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Tropane alkaloids from *Erythroxylum zeylanicum* O.E. Schulz (Erythroxylaceae)

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Abstract

Six tropane alkaloids were isolated from the Sri Lankan endemic plant *Erythroxylum zeylanicum* O.E. Schulz (Erythroxylaceae) and structurally elucidated by NMR and MS measurements. Three of them, erythrozeylanines A [1*R*,3*R*,5*S*,6*R*-6-acetoxy-3-(3′,4′,5′-trimethoxybenzoyloxy)tropane], B [*cis*-3β-(cinnamoyloxy)tropane], and C [*cis*-6β-acetoxy-3α-(cinnamoyloxy)tropane] are new, whereas the others have already been found in other *Erythroxylum* species. For the first time, the absolute configuration of a tropane alkaloid (erythrozeylanine A) has been determined by quantum chemical CD calculations. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Erythroxylum zeylanicum*; Erythroxylaceae; Structural elucidation; Tropane alkaloids; Erythrozeylanine A [1*R*,3*R*,5*S*,6*R*-6-acetoxy-3-(3′,4′,5′-trimethoxybenzoyloxy)tropane]; Erythrozeylanine B [*cis*-3β-(cinnamoyloxy)tropane]; Erythrozeylanine C [*cis*-6β-acetoxy-3α-(cinnamoyloxy)tropane]; 3α-(3′,4′,5′-Trimethoxybenzoyloxy)tropane; *trans*-3β-(Cinnamoyloxy)tropane; *trans*-6β-Acetoxy-3α-(cinnamoyloxy)tropane; CD calculations

1. Introduction

Among the ca. 250 species of the genus *Erythroxylum* (Erythroxylaceae), which are all distributed throughout the tropics (Brachet, Muñoz, Gupta, Veuthey & Christen, 1997), five are found in Sri Lanka, but only one, *E. zeylanicum*, is endemic to this country (Wadhwa & Weerasooriya, 1996). The plant is a branched shrub about 3 m tall with lanceolate leaves and small dark-red fruits (Schulz, 1907). The leaves are used as an anthelmintic against round worms in traditional medicine (Jajaweera, 1980). No phytochemical investigation on this species has so far been reported, although alkaloids of unknown structure were detected on TLC (Sultanbawa et al., 1978), thus making *E. zeylanicum* a rewarding research subject. Among the other four Sri Lankan *Erythroxylum* species, only *E. monogynum* has been analyzed phytochemically, leading to the isolation of several tropane alkaloids (e.g. Agar, Evans & Treagust, 1974; Agar & Evans, 1976). In this paper, we describe the isolation and structural elucidation of six alkaloids with a tropane skeleton, viz. erythrozeylanine A [**1**, alias 1*R*,3*R*,5*S*,6*R*-6-acetoxy-3-(3′,4′,5′-trimethoxybenzoyloxy)tropane], 3α-(3′,4′,5′-trimethoxybenzoyloxy)tropane (**2**), erythrozeylanine B [**3a**, alias *cis*-3β-(cinnamoyloxy)tropane], *trans*-3β-(cinnamoyloxy)tropane (**3b**), erythrozeylanine C [**4a**, alias *cis*-6β-acetoxy-3α-(cinnamoyloxy)tropane], and *trans*-6β-acetoxy-3α-(cinnamoyloxy)tropane (**4b**) (see Fig. 1); of which, **1**, **3a**, and **4a** are new. Since high resolution NMR data are not available in the literature for the known isolated alkaloids **2**, **3b**, and **4b** as well, they are given in this paper. Furthermore, we describe the first determi-

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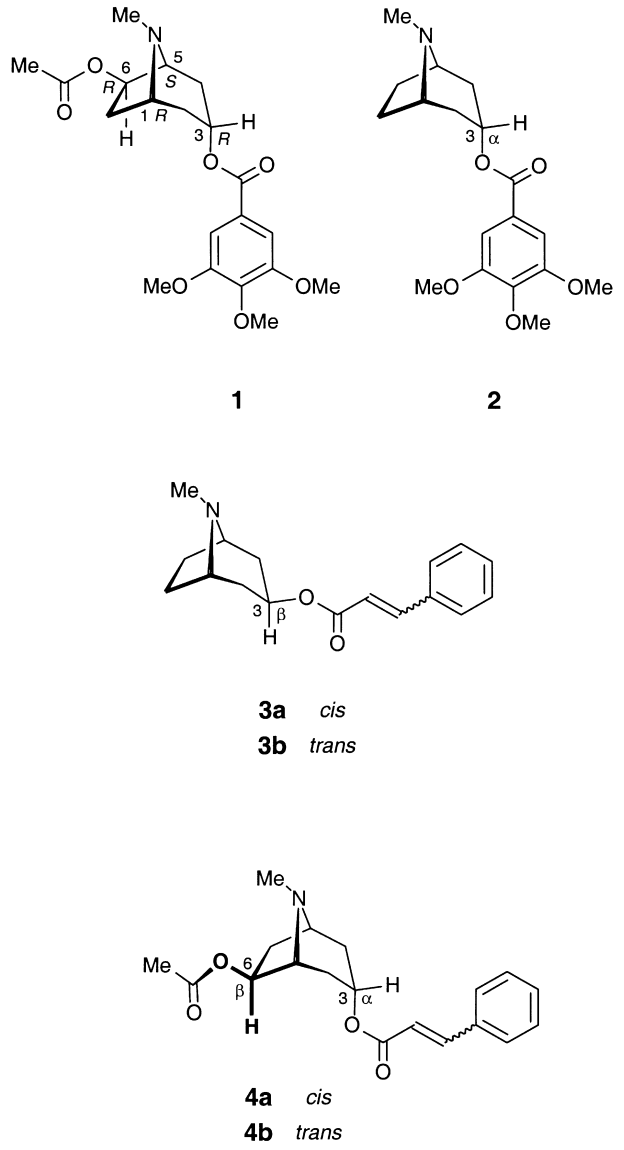


Fig. 1. Tropane alkaloids of *E. zeylanicum* (**1**–**4**). The compounds **1**, **3a**, and **4a** are new.

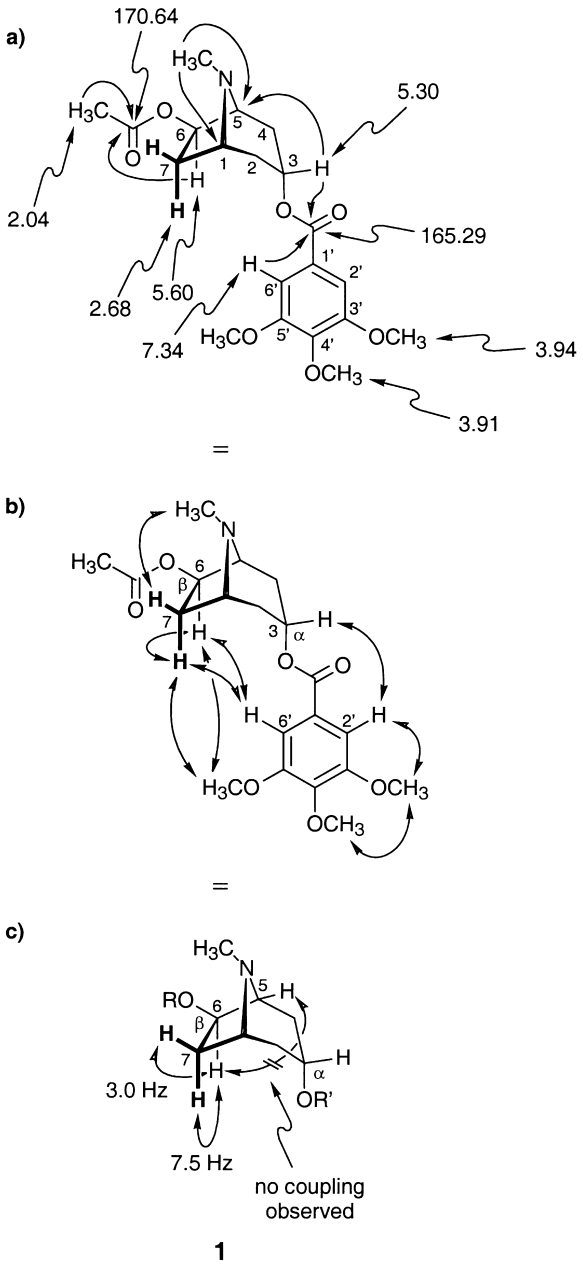


Fig. 2. Structure of erythrozeylanine A (**1**), as deduced from chemical shifts and selected HMBC interactions (a) as well as ROESY interactions (b); additional support for the relative configuration by the NMR coupling constants (c). At this stage, the absolute configuration drawn is still arbitrary.

nation of the absolute configuration of a tropane alkaloid (**1**) by semiempirical CD calculations.

2. Results and discussion

The CH_2Cl_2 extract of the roots of *E. zeylanicum* was extracted with 2 N HCl to separate the alkaloids from non-basic compounds. The alkaloid portion was then subjected to separation on MPLC, consecutively yielding three fractions, of which the first one was purified by flash CC. The mass peak (*m*/*z* 393) of the first compound, together with the HREIMS, hinted at a molecular formula of $C_{20}H_{27}NO_7$. The ^{1}H-NMR spectrum exhibited typical signals of a tropane alkaloid with an ester-linked aromatic acid and another side chain (see Fig. 2(a)). The triplet at 5.30 ppm was, due to its low-field position, attributed to H-3, which is neighbored by an oxygen substituent in almost all tropane alkaloids. By Heteronuclear Multiple Bond Correlation (HMBC) measurements and Rotating Frame Overhauser Enhancement Spectroscopy (ROESY) as depicted in Fig. 2(a) and (b), the initial assumption of

the presence of a tropane skeleton was confirmed. The aromatic moiety turned out to be a 3,4,5-trimethoxybenzoate by virtue of a singlet at 7.34 ppm corresponding to two protons and two singlets at 3.91 and 3.94 ppm, integrating as three and six protons, respectively. This was further confirmed by Heteronuclear Multiple Quantum Correlation (HMQC) and HMBC experiments. HMBC correlations between H-3 and the carbonyl signal at 165.29 ppm located this substituent at C-3 of the tropane moiety. The ^{1}H-NMR signal at 2.04 ppm was attributed to an *O*-acetyl group, which was analogously proven to be situated at C-6 by an HMBC correlation between H-6 (5.60 ppm) and the ^{13}C-NMR signal at 170.64 ppm. The constitution of the present alkaloid is hence 6-acetoxy-(3′,4′,5′-trimethoxybenzoyloxy)tropane. Since no compound with this constitution has yet been known in nature, the compound, subsequently named erythrozeylanine A, must be new.

The next structural detail of erythrozeylanine A to be established was the stereochemical orientation of the two substituents relative to the nitrogen containing bridge. As per general experience (e.g. Agar & Evans, 1976; Al-Said, Evans & Grout, 1989b), a triplet splitting of the H-3 signal is indicative of an α (i.e. *endo*) position of that substituent, which was also observed here. This attribution was supported by ROESY interactions between protons of the aromatic system on one hand (H-2′/H-6′, 7.34 ppm, and methoxy protons, 3.94 ppm) and H-6/H_{endo}-7 (5.60 and 2.68 ppm, respectively) on the other, thus establishing accurately that these protons must be situated *endo*, i.e. below the 'roof' formed by the tropane skeleton (see Fig. 2(b)). This, likewise, provided evidence for a β (i.e. *exo*) arrangement of the acetoxy group at C-6, which was supported by analysis of the coupling constants (see Fig. 2(c)). Since the two couplings of H-6 had to be attributed to the two H-7 protons, there is no coupling left between H-6 and H-5, which can be easily explained by the ca. 90° dihedral angle between H-5 and H_{endo}-6, according to the Karplus equation. By contrast, a coupling to H-5 should be expected for a proton located *exo* at C-6. Therefore, the new alkaloid erythrozeylanine A has to be regarded as a 6β-acetoxy-3α-(3′,4′,5′-trimethoxybenzoyloxy)tropane (**1**).

In the literature, only few tropane alkaloids have been investigated successfully for their absolute configuration (e.g., Lounasmaa, 1988), mainly by tedious chemical and degradative work. An investigation of the circular dichroism (CD) of **1** seemed not to be too promising initially, because of the low ellipticities experimentally found. Moreover, the chromophore (the trimethoxybenzoic acid ester) is attached in a conformationally flexible way to the tropane core of the molecule, with the deciding stereocenter quite far from the aromatic system. Nonetheless, we tried to establish the

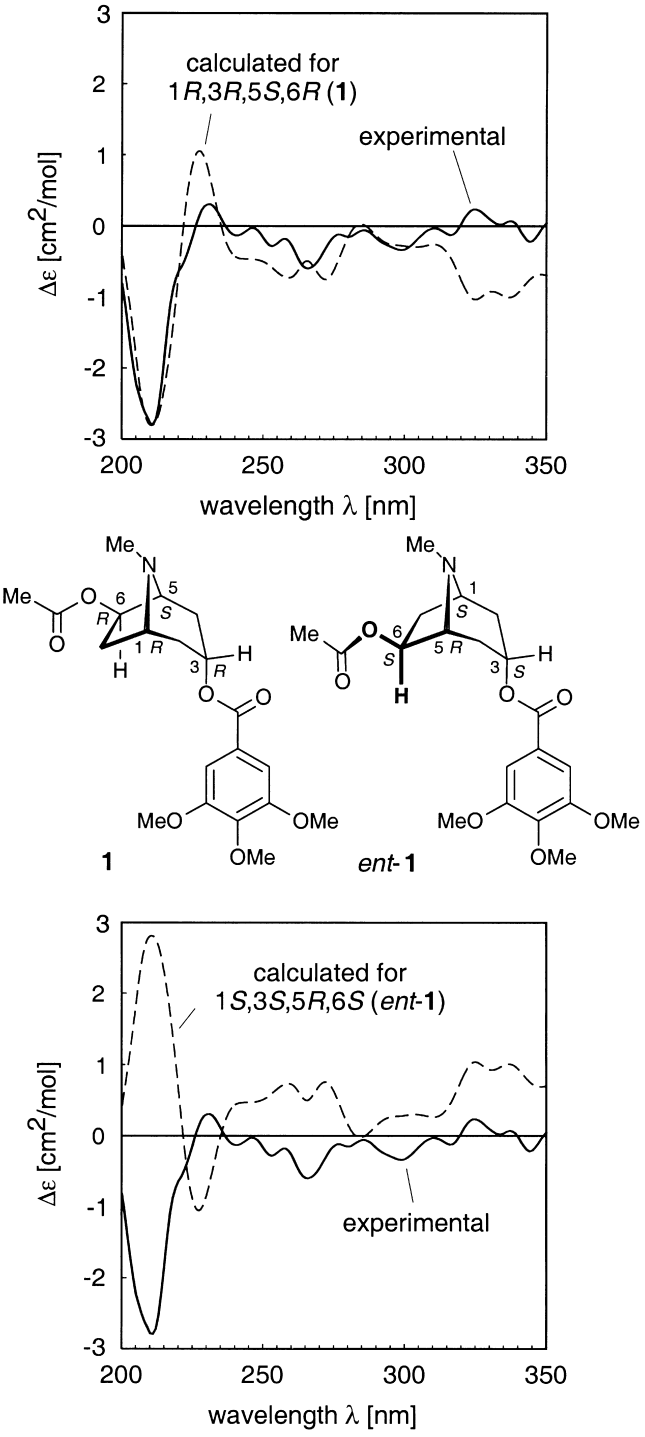


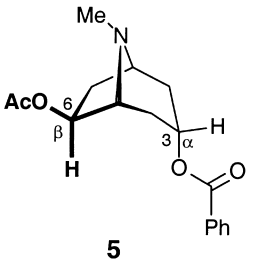
Fig. 3. Calculated CD spectra of **1** and *ent*-**1** in comparison with the experimental one.

absolute configuration of **1** by quantum chemical calculations and comparison of calculated and experimental CD spectrum, a particularly valuable tool as shown earlier in our group, in the case of numerous different classes of compounds with axial, planar, or centrochirality (Bringmann & Busemann, 1998; Tochtermann, Kuckling, Meints, Kraus & Bringmann, 1999).

Because of the molecular flexibility of the chromophore part, the CD calculations were not based on a Boltzmann-weighted addition of the single CD spectra as usual, but on a molecular dynamics simulation using the MM3 force field (Allinger, Yuh & Lii, 1989; Lii & Allinger, 1989), arbitrarily for 1*R*,3*R*,5*S*,6*R*-erythrozeylanine A. The simulation was carried out for 500 ps, recording the structure every 0.5 ps for further calculations.

For the 1000 collected structures, single CD spectra were calculated. The computed spectra were averaged arithmetically over the trajectory to give the theoretical overall spectrum. In order to take into account a systematic shift of the calculated CD spectrum, a 'UV correction' was carried out as introduced by our group (Bringmann & Busemann, 1998). The good agreement of the calculated overall CD spectrum for the 1*R*,3*R*,5*S*,6*R*-configured enantiomer with the experimental one (see Fig. 3) allows us an accurate attribution of the absolute configuration of **1**. This constitutes the first elucidation of the absolute configuration of a tropane alkaloid without the application of chemical (i.e. degradative or synthetic) methods.

Closely related derivatives of **1** are known from other *Erythroxylum* species, e.g. from *E. hypericifolium*, such as 6β-acetoxy-3α-(benzoyloxy)tropane (**5**) (Al-Said, Evans & Grout, 1989a) and 3α-(3′,4′,5′-trimethoxybenzoyloxy)tropane-6β-ol (**6**) (El-Iman, Evans, Grout & Ramsey, 1987), the absolute configurations of which are unknown. It seems feasible that **6** is a biogenetic precursor of **1**.



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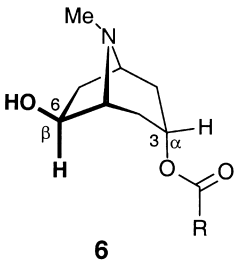
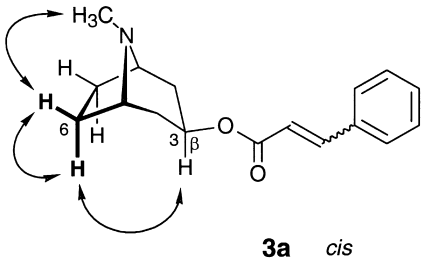


6

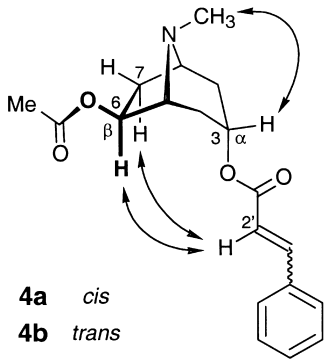
(R = 3',4',5'-$C_6H_2(OMe)_3$)

The second MPLC fraction yielded after purification by preparative TLC a compound with a mass peak at *m*/*z* 335 and a molecular formula of $C_{18}H_{25}NO_5$, as derived from HREIMS. The NMR spectra showed some resemblance to that of **1**, except for the reduced number of signals. A similar molecular framework was hence to be expected, but with a plane of symmetry in the tropane part, which lacks a C-6 substituent. The substituent at C-3 is again a 3,4,5-trimethoxybenzoyloxy group, and its orientation on the tropane skeleton was found to be *endo* (α) as described above. Hence, this alkaloid, **2**, is 3α-(3′,4′,5′-trimethoxybenzoyloxy)-tropane (see Fig. 1). The compound is already known



3a *cis*

3b *trans*



4a *cis*

4b *trans*

Fig. 4. Relative configurations of **3** and **4** as deduced from ROESY measurements.

from *E. monogynum*, a plant indigenous to India and Sri Lanka (Agar et al., 1974; Agar & Evans, 1976), and from the South African species *E. zambesiacum* (El-Iman, Evans, Grout & Ramsey, 1987; Christen, Roberts, Phillipson & Evans, 1993). Its physical and spectroscopical data were fully identical to those reported in the literature.

The third MPLC fraction, which was also purified by preparative TLC, consisted of a mixture of two compounds, both with a mass peak at *m*/*z* 271 and thus a molecular formula of $C_{17}H_{21}NO_2$, proven by HREIMS. Whereas the aliphatic region of the ^{1}H-NMR spectrum nearly matched that of **2**, the aromatic parts exhibited major deviations, indicating a different substituent at C-3. This moiety turned out to be a mixture of *cis*- and *trans*-cinnamic acid, as shown by the coupling constants of 12.6 Hz (*cis*) and 16.0 Hz (*trans*) between H-2′ and H-3′ (see Fig. 4). The reduced number of NMR signals demonstrated again the presence of a mirror plane in the tropane moiety. The cinnamoyloxy residue as the only substituent is located at C-3, but this time it is oriented upwards (β or *exo*), as can clearly be seen by the typical splitting of the H-3 signal (triplet of triplets at 600 MHz) and by ROESY interactions between H-3 and two of the four protons at C-6 and C-7, which were further con-

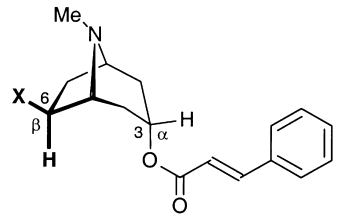
firmed to be *endo* by ROESY correlations between the two *exo* protons at C-6 and C-7 and the *N*-methyl group. Therefore, the two diastereomeric alkaloids are *cis*- and *trans*-3β-(cinnamoyloxy)tropane (**3a** and **3b**, respectively).

The *cis*-compound **3a**, erythrozeylanine B, has not yet been described as a natural product, but its formation as an isolation artefact is not entirely excluded since *cis*-cinnamic acid derivatives are rare in nature, and a photochemical *trans–cis* interconversion is known to occur easily (Harborne, 1988). By contrast, **3b** has previously been isolated from *E. hypericifolium* (Al-Said et al., 1989b), and the corresponding 3α-substituted tropane (**7**) is known from *Crossostylis sebertii* (Rhizophoraceae) (Gnecco Medina, Pusset, Pusset & Husson, 1983) and *E. monogynum* (in the latter case — as sometimes in the literature — without giving the configuration at the double bond) (Christen, Roberts, Phillipson & Evans, 1995).

The leaves and twigs, treated as described above, were found to contain another pair of diastereomeric natural products with NMR spectra similar to those of **3**. From the mass spectrum (*m*/*z* 329) and the HREIMS, a molecular formula of $C_{19}H_{23}NO_4$ was deduced. Again, a mixture of *cis–trans* isomers of tropane cinnamic esters was found to be present, now with an acetoxy group at C-6, as for **1**. The relative configuration at C-3 was assigned, as described above, from the H-3 signal, which appeared as a clear triplet (see Fig. 4). This was likewise confirmed by ROESY relationships between H-2′ and H-6 as well as H_{endo}-7, and, on the other side, between H-3 and *N*–Me. As shown for **1**, the acetoxy group is placed *exo* in both the isolated compounds, too. In conclusion, the two diastereomeric alkaloids are *cis*- and *trans*-6β-acetoxy-3α-(cinnamoyloxy)tropane (**4a** and **4b**, respectively). The CD spectrum of the mixture did not permit an accurate attribution of the absolute configuration.

Whereas the *cis*-configurated compound **4a**, subsequently named erythrozeylanine C, is a new alkaloid (again not fully excluded to be an artefact), its *trans*-analog **4b** has been found to be produced by *E. hypericifolium* (Al-Said et al., 1989b), and its presumable biosynthetic precursor 3α-(cinnamoyloxy)tropane-6β-ol (**8**) has been found in *E. australe* (El-Imam, Evans & Grout, 1988).

In conclusion, the alkaloids found in the twigs and roots of *E. zeylanicum* provide clues for a first chemotaxonomic classification of this plant species endemic to Sri Lanka, hinting at a phylogenetic affinity to *E. hypericifolium*, which is native to Réunion, an island in the Indian Ocean. For a more thorough investigation of the relationships including the other Sri Lankan *Erythroxylum* species, further phytochemical analyses on the other species remain to be conducted.



7 X = H
8 X = OH

3. Experimental

3.1. General

Optical rotations: 25°C, 10 cm cell, $CHCl_3$. IR: KBr. ^{1}H-NMR (600 MHz, Bruker) and ^{13}C-NMR (150 MHz, Bruker) were recorded in $CDCl_3$ (solvent as internal standard, δ 7.26 and δ 77.01, respectively). Proton-detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 7 Hz). EIMS: 70 eV. CC: silica gel (60–200 mesh, Merck). TLC: precoated silica gel 60 F_{254} plates (Merck). Spots were detected under UV light. Preparative TLC: precoated silica gel 60 F_{254} plates (Merck), 0.5 mm and 1 mm layer. MPLC: silica gel (32–63 mesh, Merck). HPLC (preparative): RP-18, Waters; UV detection.

3.2. Plant material

Plant material (leaves, twigs, and roots) of *E. zeylanicum* O.E. Schulz was collected at Hulandawa in the Monaragala district of Sri Lanka in March 1995 and was identified by comparison with a voucher specimen deposited at the National Herbarium, Royal Botanic Gardens, Peradeniya, Sri Lanka. A voucher specimen is deposited at the private Herbarium of the Department of Chemistry, University of Peradeniya, Sri Lanka.

3.3. Extraction and isolation

The air dried twigs and leaves (4.0 kg) and roots (3.0 kg) were treated separately. The plant material was ground and extracted with CH_2Cl_2 in a bottle shaker for two days. The extracts were evaporated in vacuo, dissolved in EtOAc and extracted several times with 2 N HCl. The combined aqueous solutions were basified with conc. NH_3 and washed with EtOAc. The organic phases were combined, dried over $MgSO_4$, and evaporated.

3.4. Erythrozeylanine A [1R,3R,5S,6R-6-acetoxy-3-(3′,4′,5′-trimethoxybenzoyloxy)tropane (**1**)]

The CH_2Cl_2 extract of the roots (65 g) was treated as described in Section 3.3. The alkaloid-containing fraction (4.16 g) was resolved subsequently by MPLC (EtOAc–MeOH, 95:5), yielding three fractions. Flash CC (CH_2Cl_2–MeOH 95:5) of the first of these fractions afforded **1** (10 mg, 0.00033%) as a colorless semisolid. $[\alpha]_D^{25}$ -22.1° ($CHCl_3$, *c* 0.3). CD: $\Delta\varepsilon_{200}$ −0.13, $\Delta\varepsilon_{205}$ -1.94, $\Delta\varepsilon_{208}$ −1.52, $\Delta\varepsilon_{211}$ -2.37, $\Delta\varepsilon_{231}$ +0.28 (EtOH, *c* 0.02). IR ν_{max} cm^{-1}: 2920 (C–H), 1720 (C═O), 1685 (C═O), 1210, 1120. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.67 (1H, *d*, *J* = 15.5 Hz, H_{endo}-2), 1.86 (1H, *d*, *J* = 15.1 Hz, H_{endo}-4), 2.04 (3H, *s*, $COCH_3$), 2.13 (1H, *m*, H_{exo}-7), 2.25 (1H, *dt*, *J* = 15.8, 3.9 Hz, H_{exo}-2), 2.28 (1H, *dt*, *J* = 16.5, 4.9 Hz, H_{exo}-4), 2.54 (3H, *s*, *N*–CH_3), 2.68 (1H, *dd*, *J* = 14.0, 7.6 Hz, H_{endo}-7), 3.23 (1H, *br.s*, H-5), 3.36 (1H, *m*, H-1), 3.91 (3H, *s*, OCH_3-4′), 3.94 (6H, *s*, OCH_3-3′ and OCH_3-5′), 5.30 (1H, *t*, *J* = 5.4 Hz, H-3), 5.60 (1H, *dd*, *J* = 7.5, 3.0 Hz, H-6), 7.34 (2H, *s*, H-2′ and H-6′). ^{13}C-NMR (150 MHz, $CDCI_3$): δ 21.30 (*C*H_3CO), 30.98 (C-4), 32.41 (C-2), 37.26 (C-7), 38.19 (N–CH_3), 56.28 (OCH_3-3′, OCH_3-5′), 58.97 (C-1), 60.90 (OCH_3-4′), 64.62 (C-5), 67.58 (C-3), 78.90 (C-6), 106.54 (C-2′ and C-6′), 125.31 (C-1′), 142.22 (C-4′), 153.09 (C-3′ and C-5′), 165.29 (COAr), 170.64 (CH_3*C*O). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m*/*z* (rel. int.): 393 $[M]^+$ (6), 303 (4), 182 $[C_{10}H_{14}O_3]^+$ (21), 138 (10), 122 $[C_8H_{12}N]^+$ (40), 96 (20), 95 $[C_6H_9N]^+$ (98), 94 $[C_6H_8N]^+$ (100), 57 (39), 55 (22), 43 $[CH_3CO]^+$ (31). HREIMS *m*/*z* 393.179 $[M]^+$ ($C_{20}H_{27}NO_7$ requires 393.179).

3.5. 3α-(3′,4′,5′-Trimethoxybenzoyloxy)tropane (**2**)

The second MPLC fraction afforded, by preparative TLC (EtOAc–NH_3–MeOH 37:1:2), the alkaloid **2** (23 mg, 0.00076%) as a colorless semisolid. ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.82 (2H, *d*, *J* = 14.8 Hz, H_{endo}-2 and H_{endo}-4), 2.07 (2H, *d*, *J* = 8.0 Hz, H_{endo}-6 and H_{endo}-7), 2.10 (2H, *m*, H_{exo}-6 and H_{exo}-7), 2.24 (2H, *ddd*, *J* = 14.8, 4.2, 4.2 Hz, H_{exo}-2 and H_{exo}-4), 2.32 (3H, *s*, *N*–CH_3), 3.18 (2H, *br.s*, H-1 and H-5), 3.90 (9H, *s*, OCH_3-3′, OCH_3-4′, and OCH_3-5′), 5.24 (1H, *t*, *J* = 5.2 Hz, H-3), 7.30 (2H, *s*, H-2′ and H-6′). ^{13}C-NMR (150 MHz, $CDCl_3$): 25.70 (C-6 and C-7), 36.52 (C-2 and C-4), 40.29 (*N*–CH_3), 56.14 (OCH_3-3′ and OCH_3-5′), 59.84 (C-1 and C-5), 60.88 (OCH_3-4′), 68.04 (C-3), 106.67 (C-2′ and C-6′), 125.79 (C-1′), 142.16 (C-4′), 152.96 (C-3′ and C-5′), 165.31 (COAr). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m*/*z* (rel. int.): 335 $[M]^+$ (29), 212 $[C_{10}H_{12}O_5]^+$ (5), 195 $[C_{10}H_{11}O_4]^+$ (9), 140 $[C_8H_{14}NO]^+$ (18), 124 $[C_8H_{14}N]^+$ (100), 94 (13), 83 (25), 82 $[C_5H_8N]^+$ (23).

3.6. Erythrozeylanine B [cis-3β-(cinnamoyloxy)tropane (**3a**)] and trans-3β-(cinnamoyloxy)tropane (**3b**)

The third MPLC fraction described in Section 3.4. was resolved by preparative TLC ($CHCl_3$–MeOH–H_2O, 7:3:1) and yielded an inseparable mixture (12 mg, 0.00040%) of **3a** and **3b** (100:38). **3a**: ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.65 (2H, *d*, *J* = 8.3 Hz, H_{endo}-6 and H_{endo}-7), 1.74 (2H, *dd*, *J* = 12.0, 2.6 Hz, H_{endo}-2 and H_{endo}-4), 1.82 (2H, *ddd*, *J* = 12.9, 6.3, 3.4 Hz, H_{exo}-2 and H_{exo}-4), 2.02 (2H, *m*, H_{exo}-6 and H_{exo}-7), 2.32 (3H, *s*, *N*–CH_3), 3.21 (2H, *dd*, *J* = 3.4, 3.4 Hz, H-1 and H-5), 5.05 (1H, *tt*, *J* = 10.9, 6.3 Hz, H-3), 5.91 (1H, *d*, *J* = 12.6 Hz, H-2′), 6.93 (1H, *d*, *J* = 12.6 Hz, H-3′), 7.32–7.38 (3H, *m*, H-5′, H-7′, and H-9′), 7.53–7.56 (2H, *m*, H-6′ and H-8′). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 26.52 (C-6 and C-7), 35.12 (C-2 and C-4), 38.37 (*N*-CH_3), 60.19 (C-1 and C-5), 67.09 (C-3), 120.19 (C-2′), 127.92 (C-6′ and C-8′), 128.88 (C-7′), 129.56 (C-5′ and C-9′), 134.92 (C-4′), 143.08 (C-3′), 165.67 (C-1′). **3b**: ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.71 (2H, *d*, *J* = 8.1 Hz, H_{endo}-6 and H_{endo}-7), 1.85 (2H, *dd*, *J* = 11.9, 2.5 Hz, H_{endo}-2 and H_{endo}-4), 1.91 (2H, *ddd*, *J* = 12.9, 6.4, 3.2 Hz, H_{exo}-2 and H_{exo}-4), 2.06 (2H, *m*, H_{exo}-6 and H_{exo}-7), 2.37 (3H, *s*, *N*-CH_3), 3.26 (2H, *dd*, *J* = 3.2, 3.2 Hz, H-1 and H-5), 5.13 (1H, *tt*, *J* = 10.8, 6.4 Hz, H-3), 6.40 (1H, *d*, *J* = 16.0 Hz, H-2′), 7.29–7.38 (3H, *m*, H-6′, H-7′, and H-8′), 7.49–7.52 (2H, *m*, H-5′ and H-9′), 7.64 (1H, *d*, *J* = 16.0 Hz, H-3′). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 26.52 (C-6 and C-7), 35.53 (C-2 and C-4), 38.57 (*N*–CH_3), 60.33 (C-1 and C-5), 67.14 (C-3), 118.50 (C-2′), 128.02 (C-5′ and C-9′), 128.82 (C-6′ and C-8′), 130.17 (C-7′), 134.42 (C-4′), 144.53 (C-3′), 166.48 (C-1′). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m*/*z*(rel. int.): 271 $[M]^+$ (15), 245 $[M–C_2H_2]^+$ (4), 131 $[C_9H_7O]^+$ (9), 124 $[C_8H_{14}N]^+$ (100), 103 $[C_8H_7]^+$ (12), 96 (40), 94 (36), 83 (65), 82 $[C_5H_8N]^+$ (99), 77 (23), 67 (26), 42 (43). HREIMS *m*/*z* 271.157 $[M^+]$ ($C_{17}H_{21}NO_2$ requires 271.157).

3.7. Erythrozeylanine C [cis-6β-acetoxy-3α-(cinnamoyloxy)tropane (**4a**)] and trans-6β-acetoxy-3α-(cinnamoyloxy)tropane (**4b**)

The CH_2Cl_2 extract of twigs and leaves (100 g) was treated as described in Section 3.3. and yielded an alkaloid fraction (1.23 g), which was fractionated consecutively by alumina CC (CH_2Cl_2–MeOH, 49:1), prepared TLC (CH_2Cl_2–MeOH, 49:1), and HPLC (MeOH–H_2O–HOAc 70:29:1), affording an inseparable oily mixture (20 mg, 0.00050%) of **4a** and **4b** (100:26). **4a**: ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.52 (1H,

d, J = 15.4 Hz, H_{endo}-2), 1.74 (1H, *d*, J = 15.5 Hz, H_{endo}-4), 2.01 (1H, *m*, H_{exo}-7), 2.03 (3H, *s*, $COCH_3$), 2.13 (1H, *m*, H_{exo}-4), 2.15 (1H, *m*, H_{exo}-2), 2.32 (1H, *dd*, J = 14.0, 7.6 Hz, H_{endo}-7), 2.47 (3H, *s*, *N*-CH_3), 3.12 (1H, *br.s*, H-5), 3.25 (1H, *dt*, J = 7.3, 3.6 Hz, H-1), 5.06 (1H, *t*, J = 5.4 Hz, H-3), 5.29 (1H, *dd*, J = 7.6, 3.0 Hz, H-6), 5.96 (1H, *d*, J = 12.6 Hz, H-2′), 6.88 (1H, *d*, J = 12.6 Hz, H-3′), 7.33 (3H, *m*, H-6′, H-7′, and H-8′), 7.58 (2H, *d*, J = 7.0 Hz, H-5′ and H-9′). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 21.36 (CH_3CO), 30.75 (C-4), 32.19 (C-2), 36.07 (C-7), 38.23 (*N*–CH_3), 58.87 (C-1), 64.92 (C-5), 66.83 (C-3), 79.06 (C-6), 120.05 (C-2′), 128.04 (C-6′ and C-8′), 129.06 (C-7′), 129.71 (C-5′ and C-9′), 134.88 (C-4′), 143.78 (C-3′), 165.21 (C-1′), 170.89 (CH_3CO). **4b**: ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.65 (1H, *d*, J = 14.8 Hz, H_{endo}-2), 1.85 (1H, d, J = 14.5 Hz, H_{endo}-4), 2.07 (3H, *s*, $COCH_3$), 2.12 (1H, *m*, H_{exo}-7), 2.21 (1H, *dt*, J = 13.3, 4.7 Hz, H_{exo}-2), 2.23 (1H, *dt*, J = 14.5, 4.6 Hz, H_{exo}-4), 2.53 (3H, *s*, *N*–CH_3), 2.64 (1H, *dd*, J = 14.0, 7.6 Hz, H_{endo}-7), 3.21 (1H, *br.s*, H-5), 3.34 (1H, *dt*, J = 7.1, 3.5 Hz, H-1), 5.17 (1H, *t*, J = 5.3 Hz, H-3), 5.57 (1H, *dd*, J = 7.5, 3.0 Hz, H-6), 6.43 (1H, *d*, J = 16.1 Hz, H-2′), 7.39 (3H, *m*, H-6′, H-7′, and H-8′), 7.59 (2H, *m*, H-5′ and H-9′), 7.72 (1H, *d*, J = 16.2 Hz, H-3′). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 21.36 (CH_3CO), 31.05 (C-4), 32.52 (C-2), 36.76 (C-7), 38.23 (*N*–CH_3), 59.03 (C-1), 64.85 (C-5), 67.03 (C-3), 79.06 (C-6), 118.35 (C-2′), 128.22 (C-5′ and C-9′), 128.90 (C-6′ and C-8′), 130.36 (C-7′), 134.34 (C-4′), 145.09 (C-3′), 165.05 (C-1′), 167.75 (CH_3CO). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m*/*z* (rel. int.): 329 [M^+] (25), 270 [M^+–H_3CCOO] (5), 243 (4), 182 [M–$C_6H_5CH{=}CHCOO]^+$ (18), 138 $[C_8H_{12}NO]^+$ (12), 131 $[C_9H_7O]^+$ (16), 122 $[C_8H_{12}N]^+$ (34), 95 (85), 94 (100), 84 (43). HREIMS *m*/*z* 329.1627 [M^+] ($C_{19}H_{23}NO_4$ requires 329.163).

3.8. Computational

The molecular dynamics simulation was performed on a SGI Octane (R10000) workstation. Bond lengths were constrained using the SHAKE algorithm (van Gunsteren & Berendsen, 1977) and a time step of 2 fs was used. The molecule was weakly coupled to a thermal bath at $T = 400$ K (Berendsen, Postma, van Gunsteren, DiNola & Haak, 1984), with a temperature relaxation time $t = 0.5$ ps. The wavefunctions for the calculation of the rotational strengths for the electronic transitions from the ground state to excited states were obtained by a CNDO/S-Cl (Downing, Del Bene & Jaffé, 1968) calculation, in which the Cl expansion takes into account the ground state and all n and π orbitals. These calculations were carried out on Linux *i*PII and *i*PIII workstations using the BDZDO/MCDSPD (Downing) program package. For a better comparison of the theoretical CD spectrum with the experimental one, a Gaussian band shape function was generated over the calculated rotational strength values. Further details will be published elsewhere.

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