Reversal of Multidrug Resistance by Tropane Alkaloids from the Stems of *Erythroxylum rotundifolium*

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Six tropane alkaloid esters were isolated from the stems of *Erythroxylum rotundifolium*. The structures of three new tropane esters, 7*β*-hydroxy-6*β*-(3,4,5-trimethoxybenzoyloxy)-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**1**), 6*β*-benzoyloxy-3α-(*Z*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**2**), and (−)-6*β*-benzoyloxy-3α-hydroxytropane (**3**), were established by spectroscopic techniques. When alkaloids **1**−**6** were evaluated against a panel of human cancer cell lines, the new compound 6*β*-benzoyloxy-3α-(*Z*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**2**) and three known compounds, 6*β*-benzoyloxy-3α-(3,4,5-trimethoxycinnamoyloxy)tropane (**4**), 6*β*-benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane-7*β*-ol (**5**), and 7*β*-acetoxy-6*β*-benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**6**), demonstrated greatest activity with multidrug-resistant oral epidermoid carcinoma (KB-V1) cells incubated in the presence of vinblastine. Thus, tropane esters of this type can reverse the multidrug-resistance phenotype, presumably by interacting with P-glycoprotein.

The tropane alkaloids occur mainly in the plant families Convolvulaceae, Erythroxylaceae, Proteaceae, Rhizophoraceae, and Solanaceae and occasionally have been reported from plants in the Brassicaceae, Euphorbiaceae, and Olacaceae.[1] Many biological and pharmacological reports on the tropane alkaloids have appeared, particularly concerning the neurochemical and physiological effects of cocaine.[2,3] The protective property of atropine against organophosphorus compounds is also well documented.[3] In addition, the therapeutic uses of atropine sulfate as an antisialogogue adjuvant in surgery and as a gastrointestinal antispasmodic are well-known.[4] Scopolamine is a further tropane alkaloid that has been subjected to numerous pharmacological investigations, including its effects on memory and hyperactivity.[3] This alkaloid is used transdermally for the prevention of motion sickness.[4]

The cytotoxic activity against cancer cell lines of tropane alkaloids does not appear to have been investigated. In an initial study on the roots of *Erythroxylum pervillei* Baill. (Erythroxylaceae) collected in Madagascar, conducted as part of an ongoing collaborative search for novel antineoplastic agents derived from plants, seven tropane alkaloid aromatic esters were found to significantly inhibit the growth of the multidrug-resistant (MDR) KB-V1 cell line in the presence of vinblastine (VLB).[5] The isolates were much less cytotoxic for normal KB cells or KB-V1 cells treated in the absence of vinblastine.[5] The mechanism involves interaction with P-glycoprotein, thus blocking drug efflux and restoring sensitivity.[6] In the present follow-up study, the stems of the Caribbean shrub *Erythroxylum rotundifolium* Lunan (Erythroxylaceae), collected in the Dominican Republic, were investigated. In this paper, we report the cytotoxicity and in vitro multidrug-resistance properties of six tropane alkaloids (**1**−**6**) isolated from this species. Three new alkaloids, 7*β*-hydroxy-6*β*-(3,4,5-trimethoxybenzoyloxy)-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**1**), 6*β*-benzoyloxy-3α-(*Z*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**2**), and the levorotatory enantiomer (−)-6*β*-benzoyloxy-3α-hydroxytropane (**3**), were elucidated using spectroscopic methods, together with three known tropane alkaloids identified by comparison with published spectroscopic data, as 6*β*-benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**4**),[7,8] 6*β*-benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane-7*β*-ol (**5**),[7] and 7*β*-acetoxy-6*β*-benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (**6**).[7]

Alkaloid **1** was assigned the molecular formula $C_{30}H_{37}NO_{11}$ from its HREIMS (*m*/*z* 587.2361). Its IR spectrum exhibited absorption bands for hydroxyl (3500 cm^{-1}), carbonyl ester (1710 cm^{-1}), α,*β*-unsaturated carbonyl ester (1641 cm^{-1}), and aromatic (1585 cm^{-1}) functionalities. The NMR spectra revealed characteristic resonances for a tropane alkaloid skeleton trisubstituted at C-3, C-6, and C-7.[7,9] The appearance and chemical shifts for H-3 (*δ* 5.26, br t, $J = 4.5$ Hz) and C-3 (δ_C 66.9) indicated that C-3 bears an acyl moiety in an α configuration in **1**.[10] The downfield chemical shifts and multiplicity for H-6 at *δ* 5.80 (d, $J = 6.0$ Hz) and its corresponding carbon signal determined by an HMQC experiment at *δ* 79.1 confirmed the occurrence of a second acyl substituent at C-6. The resonance assigned to H-7 at *δ* 4.80 was observed as a broad triplet ($J = 6.0$ Hz), due to coupling with the hydroxyl proton (*δ* 2.51, d, $J = 6.0$ Hz), and this signal changed to a doublet ($J = 6.0$ Hz) after equilibrium with D_2O in the ^1H NMR spectrum. The appearance of the corresponding ^{13}C NMR signal at *δ* 75.4 confirmed a free alcohol unit at C-7. The coupling constant value ($J = 6.0$ Hz) was in agreement with C-6 and C-7 being *exo* substituents.[7,9] In contrast, for 3α-(3,4,5-trimethoxycinnamoyloxy)-7*β*-(3,4,5-trimethoxybenzoyloxy)-6α-hydroxytropane, an alkaloid with C-6 *endo* and C-7 *exo* substituents previously isolated from the leaves and stem bark of *E. rotundifolium* collected in Cuba, protons H-6 and

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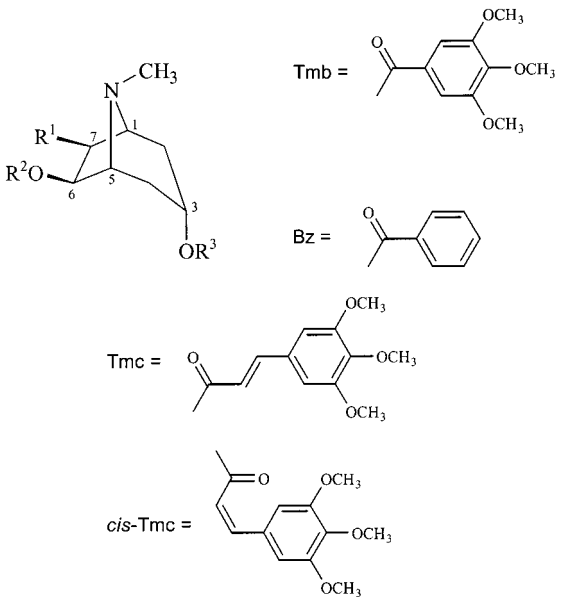
Table 1. ^{1}H NMR Spectral Data of Alkaloids **1**−**4**[a]

position	**1**	**2**	**3**	**4**
1	3.20 br s	3.36 br s	3.66 br s	3.40 br s
2ax	2.29 dd (13.5, 4.4)	2.25 m	2.46 m	2.25 m
2eq	1.69 d (13.5)	1.95 br d (15.3)	2.14 br d (14.5)	1.99 br d (15.3)
3*β*	5.26 br t (4.5)	5.11 br t (5.0)	4.18 br t (5.0)	5.22 br t (4.7)
4ax	2.34 dd (11.7, 4.2)	2.17 m	2.46 m	2.20 m
4eq	1.73 d (11.7)	1.69 br d (14.7)	1.88 br d (14.6)	1.77 br d (15.4)
5	3.43 br s	3.30 br s	3.59 br s	3.40 br s
6α	5.80 d (6.0)	5.64 dd (7.3, 2.9)	6.02 dd (7.9, 2.7)	5.90 dd (7.4, 3.0)
7α	4.80 br t (6.0)	2.51 dd (14.1, 7.3)	3.12 dd (14.0, 7.9)	2.78 dd (13.8, 7.4)
7*β*		2.26 m	2.62 m	2.30 m
NCH$_3$	2.63 s	2.56 s	2.82 s	2.61 s
OH	2.51 d (6.0)			
	Tmb	Bz	Bz	Bz
2′, 6′	7.32 s	8.00 d (7.8)	7.99 d (7.4)	8.02 d (7.9)
3′, 5′		7.43 dd (7.8, 7.8)	7.45 d (7.4, 7.4)	7.46 dd (7.9, 7.9)
4′		7.56 dd (7.8, 7.8)	7.59 d (7.4, 7.4)	7.57 dd (7.9, 7.9)
*m*OCH$_3$	3.90			
*p*OCH$_3$	3.91			
	Tmc	*cis*-Tmc		Tmc
α	6.36 d (16.0)	5.96 d (12.7)		6.36 d (16.0)
β	7.78 d (16.0)	6.88 d (12.7)		7.46 d (16.0)
2′, 6′	6.96 s	7.06 s		6.91 s
*m*OCH$_3$	3.96	3.88		3.95
*p*OCH$_3$	3.90	3.87		3.90

[a] $CDCl_3$, 300 MHz (*J* in Hz). Values are given in ppm (*δ*) using TMS as internal standard.

H-7 showed a coupling constant of 2.5 Hz.[9] The remaining ^{1}H NMR signals for the tropane nucleus in alkaloid **1** were



	R^1	R^2	R^3
1	OH	Tmb	Tmc
2	H	Bz	*cis*-Tmc
3	H	Bz	H
4	H	Bz	Tmc
5	OH	Bz	Tmc
6	OAc	Bz	Tmc

observed at *δ* 3.43 (H-5), 3.20 (H-1), 2.34/1.73 (H-4ax/eq), and 2.29/1.69 (H-2ax/eq). These signals correlated in the HMQC spectrum with the carbon signals at *δ* 62.7 (C-5), 66.1 (C-1), 27.2 (C-4), and 27.0 (C-2), respectively. The *N*-methyl substituent was observed at δ_H 2.63 and δ_C 35.2 (Tables 1 and 2).

Table 2. ^{13}C NMR Spectral Data of Alkaloids **1**−**6**[a]

position	**1**	**2**	**3**	**4**	**5**	**6**
1	66.1 d	59.9 d	61.2 d	60.0 d	66.1 d	64.7 d
2	27.0 t	33.1 t	36.1 t	34.6 t	27.1 t	30.2 t
3	66.9 d	66.8 d	62.7 d	67.0 d	66.8 d	66.5 d
4	27.2 t	34.5 t	37.3 t	33.2 t	27.3 t	30.2 t
5	62.7 d	65.9 d	68.5 d	65.7 d	62.8 d	64.7 d
6	79.1 d	80.0 d	79.7 d	80.1 d	78.6 d	77.6 d
7	75.4 d	35.7 t	36.2 t	36.5 t	75.4 d	77.5 d
NCH$_3$	35.2 q	40.2 q	40.2 q	40.1 q	35.3 q	38.0 q
	Tmb	Bz	Bz	Bz	Bz	Bz
C=O	166.4 s	166.3 s	166.9 s	166.4 s	166.5 s	165.5 s
1′	124.9 s	130.3 s	131.4 s	130.3 s	129.8 s	129.7 s
2′, 6′	107.0 d	129.4 d	130.6 d	129.5 d	129.5 d	129.4 d
3′, 5′	153.0 s	128.4 d	129.7 d	128.3 d	128.4 d	128.4 d
4′	142.5 s	132.9 d	134.5 d	133.0 d	133.1 d	133.1 d
*m*OCH$_3$	56.2 q					
*p*OCH$_3$	61.0 q					
	Tmc	*cis*-Tmc		Tmc	Tmc	Tmc
C=O	165.7 s	165.2 s		165.9 s	165.6 s	165.8 s
α	117.1 d	119.2 d		117.4 d	117.0 d	116.8 d
β	145.6 d	143.8 d		145.3 d	145.5 d	145.9 d
1′	129.8 s	130.2 s		129.8 s	129.7 s	129.7 s
2′, 6′	105.6 d	107.8 d		105.4 d	105.5 d	105.6 d
3′, 5′	153.4 s	152.7 s		153.4 s	153.3 s	153.3 s
4′	140.2 s	139.9 s		140.0 s	140.1 s	140.0 s
*m*OCH$_3$	56.2 q	56.1 q		56.2 q	56.2 q	56.1 q
*p*OCH$_3$	61.0 q	60.9 q		60.9 q	60.9 q	60.9 q
						acetate
CH_3						20.9 q
C=O						170.0 s

[a] $CDCl_3$, 75 MHz; values are given in ppm (*δ*) using TMS as internal standard. All assignments are based on 2D NMR measurements (HMBC, HMQC). Multiplicities were determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3).

The two acyl moieties of **1** were identified as 3,4,5-trimethoxybenzoyl (Tmb) and 3,4,5-trimethoxycinnamoyl (Tmc) units as follows: two singlets at *δ* 7.32 (2H) and 6.96 (2H) were attributed to the *ortho* aromatic protons of the benzoyl and cinnamoyl moieties, respectively,[9] while two olefinic protons in a *trans* arrangement (*J* = 16.0 Hz) at *δ*

7.78 (Hβ) and 6.36 (Hα) were assigned to the cinnamoyl residue, and signals for 18H (6 × $-OCH_3$, δ 3.90−3.96) confirmed the presence of three methoxyl substituents symmetrically distributed in each unit.[5,9] Further support for the structure of alkaloid **1** was the presence of fragment peaks in the EIMS at *m/z* 375 ($M^+ - TmbA$, 4%) and *m/z* 350 ($M^+ - TmcO$ 14%). The positions of attachment of the Tmc and Tmb moieties on the tropane nucleus were determined by analysis of the HMBC spectrum. The protons H-β (δ 7.78), H-α (δ 6.36), and H-3 (δ 5.26) correlated with the carbon signal at δ 165.7, placing the Tmc moiety at the C-3 position. On the other hand, the *ortho* protons in the Tmb unit (δ 7.32) and H-6 (δ 5.80) showed correlations with the other carbonyl signal at δ 166.4, confirming the position of the Tmb group at C-6. Thus, on the basis of the evidence discussed above, alkaloid **1** was characterized structurally as 7β-hydroxy-6β-(3,4,5-trimethoxybenzoyloxy)-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane.

Alkaloid **2** was isolated as a white amorphous solid, mp 33−35 °C, having a molecular formula of $C_{27}H_{31}NO_7$ as determined by HRFABMS. Its IR spectrum showed absorption bands for carbonyl ester (1717 cm^{-1}), α,β-unsaturated carbonyl ester (1648 cm^{-1}), and aromatic (1595 cm^{-1}) functionalities. The ^1H NMR spectrum of **2** revealed characteristic resonances for a tropane alkaloid skeleton disubstituted at C-3 (δ 5.11, br t, J = 5.0 Hz) and C-6 (δ 5.64, dd, J = 7.3, 2.9 Hz).[11,12] The downfield chemical shift and multiplicity of the H-3 signal indicated an acyl moiety in an α configuration at C-3, and the signal for H-6 was consistent with the presence of an *exo* acyl substituent at C-6.[5,9] Additional signals were observed for the disubstituted tropane nucleus, with H-1 and H-5 appearing as broad singlets at δ 3.36 and 3.30, respectively, and H-7α as a doublet of doublets (δ 2.51, J = 14.1, 7.3 Hz). The H-2ax, H-4ax, and H-7β resonances were observed at δ 2.17−2.26 (3H, m), with the H-2eq and H-4eq chemical shifts appearing as doublets at δ 1.95 (J = 15.3 Hz) and 1.69 (J = 14.7 Hz), respectively. The ^{13}C NMR profile of the aliphatic carbons in **2** was also consistent with a C-3 and C-6 disubstituted tropane nucleus. These signals were determined by an HMQC experiment to be δ 80.0 (C-7), 66.8 (C-3), 65.9 (C-5), 59.9 (C-1), 35.7 (C-7), 34.5 (C-4), and 33.1 (C-2), respectively. The *N*-CH_3 group signals appeared at δ_H 2.56 and δ_C 40.2 (Tables 1 and 2).

In the ^1H NMR spectrum, one of the acyl moieties of **2** was represented by a singlet resonance at δ 7.06 (2H), two olefinic protons as doublets with a coupling constant of 12.7 Hz at δ 6.88 (H-β) and 5.96 (H-α), and three methoxyl groups at δ 3.87−3.89 (9H), in accordance with this ester unit being a symmetrically trisubstituted cinnamoyl moiety with *cis* stereochemistry (*cis*-Tmc). The chemical shift and coupling constants for the proton signals in this unit were in agreement with a (*Z*)-3,4,5-trimethoxycinnamoyl group.[13] The ^{13}C NMR resonances were determined by an HMQC experiment to be δ 143.8 (C-β), 119.2 (C-α), 107.8 (2C, C-2′, 6′), 60.9 (C-*p*OCH_3), and 56.1 (2C-*m*OCH_3). An HMBC experiment showed correlations from the olefinic protons (δ 6.88 and 5.96) and H-3 (δ 5.11) to the carbonyl at δ 165.2, thereby placing the *cis*-Tmc moiety at C-3. Other HMBC correlations for H-2′, 6′ (δ 7.06) and the methoxyl groups led to the assignment of C-3′ and C-5′ at δ 152.7, C-4′ at δ 139.9, and C-1′ at δ 130.2. The second acyl unit was determined to be a benzoyl (Bz) unit according to the ^1H NMR spectral evidence of a doublet at δ 8.00 (2H, J = 7.8 Hz, H-2′, 6′), a doublet of doublets at δ 7.56 (J = 7.8, 7.8 Hz, H-4′), and a doublet of doublets at δ 7.43 (2H, J = 7.8, 7.8 Hz, H-3′, H-5′). The ^{13}C NMR signals for the benzoyl moiety were observed at δ 129.4 (2C, C-2′, C-6′), 132.9 (C-4′), and 128.4 (2C, C-3′, 5′). HMBC correlations were observed between H-2′/H-6′ (δ 8.00) and δ 130.2 (C-1′) and the carbonyl at δ 166.3. The H-6 (δ 5.64) signal showed a connectivity with the same carbonyl carbon indicating that the benzoyl group was located at C-6. Thus, the structure proposed for the new alkaloid **2** is 6β-benzoyloxy-3α-(*Z*)-(3,4,5-trimethoxycinnamoyloxy)tropane.

The NMR properties of alkaloid **2** were similar to those of **4**. Characterization of the latter compound using spectroscopic methods led to its identification as 6β-benzoyloxy-3α-(3,4,5-trimethoxycinnamoyloxy)tropane. This compound was described previously from *Erythroxylum monogynum* Roxb.[8] and *E. zambesiacum* N. Robson,[7] where the structure was proposed by MS analysis and corroborated with synthetic methods. The major ^1H NMR differences between **2** and **4** were the chemical shift and coupling constants for the vinylic protons. The Tmc unit of alkaloid **4** showed resonances for δ H-α/β 6.36/7.46 (J = 16.0 Hz) versus δ H-α/β 5.96/6.88 (J = 12.7 Hz) for the *cis*-Tmc unit in **2**. There is only one report of tropane alkaloid *cis*-cinnamoyl esters, and the authors considered that these compounds were extraction artifacts, since *cis*-cinnamic acid derivatives are not common and the photochemical *trans/cis* interconversion occurs easily.[14]

Alkaloid **3** was isolated as a white amorphous solid, with mp 155−157 °C. Its molecular formula $C_{15}H_{19}NO_3$ was determined by HREIMS. The IR spectrum showed absorption bands for hydroxyl (3400 cm^{-1}), carbonyl ester (1710 cm^{-1}), and aromatic (1585 cm^{-1}) functionalities. The ^1H NMR spectrum of **3** showed a profile corresponding to a tropane alkaloid skeleton disubstituted at C-3 (δ 4.18, br t, J = 5.0 Hz) and C-6 (δ 6.02, dd, J = 7.9, 2.7 Hz).[11,12] The downfield chemical shifts and multiplicities of the H-3 and H-6 signals indicated hydroxyl substitution in an α configuration at C-3 and an *exo* acyl substituent at C-6.[5,9] The acyl unit was determined to be benzoyl from a doublet at δ 7.99 (2H, J = 7.4 Hz, H-2′, 6′), a doublet of doublets at δ 7.59 (J = 7.4, 7.4 Hz, H-4′), and a doublet of doublets at δ 7.45 (2H, J = 7.4, 7.4 Hz, H-3′, H-5′) in the ^1H NMR spectrum (Table 1). The NMR data (Tables 1 and 2) were in agreement with the structure 6β-benzoyloxy-3α-hydroxytropane, an alkaloid previously isolated from the rootbark of *Erythroxylum zambesiacum*[7] and the leaves of *Knightia strobilina* Labill. (Proteaceae).[15] The optical rotation of compound **3** was −11°, in contrast to the dextrorotatory value reported earlier ($[\alpha]_D$ +8°).[15] Therefore, alkaloid **3** was identified as the levorotatory enantiomer, (−)-6β-benzoyloxy-3α-hydroxytropane.

The structures of alkaloids **1**−**6** are numbered clockwise starting from the bridgehead methine, and only the relative stereochemistry is shown. The *N*-methyl group is presented in an axial orientation in accord with our previous single-crystal X-ray crystallographic analysis of tropane-3α,6β,7β-triol 3-phenylacetate.[5] ^1H NMR data were measured for the first time for alkaloid **4**, with ^{13}C NMR assignments also made for alkaloids **4**−**6** (Tables 1 and 2).

Tropane alkaloids **1**−**6** were evaluated against a panel of human tumor cell lines. Compounds **2** and **4**−**6** exhibited potent activity and demonstrated selectivity for the multidrug-resistant KB-V1 cells (ED_{50}, 0.2−1.1 μg/mL) in the presence of vinblastine (1.0 μg/mL), while exhibiting 10-fold less cytotoxicity with KB-V1 cells in the absence of vinblastine and no significant activity with the parental KB cells (Table 3). These data clearly reveal a reversion of resistance to vinblastine in KB-V1 cells.

Table 3. Cytotoxicity of Alkaloids **1**−**6** against Human Cancer Cell Lines[a,b]

compd	BC1	Lu1	Col2	KB	KB-V1$^+$	KB-V1$^-$	LNCaP	ASK
1	6.1	14.6	10.4	12.6	6.6	20	15.0	*c*
2	>20	>20	NT[d]	>20	0.3	3.5	NT	NT
3	>20	>20	NT	>20	>20	>20	NT	NT
4	15.3	>20	5.1	10.3	0.2	3.6	10.0	*c*
5	>20	>20	>20	>20	0.3	2.6	6.7	*c*
6	14.6	>20	>20	14.8	1.1	13.2	14.9	*c*

[a] Results are expressed as ED_{50} values (μg/mL). [b] Key to cancer cell lines used: BC1 = human breast cancer; Lu1 = human lung cancer; Col2 = human colon cancer; KB = human epidermoid carcinoma; KB-V1$^+$ = multidrug-resistant KB assessed in the presence of vinblastine (1 μg/mL); KB-V1$^-$ = multidrug-resistant KB assessed in the absence of vinblastine; LNCaP, hormone-dependent human prostate cancer; ASK, human astrocytoma. [c] Not active when tested at a concentration of 20 μg/mL. [d] NT, not tested.

The *cis* or *trans* stereochemistry in the trimethoxycinnamoyl ester unit, as in alkaloids **2** and **4**, respectively, appears not to affect the resultant cytotoxic activity of these tropane alkaloids. The new compounds **1** and **3** showed no significant activity for the cell lines represented in the panel at the doses used.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were taken on a JASCO FT/IR-410 spectrophotometer. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker Avance DPX-300 MHz spectrometer. Spectra were run in $CDCl_3$, with tetramethylsilane (TMS) used as internal standard. DEPT, HMQC, HMBC, and ^{1}H−^{1}H COSY NMR experiments were run using the manufacturer's software. The EIMS and HREIMS data were obtained either on a Finnigan MAT-90 instrument or a VG 7070-HF instrument. All experiments were performed in the electron-impact mode (EI) at 70 eV using a direct insertion probe.

Open column chromatography was carried out over aluminum oxide (alumina, neutral, Brockman Activity I, 60−325 mesh; Fisher Chemicals, Pittsburgh, PA) or silica gel G (Merck, 70−230 mesh) using gradient mixtures of hexane−acetone−MeOH−NH_4OH or $CHCl_3$−MeOH as solvents, respectively. TLC plates (Merck silica gel 60 F_{254} glass plates, 0.25 mm layer thickness) were visualized under UV light, using Dragendorff's or platinic chloride spray reagents.

Plant Material. The stem of *Erythroxylum rotundifolium* Lunan was collected in the Province of Barahona, Dominican Republic, in December 1995, and identified by one of us (R.G.). A voucher specimen (A2298) has been deposited at the Field Museum of Natural History, Chicago, IL, under the accession number 2169773.

Extraction and Isolation. The air-dried powdered stem of *E. rotundifolium* (2.2 kg) was extracted three times with MeOH. The resultant extract was then suspended in MeOH (500 mL) and washed with hexane (3 × 350 mL). The MeOH layer was partitioned between 50% aqueous MeOH and $CHCl_3$ (3 × 500 mL). The $CHCl_3$-soluble extract (5.4 g) was fractionated over a neutral aluminum oxide (Al_2O_3) column eluted with a gradient mixture of hexane−acetone−MeOH (10:1:0.1 → 2:1:0.1) to afford 10 fractions. Fraction 5 (240 mg) was subjected to column chromatography over silica gel using a gradient mixture solvent system of $CHCl_3$−acetone−MeOH−NH_4OH (5:1:1:0.1 → 2:1:1:0.1) to yield five subfractions. Further chromatography of subfraction 2 over an Al_2O_3 column using hexane−acetone−NH_4OH (5:1:0.1) afforded **6** (8 mg). Fraction 6 (56 mg) was chromatographed over Si gel eluting with $CHCl_3$−MeOH to yield eight subfractions. Preparative TLC on silica gel [$CHCl_3$−MeOH, (95:5)] of subfraction 4 afforded **2** (R_f 0.7, 4.0 mg) and **4** (R_f 0.55, 6.0 mg). Repeated crystallization from MeOH on fraction 7 (126 mg) yielded **5** (5 mg). From fraction 8 (210 mg) a precipitate was obtained, which was purified by crystallization from MeOH to afford **1** (42 mg). Finally, preparative TLC on silica gel ($CHCl_3$−MeOH, 85:15) of fraction 9 yielded **3** (R_f 0.3, 5.0 mg).

7*β*-Hydroxy-6*β*-(3,4,5-trimethoxybenzoyloxy)-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (1): white amorphous solid, mp 179−180 °C; $[\alpha]_D$ +9.0° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 214 (4.65), 233 sh (4.30), 272 (4.18), 306 (4.30) nm; IR (NaCl) ν_{max} 3500, 2944, 1710, 1641, 1585, 1504, 915, 745 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 587 (3) [M$^+$], 375 (4) [M$^+$ − TmbA], 350 (14) [M$^+$ − TmcO], 238 (14) [TmcA$^+$], 221 (15) [TmcO$^+$], 212 (6) [TmbA$^+$], 137 (100) [M$^+$ − TmcA − TmbA]; HREIMS *m*/*z* found 587.2361, calcd for $C_{30}H_{37}NO_{11}$, 587.2367.

6*β*-Benzoyloxy-3α-(*Z*)-(3,4,5-trimethoxycinnamoyloxy)-tropane (2): white amorphous solid; mp 33−35 °C; $[\alpha]_D$ +4.0° (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (4.49), 228 (4.54), 304 (3.85) nm; IR (NaCl) ν_{max} 2944, 1717, 1648, 1595, 1500, 914, and 745 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 481 (15) [M$^+$], 359 (6) [M$^+$ − BzA], 244 (39) [M$^+$ − TmcO], 238 (8) [TmcA$^+$], 122 (29) [BzA$^+$], 94 (100); HRFABMS (NBA) *m*/*z* found 482.2217, calcd for $C_{27}H_{31}NO_7$ + H, 482.2178.

6*β*-Benzoyloxy-3α-hydroxytropane (3): white amorphous solid; mp 155−157 °C; $[\alpha]_D$ −11° (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (3.89), 272 (3.41), 294 3.35) nm; IR (NaCl) ν_{max} 3400, 2944, 1710, 1585, 1504, 915, 745 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 261 (10) [M$^+$], 139 (3) [M$^+$ − BzA], 122 (12) [BzA$^+$], 113 (100) [M$^+$ − C(6)HBz − C(7)H$_2$]; HREIMS *m*/*z* found 261.1365, calcd for $C_{15}H_{19}NO_3$, 261.1365.

6*β*-Benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)-tropane (4): white amorphous solid; mp 54−55 °C; $[\alpha]_D$ +39° (*c* 1.0, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS data, consistent with literature values.[7,8]

6*β*-Benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)-tropane-7*β*-ol (5): white needle crystals; mp 184−187 °C; $[\alpha]_D$ +16° (*c* 1.0, $CHCl_3$); ^{1}H NMR and EIMS data, consistent with literature values;[7] ^{13}C NMR data, see Table 2.

7*β*-Acetoxy-6*β*-benzoyloxy-3α-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (6): white amorphous solid; mp 56−58 °C; $[\alpha]_D$ +2.1° (*c* 0.9, $CHCl_3$); ^{1}H NMR and EIMS data, consistent with literature values;[7] ^{13}C NMR data, see Table 2.

Bioassay Evaluation Procedures. Alkaloids **1**−**6** were evaluated for cytotoxic activity against a panel of human cancer cell lines according to established protocols.[16]

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