Modulation of the Multidrug-Resistance Phenotype by New Tropane Alkaloid Aromatic Esters from *Erythroxylum pervillei*

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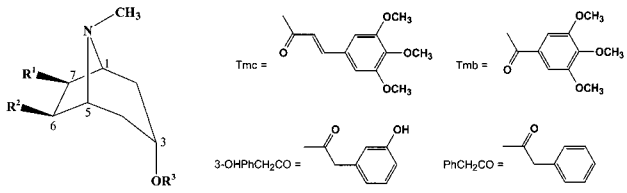
Nine tropane alkaloid aromatic esters (**1**−**9**) were isolated from the roots of *Erythroxylum pervillei* by following their potential to reverse multidrug-resistance with vinblastine-resistant oral epidermoid carcinoma (KB-V1) cells. All isolates, including seven new structures (**3**−**9**), were evaluated against a panel of human cancer cell lines, and it was found that alkaloids **3** and **5**−**9** showed the greatest activity with KB-V1 cells assessed in the presence of vinblastine, suggesting that these new compounds are potent modulators of P-glycoprotein. Confirmatory results were obtained with human ovarian adenocarcinoma (SKVLB) cells evaluated in the presence of adriamycin and synergistic studies performed with several cell lines from the NCI tumor panel. The structures of the new compounds were determined using spectroscopic techniques. Single-crystal X-ray analysis was performed on the monoester, tropane-3α,6*β*,7*β*-triol 3-phenylacetate (**1**).

Certain species from the plant genus *Erythroxylum* (family Erythroxylaceae) are used in traditional medicine to treat amenorrhea, hemorrhage, kidney disorders, influenza, sinusitis, and upset stomach, to combat fatigue and the feeling of hunger, and as stimulants.[1−3] Extracts of some *Erythroxylum* species have shown biological activity, such as antiinflammatory, analgesic, and antimicrobial effects.[3,4] Tropane alkaloids are known to be present in *Erythroxylum* species, and some compounds of this type are currently used in medicine.[5−7] However, their activity as potential anticancer agents does not appear to have been investigated so far. As part of an ongoing collaborative search for novel antineoplastic agents derived from plants, the roots of *Erythroxylum pervillei* Baill. (Erythroxylaceae) collected in Madagascar were investigated. This plant is known locally as "Tsivano" and has several folkloric uses, including as a fish poison and to treat abdominal pain and tumors.

In the present work, a chloroform-soluble extract of *E. pervillei* was found to significantly inhibit the growth of a multidrug-resistant (MDR) KB-V1 cell line[8] in the presence of vinblastine (VLB), while being much less cytotoxic for KB-V1 cells in the absence of VLB or normal KB cells. Activity-guided fractionation led to the isolation of nine tropane alkaloid aromatic esters (**1**−**9**), including seven new alkaloids, pervilleine A (**3**), pervilleine A *N*-oxide (**4**), and pervilleines B−F (**5**−**9**). In this paper we report the isolation, identification, and structural determination of these isolates, as well as their cytotoxic activity and their action as modulators of multidrug resistance.



	R^1	R^2	R^3	Other
1	OH	OH	$PhCH_2CO$	--
2	H	H	Tmb	--
3	OH	OTmc	Tmb	--
4	OH	OTmc	Tmb	N→O
5	H	OTmc	Tmb	--
6	H	OTmc	Tmc	--
7	OH	OTmc	Tmc	--
8	H	OTmc	3-$HOPhCH_2CO$	--
9	H	OTmc	$PhCH_2CO$	--

Results and Discussion

The air-dried powdered roots of *E. pervillei* were extracted with MeOH, and after removal of solvent, the residue was suspended in aqueous MeOH (9:1) and washed with hexane. The aqueous MeOH-soluble extract was then partitioned between $CHCl_3$ and 5% MeOH. The chloroform-soluble extract showed significant inhibitory activity against a vinblastine-resistant KB (oral epidermoid carcinoma) cell

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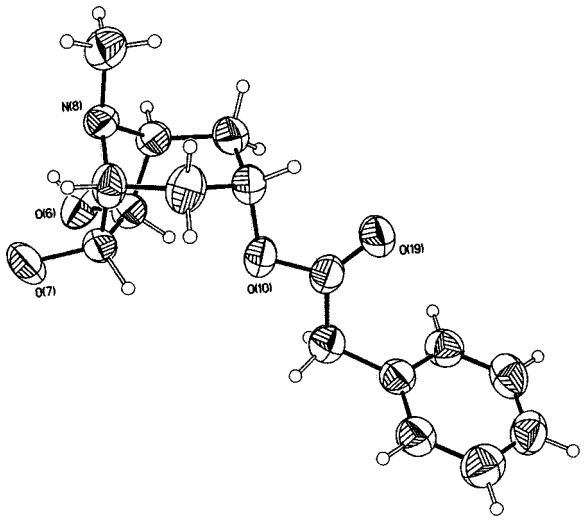

Figure 1. Perspective view of the molecular structure of **1** as determined by X-ray crystallography.

line, KB-V1, assessed in the presence of VLB (KB-V1$^+$, ED_{50} 5.4 μg/mL). The residue was then subjected to a series of cytotoxicity-guided column chromatographic purification steps over aluminum oxide, using this assay to monitor fractionation, to afford nine alkaloids (**1**−**9**). Alkaloids **1** and **2** were identified as the known alkaloids, tropane-3α,6β,7β-triol 3-phenylacetate[9] and 1αH,5αH-tropan-3α-yl 3,4,5-trimethoxybenzoate,[10] by comparison with reported data. Compounds **3**−**9** are novel tropane alkaloids with different acyl substituents, and their structures were assigned by the extensive use of ^{1}H, ^{13}C, one-dimensional selective INEPT, and 2D NMR spectroscopic techniques.

A single-crystal X-ray analysis was performed on **1**, crystallized as white needles from MeOH, to establish the stereochemistry of the *N*-methyl group. The final *R* factor was 6%, and the goodness of fit, 1.22. It was determined that in the solid state the *N*-methyl group in this alkaloid is axial (Figure 1).

Alkaloid **3** exhibited a molecular formula of $C_{30}H_{37}NO_{11}$, as determined from its high-resolution electron-impact mass spectrum (HREIMS). Its IR spectrum exhibited absorption bands at 3500 (OH), 1711 (ester), 1642 (α,β-unsaturated C=O), 1585 (aromatic C=C), 1220, and 1128 cm^{-1} (C−O). The ^{1}H NMR spectrum of **3** revealed characteristic resonances for a tropane alkaloid skeleton trisubstituted at the C-3, C-6, and C-7 positions with signals at δ 5.37 (1H, br t, ca. J = 4.5 Hz, H-3β), δ 5.72 (1H, d, J = 6.9 Hz, H-6α), and δ 4.77 (1H, d, J = 6.9 Hz, H-7α) (Table 1), respectively.[9] The downfield chemical shift and multiplicity of the H-3 signal indicated that C-3 bears an acyl moiety in an α configuration.[9−11] The above-mentioned coupling constants for the H-6α and H-7α chemical shifts accounted for the presence of *exo* substituents at the C-6 and C-7 positions, and the downfield shifts of H-6 and H-7 clearly indicated the substitution of an acyl moiety and a hydroxyl moiety at C-6 and C-7, respectively.[11] A singlet at δ 2.62 (3H) was assigned to an *N*-CH_3 group, with H-1 and H-5 appearing as broad singlets at δ 3.22 (1H) and δ 3.36 (1H), respectively, and the H-2 and H-4 resonances observed at δ 2.37 (2H, m, H-2ax and H-4ax) and δ 1.74 (2H, br d, J = 12.6 Hz, H-2eq and H-4eq) (Table 1). The ^{13}C NMR profile of the aliphatic carbons in **3** was also consistent with a C-3, C-6, C-7 trisubstituted tropane nucleus with signals at δ 67.5 (C-3), 77.7 (C-6), and 75.3 (C-7). The resonances at δ 26.4 and 26.3 were assigned to

Table 1. ^{1}H NMR Data of Alkaloids **3**−**9** (δ, $CDCl_3$, J in parentheses)[a]

proton	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	3.22 br s	3.85 br s	3.41 br d (7.4)	3.39 br d (7.2)	3.18 br s	3.27 br s	3.26 br d (6.3)
2ax	2.37 m	2.46−2.56 m	2.33 br dd (11.4, 5.4)	2.24 m	2.31 m	2.20 m	2.00−2.15 m
2eq	1.74 br d (12.6)	2.38 br d (16.9)[b]	1.72 br d (11.7)	1.69 br d (15.3)	1.69 dd (15.3, 7.3)	1.71 br d (15.2)	1.78 br d (15.2)[b]
3β	5.37 br t (4.5)	5.36 br t (4.5)	5.34 br dd (5.4, 4.5)	5.21 br t (5.0)	5.24 br t (4.5)	5.07 br t (5.2)	5.05 br t (5.2)
4ax	2.37 m	2.46−2.56 m	2.33 dd	2.26 m	2.37 m	2.13 m	2.00−2.15 m
4eq	1.74 br d (12.6)	2.31 br d (17.3)[b]	1.93 br d (12.6)	1.90 br d (15.3)	1.69 dd (15.3, 7.3)	1.45 br t (15.2)	1.48 br d (15.2)[b]
5	3.36 br s	4.32 br s	3.29 br s	3.31 br s	3.32 br s	3.27 br s	3.18 br s
6α	5.72 d (6.9)	5.97 d (6.9)	5.77 dd (7.5, 2.7)	5.73 dd (7.6, 2.7)	5.72 d (6.3)	5.66 dd (7.5, 3.0)	5.35 dd (7.5, 3.0)
7α	4.77 d (6.9)	4.81 d (6.9)	2.77 dd (13.6, 7.4)	2.72 dd (14.1, 7.6)	4.77 d (6.3)	2.41 dd (14.1, 7.6)	2.19 dd (14.1, 7.5)
7β			2.20−2.22 m	2.22−2.26 m		2.01 m	2.00−2.15 m
N-CH_3	2.62 s	3.31 s	2.61 s	2.59 s	2.60 s	2.55 s	2.50 s
Tmc				R^2/R^3	R^2/R^3		
a	6.45 d (15.8)	6.54 d (15.9)	6.37 d (15.9)	6.35/6.38 d (15.8)	6.36/6.47 d (15.9)	6.40 d (15.9)	6.37 d (15.9)
b	7.64 d (15.8)	7.67 d (15.9)	7.56 d (15.8)	7.31/7.56 d (15.8)	7.62/7.74 d (15.9)	7.66 d (15.9)	7.58 d (15.9)
ortho	6.76 s	6.77 s	6.57 s	6.74/6.88 s	6.75/6.92 s	6.77 s	6.76 s
OCH_3	3.89−4.00 (3 s)	3.88−3.97 (3 s)	3.88−3.98 (3 s)	3.88−3.94 (6 s)	3.90−3.96 (6 s)	3.89−3.90 (3 s)	3.88−3.90 (3 s)
	Tmb	Tmb	Tmb			3-$HOPhCH_2CO$	$PhCH_2CO$
CH_2						3.56 s	3.66 s
2′	7.35 s	7.36 s	7.38 s			7.19 d (2.2)	7.25−7.36 m
3′							7.25−7.36 m
4′,5′						6.80−6.84 m	7.25−7.36 m
6′	7.35 s	7.36 s				7.22 d (7.8)	7.25−7.36 m
OCH_3	3.89−4.00 (3 s)	3.88−3.97 (3 s)	3.88−3.98 (3 s)				

[a] Spectra recorded in $CDCl_3$ using TMS as internal standard. J values are given in ppm. [b] Assignments bearing the same superscript may be exchanged in each column.

Table 2. ^{13}C NMR Data of Alkaloids **3**−**9** (taken in $CDCl_3$ at 90.8 MHz using TMS as internal standard)[a]

carbon	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	65.8	76.9	59.1	59.0	65.8	64.3	65.0
2	26.4[b]	32.4	32.5	32.5	26.3[b]	32.0[b]	32.4[b]
3	67.5	62.3	67.6	67.1	66.8	67.4	67.3
4	26.3[b]	33.5	31.1	31.0	26.2[b]	30.9[b]	30.9[b]
5	62.7	78.4	64.8	64.9	62.7	58.9	58.9
6	77.7	74.9	78.9	79.3	77.8	79.9	78.9
7	75.3	74.7	37.3	37.2	75.1	37.2	35.8
N-CH_3	34.6	49.2	38.6	38.4	34.5	38.1	38.4
Tmc				R^2/R^3	R^2/R^3		
C═O	166.5	165.9	166.4	165.9/166.8	166.7/165.7	168.2	166.5
a	116.7	116.7	117.5	117.6/117.4	117.1/116.9	116.8	117.5
b	145.4	145.4	144.6	145.2/144.6	145.5/145.3	146.1	144.6
1′	129.7	129.8	129.8	129.8/129.8	129.7/129.7	129.5	129.8
2′,6′	105.2	105.1	105.1	105.4/105.1	105.5/105.2	105.4	105.2
3′,4′,5′	153.4	153.4	153.3	153.4/153.4	153.4/153.4	153.4	153.4
m-MeO	56.2[c]	56.1−56.4	56.1	56.2[d]/56.1[d]	56.2/56.2	56.2	56.1
p-MeO	61.0	62.3	61.0	61.0/60.9	60.9/61.0	61.0	61.0
	Tmb	Tmb	Tmb			3-HOPhCH_2CO	PhCH_2CO
C═O	164.8	165.0	165.3			170.1	170.4
CH_2						42.7	42.2
1′	125.0	123.7	125.3			134.7	133.8
2′	106.5	106.6	106.5			116.8	128.7
3′	153.4	153.3	153.8			156.9	129.3
4′	153.1	153.2	153.5			114.9	127.1
5′	153.4	153.3	153.8			130.0	129.3
6′	106.5	106.6	106.5			121.0	128.7
m-MeO	56.3[c]	60.9	56.1				
p-MeO	60.9	56.1	60.9				

[a] Values are given in ppm. Data are based on APT, selective INEPT, and 1D-HETCOR experiments. [b−d] Assignments bearing the same superscript may be exchanged in each column.

C-2 and C-4, respectively, while the C-1 and C-5 signals appeared, in turn, at δ 65.8 and 62.7, and finally, the *N*-CH_3 signal resonated at δ 34.6[11,12] (Table 2).

The acyl moieties of **3** were identified as 3,4,5-trimethoxycinnamoyl (Tmc) and 3,4,5-trimethoxybenzoyl (Tmb) units, with two signals at δ 6.45 (1H, d, J = 15.8 Hz) and 7.64 (1H, d, J = 15.8 Hz) assignable to the *trans* olefinic protons of the cinnamoyl residue. Two singlets at δ 6.76 (2H) and 7.35 (2H) were attributed to the *ortho* aromatic protons of the cinnamoyl and benzoyl moieties, respectively,[11] and signals for 18H (6× −OCH_3, δ 3.89−4.00) confirmed the presence of three methoxyl substituents symmetrically distributed in each unit.[11,13] Further support for the identities of these substituents was obtained from the EIMS fragments at *m*/*z* 375 (M^+ − TmbA, 6%) and *m*/*z* 349 (M^+ − TmcA 10%).[2,10,12] The NMR data (Tables 1 and 2) for the Tmc and Tmb units were in agreement with those found in the literature.[11,13,14] The positions of attachment of the Tmc and Tmb moieties to the nucleus were determined by several selective INEPT NMR[15] experiments. Thus, irradiation of H-6 (δ 5.72; $^3J_{CH}$ = 6 Hz) enhanced the carbonyl carbon signal at 166.5 ppm, which was similarly enhanced by selective irradiation of the vinylic protons at δ 7.64/6.45 (H-β/α), indicating that the Tmc residue is attached to C-6. In the same way, the Tmb moiety was placed at C-3 by irradiation of H-3 (δ 5.37) and the Tmb aromatic protons (δ 7.35) with the corresponding enhancement of the carbonyl carbon signal at δ 164.8. The *N*-methyl group was assigned as axial, by analogy with alkaloid **1**. Accordingly, alkaloid **3** was determined as the new tropane alkaloid 3α-(3,4,5-trimethoxybenzoyloxy)-6β-(*E*)-(3,4,5-trimethoxycinnamoyloxy)-7β-hydroxytropane and has been accorded the trivial name pervilleine A.

The NMR spectral data of **4** resembled those of pervilleine A (**3**), with proton signals for H-3β at δ 5.36 (1H, br t, J = 4.5 Hz), H-6α at δ 5.97 (1H, d, J = 6.9 Hz), H-7α at δ 4.81 (1H, d, J = 6.9 Hz) and their corresponding carbon signals at δ 62.3 (C-3), 74.9 (C-6), and 74.7 (C-7) (Tables 1 and 2), suggesting the presence of hydroxyl and diacyl substituents in the tropane nucleus. The acyl moieties were identified as Tmc and Tmb (Tables 1 and 2) and were placed at C-3 and C-6, respectively, by a sequence of selective INEPT experiments carried out as described for alkaloid **3**. A prominent ion at *m*/*z* 588 (56%) in the CIMS (positive-ion mode) corresponding to the pseudoempirical formula of $C_{30}H_{37}NO_{11}$ was observed for **4**, the same molecular formula determined for alkaloid **3**. If **4** were an isomer of pervilleine A (**3**), this would not satisfactorily explain the strong deshielding effects observed for H-1 (1H, br s, δ 3.85), H-2eq (1H, br d, δ 2.38), H-4eq (1H, br d, δ 2.31), and H-5 (1H, br s, δ 4.32) and the downfield shift for the *N*-CH_3 signal (δ 3.31 s). This effect was also observed for the corresponding carbons in the ^{13}C NMR spectra, which were assigned by APT and 1D- and 2D-HETCOR experiments (Table 2). The presence of a protonated molecular ion at *m*/*z* 604 ($[M + 1]^+$, 7%) in the FABMS (positive mode) showed a 16 amu difference from the molecular weight of **3**, and the decomposition of the product when subjected to EIMS suggested that compound **4** could be an *N*-oxide of pervilleine A. Wenkert and co-workers[16] have compared the ^{13}C NMR data of scopolamine *N*-oxide and scopolamine free base and observed significant changes on the chemical shifts of the neighboring carbons, leading to differences [$\Delta\delta_C$ (scopolamine *N*-oxide − scopolamine)] of +11.4 (C-1, C-5), −4.7 (C-3), −2.5 (C-6, C-7), and +9.0 (*N*-CH_3). The changes in the analogous chemical shifts observed for compound **4** and pervilleine A (**3**) were $\Delta\delta_C$ +11.1 (C-1), +15.7 (C-5), −5.2 (C-3), −2.8 (C-6), −0.6 (C-7), and +14.6 (*N*-CH_3) and were clearly indicative of *N*-oxide substitution in alkaloid **4**. While most of the $\Delta\delta_C$ values matched the literature values,[16] the small differences observed can be explained based on the unsymmetrical substitution of the tropane nuclei in **3** and **4**. Moreover, analysis of the ^1H NMR spectrum of **4** showed the *N*-CH_3 signal at δ 3.31. Huber and co-workers[17] demonstrated, by applying X-ray crystallographic and NMR

studies, a clear correlation of the configuration and the ^{1}H NMR chemical shift of the *N*-methyl group in scopolamine *N*-oxide. The axial methyl was found to resonate at δ 3.39, while the stereoisomeric equatorial methyl occurred at δ 3.60. On the basis of these observations, the *N*-methyl group in alkaloid **4** (δ 3.31) corresponded to an axial configuration. Thus, **4** was identified as 3α-(3,4,5-trimethoxybenzoyloxy)-6β-(*E*)-(3,4,5-trimethoxycinnamoyloxy)-7β-hydroxytropane *N*-oxide (pervilleine A *N*-oxide).

Alkaloid **5** also showed spectroscopic data similar to those of pervilleine A (**3**). The molecular formula was obtained as $C_{30}H_{37}NO_{10}$ by HREIMS, 16 amu lower than that of **3**, suggesting the lack of a hydroxyl group in the structure of alkaloid **5**. The ^{1}H NMR spectrum exhibited characteristic signals for a disubstituted tropane nucleus at the C-3 and C-6 positions with signals appearing for H-3β at δ 5.34 (1H, br dd, J = 5.4, 4.5 Hz), H-6α at δ 5.77 (1H, dd, J = 7.5, 2.7 Hz), and CH_2-7 at δ 2.77 (1H, dd, J = 13.6, 7.4 Hz, Hα) and δ 2.20−2.22 (1H, m, Hβ) (Table 1). The acyl moieties were deduced as Tmc and Tmb, and the positions of their attachment were determined as for alkaloid **3**. Accordingly, alkaloid **5** was identified as 3α-(3,4,5-trimethoxybenzoyloxy)-6β-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane and was given the trivial name pervilleine B.

Compound **6** was assigned a molecular formula of $C_{32}H_{39}NO_{10}$, as determined by HREIMS, and showed the same pattern of substitution as that of **5**, with resonances for H-3β at δ 5.21 (1H, br t, J = 5.0 Hz), H-6α at δ 5.73 (1H, dd, J = 7.6, 2.7 Hz), and CH_2-7 at δ 2.72 (1H, d, J = 14.1, 7.6 Hz, Hα) and δ 2.22−2.26 (1H, m, Hβ). However, signals for a Tmb moiety were not evident in the ^{1}H and ^{13}C NMR spectra of **6**, and the signals for the Tmc moiety were duplicated (Tables 1 and 2). Hence, the structure of **6** (pervilleine C) was elucidated as 3α,6β-di-[(*E*)-3,4,5-trimethoxycinnamoyloxy]tropane.

Alkaloid **7** was assigned a molecular formula of $C_{32}H_{39}NO_{11}$ (HREIMS) and showed signals for the tropane nucleus in the ^{1}H NMR spectrum similar to **3**, with signals seen for H-3β (δ 5.24; 1H, br t, J = 4.5 Hz), H-6α (δ 5.72; 1H, d, J = 6.3 Hz), and H-7α (δ 4.77; 1H, d, J = 6.3 Hz). The portion of the ^{1}H NMR spectrum depicting the acyl groups of **7** was closely comparable to equivalent data for alkaloid **6**, with resonances observed at δ 7.62/7.74 (1H each, d, J = 15.9 Hz, Hα), 6.36/6.47 (1H each, d, J = 15.9 Hz, Hβ), and 6.75/6.92 (2H each, s, H-2′, H-6′). The ^{13}C NMR spectrum also supported the presence of two Tmc moieties in **7** (Table 2). On the basis of the above evidence, the structure of **7** was therefore deduced as 3α,6β-di-[(*E*)-(3,4,5-trimethoxycinnamoyloxy)-7β-hydroxytropane] (pervilleine D).

The NMR spectral data for alkaloids **8** and **9**, when compared with the spectra for the above-mentioned analogues, exhibited values consistent with the presence of one Tmc substituent and one other acyl substituent in each alkaloid. The tropane nucleus showed NMR resonances in agreement with those of alkaloid **5**, suggesting disubstitution at the C-3 and C-6 positions. The coupling constants for H-3 (brt, J = 5.2 Hz) and H-6 (dd, J = 7.5, 3.0 Hz) indicated a β and α configuration, respectively, for these protons in **8** and **9** (Table 1). Alkaloid **8**, with a molecular formula of $C_{28}H_{33}NO_8$, as determined by HREIMS, showed in the ^{1}H NMR spectrum resonances at δ 3.56 (2H, s), 7.19 (1H, d, J = 2.2 Hz), 6.80−6.84 (2H, m), and 7.22 (1H, d, J = 7.8 Hz) and carbon signals at δ 170.1, 42.7, 134.7, 116.8, 156.9, 114.9, 130.0, and 121.0, indicating the presence of a 3-hydroxyphenylacetyl moiety.[9] Selective INEPT experiments were used to confirm the substitution patterns of the Tmc unit at C-6 and the 3-hydroxyphenylacetyl group at C-3. The molecular formula of compound **9** was determined by HREIMS as $C_{28}H_{33}NO_7$, 16 amu lower than that of **8**, suggesting there is no hydroxyl group present in **9**. Analysis of the NMR data showed that the second acyl group was a phenylacetyl unit.[9] Selective INEPT experiments led to the placement of the Tmc unit at C-6 and the phenylacetyl group at C-3 for this alkaloid. On the basis of the evidence presented above, the structures of **8** and **9** were elucidated as 3α-(3-hydroxyphenylacetoxy)-6β-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane (pervilleine E) and 3α-phenylacetoxy-6β-(*E*)-(3,4,5-trimethoxycinnamoyloxy)-tropane (pervilleine F), respectively.

The stereochemistry at the respective asymmetric carbons for pervilleines A−F (**3**, **5**−**9**) and pervilleine A *N*-oxide (**4**) was determined on the basis of NMR coupling constant observations and confirmed using molecular modeling experiments (PCMODEL Version 4.0). In this regard, different stereoisomer combinations were minimized using the MMX force field, wherein predicted coupling constants were compared with the experimental J values actually obtained finding the best match with the stereoisomers presented herein. All structures are numbered clockwise starting from the bridgehead methine and arbitrarily represent one of the two possible enantiomers. The *N*-methyl group is presented in its axial configuration for pervilleines A−F (**3**, **5**−**9**), according to the structure established for alkaloid **1** by means of X-ray crystallographic analysis (Figure 1). Moreover, in NOESY NMR experiments on both pervilleine A (**3**) and pervilleine C (**6**) clear correlations were observed between the *N*-methyl group and H-2eq and H-4eq in each case, supporting the axial orientation of the *N*-CH_3 group in these compounds. These data suggest that pervilleines B (**5**) and D−F (**7**−**9**) also have the *N*-methyl group in the axial orientation as the preferred conformation.

Alkaloids **1**−**9** were initially tested against a panel of human cancer cell lines according to established protocols.[8] Pervilleines A and F (**3** and **9**) showed both strong activity and selectivity for the multidrug-resistant KB-Vl cell line in the presence of vinblastine (ED_{50}, 0.3 and 0.2 μg/mL, respectively), while pervilleine E (**8**) exhibited selectivity, but potency was reduced by approximately one order of magnitude (ED_{50}, 1.9 μg/mL). Pervilleines B−D (**5**−**7**) displayed high potency with KB-Vl cells assessed in the presence of VLB, but were less selective. Pervilleine A *N*-oxide (**4**), as well as the known alkaloids **1** and **2**, was shown to be inactive ($ED_{50} \geq 20$ μg/mL) for all cell lines, including the KB-V1 cell line in the presence of VLB (Table 3).

Several observations concerning the structural requirements for activity within this alkaloid group may be made from the cytotoxicity panel data of alkaloids **1**−**9** shown in Table 3. When alkaloids **1**−**3** and **5**−**9** are considered specifically, a C-6 Tmc ester substituent was seen to be essential for the elicitation of significant activity against KB-V1 cells in the presence of VLB. The absence of a hydroxyl group at C-7, as in pervilleines B (**5**) and C (**6**), led to approximately the same cytotoxic potency shown by pervilleines A (**3**) and D (**7**) for KB-V1 cells in the presence of VLB, but reduced selectivity when the other cell lines in the panel are considered. Pervilleine E (**8**), esterified at C-3 with a 3-hydroxyphenylacetyl moiety, exhibited less potency and less selectivity than pervilleine D (**7**) with a Tmc ester at C-3. When the C-3 Tmc ester at C-3 [pervilleine C (**6**)] was altered to a phenylacetyl group [pervilleine

Table 3. In Vitro Cytotoxicity of Alkaloids **1**−**9** in a Human Tumor Panel[a]

	ED_{50} (μg/mL)							
compd	BC1	Lu1	Col2	KB	KB-V1$^+$	KB-V1$^-$	LNCaP	SW626
1	>20	>20	>20	>20	>20	>20	>20	>20
2	>20	>20	>20	>20	>20	>20	>20	>20
3	>20	>20	>20	>20	0.3	>20	>20	>20
4	>20	>20	>20	>20	>20	>20	>20	>20
5	9.4	3.1	1.3	>20	0.1	8.8	1.0	3.2
6	2.8	2.3	4.1	2.2	0.1	9.0	>20	1.4
7	15.0	15.1	4.9	11.0	0.2	9.0	14.1	6.5
8	>20	>20	13.4	14.7	1.9	>20	>20	8.4
9	>20	>20	4.9	>20	0.2	>20	>20	>20

[a] Key to human cancer cell lines used: BC1, breast cancer; Lu1, lung cancer; Col2, colon cancer; KB, oral epidermoid carcinoma; KB-V1$^+$, drug-resistant KB assessed in the presence of vinblastine (1 μg/mL); KB-V$^-$, drug-resistant KB assessed in the absence of vinblastine; LNCaP, hormone-dependent prostate cancer; SW626, human ovarian adenocarcinoma.

Table 4. Multidrug-Resistance Inhibition by Tropane Alkaloids **3**−**6**

	IC_{50} (μM)		
compound	SKOV3[a]	SKVLB[b]	SKVLB[c]
adriamycin	0.008	2.2	
GF120918[d]	8.1	14	0.02
pervilleine A (**3**)	>10	>10	0.65
pervilleine A *N*-oxide (**4**)	>10	>10	>10
pervilleine B (**5**)	3.8	>10	0.12
pervilleine C (**6**)	2.8	>10	0.08

[a] SKOV3 (ovarian adenocarcinoma; provided by Dr. V. Lin, Ontario Cancer Institute). [b] SKVLB (ovarian adenocarcinoma, multidrug resistant; provided by Dr. V. Lin, Ontario Cancer Institute). [c] Addition of 1 μM adriamycin. [d] *N*-{4-[2-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridone carboxamide.[19]

F (**9**)], less potency but greater selectivity resulted. Pervilleine A *N*-oxide (**4**) was not significantly active for any of the cell lines represented in the tumor panel. However, it is known that some *N*-oxides are bioreductive drugs,[18,19] so possibly compound **4** could serve as a prodrug and be reduced to release the active pervelleine A (**3**).

In Table 4, the cytotoxic activities of pervilleines A−C (**3**, **5**, **6**) and pervilleine A *N*-oxide (**4**) are compared with the acridone carboxamide derivative GF120918,[20] an MDR inhibitor, in ovarian adenocarcinoma (SKOV3) and multidrug-resistant ovarian adenocarcinoma (SKVLB) cell lines, performed as previously described.[21] When 1 μM adriamycin was added to the latter cell line, pervilleines B (**4**) and C (**5**) (IC_{50} 0.12 and 0.08 μM, respectively) mediated potent responses that were comparable to GR120918 (IC_{50} 0.02 μM). Since the data in Tables 3 and 4 indicate that the tropane alkaloid esters evaluated were less toxic for KB-V1 and SKVLB cells than their normal counterparts in the absence of any additional drug, it is possible that the compounds are themselves substrates for the MDR transporter and may exhibit a competitive mechanism for MDR inhibition.

Alkaloids **5**−**7** and **9** were further evaluated for their efficacy as potential modulators of P-glycoprotein. This assay assessed the ability of these test compounds to increase the cytotoxicity of an agent of the MDR phenotype {4,9-dihydro-3-isobutyl-2-methyl-1-(*p*-nitrophenacyl)-4,9-dioxo-1*H*-naphth[2,3-*d*]imidazolium bromide (DINIB)}, compared with an agent not of the MDR phenotype (5-fluorouracil), used as a negative control.[22,23] Evaluation of the data for the alkaloids tested indicated that they are excellent modulators of the MDR phenotype, as exemplified by their ability, at nontoxic doses, to significantly increase the toxicity of the MDR substrate DINIB, with magnitude comparable to the standard MDR modulators, verapamil and cyclosporin A (Table 5). The dose ranges selected for the pervilleines evaluated were appropriate to produce full dose−response curves (except for **7**, which induced limited toxicity at 50 ng/mL). Maximum escalation of DINIB toxicity was measured at nontoxic concentrations of the pervilleines at least 4- to 10-fold below the concentration required to produce 50% growth inhibition in the cells.

Table 5. In Vitro Multidrug-Resistance Modulation of Alkaloids **5**−**7** and **9**

	conc range (5 doses)	cell line[b]			
compound	(μg/mL)[a]	HCT-15	UO-31	CAKI-1	LOX IMVI
verapamil	12.5−0.8	696.8	432.1	369.8	185.1
cyclosporin A	6.3−0.4	719.1	642.1	273.3	72.9
pervilleine B (**5**)	12.5−0.2	607.6	248.7	268.1	33.3
pervilleine C (**6**)	3.1−0.2	946.7	444.0	332.3	32.6
pervilleine D (**7**)	50.0−3.1	758.3	562.7	387.9	13.2
pervilleine F (**9**)	25.0−1.5	714.7	364.4	342.1	59.2

[a] Concentration range tested was appropriate to produce a dose−response curve. Rank order potency of the pervilleines in these cell lines was **6** > **5** > **9** > **7**. [b] Cancer cell lines are shown in rank order of MDR1 expression and function, with HCT-15 (colon) and UO-31 (renal) being high expressers, CAKI-1 (renal) a moderate expresser, and LOX IMVI (melanoma) having no MDR1 and exhibiting no MDR phenotype. The ability of potential modulators to increase the cytotoxicity of 4,9-dihydro-3-isobutyl-2-methyl-1-(*p*-nitrophenyl)-4,9-dioxo-1*H*-naphth[2,3-*d*]imidazolium bromide (DINIB; 1×10^{-4} M) was evaluated, and results represent synergy values at a 95% confidence level. Values <25 are insignificant, 25−50 are minor, but significant, 50−100 indicate moderate synergy, and >100 indicate strong synergy and are probably important in vivo.[22,23]

In conclusion, nine aromatic tropane alkaloid esters have been isolated from the roots of *Erythroxylum pervillei*. Seven of these alkaloids are new, namely, pervilleine A (**3**), pervilleine A *N*-oxide (**4**), and pervilleines B−F (**5**−**9**). All of these new tropane derivatives were found to contain a *trans*-3,4,5-trimethoxycinnamoyloxy unit at the C-6 position, with the C-3 hydroxyl group being differentially esterified and C-7 sometimes being hydroxylated among alkaloids **3**−**9**. The known alkaloids tropane-3α,6*β*,7*β*-triol 3-phenylacetate (**1**) and 1αH,5αH-tropan-3α-yl 3,4,5-trimethoxybenzoate (**2**) were also obtained, with the configuration of the *N*-methyl group of **1** established as axial by X-ray crystallographic analysis. Several of these compounds showed substantial selectivity for a MDR human oral epidermoid (KB) cell line in the presence of vinblastine, with pervilleines A (**3**), B (**5**), C (**6**), D (**7**), and F (**9**) all showing a similar cytotoxic potency against this cell line. In a MDR human ovarian adenocarcinoma (SKVLB) cell line in the presence of adriamycin, pervilleines B (**5**) and C (**6**) reversed drug resistance with potencies comparable to the acridone carboxamide MDR modulator GR120918.[19] In a further in vitro biological test protocol, **5**−**7** and **9** were found to exhibit comparable potency to verapamil and cyclosporin A at increasing the cytotoxicity of DINIB.[22,23] As a follow-up biological study to the present investigation, the mode of action of pervilleine A (**4**) has been further characterized, and synergistic activity with verapamil has been demonstrated for this compound in the in vivo hollow fiber model using KB-V1 and KB-8-5 cells.[24]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-

Elmer 241 polarimeter. UV spectra were recorded with a Nicolet MX-1 spectrophotometer, and IR spectra were taken on a Beckman DU-7 instrument. NMR spectra were obtained in $CDCl_3$ with TMS as an internal standard. ^1H NMR and ^{13}C NMR spectra, selective INEPT, and HETCOR experiments were recorded on either a Nicolet NMC-360 or a Varian XL-300 NMR spectrometer. HMQC, HMBC, and NOESY experiments on selected compounds were run on a Bruker DRX-500 NMR spectrometer. The EIMS, HREIMS, and HRFABMS data were obtained on a Finnigan MAT-112S mass spectrometer. Open column chromatography was carried out over silica gel G (70−230 mesh; E. Merck, Darmstadt, Germany) or aluminum oxide (alumina, neutral, Brockman Activity I, 60−325 mesh; Fisher Chemicals, Pittsburgh, PA), using gradient mixtures of $CHCl_3$−acetone−28% NH_4OH or hexane−acetone−28% NH_4OH as solvents, respectively. TLC plates (silica gel 60 F_{254} glass plates, 0.25 mm layer thickness; E. Merck) were visualized under UV light and using Dragendorff's spray reagent.

Plant Material. The roots of *E. pervillei* were collected in a semiarid region in southern Madagascar in October 1992. A voucher specimen (A00362) is deposited in the John G. Searle Herbarium, Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The air-dried powdered roots of *E. pervillei* (12 kg) were extracted with MeOH two times to afford a residue (129 g), which was then suspended in MeOH−H_2O (9:1, 800 mL) and washed with hexane (3 × 500 mL). The aqueous layer was concentrated under vacuum and then partitioned between 5% MeOH (600 mL) and $CHCl_3$ (3 × 400 mL). The $CHCl_3$-soluble extract (28 g) showed significant activity against a vinblastine-resistant KB cell line (KB-V1 assessed in the presence of 1 μg/mL vinblastine; KB-V1$^+$, ED_{50} 5.4 μg/mL), while the aqueous layer was inactive against this cell line. The active extract (28 g) was absorbed on silica gel and purified by silica gel column chromatography using gradient mixtures of $CHCl_3$−acetone−28% NH_4OH (20:10:0.1 → 5:20:1) as solvent systems to yield five major alkaloid-containing mixtures (fractions 1−5). Fraction 1 (0.8 g) was purified by passage on a neutral aluminum oxide (Al_2O_3) column eluted with hexane−acetone−MeOH−Et_2NH (9:1:0.1:0.1) to afford **5** (420 mg) and **9** (450 mg). Fractions 2 and 3 were combined (4.5 g) and subjected to Al_2O_3 column chromatography using hexane−acetone−MeOH−Et_2NH gradient mixtures (6:1:0.1:0.1→3:1:0.1:0.1) to afford **1** (4 mg), **2** (450 mg), **6** (520 mg), and **8** (480 mg). Fraction 4 (1.9 g) was subjected to passage over an Al_2O_3 column using hexane−acetone−MeOH−Et_2NH (3:1:0.1:0.1) as eluent to afford **3** (500 mg), **7** (510 mg), and additional quantities of **1** (42 mg). Fraction 5 (62 mg) was purified by preparative TLC using an acetone−H_2O−28% NH_4OH (4:0.75:2 drops) mixture as solvent system to afford **4** (14 mg, R_f 0.47).

Tropane-3α,6*β*,7*β*-triol 3-phenylacetate (1): white crystals (MeOH); mp 120−122 °C; ^1H, ^{13}C NMR, and EIMS data, consistent with literature values.[9]

1αH,5αH-Tropan-3α-yl 3,4,5-trimethoxybenzoate (2): white amorphous solid; mp 238−240 °C; ^1H NMR and EIMS data, consistent with literature values.[10]

Pervilleine A [3α-(3,4,5-trimethoxybenzoyloxy)-6*β*-(*E*)-(3,4,5-trimethoxycinnamoyloxy)-7*β*-hydroxytropane] (3): white amorphous solid, softened at 46−48 °C; $[\alpha]_D$ −0.6° (*c* 0.18, $CHCl_3$); UV λ_{max} (MeOH) 302.5 nm (log ϵ 4.39); IR ν_{max} (film) 3500, 2944, 1711, 1642, 1585, 1505, 1472, 1416, 1345, 1265, 1220, 1128, 1057, 1004 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 587 (4) [M^+], 375 (6) [M^+ − TmbA], 349 (10) [M^+ − TmcA], 273 (19), 231 (29), 137 (100) [M^+ − TmcA − TmbA]; HREIMS *m/z* found 587.2360, calcd for $C_{30}H_{37}NO_{11}$, 587.2367.

Pervilleine A *N*-oxide [3α-(3,4,5-trimethoxybenzoyloxy)-6*β*-(*E*)-(3,4,5-trimethoxycinnamoyloxy)-7*β*-hydroxytropane *N*-oxide] (4): white amorphous solid, softened at 114 °C, mp 118−121 °C; $[\alpha]_D$ +1.46 ° (*c* 0.28, $CHCl_3$); UV λ_{max} (MeOH) 303 nm (log ϵ 4.24); IR ν_{max} 2944, 1717, 1688, 1588, 1500, 1468, 1420, 1338, 1278, 1220, 1130, 1004, 759 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; CIMS *m/z* 604 (6) [M + H$^+$], 588 (56) [M + H$^+$ − O], 281 (43) [M + H$^+$ − C(6)HTmc − C(7)HOH], 239 (32) [TmcA + H$^+$], 213 (100) [TmbA + H$^+$]; HRFABMS *m/z* found 604.2407 (M^+ + 1), calcd for $C_{30}H_{37}NO_{12}$ + H, 604.2394.

Pervilleine B [3α-(3,4,5-trimethoxybenzoyloxy)-6*β*-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane] (5): white amorphous solid, mp 40−42 °C; $[\alpha]_D$ −22.5° (*c* 0.25, $CHCl_3$); UV λ_{max} (MeOH) 302.5 nm (log ϵ 4.31); IR ν_{max} 2944, 1711, 1641, 1585, 1505, 1472, 1416, 1345, 1220, 1127, 1004 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 571 (50) [M^+], 360 (100) [M^+ − TmbA], 307 (3) [M^+ − C(6)HTmc − C(7)-H_2], 238 (18) [TmcA$^+$], 221 (41) [TmcO$^+$], 212 (22) [TmbA$^+$]; HREIMS *m/z* found 571.2421, calcd for $C_{30}H_{37}NO_{10}$, 571.2417.

Pervilleine C {**3α,6*β*-di-[(*E*)-(3,4,5-trimethoxycinnamoyloxy)]tropane**} **(6)**: white amorphous solid, mp 53−57 °C; $[\alpha]_D$ +29.0° (*c* 0.10, $CHCl_3$); UV λ_{max} (MeOH) 305 nm (log ϵ 4.51); IR ν_{max} 2944, 1711, 1641, 1585, 1504, 1472, 1454, 1345, 1270, 1167, 1127, 915, 745 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 597 (3) [M^+], 360 (100) [M^+ − TmbA], 333 (4) [M^+ − C(6)HTmc − C(7)H_2], 238 (18) [TmcA$^+$], 221 (41) [TmcO$^+$], 212 (22) [TmbA$^+$]; HREIMS *m/z* found 597.2552, calcd for $C_{32}H_{39}NO_{10}$, 597.2574.

Pervilleine D {**3α,6*β*-di-[(*E*)-(3,4,5-trimethoxycinnamoyloxy)]-7*β*-hydroxytropane**} **(7)**: white amorphous solid, mp 59−61 °C; $[\alpha]_D$ +9.0° (*c* 0.10, $CHCl_3$); UV λ_{max} (MeOH) 305 nm (log ϵ 4.50); IR ν_{max} 2944, 1710, 1641, 1585, 1504, 915, 745 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 613 (6) [M^+], 375 (16) [M^+ − TmcA], 333 (2) [M^+ − C(6)-HTmc − C(7)HOH], 238 (29) [TmcA$^+$], 221 (52) [TmcO$^+$], 190 (6), 154 (14), 137 (100) [M^+ − 2TmcA]; HREIMS *m/z* found 613.2526, calcd for $C_{32}H_{39}NO_{11}$, 613.2523.

Pervilleine E [3α-(3-hydroxyphenylacetoxy)-6*β*-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane] (8): white amorphous solid, mp 82−84 °C; $[\alpha]_D$ +28.0° (*c* 0.12, $CHCl_3$); UV λ_{max} (MeOH) 302 nm (log ϵ 4.25); IR ν_{max} 2944, 1710, 1642, 1579, 915 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 511 (77) [M^+], 360 (100) [M^+ − 3 − OHPhCH$_2$-CO$_2$], 247 (39) [M^+ − C(6)HTmc − C(7)H_2], 238 (17) [TmcA$^+$], 221 (39) [TmcO$^+$], 138 (45), 122 (63) [M^+ − TmcA − 3-OHPhCH$_2$CO$_2$]; HREIMS *m/z* found 511.2210, calcd for $C_{28}H_{33}NO_8$, 511.2206.

Pervilleine F [3α-phenylacetoxy-6*β*-(*E*)-(3,4,5-trimethoxycinnamoyloxy)tropane] (9): white amorphous solid, mp 95−97 °C; $[\alpha]_D$ +12.0° (*c* 0.12, $CHCl_3$); UV λ_{max} (MeOH) 304 nm (log ϵ 4.24); IR ν_{max} 2942, 1714, 1640, 1578, 1504, 744 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 495 (73) [M^+], 360 (100) [M^+ − PhCH$_2$CO$_2$], 238 (29) [TmcA$^+$], 231 (49) [M^+ − C(6)HTmcC(7)H_2], 221 (65) [TmcO$^+$], 136 (8) [PhCH$_2$CO$_2$H$^+$], 122 (70) [M^+ − TmcA − PhCH$_2$CO$_2$]; HREIMS *m/z* found 495.2260, calcd for $C_{28}H_{33}NO_7$, 495.2257.

X-ray Crystallographic Analysis of Tropane-3α,6*β*,7*β*-triol 3-Phenylacetate (1). *Crystal Data*: $C_{16}H_{21}NO_4$, M_r = 291.34, monoclinic, space group $P2_1/c$, a = 6.5574(13) Å, b = 26.577(5) Å, c = 8.448(2) Å, β = 102.08(3)°, V = 1439.7(5) Å^3 (by least-squares refinement on diffractometer angles for 16 automatically centered reflections), λ = 1.54178 Å, Z = 4, D_c = 1.344 Mg/m^{-3}, $F(000)$ = 624, μ(Cu Kα) = 0.789 mm^{-1}. Crystal dimensions: 0.5 × 0.2 × 0.2 mm. *Data Collection and Processing:* Three-dimensional, room-temperature (293 K) X-ray data were collected on a Siemens P3 diffractometer with monochromatized Cu Kα X-radiation, using the T mode with scan range (T) 3.33−57.47° plus Kα separation and a variable scan speed (4.88−14.65 min^{-1}). A total of 2158 reflections were measured (3° < 2θ < 115°, min. *hkl* 0 0−9, max. *hkl* 7 29 9); 1968 independent reflections were obtained [$R(F)$ 0.0478, Friedel opposites merged]. No absorption correction was applied. Three control reflections were monitored every 97 reflections and showed no appreciable decay during data collection. *Structure Analysis and Refinement:* Direct methods resulted in the location of all of the non-hydrogen atoms. Full-matrix least-squares refinement with anisotropic thermal parameters was used for all non-hydrogen atoms. Hydrogen atoms were refined in riding mode. Refinement converged at R = 0.0631, R_w = 0.1643. Maximum and mean shift/errors in

the final cycle of refinement were 0.0398 and 0.014, respectively. The final electron-density difference synthesis showed no peaks >0.194 or <−0.198 e Å^3. All computations were carried out using the SHELXTL for IRIS V5.03 system of programs.[25,26]

Bioassay Evaluation. Cytotoxicity Assay. Alkaloids **1**−**9** were evaluated for cytotoxic activity against a panel of human cancer cell lines, including KB-V1 in the presence and absence of VLB, according to established protocols.[8] Similar procedures were used with the ovarian cancer cell lines listed in Table 4.[21]

In Vitro Multidrug-Resistance Assay. Alkaloids **5**−**7** and **9** were evaluated for efficacy as potential modulators of P-glycoprotein according to established protocols.[22,23]

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Supporting Information Available: Tables of crystal data and structure refinement, atomic coordinates, bond lengths and bond angles, anisotropic displacement parameters, and hydrogen coordinates for tropane-3α,6β,7β-triol 3-phenylacetate (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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