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A Study of Procedures for the Identification of Heroin

For forensic purposes, it is essential for the chemist to identify drugs correctly. The method or combination of methods chosen must be able to differentiate the drug present from all other compounds, regardless of structural similarities. The proper choice of method can be achieved only with a thorough understanding of the limitations and specificity of each analytical technique available to the chemist. Virtually no literature is available on the specificity of the many procedures used for the identification of heroin (diacetylmorphine). Indeed, it has been shown [*1*] that even pyramiding certain analytical tests may not be adequate.

The identification of heroin for many years depended on a series of tests using color and microcrystal formation in an exclusion scheme [*2*]. Gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) were, and still are today, also widely used [*3*, pp. 94–102]. In recent years, a slow shift in analytical techniques toward instrumentation has occurred. These include ultraviolet (UV), infrared (IR), and mass spectroscopy (MS).

The purpose of this paper is to determine the adequacy of some of the procedures used for the identification of heroin. To determine the specificity of each procedure, 17 compounds closely related to heroin were obtained and examined by each technique. Some are legitimate drugs, others have no accepted medicinal value.

The basic structure for morphine is shown in Fig. 1. To limit the scope of this investigation to some of those compounds most likely to be wrongly identified as heroin, only three changes were allowed in the basic morphine structure. These were changes in the substituents at the C_3 and C_6 positions and changes in the position of, or existence of, the double bond in Ring A of the basic morphine structure. Table 1 identifies the structure of each compound and its molecular weight. Compounds I through VI were purchased from commercial sources. The remainder were prepared in this laboratory.

Experimental Procedures

Esters of morphine, dihydromorphine, codeine, and ethylmorphine were prepared by dissolving approximately 300 mg of the appropriate free base in 3 ml pyridine and 3 ml acetic or propionic anhydride, depending on the ester desired. The solution was allowed to stand at room temperature for 6 h in the dark. The only exception was Compound XIII, which was prepared by starting with Compound II, adding the pyridine and acetic anhydride, and maintaining the solution at 60°C for 6 h. The solutions were then diluted

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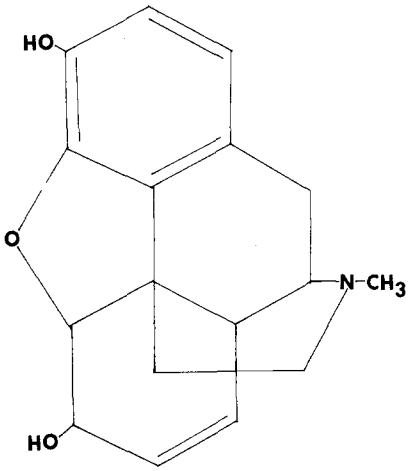

FIG. 1—*Basic structure for morphine.*

to 250 ml with water, and the esters of the free bases were extracted into 100 ml benzene after the addition of 10 g $NaHCO_3$ to the aqueous solution. The aqueous solution was discarded and the benzene solution washed twice with 250-ml portions of water. After drying the benzene by passing it through anhydrous Na_2SO_4, the solution was evaporated to dryness on a steam bath and the heating continued to the absence of pyridine vapors. The residue was dissolved in 25 ml 1*N* H_2SO_4 and extracted with 25 ml $CHCL_3$ to remove neutral, organic-soluble impurities. The amine ester was then extracted as its hydrochloride salt with two 25-ml portions of $CHCL_3$ after the addition of 5 g KCl to the aqueous solution. Evaporation of the $CHCL_3$ extracts yielded approximately 200 mg of the derivative in each case.

Compounds VII and VIII were obtained by the controlled-base hydrolysis of Compound XII and acid hydrolysis of Compound XV, respectively. Hydrochloride salts of these monoesters were prepared by dissolving the bases in methyl alcohol, bubbling HCl gas through the solution, and evaporating the solution to dryness. All derivatives were subjected to a preliminary GLC examination and found to be chromatographically pure.

Color Tests

Two color tests, the familiar Marquis test and perhaps less familiar Mecke test, were studied. The reagents were prepared according to directions given elsewhere [*3*, p. 136]. The tests were conducted on a porcelain spot-test plate with one drop of reagent added to a 0.1 to 0.2-mg sample of the hydrochloride salt of the amine. Table 2 lists the results of these tests.

Microcrystalline Tests

Reagents for the microcrystalline tests, prepared according to literature instructions, were platinum chloride in water, gold bromide in HCl [*3*, p. 136], and a saturated solution of red mercuric iodide in 10% HCl. The tests were performed according to a general procedure for aqueous microcrystalline tests [*4*] with no cover glass being used. Crystals formed were observed for speed of formation, shape, size, and color with polarized light. Table 3 shows the results of these tests.

TABLE 1—*Structure and molecular weights of compounds studied.*

Compound	Name	C_3	C_6	Position of Double Bond in Ring A	Molecular Weight
I	morphine	OH	OH	C_7	285
II	hydromorphone	OH	=O	saturated	285
III	dihydromorphine	OH	OH	saturated	287
IV	codeine	CH_3O	OH	C_7	299
V	dihydrocodeine	CH_3O	=O	saturated	299
VI	ethylmorphine	C_2H_5O	OH	C_7	313
VII	monoacetylmorphine	OH	$CH_3C(=O)-O$	C_7	327
VIII	monoacetyldihydromorphine	$CH_3C(=O)-O$	OH	saturated	329
IX	acetylester of codeine	CH_3O	$CH_3C(=O)-O$	C_7	341
X	acetylester of ethylmorphine	C_2H_5O	$CH_3C(=O)-O$	C_7	355
XI	propionyl ester of codeine	CH_3O	$C_2H_5C(=O)-O$	C_7	355
XII	diacetylmorphine (heroin)	$CH_3C(=O)-O$	$CH_3C(=O)-O$	C_7	369
XIII	pseudoheroin (acetyldihydromorphinoneenol acetate)	$CH_3C(=O)-O$	$CH_3C(=O)-O$	C_6	369
XIV	propionyl ester of ethylmorphine	C_2H_5O	$C_2H_5C(=O)-O$	C_7	369
XV	diacetyldihydromorphine	$CH_3C(=O)-O$	$CH_3C(=O)-O$	saturated	371
XVI	monoacetylmonopropionyldihydromorphine	$CH_3C(=O)-O$	$C_2H_5C(=O)-O$	saturated	385
XVII	dipropionylmorphine	$C_2H_5C(=O)-O$	$C_2H_5C(=O)-O$	C_7	397
XVIII	dipropionyldihydromorphine	$C_2H_5C(=O)-O$	$C_2H_5C(=O)-O$	saturated	399

TABLE 2—*Color tests.*

Compound	Group	Marquis Test	Mecke Test
I	1	maroon to purple	deep green
II	4	weak red	yellow-green
III	2	red to maroon	yellow to blue-green
IV	3	deep purple	deep green
V	4	weak red	yellow-green
VI	3	deep purple	deep green
VII	1	maroon to purple	deep green
VIII	2	red to maroon	yellow to blue-green
IX	3	deep purple	deep green
X	3	deep purple	deep green
XI	3	deep purple	deep green
XII	1	maroon to purple	deep green
XIII	...	maroon to purple	yellow to brown
XIV	3	deep purple	deep green
XV	2	red to maroon	yellow to blue-green
XVI	2	red to maroon	yellow to blue-green
XVII	1	maroon to purple	deep green
XVIII	2	red to maroon	yellow to blue-green

TABLE 3—*Microcrystalline test data.*

Compound	$PtCl_4$	HgI_2 in Dilute HCl	$HAuBr_4$ in HCl
I	+	+	+
II	–	–	*
III	–	–	–
IV	–	+	+
V	–	–	–
VI	+	+	+
VII	+	+	+
VIII	+	+	–
IX	–	+	*
X	+	+	+
XI	+	+	+
XIII	+	–	–
XIV	+	+	+
XV	+	–	–
XVI	–	–	+
XVII	+	*	+
XVIII	+	–	+

+ = a crystal different from that of heroin.
* = a crystal similar to that of heroin.
– = no crystal formed.

Gas-Liquid Chromatography

Two of the silicone-based stationary phases, OV-1 and OV-17, were used in this study. The columns were 6-ft (1.8-m) by 4-mm inside diameter glass. Approximate injection, column, and detector temperatures were 260, 250, and 260°C, respectively. The GLC used was a Hewlett-Packard 7620A equipped with an on-column injection system and a flame detector. Approximately 1 μg of each compound of interest was injected onto each column. Table 4 lists the results of the GLC retention studies.

TABLE 4—*Gas-liquid chromatography data: retention times relative to heroin at 250°C.*

Compound	Stationary Phase	
	3% OV-1	3% OV-17
I	0.80	0.55
II	...[a]	0.70
III	0.80	0.52
IV	0.61	0.49
V	...[a]	0.63
VI	0.65	0.47
VII	0.80	0.68
VIII	0.79	0.61
IX	0.77	0.64
X	0.83	0.68
XI	0.94	0.81
XII	1.00 (3.95)[b]	1.00 (4.50)[b]
XIII	1.00	1.13
XIV	0.99	0.85
XV	0.93	0.88
XVI	1.12	1.09
XVII	1.51	1.55
XVIII	1.34	1.32

[a] Retention time could not be determined because of excessive peak symmetry.

[b] Retention time of diacetylmorphine (heroin) in minutes.

Thin-Layer Chromatography

Four TLC systems were used in this study. Two are from literature references [*3*, p. 92, and *5*], and the others are ones known to be used in forensic laboratories. The amine hydrochloride salts were spotted onto the TLC plates from a 0.5-mg/ml solution in methyl alcohol. Authentic heroin was also spotted on each TLC plate for use in calculating relative retention values. The plates used were 5 by 10-cm, commercially prepared silica gel on glass plates. The mobile phase was allowed to ascend the full 10 cm in an unlined tank slightly larger than the plate size. Table 5 lists the results of the TLC studies.

Ultraviolet Spectroscopy

The ultraviolet absorbances of all 18 compounds were determined by dissolving a sample of each in 0.1*N* HCl and scanning on a Beckman Acta V spectrophotometer equipped with matched 1-cm cells. After scanning, 1 drop of 5*N* NaOH was added directly to the cell contents to hydrolyze all ester groups present [*6*]. After 2 min the solution was scanned again. Results of the UV scans are presented in Table 6.

Mass Spectroscopy

Electron impact (EI) ionization spectra were obtained for all 18 of the compounds studied. The compounds were dissolved in methyl alcohol at a concentration of 0.5 mg/ml and injected into an analytical GLC interfaced with a Finnigan 3000 Peak Identifier mass spectrometer. The GLC column was maintained at 250°C. The mass/charge (*m/e*) range scanned was 40 to 400 atomic mass units. Ionization potential for all scans was held constant at 70 eV.

TABLE 5—*Thin-layer chromatography data. System 1: butyl ether-ethyl ether-diethylamine (45:45:10). System 2: chloroform-dioxane-ethyl acetate-NH_4OH (25:60:10:5). System 3: chloroform-methanol (9:1). System 4: chloroform saturated with NH_3 methanol (18:1). The spray used was acidified iodoplatinate, and the TLC plates were Q-1 Quanta/Gram (Quantum Industries, Fairfield, N.J.) silica gel.*

Compound	R_R			
	System 1	System 2	System 3	System 4
I	0.16	0.44	0.17	0.21
II	0.41	0.45	0.32	0.40
III	0.12	0.38	0.07	0.20
IV	0.52	0.77	0.64	0.77
V	0.53	0.70	0.58	0.82
VI	0.66	0.78	0.60	0.79
VII	0.67	0.94	0.70	0.82
VIII	0.80	0.66	0.27	0.60
IX	1.16	1.04	0.97	1.00
X	1.24	1.05	1.00	1.02
XI	1.27	1.06	1.02	1.05
XII	1.00 (0.44)[a]	1.00 (0.85)[a]	1.00 (0.61)[a]	1.00 (0.70)[a]
XIII	1.00	1.00	0.93	1.00
XIV	1.25	1.08	1.05	1.05
XV	0.84	0.77	0.64	0.90
XVI	0.83	0.86	0.64	1.00
XVII	1.05	1.05	1.03	1.03
XVIII	0.84	0.80	0.72	0.95

[a] R_f for diacetylmorphine (heroin)

TABLE 6—*Ultraviolet data.*

Compound	Wavelength of A_{max}, base, nm	A_{max}/A_{min}, acid	A_{max}/A_{min}, base
I	297	3.05	1.58
II	292	1.98	1.48
III	297	3.98	2.34
IV	282	2.69	2.47
V	282	1.81	1.54
VI	282	2.78	2.54
VII	297	3.04	1.58
VIII	297	4.98	2.38
IX	282	2.74	2.49
X	282	2.77	2.54
XI	282	2.80	2.52
XII	297	4.50	1.60
XIII	291	2.56	1.35
XIV	282	2.89	2.51
XV	297	4.90	2.40
XVI	297	4.93	2.39
XVII	297	4.70	1.47
XVIII	297	4.89	2.37

Infrared Spectroscopy

Infrared spectra were obtained for both the free base and hydrochloride salts of all 18 compounds. The base or salt was dried and 1 mg mixed with 100 mg KBr. The mixture was pressed into 15-mm pellets. There were two exceptions to this general procedure.

The spectra of Compounds XI and XIV, whose free bases failed to crystallize even after days at room temperature, were obtained by making a smear of the neat liquid on a blank 15-mm pellet. All the pellets were scanned on a Perkin-Elmer 457A Infrared Spectrophotometer for 2.5 to 40 μm.

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectra were obtained on the free bases of all 18 compounds on a JOEL C-60HL instrument. Approximately 20 mg of the free base was dissolved in a minimum amount of deuterated chloroform for the determinations. Compounds I and III required approximately 20% D_6-dimethyl sulfoxide in deuterated chloroform to effect solvation.

Results and Discussion

Close examination of the results presented in Table 2 reveals that the color differences noted are related to functional groups present in the molecule. For the purpose of evaluation of the color tests, the 18 compounds studied can be divided into four groups. The first is morphine and esters prepared from it (C_3 and C_6 hydroxyl or ester, C_7 double bond); the second is dihydromorphine and esters prepared from it (C_3 and C_6 hydroxyl or ester, Ring A saturated); the third is codeine or ethylmorphine and esters prepared from them (C_3 ether, C_6 hydroxyl or ester, C_7 double bond); and the fourth is compounds having a C_6 ketone. Using the Marquis test, Group 1 members produced a maroon changing to purple, Group 2 members produced a red changing to a deep maroon, Group 3 members produced an immediate deep purple, and Group 4 members produced a weak, dirty red color. Compound XIII, not properly belonging to any of the four groups, produced a color similar to that produced by Group 1 members. Using the Mecke test, Groups 1 and 3 members produced a deep green color, Group 2 members produced a yellow color slowly changing to blue-green, and Group 4 members produced a yellow-green color. Compound XIII produced a yellow color slowly changing to brown.

The GLC retention data presented in Table 4 suggests that certain generalizations which apply to both stationary phases can be made. First, the morphine derivatives exhibit less mobility, that is, they have a longer retention time than their dihydromorphine counterparts. Thus, diacetylmorphine has a longer retention time than diacetyldihydromorphine. Secondly, compounds having a C_6 ketone have a longer retention time than their C_6 hydroxyl counterparts of equal molecular weight. Thus, hydromorphone has a longer retention time than morphine. Thirdly, within a morphine or dihydromorphine series, the compounds with the larger C_3 and C_6 ester groups have the longer retention time. Thus, dipropionylmorphine has a longer retention time than diacetylmorphine. Other factors such as molecular weight being equal, a compound with a C_3 ether group had a shorter retention time than one with a C_3 ester. This is well illustrated by heroin and Compound XIV, the propionyl ester of ethylmorphine.

Examination of the TLC data presented in Table 5 showed a reversal of some of the GLC generalizations. Morphine derivatives showed a greater mobility, that is, less relative retention, than their dihydromorphine counterparts. A C_6 ketone migrated farther than its C_6 hydroxyl counterpart of equal molecular weight. Increasing the size of the C_3 and C_6 ester groups within a morphine of dihydromorphine series resulted in increased mobility. Similar to the GLC generalizations, however, is the fact that a C_3 ether showed greater migration than a C_3 ester of equal molecular weight. This effect is especially noticeable using System 1.

These generalizations concerning the chromatographic behavior of the compounds studied can be useful in making some predictions. For example, using these chromatographic conditions, could dihydrocodeine (C_3 ether, C_6 hydroxyl, Ring A saturated) be mistaken for heroin? Although not included in this study, the generalizations would predict that dihydrocodeine would have less TLC migration than codeine (whose migration is already smaller than heroin) and would have a GLC retention time smaller than codeine (whose retention time is already smaller than heroin). These predictions have been experimentally verified by the author. Thus, using these conditions, dihydrocodeine could not have been mistaken for heroin.

The UV spectra were found to be useful only for group identification. For convenience of comparison, the four groups used for color test comparisons are maintained. All 18 compounds have an absorbance A maximum near 282 nm in acid. Group 1 members having a C_3 phenolic OH exhibited an A_{max}/A_{min} of approximately 3.1 in acid. Those in Group 1 having an ester at C_3 exhibited an A_{max}/A_{min} of approximately 4.6 in acid. Group 2 members having a C_3 phenolic OH exhibited an A_{max}/A_{min} of 4.0 in acid. Those in Group 2 having an ester at C_3 exhibited an A_{max}/A_{min} of approximately 4.9 in acid. All Group 3 members had an A_{max}/A_{min} of approximately 2.8 in acid. In base, after saponification of esters, all members of Groups 1 and 2 had an A_{max} occurring near 297 nm. However, Group 1 members exhibited an A_{max}/A_{min} of approximately 1.6, while Group 2 members exhibited an A_{max}/A_{min} value of approximately 2.4. All Group 3 members retained the A_{max} occurring near 282 nm and had an A_{max}/A_{min} of approximately 2.5. These observations are summarized in Table 7. Representative UV spectra of Groups 1 to 3 are shown in Fig. 2. The two members of Group 4 have dissimilar UV spectra because of different C_3 substituents. Using these observations, one would predict that the UV spectrum of dihydrocodeine would resemble that of Group 3 members, not heroin, with perhaps a larger A_{max}/A_{min} ratio. This also has been verified experimentally by the author.

TABLE 7—*Ultraviolet data by groups.*

Group 1			Group 2			Group 3		
Compound	A_{max}/A_{min}, acid	A_{max}/A_{min}, base	Compound	A_{max}/A_{min}, acid	A_{max}/A_{min}, base	Compound	A_{max}/A_{min}, acid	A_{max}/A_{min}, base
I	3.05	1.58	III	3.98	2.34	IV	2.69	2.47
VII	3.04	1.58	VIII	4.98	2.38	VI	2.78	2.56
XII	4.50	1.60	XV	4.90	2.40	IX	2.74	2.49
XVII	4.70	1.47	XVI	4.93	2.39	X	2.77	2.54
			XVIII	4.89	2.37	XI	2.80	2.52
						XIV	2.89	2.51

No correlation between molecular structure and microcrystal shape could be found. No attempt was made to describe the crystals because no descriptive terms could be found that were adequate to describe the subtle differences in crystal shape. Although the microcrystals formed using $PtCl_4$ on Compounds XII, XV, XVII, and XVIII could all be described as rosettes with yellow needles [*1*], the eye of a trained observer can detect significant differences.

The spectra obtained from both the IR and NMR studies were all mutually exclusive. The identification procedures, however, both require the heroin to be present in a relatively pure state if a valid spectrum is to be obtained. Heroin is frequently cut with a

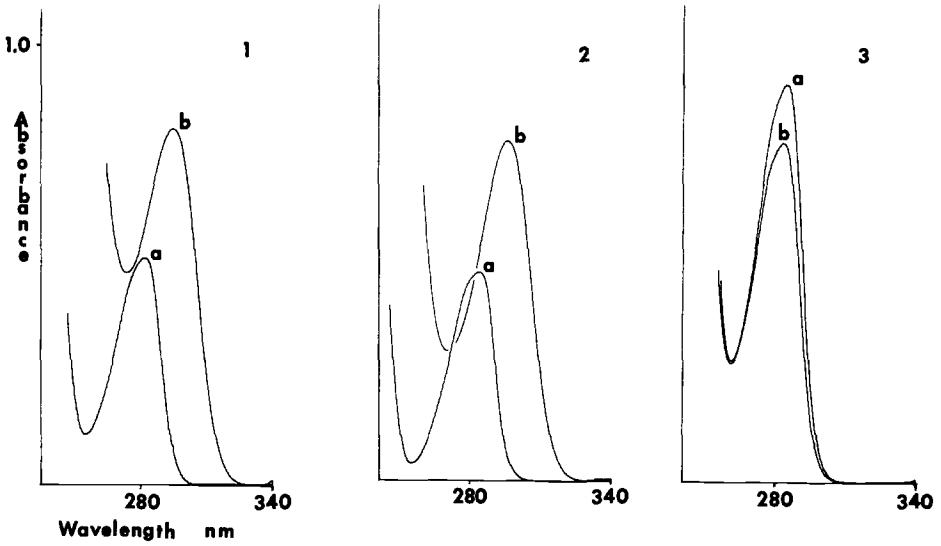

FIG. 2—*Ultraviolet spectra of* (1) *morphine,* (2) *dihydromorphine, and* (3) *codeine. Solvents are* (a) *0.1*N *HCl and* (b) *approximately 0.5*N *NaOH.*

wide variety of adulterants and diluents for street use, thereby making the heroin isolation procedure both tedious and time-consuming.

The mass spectra obtained on the 18 compounds were also mutually exclusive. A mass spectrometer interfaced with analytical GLC usually needs no preliminary sample clean up [7]. In any exhibit in which the heroin content can be quantitated by GLC, the heroin can be identified by GLC-MS. In this study, all 18 compounds gave a strong molecular ion. Thus, only the two compounds having the same molecular weight as heroin could possibly be mistaken for it. The mass spectra of these two compounds as well as heroin are shown in Fig. 3. One can easily see that, although the three compounds all have a molecular ion at *m/e* 369, their EI fragmentation patterns are easily distinguished.

Conclusion

Data are presented indicating the specificity of eight techniques used for the detection and identification of heroin. The techniques covered are color tests; microcrystal tests; GLC; TLC; and UV, IR, MS, and NMR spectroscopy. Color tests and UV spectroscopy are shown to be class-specific tests. Gas-liquid chromatography and TLC are shown to be of value for the identification of the various members of a class only when a member of that class is known to be present. Microcrystal tests are shown to be of limited specificity because no microcrystal response-molecular structure correlation could be found. Even the most specific microcrystal test ($PtCl_4$) could possibly give a positive response for heroin with a very dissimilar molecule. Infrared spectroscopy, EIMS, and NMR spectroscopy were all found to be highly specific for the identification of heroin with definite response-molecular structure relationships. Based on these observations and the fact that of the definitive tests only GC-MS does not require the heroin to be present in relatively pure form, GC-MS seems the obvious method of choice when amine mixtures are present and instrumentation is available.

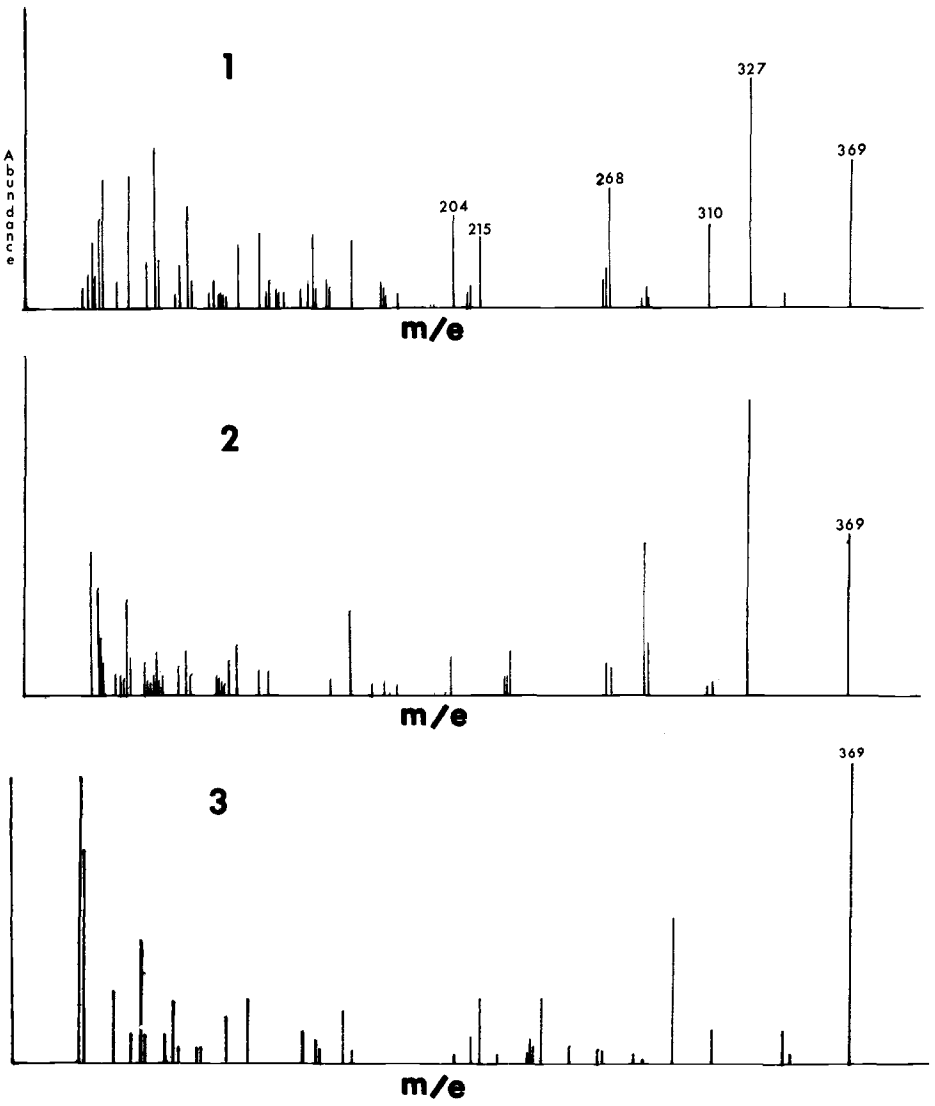

FIG. 3—*Electron impact mass spectra of* (1) *heroin,* (2) *pseudoheroin* (*acetyldihydromorphinoneenol acetate*), *and* (3) *propionyl ester of ethylmorphine.*

Summary

Heroin (diacetylmorphine) and 17 structurally related compounds were obtained, either by synthesis or by purchase from commercial sources, to evaluate heroin identification procedures presently in use in forensic laboratories. Changes in test response caused by changes in substitution at the C_3 and C_6 positions as well as changes in the position of, or absence of, the morphine C_7 double bond were studied. Methods covered by the study included four thin-layer chromatographic systems, two gas-liquid chromatographic systems, color tests, and four microcrystalline tests as well as ultraviolet, infrared, nuclear magnetic resonance, and mass spectroscopy techniques.

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