Journal of Chromatography, 504 (1990) 391–401
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 265

Isolation, identification and separation of isomeric truxillines in illicit cocaine

IRA S. LURIE*, JAMES M. MOORE, THEODORE C. KRAM and DONALD A. COOPER
Special Testing and Research Laboratory, Drug Enforcement Administration, 7704 Old Springhouse Road, McLean, VA 22102 (U.S.A.)
(First received September 11th, 1989; revised manuscript received January 3rd, 1990)

SUMMARY

Seven out of the eleven possible intact isomeric truxillines present in illicit cocaine have been isolated and identified. These truxilline alkaloids included α-, β-, γ-, δ-, ε-, ω- and *neo*-isomers. The individual truxillines were characterized via high-performance liquid chromatography–diode array detection, capillary gas chromatography–electron ionization mass spectrometry and capillary supercritical fluid chromatography–flame ionization detection. α- and β-truxilline were also identified using nuclear magnetic resonance spectrometry. Good resolution of the intact truxillines was obtained using high-performance liquid chromatography.

INTRODUCTION

The eleven alkaloidal isomeric truxillines, the structures of which are shown in Fig. 1, have been reported recently to be present in illicit cocaine at the <0.01%–1% level[1]. This is not surprising since α- and β-truxilline have been shown to exist in coca leaf[2,3]. The presence of all eleven isomeric truxillines in illicit cocaine was originally based on good indirect evidence provided mainly from the chromatographic and spectrometric analysis of the dimethyl esters of diphenylcyclobutanedicarboxylic acids formed after acid hydrolysis[1]. The truxillic and truxinic acids (isomeric diphenylcyclobutanedicarboxylic acids) are obtained from the acid hydrolysis of the intact truxillines. The detection of these trace constituents in cocaine samples could be important for intelligence purposes, in particular for the comparative analysis of exhibits.

The chromatographic analysis described by Moore *et al.*[1] involved lithium aluminum hydride reduction of the truxillines followed by derivatization with heptafluorobutyric anhydride and analysis via capillary gas chromatography with electron-capture detection (GC–ECD). Sensitivity at the low pg level on-column was obtained. Previous attempts at chromatographing the intact truxillines via capillary GC proved unsuccessful[1]. In contrast, high-performance liquid chromatography (HPLC) and capillary supercritical fluid chromatography (SFC) appeared promising

0021-9673/90/$03.50

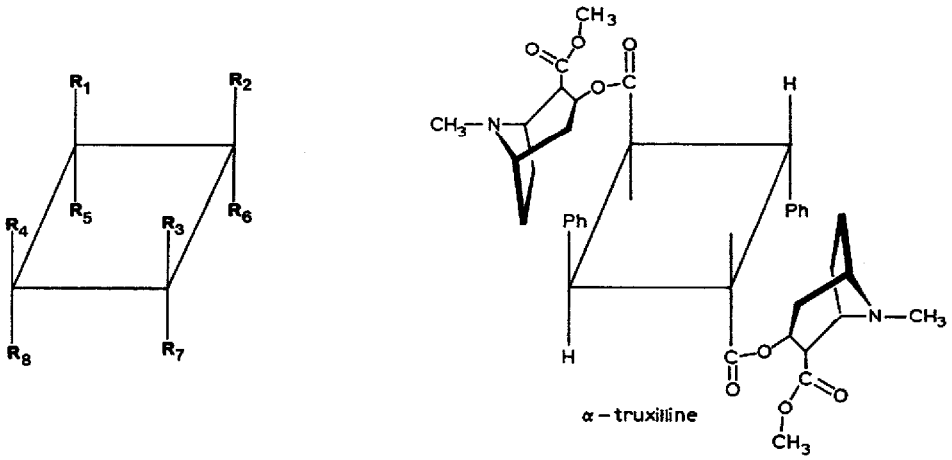

Fig. 1. Structures of the truxillines. (Left) General structure. (1) α-, $R_1 = R_7 =$ methyl ecgonine ester, $R_4 = R_6 =$ phenyl, $R_2 = R_3 = R_5 = R_8 =$ H; (2) β-, $R_5 = R_6 =$ methyl ecgonine ester, $R_3 = R_4 =$ phenyl, $R_1 = R_2 = R_7 = R_8 =$ H; (3) δ-, $R_2 = R_5 =$ methyl ecgonine ester, $R_4 = R_7 =$ phenyl, $R_1 = R_3 = R_6 = R_8 =$ H; (4) ε-, $R_5 = R_7 =$ methyl ecgonine ester, $R_2 = R_4 =$ phenyl, $R_1 = R_3 = R_6 = R_8 =$ H; (5) μ-, $R_1 = R_6 =$ methyl ecgonine ester, $R_4 = R_7 =$ phenyl, $R_2 = R_3 = R_5 = R_8 =$ H; (6) γ-, $R_1 = R_3 =$ methyl ecgonine ester, $R_4 = R_6 =$ phenyl, $R_2 = R_5 = R_7 = R_8 =$ H; (7) *neo*-, $R_2 = R_5 =$ methyl ecgonine ester, $R_3 = R_4 =$ phenyl, $R_1 = R_6 = R_7 = R_8 =$ H; (8) ζ-, $R_5 = R_6 =$ methyl ecgonine ester, $R_4 = R_7 =$ phenyl, $R_1 = R_2 = R_3 = R_8 =$ H; (9) *epi*-, $R_1 = R_7 =$ methyl ecgonine ester, $R_2 = R_4 =$ phenyl, $R_3 = R_5 = R_6 = R_8 =$ H; (10) *peri*-, $R_1 = R_3 =$ methyl ecgonine ester, $R_2 = R_4 =$ phenyl, $R_5 = R_6 = R_7 = R_8 =$ H; (11) ω-, $R_1 = R_2 =$ methyl ecgonine ester, $R_3 = R_4 =$ phenyl, $R_5 = R_6 = R_7 = R_8 =$ H. (Right) Structure of α-truxilline. Ph = phenyl.

for the direct analysis of these compounds[4,5]. Two HPLC systems have been reported for the analysis of the truxillines using reversed-phase chromatography and UV detection[4]. In these works the identities of the individual truxilline peaks were not determined. One system used dodecylsulfate as a counter ion and was good for the simultaneous separation of acids, monoprotic amines and diprotic amines in illicit cocaine. Resolution obtained for seven peaks believed to be truxillines was fair. The second system was used only for the analysis of truxillines and gave good reslution for eight peaks believed to be truxillines. UV detection afforded on-column sensitivity at the low-ng level[6]. Excellent methodology sensitivity was obtained via HPLC because of the ability to use large injection volumes. Capillary SFC analysis of a HPLC size-exclusion extract also believed to contain truxillines has been reported[5]. Sensitivity of these compounds on-column, using UV detection, was at the high-pg level[6]. Capillary SFC was used as a viable alternative to HPLC and GC because of the possibility of a high-resolution separation of the intact isomeric truxillines. In the capillary SFC system the identities of the individual truxillines were not determined.

In this study seven intact truxillines were isolated using previously reported chromatographic methods including alumina column chromatography[1], reversed-phase chromatography[4] and size-exclusion chromatography[5]. Analytical data were obtained for the individual truxillines via HPLC using a diode array detector, by capillary GC using both an electron-capture cell and a low-resolution mass spectrometry (MS) system operated in the electron ionization (EI) modes as detectors, and by

capillary SFC using a flame ionization detector. Two of the truxillines (α and β) were also analyzed via nuclear magnetic resonance (NMR) spectroscopy.

EXPERIMENTAL

Equipment

The equipment used for size-separation chromatography, analytical–preparative HPLC separations, analytical HPLC separations using diode array detection, capillary GC–ECD, capillary SFC with flame ionization detection (FID) and NMR analysis have been described elsewhere[1,4,5]. For capillary SFC analysis various 10 m × 50 μm I.D. fused-silica capillary columns were used (Lee Scientific, Salt Lake City, UT, U.S.A.). The stationary phases included SB-octyl-50 (0.25 μm film thickness), SB-methyl-100 (0.25 μm film thickness), SB-biphenyl-30 (0.25 μm film thickness), SB-cyanopropyl-25 (0.25 μm film thickness) and SB-smectic (0.15 μm film thickness).

For semi-preparative HPLC separations the same equipment used for analytical–preparative analysis was used[4] except the column consisted of a Magnum 9 ODS-3 column (25.0 cm × 9.4 mm I.D.) (Whatman, Clifton, NJ, U.S.A.).

Low-resolution EI-MS spectra were obtained on a Finnigan MAT 4630 (San Jose, CA, U.S.A.) quadrupole mass spectrometer. The column for the capillary GC–MS system consisted of a DB-1 (8 m × 0.25 mm I.D., film thickness 0.25 μm) (J & W Scientific, Rancho Cordova, CA, U.S.A.). Sample injection was accomplished using an on-column injector (J & W Scientific) and helium was used as the carrier gas at a velocity of 40 cm/s. EI data were acquired at an ionization potential of 50 eV and a source temperature of 140°C. Samples were dissolved in chloroform [some solutions contained 25% (v/v) N,O-bis(trimethylsilyl)acetamide (BSA)].

200-MHz proton NMR spectra were obtained as described previously[1]. HPLC fractions were made alkaline with sodium bicarbonate and extracted with deuterated chloroform. The organic layer was backwashed with deuterated water to diminish the presence of hydrophilic artifacts and to reduce the water proton signal. Standards were dissolved in deuterated chloroform and evaporated to dryness in a warm water bath (50°C) under a stream of nitrogen. The solution and evaporation steps were repeated as before in order to remove or otherwise diminish volatile organic solvent impurities. The residue was then taken up with deuterated chloroform and washed successively with sodium bicarbonate–deuterated water and deuterated water.

Materials

Methanol, acetonitrile and tetrahydrofuran (Burdick & Jackson, Muskegon, MI, U.S.A.) were HPLC grade. Other chemicals were reagent grade.

The HPLC mobile phases were internally mixed from solvent reservoirs containing methanol, acetonitrile, tetrahydrofuran, methylene chloride and either phosphate buffer or phosphate buffer containing 0.02 *M* sodium dodecyl sulfate, pH 2.0. The phosphate buffer was a mixture of 3480 ml water, 120 ml 2 *M* sodium hydroxide and 40.0 ml phosphoric acid.

α-Truxilline was prepared from α-truxilloyl chloride and methyl ecgonine in a procedure similar to the one used for the synthesis of *trans*-cinnamoylcocaine[7]. α-Truxilloyl chloride was prepared from α-truxillic acid and thionyl chloride.

α-Truxillic acid was synthesized and characterized as previously described[1]. The synthesized standard was characterized via proton NMR, MS, IR and UV.

β-Truxilline was prepared as above from β-truxilloyl chloride and methyl ecgonine. β-Truxinic acid was synthesized and characterized as previously described[1]. Unlike the α-truxilline synthesis, a mixture of roughly equal amounts of β- and *neo*-truxilline was produced. β-Truxilline was isolated from this mixture using alumina column chromatography, potassium permanganate oxidation and semi-preparative HPLC. The standard was characterized via proton NMR, MS, IR and UV.

Semi-preparative isolation of truxillines

An equivalent of 1.0 g of cocaine–HCl was subjected to extractions and alumina column chromatography[1].

The extract from the column was evaporated to dryness and reconstituted in 5 ml of diethyl ether. Three 1-ml portions were evaporated to dryness. Each fraction was then reconstituted in 200 μl of the HPLC starting mobile phase prior to injection of the sample onto the liquid chromatograph.

Gradient elution for HPLC analysis was used at ambient temperature with a flow-rate of 6.3 ml/min. A 60-min linear gradient from methanol–phosphate buffer (20:80) to (50:50) was used.

Analytical–preparative isolation of truxillines

An illicit cocaine–HCl sample equivalent to 1.0 g cocaine–HCl was dissolved in 20 ml 0.05 *M* sulfuric acid and then extracted with three 20-ml aliquots of methylene chloride. The solution was made basic by the addition of 20.0 ml of a saturated solution of sodium bicarbonate and then extracted with three 40-ml aliquots of methylene chloride. The combined methylene chloride extracts were concentrated to 1 ml on a water bath at 60°C under a stream of nitrogen, prior to nine 100-μl injections into the liquid chromatograph fitted with size-exclusion column.

Size-exclusion chromatography was performed as previously described[5], except the effluent was diverted to a fraction collector.

The pooled fractions of interest from size-exclusion chromatography representing compounds of higher molecular size were evaporated to dryness on a water bath at 60°C under a stream of nitrogen and reconstituted in 500 μl of starting mobile phase prior to injection into the liquid chromatograph.

A previously reported gradient system for the analysis of these compounds[4] was modified as follows. A 30-min linear gradient from methanol–phosphate buffer (20:80) to (50:50) was employed.

RESULTS AND DISCUSSION

α- and β-truxilline were isolated from an illicit cocaine–HCl exhibit using liquid–liquid extractions, alumina column chromatography and semi-preparative HPLC. The proton NMR spectra of the extracted HPLC fractions representing these compounds were consistent with their respective structures in view of spectra previously reported for the dimethyl esters of the corresponding acids (and in contrast with those reported, or otherwise calculated, for the nine other isomers[1,8–10]) and for methyl ecgonine[11] and the relative intensities of selected signals representing the acid portion of each compound to those of the methyl ecgonine moieties.

Most of the major signals observed in the two truxillines exhibited an upfield shift of 0.1 ppm or less as compared with their counterparts in the diphenylcyclobutanedicarboxylic methyl esters and in methyl ecgonine. The phenyl signals, at about 7 ppm, did not shift at all. The tritium absorbance of methyl ecgonine (3.8 ppm) exhibited a dramatic downfield shift in the formation of the truxillines, appearing at 4.5 and 4.6 ppm in the α-isomer and at 5.0 ppm in the β. Deshielding shifts of this magnitude are common for the signals of secondary protons geminal to hydroxyl groups when they undergo esterification (the "acylation shift"[12,13]).

NMR spectra of these two extracts closely matched synthesized α- and β-truxilline, respectively, as shown in Figs. 2 and 3. Minor differences noted between sample and standard, particularly upfield of 1.3 ppm, are ascribed to moisture and other impurities. Interferences of this sort are virtually unavoidable when sub-mg quantities of a compound are extracted from a large chromatographic fraction of a natural substance and spectrally compared with an identical compound synthetically prepared.

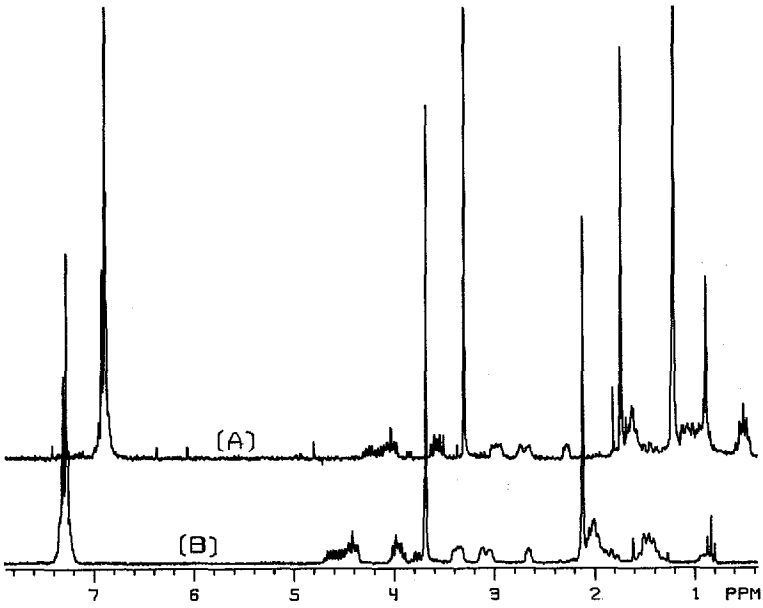

Fig. 2. 200-MHz proton NMR spectra of α-truxilline as obtained from (A) extracted HPLC fraction and (B) synthesized standard.

Seven truxillines, including α- and β-truxilline, were isolated from an illicit cocaine–HCl exhibit using liquid–liquid extractions, size-exclusion chromatography and analytical–preparative HPLC. Size-exclusion chromatography gave a cleaner extract and a more nearly quantitative recovery than alumina column chromatography. HPLC analysis of the truxilline content before and after alumina column chromatography revealed different ratios of the truxilline isomers.

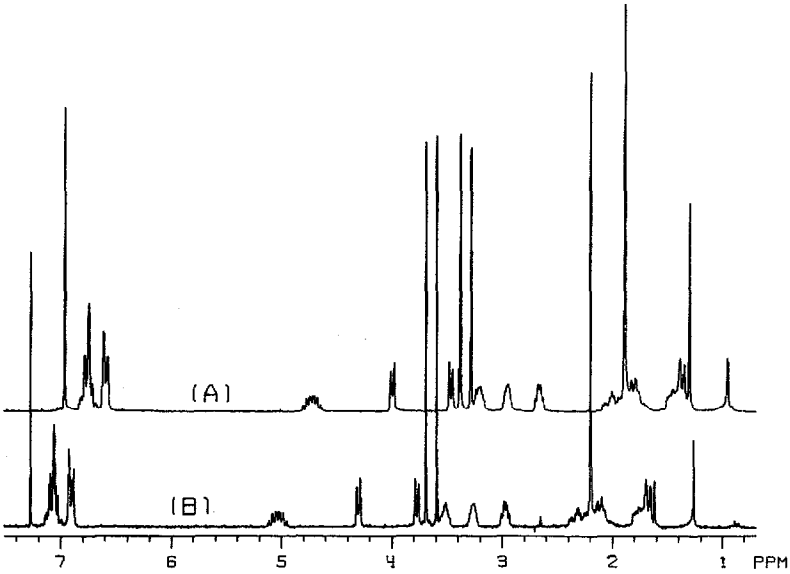

Fig. 3. 200-MHz proton NMR spectra of β-truxilline as obtained from (A) extracted HPLC fraction and (B) synthesized standard.

The seven truxillines isolated via analytical–preparative HPLC dissolved in mobile phase were reanalyzed on the same system with one major peak obtained for each fraction. In addition the seven fractions were also analyzed via HPLC and diode array detection using the previous reported system for the simultaneous analysis of acidic and basic impurities in cocaine[4]; with again only one major peak obtained. UV spectra obtained for the individual peaks using the diode array detector were consistent with compounds containing benzene rings without extended conjugation. An example of a UV spectrum obtained from an isolated fraction is shown in Fig. 4.

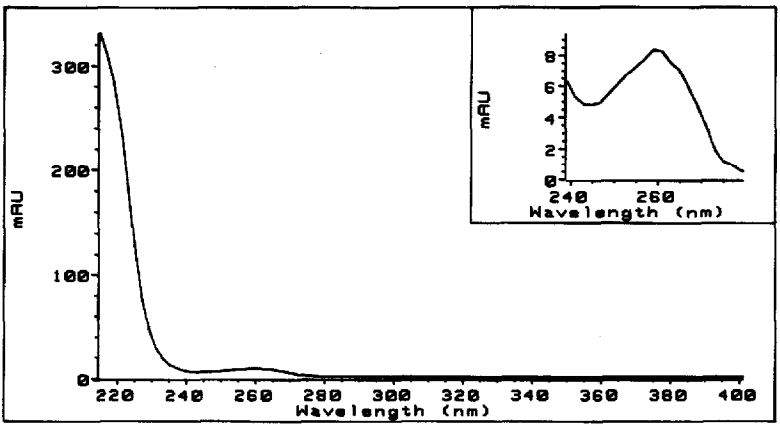

Fig. 4. Apex-UV spectrum for peak obtained from isolated fraction representing α-truxilline. Chromatographic conditions described in ref. 4.

Fractions, each representing a truxilline compound, were then made basic with saturated sodium bicarbonate and extracted with methylene chloride prior to capillary GC–ECD, capillary GC–MS and capillary SFC–FID analysis.

The isolated truxillines were subjected to lithium aluminum hydride reduction[a] followed by derivatization with heptafluorobutyric anhydride (HFBA) and capillary GC–ECD analysis[1]. Each truxilline fraction gave peaks matching ecgonine and, as expected, a truxillic or truxinic acid. The resulting data obtained on the truxilline isolates were correlated to the work of Moore *et al.*[1] which in turn allowed the identification of the respective truxillines as the α-, β-, γ-, δ-, ε-, ω- and *neo*-isomers. GC–ECD analysis also revealed that minor amounts of the *peri*- and/or *epi*- and ζ-isomers were also present in a few of the isolated fractions.

Direct spectrometric proof that the isolated fractions contained truxillines was provided by capillary GC–MS under EI conditions. Previous attempts at chromatographing intact truxillines via capillary GC using either a standard Grob injection port with a DB-1 stationary phase or an on-column injector with a DB-1701 stationary phase proved unsuccessful[1]. However, in this work it was found that a cold (less than 100°C) on-column injection of the truxilline in a solution of methylene chloride–BSA (75:25, v/v) into a fused-silica capillary column (DB-1) resulted in a chromatogram having less than 10% decomposition products of the respective truxilline[b], that is, *ca.* 90% of the truxilline chromatographed without decomposition. Without the addition of BSA, the response obtained for the truxilline was estimated to be *ca.* 25% of expected. The improved performance with BSA is believed to be due to the masking of active sites by the silylating reagent.

EI-MS data for the truxillines examined in this work are shown in Table I. Although all of the truxilline spectra are very similar, they can be distinguished from one another easily as the relative abundances of several ions are sensitive to the intra-molecular stereochemical relationships of these molecules. MS data obtained for the various suspected truxillines correlated very well with those obtained for standard α-truxilline. The capillary GC–MS data for isolated α-truxilline were in excellent agreement with those obtained for standard α-truxilline. The total ion chromatogram for the capillary GC–MS analysis of the cocaine extract before fraction collection is shown in Fig. 5. Based on a comparison of the capillary GC–MS data with the capillary GC–ECD results, small amounts of ζ-, *epi*- and *peri*-truxilline were also believed to be present.

The chromatogram of the various truxilline isomers on the reversed-phase HPLC system used to isolate these compounds is shown in Fig. 6. This system, optimized just for isomeric truxillines, was developed based on the model of Kirkland and Glajch[14]. The gradient optimization scheme was carried out using binary gradients at approximately equal solvent strengths. A phosphate buffer (pH 2.0) was used with the modifiers methanol, acetonitrile and tetrahydrofuran. The retention characteristics of the truxilline isomers on the HPLC system used for the simultaneous analysis of acidic and basic impurities in cocaine[4] are shown in Fig. 7. The latter

[a] The lithium aluminum hydride reduction of 1 mol of a truxilline results in the production of two moles of a tropanediol and 1 mol of a diphenylcyclobutanediol.

[b] The thermal decomposition products of a truxilline are 2-carbomethoxytropidine and either one or both of the two possible hydrolysis products of the respective truxillic or truxinic acid.

TABLE I

MASS SPECTRUM ABUNDANCES OF TRUXILLINES

Mass	*Relative abundance*						
	Truxilline						
	δ	*Neo*	*β*	*ε*	*ω*	*γ*	*α*
82	6370	6050	5430	5260	5770	5050	5610
83	2390	2590	2730	2100	4370	2870	3070
94	2530	2360	2050	2190	2160	2070	2160
95	430	450	310	380	380	360	340
96	1900	2110	1920	1510	1610	1940	1650
98	120	120	70	80	100	120	70
102	1	70	5	40	1	1	40
103	170	270	290	280	390	290	380
108	320	280	300	290	150	220	280
119	320	390	270	250	270	230	210
120	130	130	90	100	10	90	110
122	540	550	460	480	410	400	480
124	360	230	190	250	100	180	140
131	260	550	440	900	900	600	1220
150	600	690	430	500	60	160	140
152	410	380	190	260	170	210	210
155	160	130	150	180	110	120	170
166	140	160	90	110	10	50	110
168	250	270	300	220	290	280	420
180	350	280	230	120	120	110	170
182	10 000	10 000	10 000	10 000	10 000	10 000	10 000
194	60	60	50	10	1	1	20
198	640	540	460	470	570	480	760
233	40	60	10	70	1	40	90
234	30	50	10	20	1	30	20
238	1	90	180	20	1	1	80
239	1	1	20	1	1	1	6
298	30	1	40	20	1	1	30
313	40	60	1	1	1	50	1
329	160	190	590	140	10	70	160
330	30	80	550	20	90	10	190
432	50	1	10	1	1	1	1260
433	5000	4440	2740	5000	770	2960	1200
476	180	280	90	40	1	1	140
477	10	10	5	140	1	1	130
478	290	360	420	220	1	80	130
515	200	60	60	160	20	1	20
577	40	60	180	40	1	160	60
627	1	1	50	50	210	100	520
630	130	100	70	100	1	1	60
658	1360	1810	870	1440	2400	840	3190

chromatographic system, although not optimum for the analysis of truxillines, gives the "best" overall separation via HPLC of acids, monoprotic amines and diprotic amines present in illicit cocaine. This was accomplished using a similar optimization scheme as above with a phosphate buffer (pH 2.0) containing dodecyl sulfate[4]. Acidic

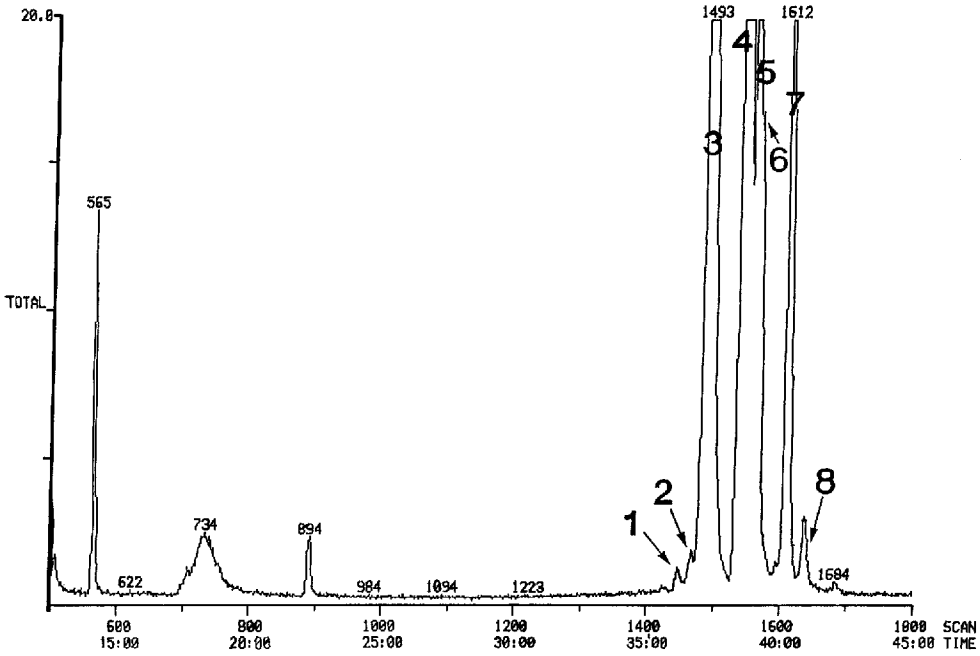

Fig. 5. Total ion chromatogram of illicit cocaine impurities including isomeric truxillines isolated via size-exclusion chromatography. Peaks: 1 = possibly ζ-truxilline; 2 = ω-truxilline; 3 = β-truxilline; 4 = *neo*-, α- and γ-truxilline; 5 = δ-truxilline; 6 = possibly *epi*- or *peri*-truxilline; 7 = ε-truxilline; 8 = *peri*- or *epi*-truxilline. A DB-1 column was used with helium carrier gas at a linear velocity of 55 cm/s. The initial temperature was held at 120°C for 2 min, programmed to 220°C at a rate of 15°C/min and finally programmed to 295°C at a rate of 2°C/min.

compounds are little affected by the addition of dodecyl sulfate while the retention of basic compounds increases, with the increase in retention for diprotic amines double that of monoprotic amines[4]. For the above HPLC systems the possibility exists based on the capillary GC–ECD and capillary GC–MS experiments that additional truxilline isomers may be present under the ε- and β-truxilline peaks.

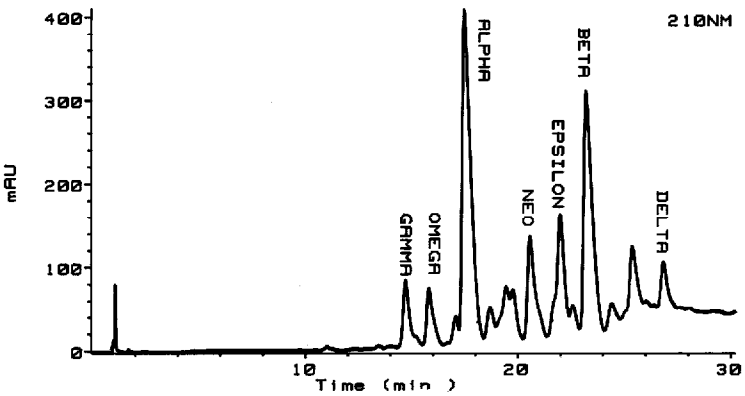

Fig. 6. HPLC of illicit cocaine impurities including isomeric truxillines isolated via size-exclusion chromatography. Chromatographic conditions are described under Experimental pertaining to analytical–preparative isolation of truxillines.

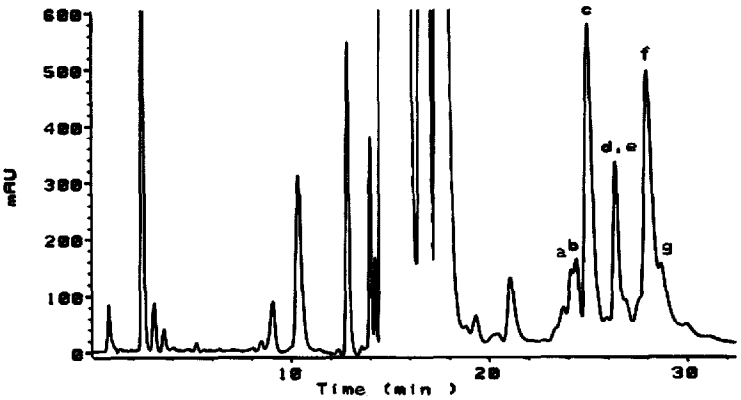

Fig. 7. HPLC at 215 nm (20-nm bandpass) of an illicit cocaine–HCl sample. Peaks: a = γ-truxilline; b = ω-truxilline; c = α-truxilline; d = *neo*-truxilline; e = ε-truxilline; f = β-truxilline; g = δ-truxilline. Chromatographic conditions and sample preparation described in ref. 4.

As shown in Figs. 6 and 7, except for *neo*- and ε-truxilline which coelute in the system containing dodecyl sulfate, identical retention order was obtained for the truxillines on two different reversed-phase systems. The retention properties of α-, β-, ε- and δ-truxillines are vastly different from what has been reported for N,N-dimethyl-

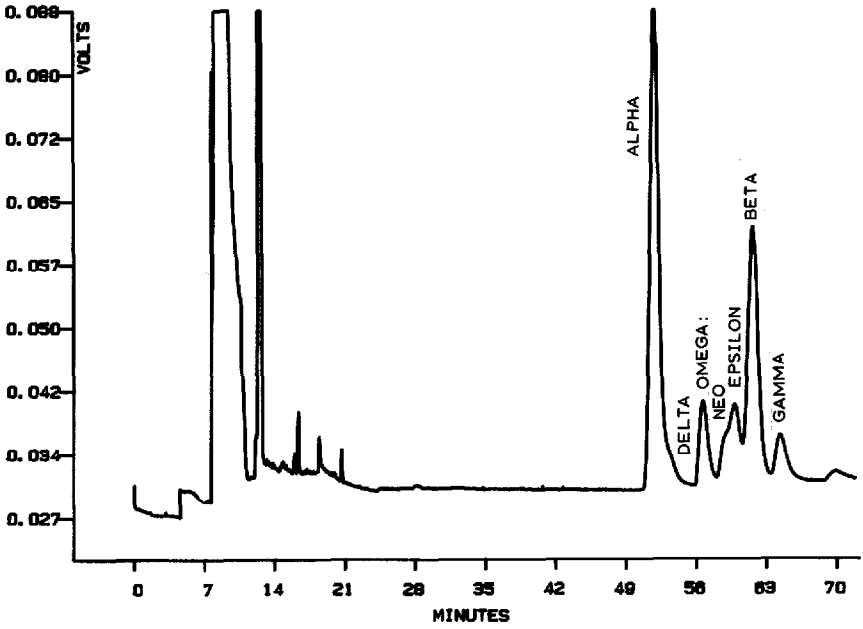

Fig. 8. Capillary SFC of illicit cocaine impurities including isomeric truxillines isolated via size-exclusion chromatography. An SB-biphenyl-30 capillary column was used with density programming for carbon dioxide at 80°C. A density ramp was employed where the initial density was held at 0.25 g/ml for 5 min, then linearly increased to 0.35 g/ml at 6 min and finally linearly increased to 0.65 g/ml at 18.5 min prior to holding the final density.

amides of the same parent truxillic and truxinic acids[15]. The separation of the diamides was explained by differences in the stereochemistry of the solute molecules which allowed various degrees of overlap of the non-polar portions with the alkyl stationary phase. It would appear the polar methyl ecgonine moiety in the truxilline molecule is a major cause of the differences in retention behavior of the truxillines and the truxillic and truxinic diamides. Solute properties such as non-polar surface area, dipole moment and charge have been shown to contribute to retention in reversed-phase chromatography[16].

Despite the use of various stationary phases and experiments at different temperatures only a partial separation of truxilline isomers was obtained via capillary SFC as shown in Fig. 8. The stationary phases investigated included a 100% methyl polysiloxane, a 50% *n*-octyl–50% methyl polysiloxane, a 25% cyanopropyl–25% phenyl–50% methyl polysiloxane, a liquid crystalline polysiloxane and a 30% biphenyl–70% methyl polysiloxane. For the latter stationary phase temperatures of 80–140°C were used.

For the various chromatographic techniques investigated, the best separation for the γ-, ω-, α-, *neo*-, ε-, β- and δ-isomers was obtained via HPLC where baseline resolution was achieved. As shown in Figs. 4, 5 and 8, vastly different retention orders were obtained for the isomeric truxillines using capillary GC, HPLC and capillary SFC. Depending on the technique, π–π, dipole–dipole, dispersion, and hydrogen-bonding interactions as well as sample volatility and sample stereochemistry can play a role in the various separation mechanisms.

ACKNOWLEDGEMENT

We gratefully acknowledge Robert Klein for useful discussions.

REFERENCES

1 J. M. Moore, D. A. Cooper, I. S. Lurie, T. C. Kram, S. Carr and J. Yeh, *J. Chromatogr.*, 410 (1987) 297.
2 O. Hesse, *Justus Liebigs Ann. Chem.*, 271 (1892) 180.
3 C. Liebermann, *Ber. Dtsch. Chem. Ges.*, 22 (1889) 782.
4 I. S. Lurie, J. M. Moore, D. A. Cooper and T. C. Kram, *J. Chromatogr.*, 405 (1987) 273.
5 I. S. Lurie, *LC · GC, Mag. Liq. Gas Chromatogr.*, 6 (1988) 1066.
6 I. S. Lurie, unpublished results.
7 J. M. Moore, *J. Assoc. Off. Anal. Chem.*, 56 (1973) 1199.
8 G. Montaudo and S. Caccamese, *J. Org. Chem.*, 38 (1973) 710.
9 S. Montaudo, S. Caccamese and V. Librando, *Org. Magn. Reson.*, 6 (1974) 1934.
10 T. C. Kram, personal communication.
11 T. Lukaszewski and W. K. Jeffery, *J. Forensic Sci.*, 25 (1980) 499.
12 L. M. Jackman, *Application of Nuclear Magnetic Resonance Spectroscopy to Organic Chemistry*, Pergamon Press, London, 1959, p. 55.
13 C. C. Culvenor, *Tetrahedron Lett.*, (1966) 1091.
14 J. J. Kirkland and J. L. Glajch, *J. Chromatogr.*, 255 (1983) 27.
15 S. Caccamese, *J. Chromatogr.*, 457 (1988) 366.
16 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.