amide bond because of resonance leading to the formation of distinct rotamers. Hence, the spectrum consisted of a mixture of rotamers resulting in many overlapping peaks producing the observed increase in complexity. To overcome this, the sample was run at increasing temperatures until coalescence of the signals was achieved. This occurred at 90°C in deuterated DMSO. Under these conditions, we obtained excellent resolution, comparable to that seen for underivatized dextromethorphan and so acquired ^{1}H, ^{13}C, correlation (COSY), HMQC, and HMBC spectra. Detailed analysis of these spectra, and with reference to published spectral data of pure dextromethorphan (18) and (+)-menthol (19), allowed full assignment of the resonances in the ^{1}H and ^{13}C spectra, as summarized in Table 1. These assignments refer to the proposed structure of the dextromethorphan derivative shown in Fig. 8.

TABLE 1—*Proton and carbon assignment for the dextromethophan derivative and the proton assignment for the levomethophan derivative.*

Assignment as in Fig. 8	Dextromethorphan Derivative		Levomethorphan Derivative
	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)
1	7.07	129.35	7.07
2	6.78	112.39	6.78
3	–	158.91	–
4	6.87	111.48	6.86
5a	1.33	36.37	1.35
5e	2.42	36.37	2.44
6a	1.23	22.20	1.24
6e	1.55	22.20	1.57
7a	1.40	26.25	1.39
7e	1.68	26.25	1.68
8a	1.03	26.33	1.03
8e	1.50	26.33	1.50
9e	4.27	50.08	4.26
10a	2.58	31.37	2.59
10e	3.11	31.37	*
11	–	128.31	–
12	–	140.50	–
13	–	37.73	–
14a	1.68	44.15	1.68
15a	1.55	41.70	1.57
15e	1.40	41.70	1.39
16a	2.52	38.40	2.50
16e	3.80	38.40	3.79
17	–	155.00	–
18a	4.54	74.83	4.54
19a	1.40	47.48	1.39
20a	0.90	34.42	†
20e	1.68	34.42	1.68
21a	1.11	24.33	1.12
21e	1.68	24.33	1.68
22a	1.50	31.50	1.50
23a	0.99	41.70	1.03
23e	1.99	41.70	2.01
24	1.93	26.99	1.86
25	0.84	17.31	0.82
25′	0.95	20.82	0.91
26	0.94	22.20	0.95
27	3.78	55.59	3.79

*This signal is obscured by a large solvent peak, and so no chemical shift could be provided.

†This signal is obscured by the large methyl doublets because of H25 and H26, and so no chemical shift could be provided.
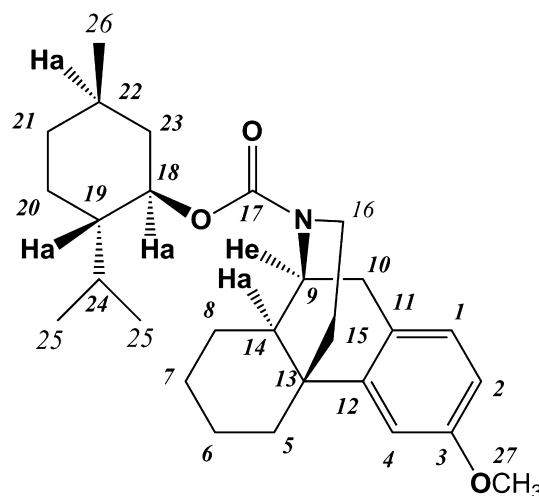

FIG. 8—*The proposed structure of the dextromethorphan derivative.*

The detailed NMR analysis confirmed the proposed structure of the dextromethorphan derivative. The derivatization procedure did not in any way alter the structures of either the methorphan or the menthyl parts of the derivative. So this derivatization method can be used with the utmost confidence in assigning the stereochemical composition of seized samples containing methorphan.

Only proton NMR analysis of the levomethorphan derivative was carried because of the small amount of the derivative available. The chemical shifts of most of the proton resonances listed in Table 1 are identical to the equivalent signals in the dextromethorphan derivative. We could see no significant difference in the chemical shifts of the proton signals because of H9e and H14a attached to the chiral carbons C9 and C14 and so could not confidently differentiate these two diastereoisomers by proton NMR.

Conclusion

Identifying the stereochemical composition of methorphan in street seizures is of great importance to law enforcement agencies as levomethorphan is a Schedule 1 controlled drug. In this study, we have shown that differentiation of the two enantiomers, dextromethorphan and levomethorphan, can be achieved under achiral GC–MS conditions following derivatization using (−)-menthyl chloroformate. Unlike other chiral derivatizing agents for amines that result in the formation of quaternary ammonium salts unsuitable for GC analysis, (−)-menthyl chloroformate gives a stable carbamate that can be chromatographed without decomposition. Under our conditions, baseline separation of the derivatives of the two enantiomers could be easily achieved. We have also successfully applied this method, to a case sample where the stereochemistry of the methorphan was initially unknown, proving conclusively that it was pure dextromethorphan.

Analysis of the mass spectra of the *dextro* and *levo* derivatives indicated that their structures remained unchanged by the derivatization process. This was subsequently confirmed by the detailed analysis of the ^{1}H, ^{13}C, and two-dimensional NMR spectra of the dextromethorphan derivative. The ^{1}H spectrum of the levomethorphan derivative was essentially identical to that of the *dextro* derivative, and we could see no significant difference in the chemical shifts of the protons at the chiral carbon centers C9 and C14.

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