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Lesser alkaloids of cocaine-bearing plants II. 3-Oxo-substituted tropane esters: detection and mass spectral characterization of minor alkaloids found in South American *Erythroxylum coca* var. *coca*

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Abstract

Alkaloid extracts from the leaves of *Erythroxylum coca* var. *coca*, grown in South America, were subjected to ion-pair chromatography and then to gas chromatographic–mass spectrometric analyses. Eight previously unreported trace level alkaloids were detected and characterized via comparison to synthesized standards, including 3α-benzoyloxytropane, 3α-phenylacetoxytropane, 3β-*cis*- and 3β-*trans*-cinnamoyloxytropane, 3β-2′-hydroxybenzoyloxytropane, 3α-3′,4′,5′-trimethoxybenzoyloxytropane, 3α- and 3β-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane. Two additional alkaloids, 3α- and 3β-3′,4′,5′-trimethoxy-*cis*-cinnamoyloxytropane, were also tentatively identified.

Keywords: *Erythroxylum coca*; Plant extracts; Alkaloids; Tropane alkaloids; Cocaine

1. Introduction

The characterization of minor alkaloids in cocaine-bearing plants and illicit cocaine are of significance in forensic drug chemistry. The detection and determination of these alkaloids along with their oxidation and hydrolytic by-products in illicit refined cocaine provides both tactical and strategic intelligence data to law enforcement. This includes (a) insights into the manufacturing processes, (b) the ability to compare multiple cocaine seizures for determination of commonality of origin and possible prosecution in drug conspiracy cases, and (c) the assessment of geographic origin [1–5].

In Part I of this series [3], we described the isolation and gas chromatographic–mass spectrometric (GC–MS) characterization of several furanoyl, pyrroloyl and nicotinoyl analogs of cocaine present in South American *Erythroxylum coca* var. *coca* (ECVC). In the present paper we describe the isolation, detection and characterization of eight previously unreported tropane esters (i.e. analogs of tropacocaine) also found in South American ECVC. These latter compounds, illustrated in Fig. 1, were recovered from the coca leaf matrix and isolated from other alkaloids by toluene extraction followed by trap and ion-pair column chromatography [4].

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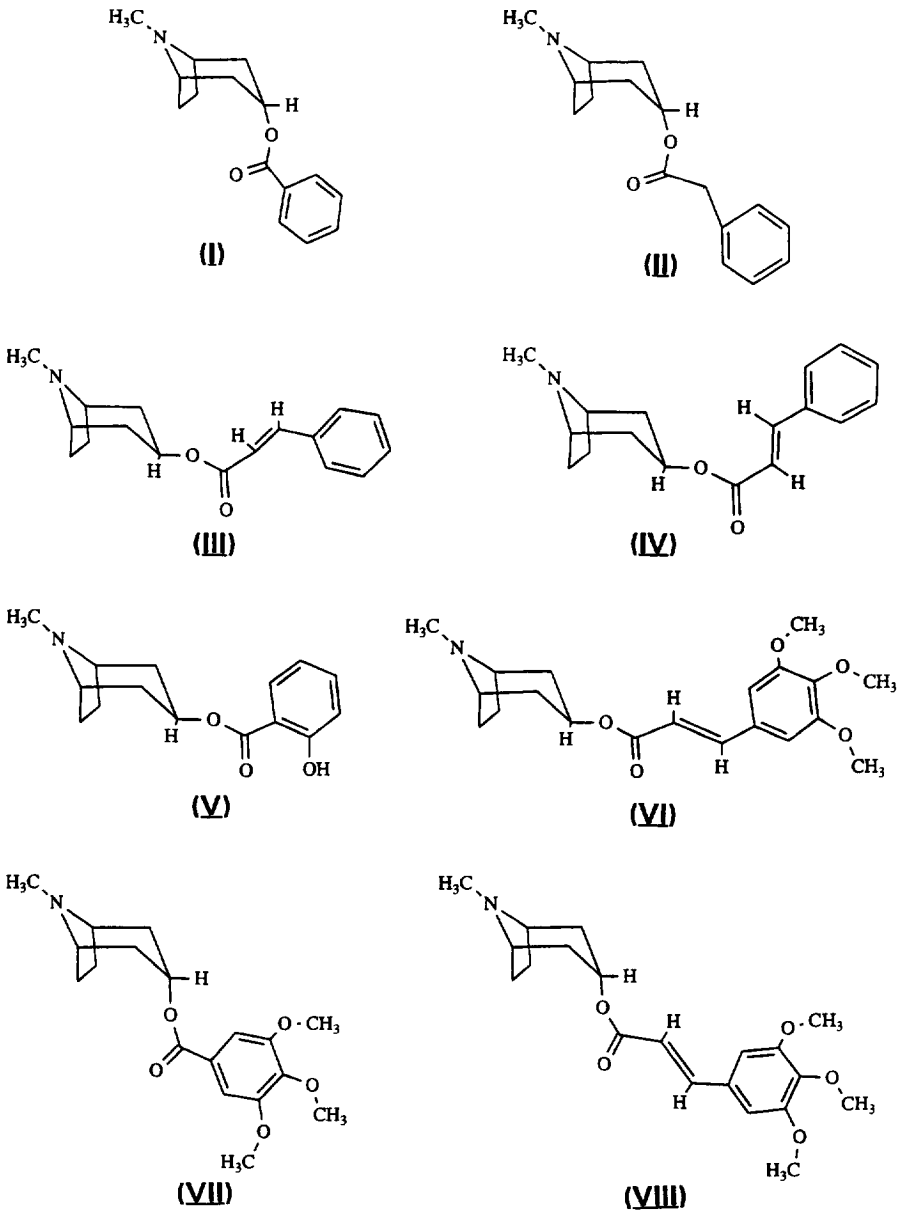


Fig. 1. Structural formulas.

Specifically, 3α-benzoyloxytropane, 3α-phenylacetoxytropane, 3β-*cis*- and 3β-*trans*-cinnamoyloxytropane, 3β-2′-hydroxybenzoyloxytropane, 3α-3′,4′,5′-trimethoxybenzoyloxytropane, 3α- and 3β-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane were characterized via GC–MS and comparison to synthesized standards.

2. Experimental

2.1. Plant material

Fresh leaves of ECVC (approximately 90 days old at the time of harvest) were collected from the Chapare Valley region of Bolivia. The leaves were

sun-dried after harvesting and stored at room temperature for three months prior to analysis.

2.2. Solvents, chemicals, standards and materials

All solvents were "distilled-in-glass" products of Burdick and Jackson (Muskegon, MI, USA). All solvents and materials used for column chromatography were prepared as previously described [5]. N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) was obtained from Pierce (Rockford, IL, USA). Standards of 3α-benzoyloxytropane, 3α-phenylacetoxytropane, 3β-*trans*-cinnamoyloxytropane, 3β-2′-hydroxybenzoyloxytropane, 3α-3′,4′,5′-trimethoxy-benzoyloxytropane, 3α-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane, 3β-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane and their positional analogs were all synthesized by the reaction of either tropine or pseudotropine with the appropriate acid chloride, as previously described [3].

2.3. Capillary gas chromatography–mass spectrometry

Mass spectra were obtained on a Hewlett-Packard Model 5972 mass-selective detector (MSD) interfaced with a Hewlett-Packard 5890 series II gas chromatograph (GC). The MSD operated under electron ionization (EI) conditions at 70 eV, a secondary electron multiplier value of 1635 and at 1.2 scans/s. The GC system was fitted with a 30 m×0.25 mm I.D. fused-silica capillary column coated with 0.25 μm DB-1 (J&W Scientific). A pressure-programmed constant linear velocity of 28.5 cm/s helium (99.999%, UHP) was used. The injection port and MSD system were maintained at 250 and 280°C, respectively. Samples were injected in the split mode (20:1) using a Hewlett-Packard Model 7673A auto-injector (2-μl injection). The oven temperature was programmed as follows: initial temperature=80°C, initial hold=5.0 min, program rate=3.0°C/min, final temperature=285°C, final hold=60.0 min.

2.4. Extraction and isolation of coca-leaf alkaloids

Dry coca leaves (2 kg) were reduced to powder in a Wiley mill to pass a 2-mm mesh sieve, then triturated with 2 l of saturated aqueous sodium bicarbonate. The triturate was equally divided between four 4-l screw-cap glass bottles, each containing 3 l of water-saturated toluene, and stirred and heated at 50°C. After 12 h, the toluene extract was isolated and extracted with 250 ml of 0.5 *M* H_2SO_4. The extract was adjusted to pH 8 with solid sodium bicarbonate and back-extracted with 2×200 ml of chloroform. The leaf material was reprocessed in the same manner two additional times to obtain 1.2 l of chloroform and the combined extracts were concentrated in vacuo to ca. 400 ml.

This concentrate was saturated with water and passed through a column (4×60 mm) packed with a mixture of 100 g Celite 545 and 60 ml of 0.9 *M* H_2SO_4. The column was washed with an additional 200 ml of water-saturated chloroform. All eluates were discarded. Alkaloids were then liberated from the column by further elution with 500 ml water-saturated chloroform containing 14 ml of diethyl amine. The resulting eluate was evaporated in vacuo to ca. 300 ml, washed with 500 ml of pH 4.0 acid phthalate buffer, dried over anhydrous sodium sulphate and evaporated in vacuo to an oily residue. This residue was reconstituted in 10 ml of water-saturated chloroform and transferred to a column packed with: bottom layer, 8 g Celite 545 mixed with 4 ml saturated aqueous sodium bicarbonate, and top layer, 80 g Celite 545 mixed with 40.0 ml of 2 *M* NaCl–1 *M* HCl solution. The column was eluted with 600 ml of water-saturated chloroform and the first 50 ml of eluate were collected and dried over anhydrous sodium sulphate. An aliquot was treated with an equal volume of MSTFA at 75°C for 30 min, and subjected to capillary GC (cGC)–MS analysis. The remaining eluate contained mostly cocaine, and was discarded.

3. Results and discussion

We have previously discussed the application and advantages of toluene extraction followed by trap and ion-pair column chromatography for the isolation of trace-level coca alkaloids from coca leaf and bulk cocaine [4,5]. Using these techniques, cocaine is virtually quantitatively (>99.9%) removed from the target compounds, thus eliminating cGC column

overloading and facilitating concentration and detection of trace-level alkaloids. A variation of this method was recently utilized for the isolation and characterization of the 3′,4′,5′-trimethoxy substituted analogs of tropacocaine, cocaine and *cis*- and *trans*-cinnamoylcocaine in coca leaves and illicit cocaine [4]. Similarly, we have isolated and characterized the 2′- and 3′-furanoyl, nicotinoyl and 2′-pyrroloyl analogs of cocaine; this latter work was reported in Part I of this series [3]. In these and numerous other studies, we have definitively established the basic mass spectral fragmentation pattern for 3α- or β-tropane esters without C-2 substituents, that is, ions at *m/z* 82, 94, 124 and 140. A previously reported ester of this type includes 3′,4′,5′-trimethoxytropacocaine (3β-3′,4′,5′-trimethoxybenzoyloxy-

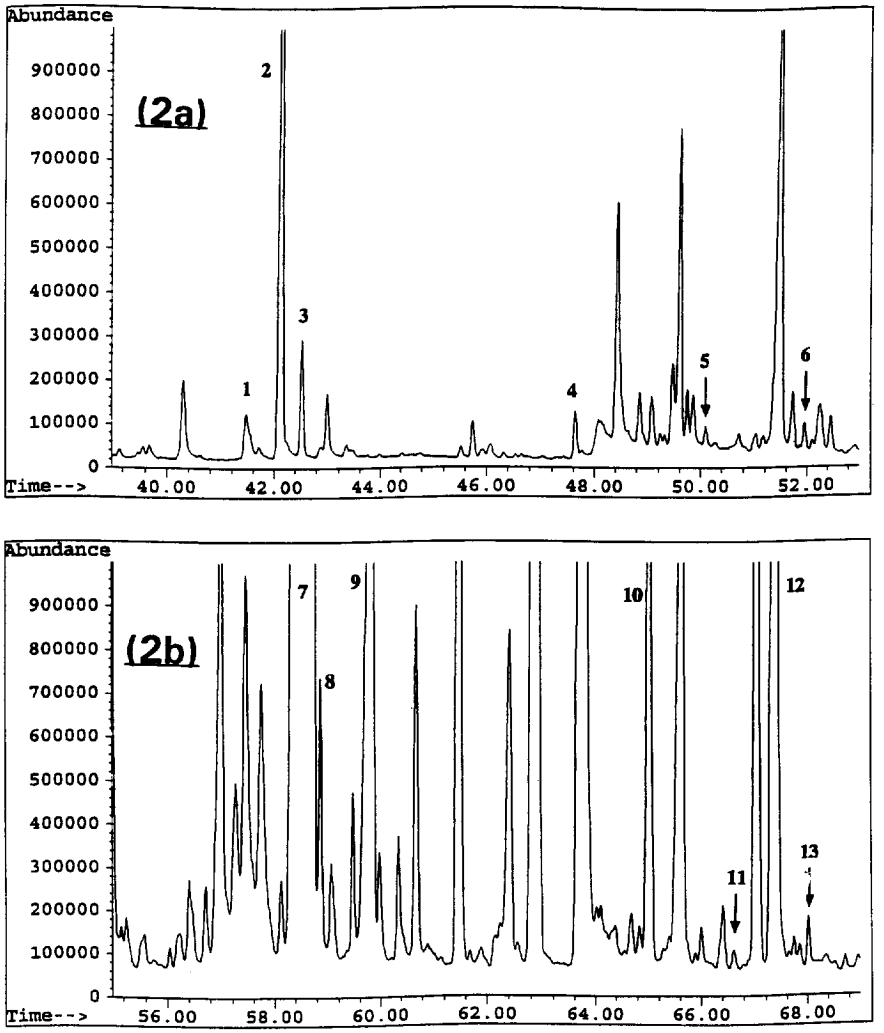


Fig. 2. (a) Partial reconstructed total ion chromatogram from ion-pair isolate. Peaks: 1=3α-benzoyloxytropane (41.55 min), 2=3β-benzoyloxytropane (42.13 min), 3=3α-phenylacetoxytropane (42.53 min), 4=3β-*cis*-cinnamoyloxytropane (47.65 min), 5=3β-2′-hydroxybenzoyloxytropane (50.10 min) and 6=3β-*trans*-cinnamoyloxytropane (51.95 min). (b) Partial reconstructed total ion chromatogram from ion-pair isolate. Peaks: 7=*trans*-cinnamoylcocaine (58.62 min), 8=3α-3′,4′,5′-trimethoxybenzoyloxytropane (58.88 min), 9=3β-3′,4′,5′-trimethoxybenzoyloxytropane and co-eluting unknown (59.65 min), 10=3′,4′,5′-trimethoxycocaine (65.02 min), 11=3α-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane (66.61 min), 12=3′,4′,5′-trimethoxy-*cis*-cinnamoylcocaine (67.38 min) and 13=3β-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane (68.03 min).

tropane) [4]. In this work, a specific eluant fraction enriched in similar esters was isolated and characterized by GC–MS. Two windows from the reconstructed total ion chromatogram displaying these compounds are illustrated in Fig. 2 and selected ions are presented in Table 1. All compounds of interest displayed the basic tropane ester fragmentation pattern. Thus, initial tentative identifications of individual components were simplified in that only the ester moiety was unknown and in fact, many of the esters were themselves familiar from prior work (i.e. benzoyl, *cis*- and *trans*-cinnamoyl, 3′,4′,5′-trimethoxybenzoyl and *cis*- and *trans*-3′,4′,5′-trimethoxycinnamoyl). Subsequent syntheses of standards (both epimers and analogs thereof) gave definitive identifications of unknowns and also allowed several structural generalizations with respect to determination of α- vs. β-configurations. To whit, the GC retention times of the β-epimers were invariably longer than the α-epimers and the ratios of the m/z 82 to m/z 83 ions typically approached 1.5 for the β-epimers vs. 0.9–1.1 for the α-epimers.

3.1. Characterization of 3-oxo-substituted tropane esters

As expected, tropacocaine (peak 2) and 3′,4′,5′-trimethoxytropacocaine (peak 9) were both easily identified in the enriched tropane ester fraction. Tropacocaine has been previously reported at a concentration of 0.16% (w/w) relative to cocaine for these leaves [5]. The relative concentrations of all other tropane esters identified in this study were therefore determined to be less than 0.1% (w/w) relative to cocaine based on their relative peak areas vs. tropacocaine.

Peak 1 gave a very similar mass spectrum, illustrated in Fig. 3a, to that of tropacocaine (peak 2), and was therefore immediately suspected to be its positional analog. Comparison of the mass spectrum and GC retention time to the standard 3α-benzoyloxytropane (I) confirmed that it was the α-epimer. This alkaloid has been previously reported in greenhouse-cultivated ECVC [5].

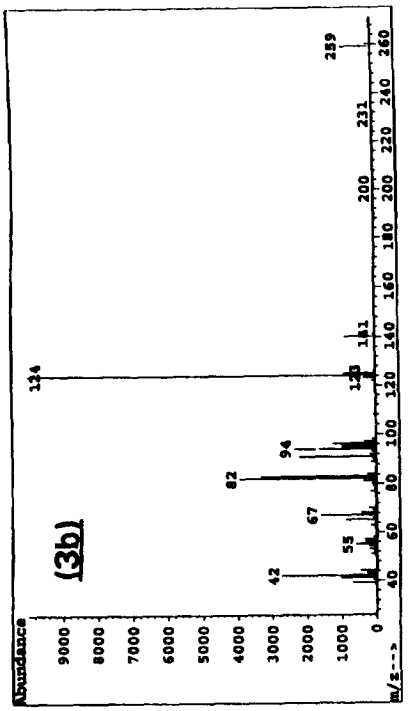
The mass spectrum of peak 3, illustrated in Fig. 3b, was markedly similar to the benzoyloxytropanes (peaks 1 and 2) but gave a molecule ion at m/z 259. The molecule ion difference of 14 suggested the compound was either 3α- or 3β-phenylacetoxytropane (or less likely, a methyl-substituted benzoyloxytropane). The relative abundance of m/z 83 vs. m/z 82 was indicative of a 3α-substitution. Comparison of the spectrum and retention time to both synthesized standards of phenylacetoxytropane confirmed that it was the 3α-epimer (II). This alkaloid has been previously detected in *E. dekindtii* [6] and *E. hypericifolium* [7]. Close inspection of the chromatogram did not indicate any 3β-phenylacetoxytropane.

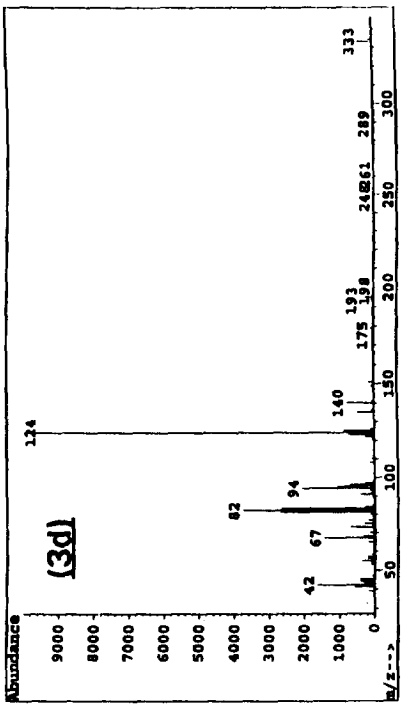
Peaks 4 and 6 gave virtually identical mass spectra, illustrated in Fig. 3c, suggesting that they were isomers. Each gave a molecule ion at m/z 271, a mass difference of 26 from tropacocaine, suggesting cinnamoyl moieties. The appearance of ion m/z 131, combined with an ion at m/z 140, supported a cinnamoyl ester, while the relative abundance of m/z 82 vs. m/z 83 was indicative of a 3β-substitution. Syntheses and comparison of 3α-

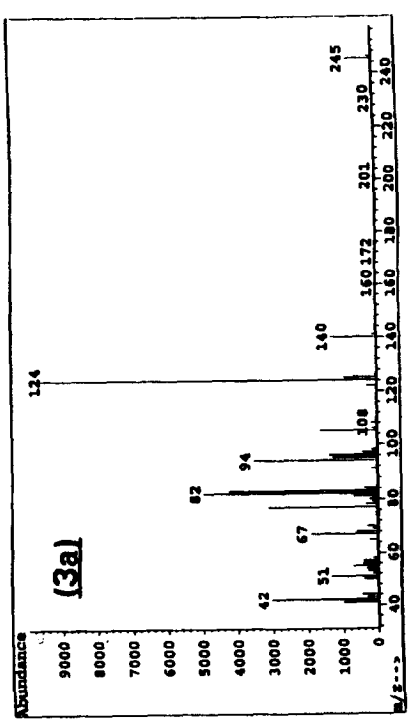
Table 1
GC–MS data from ion-pair isolate[a]

Alkaloid	Peak No.	GC: t_R (min)	MS: Fig.	m/z[b]	M^+
I	1	41.55	3a	124, 82, 83, 94, 77, 140, 67	245
II	3	42.53	3b	124, 82, 83, 91, 42, 140, 67	259
III	4	47.65	3c	124, 82, 83, 94, 96, 140, 42	271
IV	6	51.95	3c	124, 82, 83, 94, 96, 140, 42	271
V	5	50.10	3d	124, 82, 81, 83, 94, 140, 73	333
VI	13	68.03	3g	124, 82, 83, 94, 96, 140, 221	361
VII	8	58.88	3e	124, 83, 82, 94, 140, 195, 212	335
VIII	11	66.61	3f	124, 96, 83, 82, 94, 140, 221	361

[a] Conditions given in Section 2.
[b] Selected ions of significant abundance.


(3b)
Abundance
m/z-->


(3d)
Abundance
m/z-->


(3a)
Abundance
m/z-->

(3c)
Abundance
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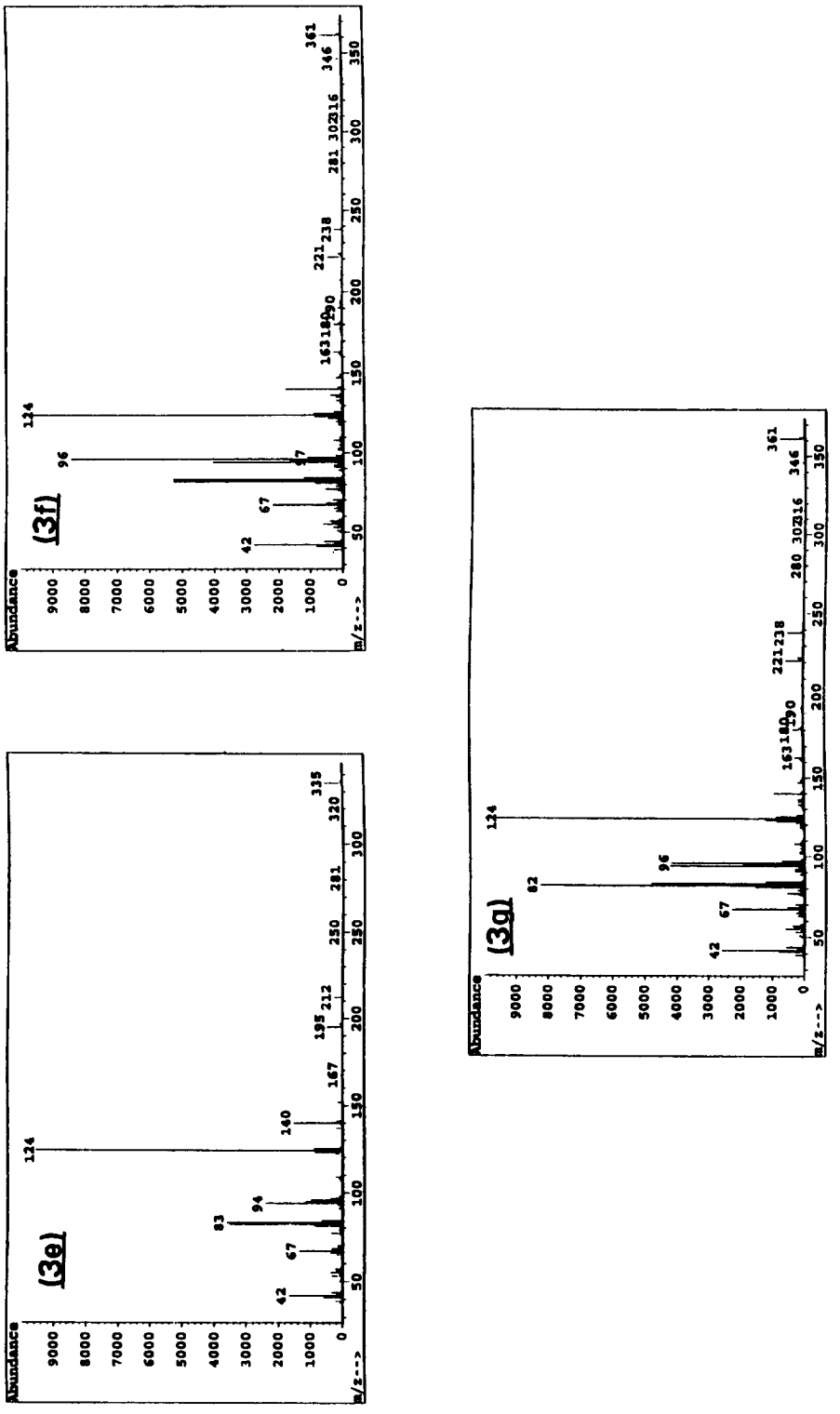

Fig. 3. Electron ionization mass spectrum of (a) 3α-benzoyloxytropane, (b) 3α-phenylacetoxytropane, (c) 3β-*cis*- or 3β-*trans*-cinnamoyloxytropane, (d) 3β-2′-hydroxybenzoyloxytropane (*ortho*-hydroxytropacocaine), (e) 3α-3′,4′,5′-trimethoxybenzoyloxytropane, (f) 3α-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane and (g) 3β-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane.

and 3β-*trans*-cinnamoyloxytropane confirmed that peak 6 was the 3β-*trans*-isomer (IV). Peak 4 was therefore identified as 3β-*cis*-cinnamoyloxytropane (III) based on its mass spectrum and relative retention time to the *trans*-isomer. Both of these alkaloids are new alkaloids. A 3β-cinnamoyloxytropane had been previously identified in *E. hypericifolium* [7], however, its absolute configuration (*cis*- vs. *trans*-) was not determined. Inspection of the chromatogram did not indicate the presence of either the *cis*- or *trans*-3α-epimers.

The mass spectrum of peak 5, illustrated in Fig. 3d, displayed a molecule ion at *m/z* 333 and a fragment ion *m/z* 73, indicating a trimethylsilylated hydroxy substituted benzoyloxytropane. Relative abundances of ions *m/z* 82, 94, 124 and 140 confirmed that the hydroxy substitution was not on the tropane moiety [8], but rather at the *ortho*-, *meta*- or *para*-positions of 3α- or 3β-benzoyloxytropane. Peak 5 was characterized as 3β-2′-hydroxybenzoyloxy-tropane (V) (*ortho*-hydroxytropacocaine) after comparison to the synthesized standards of all six positional isomers. None of the remaining five isomers were identified in the chromatogram. 3β-2′-hydroxybenzoyloxytropane is a new alkaloid.

Peak 8 gave a mass spectrum, illustrated in Fig. 3e, that was very similar to 3′,4′,5′-trimethoxytropacocaine [4], again immediately suggesting that it was the 3α-epimer. Peak 8 was characterized as 3α-3′,4′,5′-trimethoxybenzoyloxytropane (VII) by comparison to the synthesized standard. This alkaloid has been previously detected in *E. monogynum* [9].

Peaks 11 and 13 gave similar mass spectra, illustrated in Fig. 3f and 3g, respectively, suggesting they were isomers. Each gave a molecule ion at *m/z* 361, a mass difference of 90 Da from 3β-*cis*- and 3β-*trans*-cinnamoyloxytropane, suggesting trimethoxy-substituted analogs. Ions *m/z* 221 and *m/z* 238 further supported the presence of trimethoxycinnamoyloxy moieties. The relative abundance of *m/z* 82 vs. *m/z* 83 was indicative of a 3α- and 3β-substitution for peaks 11 and 13, respectively. Comparison with synthesized standards confirmed that peaks 11 and 13 were 3α-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane (VIII) and 3β-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane (VI), respectively. Inspection of the chromatogram did indicate the presence of trace amounts of the 3α- and 3β-*cis*-isomers (61.70 and 62.18 min). However, they are only tentatively identified due to extraneous spectral data from co-eluting compounds. 3α-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane has been reported previously in *E. monogynum* [9] and *E. ellipticum* [10]. 3β-3′,4′,5′-trimethoxy-*trans*-cinnamoyloxytropane, 3β-3′,4′,5′-trimethoxy-*cis*-cinnamoyloxytropane and 3α-3′,4′,5′-trimethoxy-*cis*-cinnamoyloxytropane are new alkaloids.

4. Conclusions

A detailed methodology for the isolation and characterization of eight previously unreported and two tentatively reported 3-oxo-substituted tropane esters in South American ECVC is presented. Six of these are reported as new alkaloids. Detection of 3α-oxo-substituted tropane esters are reported for the first time in this leaf. The use of trap and ion-pair chromatography effectively removed cocaine from these minor alkaloids, enhancing their detectability and characterization. The title compounds were present at levels less than 0.1% (w/w) relative to cocaine. Application of this methodology to illicit cocaine samples should enhance common source determinations.

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