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Lesser alkaloids of cocaine-bearing plants III. 2-Carbomethoxy-3-oxo substituted tropane esters: detection and gas chromatographic–mass spectrometric characterization of new minor alkaloids found in South American *Erythroxylum coca* var. *coca*

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Abstract

Crude alkaloid extracts from the leaves of South American *Erythroxylum coca* var. *coca* were subjected to alumina chromatography and recrystallization to isolate fractions containing eleven previously unreported 2-carbomethoxy-3-oxo substituted tropane alkaloids. Specifically, the butyroyl-, 2-methylbutyroyl-, isovaleroyl-, senecioyl-, tigloyl-, *trans*-4-hexenoyl-, hexanoyl-, *trans*-3-hexenoyl-, *trans*-2-hexenoyl-, *trans*-3-heptenoyl- and *trans*-, *trans*-2,4-hexadienoyl-ecgonine methyl esters were characterized via electron and chemical ionization gas chromatographic–mass spectrometric analyses and comparison to synthesized standards. Three additional alkaloids similar to the aforementioned esters were also presumptively identified as heptadienoylecgonine methyl ester (geometry not determined) and *cis*-, *trans*- and *trans*-, *cis*-2,4-hexadienoylecgonine methyl ester.

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

1. Introduction

The leaves of the coca plant, *Erythroxylum coca* var. *coca* (ECVC), are the predominant source of cocaine. The crude illicit processing of this leaf provides cocaine containing numerous trace-level tropane alkaloids; i.e., at concentrations less than one percent relative to cocaine [1]. The detection of these trace-level alkaloids in refined illicit cocaine provides a basis for comparative analyses and origin determinations [2]. A number of recent studies have reported new trace-level tropane alkaloids in both coca leaf and refined illicit cocaine base and cocaine hydrochloride [3–8].

The first two studies in this series described the characterization of heteroaroyl analogs of cocaine and 3-oxo substituted tropane analogs found in extracts of ECVC [7,8]. This report concerns the preparative isolation, detection and characterization of eleven new alkane/alkene ester analogs of cocaine. Specifically, the butyroyl-, 2-methylbutyroyl-, isovaleroyl-, senecioyl-, tigloyl-, *trans*-

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4-hexenoyl-, hexanoyl-, *trans*-3-hexenoyl-, *trans*-2-hexenoyl-, *trans*-3-heptenoyl- and *trans*-, *trans*-2,4-hexadienoylecgonine methyl esters were characterized by comparison of their electron ionization (EI) and chemical ionization (CI)–gas chromatography–mass spectra with their synthesized standards. Three additional alkaloids, including a heptadienoylecgonine methyl ester, and *cis*-, *trans*- and *trans*-, *cis*-2,4-hexadienoylecgonine methyl ester, were also tentatively identified.

2. Experimental

2.1. Coca-leaf extract, solvents, chemicals, chromatographic materials, precursors and standards

A crude South American coca leaf extract (*E. Harz*) was supplied by Stepan Chemical Co., Maywood, NJ, USA and was derived from Bolivian and/or Peruvian coca (presumed to be ECVC). All other chemicals, solvents and materials utilized in this study were essentially identical to those reported in Parts I and II [7,8]. The precursor acid chlorides or carboxylic acids were products of Aldrich Chemical (Milwaukee, WI, USA) or TCI America (Portland, OR, USA). The title alkaloids and related compounds were synthesized and isolated from ecgonine methyl ester using the previously reported procedures [7,8].

2.2. Capillary gas chromatography–mass spectrometry (cGC–MS)

Two cGC–MS systems were used in this study; experimental parameters and conditions have been previously described [7]. All mass spectra were normalized.

2.3. Isolation of minor alkaloids from E. Harz

Approximately 200 g of *E. Harz* (equivalent to ca. 80 g cocaine) was dissolved into 500 ml of chloroform and extracted with 2000 ml of pH 4.0 acid phthalate buffer. After adjusting the buffer back to pH 4.0 via addition of concentrated HCl, it was then extracted with chloroform (4×200 ml, discarded), adjusted to pH 8 with 200 g sodium bicarbonate and extracted with chloroform (4×200 ml). The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to a residue; this was reconstituted in 750 ml of chloroform and extracted with an equal volume of pH 4.0 buffer. The buffer phase was extracted with additional chloroform (3×200 ml, discarded). The buffer was then adjusted to pH 8 with 100 g sodium bicarbonate and extracted with chloroform (3×200 ml). The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to a residue.

This residue was reconstituted in a minimal volume of chloroform and transferred to a glass chromatographic column (ca. 965×50 mm I.D.) packed with 600 g of basic alumina (4.0% H_2O). Sufficient chloroform was added to collect 600 ml of eluate (Fraction EH-1A) followed by 600 ml of chloroform–acetone (85:15) (Fraction EH-1B). Each fraction was dried over anhydrous sodium sulfate, filtered and evaporated in vacuo to a residue.

The resulting residues were then recrystallized from a minimal volume of hot hexane–methylene chloride (9:1), with cocaine being the major crystallization component. The mother liquors were reduced to dryness and recrystallized twice more in the same manner to give final mother liquors containing enhanced concentrations of the titled alkaloids (Fractions EH-2A and EH-2B).

3. Results and discussion

Recrystallization of alumina fractions EH-1A and EH-1B markedly enhanced the levels of many of the minor alkaloids in the mother liquors relative to cocaine. Cocaine was the major product removed by crystallization, with 2′-furanoylecgonine methyl ester comprising the most abundant co-crystallized minor alkaloid. The mother liquors (Fractions EH-2A and EH-2B) were subjected to cGC–EI–MS and cGC–CI–MS analyses, and found to contain fourteen new tropane alkaloids, as illustrated in Fig. 1.

Fraction EH-2B contained the highest concentrations of minor alkaloids. A window exhibiting peaks of interest in the reconstructed total EI chromatogram is illustrated in Fig. 2. The enumerated

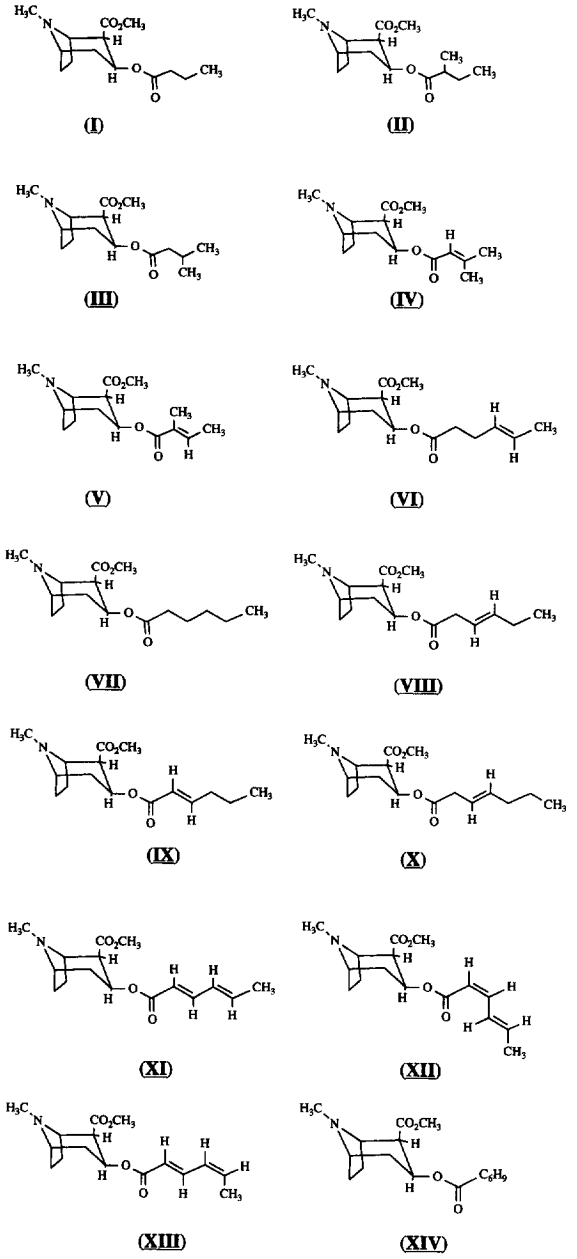

Fig. 1. Structural formulas.

peaks provided mass spectra with several ions analogous to cocaine. Specifically, each contained the characteristic fragment ions *m/z* 82, 94, 96, 182 and 198, which are indicative of intact 2-carbomethoxy-3-oxo substituted tropane alkaloids. The relative

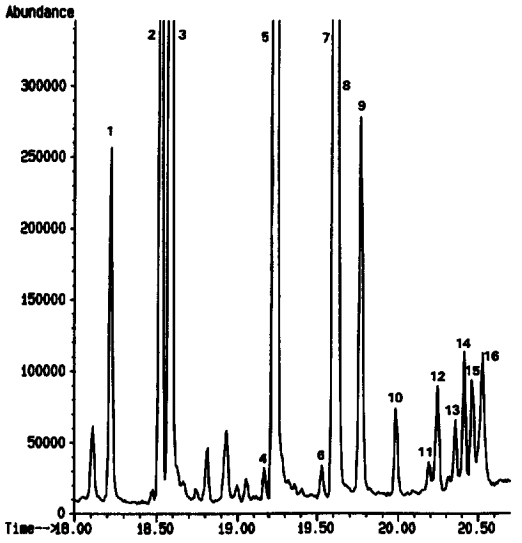

Fig. 2. Partial reconstructed total electron ionization chromatogram from Fraction EH-2B. Peaks: 1=butyroylecgonine methyl ester, 2=2-methylbutyroylecgonine methyl ester, 3=isovaleroylecgonine methyl ester, 4=senecioylecgonine methyl ester, 5=tigloylecgonine methyl ester, 6=*trans*-4-hexenoylecgonine methyl ester, 7=hexanoylecgonine methyl ester, 8=*trans*-3-hexenoylecgonine methyl ester, 9=3′-furoylecgonine methyl ester, 10=*trans*-2-hexenoylecgonine methyl ester, 11=2′-furoylecgonine methyl ester, 12=*trans*-3-heptenoylecgonine methyl ester, 13 and 14=*cis*-, *trans*-2,4-hexadienoylecgonine methyl ester or *trans*-, *cis*-2,4-hexadienoylecgonine methyl ester, 15=*trans*-, *trans*-2,4-hexadienoylecgonine methyl ester and 16=heptadienoylecgonine methyl ester.

abundances of significant ions at *m/z* 82 and 83 (82>83) were consistent with a 3β-oxo substituent for each compound (3α-oxo substituents have ratios of 82≤83) [8]. In addition, each contained ions at *m/z* 152 and 155 in ratios (ca. 1:1) indicative of 2β-carbomethoxy-3-oxo substituted tropanes (2α-epimers have ratios of approximately 1:5) [9]. Given that the major spectral differences between the titled alkaloids versus cocaine were the molecule ions, it was clear that these alkaloids differed from cocaine only in their C-3 substituents. This facilitated their structural elucidation. In each case, the tentatively identified alkaloid was confirmed by comparison with its synthesized standard. The mass spectrum and GC retention time of each alkaloid was virtually identical to its standard, and differed from its synthesized isomers; retention times and selected ions are given in Table 1.

Table 1
GC–MS data from Fraction EH-2B

Alkaloid	Peak No.	GC t_R	MS Fig.	m/z[a]	M^+
I	1	18.22	3a	42, 43, 55, 71, 82, 94, 96, 182, 198	269
II	2	18.57	3b	41, 42, 57, 82, 94, 96, 182, 198, 252	283
III	3	18.58	3c	41, 42, 57, 82, 94, 96, 182, 198, 252	283
IV	4	19.17	3d	42, 55, 82, 94, 96, 182, 198, 238, 250	281
V	5	19.24	3e	42, 55, 82, 94, 96, 182, 198, 250, 266	281
VI	6	19.53	3f	41, 42, 55, 82, 94, 96, 182, 198, 264	295
VII	7	19.61	3g	42, 43, 55, 82, 94, 96, 182, 198, 266	297
VIII	8	19.63	3h	41, 42, 55, 82, 94, 96, 182, 198, 264	295
IX	10	19.98	3i	42, 55, 82, 94, 96, 182, 198, 238, 266	295
X	12	20.25	3j	41, 42, 55, 82, 94, 96, 182, 198, 280	309
XII[b]	13	20.36	3k	42, 55, 69, 82, 94, 96, 182, 198, 238	293
XIII[b]	14	20.41	3l	42, 55, 69, 82, 94, 96, 182, 198, 238	293
XI	15	20.46	3m	42, 55, 69, 82, 94, 96, 182, 198, 238	293
XIV	16	20.53	3n	42, 53, 82, 94, 96, 109, 182	307

[a] Selected ions of significant abundance.
[b] The order of these alkaloids may be reversed.

3.1. Structural characterization of titled alkaloids

The EI mass spectrum of peak 1, illustrated in Fig. 3a, produced a moderately intense molecule ion at 269 Da. The molecule ions for this and all subsequent target compounds were confirmed by CI–MS. The mass difference between the molecule ion and that of cocaine was −34 Da, suggesting a 4-carbon ester ($C_4H_7O_2$) at C-3, either butyroyl or isobutyroyl. Fragment ions supporting these esters were found at m/z 43 ($C_3H_7^+$) and m/z 71 ($C_4H_7O^+$). Peak 1 was identified as butyroylecgonine methyl ester (I) by comparison to butyroyl- and isobutyroylecgonine methyl ester standards.

Peak 2 yielded an EI mass spectrum, illustrated in Fig. 3b, giving a moderately intense molecule ion at 283 Da. The mass difference between this compound and (I) was +14 Da, suggesting the 5-carbon homolog ($C_5H_9O_2$) at C-3. A fragment ion at m/z 57 ($C_4H_9^+$) supported this ester. Peak 2 was characterized as 2-methylbutyroylecgonine methyl ester (II) by comparison to 2-methylbutyroyl-, valeroyl- and isovaleroylecgonine methyl ester.

The EI mass spectrum of peak 3, illustrated in Fig. 3c, was virtually identical to that of (II) (peak 2). The only discernable differences in the EI spectra were the relative abundances of ions m/z 42 and m/z 57, suggesting this compound was a C-3 isomer of II. Peak 3 was characterized as isovaleroylecgonine methyl ester (III).

Peak 4 yielded an EI mass spectrum, illustrated in Fig. 3d, giving a molecule ion at 281 Da. The mass difference between this compound and (II) and (III) was −2 Da, suggesting an unsaturated 5-carbon analog ($C_5H_7O_2$) at C-3. A fragment ion found at m/z 55 ($C_4H_7^+$), due from cleavage alpha to the carbonyl, supported this analog. Additionally, the presence of a fragment ion at m/z 238 is indicative of tropane alkaloids bearing alpha-beta unsaturated esters with a proton on the alpha carbon, e.g., the cinnamoyltropanes. Of the three possible geometric conformations, only the senecioyl ester contains a proton on the alpha carbon; the tigloyl and angeloyl esters both have a methyl on the alpha carbon. Peak 4 was characterized as senecioylecgonine methyl ester (IV).

The EI mass spectra of peak 5, illustrated in Fig. 3e, was quite similar to that of (IV), suggesting either the tigloyl or angeloyl analogs. The absence of the m/z 238 ion also suggested an alpha-beta unsaturated ester with a methyl on the alpha carbon (i.e., consistent with either the tigloyl or angeloyl ester). Peak 5 was characterized as tigloylecgonine methyl ester (V).

Peak 6 yielded an EI mass spectrum, illustrated in Fig. 3f, giving a molecule ion at 295 Da. The mass

difference between this compound and (IV) and (V) was +14 Da, suggesting an unsaturated 6 carbon analog. Fragment ions found at *m/z* 41 ($C_3H_5^+$), *m/z* 55 ($C_4H_7^+$) and *m/z* 69 ($C_5H_9^+$) supported this analog. The absence of the *m/z* 238 ion indicated that the unsaturated position was either not alpha-beta to the carbonyl or was alpha-beta with an alkyl substituent at the alpha position. Peak 6 was characterized as *trans*-4-hexenoylecgonine methyl ester (VI) by comparison to 2-, 3-, 4- and 5-*trans*-hexenoyl-, 2-methyl-2-pentenoyl- and cyclopentanoylecgonine methyl ester. No evidence of the *cis*-isomer was found in the chromatogram.

The EI mass spectrum of peak 7, illustrated in Fig. 3g, produced a moderately intense molecule ion at 297 Da. The mass difference between this compound and (VI) was +2 Da, suggesting a saturated six-carbon ester ($C_6H_{11}O_2$) or a keto-substituted 5 carbon ester ($C_5H_7O_3$) at C-3 as possibilities. Peak 7 was characterized as hexanoylecgonine methyl ester (VII) by comparison to the 2-oxo-pentanoyl, 3-oxo-pentanoyl, 3-methyl-2-oxobutanoyl, tetrahyro-2-furanoyl, tetrahydro-3-furanoyl, levulinoyl, 2-methylvaleroyl, 3-methylvaleroyl, 4-methylvaleroyl, 2-ethylbutyroyl, *tert.*-butylacetoxyl and 2,2-dimethylbutyroyl analogs.

Peak 8 eluted on the tailing edge of peak 7. Its EI mass spectrum, illustrated in Fig. 3h, gave a moderately intense molecule ion at 295 Da. Its mass spectrum was very similar to VI with slight differences in the relative abundance of ions *m/z* 41 and *m/z* 42, suggesting that this compound was an isomer of VI. Peak 8 was characterized as *trans*-3-hexenoylecgonine methyl ester (VIII). No evidence of the *cis*-isomer was found in the chromatogram.

Peak 9 (Fig. 2) has been previously characterized as 3′-furanoylecgonine methyl ester [7].

Peak 10 yielded an EI mass spectrum, illustrated in Fig. 3i, giving a moderately intense molecule ion at 295 Da. The spectrum was very similar to VI and VIII, indicating another unsaturated six-carbon ester. The presence of a fragment ion at *m/z* 238 indicated an alpha-beta unsaturated ester with a proton on the alpha carbon. Peak 10 was characterized as *trans*-2-hexenoylecgonine methyl ester (IX). No evidence of the *cis*-isomer was found in the chromatogram.

Peak 11 (Fig. 2) has been previously characterized as 2′-furanoylecgonine methyl ester [7].

The EI mass spectrum of peak 12, illustrated in Fig. 3j, produced a moderately intense molecule ion at 309 Da. The mass difference between this compound and VI, VIII, and IX was +14 Da, suggesting either an unsaturated seven-carbon ester ($C_7H_{11}O_2$) or a thiophenoyl ester as possibilities. However, the two possible thiophene esters would be expected to give rise to a relatively intense *m/z* 111 ion ($C_5H_3SO^+$), which was not present, thus supporting an unsaturated seven carbon ester. In fact, the synthesized standards of the 2′- and 3′-thiophenoyl esters each gave rise to an *m/z* 111 ion, thereby discounting them. Peak 11 was characterized as *trans*-3-heptenoylecgonine methyl ester (X) by comparison to 2-, 3-, and 6-*trans*-heptenoylecgonine methyl ester. No evidence of the *cis*-isomer was found in the chromatogram.

Peaks 13, 14 and 15 provided EI mass spectra, illustrated in Fig. 3k–m, respectively, giving molecule ions at *m/z* 293. The spectra were virtually identical, indicating that the compounds were isomers. The mass difference between these compounds and VI, VIII, and IX was −2 Da, suggesting six-carbon diene esters ($C_6H_7O_2$). The presence of an *m/z* 238 ion in each of the three spectra indicated alpha-beta unsaturation with protons on the alpha carbons. The most logical and stable esters would be the 2,4-dienes; i.e., in conjugation with the carbonyl. Synthesized standards of the *trans*-, *trans*- (XI), *cis*-, *trans*-(XII) and *trans*-, *cis*-2,4- (XIII) hexadienoylecgonine methyl esters were produced from crude *trans*-, *trans*-2, 4-hexadienoic acid (which contained lesser amounts of the *cis*-, *trans*- and *trans*-, *cis*-isomers). Peak 15 was characterized as the *trans*-, *trans*-isomer (XI). Peaks 13 and 14 were tentatively identified as the *cis*-, *trans*-(XII) and *trans*-, *cis*-(XIII) isomers, respectively, based on their analytical data; their actual elution order was uncertain.

The EI mass spectrum of peak 16, illustrated in Fig. 3n, gave a molecule ion at 307 Da. The mass differences between this compound and X and XI was −2, and XII and XIII was +14 Da, respectively, suggesting a seven-carbon diene ester ($C_7H_9O_2$) as one possibility. However, the lack of any commercially available heptadienoic acid precursors precluded definitive characterization. Peak 16 is therefore tentatively characterized as a heptadienoyl ester

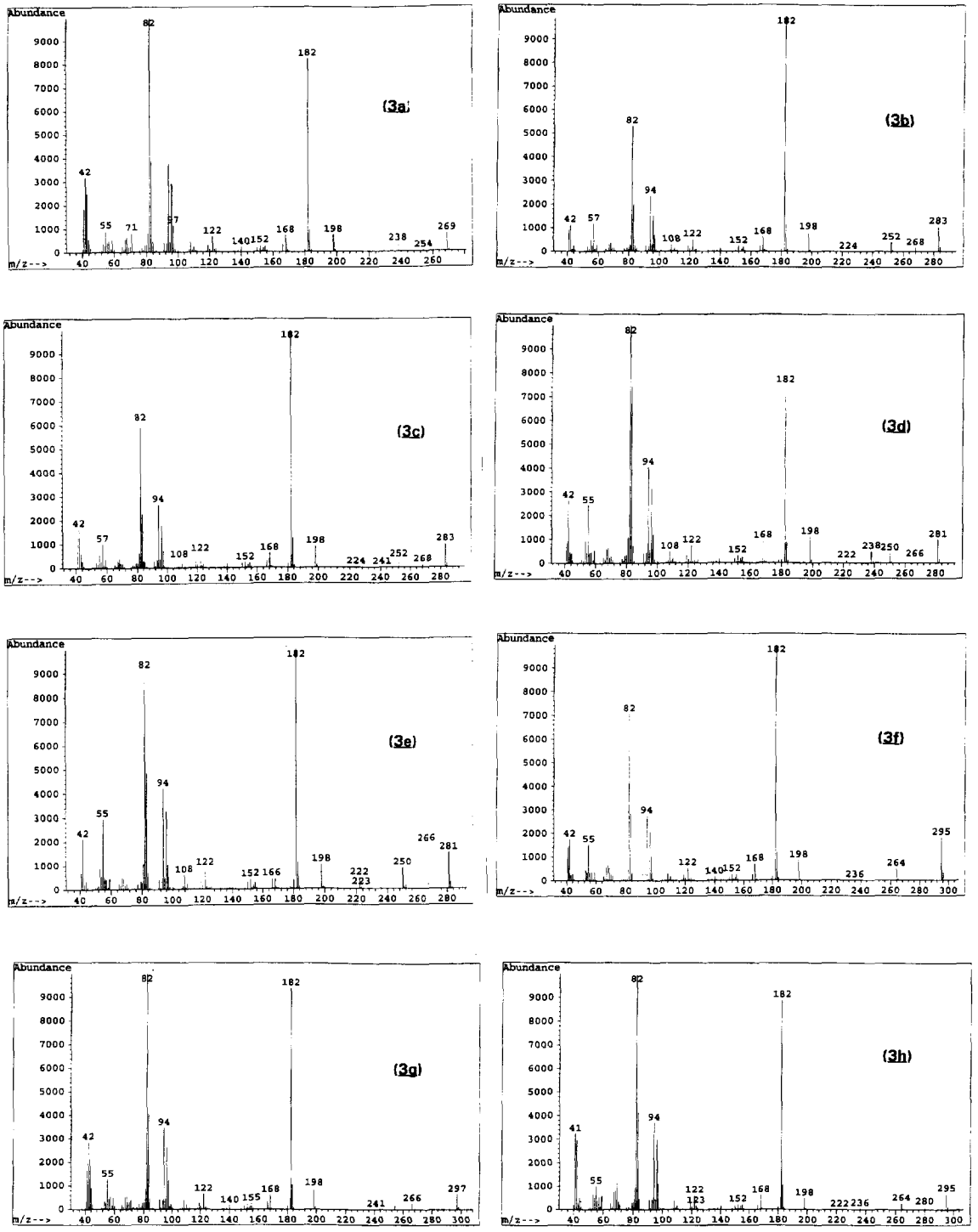

Fig. 3. Electron ionization mass spectrum of (a) butyroylecgonine methyl ester, (b) 2-methylbutyroylecgonine methyl ester, (c) isovaleroylecgonine methyl ester, (d) senecioylecgonine methyl ester, (e) tigloylecgonine methyl ester, (f) *trans*-4-hexenoylecgonine methyl ester, (g) hexanoylecgonine methyl ester (h) *trans*-3-hexenoylecgonine methyl ester, (i) *trans*-2-hexenoylecgonine methyl ester, (j) *trans*-3-heptenoylecgonine methyl ester, (k) either *cis*-, *trans*-2,4-hexadienoylecgonine methyl ester or *trans*-, *cis*-2,4-hexadienoylecgonine methyl ester, (l) either *cis*-, *trans*-2,4-hexadienoylecgonine methyl ester or *trans*-, *cis*-2,4-hexadienoylecgonine methyl ester, (m) *trans*-, *trans*-2,4-hexadienoylecgonine methyl ester, (n) heptadienoylecgonine methyl ester.

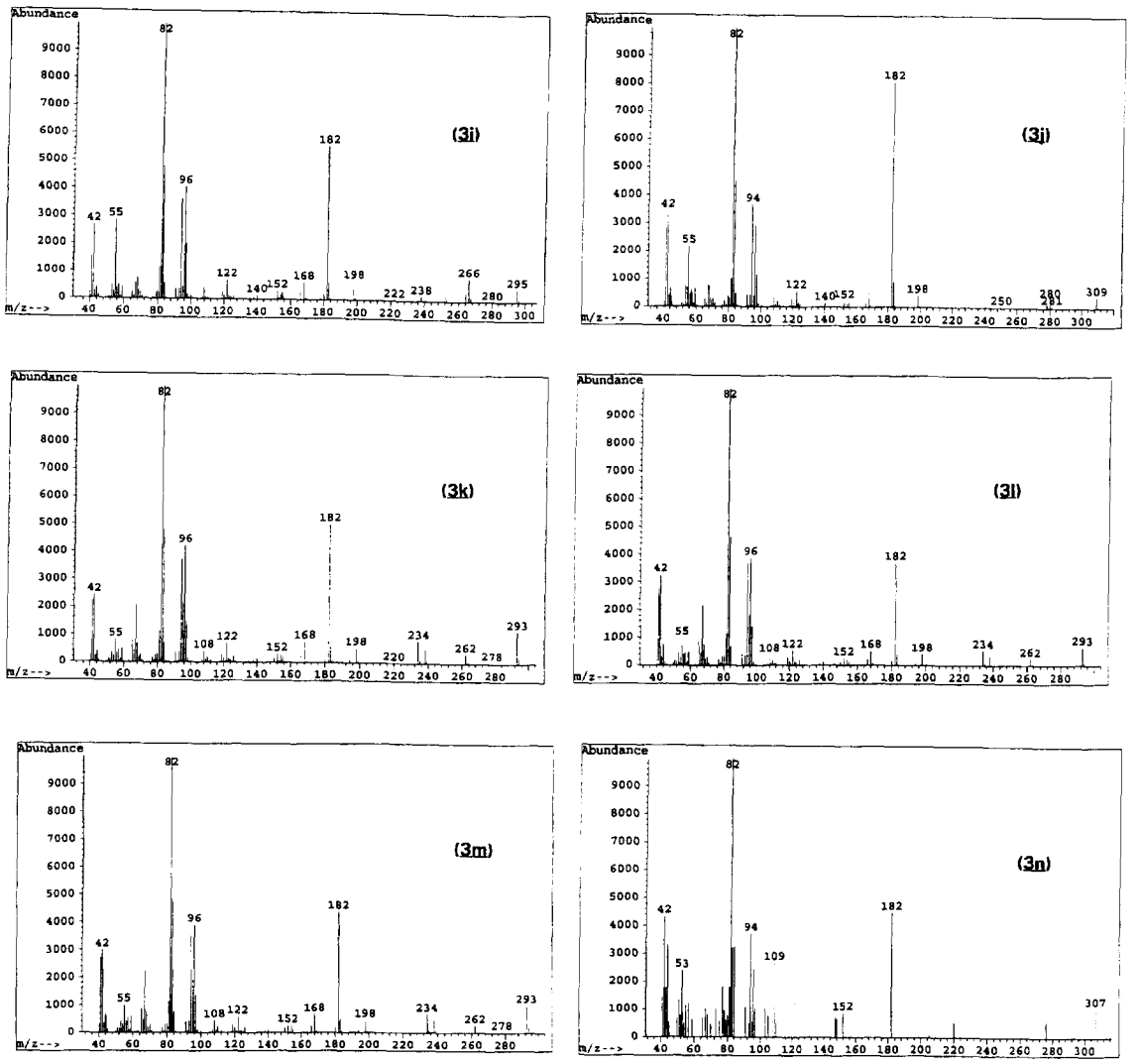

Fig. 3. (*continued*)

of ecgonine methyl ester (XIV). The geometry of this compound was not determined; however, the *trans*-, *trans*-2,4-isomer is the most logical and likely candidate.

4. Summary

Fourteen new trace-level alkaloids found in the crude extract of South American *Erythroxylum coca* var. *coca* were preparatively isolated from cocaine. These saturated and unsaturated ester analogs of cocaine at C-3 are reported for the first time. The titled alkaloids were characterized (three tentatively) by comparison of their gas chromatographic–electron and chemical ionization mass spectra with their synthesized standards.

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