Tropane Alkaloids from the Bark of *Erythroxylum vacciniifolium*

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Eight new tropane alkaloids (**1**−**8**) were isolated from the bark of "catuaba", a Brazilian endemic plant *Erythroxylum vacciniifolium* Martius. Their structures were determined by high-resolution mass spectrometry and multidimensional NMR spectroscopy.

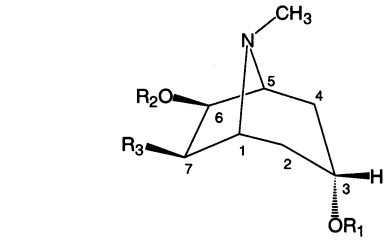
"Catuaba" is the name given to a popular herbal medicine from Brazil. Plants of different genera, from the Erythroxylaceae, Bignoniaceae, Sapotaceae, Euphorbiaceae, Myrtaceae, Meliaceae, Apocynaceae, or Burseraceae, have been referred to as "catuaba", and these have been attributed with aphrodisiac and tonic properties.[1] "Catuaba" from the northeast of Brazil has been associated with the genus *Erythroxylum*.[2,3] This genus, the largest of the four Erythroxylaceae genera, has some 250 species, which are widely distributed in tropical regions of South America, Africa, and the island of Madagascar. Although it is a rich source of tropane alkaloids and the different plants are widely used in native medicine, the genus *Erythroxylum*, apart from the cocaine-producing species, has not been examined systematically by modern analytical methods.[4] In this genus, three species, *E. vacciniifolium*, *E. subracemosum*, and *E. catuaba*, are traditionally described as "catuaba", which generates some confusion.[1]

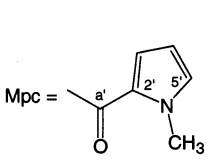
A sample of "catuaba", assigned the name *Erythroxylum vacciniifolium*, from Paraíba, has been investigated in our ongoing search for new bioactive compounds from higher plants. *E. vacciniifolium* and *E. catuaba*, long valued by local populations as aphrodisiacs and central nervous system stimulants,[5,6] have recently been the focus of great public interest because of use of the bark as a remedy for erectile dysfunction. Despite widespread commercialization of "catuaba", only a few pharmacological and phytochemical studies have been reported on *E. vacciniifolium* and *E. catuaba*. One describes the isolation and structure elucidation of three tropane alkaloids from *E. vacciniifolium* (catuabines A, B, and C).[6] Another shows hot water and alkaline extracts to have an effect on human immunodeficiency virus (HIV) and suggests a medicinal potential of *E. catuaba* against opportunistic infections in HIV patients.[7]

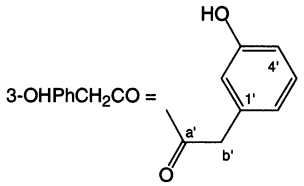
To evaluate its activities and because of the chemotaxonomic importance of tropane alkaloids, a phytochemical investigation of the alkaloid extract of the bark of *E. vacciniifolium* was undertaken. In this paper, we report the isolation, identification, and structure elucidation of eight new tropane alkaloid aromatic esters (**1**−**8**).

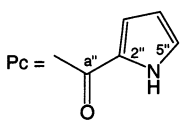
Results and Discussion

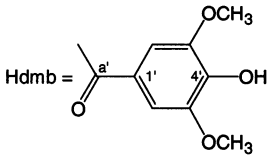
With the aim of obtaining an enriched alkaloid extract, the powdered stem bark of *E. vacciniifolium* was moistened with concentrated NH_4OH and extracted with $CHCl_3$. Analysis of this extract by LC-hyphenated techniques showed the presence of novel tropane alkaloids. Compounds **1**−**8** were purified by medium-pressure liquid chromatography (MPLC).











	R_1	R_2	R_3
1	Mpc	Pc	H
2	Mpc	Pc	OH
3	Mpc	Mpc	H
4	Mpc	Mpc	OH
5	Mpc	Mpc	CH_3COO
6	Hdmb	Mpc	H
7	Hdmb	Mpc	OH
8	3-$OHPhCH_2CO$	H	OH

Catuabine D (**1**) was isolated as a white amorphous powder. High-resolution electrospray ion cyclotron resonance mass spectroscopic analysis (HRESMS) of this compound suggested a molecular formula of $C_{19}H_{23}N_3O_4$, implicating 10 centers of unsaturation and/or ring structures. The ^{13}C NMR spectrum recorded in chloroform-*d* indicated 10 sp^2-hybridized carbon atoms (Table 1), of which six had protons attached and four were nonprotonated. The IR spectrum of **1** exhibited a large absorption band at 1700 cm^{-1}, indicating the presence of ester group-

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Table 1. ^{13}C NMR Data of Alkaloids **1**−**8**[a]

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	65.5	64.6	60.5	63.7	65.4	60.3	68.0	65.5
2	33.7	29.3	35.0	28.1	31.4	34.8	30.0	27.2
3	65.9	65.5	65.5	65.9	65.6	67.0	65.8	66.9
4	32.2	29.3	33.4	28.2	31.2	33.5	30.1	27.2
5	59.6	67.4	66.4	66.5	64.8	65.8	65.0	65.5
6	79.1	76.5	78.2	77.6	76.5	78.0	75.9	73.3
7	36.4	74.6	35.6	75.9	77.7	36.2	74.3	73.3
N-CH_3	39.2	37.2	40.4	36.1	38.9	40.3	37.9	35.1
	Mpc	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Hdmb**	**Hdmb**	**3-OHPhCH$_2$CO**
1′						120.7	119.5	134.5
2′	122.5	121.8	122.4	122.3	122.2	106.6	106.5	115.6
3′	117.8	118.5	117.8	118.2	118.5	147.0	147.2	156.6
4′	108.2	110.1	108.2	108.3	108.5	139.7	140.3	113.8
5′	129.7	124.0	129.8	129.9	129.9	147.0	147.2	129.2
6′						106.6	106.5	119.9
N-CH_3	36.8	36.3	36.7	36.7	36.7			
a′	160.3	160.2	160.2	160.2	160.1	165.6	165.4	170.4
b′								41.4
O-CH_3						56.3	55.9	
	Pc	**Pc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	
2″	123.0	121.4	122.4	122.0	122.1	122.2	121.2	
3″	115.3	116.0	117.9	118.3	117.9	117.8	118.1	
4″	110.3	107.9	107.9	107.9	107.8	107.8	107.7	
5″	122.9	130.0	129.7	129.8	129.6	129.7	129.9	
N-CH_3			36.7	36.7	36.7	36.5	36.0	
a″	160.9	160.7	160.8	160.8	160.3	160.6	160.0	
					CH_3COOH			
a‴					170.2			
b‴					20.8			

[a] Spectra recorded in $CDCl_3$ at 125.70 MHz using $CHCl_3$ as internal standard, δ values given in ppm. The ^{13}C assignments were ascertained by DEPT, HSQC, and HMBC experiments.

(s). According to the number of sp^2-hybridized carbons, two ester functions associated with chemical shifts at δ_C 160.9 and 160.3 and four ethylenic groups were required. These six centers of unsaturation suggested consequently the presence of four rings in the molecule. A gDQF-COSY NMR experiment on **1** indicated four isolated spin systems corresponding to the four different rings. The ^1H NMR spectrum of one of these rings exhibited typical resonances for a methylpyrrole substructure with signals at δ_H 3.94 (N-CH_3), 6.15 (H-4′), 6.80 (H-5′), and 7.08 (H-3′), respectively. The gHMBC spectrum showed characteristic long-range $^1H-^{13}C$ correlations between the methyl group at δ_H 3.94 (N-CH_3) and two carbons of the pyrrole ring at δ_C 122.5 (C-2′) and 129.7 (C-5′). In addition, the gHMBC spectrum exhibited a correlation between the pyrrole proton at δ_H 7.08 (H-3′) and the carbonyl carbon at δ_C 160.3 (C-a′), indicating then the substitution of the methylpyrrole moiety at the 2′-position by an ester group. Another spin system deduced from the gDQF-COSY experiment was associated with a second pyrrole substructure characterized by resonance signals at δ_H 6.25 (H-4″), 6.94 (H-5″), 6.91 (H-3″), and 9.58 (H-N), respectively. Deduced from the observation of a long-range $^1H-^{13}C$ correlation between H-3″ and the carbonyl carbon at δ_C 160.9 (C-a″) by a gHMBC experiment on **1**, the 2-position of this second pyrrole substructure was also substituted by an ester moiety. The two other remaining spin systems belonged to the tropane alkaloid skeleton. The ^1H NMR spectrum of **1** exhibited a singlet signal at δ_H 2.58 attributed to the protons of a methyl group bonded to the nitrogen. The gHMBC experiment showed $^1H-^{13}C$ correlations between these methyl protons and two methine moieties at δ_C 65.5 and 59.6 corresponding to C-1 and C-5, respectively. According to the gHSQC spectrum, these two methine carbons were linked to protons with resonances at δ_H 3.40 (H-1) and 3.34 (H-5). The gDQF-COSY experiment showed that the two methine protons belonged to a first spin system of five protons arranged in a 2-oxypropyl substructure with signals at δ_H 1.74 (H_{endo}-2), 1.96 (H_{endo}-4), 2.22 (H_{exo}-2), 2.25 (H_{exo}-4), and 5.23 (H-3β). In addition, the methine proton H-1 was linked to a second spin system of three coupled protons characterized by chemical shifts at δ_H 5.81 (H-6α), 2.28 (H-7β), and 2.76 (H-7α). The exact substitution of the tropane alkaloid skeleton was then confirmed by examination of gHMBC spectra: the resonance at δ_H 5.81 (H-6α) showed a long-range $^1H-^{13}C$ correlation with a methylene group at δ_C 32.2 (C-4), and the signals at δ_H 2.28 (H-7β) and 2.76 (H-7α) correlated with the carbon at δ_C 33.7 (C-2). To conclude, the long-range $^1H-^{13}C$ correlations between the H-3 and H-6 protons and the carbonyl carbons C-a′ and C-a″ of the two pyrrole substructures, respectively, led to the general structure 3-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-6-(1*H*-pyrrol-2-ylcarbonyloxy)tropane.

The relative configuration of **1** was established in order to give the stereochemical orientation of the two substituents relative to the nitrogen-containing bridge. The multiplicity (triplet) of the H-3 signal with the coupling constant ($J = 4.9$ Hz) indicated the α-orientation (i.e., *endo*) of the substituent at C-3.[8−10] The arrangement of the substituent at C-6 was established by the analysis of the coupling constants of the H-6, H-7, and H-5 protons. The H-6 proton showed two couplings (7.3, 2.9 Hz) with the two H-7 protons, and it did not present any coupling with the vicinal H-5 proton. This observation implied a β-orientation of the substituent and a dihedral angle close to 90° between H-5 and H-6α.[11,12] Thus, catuabine D (**1**) is 3α-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-6β-(1*H*-pyrrol-2-ylcarbonyloxy)-tropane.

Compound **2** was purified as a white amorphous powder and was assigned a molecular formula of $C_{19}H_{23}N_3O_5$, as determined by HRESMS. The EI mass spectrum showed a molecular ion at *m*/*z* 373, 16 amu higher than that of **1**, suggesting the occurrence of an additional oxygen atom in the structure of alkaloid **2**. The NMR data of those two compounds were also closely related, indicating the pres-

Table 2. ^{1}H NMR Data of Alkaloids **1**−**8**[a]

proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	3.40 br s	3.56 br s	3.50 br s	3.33 br s	3.35 br s	3.45 br s	3.50 br s	3.02 br s
2_{exo}	2.22 br dd (15.1, 4.9)	2.43 m	2.34 m	2.27 br dd (17.1, 4.9)	2.28 br dd (15.1, 5.1)	2.29 br dd (15.1, 4.9)	2.55 m	2.17 dd (15.6, 4.9)
2_{endo}	1.74 d (15.1)	1.93 d (16.1)	1.82 d (15.1)	1.72 d (17.1)	1.87 d (15.1)	1.86 d (15.1)	2.04 d (13.7)	1.52 d (15.6)
3β	5.23 t (4.9)	5.27 t (4.9)	5.23 t (4.9)	5.24 t (4.9)	5.28 t (5.1)	5.31 t (4.9)	5.36 t (4.9)	4.97 t (4.9)
4_{exo}	2.25 br dd (15.1, 4.9)	2.47 m	2.34 m	2.29 br dd (18.1, 4.9)	2.25 br dd (14.6, 5.1)	2.32 br dd (15.6, 4.9)	2.58 m	2.17 dd (15.6, 4.9)
4_{endo}	1.96 d (15.1)	1.96 d (15.6)	2.06 d (15.6)	1.76 d (18.1)	1.92 d (14.6)	2.07 d (15.6)	2.07 d (15.1)	1.52 d (15.6)
5	3.34 br s	3.38 br s	3.44 br s	3.20 br s	3.35 br s	3.40 br s	3.72 br s	3.02 br s
6α	5.81 dd (7.3, 2.9)	5.80 d (6.4)	5.77 dd (7.6, 3.2)	5.71 d (5.9)	5.88 d (6.3)	5.86 dd (7.3, 3.2)	5.91 d (6.4)	4.18 s
7α	2.76 dd (13.9, 7.3)	4.88 d (6.4)	2.77 dd (14.4, 7.6)	4.79 d (5.9)	5.81 d (6.3)	2.77 dd (14.2, 7.3)	4.90 d (6.4)	4.18 s
7β	2.28 m		2.34 m			2.30 m		
N-CH_3	2.58 s	2.78 s	2.63 s	2.61 s	2.65 s	2.62 s	2.90 s	2.51 s
	Mpc	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Hdmb**	**Hdmb**	**3-OHPhCH$_2$CO**
2′						7.39 s	7.39 s	6.74 m
3′	7.08 dd (3.9, 2.0)	7.07 dd (3.9, 1.5)	7.05 dd (3.9, 2.0)	7.13 dd (3.9, 2.0)	7.22 dd (3.9, 2.0)			
4′	6.15 dd (3.9, 2.4)	6.30 dd (3.9, 2.4)	6.16 dd (3.9, 2.4)	6.18 dd (3.9, 2.0)	6.18 dd (3.9, 2.0)			6.76 m
5′	6.80 t (2.0)	7.06 dd (2.4, 1.5)	6.80 t (2.4, 2.0)	6.81 t (2.0)	6.80 t (2.0)			7.18 dd (8.8, 7.3)
6′						7.39 s	7.39 s	6.76 m
N-CH_3 b′	3.94 s	3.95 s	3.93 s	3.93 s	3.92 s			3.55 s
OCH_3						3.99 s	4.01 s	
	Pc	**Pc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	
3″	6.91 dd (3.9, 2.0)	7.10 dd (3.9, 2.0)	6.91 dd (3.9, 1.5)	7.03 dd (3.9, 2.0)	6.93 dd (3.9, 2.0)	6.91 dd (3.9, 2.0)	7.04 dd (3.9, 2.0)	
4″	6.25 dd (3.9, 2.0)	6.13 dd (3.9, 2.4)	6.10 dd (3.9, 2.4)	6.11 dd (3.9, 2.0)	6.09 dd (3.9, 2.0)	6.11 dd (3.9, 2.0)	6.13 dd (3.9, 2.0)	
5″	6.94 dd (3.9, 2.0)	6.86 t (2.0)	6.79 t (2.4, 2.0)	6.79 t (2.0)	6.78 t (2.0)	6.81 t (2.0)	6.87 t (2.0)	
N-H	9.58 br s							
N-CH_3			3.94 s	3.94 s	3.93 s	3.90 s	3.92 s	
					CH_3COO			
b‴					2.04 s			

[a] Spectra recorded in $CDCl_3$ at 499.87 MHz using TMS as internal standard, δ values given in ppm, J values in parentheses given in Hz.

ence in **2** of a central tropane moiety esterified by two methylpyrrole (Mpc) or pyrrole (Pc) acids. By the analysis of the gHMBC spectrum, the methylpyrrole and pyrrole esters were located, as for catuabine D, at positions C-3 and C-6, respectively. Except for proton H-7, the ^{1}H NMR spectrum of the tropane nucleus of **2** exhibited chemical shifts similar to **1** (Table 2). Actually, this spectrum showed typical resonances for a tropane alkaloid skeleton trisubstituted at the C-3, C-6, and C-7 positions with signals at δ_H 5.27 (H-3β), 5.80 (H-6α), and 4.88 (H-7α), respectively. According to the molecular formula and the values of the chemical shifts associated with the 7-position (δ_H 4.88 and δ_C 74.6), a hydroxyl moiety was required at this location. The α-orientation of the esterifying group in C-3 was resolved from the multiplicity (triplet) and the coupling constant (J = 4.9 Hz) of the H-3 proton signal.[8,10] The α-stereochemistry of protons at C-6 and C-7 was deduced by the lack of any coupling constant with vicinal protons H-5 and H-1, respectively. Hence, **2** (7β-hydroxycatuabine D) was deduced as 3α-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-6β-(1*H*-pyrrol-2-ylcarbonyloxy)-7β-hydroxytropane.

Alkaloid **3** was purified as a white amorphous powder and also exhibited spectroscopic data similar to those of **1**. The molecular formula was obtained as $C_{20}H_{25}N_3O_4$. The EI mass spectrum showed a molecular ion 14 amu higher than that of **1** and a similar fragmentation pattern, suggesting the presence of an additional methyl group in compound **3**. The NMR data strongly resembled those of **1**, consistent with a general structure containing a central tropane moiety dioxygenated at C-3 and C-6 and esterified by two methylpyrrole acids. The signal integrations of the ^{1}H NMR spectrum confirmed the presence of two methyl groups, one at δ_H 3.93 (3H, s, N-CH_3) and the other at δ_H 3.94 (3H, s, N-CH_3), linked to the nitrogen atoms of the two pyrrole rings. Analysis of the gDQF-COSY spectrum showed two distinct spin systems for the substituents, allowing assignment of each proton to the corresponding methylpyrrole ester and positioning of the two Mpc at positions C-3 and C-6, according to the gHMBC experiment. The relative configuration of **3** was identical to that of **1**. On the basis of the above evidence, the structure of **3** (catuabine E) was elucidated as 3α,6β-di(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)tropane.

Compound **4** was isolated as a white amorphous powder and gave a molecular formula of $C_{20}H_{25}N_3O_5$, according to a HRESMS experiment. Analysis of the NMR data of **4** indicated strong similarity to **2** and **3**. The ^{1}H NMR spectrum of the tropane moiety exhibited the same pattern of substitution as that of **2**, with signals at δ_H 5.24 (H-3β), 5.71 (H-6α), and 4.79 (H-7α). The other part of the ^{1}H NMR spectrum describing the acyl moieties was closely comparable to equivalent data of alkaloid **3** and indicated the presence of two Mpc groups as substituents. The positions of their attachment were determined by analyzing gDQF-

COSY and gHMBC spectra as for compound **3**. The relative configuration of **4** was the same as that for **2**. The triplet for the proton at C-3 ($J = 4.9$ Hz) and the doublets for the protons at C-6 and C-7 (each $J = 5.9$ Hz) indicated α-, *β*-, and *β*-orientations of the substituents, respectively. Thus, alkaloid **4** was 3α,6*β*-di(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-7*β*-hydroxytropane, named 7*β*-hydroxycatuabine E.

Alkaloid **5** was isolated as a white amorphous powder. The HRESMS analysis implied a molecular formula of $C_{22}H_{27}N_3O_6$, and the EIMS experiment showed a $[M]^+$ molecular ion at *m/z* 429. Similarity of fragmentation pattern and a molecular mass of 42 amu higher than that of **4** suggested a core structure similar to 7*β*-hydroxycatuabine E with an additional acetyl group. Analyses of the NMR data indicated a close resemblance of the structure of **5** to that of compound **4**. The presence of two Mpc moieties was established after analysis of the ^{1}H NMR spectrum, which was superimposable with **4** between δ_H 6.0 and 7.3. The assignment of each proton to the respective spin system was achieved by a gDQF-COSY experiment, and the linkage of the esters to the tropane moiety was established by analyzing the gHMBC spectrum, as for the other compounds. The remainder of the ^{1}H NMR spectrum showed typical chemical shifts for a trisubstituted tropane skeleton with resonances at δ_H 5.28 (H-3*β*), 5.88 (H-6α), and 5.81 (H-7α). The signal at δ_H 5.81, typically shifted downfield with respect to nonesterified compounds, like **2** or **4**, indicated esterification at C-7 (δ_C 77.7) rather than a free alcohol.[9,11,13] The substituent at C-7 was shown to be an *O*-acetyl group by the signals at δ_H 2.04 (3H, s, H-b‴), δ_C 170.2 (C-a‴), and δ_C 20.8 (C-b‴). Its position was confirmed by a gHMBC correlation between H-7 and C-a‴. The relative configuration of **5** was the same as for the other two trioxygenated tropane alkaloids **2** and **4**. Thus, compound **5** was elucidated as 7*β*-acetoxy-3α,6*β*-di(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)tropane and subsequently named 7*β*-acetylcatuabine E.

Compound **6** was obtained as an amorphous solid. Its molecular formula $C_{23}H_{28}N_2O_7$ was determined by HRESMS. The EIMS gave a molecular ion at *m/z* 444 and a fragmentation pattern partially similar to that of the other isolated compounds. The appearance of a new fragment ion (*m/z* 181) suggested the presence of a novel substituent linked to the tropane nucleus. An extensive NMR analysis indicated that compound **6** was closely related to **3** since the NMR data associated with the tropane moiety and the methylpyrrole ester observed for the two compounds were superimposable. The other elements of their NMR data exhibited differences only in the nature of their substituents at the 3-position of the tropane nucleus. gHSQC and gHMBC experiments gave evidence for a trioxygenated benzoyl moiety in alkaloid **6**. Indeed, the remaining aromatic signal at δ_H 7.39 (2H, *s*, H-2′ and H-6′), which integrated for two protons, presented long-range ^{1}H−^{13}C couplings with only three aromatic carbons at δ_C 120.7, 139.7, and 147.0. These elements, corroborated by the molecular formula, suggested symmetry in this aromatic ring. The exact positions of the two methoxyl groups at δ_H 3.99 (6H, s, 3′-OMe and 5′-OMe) and the undetectable hydroxyl phenolic group were deduced by observation of a long-range ^{1}H−^{13}C coupling between the signal at δ_H 7.39 and an ester carbonyl at δ_C 165.6 (C-a′), showing the protons to be at the 2′- and 6′-positions of the aromatic ring. Thus, this substructure was a 4-hydroxy-3,5-dimethoxybenzoyloxy moiety, and its substitution at the 3-position of the tropane nucleus was confirmed by the long-range ^{1}H−^{13}C coupling between H-3 and C-a′. The relative configuration of compound **6** was the same as the other isolated alkaloids. Actually, the coupling constants for H-3 (t, $J = 4.9$ Hz) and the absence of coupling between H-6 and H-5 designated *β*- and α-orientations, respectively, for these protons. On the basis of the above evidence, compound **6**, or catuabine F, was identified as 3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)-6*β*-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)tropane.

Compound **7** was purified as an amorphous solid. HRESMS analysis of its pseudomolecular ion $[M + H]^+$ indicated the molecular formula as $C_{23}H_{29}N_2O_8$. The EIMS exhibited a molecular ion at *m/z* 460, 16 amu higher than that of **6**. The fragmentation pattern was akin to that of **6**, suggesting a similar core structure with the addition of a hydroxyl group. The analysis of NMR data demonstrated that compounds **7** and **6** were two alkaloids with the same substituents but with a different tropane core skeleton. The ^{1}H and ^{13}C NMR spectra of the ester substructures of these two alkaloids were superimposable, while the spectral region of their tropane centers showed diverse substitution patterns. The ^{1}H NMR spectrum of **7** showed typical resonances for a trioxygenated tropane with signals at δ_H 5.36 (H-3*β*), 5.91 (H-6α), and 4.90 (H-7α). The position of the substituents was determined by analysis of the long-range ^{1}H−^{13}C correlations as for compound **6**. The relative configuration of **7** was established by the multiplicity and the couplings of the protons at C-3, C-6, and C-7, giving results identical to that of the other compounds isolated. Thus, compound **7** (7*β*-hydroxycatuabine F) was identified as 3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)-6*β*-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-7*β*-hydroxytropane.

The molecular formula of **8** was established as $C_{16}H_{22}NO_5$ by HRESMS. The NMR data of **8** differed from those of the other tropanes. In fact, only 13 signals were observed in its ^{13}C NMR spectrum, indicating a certain symmetry in the molecule. In addition, NMR data of **8** exhibited typical resonances for a 3-hydroxyphenylacetoxy substructure.[11,14] Long-range ^{1}H−^{13}C couplings deduced from the HMBC spectrum of **8** revealed links between the phenol moiety, the methylene group, and the carbonyl of an ester function (δ_C 170.4). The presence of a 3-hydroxyphenylacetoxy substructure was further inferred from the ions observed in the EIMS at *m/z* 156 $[M - 3\text{-OHPhCH}_2\text{CO}]^+$ and 107 $[3\text{-OHPhCH}_2]^+$. Then, deduced from the reduced number of the remaining signals observed in the ^{1}H and ^{13}C NMR spectra of **8**, the tropane substructure was characterized by the presence of a plane of symmetry. This fact was also corroborated by the integration values in the ^{1}H spectrum calculated for the tropanic signals at δ_H 1.52 (H_{endo}-2 and H_{endo}-4), 2.17 (H_{exo}-2 and H_{exo}-4), 2.51 (N-CH_3), 3.02 (H-1 and H-5), 4.18 (H-6α and H-7α), and 4.97 (H-3*β*). Of note was the equivalence of positions C-6 and C-7, both substituted by a hydroxyl function. No coupling constant was observed between H-6 (or H-7) and the vicinal proton H-5 (or H-1), implying an α-orientation of the two hydroxyl moieties. The long-range ^{1}H−^{13}C coupling between H-3 and the ester carbonyl at δ_C 170.4 (C-a′) showed attachment of the 3-hydroxyphenylacetoxy moiety to the 3-position of the tropane nucleus. Thus, the structure of alkaloid **8** was elucidated as 3α-(3-hydroxyphenylacetoxy)-6*β*,7*β*-dihydroxytropane, a new compound (catuabine G).

The tropane N-CH_3 group stereochemistry was established by NOESY NMR experiments. The clear NOESY correlations between the N-CH_3 group and the H_{exo}-2 and H_{exo}-4 in each case indicated an axial orientation of the N-CH_3 group in all isolated compounds. In some cases, the

NOESY correlations between the N-CH_3 group and the H-3 confirmed the α-orientation of the substituent at C-3.

The tropane alkaloids isolated (**1**−**8**) are interesting for their ester moieties, which are unique to the species of the studied genus, *E. vacciniifolium*. The tropane moieties of alkaloids do not show intrageneric chemotaxonomic characteristics, but esterifying acids are often distinguishing features of certain species.[9,15,16] Pyrrole-2-carboxylic and 1-methyl-pyrrole-2-carboxylic acid have been found only in this species (section Archerythroxylum),[6,17] while the 3-hydroxyphenylacetyl moiety found in alkaloid **8** has been reported in two different species, *E. hypericifolium* and *E. pervillei*, both belonging to the section Venelia.[11,14] Although the 3,4,5-trimethoxybenzoic acid substituent is very common in the *Erythroxylum* genus, the 4-hydroxy-3,5-dimethoxybenzoyl unit found in compounds **6** and **7** has to be considered unique for the time being. In conclusion, the alkaloids reported here from *E. vacciniifolium* represent a useful contribution to the chemotaxonomy of the genus.

Experimental Section

General Experimental Procedures. Optical rotations were determined using a Perkin-Elmer 241 polarimeter (EtOH, *c* in g/100 mL, 25 °C, 10 cm cell). UV spectra were measured on a Perkin-Elmer Lambda 20 spectrophotometer, and IR spectra were obtained on a Perkin-Elmer 1600 FTIR instrument. ^{1}H and ^{13}C NMR were recorded on a Varian Inova 500 spectrometer (499.87 and 125.70 MHz, respectively) in $CDCl_3$ with Me_4Si as an internal standard. Complete assignment was performed on the basis of 2D experiments (DEPT, gradient COSY, gradient HSQC, gradient HMBC, NOESY). Mass spectra were obtained on a Finnigan-MAT/TSQ-700 triple stage quadrupole instrument; EIMS: 70 eV; D/CI-MS: NH_3, positive ion mode. HRESMS data were obtained on a Bruker FTMS 4.7T BioAPEX II. Analytical HPLC was carried out on a HP 1100 system equipped with a photodiode array detector (Agilent Technologies). Extracts and fractions were analyzed on a Nucleosil 100-5 C_{18} AB column (125 × 4.6 mm i.d., 5 μm; Macherey-Nagel). MPLC separation was done using a Büchi 681 pump equipped with a Knauer UV detector and Lichroprep C_{18} as stationary phase (460 × 70 mm or 460 × 36 mm, 15−25 μm, Merck), with a gradient mixture of acetonitrile−water−triethylamine (2 mM) as mobile phase. TLC: silica gel 60 F_{254} Al sheets (Merck), detection at 254 nm, and with Dragendorff's spray reagent.

Plant Material. The stem bark of *Erythroxylum vacciniifolium* was collected in Buraquinho rain forest (João Pessoa, Paraíba, Brazil), in August 2000. A voucher specimen was deposited at the HLPQN (Herbarium do Laboratorio de Química de Produtos Naturais), Universidade Federal de Paraíba, 58059 João Pessoa, Paraíba, Brazil (JPB.-No. 152) and identified by Prof. Zoraide Maria de Medeiros Gouveia of the Department of Science of Nature, University of Paraíba, Brazil, and Dr. Douglas C. Daly, The New York Botanical Garden.

Extraction and Isolation. Stem bark (840 g) was pulverized, moistened with 20 mL of concentrated NH_4OH, and exhaustively extracted with $CHCl_3$ (3 × 24 h; each 3 L). After filtration of the extracts, $CHCl_3$ was removed by rotary evaporation under vacuum to give 15.7 g of the $CHCl_3$ extract. This extract (10 g) was fractionated by MPLC with MeCN−H_2O−2 mM Et_3N (460 × 70 mm, flow rate 5.0 mL/min, gradient MeCN 5% to 100% in 3 days, UV detection at 280 nm) to give 13 fractions (A to M). Fraction L yielded compound **1** (813 mg). Fraction F was rechromatographed by MPLC with MeCN−H_2O−2 mM Et_3N (460 × 36 mm, flow rate 3.4 mL/min, gradient MeCN 5% to 15% in 24 h, UV detection at 280 nm) to give five fractions (F1 to F5). Fraction F2 yielded compound **8** (180 mg). Fraction J was purified by MPLC with MeCN−H_2O−2 mM Et_3N (460 × 36 mm, flow rate 3.0 mL/min, gradient MeCN 5% to 25% in 2.5 days, UV detection at 280 nm)) to give eight fractions (J1 to J8). Fractions J1, J3, and J7 yielded respectively compounds **7** (13 mg), **6** (33 mg), and **2** (8 mg). Fraction K was separated by MPLC with MeCN−H_2O−2 mM Et_3N (460 × 36 mm, flow rate 5.0 mL/min, gradient MeCN 15% to 60% in 4 h, UV detection at 280 nm) to give nine fractions (K1 to K9). Fraction K6 yielded compound **4** (631 mg), and fraction K8 yielded compound **5** (232 mg). Fraction M was purified by MPLC MeCN−H_2O−2 mM Et_3N (460 × 36 mm, flow rate 5.0 mL/min, gradient MeCN 15% to 60% in 19 h, UV detection at 280 nm) to afford compound **3** (727 mg).

Catuabine D [3α-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-6*β*-(1*H*-pyrrol-2-ylcarbonyloxy)tropane] (1): amorphous white solid; $[\alpha]_D$ −32.0° (*c* 0.58, EtOH); UV (EtOH) λ_{max} (log ϵ) 266.8 nm (4.38); IR ν_{max} (KBr) 2935 (C−H), 1700 (C═O),1410, 1320, 1245, 1110, 740; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* (rel int) 357 (55) $[M]^+$, 264 (18) [M + H − Pc]$^+$, 249 (15) [M − Mpc]$^+$, 233 (80) [M − MpcO]$^+$, 232 (75) [M + H − MpcO]$^+$, 140 (40) [M + H − Pc − MpcO]$^+$, 138 (32) [M + H − Pc − MpcO]$^+$, 122 (100) [M − PcO − MpcOH]$^+$, 108 (80) $[Mpc]^+$, 95 (100) $[C_6H_9N]^+$, 86 (95), 81 (35) $[C_5H_7N]^+$; D/CI-MS *m*/z 358 $[M + H]^+$; HRESMS *m*/*z* 358.1768 $[M + H]^+$ (calcd for $C_{19}H_{24}N_3O_4$, 358.1762).

7*β*-Hydroxycatuabine D [3α-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-6*β*-(1*H*-pyrrol-2-ylcarbonyloxy)-7*β*-hydroxytropane] (2): amorphous white solid; $[\alpha]_D$ −2.8° (*c* 0.40, EtOH); UV (EtOH) λ_{max} (log ϵ) 267.2 nm (3.92); ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* (rel int) 373 (10) $[M]^+$, 263 (7) [M − PcO]$^+$, 248 (10) [M − MpcOH]$^+$, 122 (10) [M − PcO − MpcO − OH]$^+$, 108 (45) $[Mpc]^+$, 95 (50) $[C_6H_9N]^+$, 94 (100) $[C_6H_8N]^+$, 81 (15) $[C_5H_7N]^+$; D/CI-MS *m*/z 374 [M + H]$^+$; HRESMS *m*/*z* 374.1709 $[M + H]^+$ (calcd for $C_{19}H_{24}N_3O_5$, 374.1710).

Catuabine E [3α,6*β*-di(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)tropane] (3): amorphous white solid; $[\alpha]_D$ −35.4° (*c* 0.57, EtOH); UV (EtOH) λ_{max} (log ϵ) 267.6 nm (4.38); IR ν_{max} (KBr) 2945 (C−H), 1695 (C═O),1415, 1320, 1245, 1115, 740; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* (rel int) 371 (30) $[M]^+$, 263 (5) [M − Mpc]$^+$, 247 (45) [M − MpcO]$^+$, 138 (15) [M − Mpc − MpcOH]$^+$, 122 (32) [M − MpcO − MpcOH]$^+$, 108 (55) $[Mpc]^+$, 95 (82) $[C_6H_9N]^+$, 94 (100) $[C_6H_8N]^+$, 86 (24), 81 (15) $[C_5H_7N]^+$; D/CI-MS *m*/z 372 [M + H]$^+$; HRESMS *m*/*z* 372.1924 $[M + H]^+$ (calcd for $C_{20}H_{26}N_3O_4$, 372.1918).

7*β*-Hydroxycatuabine E [3α,6*β*-di(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-7*β*-hydroxytropane] (4): amorphous white solid; $[\alpha]_D$ 0.8° (*c* 0.57, EtOH); UV (EtOH) λ_{max} (log ϵ) 267.5 nm (4.41); IR ν_{max} (KBr) 3500 (−OH), 2945 (C−H), 1695 (C═O), 1410, 1320, 1250, 1115, 740; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* (rel int) 387 (10) $[M]^+$, 262 (60) [M − MpcOH]$^+$, 154 (16) [M − Mpc − MpcOH]$^+$, 138 (80) [M − MpcO − MpcOH]$^+$, 137 (100) [M − MpcOH − MpcOH]$^+$, 122 (10) [M − MpcO − MpcO − OH]$^+$, 108 (100) $[Mpc]^+$, 94 (100) $[C_6H_8N]^+$, 81 (15) $[C_5H_7N]^+$; D/CI-MS *m*/z 388 $[M + H]^+$; HRESMS *m*/*z* 388.1869 $[M + H]^+$ (calcd for $C_{20}H_{26}N_3O_5$, 388.1867).

7*β*-Acetylcatuabine E [7*β*-acetoxy-3α,6*β*-di(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)tropane] (5): amorphous white solid; $[\alpha]_D$ −36.8° (*c* 0.56, EtOH); UV (EtOH) λ_{max} (log ϵ) 268.5 nm (4.45); IR ν_{max} (KBr) 2945 (C−H), 1745 (C═O), 1700 (C═O), 1410, 1320, 1250, 1110, 745; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* (rel int) 429 (80) $[M]^+$, 321 (15) [M − Mpc]$^+$, 305 (100) [M − MpcO]$^+$, 245 (73) [M − CH_3COOH − MpcO]$^+$, 196 (20) [M − Mpc − MpcOH]$^+$, 180 (80) [M − MpcO − MpcOH]$^+$, 138 (32) [M − CH_3COO − Mpc − MpcO]$^+$, 122 (16) [M − CH_3COO − MpcO − MpcO]$^+$, 108 (100) $[Mpc]^+$, 94 (100) $[C_6H_8N]^+$, 81 (15) $[C_5H_7N]^+$; D/CI-MS *m*/z 358 [M + H]$^+$; D/CI-MS *m*/z 430 [M + H]$^+$; HRESMS *m*/*z* 430.1967 [M + H]$^+$ (calcd for $C_{22}H_{28}N_3O_6$, 430.1973).

Catuabine F [3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)-6*β*-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)tropane] (6): amorphous white solid; $[\alpha]_D$ −32.8° (*c* 0.46, EtOH); UV (EtOH) λ_{max} (log ϵ) 220.7 (4.27), 270.0 (4.30), 293.4 sh nm (3.85); IR ν_{max} (KBr) 3420, 2945 (C−H), 1700 (C═O), 1415, 1330, 1215, 1110, 745; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2;

EIMS *m/z* (rel int) 444 (10) $[M]^+$, 247 (22) $[M - HdmbO]^+$, 181 (10) $[Hdmb]^+$, 138 (15) $[M - Hdmb - MpcOH]^+$, 122 (24) $[M - HdmbO - MpcOH]^+$, 108 (28) $[Mpc]^+$, 94 (100) $[C_6H_8N]^+$, 81 (15) $[C_5H_7N]^+$; D/CI-MS *m/z* 445 $[M + H]^+$; HRESMS *m/z* 445.1968 $[M + H]^+$ (calcd for $C_{23}H_{29}N_2O_7$, 445.1969).

7*β*-Hydroxycatuabine F [3α-(4-hydroxy-3,5-dimethoxybenzoyloxy)-6*β*-(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-7*β*-hydroxytropane] (7): amorphous white solid; $[\alpha]_D$ −2.6° (*c* 0.44, EtOH); UV (EtOH) λ_{max} (log ϵ) 220.7 (4.16), 269.3 (4.13), 293.4 sh nm (3.71); IR ν_{max} (KBr) 3445 (−OH), 2945 (C−H), 1700 (C═O), 1415, 1330, 1220, 1110, 745; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 460 (10) $[M]^+$, 335 (10) $[M - MpcOH]^+$, 263 (10) $[M - HdmbO]^+$, 181 (12) $[Hdmb]^+$, 138 (60) $[M - HdmbO - MpcOH]^+$, 122 (10) $[M - HdmbO - MpcO - OH]^+$, 108 (40) $[Mpc]^+$, 94 (100) $[C_6H_8N]^+$, 81 (15) $[C_5H_7N]^+$; D/CI-MS *m/z* 461 $[M + H]^+$; HRESMS *m/z* 461.1916 $[M + H]^+$ (calcd for $C_{23}H_{29}N_2O_8$, 461.1918).

Catuabine G [3α-(3-hydroxyphenylacetoxy)-6*β*,7*β*-dihydroxytropane] (8): amorphous white solid; $[\alpha]_D$ +0.0° (*c* 0.57, EtOH); UV (EtOH) λ_{max} (log ϵ) 217.7 (4.35), 276.3 nm (3.85); IR ν_{max} (KBr) 3455 (−OH), 2930 (C−H), 1735 (C═O), 690; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 307 (10) $[M]^+$, 247 (15) $[M - OHCH{=}CHOH]^+$, 156 (10) $[M - 3\text{-}OHPhCH_2CO]^+$, 107 (20) $[3\text{-}OHPhCH_2]^+$, 95 (55) $[C_6H_9N]^+$, 94 (100) $[C_6H_8N]^+$; D/CI-MS *m/z* 308 $[M + H]^+$; HRESMS *m/z* 308.1493 $[M + H]^+$ (calcd for $C_{16}H_{22}NO_5$, 308.1492).

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