Methylpyrrole Tropane Alkaloids from the Bark of *Erythroxylum vacciniifolium*

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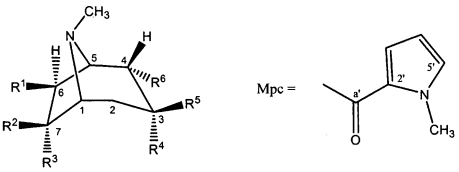
Nine new tropane alkaloids substituted by a methylpyrrole moiety were isolated from the bark of *Erythroxylum vacciniifolium*, a Brazilian endemic plant used in traditional medicine and locally known as *catuaba*. All compounds were elucidated as tropanediol or -triol alkaloids esterified by at least one 1-methyl-1*H*-pyrrole-2-carboxylic acid. One of the isolated compounds was identified as a tropane alkaloid *N*-oxide. Their structures were determined by high-resolution mass spectrometry and multidimensional NMR spectroscopy.

In the course of a study of medicinal plants from Brazil, a sample of *catuaba* from Paraíba, assigned the name *Erythroxylum vacciniifolium* Mart. (Erythroxylaceae), has been investigated. For over a century, *catuaba* has been one of the most popular herbal remedies in Brazil, and it has been attributed in local traditional medicine with aphrodisiac and tonic properties.[1,2] Several plants having similar properties from different families have been referred to as *catuaba*, and the true identity of this remedy remains uncertain.[2] In the northeastern region of Brazil, *catuaba* has been associated with three species of *Erythroxylum*, among them *E. vacciniifolium*.[3,4] Recently, this plant has been the focus of great public interest because of use of the bark as a remedy for erectile dysfunction. The genus *Erythroxylum* is the largest of the four Erythroxylaceae genera and has some 250 species, which are broadly distributed in tropical regions of South America, Africa, and the island of Madagascar. This genus, apart from the cocaine-producing species, has not been examined systematically by modern analytical methods, even though the different plants are widely used in native medicine and they are a well-known source of tropane alkaloids.[5]

Previous studies reported the isolation and identification of three tropane alkaloids (catuabines A, B, and C) from *E. vacciniifolium*.[6,7] A dereplication of a crude extract of this species with LC-hyphenated techniques was performed, and 24 putative new tropane alkaloids were fully or partially identified on-line.[8] Therefore, the isolation and structural characterization of these compounds was carried out and a first series of eight original tropane alkaloids were elucidated as catuabines D to G and their derivatives.[9] These tropane alkaloids are interesting for their ester moieties (1*H*-pyrrole-2-carboxylic- and 1-methyl-1*H*-pyrrole-2-carboxylic acid), which are unique to the species *E. vacciniifolium* (section Archerythroxylum). The isolation, identification, and structural elucidation of a series of new tropane alkaloids (**1**−**9**) are reported in this paper.

Chart 1



	R^1	R^2	R^3	R^4	R^5	R^6	Other
1	OMpc	H	H	OH	H	H	
2	OMpc	H	H	H	OH	H	
3	OMpc	OH	H	OH	H	H	
4[a]	OMpc	H	OH	OH	H	H	
5	OH	H	H	OMpc	H	H	
6	OH	OH	H	OMpc	H	H	
7	H	H	H	OH	H	OMpc	
8	OH	H	H	H	H	OMpc	
9	OMpc	H	H	OMpc	H	H	N → O

[a] For compound **4**, the methyl group of the tropane skeleton is orientated towards R^1 and R^2.

Results and Discussion

The powdered stem bark of *E. vacciniifolium* was moistened with concentrated NH_4OH and extracted with chloroform. The enriched alkaloid extract was then subjected to a series of column chromatographic purification steps (medium-pressure liquid chromatography, MPLC) to afford pure alkaloids **1**−**9**.

Compound **1** was isolated as a white amorphous powder. High-resolution electrospray ion cyclotron resonance mass spectrometric analysis (HRESMS) of this compound suggested a molecular formula of $C_{14}H_{20}N_2O_3$, implicating six centers of unsaturation and/or ring structures in the molecule. The ^{1}H NMR spectrum of **1** exhibited typical resonances for a methylpyrrole substructure with signals at δ_H 3.93 (3H, s, N-CH_3), δ_H 6.09 (1H, dd, J = 3.9, 2.4 Hz, H-4′), δ_H 6.77 (1H, t, J = 2.0 Hz, H-5′), and δ_H 6.90 (1H, dd, J = 3.9, 2.0 Hz, H-3′).[9] Long-range ^{1}H−^{13}C couplings deduced from the gHMBC spectrum of **1** revealed that the pyrrolic proton at δ_H 6.90 (H-3′) correlated with a carbonyl carbon at δ_C 161.2 (C-a′), indicating the substitution of the methylpyrrole moiety at the 2′-position by an ester group. The UV spectrum of **1** showed an absorption maximum at 267 nm, supporting the presence of the 2-(carbonyloxy)-methylpyrrole chromophore.[9] According to the molecular formula of **1**, the remaining moiety was characterized by the presence of one nitrogen atom and two ring structures.

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Table 1. ^{13}C NMR Data of Alkaloids **1**–**9**[a]

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	60.4	61.4	66.8	62.0	59.3	66.1	60.5	59.8	72.5
2	37.7	37.4	30.9	28.4	31.2	28.0	38.15	25.7	34.5
3	64.1	62.4	64.0	62.7	65.3	65.3	67.3	23.8	61.2
4	36.8	35.9	31.1	32.5	29.6	28.0	77.3	67.3	33.4
5	66.6	67.3	64.1	64.0	67.6	66.1	63.1	70.9	76.6
6	79.2	76.2	77.7	84.6	74.8	73.5	22.5	72.6	74.2
7	35.6	35.6	75.8	78.9	39.2	73.5	26.7	39.8	35.0
N-CH_3	40.6	39.1	36.1	37.6	37.0	35.7	38.7	37.8	48.9
	Mpc	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**
2′	122.8	121.7	122.1	122.0	122.2	121.7	122.0	122.5	121.6
3′	117.7	118.2	118.3	118.6	117.6	117.3	118.3	118.0	118.5
4′	107.7	108.0	107.9	107.9	108.0	107.6	107.9	107.8	108.6
5′	129.4	130.2	129.8	129.9	130.0	129.8	129.9	129.6	130.6
N-CH_3	36.7	36.7	36.8	36.7	36.6	36.0	36.8	36.8	36.8
a′	161.2	160.5	161.0	161.6	160.2	159.9	161.3	160.4	159.6
									Mpc
2″									122.5
3″									119.3
4″									108.1
5″									129.7
N-CH_3									37.1
a″									161.3

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

Two methine groups and one methyl group linked to the nitrogen atom were recorded at δ_H 3.26 (H-5), 3.35 (H-1), 2.55 (N-CH_3) and δ_C 66.6 (C-5), 60.4 (C-1), 40.6 (N-CH_3), suggesting their inclusion in a tropane skeleton. The observation in the ^1H NMR data of three methylene moieties at δ_H 1.66, 2.12 (2H, H-2), 1.91, 2.16 (2H, H-4), and 2.24, 2.75 (2H, H-7) and two deshielded methine groups at δ_H 4.08 (H-3) and 5.79 (H-6) completed the characterization of the tropane core skeleton. The substitution pattern of the alkaloid substructure was deduced by extensive NMR analysis with gDQF-COSY, gHMBC, and NOESY experiments. A long-range $^1H-^{13}C$ coupling associating the deshielded methine at δ_H 5.79 (H-6) and the carbon of the carbonyl function at δ_C 161.2 (C-a′) implicated the location of the ester moiety at the 6-position of the tropane nucleus. The remaining oxygen atom was associated with the 3-position of the alkaloid because of its characteristic chemical shifts at δ_H 4.08 (H-3) and δ_C 64.1 (C-3). The presence of one exchangeable proton in the molecule was confirmed by its LC-MS analysis using D_2O as eluent. An increase of 2 Da, due to the exchangeable hydroxyl function and the ionizing deuteron, was in fact observed in the deuterated molecule ($[M_D + D]^+$ 267 Da) when compared to the corresponding protonated molecule ($[M_H + H]^+$ 265 Da).[8]

The stereochemical orientation of the substituents relative to the nitrogen-containing bridge was deduced according to the multiplicity and coupling of the corresponding signals. The H-3 signal showed a triplet and coupling constant J of 4.9 Hz, indicating the α-orientation (i.e., *endo*) of the substituent at C-3.[10,11] The arrangement of the substituent at C-6 was established by the analysis of the coupling constants of H-6, H-7, and H-5. H-6 of the disubstituted tropane alkaloid showed two couplings (J = 7.3, 3.4) with the two H-7 protons, and it did not exhibit any coupling with the vicinal H-5 proton. This observation implied a β-orientation (i.e., *exo*) of the substituent and a dihedral angle close to 90° between H-5 and H-6α.[12−14] The relative configuration of the tropane substructure was also corroborated by analysis of its NOESY spectrum. The presence of a NOESY interaction between H-3 and H-6β confirmed that these were located on the same side of the molecule (β-position). Absolute configurations of **1** and the other isolates were not determined due to lack of material. Thus, the structure of catuabine H (**1**) is 3α-hydroxy-6β-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane.

Compound **2** was purified as a white amorphous powder and was assigned the same molecular formula as **1**, $C_{14}H_{20}N_2O_3$. The spectroscopic data of **2**, similar to those of **1** (Tables 1 and 2), were consistent with a general structure containing a central tropane moiety dioxygenated at C-3 and C-6 and esterified by one methylpyrrole acid in the 6-position. The main difference between the NMR data of compounds **1** and **2** concerned the multiplicity (triplet and doubled triplet, respectively) and the coupling constants (J = 4.9 Hz and J = 10.8 and 5.9 Hz, respectively) of their H-3 proton signals. The modification of the multiplicity of H-3 according to its relative configuration is well-known for the tropane alkaloid series.[10,15,16] As reported for **1**, the α-orientation of the hydroxyl group in C-3 was associated with a triplet multiplicity, while the doubled-triplet multiplicity of H-3 for compound **2** was related to the β-orientation of the substituent. The presence of NOE interactions between the protons H-6 and H-3 confirmed that these groups were located on the same side of the molecule (α-position). Hence, the structure of **2** (isocatuabine H) was deduced as 3β-hydroxy-6β-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane.

Alkaloid **3** was purified as a white amorphous powder and also exhibited spectroscopic data similar to those of catuabine H (**1**). The HRES mass spectrum of this compound showed a protonated molecular ion at *m/z* 281.1492, 15.9946 amu higher than that of **1**, suggesting the presence of an additional oxygen atom ($C_{14}H_{20}N_2O_4$). The NMR data of these two compounds were also closely related, indicating the presence in **3** of a central tropane moiety esterified by one methylpyrrole subunit. Except for proton H-7, the ^1H NMR spectrum of the tropane nucleus of **3** exhibited chemical shifts similar to those of **1** (Table 2). Actually, this spectrum showed typical resonances for a tropane alkaloid skeleton trisubstituted at C-3, C-6, and also C-7 with signals at δ_H 4.11 (1H, t, J = 4.9 Hz, H-3β), 5.75 (1H, d, J = 6.4 Hz, H-6α), and 4.84 (1H, d, J = 6.4 Hz, H-7α), respectively. According to the molecular formula and the values of the chemical shifts associated with the 7-position (δ_H 4.84 and δ_C 75.8), a hydroxyl substituent was required

Table 2. ^{1}H NMR Data of Alkaloids **1**−**9**[a]

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	3.35 m (3.9, 3.4)	3.60 br s	3.16 br s	3.35 m (6.4)	3.48 m (3.4)	3.21 br s	3.26 br d	3.33 br d (6.4)	4.07 br s
2_{exo}	2.12 m (4.9)	1.88 m	2.20 m	2.17 dt (15.1, 4.9)	2.32 m	2.32 m	1.86 m	1.88 m	2.53 m
2_{endo}	1.66 d (14.7)	1.88 m	1.61 d (17.6)	1.89 d (15.1)	1.69 d (15.6)	1.76 d (15.6)	1.96 m	1.33 m	2.20 d (16.6)
3α		3.78 tt (10.8, 5.9)						1.36 m (6.4)	
3*β*	4.08 t (4.9)		4.11 t (4.9)	4.08 t (4.9)	5.15 t (5.4)	5.13 t (4.9)	3.87 dd (8.8)	1.97 m	5.27 t (5.4)
4_{exo}	2.16 m 4.9	2.14 m	2.20 m	2.20 dt (15.1, 4.9)	2.33 m	2.32 m	4.98 dd (8.8, 3.9)	5.12 m (7.3, 3.4)	2.51 m
4_{endo}	1.91 d (14.7)	1.88 m	1.64 d (17.6)	1.84 d (15.1)	1.83 d (15.6)	1.76 d (15.6)			2.51 m
5	3.26 br s	3.51 br s	3.27 br s	3.11 br s	3.23 br s	3.21 br s	3.37 br d	3.18 d (3.4)	4.07 br s
6α	5.79 dd (7.3, 3.4)	5.24 dd (5.4)	5.75 d (6.4)	5.42 d (2.4)	4.72 dd (7.3, 2.9)	4.62 s	1.92 m	4.55 dd (7.3, 3.4)	5.86 dd (8.3, 3.4)
6*β*							1.92 m		
7α	2.75 dd (13.7, 7.3)	2.33 d (5.4)	4.84 d (6.4)		2.75 dd (14.2, 7.3)	4.62 s	1.59 m	2.09 m (7.3)	2.74 dd (13.2, 8.8)
7*β*	2.24 m	2.32 d (3.4)		4.62 d (6.4)	2.08 dd (14.2, 7.3)		2.08 m	2.05 m (3.4)	3.19 m
N-CH_3	2.55 s	2.67 s	2.58 s	2.54 s	2.68 s	2.63 s	2.42 s	2.63 s	3.42 s
	Mpc	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**	**Mpc**
3′	6.90 dd (3.9, 2.0)	6.90 dd (3.9, 2.0)	7.02 dd (3.9, 2.0)	7.00 dd (3.9, 2.0)	6.91 dd (3.9, 2.0)	6.90 br s	6.90 dd (3.9, 2.0)	6.95 dd (3.9, 2.0)	7.07 dd (3.9, 1.5)
4′	6.09 dd (3.9, 2.4)	6.11 dd (3.9, 2.4)	6.10 dd (3.9, 2.4)	6.11 dd (3.9, 2.4)	6.14 dd (3.9, 2.4)	6.13 br s	6.11 dd (3.9, 2.4)	6.10 dd (3.9, 2.4)	6.19 dd (3.9, 2.4)
5′	6.77 t (2.0)	6.82 t (2.0)	6.79 t (2.0)	6.81 t (2.0)	6.83 dd (2.4, 2.0)	6.88 br s	6.80 dd (3.9, 2.0)	6.78 dd (2.4, 2.0)	6.86 t (2.0)
N-CH_3	3.93 s	3.92 s	3.92 s	3.92 s	3.93 s	3.93 s	3.91 s	3.92 s	3.96 s
									Mpc
3″									7.12 dd (3.9, 1.5)
4″									6.11 dd (3.9, 2.4)
5″									6.78 t (2.0)
N-CH_3									3.95 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.

at this location. The α-orientation of the hydroxyl group in C-3 was resolved from the multiplicity (triplet) and the coupling constant (J = 4.9 Hz) of the H-3 proton signal. The α-orientation at C-6 and C-7 positions was deduced by the lack of any coupling constant with vicinal protons H-5 and H-1, respectively, and confirmed by analysis of the NOESY spectrum. Hence, the structure of **3** (7*β*-hydroxycatuabine H) was deduced as 3α,7*β*-dihydroxy-6*β*-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane.

Compound **4**, purified as a white amorphous solid, had the same molecular formula as **3** ($C_{14}H_{20}N_2O_4$) as deduced from HRESMS analysis. The NMR data strongly resembled those of **3**, consistent with a general structure containing a central tropane moiety trioxygenated at C-3, C-6, and C-7 and esterified by a methylpyrrole acid in the 6-position. NMR analysis of the two compounds revealed only slight differences, essentially concerning the multiplicity and the couplings of some proton signals of the tropane core skeleton, except for C-6, which gave a downfield-shifted signal at δ_C 84.6, probably due to the inversion of the hydroxyl substituent at C-7. The absence of any coupling constant between H-6 and H-7 in compound **4** suggested a vicinal angular relationship around 90° for the two protons. In addition, unlike the previous compounds, a coupling constant was observed between H-7 and H-1 (J = 6.4 Hz), indicating a dihedral angle close to 0° between the two protons. The presence of NOE interactions between the protons N-CH_3 and H-7 confirmed the *β*-orientation of the latter proton. In addition, the NOE association suggested that compound **4** differs from **1**−**3** by the orientation of the tropanic methyl group. On the basis of the above evidence, compound **4**, or 7α-hydroxycatuabine H, was identified as 3α,7α-dihydroxy-6*β*-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]-tropane.

Compound **5** was isolated as a white amorphous powder. According to an HRESMS experiment, it gave a molecular formula of $C_{14}H_{20}N_2O_3$, corresponding to that observed for compounds **1** and **2**. The NMR spectroscopic data of **5** were consistent with a C-3 and C-6 dioxygenated tropane alkaloid substituted by a methylpyrrole ester. Nevertheless, in this molecule, the signal at δ_H 5.15, typically shifted downfield with respect to nonesterified compounds, like **1** and **2**, indicated esterification at C-3 (δ_C 65.3) rather than a free alcohol.[11,13,17] Conversely, H-6 exhibited a resonance at δ_H 4.72, indicating a hydroxyl group at C-6 (δ_C 74.8). A long-range ^{1}H−^{13}C correlation between the oxygenated methine at δ_H 5.15 (H-3) and the carbonyl signal at δ_C 160.2 (C-a′) confirmed that the pyrrole unit substituted the tropane moiety at C-3. By analysis of its NOESY spectrum, the relative configuration of **5** was assigned as in **1**. This configuration was corroborated according to the multiplicity of H-3 and coupling between H-6 and H-5. Thus, the structure of catuabine I (**5**) is 6*β*-hydroxy-3α-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane.

The molecular formula of compound **6** was established as $C_{14}H_{20}N_2O_4$ by HRESMS, suggesting the occurrence of

an additional oxygen atom in the alkaloid compared to compound **5**. The observation of only 11 signals in the ^{13}C NMR spectrum of **6** indicated a symmetrical part in the molecule. This suggested the equivalence of the C-6 and C-7 positions, and according to the NMR data, the additional oxygen was located at the 7-position. Thus, the tropane moiety was substituted by two hydroxyl functions in positions C-6 and C-7. No coupling constant was observed between H-6 (or H-7) and the vicinal H-5 (or H-1), implicating a β-orientation of the two hydroxyl groups. As observed for compound **5**, the gHMBC data of **6** were consistent with the substitution of the tropane moiety at the C-3 position by a methylpyrrole ester. The structure of alkaloid **6** was elucidated as 6β,7β-dihydroxy-3α-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane, a new compound named 7β-hydroxycatuabine I.

HRESMS experiments of alkaloid **7** gave $C_{14}H_{20}N_2O_3$ as the molecular formula. This compound exhibited spectroscopic data similar to those of the other isolated tropane alkaloids. As previously reported for compound **1**, analysis of the gHMBC spectrum of **7** led to the identification of the two methine groups at δ_H 3.26 (H-1) and 3.37 (H-5). Their linkage to the nitrogen atom was confirmed by the observation of long-range $^1H-^{13}C$ correlations between the protons at δ_H 2.54 (3H, s, N-CH_3) and C-1 (δ_C 60.5) and C-5 (63.1). Nevertheless, in contrast to compounds **1**−**6**, the gDQF-COSY spectrum of **7** showed a partial spin system implicating two oxygenated methine moieties, at δ_H 4.98 and 3.87, and one methylene group at δ_H 1.86 and 1.96. This observation suggested that these three latter structural elements were located on the six-membered ring of the tropane skeleton. Analysis of the remaining NMR data corroborated the presence of two saturated methylene groups at the 6- and 7-positions of the tropane core skeleton. A long-range $^1H-^{13}C$ correlation between the oxygenated methine at δ_H 4.98 (H-4) and the carbonyl signal at δ_C 161.3 (C-a′) indicated that the methylpyrrolic moiety substituted the C-4 position of the tropane. The signal at δ_H 4.98, typically shifted downfield, indicated an esterification at C-4 (δ_C 77.3) rather than a hydroxyl group. The relative configuration of **7** was deduced by a NOESY experiment and the recorded coupling constants. Concerning H-3 and H-4, their coupling constant was measured at 8.8 Hz, supporting a dihedral angle close to 0° or 180° between the two methine groups. The observation of a spatial proximity between H-3 and H-4 removed the 180° dihedral angle possibility. In 1971, Johns and co-workers reported the isolation of a new nortropane alkaloid substitued at C-2 and C-3 from the leaves of *Peripentadenia mearsii* (Euphorbiaceae).[18] They considered the 9.0 Hz coupling between H-2 and H-3 as a *trans* diaxial arrangement of the two protons and elucidated the structure as 2α-benzoyloxy-3β-hydroxynortropane. On the basis of the above evidence, the structure of **7** (vaccinine A) was elucidated as 3α-hydroxy-4α-[(1-methyl-1*H*-pyrrol-2-yl)-carbonyloxy]tropane.

Compound **8** had the same molecular formula as **1**, **2**, **5**, and **7** ($C_{14}H_{20}N_2O_3$), as deduced from HRESMS analysis. Its spectroscopic data were consistent with a dioxygenated tropane structure substituted by a methylpyrrole ester. The gDQF-COSY spectrum of **8** suggested that the substituents were located adjacent to the *N*-methine group at δ_H 3.18 (H-5), implicating oxygenation of the 4- and 6-positions of the tropane skeleton. The methylpyrrole ester position was deduced from the long-range $^1H-^{13}C$ correlation between H-4 (δ_H 5.12) and C-a′ (δ_C 160.4) and the downfield shift of H-4. The presence of NOE interactions between the N-CH_3 protons and H-4 indicated that these groups were on the same side of the molecule (β-orientation of H-4). The relative configuration of C-6 was elucidated according to the absence of coupling constants with H-5 and the NOESY experiment. Hence, the structure of **8** (vaccinine B) was deduced as 6α-hydroxy-4α-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane.

Compound **9** was purified as an amorphous solid. HRESMS analysis of its pseudomolecular ion $[M + H]^+$ indicated the molecular formula as $C_{20}H_{26}N_3O_5$. The analysis of NMR data demonstrated that compound **9** was a dioxygenated tropane substituted by two methylpyrrole esters. The proton sequences and the carbon atom locations, respectively deduced by gDQF-COSY and gHMBC experiments, implicated the 3- and 6-positions for the two ester moieties of the tropane alkaloid. When compared to catuabine E [3α,6β-di(1-methyl-1*H*-pyrrol-2-ylcarbonyloxy)-tropane],[9] compound **9** seemed to have an additional oxygen atom (16 amu excess of molecular weight). Several ^1H and ^{13}C chemical shifts appeared to be strongly deshielded, especially the two methine groups linked to the nitrogen atom [δ_H 4.07 (H-1 and H-5) and δ_C 72.5 (C-1), 76.6 (C-5)] and the N-CH_3 moiety [δ_H 3.42 and δ_C 48.9]. According to the literature,[19−21] the deshielding effect could be explained by the presence of an *N*-oxide function. Moreover, no exchangeable protons were detected in the molecule by LC-MS analysis using D_2O as eluent,[8] confirming the presence of the *N*-oxide group. The additional oxygen atom was then located on the N-CH_3 moiety of the saturated five-membered ring, and **9** was considered as a tropane alkaloid *N*-oxide. The relative configuration was deduced by the interpretation of the recorded H-3 multiplicity and H-6 couplings, and it was confirmed by the NOESY experiment. From the evidence of the aforementioned spectroscopic data, compound **9** (catuabine E *N*-oxide) was identified as 3α,6β-di[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane *N*-oxide.

Nine methylpyrrole tropane alkaloids have been isolated from the bark of *E. vacciniifolium*. All of these alkaloids are new, namely, catuabines H (**1**, **2**), catuabine I (**5**), their hydroxy derivatives (**3**, **4**, **6**), vaccinines A and B (**7**, **8**), and catuabine E *N*-oxide (**9**). These nine alkaloids are additional to the 11 already isolated from this plant in earlier studies.[6,7,9] The originality of these compounds resides in their ester unit (1-methyl-1*H*-pyrrole-2-carboxylic acid), which is unique for a tropane alkaloid and to the species *E. vacciniifolium* (section Archerythroxylum). Esterifying groups are often characteristic features of certain species, while the tropane moieties do not themselves show intrageneric chemotaxonomic distinctiveness.[22−24] All catuabines isolated during this and previous studies are tropane alkaloids di- or trioxygenated at the 3-, 6-, or 7-position.[6,7,9] Vaccinines constitute a new series of tropane alkaloids, as they are dioxygenated at the 3-, 4-, or 6-position. The esterifying unit of vaccinines, the same as for catuabines, is placed at C-4, while the hydroxyl group can be at the C-3 or C-6 position. Tropanes with esters at the C-2 or C-4 position are rare in nature, whereas carboxyl groups (e.g., ecgonine derivatives) and hydroxyl groups (e.g., calystegines) at the same locations are common. In conclusion, this study illustrates that *E. vacciniifolium* is a source of original tropane alkaloids, unique for their esterifying acids and ester location.

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 spectra 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 and 460 × 36 mm, 15−25 μm, Merck), with a gradient mixture of acetonitrile/water/triethylamine (2 mM) as mobile phase.

Plant Material. The stem bark of *Erythroxylum vaccinii-folium* was collected in the 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. Powdered stem bark (840 g) was moistened with 20 mL of concentrated NH_3 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 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 F5 yielded compound **4** (117 mg). Fraction G was purified by MPLC MeCN/H_2O/2 mM Et_3N (460 × 36 mm, flow rate: 6.6 mL/min, gradient: MeCN 5% to 10% in 8 h, UV detection at 280 nm) to afford compound **3** (198 mg). Fraction H was separated by MPLC with MeCN/H_2O/2 mM Et_3N (460 × 36 mm, flow rate: 3.4 mL/min, gradient: MeCN 5% to 30% in 47 h, UV detection at 280 nm) to give 10 fractions (H1 to H10). Fractions H7 and H10 yielded compounds **2** (17 mg) and **7** (86 mg), respectively. Fraction I was purified by MPLC MeCN/H_2O/2 mM Et_3N (460 × 36 mm, flow rate: 5.0 mL/min, gradient: MeCN 5% to 20% in 20 h, UV detection at 280 nm) to afford compound **8** (543 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). Fraction J6 yielded compound **9** (37 mg). Fraction J4 was rechromatographed by HPLC with MeCN/H_2O/2 mM Et_3N (Nucleosil 100-5 C_{18} AB, 125 × 8 mm, 5 μm, flow rate: 2.0 mL/min, isocratic: MeCN 13% for 30 min, UV detection at 272 nm) to afford compounds **1** (22 mg) and **5** (7 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 K3 yielded compound **6** (81 mg).

Catuabine H [3α-hydroxy-6*β*-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (1): amorphous white solid; $[\alpha]_D^{20}$ −9.6° (*c* 0.46, EtOH); UV (EtOH) λ_{max} (log ϵ) 267 nm (4.10); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 264 (25) $[M]^+$, 113 (100) $[M - Mpc - C_2H_3O]^+$, 108 (30) $[Mpc]^+$, 96 (40) $[C_6H_{10}N]^+$, 94 (35) $[C_6H_8N]^+$, 82 (10) $[C_5H_8N]^+$; D/CI-MS *m/z* 265 $[M + H]^+$; HRESMS *m/z* 265.1546 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_3$, 265.1547).

3-α*H*-Catuabine H [3*β*-hydroxy-6*β*-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (2): amorphous white solid; $[\alpha]_D^{20}$ −8.0° (*c* 0.41, EtOH); UV (EtOH) λ_{max} (log ϵ) 266.6 nm (3.96); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 264 (20) $[M]^+$, 156 (20) $[M - Mpc]^+$, 139 (10) $[M - MpcOH]^+$, 122 (10) $[M - MpcOH - OH]^+$, 113 (100) $[M - Mpc - C_2H_3O]^+$, 108 (40) $[Mpc]^+$, 96 (40) $[C_6H_{10}N]^+$, 94 (35) $[C_6H_8N]^+$, 82 (10) $[C_5H_8N]^+$; D/CI-MS *m/z* 265 $[M + H]^+$; HRESMS *m/z* 265.1552 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_3$, 265.1547).

7*β*-Hydroxycatuabine H [3α,*β*-dihydroxy-6*β*-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (3): amorphous white solid; $[\alpha]_D^{20}$ −1.5° (*c* 0.53, EtOH); UV (EtOH) λ_{max} (log ϵ) 266.9 nm (4.04); IR ν_{max} (KBr) 3460 (−OH), 2930 (C−H), 1705 (C=O), 1415, 1330, 1240, 1110, 750; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 280 (10) $[M]^+$, 156 (25) $[M - MpcO]^+$, 155 (60) $[M - MpcOH]^+$, 138 (10) $[M - MpcOH - OH]^+$, 127 (35) $[M - Mpc - C_2H_5O]^+$, 113 (100) $[M - MpcO - C_2H_3O]^+$, 108 (80) $[Mpc]^+$, 96 (45) $[C_6H_{10}N]^+$, 94 (25) $[C_6H_8N]^+$, 82 (20) $[C_5H_8N]^+$; D/CI-MS *m/z* 281 $[M + H]^+$; HRESMS *m/z* 281.1492 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_4$, 281.1496).

7α-Hydroxycatuabine H [3α,7α-dihydroxy-6*β*-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (4): amorphous white solid; $[\alpha]_D^{20}$ −40.7° (*c* 0.57, EtOH); UV (EtOH) λ_{max} (log ϵ) 268.1 nm (4.11); IR ν_{max} (KBr) 3390 (−OH), 2935 (C−H), 1700 (C=O), 1415, 1320, 1250, 1110, 745; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 280 (10) $[M]^+$, 156 (25) $[M - MpcO]^+$, 155 (45) $[M - MpcOH]^+$, 138 (10) $[M - MpcOH - OH]^+$, 127 (35) $[M - Mpc - C_2H_5O]^+$, 113 (100) $[M - MpcO - C_2H_3O]^+$, 108 (80) $[Mpc]^+$, 96 (55) $[C_6H_{10}N]^+$, 94 (30) $[C_6H_8N]^+$, 82 (20) $[C_5H_8N]^+$; D/CI-MS *m/z* 281 $[M + H]^+$; HRESMS *m/z* 281.1501 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_4$, 281.1496).

Catuabine I [6*β*-hydroxy-3α-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (5): amorphous white solid; $[\alpha]_D^{20}$ −1.1° (*c* 0.39, EtOH); UV (EtOH) λ_{max} (log ϵ) 267.1 nm (4.02); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 264 (10) $[M]^+$, 140 (25) $[M - MpcO]^+$, 139 (15) $[M - MpcOH]^+$, 108 (15) $[Mpc]^+$, 96 (15) $[C_6H_{10}N]^+$, 95 (60) $[C_6H_9N]^+$, 94 (100) $[C_6H_8N]^+$, 82 (10) $[C_5H_8N]^+$; D/CI-MS *m/z* 265 $[M + H]^+$; HRESMS *m/z* 265.1546 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_3$, 265.1547).

7*β*-Hydroxycatuabine I [6*β*,7*β*-dihydroxy-3α-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (6): amorphous white solid; $[\alpha]_D^{20}$ −0.0° (*c* 0.60, EtOH); UV (EtOH) λ_{max} (log ϵ) 267.4 nm (4.13); IR ν_{max} (KBr) 3500 (−OH), 2930 (C−H), 1705 (C=O), 1415, 1320, 1250, 1100, 745; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 280 (20) $[M]^+$, 156 (15) $[M - MpcO]^+$, 155 (65) $[M - MpcOH]^+$, 138 (20) $[M - MpcOH - OH]^+$, 127 (25) $[M - Mpc - C_2H_5O]^+$, 113 (100) $[M - MpcO - C_2H_3O]^+$, 108 (70) $[Mpc]^+$, 96 (15) $[C_6H_{10}N]^+$, 94 (45) $[C_6H_8N]^+$, 82 (30) $[C_5H_8N]^+$; D/CI-MS *m/z* 281 $[M + H]^+$; HRESMS *m/z* 281.1493 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_4$, 281.1496).

Vaccinine A [3α-hydroxy-4α-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (7): amorphous white solid; $[\alpha]_D^{20}$ 37.1° (*c* 0.51, EtOH); UV (EtOH) λ_{max} (log ϵ) 268.1 nm (4.19); IR ν_{max} (KBr) 3390 (−OH), 2940 (C−H), 1695 (C=O), 1415, 1320, 1245, 1110, 740; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 264 (25) $[M]^+$, 156 (20) $[M - Mpc]^+$, 140 (30) $[M - MpcO]^+$, 139 (20) $[M - MpcOH]^+$, 108 (70) $[Mpc]^+$, 96 (40) $[C_6H_{10}N]^+$, 94 (20) $[C_6H_8N]^+$, 82 (100) $[C_5H_8N]^+$; D/CI-MS *m/z* 265 $[M + H]^+$; HRESMS *m/z* 265.1547 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_3$, 265.1547).

Vaccinine B [6α-hydroxy-4α-[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane] (8): amorphous white solid; $[\alpha]_D^{20}$ −2.0° (*c* 0.47, EtOH); UV (EtOH) λ_{max} (log ϵ) 266.9 nm (4.10); IR ν_{max} (KBr) 3140 (−OH), 2950 (C−H), 1690 (C=O), 1415, 1330, 1245, 1120, 745; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* (rel int) 264 (55) $[M]^+$, 156 (50) $[M - Mpc]^+$, 140 (20) $[M - MpcO]^+$, 139 (10) $[M - MpcOH]^+$, 108 (80) $[Mpc]^+$, 96 (30) $[C_6H_{10}N]^+$, 94 (10) $[C_6H_8N]^+$, 82 (100) $[C_5H_8N]^+$;

D/CI-MS *m*/*z* 265 $[M + H]^+$; HRESMS *m*/*z* 265.1549 $[M + H]^+$ (calcd for $C_{14}H_{21}N_2O_3$, 265.1547).

Catuabine E *N*-oxide [3α,6*β*-di[(1-methyl-1*H*-pyrrol-2-yl)carbonyloxy]tropane *N*-oxide] (9): amorphous white solid; $[\alpha]_D^{20}$ −7.3° (*c* 0.33, EtOH); UV (EtOH) λ_{max} (log ϵ) 268.2 nm (4.43); ^1H and ^{13}C NMR data, see Tables 1 and 2; D/CI-MS *m*/*z* 388 $[M + H]^+$; HRESMS *m*/*z* 388.1875 $[M + H]^+$ (calcd for $C_{20}H_{26}N_3O_5$, 388.1867).

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