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Antiparasitic Compounds from *Cupania cinerea* with Activities against *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense*

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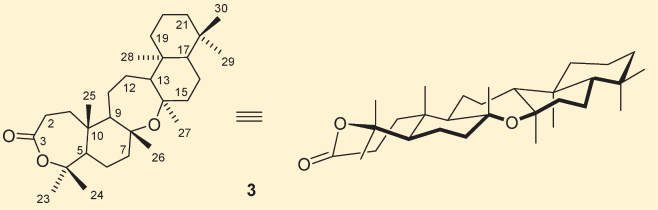
Supporting Information

ABSTRACT: In a survey of plants from Ecuador with antiprotozoal activity, *Cupania cinerea* was found to show significant in vitro activity against the *Plasmodium falciparum* K1 strain and *Trypanosoma brucei rhodesiense*. Subsequently, activity-guided isolation of the *n*-hexane and dichloromethane extracts from the bark of *C. cinerea* afforded two diterpene glycosides (**1** and **2**), named cupacinoside and 6′-de-*O*-acetylcupacinoside, and a lactonized triterpene bearing an oxepin moiety named cupacinoxepin (**3**), together with the known compounds scopoletin (**4**), caryophyllene oxide (**5**), two bisabolane sesquiterpenes (**6** and **7**), lichexanthone (**8**), gustastatin (**9**), lupenone (**10**), betulone (**11**), 17β,21β-epoxyhopan-3-one (**12**), taraxerol (**13**), and taraxerone (**14**). For compound **3**, X-ray crystallography was employed to elucidate the relative configuration. For cupacinosides (**1**) and (**2**) and cupacinoxepin (**3**), in vitro activities against the *P. falciparum* K1 strain (IC_{50} **1**, 1.3; **2**, 1.8; and **3**, 8.7 μM) and *T. b. rhodesiense* (IC_{50} **1**, 4.5; **2**, 15.8; and **3**, 71.6 μM) were found. Cytotoxicity toward L-6 cells is discussed for all the compounds isolated.



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The genus *Cupania* (Sapindaceae) consists of ca. 45 species distributed throughout tropical America from Honduras to Uruguay and in the Caribbean.[1−4] Most *Cupania* species grow as trees or shrubs and are used locally as timber (loblolly tree) in construction, in carpentry, and for the paper industry.[3,5,6] *Cupania cinerea* Poepp. (Kichwa name palo-meta muyu, Spanish guabillo) is a shrub or tree growing at elevations between 0 and 2000 m.[1,7] Information about the traditional uses of *C. cinerea* and its secondary metabolites has not yet been reported. Ethnobotanical and pharmacological properties attributed to species of *Cupania* other than *C. cinerea* refer to the treatment of stomach problems (e.g., dysentery), coughs, and fever, as well as to treat inflammatory, protozoal, and bacterial diseases.[8−16] Common constituents that have been identified in the genus *Cupania* include sterols, triterpenes, diterpene glycosides, and a polyprenol.[9,10,17]

During a recent evaluation of antiprotozoal activity of plants traditionally used for medicine in Ecuador, the *n*-hexane and dichloromethane extracts of the bark of *C. cinerea* showed promising in vitro activity against *Plasmodium falciparum* K1 strain and *Trypanosoma brucei rhodesiense*.[19] To date, antiplasmodial and leishmanicidal activities have been reported from the *n*-hexane extract of the leaves of *Cupania vernalis*, which were attributed to the general cytotoxicity of the diterpene glycosides present in this plant.[14,15] The present article describes the bioassay-guided isolation, structure elucidation, antiprotozoal evaluation, and the assessment of cytotoxicity of three new compounds, namely, cupacinoside (**1**), 6′-de-*O*-acetylcupacinoside (**2**), and cupacinoxepin (**3**), together with the known compounds scopoletin (**4**), caryophyllene oxide (**5**), two bisabolane sesquiterpenes (**6** and **7**), lichexanthone (**8**), gustastatin (**9**), lupenone (**10**), betulone (**11**),

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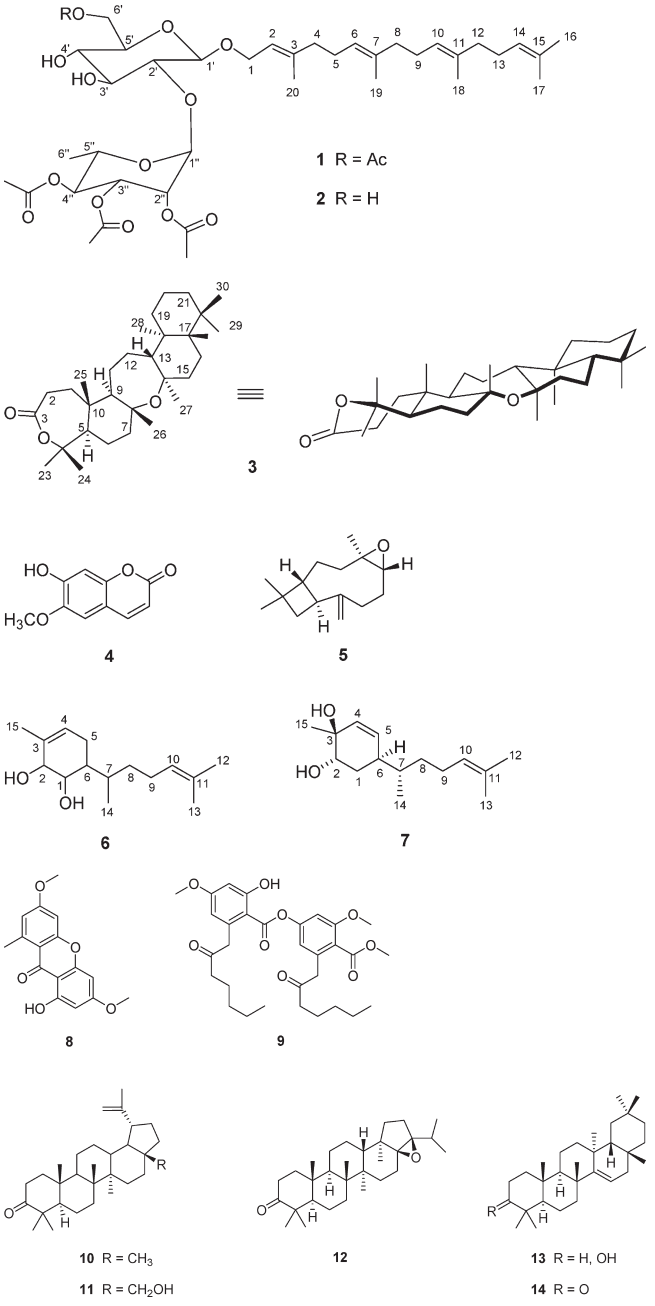
17β,21β-epoxyhopan-3-one (**12**), taraxerol (**13**), and taraxerone (**14**) from *C. cinerea*.



RESULTS AND DISCUSSION

The *n*-hexane and dichloromethane extracts of the bark of *C. cinerea* were subjected to chromatographic separation, which led to the isolation of two new diterpene glycosides (**1**, **2**), a new triterpene (**3**), and 11 known compounds. The identification of the compounds was performed using combined NMR and MS analyses, and in the case of cupacinoxepin (**3**) the structure and the relative configuration were confirmed by X-ray crystallography.

Cupacinoside (**1**) was isolated as a yellowish oil. The positive ion HRESIMS of **1** showed a cationized molecule with sodium at *m*/*z* 789.3975, corresponding to $[M + Na]^+$, and the negative ion ESIMS yielded an $[M + CH_3COO]^-$ ion at *m*/*z* 825.45, thus indicating a molecular formula of $C_{40}H_{62}O_{14}$ (calcd 766.4140). The ^1H, ^{13}C, and HSQC NMR spectra showed the presence of four *O*-acetyl groups and two anomeric protons, indicating the existence of a disaccharide unit, together with five methyl groups reminiscent of the presence of a geranylgeraniol unit. COSY correlations between H-1′, H-2′, H-3′, H-4′, H-5′, and CH_2-6′ as well as between H-2″, H-3″, H-4″, H-5″, and H-6″ together with the analysis of the homonuclear coupling constants allowed the identification of the sugar moieties as glucopyranose and rhamnopyranose units, respectively. The appearance of the proton resonances of H-2′, H-3′, and H-4′ as triplets, together with a large three-bond homonuclear coupling constant, indicated the presence of the β-form of the glucopyranose. Selective irradiation of H-1″ of the rhamnose did not lead to an NOE signal at positions H-3″ and H-5″. Therefore, the orientation of H-1″ is equatorial, as in the α-form of rhamnopyranose. HMBC correlations between H-1′ and C-1 as well as between H-2′ and C-1″ revealed a linkage between the glucopyranose and the isoprene chain and the rhamnopyranosyl moiety, respectively. The positions of the four acetyl groups were assigned by HMBC correlations from H-6′, H-2″, H-3″, and H-4″ to the carbonyl of each of the attached acetyl units. The assignment of the NMR resonances of the side chain led to the identification of the C_{20} isoprenoid unit as geranylgeraniol, which was further confirmed by comparison of the ^1H and ^{13}C NMR data with vernanolide, a geranylgeraniol disaccharide isolated from *C. vernalis*,[17] thus leading to the structure assigned as shown. Compound **1** was given the name cupacinoside. To determine the configuration of the sugars, cupacinoside was subjected to acid hydrolysis and the cleaved sugar moieties were derivatized. The configuration of the sugars was determined to be D-glucose and L-rhamnose using capillary electrophoresis.[18] ^1H and ^{13}C NMR shift values are given in Table 1.

Compound **2** was isolated as a yellowish oil. As with cupacinoside (**1**), the positive ion HRESIMS showed a cationized molecule with sodium at *m*/*z* 747.3824, corresponding to $[M + Na]^+$, whereas the negative ion ESIMS yielded an $[M + CH_3COO]^-$ ion at *m*/*z* 783.42, indicating a molecular formula of $C_{38}H_{60}O_{13}$ (calcd 724.4034). The ^1H, ^{13}C, and HSQC NMR spectra of compound **2** were similar to those of compound **1**, but showed the presence of only three *O*-acetyl groups. The study of HSQC and HMBC correlations, together with NOE data, led to the identification of **2** as shown. The new compound is assigned as 6′-de-*O*-acetylcupacinoside, which differs from compound 1 only by the absence of an acetyl moiety at C-6′ of the glucopyranoside moiety. The ^1H and ^{13}C NMR shift values are shown in Table 1. Relevant mass fragments of compounds **1** and **2** from extensive CID analysis are given in the Supporting Information.

Compound **3** was isolated as white, needle-shaped crystals. The HREIMS showed an $[M]^{+\bullet}$ ion peak at *m*/*z* 458.3758, indicating a molecular formula of $C_{30}H_{50}O_3$ (calcd 458.3760). The ^1H, ^{13}C, and HSQC NMR spectra revealed the presence of a triterpene skeleton. Overlapping signals in the proton spectrum impeded the determination of multiplicities as well as the deduction of complete structural information from NOE experiments, but indicated the presence of a pseudosymmetric structure. HSQC and HMBC analysis confirmed the presence of 30 carbon signals, some of which were overlapping. HMBC correlations based on couplings obtained for the eight methyl groups allowed the establishment of the core structure of the molecule. A correlation from the protons of CH_3-23 and CH_3-24 to a

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1 and 2 (600 and 150 MHz, in $CDCl_3$)

	1		2	
	δ_H mult. (J, Hz)	δ_C	δ_H mult. (J, Hz)	δ_C
isoprene chain				
1	a 4.36 dd (6.8, 11.6)	65.9 t	a 4.37 dd (6.8, 11.6)	66.1 t
	b 4.15 dd (7.6, 11.6)		b 4.14 dd (7.6, 11.6)	
2	5.36 t (7.2)	119.4 d	5.35 t (7.2)	119.4 d
3		141.4 s		141.3 s
4	1.97−2.04[e]	39.7[a] t	1.97−2.04[e]	39.7[a] t
5	2.05−2.10[e]	26.4[b] t	2.05−2.10[e]	26.4[b] t
6	5.11[e]	123.6[c] d	5.10[e]	123.6[c] d
7		135.5[d] s		135.5[d] s
8	1.97−2.04[e]	39.7[a] t	1.97−2.04[e]	39.7[a] t
9	2.05−2.10[e]	26.7[b] t	2.05−2.10[e]	26.6[b] t
10	5.11[e]	124.1[c] d	5.10[e]	124.1[c] d
11		135.0[d] s		135.0[d] s
12	1.97−2.04[e]	39.7[a] t	1.96−2.04[e]	39.7[a] t
13	2.05−2.10[e]	26.7[b] t	2.05−2.10[e]	26.7[b] t
14	5.09[e]	124.4 d	5.10[e]	124.3 d
15		131.3 s		131.3 s
16	1.59 s	17.7 q	1.59 s	17.7 q
17	1.68 s	25.7 q	1.68 s	25.7 q
18	1.59 s	16.0 q	1.59 s	16.0 q
19	1.59 s	16.0	1.59 s	16.0
20	1.69 s	16.5 q	1.69 s	16.5 q
β-glucose				
1′	4.40 d (8.0)	100.3 d	4.43 d (8.0)	100.5 d
2′	3.51 t (8.4)	77.7 d	3.47 t (8.0)	78.2 d
3′	3.64 t (9.2)	77.1 d	3.64 t (9.2)	77.3 d
4′	3.35 t (9.2)	70.1 d	3.54 t (9.2)	70.3 d
5′	3.40[e]	73.7 d	3.32[e]	75.1 d
6′	a 4.47 dd (4.0, 12.0)	63.3 t	a 3.88[e]	62.2 t
	b 4.29 br d (12.0)		b 3.84[e]	
6′-OAc (CH_3)	2.11 s	20.9 q		
6′-OAc (C=O)		172.0 s		
α-rhamnose				
1″	5.18 s	97.9 d	5.16 s	97.9 d
2″	5.31 br s	69.8 d	5.31 dd (1.6, 3.2)	69.9 d
2‴-OAc (CH_3)	2.14 s	21.0 q	2.15 s	21.0 q
2″-OAc (C=O)		170.6 s		170.8 s
3″	5.24 dd (3.2, 10.0)	69.4 d	5.22 dd (3.2, 10.0)	69.4 d
3‴-OAc (CH_3)	1.99 s	20.78 q	2.00 s	20.8 q
3″-OAc (C=O)		170.4 s		170.5 s
4″	5.07[e]	71.0 d	5.06[e]	70.9 d
4‴-OAc (CH_3)	2.03 s	20.8 q	2.04 s	20.8 q
4″-OAc (C=O)		170.0 s		170.0 s
5″	4.27 br s	66.5 d	4.25 dq (6.5, 10.0)	66.5 d
6″	1.16 d (6.4)	17.1 q	1.16 d (6.4)	17.1 q

[a,b,c,d] Interchangeable signals. [e] Signals overlapping.

carbonyl resonance at 175.0 ppm indicated a lactone structure in the A ring. The carbon shifts of C-8 and C-14 (78.8 and 80.4 ppm) together with a missing HMBC correlation from H-26 to C-14, as well as from H-27 to C-8, indicated the insertion of an oxygen atom between C-8 and C-14 in the C ring. Intriguingly, the protons of CH_3-30 (carbon shift at 33.5 ppm) and CH_3-29 showed HMBC correlations to a carbon at 33.5 ppm, indicating that C-22 has the same chemical shift as C-30. The protons CH_3-29 and CH_3-30 showed HMBC correlations to C-17 (56.2 ppm), which were also observed for the protons CH_3-28. Similar HMBC

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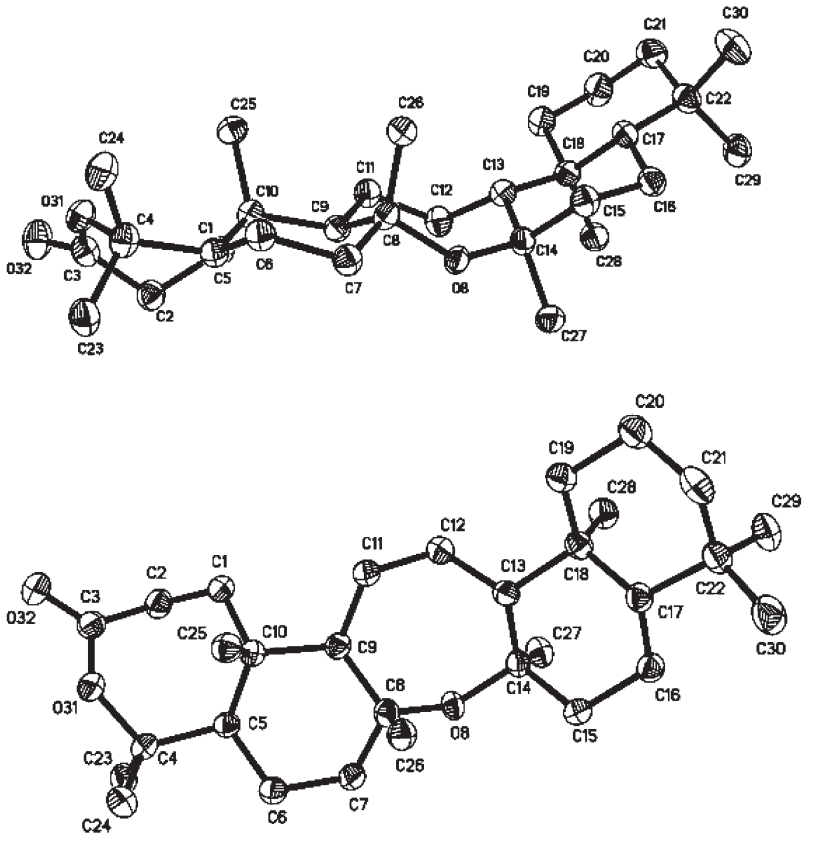

Figure 1. ORTEP plot of one possible enantiomer of compound **3**. Displacement ellipsoids are drawn at a 65% probability level, and hydrogens are omitted for clarity.

correlations were found from the methyl groups at carbons 23, 24, and 25 to C-5 in the A ring. The arrangement of the eight methyl groups, together with an oxygen inclusion in ring C, is similar to that seen in the scaffold of the triterpene onoceranoxide, which is found in ferns of the genus *Lemmaphyllum* and *Polypodium*.[20,21] However, the combination of a seven-membered lactonized A ring and an oxygen inclusion in the C ring is novel. With regard to the relative configuration, NOE correlations between the protons CH_3-28 and CH_3-27, between CH_3-25 and CH_3-24, and between CH_3-26 and CH_3-25 indicated that the respective groups are each located on the same side of the plane given by the annelated rings A−E. Due to the more complex configurations that could be realized in a seven-membered oxepin ring, NOE signals between CH_3-26 and H-13 as well as between CH_3-27 and H-9 could not be used. However, the absence of an NOE correlation between CH_3-26 and CH_3-27 suggests *trans* positioning.

In accordance with Hiroyuki et al.[20] and for biogenetic reasons, it was assumed that the most likely configuration of the rings is an all-*trans* configuration. A *cis* fusion of the rings B−C and C−D, respectively, would also be in agreement with the NMR data. The latter would indicate a pseudosymmetric structure of two enantiomeric subunits, which from a biogenetic viewpoint appears to be unlikely. To settle the question of the relative configuration of compound **3**, an X-ray crystallographic analysis was undertaken. The relative configuration of the stereogenic centers in **3** obtained by X-ray analysis is given in Figure 1. Compound **3** was given the name cupacinoxepin. The ^{1}H and ^{13}C NMR shift values are shown in Table 2.

Compound **6** was isolated as a light yellow oil. HRESIMS yielded a cationized molecule with sodium, $[M + Na]^+$, at m/z 261.1787, and EIMS showed an $[M - H_2O]^{+\bullet}$ ion peak at m/z 220.2, indicating a molecular formula of $C_{15}H_{26}O_2$ (calcd 238.1933). The ^{1}H, ^{13}C, and HSQC NMR spectra indicated the presence of four methyl, three methylene, six methine, including two olefinic protons at 5.13 ppm, and two quaternary carbons. COSY correlations between H-7 and H-8, H-9, and H-10, as well as between H-1 and H-6, and H-5b led to the establishment of the different spin systems. HMBC correlations from H-12 to C-10, C-11, and C-13, from H-14 to C-6, C-7, and C-8, and from H-15 to C-2, C-3, and C-4 were used to assign the positions of the CH_3 groups and the two double bonds formed by C-3 (136.7 ppm) and C-4 (130.0 ppm) as well as C-10 (124.6 ppm) and C-11 (131.4 ppm). The ^{13}C NMR shift values of C-1 (69.2 ppm) and C-2 (68.0 ppm) suggested the presence of a glycol moiety. No further attempts were performed to determine the relative or absolute configuration as the crucial signals, that is, H-1/H-2 were overlapping and suitable reference data could not be found. A database search indicated that compound **6** was mentioned in Wang et al.[24] as a known compound without further citation; however, neither the chemical formula nor ^{1}H and/or ^{13}C NMR shift values could be extracted from internationally accessible journals. The ^{1}H and ^{13}C NMR shift values are therefore given below.

The identity of the known compounds (**4**, **5**, **7**−**14**) was established by NMR and MS data comparison with those given in the literature. For compound **7**, ^{1}H and ^{13}C NMR shift values

Table 2. ^{1}H and ^{13}C NMR Data of Compound 3 (600 and 150 MHz, in $CDCl_3$)

no.	δ_H mult. (*J*, Hz)	δ_C	no.	δ_H mult. (*J*, Hz)	δ_C
1	a 1.66 m	40.2 t	16	a 1.22[a]	20.8 t
	b 1.89[a]			b 1.60[a]	
2	a 2.66 dt (4.2, 14.4)	32.4 t	17	0.87[a]	56.2 d
	b 2.48 dt (3.6, 14.4)		18		39.3 s
3		175.0 s	19	a 1.77[a]	40.5 t
4		85.8 s		b 0.89[a]	
5	1.87[a]	52.1 d	20	a 1.58[a]	18.9 t
6	1.48−1.56[a]	25.9 t		b 1.44[a]	
7	1.74[a]	43.7	21	a 1.36[a]	42.0 t
	1.55[a]			b 1.13 dt (3.6, 13.2)	
8		78.8 s	22		33.5 s
9	1.51[a]	61.2 d	23	1.48 s	30.7 q
10		41.0 s	24	1.36 s	27.3 q
11	a 1.73[a]	26.4 t	25	1.00 s	18.3 q
	b 1.35[a]		26	1.29 s	24.1 q
12	1.84[a]	25.1 t	27	1.25 s	25.4 q
	1.22[a]		28	0.74 s	15.9 q
13	1.41[a]	60.8 d	29	0.77 s	21.6 q
14		80.4 s	30	0.85 s	33.5 q
15	a 1.76[a]	45.3 t			
	b 1.55[a]				

[a] Signals overlapping.

Table 3. Activities of the Compounds 1−14 against *P. falciparum*, *T. b. rhodesiense* and L-6 Cells[a]

	IC_{50}		
sample	*T.b.rhod.*	*P. falc.*	L-6 cells
cupacinoside (**1**)	4.6	1.3	11.6
6′-de-*O*-acetylcupacinoside (**2**)	15.8	2.1	8.7
cupacinoxepin (**3**)	71.6	8.7	>90
scopoletin (**4**)	>90	>25	>90
caryophyllene oxide (**5**)	>90	>25	>90
bisabolane (**6**)	38.2	>20	>90
bisabolane (**7**)	61.3	>20	>90
lichexanthone (**8**)	>90	>20	52.1
gustastatin (**9**)	19.4	>9	>90
lupenone (**10**)	>90	4.7	>90
betulone (**11**)	18.9	3.0	22.2
17β,21β-epoxyhopan-3-one (**12**)	>90	>10	>90
taraxerol (**13**)	10.5	8.5	>90
taraxerone (**14**)	>90	>10	>90
melarsoprol[b]	0.01		
chloroquine[b]		0.22	
podophyllotoxin[b]			0.012

[a] Each sample was tested in two independent assays. Replicate values are within a factor of 2. Data are expressed in μM. [b] Positive control.

and optical rotation data were in agreement with data published by Sy and Brown.[22,23]

The results of the antiplasmodial and antitrypanosomal activities, as well as the cytotoxic effect against L-6 cells of all isolated compounds, are presented in Table 3. Cupacinoside (**1**) and taraxerol (**13**) showed IC_{50} values of <10 μM against *T. b. rhodesiense*, with taraxerol (**13**) exhibiting only low cytotoxicity. This is the first report of antitrypanosomal activity of the well-known triterpene taraxerol. With respect to *P. falciparum*, several compounds showed medium or weak activity, and selectivity was observed only for taraxerol (**13**), lupenone (**10**), and cupacinoxepin (**3**). Cupacinoside (**1**) and 6′-de-*O*-acetylcupacinoside (**2**) exhibited general cytotoxicity, which could be attributed to the surfactant properties due to the presence of geranylgeraniol glycosides.[17,24]

Lichexanthone (**8**) and gustastatin (**9**), both resembling orcinol derivatives, have not yet been described for Sapindaceae. Their structures suggest that these compounds may result from lichen biosynthetic pathways. A detailed analysis of bark segments taken from finely sliced material revealed that lichexanthone and gustastatin occurred only in the very outer layer of the bark. In consideration of the fact that tropical woody species are often covered by epiphytic lichens, we propose that compounds **8** and **9** are actually lichen secondary metabolites.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ or MeOH on a Perkin-Elmer 341 polarimeter. UV/VIS spectra were recorded on a UV-160A Shimadzu spectrophotometer. IR spectra were taken from a sample film on Si using a Perkin-Elmer 281 spectrophotometer. 1D and 2D NMR spectra (^{1}H, ^{13}C, NOE, COSY, HMBC, and HSQC) were recorded at 298 K on a Varian UNITY INOVA-600 MHz spectrometer using $CDCl_3$ or CD_3OD as solvent with TMS as internal standard. HREIMS were measured on a Waters Micromass AutoSpec Ultima magnetic sector instrument. EIMS were recorded on a Hewlett-Packard HP 6890 instrument fitted with a HP 7890 detector. HRESIMS spectra were recorded on an ESI-Qq-TOF mass spectrometer (micrOTOF-Q II, Bruker Daltonics, Bremen, Germany). For all compounds analyzed on this system (**1**, **2**, **6**, and **7**), the expected sum formula was ranked #1 by the SmartFormula algorithm based on the mass accuracy and True Isotope Pattern analysis. Positive and negative ion mode ESIMS^n spectra were obtained on a 3D ion trap mass spectrometer (HCT, Bruker Daltonics). LC-ESIMS was performed in positive ion mode on a Thermo Finnigan LQ Deca XP^{PLUS} mass spectrometer with autosampler using a SB-C18 Zorbax column (3.5 μm; 150 × 2.1 mm; Agilent Technologies) equipped with a guard column at a flow rate of 300 μL/min using an ACN/H_2O (10:90 → 90:10) gradient in water.

X-ray Crystallographic Analysis of Cupacinoxepin (3). Crystals of compound **3** were grown by slow evaporation from a 4:1 (v/v) mixture of MeCN/CH_2Cl_2 in an NMR tube. Crystals formed within 2−3 months. A needle-like crystal measuring 0.06 × 0.06 × 0.9 mm was mounted in a hair loop and flash-cooled in liquid nitrogen, and data were collected at the X12 beamline (EMBL outstation at the DORIS storage ring, DESY, Hamburg) equipped with a MARMosaic 225 mm CCD detector, focusing mirror, and double-crystal Si(111) monochromator set at a wavelength of 0.801 Å. Data sets were processed and scaled with DENZO and SCALEPACK.[25] The crystal was tetragonal, space group $P4_2$, with cell parameters $a = b = 18.293(3)$ Å, $c = 7.391(2)$ Å, $V = 2473.3(7)$ Å^3, $Z = 4$, $\rho_{calc} = 1.232$ g/cm^3. The number of measured and unique reflections was 16686 and 2472, respectively, yielding an overall redundancy of 6.7, a completeness of 97.3%, and a linear R_{merge} of 5.8%. The structure was solved by direct methods using SHELXS and refined using SHELXL.[26] All non-hydrogen atoms were refined with anisotropic temperature coefficients, and the hydrogens are refined with a riding model (i.e., with fixed geometry and isotropic

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temperature coefficients, except for the methyl groups, for which also the proper torsion angle was refined). The final refinement using 2203 reflections with $F > 4\sigma(F)$ or all 2472 unique reflections yielded *R*-factors of $R_1 = 3.6\%$ or 4.2%, respectively, $R_w = 9.5\%$, and an essentially featureless final difference-Fourier map (highest peak 0.17 $e^-/Å^3$, deepest hole −0.22 $e^-/Å^3$). The Flack parameter[27] $x = 0.60(1.28)$ could not be used for the determination of the absolute chirality because of the weak anomalous signal leading to the high uncertainty of the parameter.

The crystallographic data for compound **3** are deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 776126. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Chromatography. Preparative HPLC separations were done using a Varian R PrepStar model SD-1 equipped with a Dynamax R absorbance detector model UV-1 and a Dynamax R solvent delivery system model SD-1. Compound mixtures were separated on VDSpher 100 C18 HPLC preparative column (10 μm, 250 × 25 mm). Semipreparative and analytical HPLC separations were performed using an Agilent 1100 Series system equipped with a diode-array UV detector. Preparative and semipreparative HPLC separations were performed using a VDSpher 100 C18 (7 μm, 250 × 25 mm) and a LiChrosorb RP-18 (7 μm, 250 × 10 mm, Merck, Darmstadt) column, respectively. Analytical HPLC-DAD analysis was performed using a SB-C18 Zorbax column (3.5 μm; 150 × 2.1 mm; Agilent Technologies) equipped with guard column at a flow rate of 300 μL/min using an MeCN/H_2O (10:90 → 90:10) gradient.

Crude fractionation was performed on a fast centrifugal partition chromatograph (FCPC) equipped with a rotor of 200 mL capacity, a high-pressure pump delivering 0.025−25 mL/min, an injection loop of 10 mL, a UV/VIS detector with flow cell, and a fraction collector (FCPC-Kromaton-201, AlphaChrom OHG). For TLC and PTLC analysis, precoated Si60 F254 plates and glass TLC plates silica gel 60 F254 20 × 20 cm (2 mm), both from Merck, Darmstadt, were used. Detection was performed under UV at 254 and 366 nm and by spraying with vanillin−H_2SO_4 reagent and heating. The SPE separations were carried out on Isolute C18 (EC) cartridges of 10 and 2 g from Biotage and AccuBONDII ODC (C18) SPE of 1 g from Agilent Technologies.

Plant Material. *C. cinerea* was collected during August−September 2006 (first collection) and December 2007−January 2008 (second collection) at the commune Pacto, area El Progreso, ca. 10 km off the main road, in a forest gap located close to the Pillallí River, in the province Pichincha, Ecuador, at 00°16′43″ S, 78°56′29″ O. The plant was identified by Carlos Morales Cabrera, and a voucher (No. 0223317) was deposited in the Herbario Nacional del Ecuador in Quito.

***Plasmodium falciparum* in Vitro Assay.** Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H]hypoxanthine incorporation assay was used.[28] Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi of [^{3}H]hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute per well at each drug concentration and expressed as percentage of the untreated controls. From the sigmoidal inhibition curves IC_{50} values were calculated.

***Trypanosoma brucei rhodesiense* in Vitro Assay.** Minimum essential medium with Earle's salts (50 μL) supplemented with 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate, and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. Then, 10^4 bloodstream cell forms of *T. b. rhodesiense* STIB 900 in 50 μL were added to each well, and the plate was incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Resazurin solution (10 μL; 12.5 mg of resazurin dissolved in 100 mL of distilled H_2O) was added to each well, and incubation continued for a further 2−4 h. The plate was read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Corporation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm.[29] Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic program Softmax Pro (Molecular Devices Corporation, Sunnyvale, CA, USA), which was used to calculate IC_{50} values from the sigmoidal inhibition curves.

Cytotoxicity Assay on L-6 Cells. The rat skeletal myoblast cell line (L-6 cells) was used to assess cytotoxicity. The cells were grown in RPMI 1640 medium supplement with 1% L-glutamine (200 nM) and 10% fetal bovine serum at 37 °C in 5% CO_2 under air. The assay was performed in 96-well microtiter plates, each well receiving 100 μL of culture medium with ca. 4×10^4 cells. After 24 h, the medium was removed from all wells, and serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. Each drug was tested in duplicate. After 72 h of incubation, the plates were inspected under an inverted microscope to ensure growth of the controls and sterile conditions. Then, 10 μL of Alamar blue (12.5 mg of resazurin dissolved in 100 mL of distilled H_2O) was added to each well, and the plates were incubated for another 2 h, followed by evaluation of the plates with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Corporation) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. IC_{50} values were determined using the microplate reader software Softmax Pro (Molecular Devices Corporation).

Extraction and Isolation. The dried and powdered bark (1210 g) of *C. cinerea* was extracted subsequently with *n*-hexane, CH_2Cl_2, and MeOH by percolation to yield 10.5, 9.6, and 25.2 g of extract, respectively. The *n*-hexane and CH_2Cl_2 extracts, which showed activity against the *P. falciparum* K1 strain and *T. b. rhodesiense*, were selected for further isolation.

The crude *n*-hexane extract was fractionated on silica gel (VLC) using a gradient of *n*-hexane/EtOAc (each of 450 mL) from 100% *n*-hexane stepwise to 50% EtOAc in *n*-hexane, 100% EtOAc, and 100% MeOH and combined based on TLC analysis to obtain 10 fractions. The activity was found in fractions F-3, F-6, F-7, and F-8, corresponding to 10%, 30%, 40%, and 50% EtOAc in *n*-hexane, respectively. Additionally, from VLC fraction F-4 (536 mg) approximately 100 mg of taraxerol (**13**)[30] precipitated from EtOAc while drying the fraction.

VLC fraction F-3 (1.68 g) was partitioned by FCPC (elution-extrusion mode) using *n*-hexane/EtOAc/MeOH/H_2O (9:1:9:1) in descending mode (switching volume 320 mL, 1000 rpm, 4 mL/min flow rate). On the basis of TLC analysis, 11 fractions were combined. From FCPC fraction 4 (5 mg), 1.0 mg of lichexanthone (**8**)[31,32] was separated from an *n*-hexane and MeOH(aq) mixture (10:1:9). FCPC fraction 5 (57.4 mg) was further fractionated by FCPC (elution-extrusion mode) using MeCN/TBME/*n*-hexane (10:1:10) in descending mode (288 mL, 800 rpm, 4 mL/min), yielding three fractions. Subfraction 2 (46 mg) was further fractionated on PTLC using *n*-hexane/EtOAc (85:15) to yield 3.4 mg of taraxerone (**14**).[30] FCPC fraction 6 (82.2 mg) contained caryophyllene oxide (**5**).[33] From FCPC fraction 7 (56 mg), 13.3 mg of 17β,21β-epoxyhopan-3-one (**12**)[34] precipitated from MeOH. FCPC fractions 9 (152 mg) and 10 (238 mg) were fractionated on SPE-C18 cartridges (1 and 2 g, respectively) using MeCN, MeOH, Et_2O, and *n*-hexane gradient steps. In both cases, subfraction 2 (39 and 75 mg, respectively) was further separated by PTLC using $CHCl_3$/HCOOH (0.1%) to yield 11.8 and 50.5 mg of lupenone (**10**).[35]

From VLC fraction F-6 (283 mg), 7.1 mg of cupacinoxepin (**3**) was obtained as a precipitate from EtOAc and by recrystallization from $CHCl_3$. The remaining material was fractionated by SPE C-18 (2 g) using a MeOH/H_2O gradient from 50:50 to 100:0 and CH_2Cl_2 to yield four subfractions. Subfraction 3 (184 mg) was separated by preparative

HPLC using MeCN/H_2O under isocratic conditions (85:15) to yield 4.9 mg of lichexanthone (**8**)[31,32] (t_R approximately 25 min). The residual HPLC eluent was evaporated (92 mg) and further fractionated by PTLC using *n*-hexane/acetone (8:2), which furnished 26.8 mg of betulone (**11**).[36,37]

From VLC fraction F-7 (409 mg), 4.3 mg of gustastatin (**9**)[38] precipitated from EtOAc. The rest was fractionated on an SPE C-18 (2 g) using a MeOH/H_2O gradient from 50:50 to 100:0 and CH_2Cl_2 to yield seven subfractions. Subfraction 3 (62 mg) was further fractionated by semipreparative HPLC under isocratic conditions using MeCN/H_2O (70:30) to yield again 4.0 mg of gustastatin (**9**)[38] (t_R 50.4 min). Subfraction 5 (78 mg) was fractionated using PTLC with *n*-hexane/acetone (8:2) and afforded 2.0 mg of betulone (**11**).[36,37]

VLC fraction F-8 (969 mg) was partitioned by FCPC (elution-extrusion mode) using *n*-hexane/EtOAc/MeOH-H_2O (6:4:6:4) in ascending mode (270 mL, 800 rpm, 4 mL/min), to yield 11 fractions. FCPC fractions 4 (120 mg) and 5 (79 mg) were separated on an SPE-C18 cartridge (1 g) using a gradient of MeCN/H_2O from 50:50 to 100:0 and MeOH, yielding 4 subfractions each. Subfractions 2 (29 mg) and 3 (74 mg) from FCPC fraction 4 and subfraction 3 (60 mg) from FCPC fraction 5 were separated on semipreparative HPLC using ACN/H_2O under isocratic conditions (85:15) to yield 14.9, 42.0, and 24.3 mg of cupacinoside (**1**) (t_R 17.87−19.19 min), respectively. FCPC fraction 7 (133 mg) was fractionated on an SPE-C18 cartridge (1 g) using an ACN/H_2O gradient from 50:50 to 100:0 and MeOH to yield four fractions. Subfraction 2 (47.3 mg) was further purified by semipreparative HPLC using ACN/H_2O under isocratic conditions (85:15) to furnish 21.1 mg of 6′-de-*O*-acetylcupacinoside (**2**) (t_R 13.37 min).

The crude CH_2Cl_2 extract of *C. cinerea* was fractionated on silica gel (VLC) using a gradient of *n*-hexane/EtOAc (each of 510 mL) from 100% *n*-hexane stepwise to 50% EtOAc in *n*-hexane, 100% EtOAc, and 100% MeOH and combined based on TLC analysis to obtain 12 fractions. The activity was found in fraction F-10 (1.52 g).

Most of VLC fraction F-10 (approximately 1.3 g) was partitioned by FCPC (elution-extrusion mode) using *n*-hexane/EtOAc/MeOH/H_2O (6:4:5:5) in descending mode (210 mL, 800 rpm, 3 mL/min), to yield 12 fractions. FCPC fraction 3 (55 mg) was fractionated using semipreparative HPLC with ACN/H_2O under isocratic conditions (85:15) and yielded 5.8 mg of scopoletin (**4**).[38] FCPC fraction 10 (845 mg) was fractionated by SPE-C18 cartridge (10 g) using MeCN/H_2O (75:25, 100:0), MeOH, and CH_2Cl_2 from which four subfractions were obtained (ca. 400 mg was loaded in the SPE cartridge each time). Subfractions 2 and 3 were further purified. Subfraction 2 (298 mg) was fractionated using preparative HPLC using MeCN/H_2O isocratic at 65:35 to obtain four fractions. Fraction 3 (142 mg) was further fractionated with PTLC using CH_2Cl_2/MeOH (95:5), yielding four fractions. PTLC fraction 3 (40 mg) was finally purified using semipreparative HPLC with MeCN/H_2O isocratic at 91:9 to afford 6.5 mg of 6′-de-*O*-acetylcupacinoside (**2**) (t_R 19.16 min). Subfraction 3 (113 mg) was fractionated with PTLC using CH_2Cl_2/MeOH (95:5) to obtain four PTLC fractions. PTLC fraction 1 (36 mg) was separated using semipreparative HPLC under isocratic conditions of MeCN/H_2O (91:9) to furnish 4.0 mg of cupacinoside (**1**) (t_R 30.23 min) and 4.5 mg of 6′-de-O-acetylcupacinoside (**2**) (t_R 19.93 min).

The remaining VLC fraction F-10 (264 mg) was separated on an SPE C-18 cartridge (2 g) using a MeOH/H_2O gradient from 50:50 to 100:0 and CH_2Cl_2 to yield five fractions. Subfraction 2 (31 mg) was further fractionated by PTLC using *n*-hexane/acetone (8:2) to afford 5.8 mg of compound **6** and 9.5 mg of **7**.

Cupacinoside 1-O-[2″,3″,4″-tri-O-acetyl-α-L-rhamnopyranosyl-(1″→2′)-6′-O-acetyl-β-D-glucopyranosyl]-(2E,7E,12E)-(3,8,13,18)-tetramethyleicosatetra-(2,7,12,17)-ene (**1**): yellowish oil; $[\alpha]_D^{22}$ −61.3 (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.4) nm; IR (film on Si) ν_{max} 3423, 2925, 2362, 1751, 1449, 1370, 1226, 1137, 1077, 1048, 980, 895, 739, 610 cm^{-1}, ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 789.3975 $[M + Na]^+$ (calcd for $C_{40}H_{62}O_{14}Na$, 789.4032); ESIMS *m/z* 825.45 $[M + CH_3COO]^-$ (100).

6′-De-O-acetylcupacinoside 1-O-[2″,3″,4″-tri-O-acetyl-α-L-rhamnopyranosyl-(1″→2′)-β-D-glucopyranosyl]-(2E,7E,12E)-(3,8,13,18)-tetramethyleicosatetra-(2,7,12,17)-ene (**2**): yellowish oil; $[\alpha]_D^{22}$ −49.9 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.4) nm; IR (film on Si) ν_{max} 3422, 1738, 1671, 1626, 1398, 1261, 1152, 1079, 1012, 863, 715 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 747.3824 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_{13}Na$, 747.3926); ESIMS *m/z* 783.42 $[M + CH_3COO]^-$ (100).

Cupacinoxepin (5aR,8aR,9aR,10aS,13bR,14bR,17bS,18bS)-4,4,8a,10a,14b,18b,22,22-octamethylperhydronaphtho[1,2-e]benzo[1,2-b:4,3-c′]dioxepin-3-one (**3**): needle-shaped crystals; $[\alpha]_D^{22}$ +56.4 (*c* 1.3, $CHCl_3$); IR (film on Si) ν_{max} 1734, 1458, 1374, 1127, 965, 889, 816, 738, 610, 566 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HREIMS *m/z* 458.3758 (7) $[M]^{+\bullet}$ (calcd for $C_{30}H_{50}O_3$, 458.3754), 440.3651 (7), 332.2708 (6), 235.1693 (6), 218.2033 (33), 191.1795 (100), 137.1307 (42), 109.0984 (52), 95.0830 (61), 81.0681 (69), 69.0697 (77), 55.0595 (56), 43.0325 (62).

Scopoletin (**4**): light yellow powder, ^1H and ^{13}C NMR ($CDCl_3$) compared with ref 39; GC EIMS $C_{10}H_8O_4$ *m/z* (%) 192.1 (100) $[M]^{+\bullet}$, 177.0 (55) $[M - CH_3]^+$, 164.1 (27), 149.1 (45), 121.1 (18), 69.0 (25).

Caryophyllene oxide (**5**): orange oil; ^1H and ^{13}C NMR ($CDCl_3$) compared with ref 33; GC EIMS $C_{15}H_{24}O$ *m/z* (%) 220.2 (7) $[M]^+$, 202.2 (26) $[M - H_2O]^{+\bullet}$, 187.2 (37), 173.2 (15), 161.2 (37), 91.1 (92), 79.1 (9); 41.1 (100).

1,2-Dihydroxybisabola-3,10-diene (**6**): light yellow oil (5.8 mg); $[\alpha]_D^{22}$ −20.6 (*c* 0.5, $CHCl_3$); IR (film on Si) ν_{max} 3262, 2961, 2913, 2361, 1450, 1377, 1312, 1066, 1027, 985, 885, 818, 739, 611 cm^{-1}; ^1H NMR (CDCl, 600 MHz) δ 5.54 (1H, s, H-4), 5.13 (1H, br t, *J* = 6.0 Hz, H-10), 3.97 (1H, overlapped, H-2), 3.96 (1H, overlapped, H-1), 2.03 (2H, overlapped, H-9), 1.99 (1H, m, H-7), 1.81 (3H, s, H-15), 1.73 (1H, m, H-5), 1.68 (3H, s, H-12), 1.65 (1H, overlapped, H-6), 1.61 (3H, s, H-13), 1.45 (1H, dt, *J* = 3.6, 13.8 Hz, H-6), 1.33 (2H, overlapped, H-8), 0.82 (3H, d, *J* = 7.2 Hz, H-14); ^{13}C NMR (CDCl, 150 MHz) δ 136.7 (CH, C-3), 131.4 (C, C-11), 130.0 (CH, C-4), 124.6 (CH, C-10), 69.2 (CH, C-1), 68.0 (CH, C-2), 40.6 (CH, C-6), 35.2 (CH_2, C-8), 30.5 (CH, C-7), 29.8 (CH_2, C-5), 26.1 (CH_2, C-9), 25.7 (CH_3, C-12), 20.5 (CH_3, C-15), 17.7 (CH_3, C-13), 14.4 (CH_3, C-14); EIMS $C_{15}H_{26}O_2$ *m/z* (%) 220.2 (3) $[M - H_2O]^{+\bullet}$, 202.1 (18) $[M - 2H_2O]^{+\bullet}$, 145.1 (27), 132.1 (68), 119.1 (100), 109.1 (68), 91.1 (43), 69.1 (46), 55.1 (32), 41.1 (52); HRESIMS *m/z* 261.1787 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1825). The compound is mentioned in the abstract of Wang et al.[22]

2,3-Dihydroxybisabola-4,10-diene (**7**): slightly yellow oil (9.5 mg); $[\alpha]_D^{22}$ −62.1 (*c* 0.8, $CHCl_3$); IR (film on Si) ν_{max} 3385, 2925, 2361, 2342, 1717, 1653, 1455, 1378, 1065, 885, 737, 611, 566 cm^{-1}; ^1H and ^{13}C NMR data in very good agreement with ref 22; EIMS $C_{15}H_{26}O_2$ *m/z* (%) 220.2 (4) $[M - H_2O]^{+\bullet}$, 202.2 (4) $[M - 2H_2O]^{+\bullet}$, 177.2 (39), 151.1 (19), 135.1 (36), 119.1 (30), 109.1 (100), 93.1 (47), 81.1 (35), 69.1 (94), 55.1 (37), 41.1 (72); HRESIMS *m/z* 261.1770 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1825).

Lichexanthone (**8**): white powder; ^1H and ^{13}C NMR ($CDCl_3$) compared with refs 31 and 34; GC EIMS $C_{16}H_{14}O_5$ *m/z* (%) 286.1 (100) $[M]^{+\bullet}$, 257.1 $[M - CHO]^+$ (50), 243.0 (9), 227.1 (6), 129.0 (7).

Gustastatin (**9**): slightly yellow powder; ^1H and ^{13}C NMR ($CDCl_3$) compared with ref 38; ESIMS $C_{31}H_{40}O_9$ *m/z* (%) 557.12 (13) $[M + H]^+$, 574.05 (100) $[M + NH_4]^+$, 579.04 (7) $[M + Na]^+$, 1129.78 (95) $[2\ M + NH_4]^+$.

Lupenone (**10**): white powder; ^1H and ^{13}C NMR ($CDCl_3$) compared with ref 35; GC EIMS $C_{30}H_{48}O$ *m/z* (%) 424.4 (52) $[M]^{+\bullet}$, 409.5 (36) $[M - CH_3]^+$, 313.3 (38), 245.2 (30), 205.2 (100), 189.2 (55), 109.1 (88), 95.5 (86), 81.1 (79).

Betulone (**11**): pale yellow powder; ^1H and ^{13}C NMR ($CDCl_3$) compared with refs 35 and 37; GC EIMS $C_{30}H_{48}O_2$ *m/z* (%) 440.4 (19)

$[M]^{+\bullet}$, 409.4 (100) $[M - CH_2OH]^+$, 315.3 (12), 286.1 (15), 245.2 (43), 203.2 (93).

17β,21β-Epoxyhopan-3-one (**12**): white powder; ^1H and ^{13}C NMR ($CDCl_3$) compared with ref 34; GC EIMS $C_{30}H_{48}O_2$ *m/z* (%) 440.4 (20) $[M]^{+\bullet}$, 425.1 (4) $[M - CH_3]^+$, 397.2 (10), 281.1 (24), 207.0 (100).

Taraxerol (**13**): white needle crystals; ^1H and ^{13}C NMR ($CDCl_3$) compared with ref 30; GC EIMS $C_{30}H_{50}O$ *m/z* (%) 426.4 (18) $[M]^{+\bullet}$; 411.4 (14) $[M - CH_3]^+$, 302.3 (45), 204.2 (100).

Taraxerone (**14**): yellowish powder; ^1H and ^{13}C NMR ($CDCl_3$) compared with ref 30; GC EIMS $C_{30}H_{48}O$ *m/z* (%) 424.4 (37) $[M]^{+\bullet}$, 409.4 (27) $[M - CH_3]^+$, 300.1 (98), 204.2 (100).

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of compounds **1**−**3**, **6**, and **7**. This information is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Stevens, W. D., Ulloa Ulloa, C., Pool, A., Montiel, O. M. *Flora de Nicaragua*, Vol. 85; Missouri Botanical Garden Press: St. Louis, MO, 2001; pp 2310−2311.

(2) Hueck, K., Seibert, P. *Vegetationskarte von Südamerika, Mapa de la Vegetación de America del Sur, Vol.* IIa; Gustav Fischer Verlag: Stuttgart, 1981; pp 1−66.

(3) Mabberley, D. J. *The Plant-Book*; Cambridge University Press: Cambridge, 1997; p 200.

(4) Klaassen, R. *Wood Anatomy of the Sapindaceae*, Vol. Supplement 2; IAWA Journal: Leiden, 1999; pp 11−17, 80, 164−166.

(5) De la Torre, L.; Navarrete, H.; Muriel, P.; Macía, M. J.; Balslev, H. *Enciclopedia de las Plantas Útiles del Ecuador*; Pontificia Universidad Católica del Ecuador, Universidad de Aarhus: Quito, 2007; p 566.

(6) Little , E. L., Jr.; Dixon, R. G. *Árboles comunes de la Provincia de Esmeraldas-Ecuador*; Programa de las Naciones Unidas para el desarrollo y la Organización de la Naciones Unidas para la Agricultura y la Alimentación: Roma, 1983; pp 330−331.

(7) Jørgensen, P. M., León-Yánez, S. *Catalogue of the Vascular Plants of Ecuador. Monogr. Syst. Bot.* Vol. 75; Missouri Botanical Garden, 1999; pp 1−1182.

(8) Morton, J. F. *Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan*; CC Thomas Publisher: Springfield, IL, 1981; pp 487−488.

(9) Setzer, W. N.; Vogler, B.; Schmidt, J. M.; Petty, J. L.; Haber, W. A. *Planta Med.* **2005**, *71*, 686–688.

(10) Sakane, W.; Hara, N.; Fujimoto, Y.; Takaishi, Y.; Acuna, R.; Osorio, C.; Duque, C. *Chem. Pharm. Bull.* **2005**, *53*, 1037–1039.

(11) Zamora-Martinez, M. C.; de Pascual Pola, C. N. *J. Ethnopharmacol.* **1992**, *35*, 229–257.

(12) De Lima, M. R. F.; de Souza Luna, J.; dos Santos Aldenir, F.; Caño de Andrade, M. C.; Goulart Sant'Ana, A. E.; Genet, J.-P.; Marquez, B.; Neuville, L.; Moreau, N. *J. Ethnopharmacol.* **2006**, *105*, 137–147.

(13) Napolitano, D. R.; Mineo, J. R.; de Souza, M. A.; de Paula, J. E.; Espindola, L. S.; Espindola, F. S. *J. Ethnopharmacol.* **2005**, *99*, 37–41.

(14) De Mesquita, M. L.; Desrivot, J.; Bories, C.; Fournet, A.; de Paula, J. E.; Grellier, P.; Espindola, L. S. *Mem. I. Oswaldo Cruz* **2005**, *100*, 783–787.

(15) De Mesquita, M. L.; Grellier, P.; Mambu, L.; de Paula, J. E.; Espindola, L. S. *J. Ethnopharmacol.* **2007**, *110*, 165–170.

(16) Peraza-Sanchez, S. R.; Cen-Pacheco, F.; Noh-Chimal, A.; May-Pat, F.; Sima-Polanco, P.; Dumonteil, E.; Garcia-Miss, M. R. *Fitoterapia* **2007**, *78*, 315–318.

(17) Cavalcanti, S. B. T.; Teles, H. L.; Silva, D. H. S.; Furlan, M.; Young, M. C. M.; Bolzani, V. S. *J. Brazil. Chem. Soc.* **2001**, *12*, 413–416.

(18) Stefansson, M.; Novotny, M. *J. Am. Chem. Soc.* **1993**, *115*, 11573–11580.

(19) Gachet, M. S.; Salazar Lecaro, J.; Kaiser, M.; Brun, R.; Navarrete, H.; Muñoz, R. A.; Bauer, R.; Schühly, W. *J. Ethnopharmacol.* **2010**, *128*, 184–197.

(20) Hiroyuki, A.; Kenji, S.; Kazuo, M. *Chem. Pharm. Bull.* **1982**, *30*, 2272–2274.

(21) Arai, Y.; Yamaide, M.; Yamazaki, S.; Ageta, H. *Phytochemistry* **1991**, *30*, 3369–3377.

(22) Wang, L. Y.; Zhang, M.; Zhang, C.-F.; Wang, Z.-T. *Acta Pharm. Sin.* **2008**, *43*, 724–727.

(23) Sy, L.-K.; Brown, G. D. *Phytochemistry* **1997**, *45*, 537–544.

(24) Holmberg, K. *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 148–159.

(25) Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307–326.

(26) Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112–122.

(27) Flack, H. D. *Acta Crystallogr. A* **1983**, *39*, 876–881.

(28) Matile, H.; Pink, J. R. L. . In *Immunological Methods*; Lefkovits, I., Pernis, B., Ed.; Academic Press: San Diego, 1990; pp 221−234.

(29) Räz, B.; Iten, M.; Grether-Buhler, Y.; Kaminsky, R.; Brun, R. *Acta Trop.* **1997**, *68*, 139–147.

(30) Sakurai, N.; Yaguchi, Y.; Inoue, T. *Phytochemistry* **1986**, *26*, 217–219.

(31) Jimenez, C.; Marcos, M.; Villaverde, M. C.; Riguera, R.; Castedo, L.; Stermitz, F. *Phytochemistry* **1989**, *28*, 1992–1993.

(32) Carvalho, A. E.; Alcantara, G. B.; Oliveira, S. M.; Micheletti, A. C.; Honda, N. K.; Maia, G. *Electrochim. Acta* **2009**, *54*, 2290–2297.

(33) Harper, J. K.; McGeorge, G.; Grant, D. M. *Magn. Reson. Chem.* **1998**, *36*, S135–S144.

(34) Kakuda, R.; Iijima, T.; Yaoita, Y.; Machida, K.; Kikuchi, M. *Phytochemistry* **2002**, *59*, 791–794.

(35) Carpenter, R. C.; Sotheeswaran, S.; Sultanbawa, M. U. S.; Ternai, B. *Org. Magn. Reson.* **1980**, *14*, 462–465.

(36) Sholichin, M.; Yamasaki, K.; Kasai, R.; Tanaka, O. *Chem. Pharm. Bull.* **1980**, *28*, 1006–1008.

(37) Núñez, M. J.; Reyes, C. P.; Jiménez, I. A.; Moujir, L.; Bazzocchi, I. L. *J. Nat. Prod.* **2005**, *68*, 1018–1021.

(38) Pettit, G. R.; Zhang, Q.; Pinilla, V.; Herald, D. L.; Doubek, D. L.; Duke, J. A. *J. Nat. Prod.* **2004**, *67*, 983–985.

(39) Razdan, T. K.; Qadri, B.; Harkar, S.; Waight, E. S. *Phytochemistry* **1987**, *26*, 2063–2069.