Ethyl Esters of Arylhydroxy- and Arylhydroxymethoxycocaines in the Urines of Simultaneous Cocaine and Ethanol Users

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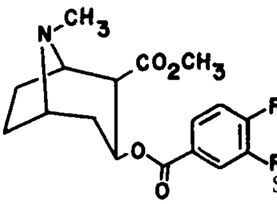
Abstract

3′-Hydroxy- and 4′-hydroxybenzoylecgonine ethyl ester *(IIb* and *IIc)* and 4′-hydroxy-3′-methoxybenzoylecgonine ethyl ester *(IIh)* were identified in basic extracts of urine samples from patients who had used cocaine and ethanol concurrently. Identification was based on comparison of patient gas chromatography and mass spectrometry data with those of synthesized standards. These findings corroborate earlier work in which the corresponding methyl esters of these metabolites were found in the same urine samples.

Introduction

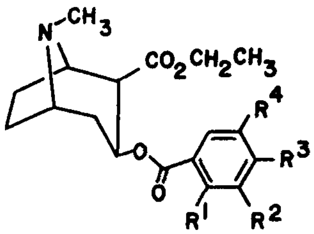
It has been known for some time that the carbomethoxy group of cocaine is partially transesterified by ethanol when cocaine and alcohol are taken together. Both benzoylecgonine ethyl ester (ethyl cocaine or cocaethylene) and ethylecgonine were found in the urine in these cases (1). The accompanying papers (2,3) have described the identification of 3′-hydroxy- and 4′-hydroxybenzoylecgonine methyl ester (3′-hydroxy- and 4′-hydroxycocaine; *Ia* and *Ib*) and 4′-hydroxy-3′-methoxybenzoylecgonine methyl ester (4′-hydroxy-3′-methoxycocaine; *Ic*) in the urines of cocaine users (Scheme 1). During examination of two of these



Ia: $R^1 = OH$; $R^2 = H$
Ib: $R^1 = H$; $R^2 = OH$
Ic: $R^1 = OCH_3$; $R^2 = OH$

Scheme 1

samples (both from patients who had used alcohol and cocaine concurrently), three previously unreported cocaine-derived compounds were encountered. Examination of the mass spectra of these compounds and their comparison with those of synthesized standards identified them as the ethyl analogs of *Ia-Ic*: 3′-hydroxy- and 4′-hydroxybenzoylecgonine ethyl ester (3′-hydroxy- and 4′-hydroxycocaethylene, *IIb* and *IIc*, respectively) and 4′-hydroxy-3′-methoxybenzoylecgonine ethyl ester (4′-hydroxy-3′-methoxycocaethylene; *IIh)* (Scheme 2).



IIa: $R^1 = OH$; $R^2 = R^3 = R^4 = H$
IIb: $R^1 = R^3 = R^4 = H$; $R^2 = OH$
IIc: $R^1 = R^2 = R^4 = H$; $R^3 = OH$
IId: $R^1 = OH$; $R^2 = OCH_3$; $R^3 = R^4 = H$
IIe: $R^1 = OH$; $R^2 = R^4 = H$; $R^3 = OCH_3$
IIf: $R^1 = OH$; $R^2 = R^3 = H$; $R^4 = OCH_3$
IIg: $R^1 = R^4 = H$; $R^2 = OH$; $R^3 = OCH_3$
IIh: $R^1 = R^4 = H$; $R^2 = OCH_3$; $R^3 = OH$

Scheme 2

Case Histories

The case histories of Patient A, a 32-year-old male, and Patient B, a 20-year-old male, are described in detail in the preceeding paper (3).

Materials and Methods

Instrumentation

Gas-liquid chromatography (GLC) analyses were carried out under three sets of column conditions, described fully elsewhere (2). GC System A consisted of a 6-ft × ¼-in i.d., 3% OV-101 on 80/100 mesh GasChrom Q column held isothermally at 250°C. GC System B was a 5-ft × ⅛-in i.d., 3% SP-2250 on 80/100 mesh Supelcoport column which, after solventless injection of the sample (2), was held isothermally at 205°C for 4 min, and then the temperature increased to 305 °C over 26.1 min. In GC System C, a 5-ft × ⅛-in i.d., 3% OV-101 on 80/100 mesh HP Chromosorb W column was held at 150°C for 1 min after injection, and then the temperature increased to 250°C over 8 min and held at 250°C for 7 min. Both columns B and C were interfaced (glass jet separator at 250°C) with Dupont DP-102 mass spectrometers.

Mass spectra were collected in the electron impact mode (75 eV) over a mass range of 35 to 400 amu at a rate of 125 amu/sec with the source at 200°C.

Infrared (IR) spectra were run in 0.5 to 5% solutions in spectrograde carbon tetrachloride (Fisher Scientific) in 0.5-mm potassium bromide cells using a matched cell as reference. Spectra were recorded on a Sargent-Welch 3-330 ratio recording IR Spectrophotometer from 4000 to 600 cm^{-1} using 7 or 20 min scans depending on the concentration of the sample.

Analysis of Urine

The extraction procedure used for the urine samples was as described previously (2).

Synthesis of Standards

Ethylecgonine was prepared in a manner analogous to that described for methylecgonine (2). Ecgonine hydrochloride (0.5 g) was dissolved in 4 mL of Punctilious™ ethanol (U.S. Industrial Chemicals), through which dry HCl gas had just previously been bubbled for ca. 1 min. This mixture was heated in an oil bath at 85° to 90°C until all of the solid had disappeared (ca. 2 hr). The solvent was evaporated under a stream of air in a hot water bath, and then residual water and ethanol were removed overnight in a vacuum dessicator. Even at this point, the ethylecgonine hydrochloride remained a glassy semiliquid; thus, it was converted to the free-base by extraction from carbonate solution with chloroform, dried through anhydrous sodium sulfate, and evaporated carefully under a stream of air (water bath < 40°C). The free-base was stored as a solution in pesticide grade benzene in a tightly stoppered container at 5°C for several weeks without noticeable deterioration. IR and mass spectral data were entirely consistent with ethylecgonine, although GLC analysis of the product showed minor contamination (5 to 10%) from methylecgonine and ethylecgonidine. No attempt was made to remove impurities at this point.

Arylhydroxybenzoylecgonine ethyl esters and arylhydroxymethoxybenzoylecgonine ethyl esters were prepared by condensation of ethylecgonine base with the appropriately substituted benzoic acids (Aldrich Chemical) in the presence of thionyl chloride. The reactions proceeded exactly as those of the methyl analogs (2,3) and were worked up in an analogous manner.

GLC of the reaction mixtures after extraction revealed mostly unreacted ethylecgonine, ethylecgonidine, and a single higher-boiling component. Preparative thin-layer chromatography (TLC) on silica gel GF-254 (Analtech), using 100:1.5 methanol:ammonium hydroxide as solvent, gave a single band in each case which showed both light blue-violet to deep violet fluorescence under UV light (300 nm) and a positive (violet-brown) reaction to acidified iodoplatinate spray (4). The R_f values for all arylhydroxycocaethylenes and arylhydroxymethoxycocaethylenes were similar to those of the methyl analogs (2,3). After scraping the desired band from the TLC plate, it was added to 2 to 3 mL of 0.05*N* sulfuric acid. Most of these compounds proved extremely labile in the presence of both silica and acid, hence the mixtures were centrifuged immediately and the acid layer drawn off. The aqueous layer was washed rapidly with 1 mL of chloroform, made basic with solid sodium carbonate, and then extracted with chloroform and dried through sodium sulfate. The purities of individual substituted cocaethylenes ranged from ca. 85% (3′- and 4′-hydroxy) to > 95% by GLC, with ethylecgonine and ethylecgonidine (this time from decomposition during the TLC workup) the only major contaminants. These impurities did not appear to interfere significantly with subsequent spectral identification.

IR, GLC, and mass spectral data were all totally consistent with the proposed structures *IIa–IIh*. The IR spectra of these compounds were extraordinarily similar to those of the corresponding methyl analogs, especially in the functional group regions (Table I). The absorbances listed differed without exception < 10 cm^{-1} (and in nearly every case < 5 cm^{-1}) from corresponding peaks in the spectra of the methyl analogs and exhibited similar characteristic peak shapes and intensities. Also, the order of elution of these compounds by GLC (Table II) was essentially the same as that of the methyl analogs; in fact, when the t_R of these compounds were calculated relative to coca-

Table I. Infrared Absorbances*

Compound	-OH	-C=O	Aromatic -C=C-	Benzoyl -C-O-	Other
Cocaethylene	—	1751 ms	—	1280 vs	—
		1719 ms			
IIa	3420 w	1748 s	1671 s	1300 vs	1211 s
	3200 w,br	1721 ms	1612 m		
IIb	3608 w	1749 ms	1689 sh	1290 vs	1223 s
	3400 w,br	1719 s	1600 m		
IIc	3605 w	1748 m	1608 m	1275 s,br	1195 s
	3400 w,br	1723 sh	1510 m		
		1712 s			
IId	3405 w	1748 ms	1669 ms	1240 vs	1068 s
	3190 w,br	1722 m	1460 s		
IIe	3405 w	1749 s	1661 s	1250 vs	1140 vs
	3170 w,br	1720 m	1620 s		
			1500 m		
IIf	3430 w	1749 ms	1677 ms	1282 s	1227 vs
	3240 w,br	1725 m	1619 m		
			1490 s		
IIg	3562 m	1750 ms	1619 w	1282 vs	1204 s
	—	1713 s	1508 m		
IIh	3550 m	1749 m	1600 m	1281 vs	1211 s
	—	1710 s	1509 m		1034 s

*Frequencies in cm^{-1}; peak size and shape abbreviations: w = weak, m = medium, ms = medium strong, s = strong, vs = very strong, br = broad, and sh = shoulder

Table II. Relative GLC Retention Data*

Sample	GC System A	GC System B	GC System C
Cocaethylene	1.18 (1.00)	1.09 (1.00)	1.05 (1.00)
IIa	1.62 (1.37)	1.27 (1.18)	1.15 (1.10)
IIb	2.45 (2.08)	1.72 (1.57)	1.34 (1.28)
IIc	2.84 (2.41)	1.77 (1.64)	1.39 (1.32)
IId	3.06 (2.59)	1.74 (1.60)	1.48 (1.41)
IIe	3.25 (2.75)	1.77 (1.64)	1.52 (1.45)
IIf	2.77 (2.35)	1.59 (1.46)	1.42 (1.35)
IIg	4.25 (3.60)	1.97 (1.81)	1.70 (1.61)
IIh	3.32 (2.81)	1.85 (1.71)	1.51 (1.44)
Patient A, Cocaethylene	—	—	1.04 (0.99)
Patient A, MW 333, #1	—	—	1.32 (1.27)
Patient A, MW 333, #2	—	—	1.37 (1.32)
Patient A, MW 363	—	—	1.47 (1.41)
Patient B, Cocaethylene	—	1.07 (1.02)	—
Patient B, MW 333, #1	—	1.72 (1.61)	—
Patient B, MW 333, #2	—	1.81 (1.69)	—
Patient B, MW 363	—	1.85 (1.73)	—

MW = Molecular Weight
*Retention times relative to cocaine IS; retention times calculated relative to cocaethylene are in parentheses.

ethylene rather than cocaine, they were nearly identical to those of the methyl analogs relative to cocaine (2,3). The mass spectral fragmentations of these compounds are discussed below.

Results and Discussion

GLC analysis of the basic extract of the urine from Patient A showed nearly every previously reported cocaine metabolite (Figure 1a). It was, therefore, surprising when the small peak just beyond benzoylecgonine gave yet another unfamiliar cocaine-like mass spectrum. The molecular ion at m/z 333 intimated possible transesterification of one of the arylhydroxycocaines with ethanol. After locating the arylsubstituted cocaine metabolites (m/z 319 and 349) by mass chromatography (5) (Figures 1b and 1d), a similar search for m/z 333 metabolites revealed two peaks, the one just mentioned plus another peak buried along with 4′-hydroxycocaine under the benzoylecgonine peak (Figure 1c). A further search for possible transesterified hydroxymethoxycocaines (m/z 363) gave a single peak that had been essentially invisible in the total ion chromatogram (Figure 1e). The mass spectra of these compounds, derived after similar treatment of the data from Patient B, are shown in Figures 2a-2c.

The large peaks at m/z 82, 196, and 212 in the mass spectra of these three compounds identified them immediately as cocaethylene derivatives (compare with Figure 2d). The 16 amu shift of both the molecular ion and the m/z 272 peaks (the latter resulting from loss of ethoxide) in the cocaethylene spectrum to m/z 333 and 288 in the spectra of the first two compounds, and the corresponding shift of 46 amu for these peaks to m/z 363 and 318 in the spectrum of the third compound, were entirely consistent with arylhydroxy and arylhydroxymethoxy derivatives of cocaethylene (Figure 3). Both possibilities seemed reasonable in light of previous work with these samples (2,3)

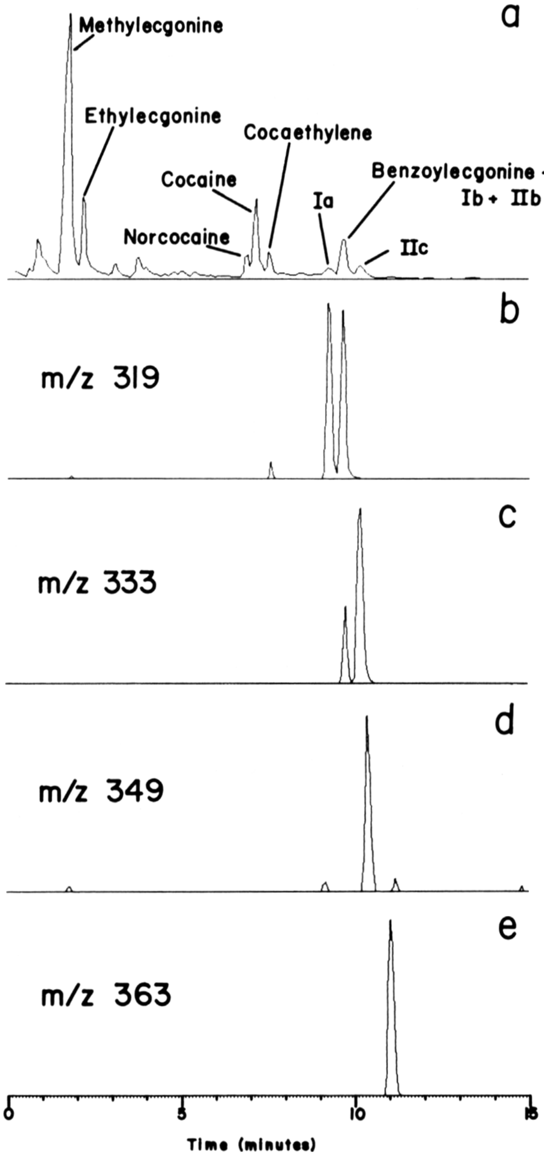


Figure 1. Chromatograms: a) total ion chromatogram of basic extract from urine of Patient A; b) mass chromatogram for arylhydroxycocaines, largest peak with 67,000 ion counts; c) mass chromatogram for arylhydroxycocaethylenes, largest peak with 87,000 ion counts; d) mass chromatogram for arylhydroxymethoxycocaines, 1900 ion counts; and e) mass chromatogram for arylhydroxymethoxycocaethylenes, 2400 ion counts.

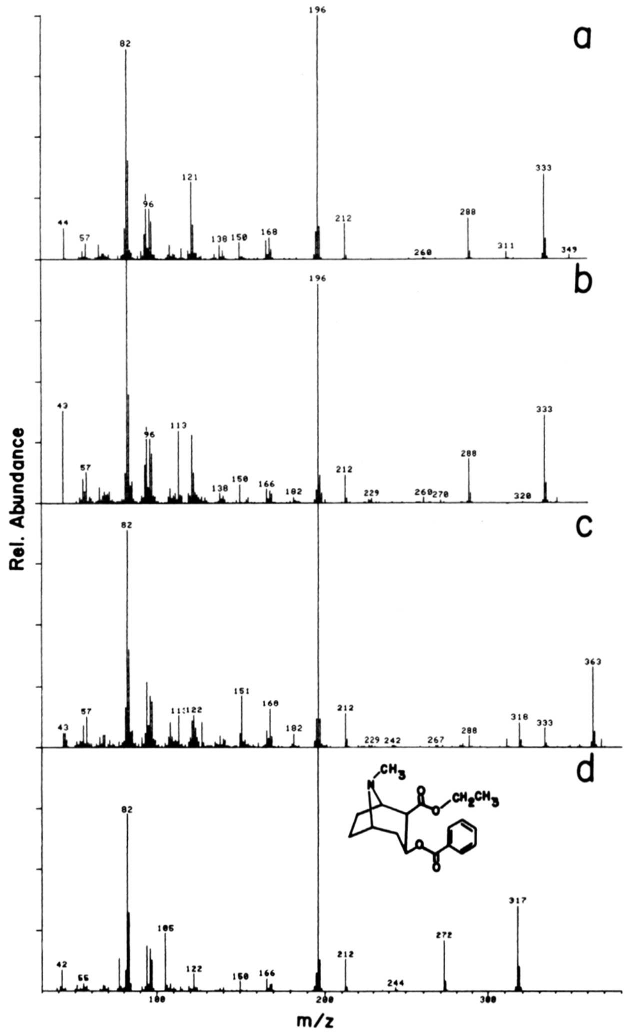


Figure 2. Mass spectra from Patient B urine: a) and b) MW 333 metabolites; c) MW 363 metabolite; and d) mass spectrum of synthesized cocaethylene.

and the previous report of transesterification of other cocaine metabolites in cocaine/alcohol users (1).

Unequivocal identification of these new compounds demanded the synthesis and characterization of all possible positional isomers. Although this was accomplished easily with the three arylhydroxycocaethylenes, only five of the necessary ten hydroxymethoxybenzoic acids were available commercially. The remainder conceivably could be accessed through tedious syntheses, but this did not seem warranted based on previous experience (2). Preparation of all of these compounds proceeded in low yields as before.

Arylhydroxycocaethylenes

Mass spectra of the three synthesized arylhydroxycocaethylenes (*IIa-IIc*) are shown in Figure 4. Although the spectra are quite similar, several characteristic differences were noted that corresponded well with those observed in the arylhydroxycocaine series (3). Most significantly, the *ortho*-hydroxy isomer differed noticeably from the other two, both in relative t_R (Table II) and in the ratios of certain characteristic ions in their mass spectra (Table III), especially those reflecting losses of ethoxide and of the benzoic acid moiety. Only the *ortho* isomer can both undergo γ-hydrogen loss of the benzoic acid group (yielding larger m/z 168 and 120 peaks), and stabilize the molecular ion toward ethoxide loss (large m/z 333/288 ratio) and ethylecgonyl loss (smaller m/z 121 peak) through intramolecular hydrogen bonding (3).

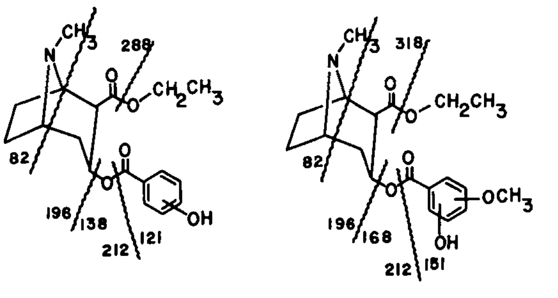


Figure 3. Mass spectral fragmentations of arylhydroxycocaethylenes and arylhydroxymethoxycocaethylenes

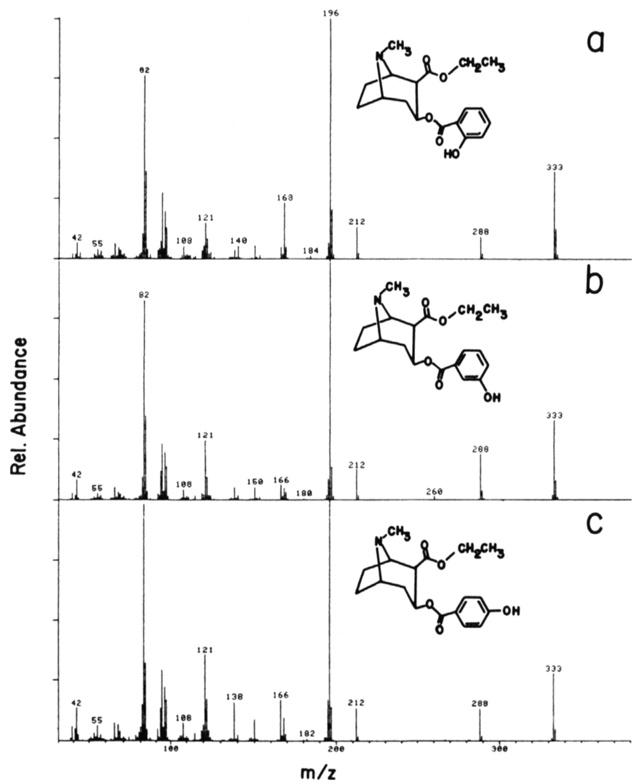


Figure 4. Mass spectra of synthesized arylhydroxycocaethylenes, *IIa-IIc* (a-c)

The relative t_R of the molecular weight (MW) 333 compounds in the two urine samples were very close to those of the synthesized *IIb* and *IIc*. The mass spectral ratios corroborated this identification. Although some of the mass spectral data for the individual metabolites did not conclusively distinguish between the 3′-hydroxy and 4′-hydroxy isomers, it was found that the ratios given in Table III were not different enough statistically to allow this distinction on the basis of mass spectrometry alone. Rather, the combination of GLC t_R and mass spectral data was necessary to distinguish all three isomers.

Arylhydroxymethoxycocaethylenes

Mass spectra of the five synthesized arylhydroxymethoxycocaethylenes (*IId-IIh*) are shown in Figure 5. These spectra also primarily reflect differences between isomers that have, or do not have, a 2′-hydroxy group. Isomers with 2′-hydroxy groups showed large m/z 363/318 ratios (decreased ethoxide loss) and relatively larger proportions of m/z 168 and 150 ions (γ-hydrogen loss of the benzoic acid portion) (Table IV). Isomers which were not safely separable by mass spectrometry alone were distinguishable by GLC, and vice versa.

The data from the single MW 363 compound in the urine samples fit most closely those of *IIh*, corroborating the previous identification of *Ic* in these samples as well (2). Unfortunately, sample deterioration and other testing precluded confirmation of these identifications by mixed GLC injections.

Curiously, the apparent concentrations of the hydroxy- and hydroxymethoxycocaethylenes were greater than those of the corresponding cocaine derivatives, according to the crude quantitation by mass chromatography shown in Figure 1. This contrasts with the relative concentrations of methyl and ethyl ecgonine, and of cocaine and cocaethylene, seen in the total ion chromatogram for this sample. The reason for this difference is not apparent, although it may imply conversion of cocaine to cocaethylene prior to ring oxidation, since the sterically more congested arylhydroxy- and arylhydroxymethoxycocaines would be expected to transesterify more slowly than cocaine itself (6).

Table III. Diagnostic Mass Spectral Ratios for Arylhydroxycocaethylenes

Compound	333/288	166/168	138/140	121/120
2′-OH (*IIa*)	5.09	0.21	0.73	2.64
3′-OH (*IIb*)	1.74	1.32	2.81	10.9
4′-OH (*IIc*)	2.55	1.46	5.15	9.2
Patient A, MW 333, #1*	—	—	—	—
Patient A, MW 333, #2	1.91	1.05	1.72	15.3
Patient B, MW 333, #1	1.96	1.09	1.35	9.3
Patient B, MW 333, #2	2.08	0.89	1.59	13.1

*Spectrum too contaminated for meaningful comparisons

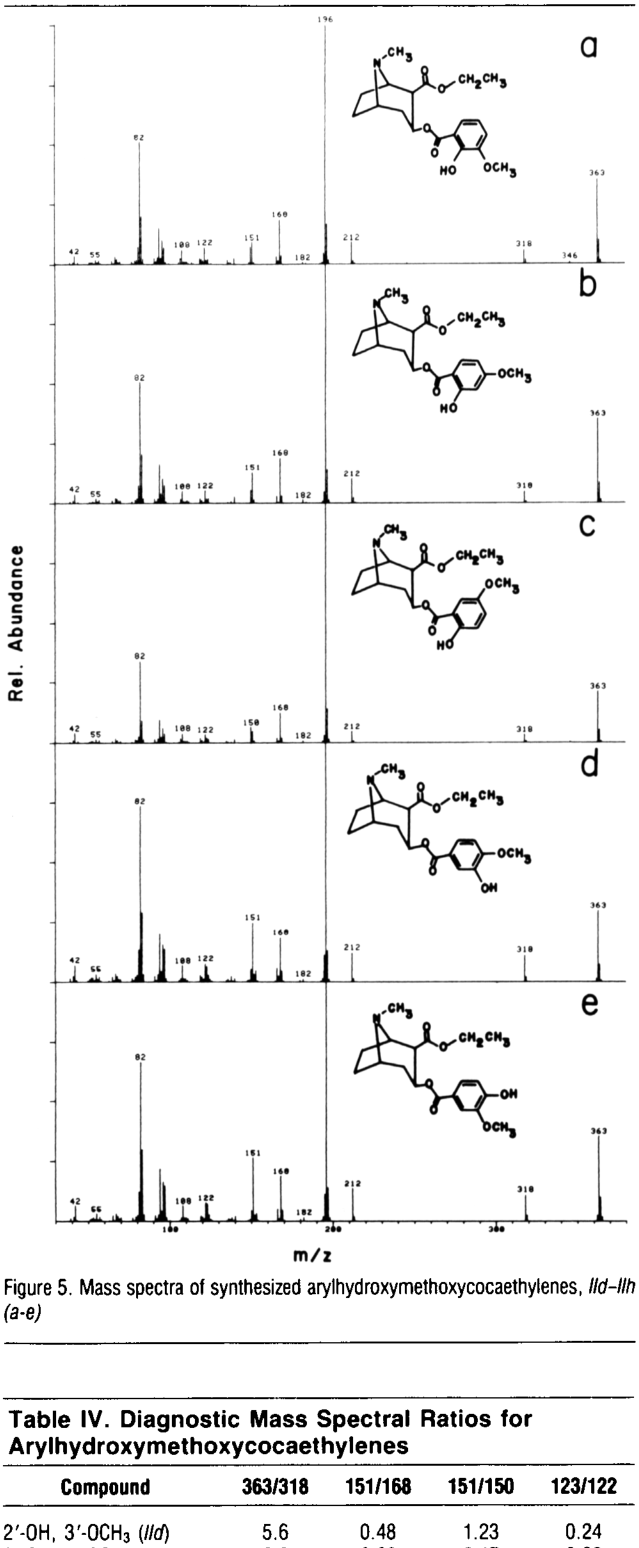


Figure 5. Mass spectra of synthesized arylhydroxymethoxycocaethylenes, *IId-IIh (a-e)*

Table IV. Diagnostic Mass Spectral Ratios for Arylhydroxymethoxycocaethylenes

Compound	363/318	151/168	151/150	123/122
2'-OH, 3'-OCH3 (*IId*)	5.6	0.48	1.23	0.24
2'-OH, 4'-OCH3 (*IIe*)	6.8	0.66	2.17	0.32
2'-OH, 5'-OCH3 (*IIf*)	8.0	0.48	0.86	0.68
3'-OH, 4'-OCH3 (*IIg*)	2.9	1.29	4.36	0.87
4'-OH, 3'-OCH3 (*IIh*)	3.3	1.40	5.68	0.94
Patient A, MW 363	2.7	1.14	4.84	0.5*
Patient B, MW 363	3.3	1.36	3.56	0.6*

*Spectrum contaminated in this area making values uncertain

The ultimate source of the cocaethylene derivatives in these patients' samples is not obvious either. It seems unlikely, however, that they are artifacts of the sample extraction procedure, since the extracting solvent contained a considerable excess of isopropanol (2). Transesterification of cocaine and its metabolites by isopropanol would be expected to some degree if that were true, but none was observed.

Conversion of cocaines to cocaethylenes in the urine without enzyme participation cannot be ruled out, since cocaethylene has been observed *in vitro* when cocaine is handled in acidic ethanol solution (7). Similarly, in biological samples, benzoylecgonine was found to esterify to some extent to cocaine in methanolic solution (8). However, such conditions seem much more forcing for transesterification (9) than those present in these urine samples, even considering the high (0.1 to 0.2%) alcohol concentrations. While one might expect cocaine "passively" to hydrolyze under these conditions (10,11), ester exchange seems much less reasonable. A more likely sight for transesterification to occur is in the stomach, where a considerable excess of acidic ethanol would be present. However, no information was received with either of these samples to indicate oral ingestion and, at least in this country, such a mode of administration is quite rare (12). Thus, these cocaethylene derivatives may, in fact, be true cocaine metabolites.

Acknowledgments

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References

1. F.K. Rafla and R.L. Epstein. Identification of cocaine and its metabolites in human urine in the presence of ethyl alcohol. *J. Anal. Toxicol.* **3:** 59-63 (1979).
2. R.M. Smith, M.A. Poquette, and P.J. Smith. Hydroxymethoxybenzoylmethylecgonines: New metabolites of cocaine from human urine. *J. Anal. Toxicol.* **8:** 29-34 (1984).
3. R.M. Smith. Arylhydroxy metabolites of cocaine in the urine of cocaine users. *J. Anal. Toxicol.* **8:** 35-37 (1984).
4. E.C.G. Clarke. *Isolation and Identification of Drugs.* The Pharmaceutical Press, London, 1969, p. 801.
5. R.A. Hites and K. Biemann. Computer evaluation of continuously scanned mass spectra of gas chromatographic effluents. *Anal. Chem.* **42:** 855-60 (1970).
6. E.S. Gould. *Mechanism and Structure in Organic Chemistry.* Holt, Rinehart and Winston, New York, 1959, p. 321.
7. T. Lukaszewski and W.K. Jeffery. Impurities and artifacts in illicit cocaine. *J. Forensic Sci.* **25:** 499-507 (1980).
8. R.L. Foltz, A.F. Fentiman, Jr., and R.B. Foltz. *GC/MS Assays for Abused Drugs in Body Fluids.* NIDA Res. Monograph #32. Rockville, Maryland, 1977, p. 105.
9. L.F. Fieser and M. Fieser. *Organic Chemistry.* Reinhold Publishing Corp., New York, 1956, p. 178.
10. S.R. Findlay. The three-dimensional structure of the cocaines. Part I. Cocaine and pseudococaine. *J. Amer. Chem. Soc.* **76:** 2855-862 (1954).
11. J.M. Moore. Identification of *cis-* and *trans-*cinnamoylcocaine in illicit cocaine seizures. *J. Assoc. Off. Anal. Chem.* **56:** 1199-205 (1973).
12. C. Van Dyke, P. Jatlow, J. Ungerer, P.G. Baresh, and R. Byck. Oral cocaine: Plasma concentrations and central effects. *Science* **200:** 211-13 (1978).

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