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POSITIVE IDENTIFICATION AND QUANTITATION OF ISOMERIC COCAINES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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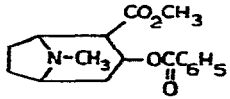
SUMMARY

A high-performance liquid chromatographic procedure for separating and identifying the four isomeric cocaines has been developed. Use of this procedure with an internal standard allows for the determination of the quantity of any isomeric cocaine in an unknown sample. The pitfalls and problems encountered in the use of gas chromatography and mass spectrometry in the analysis of cocaines and ecgonine methyl esters are discussed.

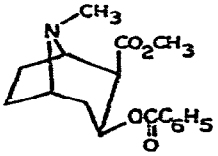
INTRODUCTION

That 3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester (I) is capable of existing in four diastereomeric configurations was

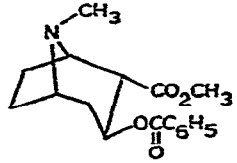


I

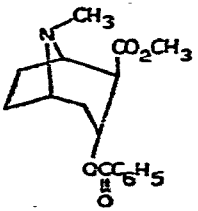
recognized by Willstätter *et al.*[1] early in this century. All four compounds are known and possess the trivial names shown below (the nomenclature of Sinnema *et al.*[2] will be used):



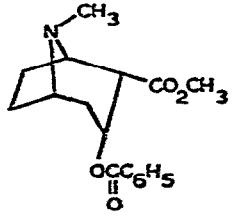
Cocaine
C



Pseudococaine
ΨC

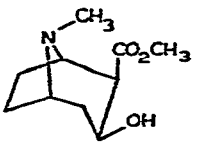


Allococaine
AC

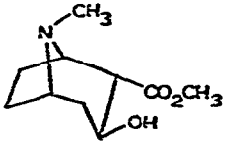


Allopseudococaine
AΨC

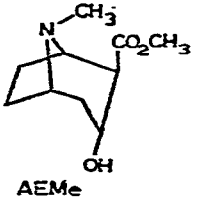
At present cocaine, which is a potent stimulant, is one of the most highly abused controlled drugs. Consequently the action and metabolism of cocaine and its congeners are under extensive investigation. Analytical procedures for cocaine and pseudococaine are available, but no method for the positive identification and quantitation of the four isomeric cocaines has been reported. We therefore undertook to develop such a method, and in the course of our investigations we discovered several anomalies of these systems leading to pitfalls in the application of routine analytical procedures. To aid investigators in the analysis of metabolites we examined the ecgonine methyl esters as well. In this paper we report a rapid, quantitative high-performance liquid chromatographic (HPLC) procedure for the analysis of cocaines and discuss problems involved in the gas chromatographic (GC) and mass spectrometric (MS) analyses of these compounds.



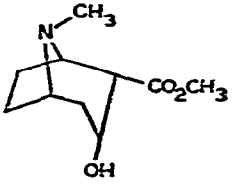
Ecgonine Methyl Ester (EMe)



ΨEMe



AEMe



AΨEMe

MATERIALS AND METHODS

Instrumentation

GC analyses were carried out using a Varian Series 1400 gas chromatograph with a flame-ionization detector (FID). The columns used were 1.8 m × 2 mm I.D. glass, packed with 3% SP 2250-DB (Supelco) or 2% OV-17. The detector temperature was 250 °C, and the injector temperature was 210 °C. Gas flow-rates were adjusted to 300 ml/min for air, 30 ml/min for hydrogen and 30 ml/min for nitrogen at ambient temperature.

Combination GC–MS analyses were performed using either a Finnigan 3300 GC–MS or a LKB 2091 EI/CI GC–MS, both operating in the chemical-ionization (CI) mode, using iso-butane or NH_3, as indicated. The GC conditions used correspond to those described above.

Ultraviolet spectra were determined on a Cary 14 spectrophotometer in 1-cm quartz cells.

HPLC analyses were carried out using a Waters Assoc. high pressure liquid chromatograph consisting of two constant flow pumps (Model 6000A) controlled electronically by a solvent programmer (Model 660), a septumless nonstop-flow high-

pressure injector (Model U6-K) and a variable wavelength UV detector (Model 450). A 250 × 4.6 mm I.D. stainless-steel column packed with Partisil 10 (Whatman Partisil 10 PXS) was used. The solvents used were: heptane, HPLC grade (Burdick & Jackson Labs., Muskegon, MI, U.S.A.); isopropanol, reagent grade (Fisher, Pittsburgh, PA, U.S.A.) 1 μ filtered; diethylamine, reagent grade (Fisher) distilled and 1 μ filtered.

Materials

Cocaine was obtained from the National Institute on Drug Abuse. It was converted to ecgonine methyl ester using literature methods[3,4] and to pseudoecgonine methyl ester (ψEMe) by the method of Findlay[4]. Benzoylation of ψEMe was carried out using benzoic anhydride and 1,5-diazabicyclo[5.4.0]undec-5-ene in benzene[5]. Allococaine, allopseudococaine, alloecgonine methyl ester and allopseudoecgonine methyl ester were all prepared following literature procedures[2]. All the compounds prepared were fully characterized and had physical parameters in agreement with the literature. The HPLC standard N,N-dibenzylbenzamide was available from another study[6].

Procedure

HPLC conditions: cocaine samples were analyzed on Partisil 10-PXS, eluting with isopropanol in heptane containing diethylamine (25:75:0.1) at a flow-rate of 0.48 ml/min increasing approximately exponentially to 4 ml/min over 12 min (program No. 10). The UV detector was set at 230 nm.

Each cocaine isomer was calibrated *vs.* the internal standard (N,N-dibenzylbenzamide) by dissolving an accurately weighed sample of the cocaine in a known volume of a solution of N,N-dibenzylbenzamide in isopropanol. Four such solutions ranging from 1×10^{-3} to 9×10^{-3} mmol cocaine/mg standard were prepared for each cocaine isomer. Each solution was analyzed at least three times by HPLC. The peak areas were determined by triangulation, and the area ratio (cocaine/std) was related to the concentration ratio (mmol cocaine/mg std.) by linear regression analysis.

The analytical procedure for an unknown sample is as follows: the sample is dissolved in a known volume of a standard solution of N,N-dibenzylbenzamide ($\approx$1 mg/ml) in isopropanol, and the resulting solution is analyzed by HPLC. The weight of the cocaine isomer(s) in the standard solution is then calculated using the expression:

$$y = ax + b$$

in which y = mmol cocaine/mg standard and x = area ratio for cocaine/standard.

RESULTS

Gas chromatography

Routine GC analysis of the cocaines revealed that, whereas cocaine (C) and ψ-cocaine (ψC) gave sharp single peaks at temperatures above 200° on low load OV-17 or SP-2250 columns, allococaine (AC) and allopseudococaine (AψC), under the same conditions, gave signals of extremely short retention time, eluting immediately after

the solvent. Carrying out the GC analysis isothermally at 140° for 10 min and programming at 10 °C/min to 240° again gave sharp signals for C and φC above 240°. AC and AφC, on the other hand, gave sharp signals of short retention time (9.5 and 9.0 min, respectively) with very broad peaks eluting after 23 min. The situation is summarized in Table I.

TABLE I

GC BEHAVIOR OF COCAINES

Retention times observed using 6 ft. 3% SP-2250-BD in glass columns and *m/e* values obtained using CI/NH_3

Compound	*Retention time (min)*		
	240 °C	*210 °C (m/e)*	*140 °C/10 min then 10 °C/min to 240 °C*
Cocaine	6.0	18.8 (304)	28.8
φ-Cocaine	5.8	17.1 (304)	28.0
Allococaine	—	1.2 (182)	9.5, 23 broad
Allo-φ-cocaine	—	1.2 (182)	9.0, 23 broad

GC of the ecgonine methyl esters at 140 °C showed alloecgonine methyl ester (AEMe) to have two peaks whose ratio varied with the particular analytical conditions, *e.g.* injector temperature, sample size. The other three ecgonine methyl esters gave sharp single peaks (Table II).

TABLE II

GC BEHAVIOR OF ECGONINE METHYL ESTERS

Compound	*Retention time (min)*	
	2% OV-17 at 130 °C	*3% SP-2250-BD at 140 °C*
Ecgonine methyl ester	8.6	5.5
φ-Ecgonine methyl ester	9.9	7.0
Alloecgonine methyl ester	5.3, 12.5	3,6, 9.1
Allo-φ-ecgonine methyl ester	8.1	5.0

High-performance liquid chromatography

A chromatogram of the results of HPLC analysis of the isomeric cocaines is shown in Fig. 1. Excellent separation was obtained using Partisil 10 with heptane–isopropanol–diethylamine (75:25:0.1) as eluent. A calibration curve using dibenzylbenzamide as internal standard gave a good least squares linear fit; the constants along with the retention times are shown in Table III.

Ultraviolet spectra

Since HPLC analysis was performed using UV detection, the UV spectra of the cocaines were recorded in the eluent system used. The results are shown in Table IV.

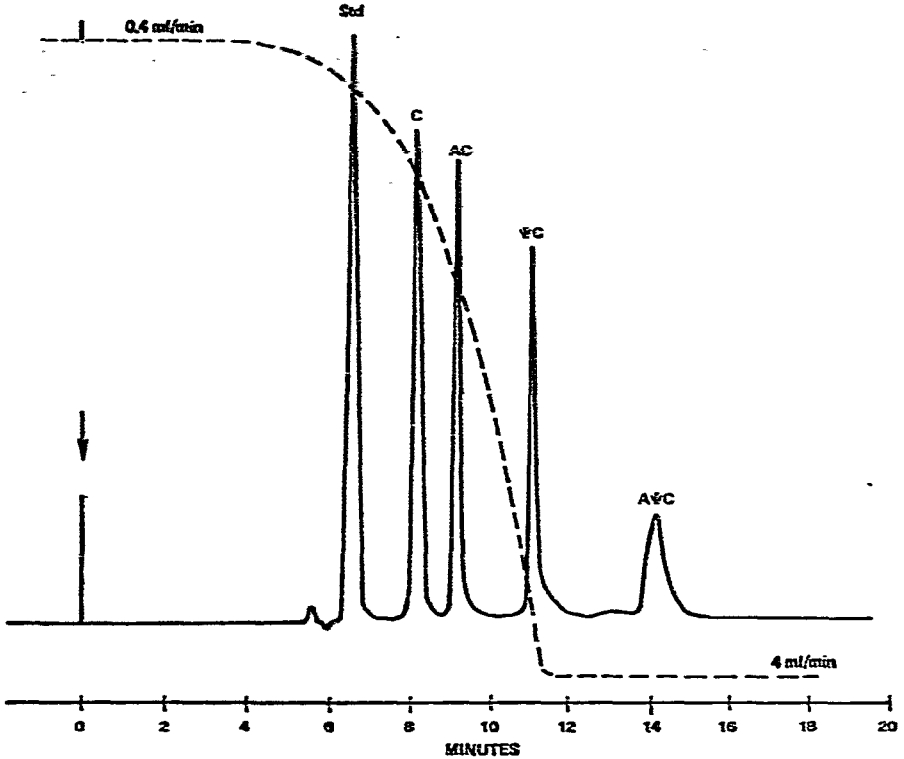


Fig. 1. Separation of isomeric cocaines by HPLC using Partisil 10 PXS. Solvent: 75% heptane, 25% isopropanol with 0.1% diethylamine. The flow program is indicated by the dashed line. The solution was made up of 0.822 mg C·HCl, 1.982 mg ψC·HCl, 1.000 mg AC and 2.030 AψC·HCl in 1 ml isopropanol containing 1.068 mg N,N-dibenzylbenzamide (std).

TABLE III

LINEAR FIT FOR ISOMERIC COCAINES

The linear fit parameters for $y = ax + b$, in which y = mmol of compound/mg of standard and x = observed area ratio, compound/standard, in HPLC analysis. The HPLC conditions are described in the Materials and methods section.

Compound	$a \times 10^3$	$b \times 10^4$	r
Cocaine	3.605	−5.959	0.996
ψ-Cocaine	8.299	0.618	1.000
Allococaine	4.413	−2.340	0.999
Allo-ψ-cocaine	11.12	5.000	0.994

TABLE IV

UV SPECTRA OF COCAINES

The spectra were recorded in the solvent system used for HPLC: heptane–isopropanol–diethylamine (75:25:0.1).

Compound	$\lambda_{max.}$	$\varepsilon \times 10^{-4}$
Cocaine	228.0	1.02
ψ-Cocaine	226.5	1.62
Allococaine	226.0	1.22
Allo-ψ-cocaine	228.0	1.16

Stability

The four isomeric cocaines were found to be stable in isopropanol solutions (1 mg/0.1 ml) for at least one week. Allococaine, however, decomposed in chloroform solution.

The ecgonine methyl esters were stable as the hydrochloride salts, but the free bases were susceptible to base catalyzed isomerization. Alloecgonine methyl ester was particularly unstable; a chloroform solution formed a substantial amount of AψEMe within two days.

Mass spectrometry

Analysis of the cocaines by GC–MS (CI/isobutane) showed the cocaine and ψ-cocaine signals to have *m/e* 304. By contrast, the signals observed for allococaine and allo-ψ-cocaine had *m/e* 182. The results of direct probe CI-MS using isobutane and ammonia are shown in Table V.

TABLE V

MASS SPECTRA OF COCAINES

The major mass spectral fragments (%) of cocaines observed in the CI mode with isobutane (i-Bu) and ammonia (NH_3) matrices.

m/e	*Compound*							
	Cocaine		*ψ-Cocaine*		*Allocaine*		*Allo-ψ-cocaine*	
	Matrix							
	i-Bu	NH_3	*i-Bu*	NH_3	*i-Bu*	NH_3	*i-Bu*	NH_3
305	19	19	3	19	19	19	3	19
304	100	100	19	100	100	100	15	100
303	24	24	16	33	25	26	14	29
183	7	5	11	9	7	6	11	10
182	54	46	100	76	61	52	100	92
181		4	2	5		4	4	10

GC–MS analysis of the ecgonine methyl esters (CI/isobutane) showed the expected *m/e* 200 for all of the isomers, but the fast eluting peak observed for the allo isomer had *m/e* 182. The mass spectra of directly introduced ecgonine methyl esters showed small amounts of fragments of *m/e* 182 (Table VI).

DISCUSSION

The GC behavior of cocaines (Table I) clearly indicates that GC is not an appropriate analytical method for their positive identification, quantitation or purity determination. Thus, cocaine and ψ-cocaine do not separate very well, but, more significantly, allo- and allo-ψ-cocaine appear to give rise only to decomposition products. A clue to the nature of these products is found in the *m/e* value, 182, observed for the decomposition products. This value corresponds to a protonated ion arising from the loss of a fragment of mass 122 from the cocaine (mol.wt. 303),

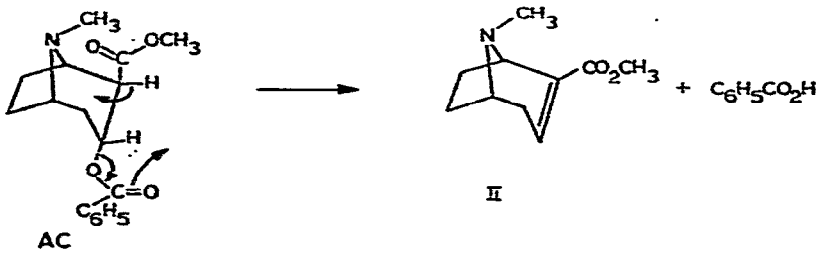
TABLE VI

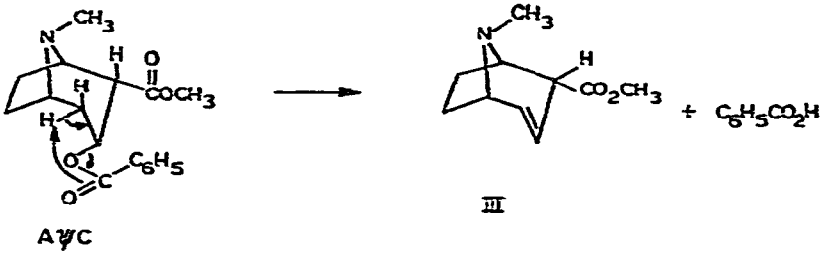
MASS SPECTRA OF ECGONINE METHYL ESTERS

The major mass spectral fragments (CI/isobutane) of GC signals (2% OV-17) and of directly introduced ecgonine methyl esters. The GC retention times are shown in Table III.

Compound	*m/e*	*Relative abundance (%)*	
		GC	*Direct probe*
Ecgonine methyl ester	201	20	16
	200	100	100
	199	12	6
	182	3	3
ψ-Ecgonine methyl ester	201	15	14
	200	96	100
	199	14	7
	182	100	15
Alloecgonine methyl ester			
(1st GC signal)	183	11	
	182	100	
	181	10	
	137	53	
(2nd GC signal)	201	15	16
	200	100	100
	199	14	10
	182	18	12
Allo-ψ-ecgonine methyl ester	201	11	12
	200	100	100
	199	5	25
	182	15	25

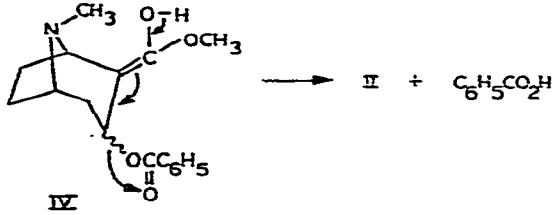
which is very suggestive of benzoic acid. In fact, injection of benzoic acid under analogous conditions gives rise to a broad GC signal of the observed retention time. Coinjection shows that it corresponds to the signal of retention time 23 min observed in the GC of allo and allo-ψ-cocaine (Table I). Thus, it appears that pyrolytic elimination of benzoic acid is taking place. Such eliminations have been shown to have *cis* stereochemistry[7]. Therefore, elimination of benzoic acid from allococaine would lead predominantly to the conjugated carbomethoxytropidine (II) and elimination from allo-ψ-cocaine will lead to the unconjugated carbomethoxytropidine (III). In fact, different retention times are observed for the GC signals with $m/e = 182$ arising from allo- and allo-ψ-cocaine.





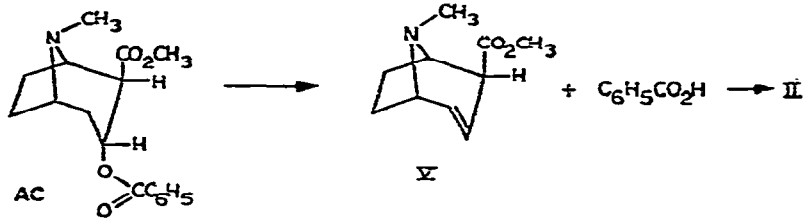
AψC III

Mass spectral analysis of cocaines can be similarly misleading. Inspection of Table V reveals that loss of 122 from the protonated molecular ion 304 represents an important fragmentation pathway for all cocaines, even in an ammonia matrix. Strikingly, the isobutane spectra of ψC and AψC show only insignificant 304 signals, which could easily lead to erroneous identification of the compound in question. Apparently, once again, elimination of benzoic acid is facile. In this case, however, elimination is probably not entirely pyrolytic since it is partially supressed in an ammonia matrix, and it takes place in ψC and AψC rather than in AC and AψC. The common feature of ψC and AψC is the equatorial 2-carbomethoxy group, and it is therefore tempting to suggest that elimination of benzoic acid is related to the ease of its enolization in the matrix followed by loss of benzoic acid from the enol (IV).



IV

An additional elimination problem is encountered in the handling of AC. Analysis of solutions of AC in $CHCl_3$ (≈1 mg/0.1 ml) by HPLC showed about one-half the AC to have disappeared after *ca.* 2 h, with the concommitant appearance of a new compound V of $m/e = 182$, different in GC retention time from II or III. At the end of 12 h AC could no longer be detected, and the signal due to V had decreased in favor of the signal due to II. Eventually V was completely transformed into II. These observations suggest that V may be a geometric isomer of the unconjugated 2-carbomethoxytropidine III. It is formed by elimination and then rearranges to the more stable conjugated carbomethoxytropidine II.



AC V

A somewhat analogous situation obtains with the ecgonine methyl ester isomers. GC analysis is obviously unsuitable for AEMe since it gives rise to two signals (Table II). The faster eluting of these has *m/e* 182 (Table VI), which corresponds to a protonated ion arising by the loss of water (18) from the molecular ion of AEMe (199). Although only one GC signal is observed for ψEMe, this signal has a very substantial *m/e* of 182 (see Table VI). That this is not due to fragmentation is shown by the *m/e* values obtained when ψEMe is introduced directly (Table VI). Thus, it appears that elimination of water is also taking place in this case, but the resulting olefin presumably coelutes with the ester. It is interesting to note that in the case of the ecgonine methyl esters the two compounds which appear to undergo dehydration are those in which the 2,3 substituents are *trans* to each other.

From the above discussion it is obvious that a different method for identification and quantitation for cocaines is required. Such a method has been developed using HPLC techniques. The cocaines are detectable by UV due to the benzoate ester chromophore (Table IV), and it was found that use of isopropanol in heptane with 0.1% diethylamine gave baseline separation of the isomeric cocaines (Fig. 1). A non-linear flow program (0.48 ml/min $\rightarrow$ 4 ml/min) was used in order to accelerate the elution of the slowest eluting component, AψC. Use of an internal standard allowed the calculation of the amount of any cocaine isomer present in solution.

Using this analytical system, we were able to determine that solutions of all four cocaine isomers in isopropanol were stable for at least 1 week. Solutions of C and ψC were stable for 6 months, but solutions of AC and AψC were totally decomposed after this long time.

CONCLUSIONS

The detection, positive identification and quantitation of cocaines can be carried out conveniently and accurately by use of the HPLC system described. Conclusions based on the use of other analytical methods may be incorrect for cocaines and for ecgonine methyl esters. Solutions of these compounds are fairly unstable, particularly those of the allo- and allopseudo-configuration. The major decomposition pathway seems to be elimination of benzoic acid or water from the cocaine or the ecgonine methyl ester, respectively.

ACKNOWLEDGEMENTS

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