*Journal of Chromatography*, 154 (1978) 76–83


CHROM. 10,869

## Note

# Identification of $O^3$-monoacetylmorphine in illicit heroin using gas chromatography–electron-capture detection and mass spectrometry*

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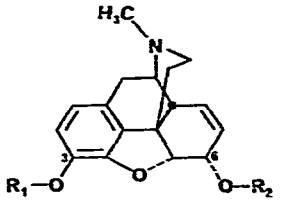
(Received December 27th, 1977)

Illicit drugs that are produced clandestinely often contain trace impurities. The identification and quantitation of these impurities is of importance for forensic purposes, especially in providing evidence of conspiracy[1–6]. The trace compounds in illicit heroin which are used for forensic purposes include codeine and morphine and their acetylated by-products, namely, acetylcodeine, $O^6$-monoacetylmorphine (Ia) and $O^3$-monoacetylmorphine (Ib). Previous methods used to quantitate these substances have utilized gas chromatography with flame ionization detection (FID)[1,6]. The presence of the isomer Ib was not established by this procedure, however, due to its co-elution with major heroin by-products, as well as its low levels in heroin. In addition, other studies have not satisfactorily demonstrated the presence of Ib in illicit heroin[7–11]. Most of the derivative Ia results from hydrolysis of heroin (Ie),




(I)

(a) $R_1$ = H ; $R_2$ = $COCH_3$

(b) $R_1$ = $COCH_3$ ; $R_2$ = H

(c) $R_1$ = $COC_3F_7$ ; $R_2$ = $COCH_3$

(d) $R_1$ = $COCH_3$ ; $R_2$ = $COC_3F_7$

(e) $R_1$ = $COCH_3$ ; $R_2$ = $COCH_3$

* Presented in part at the 25th Annual Conference on Mass Spectrometry and Allied Topics, Washington, D.C., May 29–June 3, 1977, and at the First Joint Meeting of the Mid-Atlantic Association of Forensic Scientists and Northeast Association of Forensic Scientists, Mt. Laurel, N.J., April 15–16, 1977.

whereas most Ib results from the incomplete esterification of morphine with acetic anhydride[12].

In order to allow for the accurate quantitation of trace-level impurities in illicit heroin, gas chromatography–electron-capture detection (GC–ECD) methodology has been developed recently for codeine, morphine and $O^6$-monoacetylmorphine[13]. During the utilization of this method, a number of unidentified peaks appeared in the gas chromatograms of all of the illicit heroin samples examined. One of these peaks resulted from the presence of Ib.

In the work described here the samples were derivatized with heptafluorobutyric anhydride (HFBA) followed by GC–ECD quantitation. Earlier studies have reported the excellent response of ECD towards perfluorinated compounds[14,15]. Following the quantitative analysis, mass spectral characterization of the $O^6$- and $O^3$-monoacetylmorphine derivatives (Ic, Id) was accomplished, applying the approach of Anggard and Sedvall[16] used for the ECD quantitation of catecholamine metabolites.

## EXPERIMENTAL

### *Apparatus*

The quantitative analysis was done on a Perkin-Elmer 990 gas chromatograph equipped with a $^{63}Ni$ ECD and interfaced with an Infotronics CRS 208 integrator. The gas chromatograph was fitted with a 6 ft. × 4 mm I.D. glass column packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh). The column, injection and detector temperatures were maintained at *ca.* 230, 275 and 300°, respectively. Nitrogen was used as carrier gas at a flow-rate of 100–120 ml/min.

GC–mass spectrometry (MS) studies were performed on a Finnigan 3100D GC–MS quadrupole instrument equipped with a jet separator. The GC–MS instrument was fitted with a 3 ft. × 2 mm I.D. glass column packed with 3% OV-17 on Gas-Chrom Q (80–100 mesh). Other parameters: emission current, 0.5 mA; electron energy, 70 eV; preamplifier sensitivity, $10^{-7}$ A/V; electron multiplier, 2.8 kV. The column was programmed from 180° with a helium carrier gas flow-rate of 25 ml/min.

### *Solvents, chemicals and chromatographic materials*

The solvents used in this study were distilled in glass, and all of the chemicals were of reagent grade quality. Chromatographic materials were obtained from the usual commercial sources. The standard of $O^3$-monoacetylmorphine was obtained from the Division of Pharmaceutical Chemistry, Food and Drug Administration (Washington, D.C., U.S.A.) and prepared by the method of Welsh[7]. All of the other standards were supplied by the Special Testing and Research Laboratory, Drug Enforcement Administration (McLean, Va., U.S.A.).

### *Quantitative analysis*

A quantity of 1–10 mg of heroin was weighed accurately in duplicate into the bottom of a 13-ml conical centrifuge tube. 1.0 ml of acetonitrile (containing 0.20 mg/ml of chlorpromazine hydrochloride internal standard) was added and the tube was swirled to dissolve the sample. Then 50 μl of HFBA were added and the tube was agitated to dissolve the HFBA and swirled occasionally for 15 min. Finally, 2.0 ml of

light petroleum and 5.0 ml of saturated sodium bicarbonate (pH 8.7; prepared fresh daily) were added. The tube was shaken vigorously for 5–10 sec, vented carefully and then centrifuged to clarify the layers. About 2–3 $\mu$l of the petroleum layer were injected into the gas chromatograph under the conditions described earlier. If necessary, further dilutions were made with petroleum.

A working standard curve of $O^3$-monoacetylmorphine was established by treating varying amounts (10–200 $\mu$g) of it with HFBA and proceeding with the sample analysis as above. All of the calculations were based on relative peak areas of the $O^3$-monoacetylmorphine–HFB and chlorpromazine hydrochloride internal standard.

*GC–MS analysis*

The derivatization method was modified for GC–MS analysis. The sample (*ca.* 30 mg) was dissolved in 3.0 ml of acetonitrile in a 13-ml glass-stoppered conical centrifuge tube. About 50 $\mu$l of HFBA were added and the solution was allowed to react at room temperature for 5 min. About 6 ml of light petroleum followed by 5 ml of saturated sodium bicarbonate solution were then added. The mixture was shaken for *ca.* 10 sec to extract the derivatized heroin impurities into the petroleum layer. The latter was then removed and evaporated to dryness. The sample was

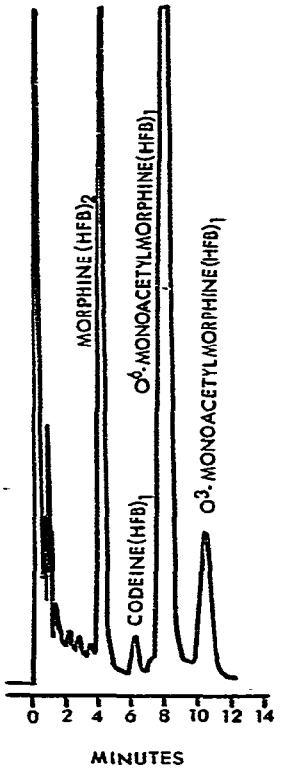



Fig. 1. Typical gas chromatogram indicating the presence of the heptafluorobutyrates of codeine, morphine, $O^6$-monoacetylmorphine and $O^3$-monoacetylmorphine.

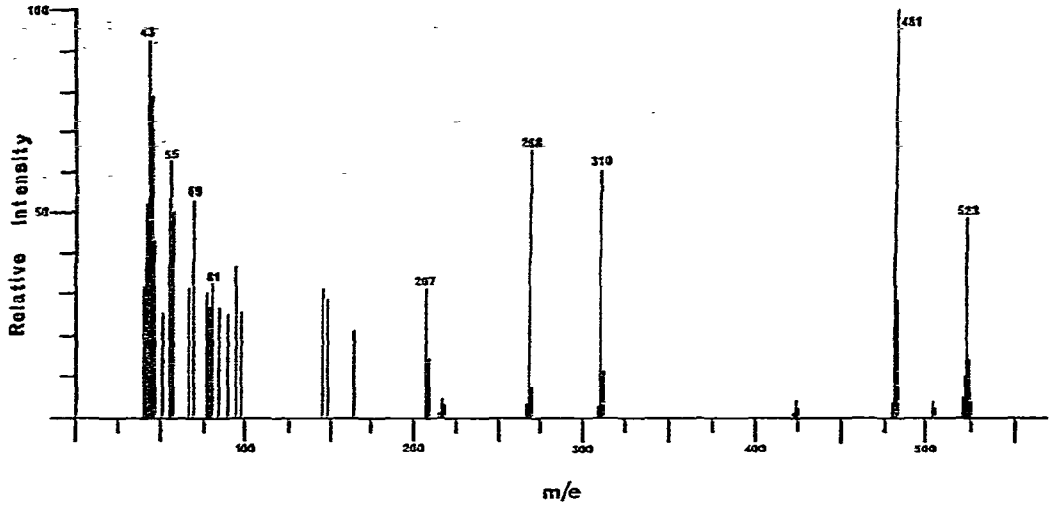



Fig. 2. Mass spectrum of $O^3$-monoacetylmorphine–HFB (Id).

redissolved in a small volume of anhydrous diethyl ether and injected into the GC–MS instrument. The procedure was used on standard $O^3$- and $O^6$-monoacetylmorphine, as well as on several illicit heroin samples.

## DISCUSSION

### *Quantitative analysis*

About twenty illicit, "uncut", brown and white heroin samples were subjected to analysis (see Fig. 1 for a typical sample chromatogram). All of the samples contained Ib, varying in content from 0.1 to 2%. In most of the samples the contents of

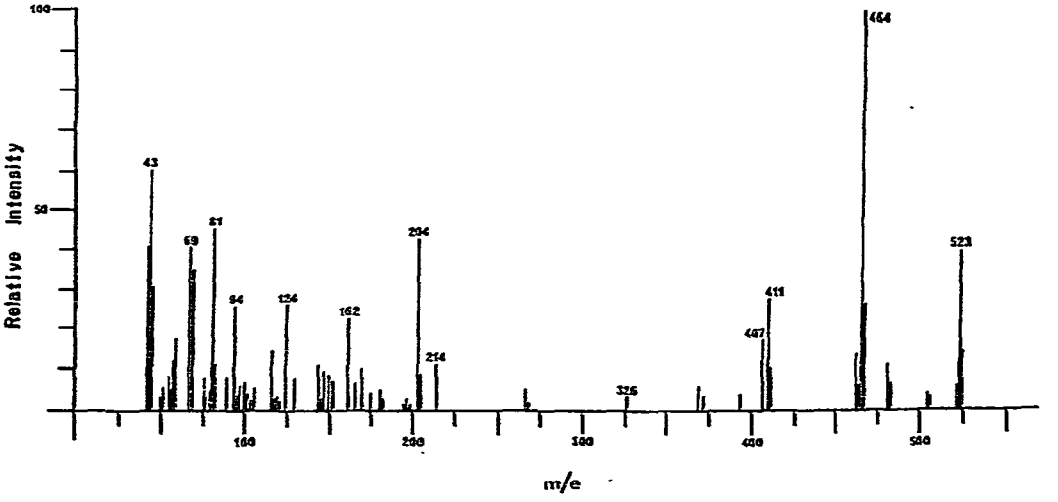



Fig. 3. Mass spectrum of $O^6$-monoacetylmorphine–HFB (Ic).

Ia and acetylcodeine varied from 3 to 15%, those of morphine and codeine varied from 0.01 to 0.5%. It was interesting that, even in illicit samples of very high purity, Ib could be detected and readily quantitated.

Most of the Ia in illicit heroin is due primarily to the ready cleavage of the $O^3$-acetyl group in heroin during hydrochloride formation and solvent removal. On the other hand, the majority of Ib is due to incomplete acetylation of morphine during the heroin synthesis. It has been observed that the formation of Ib is largely dependent upon the length and temperature of the reaction when using acetic anhydride. The product of reaction of morphine and acetic anhydride at room temperature is primarily $O^3$-monoacetylmorphine[12].

It is evident from Fig. 1 that an accurate quantitation of Ib can be dependent upon the amount of Ia in the sample. Since Ib elutes closely after Ia, large quantities of the latter can tail into the former, thus affecting the accuracy of the quantitation. Therefore, the chromatographic conditions may have to be modified in order to effect an accurate quantitation of Ib in some samples.

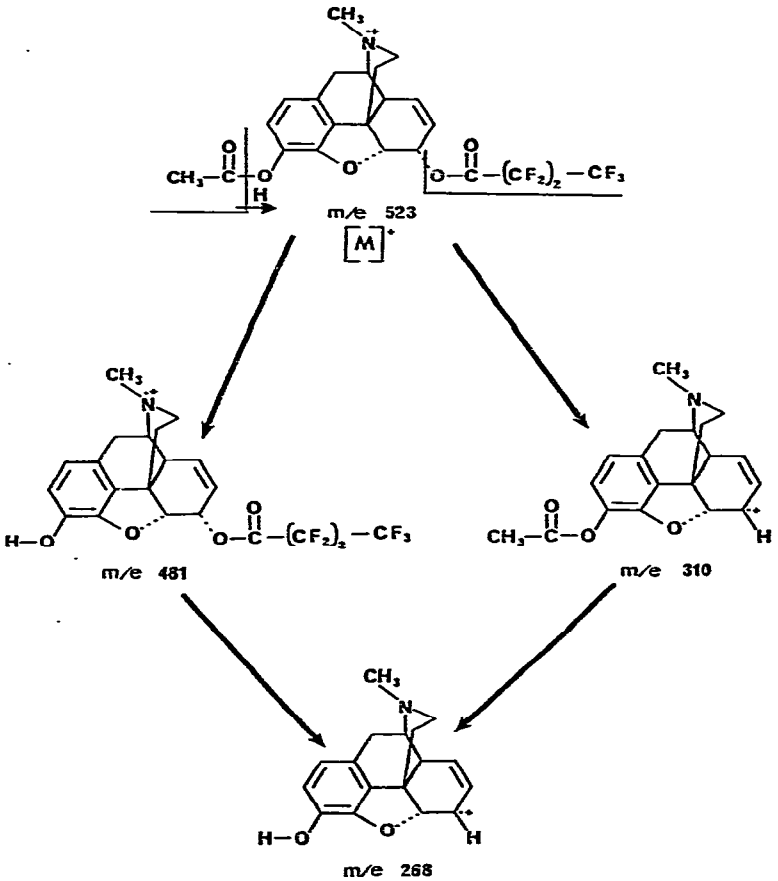



Fig. 4. Fragmentation of the $O^5$-acetate and $O^6$-heptafluorobutyrate esters of $O^3$-monoacetylmorphine-HFB (Id), showing the formation of the major ions.

The HFBA derivatives in light petroleum were stable for several hours. However, it is recommended that, upon derivative formation, the analysis be completed without delay.

*Mass fragmentation of $O^3$-monoacetylmorphine–HFB (Id) and $O^6$-monoacetylmorphine–HFB (Ic)*

The mass spectra of standard samples of Ib and Ia after derivatization with heptafluorobutyric anhydride were obtained (Figs. 2 and 3). The perfluorinated derivatives were then observed by GC–MS in illicit heroin. The presence of the perfluorinated group was supported by strong fragments at *m/e* 31 due to the $[CF]^+$ fragment, *m/e* 69 due to the $[CF_3]^+$ fragment and *m/e* 81 due to the $[C_2F_3]^+$ fragment. Both Id and Ic produced readily recognizable molecular ions at *m/e* 523. However, fragmentation of the ester groups (acetate and heptafluorobutyrate) at different positions in the molecules produced different major ions which could in turn be used to distinguish the two isomers.

$O^3$-Monoacetylmorphine–HFB produced a prominent ion at *m/e* 481 $[M-(CH_2CO)]^+$ from $O^3$-acetate fragmentation, *m/e* 310 $[M-(CF_3CF_2CF_2CO_2)]^+$ from fragmentation of the $O^6$-perfluorinated moiety and *m/e* 268 $[M-(CH_2CO + CF_3CF_2CF_2CO_2)]^+$, resulting from both fragmentation processes (Fig. 4). The $O^6$-acetyl group of $O^6$-monoacetylmorphine–HFB fragmented to give a base peak at *m/e* 464 $[M-CH_3CO_2]^+$ (Fig. 5). The heptafluorobutyryl portion at the $O^3$ position fragmented to give *m/e* 326 $[M-(CF_3CF_2CF_2CO)]^+$, although only of minor intensity. Cleavage of the ethanamine bridge in the morphine moiety, $[M-(CH_3CO_2 + CH_2=CH-NHCH_3)]^+$, would produce the fragment at *m/e* 407 (ref. 17) (Fig. 5). Finally, the fragment at *m/e* 411 could reasonably have formed via the retro Diels–

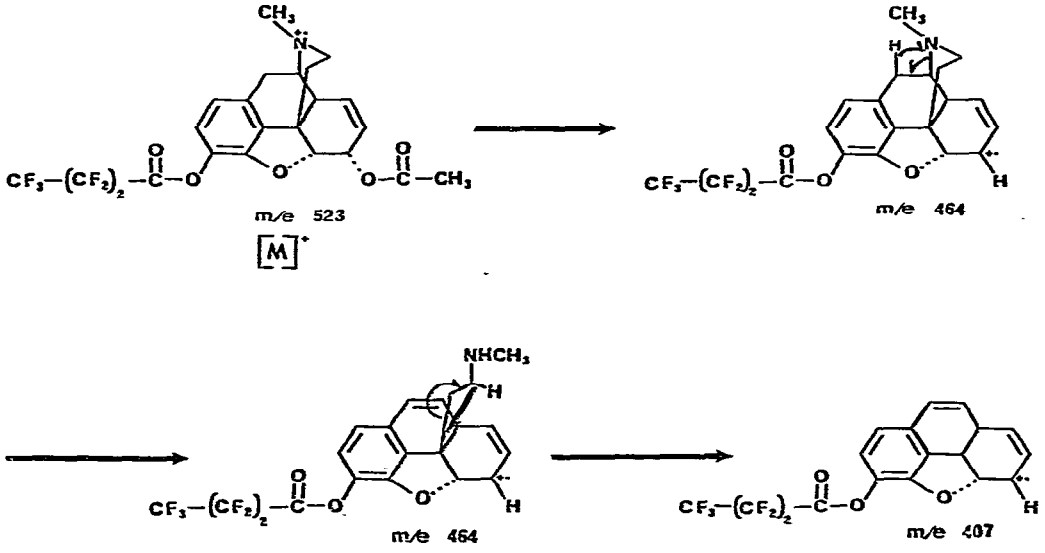



Fig. 5. Fragmentation of the $O^6$-acetate and ethanamine bridge of $O^6$-monoacetylmorphine–HFB (Ic), showing the formation of the major ions.

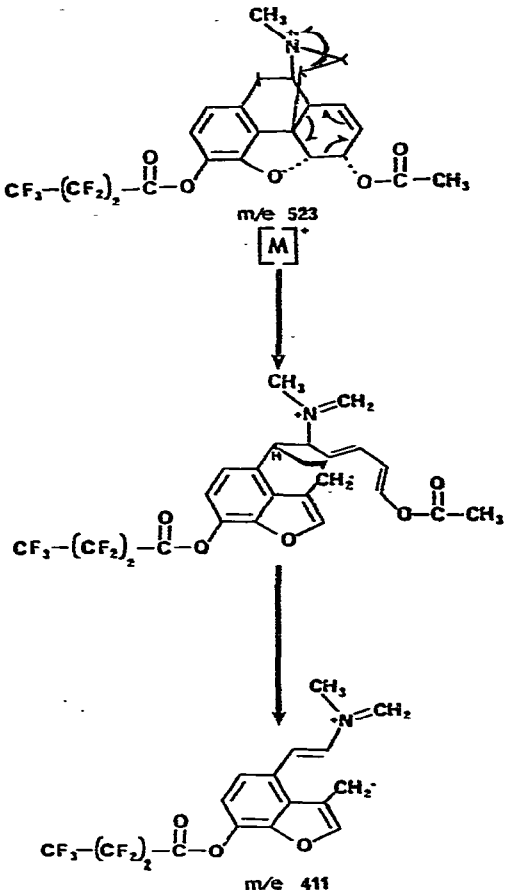



Fig. 6. Formation of the ion at *m/e* 411 from $O^6$-monoacetylmorphine–HFB (Ic) via a retro Diels–Alder mechanism.

Alder route shown by Audier *et al.*[18] (Fig. 6). This involves loss of the acetoxyl group and ring C carbon atoms 6, 7, 8 and 14 ($-C_6H_8O_2$; 112 a.m.u.). The resulting fragment then retains the heptafluorobutyrate at the $O^3$ position.

## REFERENCES

1 E. P. J. van der Slooten and H. J. van der Helm, *Forensic Sci.*, 6 (1975) 83.
2 J. M. Moore, *J. Chromatogr.*, 101 (1974) 215.
3 R. B. Barron, A. V. Kruegel, J. M. Moore and T. C. Kram, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 1147.
4 J. M. Moore, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1199.
5 T. C. Kram and A. V. Kruegel, *J. Forensic Sci.*, 22 (1977) 40.
6 S. P. Sobol and A. R. Sperling, *Forensic Science Symposium, Amer. Chem. Soc. National Meeting, Atlantic City, N.J., Sept. 1974, Amer. Chem. Soc. Symp. Ser.*, 18 (1975) 170.
7 L. H. Welsh, *J. Org. Chem.*, 19 (1954) 1409.
8 G. R. Nakamura, *J. Forensic Sci.*, 5 (1960) 259.
9 C. C. Fulton, *Microchem. J.*, 6 (1962) 51.

10 M. Lerner and A. Mills, *U.N. Bulletin on Narcotics*, 15 (1962) 37.
11 M. Klein, in A. Frigerio and E. L. Ghisalberti (Editors), *Mass Spectrometry in Drug Metabolism*, Plenum, New York, 1977, pp. 449–464.
12 J. M. Moore and M. Klein, unpublished results.
13 J. M. Moore, *J. Chromatogr.*, 147 (1978) 327.
14 F. Kawahara, *Anal. Chem.*, 40 (1968) 1009.
15 F. Kawahara, *Anal. Chem.*, 40 (1968) 2073.
16 E. Anggard and G. Sedvall, *Anal. Chem.*, 41 (1969) 1250.
17 A. Mandelbaum and D. Ginsburg, *Tetrahedron Lett.*, (1965) 2479.
18 H. Audier, M. Fetizon, D. Ginsburg, A. Mandelbaum and T. Rull, *Tetrahedron Lett.*, (1965) 13.