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The Identification of Heroin and Three Structurally Related Isoheroins

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ABSTRACT: Heroin and three structurally related isomers were studied. The configurational assignments of the structural isomers supported from nuclear magnetic resonance and mass spectrometry data are discussed. Infrared spectroscopy spectra are also presented. Gas chromatography procedures using packed and capillary systems demonstrated how the isomers could be best fully resolved.

KEYWORDS: toxicology, heroin, nuclear magnetic resonance, spectroscopic analysis

Earlier publications have reported the existence of structurally related isomers of morphine. In a three-part series, Schryver and Lees reported the existence of alpha, beta, and gamma isomorphines afforded from chloromorphide and bromomorphide upon hydrolysis with mild acetic acid [*1-3*]. Their conclusions were based primarily on the different physical properties exhibited by the isomers.

This paper is concerned with the acetylated derivatives of the isomorphines and morphine. The structures shown in Fig. 1 illustrate the nomenclature used to represent the numbering and designation for the C-6 and C-8 epimers. Alpha-isoheroin (II) whose -OR group at C-6 has been rotated is an epimer of heroin. Gamma-isoheroin (III) and beta-isoheroin (IV) are epimers with the -OR group at C-8 and the double bond positioned at C-6.

In this report we present data from two gas liquid chromatography (GLC) systems and spectral data from nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and electron impact mass spectometry (MS) that can help the forensic chemist to distinguish the heroin isomers properly. In addition, we discuss the NMR and MS data used to support the configurational assignments of the gamma and beta isomers.

Experimental Procedure

The GLC work was performed on a Hewlett Packard 5880A equipped with on-column injector ports and flame ionization detectors. The chromatographic packings were purchased from Alltech Associates and were 3% by weight of stationary phase on Chromosorb Q 100-120 mesh. The glass columns were packed in the laboratory and were 6-ft (2-m) long by 4-mm (0.16-in.) inside diameter (ID). The bonded fused silica open tubular capillary column, 25-m (82.5-ft) long by 0.25-mm (0.01-in.) ID bonded with 0.2 μm of polydimethylsiloxane (SE-30, OV-1), was obtained from Alltech Associates. Trimethylanilinium

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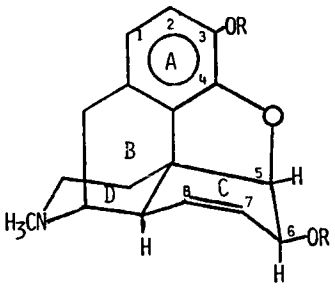
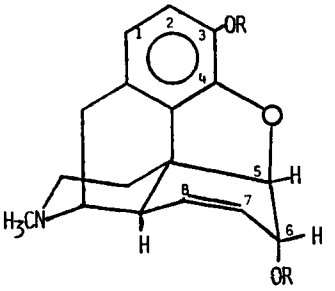
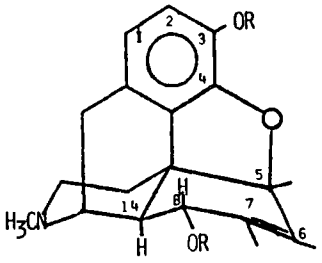


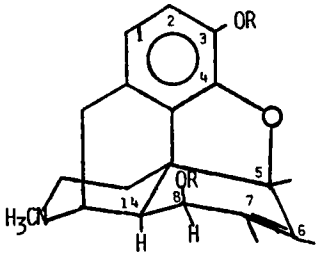
HEROIN (I)
MORPHINE (V)



ALPHA-ISOHEROIN (II)
ALPHA-ISOMORPHINE (VI)



GAMMA-ISOHEROIN (III)
GAMMA-ISOMORPHINE (VII)



BETA-ISOHEROIN (IV)
BETA-ISOMORPHINE (VIII)

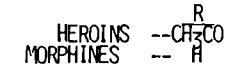


FIG. 1—*Structures of diacetylmorphine isomers.*

hydroxide (TMAH) 0.2*M* in methanol, used for the purpose of methyl alkylation, was purchased from Regis Chemical Company.

Alpha-isoheroin (II) was synthesized by the inversion of morphine using dimethylformamide dineophental acetal as described in a method by Barber and Rapoport [*4*] for the synthesis of isocodeine. Compound II was isolated by partition chromatography using 0.1*N* hydrochloric acid (HCL) as the stationary phase and water-washed chloroform as the mobile phase.

Gamma-isomorphine (VII) and beta-isomorphine (VIII) were synthesized from chloromorphide. Chloromorphide was prepared by refluxing morphine, dried at 110°C with phosphorous trichloride as described in a method by Schryver and Lees [*1*]. Purification of the crude product yielded a white crystalline powder (GC/MS *m/e* 303; melting point [m.p.] 191; lit. m.p. 190) [*1*]. Compounds VII and VIII were prepared by hydrolyzing chloromorphide with 1.0% acetic acid (2 g of chloromorphide to 15 mL of 1.0% acetic acid). The

solution was concentrated to a syrup on a steam bath. Ethanol was added repeatedly, followed by continued evaporation to enhance crystallization. The morphine isomers were isolated by adsorption column chromatography using aluminum oxide. Chloroform eluted beta-isomorphine, while gamma-isomorphine retained on the column. Isopropyl alcohol-cloroform (1:99) solution was required to elute Compound VII.

The heroin isomers were then prepared from the isomorphines by the acetylation. A Varian 390 (90-MHz) NMR was used to record spectra of the freebase isomers in deuterated chloroform with tetramethylsilane as reference. The mass spectrometry spectra were obtained on a Hewlett Packard Model 5985 GC/MS system interfaced with a Hewlett Packard Model 7920 data-processing system. All infrared spectra were recorded from potassium bromide discs using a Perkin Elmer Model 283B grating infrared spectrophotometer. Each isomer was dissolved in chloroform and passed through 1 g of aluminum oxide packed in a 5 3/4-in. (14.6-cm) pasteur pipet. Chloroform was removed by evaporation, and crystallization was enhanced by repeated addition and continued evaporation of petroleum ether.

Results and Discussion

Gas Chromatography

Tables 1 through 3 show the retention data of the isomers of heroin, morphine, and bis-trimethylsilylacetamide (BSA) derivatives of the morphine isomers obtained by capillary and packed column systems. Cocaine hydrochloride was used as reference to assign relative retentions (RRT). Retention index (RI) valves were assigned from linear retention data generated by a series of *n*-alkane hydrocarbon references and each isomer compound. The isomers were tested individually, as well as in mixtures to recognize baseline resolutions.

The packed column systems proved to be inadequate to resolve totally a mixture of heroin or morphine and their respective isomers. Table 1 lists the retention data obtained from the packed column systems. The OV-1 phase showed heroin eluting at 5.55 min. Beta-isoheroin gave the shortest retention of the heroin isomers at 4.83 min. A difference of 0.72 min between Compounds I and IV allowed a peak resolution of $R = 1.8$. Gamma-isoheroin expressed a peak resolution of $R = 1.2$ in relation to heroin. A peak resolution factor of 1.5

TABLE 1—*Results obtained by GLC. Column temperature = 245°C; packed column systems = 1.8 m by 4 mm; carrier gas-nitrogen = 40 mL/min.*

Compound	*RT*(min)	*RRT*	*RI*
	3% OV-1		
heroin (I)	5.55	2.63	2617
alpha-isoheroin (II)	5.23	2.48	2593
gamma-isoheroin (III)	5.00	2.37	2575
beta-isoheroin (IV)	4.83	2.29	2561
morphine (V)	3.62	1.72	2446
alpha-isomorphine (VI)	3.69	1.75	2453
gamma-isomorphine (VII)	3.69	1.74	2453
beta-isomorphine (VIII)	3.29	1.52	2407
	3% OV-225		
heroin (I)	22.2	5.02	3838
alpha-isoheroin (II)	19.04	4.31	3762
gamma-isoheroin (III)	17.39	3.93	3715
beta-isoheroin (IV)	17.29	3.90	3718

TABLE 2—*GLC results of morphine isomers—BSA derivatives, column temperature = 235°C; packed column systems = 1.8 m by 4 mm, carrier gas nitrogen = 40 mL/min.*

Compound	*RT*(min)	*RRT*
3% OV-1		
morphine-TMS[a] (V)	6.09	2.26
alpha-isomorphine-TMS[a] (VI)	4.87	1.8
beta-isomorphine-TMS (VIII)	4.88	1.81
gamma-isomorphine-TMS (VII)	4.95	1.83
3% OV-225		
morphine-TMS[a] (V)	5.89	1.11
alpha-isomorphine-TMS (VI)	4.05	0.77
beta-isomorphine-TMS (VIII)	5.96	1.13
gamma-isomorphine-TMS (VII)	4.22	0.80

[a]TMS = trimethylsilyl derivatives.

TABLE 3—*Results obtained by high-resolution GLC. Column temperature = 240°C; 25-m bonded FSOT; helium carrier gas; average velocity = 41.4 cm/s; split ratio = 60:1.*

Compound	*RT*(min)	*RRT*	*RI*
heroin (I)	6.09	2.34	2598
alpha-heroin (II)	5.8	2.23	2577
gamma-isoheroin (III)	5.57	2.14	2560
beta-isoheroin (IV)	5.34	2.05	2541
morphine (V)	4.07	1.57	2425
alpha-isomorphine (VI)	4.08	1.57	2426
gamma-isomorphine (VII)	4.11	1.58	2430
beta-isomorphine (VIII)	3.73	1.40	2388
BSA Derivatives			
morphine	4.89	1.88	
alpha-isomorphine-TMS	4.06	1.56	
beta-isomorphine-TMS	4.11	1.58	
gamma-isomorphine-TMS	4.11	1.58	

allows for 99.7% resolution [5]. On OV-225, heroin was easily resolved from Compounds II, III, and IV.

Chromatographic elution of the morphines on OV-225 was erractic. The high polarity of morphine, its isomers, and OV-225 phase resulted in nonreproducible data. Derivatization by the process of silylation using BSA generated useable data that are given in Table 2. The paired stereoisomers (V and VI, VII and VIII) were resolved to baseline, but a mixture of the four isomers presented co-elution problems.

The high-resolution capillary system allowed for baseline separation of the heroin isomers at 240°C (Fig. 2). Table 3 lists the retention data obtained from the bonded FSOT column. A chromatogram of morphine, Compounds VI, and VIII yielded partially overlapping peaks, while Compound VII was fully resolved. The BSA derivatives eluted in a similar fashion with baseline resolution of Compound V from the other overlapping isomers.

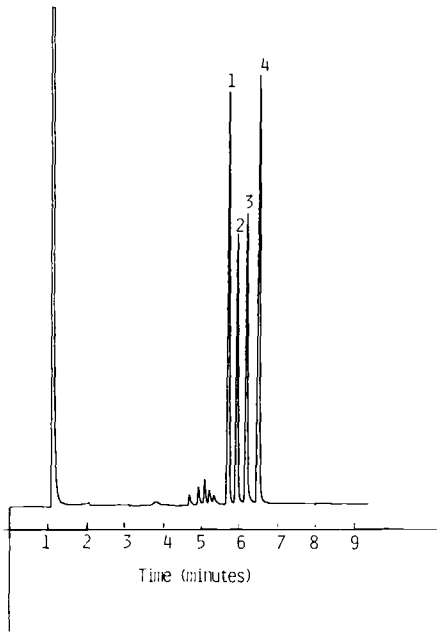


FIG. 2—*Capillary gas chromatogram of heroin isomers at 240°C;* 1 = *beta-isoheroin,* 2 = *gamma-isoheroin,* 3 = *alpha-isoheroin,* 4 = *heroin.*

Nuclear Magnetic Resonance

The axially oriented acetoxy at C-6 of alpha-isoheroin (Fig. 3) was characterized by Beazley [*6*]. The C-6 proton centered at 5.2 ppm and the somewhat shielded C-5 proton at 4.85 ppm easily distinguishes Compound II from the other heroin isomers.

Table 4 lists the chemical shifts and coupling constants for the protons about Ring C for gamma-isomorphine and beta-isomorphine. The C-8 protons in Compounds VII (3.60 ppm) and VIII (4.1 ppm) showed a chemical shift difference of 0.5 ppm. The upshift of the C-8 proton in VII suggests an axially oriented proton influenced by aromatic ring currents [*7*]. In beta-isomorphine, the equatorial C-8 proton is oriented away from the aromatic ring currents. The configurational assignments of the C-8 protons for VII and VIII are analogous to the configurational assignments established by Batterham et al. [*7*] for pseudocodeine and allopseudocodeine. Pseudocodeine and allopseudocodeine are analogous designations for gamma-isocodeine and beta-isocodeine, the methylated ethers of gamma-isomorphine and beta-isomorphine.

Acetylation of Compounds VII and VIII yielded gamma-isoheroin (Fig. 4) and beta-isoheroin (Fig. 5). The C-8 protons of III and IV were deshielded to 4.85 ppm and 5.36 ppm, respectively. Downshifts of 1 to 2 ppm are commonly observed for alpha protons of acetylated secondary alcohols [*8*].

Further, suggesting an axial configuration of the C-8 proton of Compound III is the dehidral relationship of protons C-8 and C-14. The large coupling J = 9.0 Hz, consistent with the Karplus equation [*9*], would indicate an axial-axial configuration of the vicinal C-8 and C-14 protons. And in beta-isoherion, the overlapping multiplet at 5.36 ppm; coupling J less than 4 Hz would indicate an equatorial-axial configuration of the vicinal C-8 and C-14 protons.

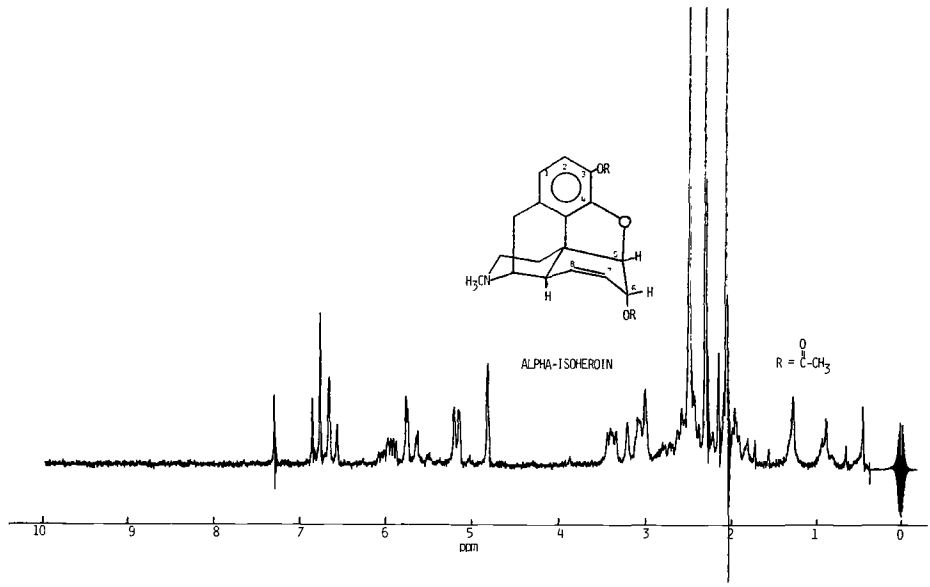


FIG. 3—*NMR spectrum of alpha-isoheroin.*

TABLE 4—*NMR spectra of gamma-isomorphine and beta-isomorphine.*

	Proton Number, ppm					*J* Constants, Hz			
Compound	5	6	7	8	14	5,6	6,7	7,8	8,14
gamma-isomorphine (VII)	4.93	5.58	5.88	3.60	2.58	3.3	10.5	1.5	9.3
beta-isomorphine (VIII)	4.88	5.74	6.01	4.10	"	3.0	10.0	6.0	5.2

"Overlapping with N-CH_3 resonance.

Mass Spectrometry

Electron impact (EI) GC/MS analysis of the heroin isomers produced the expected molecular ion at *m/e* 369 (Figs. 6 to 9). The fragmentation patterns of the heroin isomers showed strong similarities. As shown in Table 5, however, the isomers can be easily distinguished by the relative abundance or absence of certain ion fragments.

To support further the configurational assignment of gamma-isoheroin, we compared GC/MS spectra of TMAH derivatives of Compounds III, IV, and acetylpseudocodeine (Figs. 10 to 12). Acetylpseudocodeine was synthesized from codeine via the methods used to prepare the gamma and beta isoheroin isomers (base noncrys, hydrochloride m.p. 173 to 184; lit., base noncrys, hydrochloride m.p. 174 to 180) [*10*]. The methylated products of III and acetylpseudocodeine compared identically. The TMAH derivative MS spectrum of IV displayed obvious fragmentation that plainly distinguishes it from the gamma isomer. Its low relative abundance of the molecular ion could be attributed to the less favorable bulky alkyl group in the axial position.

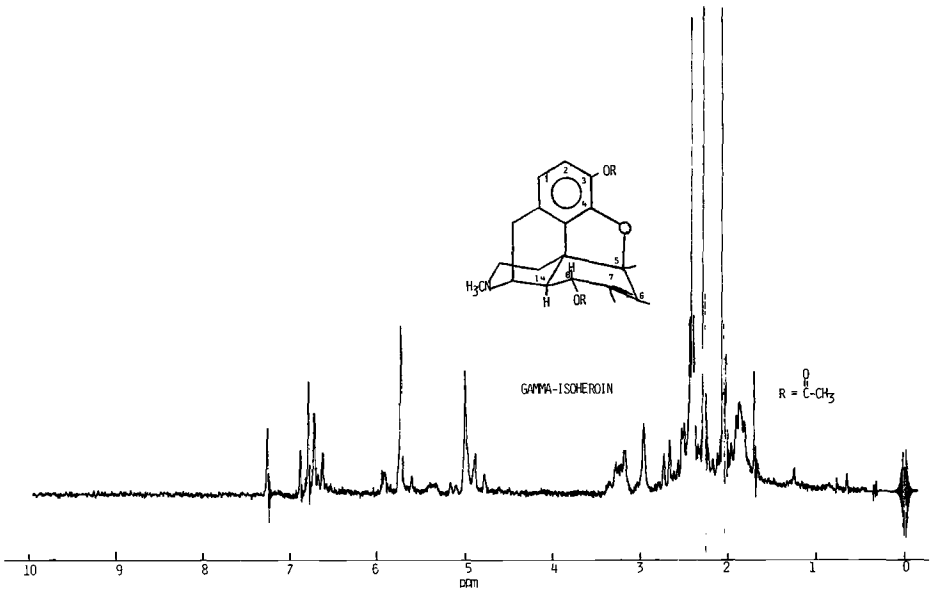


FIG. 4—*NMR spectrum of gamma-isoheroin.*

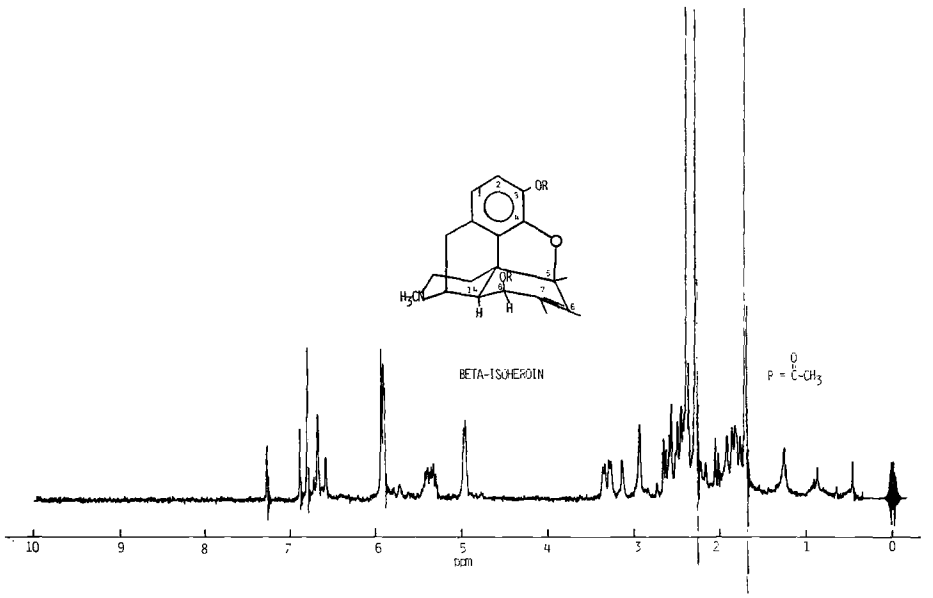


FIG. 5—*NMR spectrum of beta-isoheroin.*

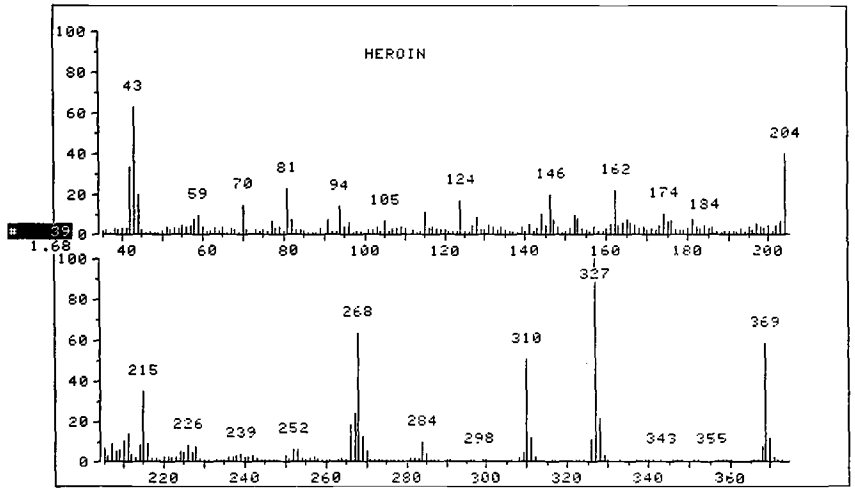


FIG. 6—*EI mass spectrum of heroin.*

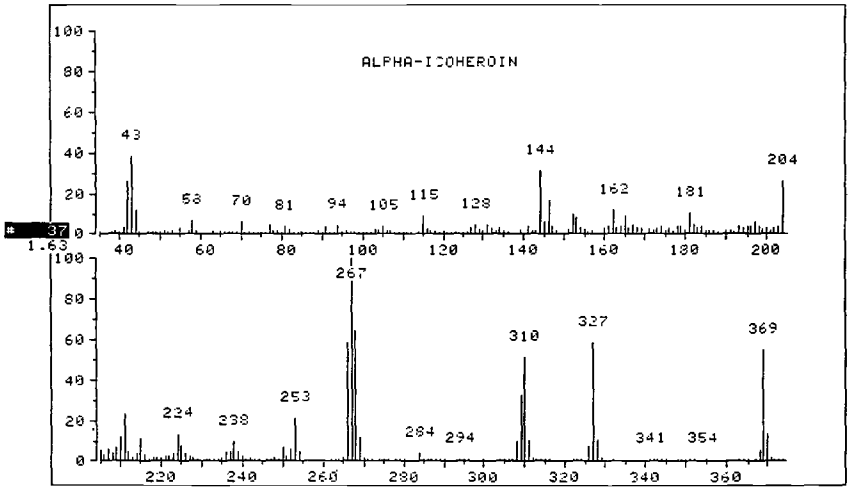


FIG. 7—*EI mass spectrum of alpha-isoheroin.*

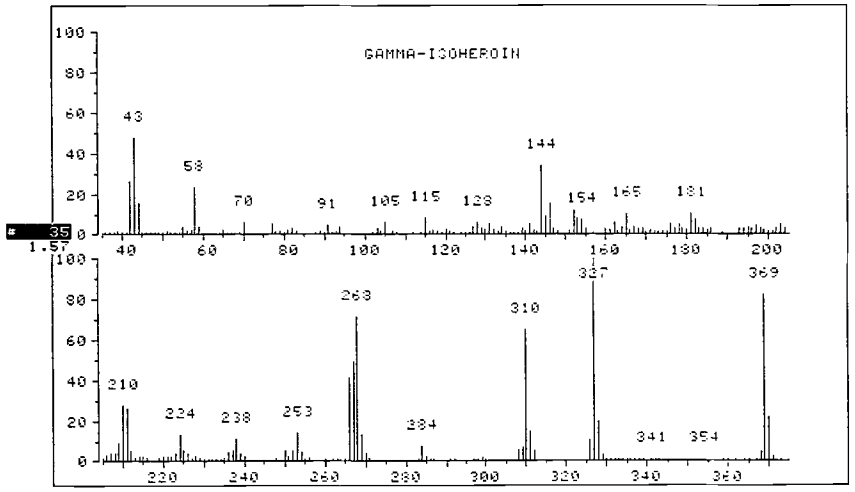


FIG. 8—*EI mass spectrum of gamma-isoheroin.*

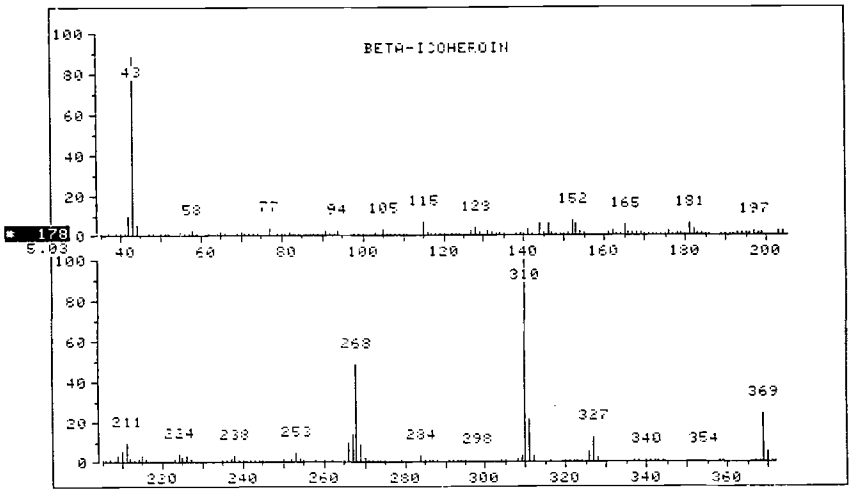


FIG. 9—*EI mass spectrum of beta-isoheroin.*

TABLE 5—*Major mass spectral ion fragments observed using EI GC/MS system; 12-m bonded FSOT, OV-1, 0.2 μm; column temperature = 200°C; helium as carrier gas.*

	Relative Abundance			
M/E	Heroin	Alpha-Isoheroin	Beta-Isoheroin	Gamma-Isoheroin
369	58.4	54.8	18.1	82.0
327	100.0	58.5	10.9	100.0
310	51.0	51.8	100.0	65.2
268	63.9	64.1	47.1	71.7
267	23.8	100.0	15.7	49.6
215	35.2	11.3	3.8	2.3
210	10.3	12.1	6.3	27.6
204	39.8	26.1	2.8	3.3
146	19.9	16.8	10.0	15.8
144	10.5	31.4	8.9	33.8

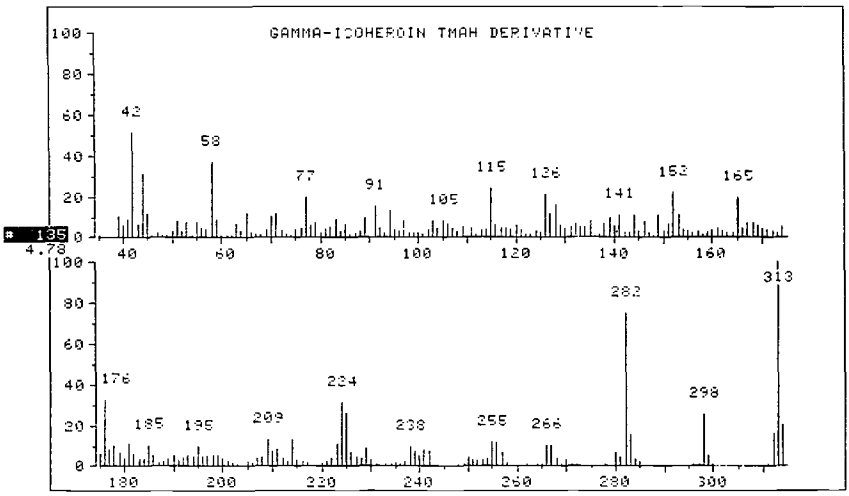


FIG. 10—*EI mass spectrum of gamma-isoheroin TMAH derivative.*

Infrared Spectroscopy

The infrared spectra of the freebase heroin isomers are shown in Figs. 13 through 16. Minor dissimilarities in their spectra can be seen on the absorption bands centered around 1400 reciprocal centimetres. The major differences, however, occurred in the 1200 to 400 reciprocal centimetre region.

Conclusion

Capillary gas chromatography was found to be the most suitable systems for separating the four heroin isomers studied. Spectra from NMR, IR, and GC/MS showed definitive differences for conclusive identification.

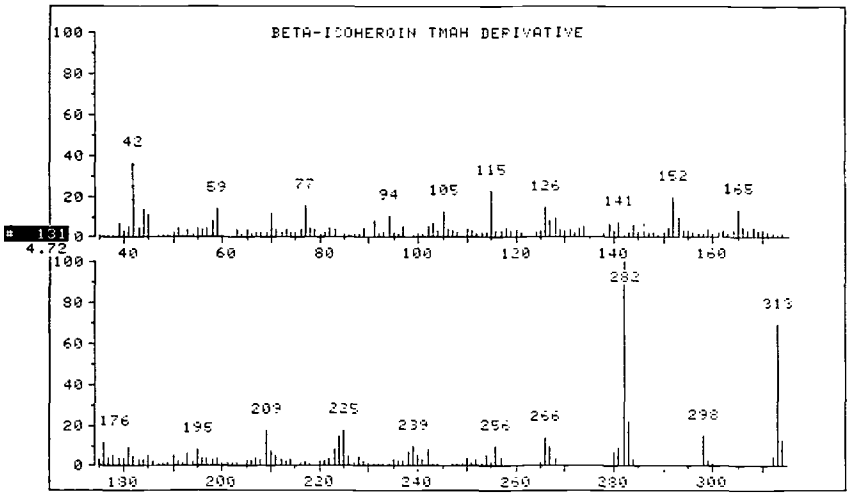


FIG. 11—*EI mass spectrum of beta-isoheroin TMAH derivative.*

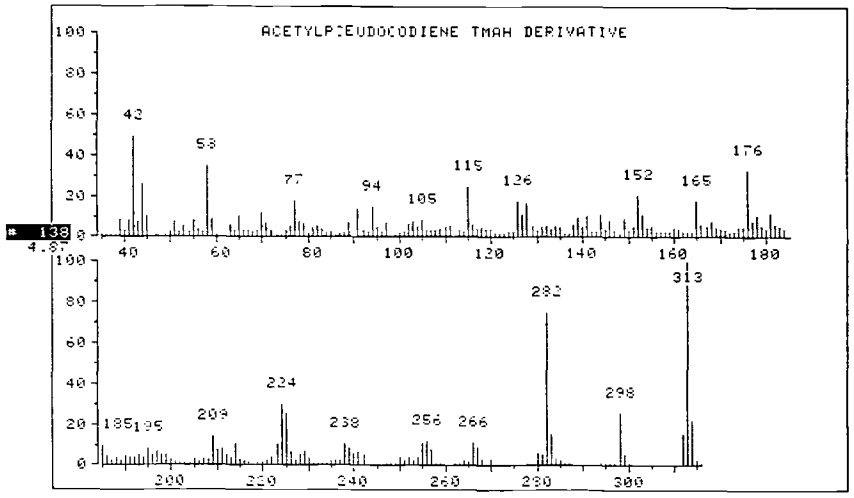


FIG. 12—*EI mass spectrum of acetylpseudocodeine TMAH derivative.*

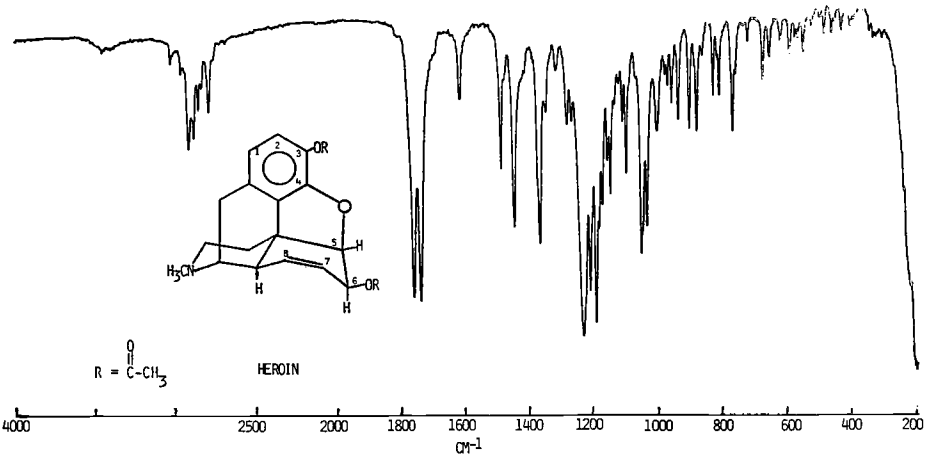


FIG. 13—*IR spectrum of heroin.*

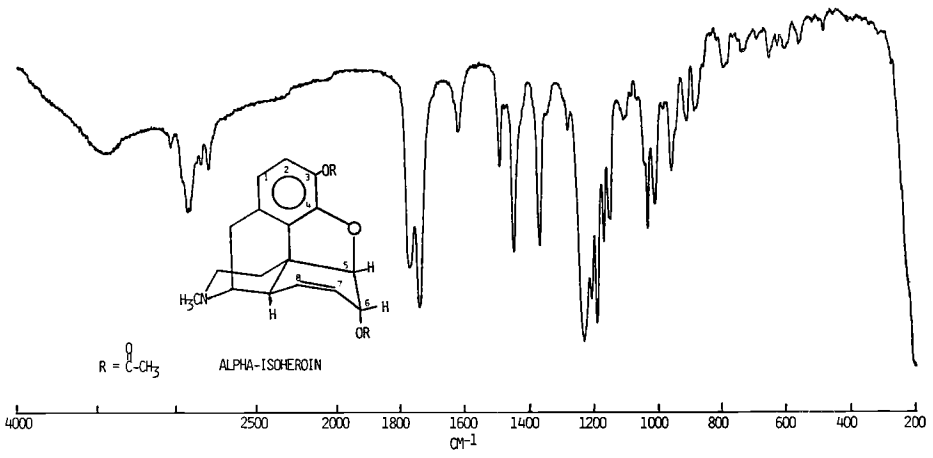


FIG. 14—*IR spectrum of alpha-isoheroin.*

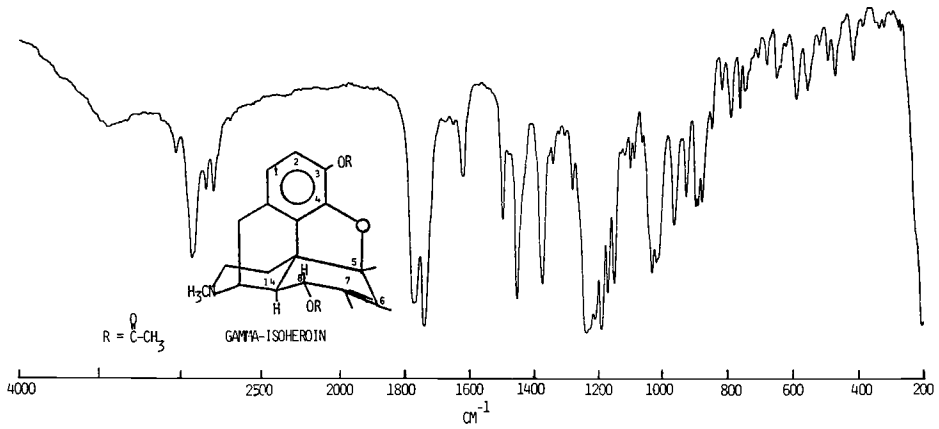


FIG. 15—*IR spectrum of gamma-isoheroin.*

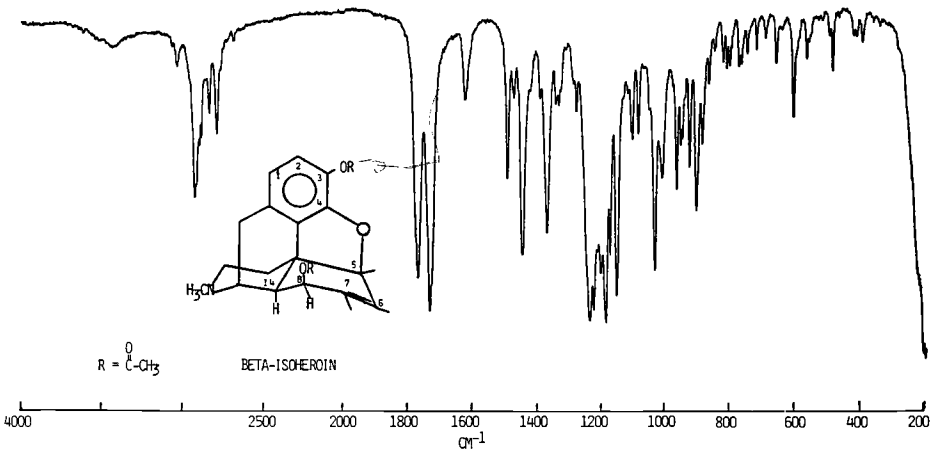


FIG. 16—*IR spectrum of beta-isoheroin.*

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