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Tropane alkaloids from the leaves and stem bark of *Erythroxylon alaternifolium* and *Erythroxylon rotundifolium*

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Abstract

A novel 3α,6β,7β-triol tropane alkaloid esterified by two benzoyl residues was isolated from the leaves of the endemic cuban species, *Erythroxylon alaternifolium*. Another novel 3α,6α,7β-triol tropane alkaloid esterified by trimethoxycinnamoyl and trimethoxybenzoyl residues was isolated from the leaves and stem bark of a second endemic cuban species, *Erythroxylon rotundifolium*. Their structures were elucidated as 3α,7β-dibenzoyloxy-6β-hydroxy-tropane and 3α-(3,4,5-trimethoxy-cinnamoyloxy)-7β-(3,4,5-trimethoxybenzoyloxy)-6α-hydroxy-tropane by spectroscopic methods including 2D-NMR techniques.

Keywords: *Erythroxylon alaternifolium*; *Erythroxylon rotundifolium*; Erythroxylaceae; Leaves; Stem bark; Tropane alkaloids; 3α,7β-dibenzoyloxy-6β-hydroxy-tropane; Alaternifoline; 3α-(3,4,5-trimethoxy-cinnamoyloxy)-7β-(3,4,5-trimethoxybenzoyloxy)-6α-hydroxy-tropane; Erythrorotundine

1. Introduction

Erythroxylon alaternifolium A. Rich and *Erythroxylon rotundifolium* Lunan (Erythroxylaceae) are shrubs growing in Cuba. The family Erythroxylaceae is present in Cuban flora with 22 species and three varieties, 16 of which are endemic in Cuba (Hnoss, 1951; Carcelen Campana, 1996). Tropane alkaloids, with well-known pharmacological activities (Lounasmaa, 1988; Fodor and Dharanipragada, 1992) are known to occur in Erythroxylaceae (Lounasmaa, 1988; Fodor and Dharanipragada, 1992). No mention of local uses or reports concerning the phytochemical composition of *E. alaternifolium* and *E. rotundifolium* have been found in the literature.

In view of the biological and chemotaxonomic importance of tropane alkaloids we have undertaken a phytochemical research on plants of the genus *Erythroxylon* endemic in Cuba. As a part of survey involving the presence of tropane alkaloids in Erythroxylaceous plants endemic in Cuba, here we report on the isolation and structural determination of compound **1**, a novel 3α,6β,7β-triol tropane alkaloid esterified by benzoyl residues at position 3 and 7 from the leaves of *E. alaternifolium* and of compound **2**, a novel 3α,6α,7β-triol tropane alkaloid esterified by trimethoxycinnamoyl and trimethoxybenzoyl residues at position 3 and 7 from the leaves and stem bark of *E. rotundifolium*.

2. Results and discussion

Compound **1** was purified by column chromatography from the total alkaloid fraction obtained from

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the leaves of *E. alaternifolium*. Compound **1** was a white crystalline substance with molecular formula $C_{22}H_{23}NO_5$ assigned by mass spectrometry and DEPT ^{13}C-NMR analyses. The EI mass spectrum showed the molecular ion at *m/z* 381 and a fragmentation pattern with peaks at *m/z* 276 $[M - COC_6H_5]^+$, 260 $[M - CO_2C_6H_5]^+$, and 94 [*N*-methyl–pyridinium] consistent with an ester derivative of a 3,6,7-tropanetriol (Al-Said et al., 1986). Its IR spectrum showed absorptions due to hydroxyl group (ν_{max} at 3450 cm^{-1}), olefinic CH (ν_{max} at 2960 cm^{-1}), ester carbonyl (ν_{max} at 1740 cm^{-1}), supporting the presence of ester functions, a free hydroxyl groups and aromatic rings.

The general structure of the tropane moiety was based on the following evidence. The ^{1}H-NMR spectrum of **1** (Table 1) showed 12 proton signals: a methyl (δ 2.61) linked to nitrogen, two $-CH_2$ (δ 1.78, 2.33 and 1.71, 2.29), two -CH (δ 3.39 and 3.19) and three–CHOH groups (δ 4.83, 5.34 and 5.80) whose chemical shifts indicated a 3,6,7-tropanetriol skeleton like in teloidine and its ester derivatives (El-Imam et al., 1987; Agar and Evans, 1976). The proton spectrum also displayed signals in the aromatic zone integrating for 10 protons and ascribable to two aromatic acid residues.

The ^{13}C-NMR spectrum of compound **1** revealed the presence of a substituted 3,6,7-tropanetriol skeleton through signals ascribable to a methyl, two methylene, two methyne and three oxymethyne (δ 67.44, 75.60, 78.63) carbons; it also allowed identification of two benzoyl moieties (Table 1).

NMR studies at high field (600 MHz) with extensive use of 2D techniques led to the proposed structure. ^{1}H–^{1}H COSY spectrum indicated two isolated spin systems: a first spin system of five coupled protons was assigned with the aid of the HSQC spectrum to H-2, H-2′, H-3, H-4, H-4′; the second one to H-6, H-7 (each *d*, $J = 6.5$ Hz). Since in all natural alkaloids where there are *exo* substituents at C-6 and C-7, the remaining *endo* protons showed coupling of ca. $J = 0$ Hz with the vicinal bridgehead protons due to a dihedral angle close to 90° (bridgehead and *endo* protons) (Al-Said et al., 1986), the β-stereochemistry of the –OH groups at C-6 and C-7 was derived by the observation that the two couples of vicinal protons H-6, H-5 and H-7, H-1 do not display any coupling. The coupling constant ($J = 5.0$ Hz) measurement of H-3 signal established the α-orientation of the –OH substituent at C-3 (El-Imam et al., 1987; Agar and Evans, 1976). The absolute configuration of **1** remains undetermined. The proton and carbon signals of the oxymethyne groups at C-3 and C-7 were typically shifted downfield (δ_H 5.34 and 5.80; δ_C 67.44 and 78.63, respectively) with respect to unesterified model compounds, like teloidine (El-Imam et al., 1987; Agar and Evans, 1976) showing that the benzoyl moieties were linked to these positions at the tropane nucleus.

Table 1
^{1}H- and ^{13}C-NMR data of alaternifoline (**1**) (600 MHz)[a]

Position	δ_C	δ_H	Connectivities by HMBC
1	63.08	3.39 *br s*	C-5; C-7
2	27.58	1.78 *dd* (14.0, 1.0)	C-3; C-4
2′		2.33 *dd* (14.0, 4.0, 2.5)	C-1; C-7
3β	67.44	5.34 *t* (5.0)	C-1; C-4; C-5; 166.51
4	27.43	1.71 *dd* (14.0, 1.0)	C-2; C-3
4′		2.29 *ddd* (14.0, 4.0, 2.5)	C-5; C-6
5	66.24	3.19 *br s*	C-1; C-7
6α	75.60	4.83 *d* (6.5)	C-1; C-4; C-5; C-7
7α	78.63	5.80 *d* (6.5)	C-2; C-5; C-6; 166.70
Me	35.61	2.61 *s*	C-1; C-5
Benzoyl at C-3			
C=O	166.51		
1′	130.60		
2′ and 6′	129.31	8.11 *d* (8.0)	C-1′; 166.51
3′ and 5′	128.73	7.49 *t* (8.0)	
4′	132.49	7.57 *t* (8.0)	
Benzoyl at C-6			
C=O	167.70		
1″	130.60		
2″ and 6″	129.31	8.07 *d* (8.0)	C-1″; 167.70
3″ and 5″	128.73	7.53 *t* (8.0)	
4″	132.49	7.40 *t* (8.0)	

[a] Chemical shift values (in CD_3OD) are in ppm from TMS and *J* values in Hz are presented in parentheses. All signals were assigned by ^{1}H–^{1}H DQF-COSY, ^{1}H–^{13}C HSQC and HMBC studies.

Additional proof of the structure of compound **1** was provided by the observation of long-range ^{1}H–^{13}C chemical shift correlations in the HMBC spectrum. The positions of the benzoyl residues were confirmed by the clear long-range correlation peaks between the carbonyl carbons (δ 166.51 and 167.70) of the two benzoyl residues and H-3 and H-7 signals, respectively, of the tropane nucleus. H-3 signal also showed correlations with C-2 and C-1 and H-7 with C-6 and C-1 signals. These and other diagnostic correlations summarized in Table 1 confirmed the proposed structure. Thus, compound **1** was 3α,7β-dibenzoyloxy,6β-hydroxy-tropane and was named alaternifoline.

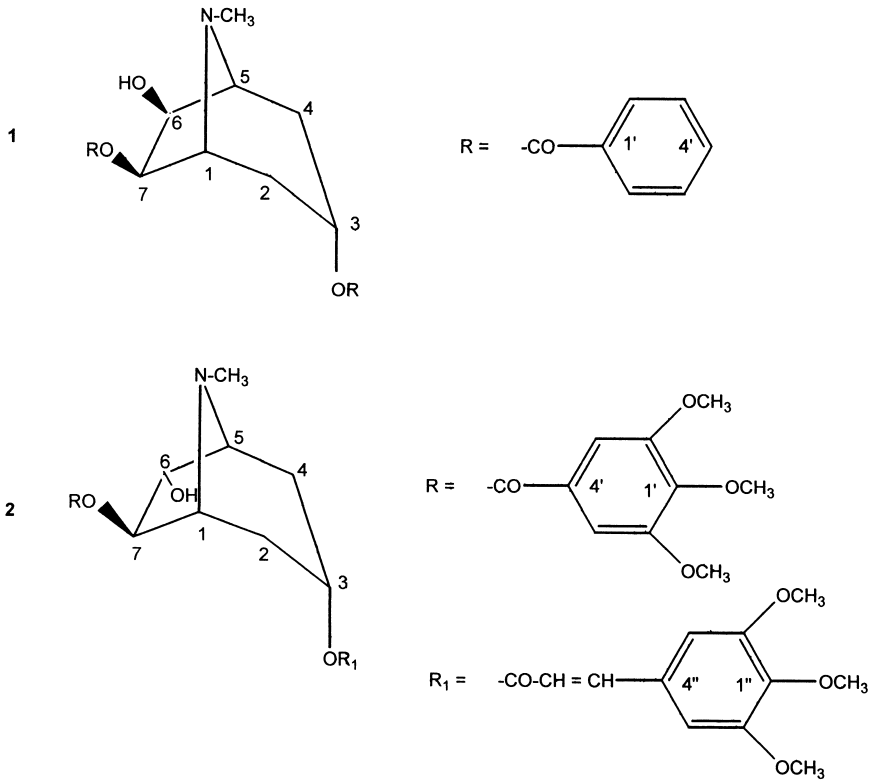
Compound **2** was purified by column chromatography from the total alkaloid fraction obtained by the leaves and stem bark of *E. rotundifolium* and crystallized as white crystals from ethanol. Mass spectrometry and ^{13}C and DEPT ^{13}C-NMR analyses gave the molecular formula $C_{30}H_{37}NO_{11}$. The EI mass spectrum showed the molecular ion at *m/z* 587, together with the well-established characteristic fragmentation pattern of a diester of 3,6,7-tropanetriol: *m/z* 392 [M — $(MeO)_3C_6H_2CO]^+$, *m/z* 333 $[(MeO)_3C_6H_2CH{=}CH\text{-}COOC_6H_{10}N]^+$ arising from a cinnamoyl substituent at C-3 of teloidine and *m/z* 94 (*N*-methyl–pyridinium) (Al-Said et al., 1986; El-Imam et al., 1987; Agar and Evans, 1976; Jenet-Siems et al., 1998; Evans and Wooley, 1978). The ions at *m/z* 221 $[(MeO)_3C_6H_2CH{=}CHCO]^+$, and 195 $[(MeO)_3C_6H_2CO]^+$ gave the two different esterifying acids as $C_{12}H_{14}O_5$ and $C_{10}H_{12}O_5$. The presence of ester functions and free hydroxyl group was supported by the IR spectrum (hydroxyl group, ν_{max} at 3450 cm^{-1}; ester carbonyls ν_{max} at 1740 and 1725 cm^{-1}).

NMR spectra gave features characteristic of a disubstituted triol tropane nucleus possessing two esterifying aromatic moieties. The identity of the acidic moities was established as 3,4,5-trimethoxybenzoyl and 3,4,5-trimethoxycinnamoyl by ^{1}H-NMR which showed singlet signal at δ 7.35 for trimethoxybenzoyl and singlet signal at 6.94 (each integrating for two protons), together with vinylic proton signals of a *trans* double bond at δ 7.67 and 6.35 ($J_{trans} = 16.0$ Hz) for trimethoxycinnamoyl and by ^{13}C-NMR data (Table 2). The ^{13}C-NMR spectrum for the tropane moiety of compound **2** revealed signals for a methyl, two methyl-

Table 2
^{1}H-and ^{13}C-NMR data of erythrorotundine (**2**) (600 MHz[a])

Position	δ_C	δ_H	Connectivities by HMBC
1	63.52	3.59 *br s*	
2	28.51	1.85 *dd* (14.0, 1.0)	
2′		2.43 *dd* (14.0, 4.0, 2.5)	
3β	66.50	5.27 *t* (5.0)	C-1; C-4; C-5; 166.1
4	28.50	1.80 *dd* (14.0, 1.0)	
4′		2.38 *ddd* (14.0, 5.0, 2.5)	
5	61.00	3.35 *m*	
6	68.13	4.86 *t* (2.5)	
7	75.60	5.85 *d* (2.5)	
Me	35.05	2.72 *s*	
Trimethoxybenzoyl at C-3			
OMe0	56.05	3.95 *s*	C-2′/C-6′
C═O	166.51		
1′	152.50		
2′ and 6′	153.00		
3′ and 5′	106.50	7.35 *d* (2.0)	166.51; C-4′
4′	125.00		
1,2,6-Trimethoxy-cinnamoyl at C-6			
OMe	56.05	3.89 *s*	C-2″/C-6″
C═O	166.10		
1″	152.50		
2″ and 6″	153.00		
3″ and 5″	106.0	6.94 *d* (2.0)	C-2″/C-6″; C-7″; C-4″
4″	130.0		
7″	146.0	7.67 *d* (16.0)	C-3″/C-5″;C-8″;C-4″;
	166.1		
8″	117.0	6.35 *d* (16.0)	C-4″; 166.1

[a] Chemical shift values (in CD_3OD) are in ppm from TMS and *J* values in Hz are presented in parentheses. All signals were assigned by ^{1}H–^{1}H DQF-COSY, ^{1}H–^{13}C HSQC and HMBC studies.



ene, two methyne and three oxymethyne (δ 66.50, 75.60, 68.13) similar to those observed in compounds **1** and other substituted 3,6,7-tropanetriol alkaloids (Al-Said et al., 1986; El-Imam et al., 1987; Ishimaru and Shimomura, 1989). The ^{1}H-NMR spectrum of **2** (Table 2) showed signals for a *N*-methyl (δ 2.72), two $-CH_2$ (δ 1.80, 2.38 and 1.85, 2.43), two –CH (δ 3.35 and 3.59) and three–CHOH (δ 4.86, 5.27 and 5.85) of a tropane nucleus closely correlated to those of alaternifoline (**1**). A combination of ^{1}H–^{1}H COSY and ^{1}H–^{13}C HSQC spectra led to identification of two sequences: H-2, H-2′, H-3, H-4, H-4′; and H-6, H-7 (H-7, δ 5.85, *d*, $J = 2.5$ Hz; H-6, δ 4.86, *t*, $J = 2.5$ Hz). The signals at δ 5.85 and 5.27, typically shifted downfield with respect to unesterified model compounds, like teloidine (Al-Said et al., 1986; El-Imam et al., 1987), indicated an esterification at C-7 (δ_C 75.60) and at C-3 (δ_C 66.50). The 3α-orientation of the esterifying group was demonstrated by the form of signal (*t*) and coupling constant ($J = 5.0$ Hz) of H-3 (δ 5.27) similar in chemical shift and multiplicity to H-3β proton in alaternifoline (**1**) and all 3α-hydroxysubstituted tropane (Al-Said et al., 1986; El-Imam et al., 1987; Agar and Evans, 1976). The occurrence of H-6 proton at δ 4.86 confirmed this substituent as the free alcohol rather than as an ester. Major differences in the spectra of **2** with respect to **1** were multiplicity and coupling constant of H-6 (*t*, $J = 2.5$ Hz) and H-7 (*d*, $J = 2.5$ Hz) signals different from H-7α and H-6α (each *d*, $J = 6.5$ Hz) of alaternifoline (**1**), and the presence of a complex multiplet centered on δ 3.35 for the bridgehead proton H-5. The small coupling constant ($J = 2.5$ Hz) detected between H-5 and H-6 protons indicated an *exo* (β) proton at C-6 in **2**; thus the –OH substituent at C-6 must be in the α-configuration. The stereochemistry at C-7 was in agreement with the observation that the two vicinal protons H-7 and H-1 do not display any coupling, due to a dihedral angle close to 90° between H-1 bridgehead and H-7 *endo* protons (Al-Said et al., 1986); thus, the –OH at C-7 must be in the β-configuration, whereas the absolute configuration of compound **2** remains undetermined like in compound **1**.

Further support to the structure of compound **2** was provided by the observation of long-range ^{1}H–^{13}C chemical shift correlations in the HMBC spectrum. The linkage of the 3,4,5-trimethoxycinnamoyl residue at C-3 was indicated by the correlation of the carbonyl signal at δ 166.10 with H-3 (δ 5.27) of the tropane nucleus, as well as with H-7″ and H-8″, H-3 also showed correlations with C-1 and C-5. The carbonyl carbon at δ 166.50 of the 3,4,5-trimethoxybenzoyl residue gave correlations with H-3′/H-5′ signals at δ 7.35. Other diagnostic correlations, summarized in

Table 2, were seen between the –OMe signals of the trimethoxybenzoyl and C-2′/C-6′ carbon signals and between the –OMe of the trimethoxycinnamoyl and C-2″/C-6″ carbon signals. Thus, compound **2**, named erythrorotundine, was 3α-(3,4,5-trimethoxy-cinnamoyloxy)-7β-(3,4,5-trimethoxybenzoyloxy)-6α-hydroxy-tropane.

The new alkaloids reported here from *E. alaternifolium* and *E. rotundifolium* can provide data for the chemotaxonomic assessment of the two species.

3. Experimental

NMR: CD_3OD, Bruker DRX-600 spectrometer; DEPT, ^1H–^1H COSY, HSQC, and HMBC experiments were performed using the UXNMR software package; chemical shifts are expressed in δ (ppm) referring to the solvent peaks: δ_H 3.34 and δ_C 49.0 for CD_3OD. Optical rotations were measured on a Perkin–Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10 cm microcell. Column and thin layer chromatographies were carried out on silica gel or active neutral aluminium oxide; spots were detected spraying with Dragendorff's reagent. Mp was determined in HMK Franz Kuesher, and was uncorrected. IR was obtained in KBr tablets and EIMS at 70 eV.

3.1. Plant material

The leaves of *E. alaternifolium* A. Rich were collected in "Loma de la Coca", Campo Florido, Havana, Cuba in December 1996; the leaves and stem bark of *E. rotundifolium* Lunan were collected at "Maysi", Guantanamo, Cuba in March 1998 and they were identified by Ing. R. Oviedo Prieto. A voucher specimen of both plants is deposited at the Herbarium of the Institute of Ecology and Systematics, HAC, CITMA, Havana.

3.2. Extraction and isolation

The dried and powdered leaves of *E. alaternifolium* (1.6 kg) were extracted with EtOH at room temperature (four times for 24 h) to give 50 g of residue. This was partitioned between 0.5 N HCl and *n*-hexane/benzene (1:1). The aqueous layer was adjusted to pH 9 with $KHCO_3$ and extracted with $CHCl_3$/EtOH (2:1). The chloroform/ethanol layer, after evaporation of the solvent to dryness, afforded the total crude alkaloid fraction (TCA 5.52 g). The TCA was subjected to CC over silica gel, eluting with $CHCl_3$ and increasing amounts of EtOH. Elution with $CHCl_3$/EtOH (98:2) gave compound **1**. This was crystallized from EtOH to give 30.7 mg of pure compound.

The dried and powdered leaves and stem bark of *E. rotundifolium* (1.9 kg) were extracted with EtOH at room temperature (four times for 24 h) to give 40 g of residue. This was partitioned between 0.5 N HCl and *n*-hexane/benzene (1:1). The aqueous layer was adjusted to pH 9 with $KHCO_3$ and extracted with $CHCl_3$/EtOH (2:1). The $CHCl_3$/EtOH layer, after evaporation of the solvent to dryness, afforded the total crude alkaloid fraction (TCA 9.12 g). The TCA was subjected to CC over active neutral aluminium oxide, eluting with *n*-hexane/$CHCl_3$, $CHCl_3$ and increasing amounts of EtOH. Elution with $CHCl_3$/EtOH (98:2) gave compound **2**. This was crystallized from EtOH to give 10.1 mg of pure compound.

Compound **1**. mp 220°C; $[\alpha]_D^{25} = +10$ (EtOH, *c* 0.1); IR ν_{max}^{KBr} (cm^{-1}) 3430 (–OH), 2960 (olefinic CH), 1740 (C═O), 1460, 1290 (C–OC), 1130 (C–OH), 790, 750, 725, 700 (aromatic, bending); EIMS (probe) 70 eV (rel. int.) *m/z* 381 $[M]^+$, 276 $[M - COC_6H_5]^+$ (0.7), 260 $[M - CO_2C_6H_5]^+$ (4), 218 $[260 - C_3H_6]^+$ (2), 172 [$C_8H_{14}NO_3$, teloidine unit] (8), 122 [$HOCOC_6H_5$] (12), 105 [$–COC_6H_5$] (40) and 94 [*N*-methyl–pyridinium] (100); for ^1H- and ^{13}C-NMR: see Table 1.

Compound **2**. Mp 176°C; $[\alpha]_D^{25} = -20.8$ (EtOH, *c* 0.1); IR ν_{max}KBr (cm^{-1}) 3450 (–OH), 2930 (olefinic CH), 1740 (C═O), 1725 (C═O), 1580, 1500, 1450 (benzene rings), 1110 (C–OC), 790, 750, 725, 700 (aromatic, bending); EIMS (probe) 70 eV (rel. int.) *m/z* 587 $[M]^+$, 392 $[M - (MeO)_3C_6H_2CO]^+$ (8), 376 $[M - (MeO)_3C_6H_2COO]^+$ (4), 333 $[(MeO)_3C_6H_2CH{=}CH\text{-}COOC_6H_{10}N]^+$ (2), 221 $[(MeO)_3C_6H_2CH{=}CHCO]^+$ (8), 212 $[(MeO)_3C_6H_2COOH]^+$ (14), 195 $[(MeO)_3C_6H_2CO]^+$ (52), 172 [$C_8H_{14}NO_3$, teloidine unit] (10), 94 [*N*-methyl–pyridinium] (100); for ^1H- and ^{13}C-NMR: see Table 2.

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