Geranins A and B, New Antiprotozoal A-Type Proanthocyanidins from *Geranium niveum*†

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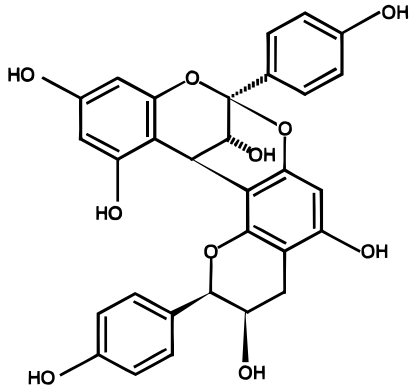
Bioassay-guided fractionation of the antiprotozoal extract of *Geranium niveum* led to the isolation of two new A-type proanthocyanidins, *epi*-afzelechin-(4β→8,2β→*O*→7)-afzelechin (**1**) and *epi*-catechin-(4β→8,2β→*O*→7)-afzelechin (**2**). Compounds **1** and **2** were given the trivial names of geranins A and B, respectively. In addition, five known compounds, mahuannin B (**3**), reynoutrin (**4**), hyperin (**5**), methyl gallate (**6**), and 3-β-caffeoyl-12-oleanen-28-oic acid (**7**), were obtained. The structures of the new proanthocyanidins were elucidated by spectroscopic and chemical methods as well as CD measurements. Compounds **1**, **2**, and **4**−**7** were tested against axenically grown trophozoites of *Giardia lamblia* and *Entamoeba histolytica*.

Geranium niveum S. Watson (Geraniaceae) is a silvery canescent-leaved herb which grows along the dry stream banks and grassy meadows of the pine−oak forests in the high mountains of western Chihuahua, Mexico. The Tarahumara Indians call this perennial herb "makiki" and employ the decoction of the roots as an antifebrile, a purgative, and as a remedy for kidney pain.[1−3]

In a preliminary screening conducted to evaluate the antiprotozoal activity of some Mexican medicinal plants we found that a $CHCl_3$−MeOH (1:1) extract prepared from the roots of *G. niveum* exhibited antiprotozoal activity against axenically grown trophozoites of *Giardia lamblia* and *Entamoeba histolytica*.[4] In this paper we describe the isolation and characterization of the major antiprotozoal substances from the roots of *G. niveum*.



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Results and Discussion

A sample of dry *G. niveum* roots was exhaustively extracted with $CHCl_3$−MeOH (1:1). The crude active extract was subjected to sequential solvent partition with $CHCl_3$ and EtOAc. The resulting fractions were tested for their ability to inhibit the growth of trophozoites of *G. lamblia* and *E. histolytica*.[4−6] The highest level of activity was found in the EtOAc fraction (see Experimental Section). The active fraction was separated by sequential column chromatography using Si gel with increasing solvent polarity, gel permeation (Sephadex LH-20) and HPLC to yield three A-type proanthocyanidins, *epi*-afzelechin-(4β→8,2β→*O*→7)-afzelechin (**1**), *epi*-catechin-(4β→8,2β→*O*→7)-afzelechin (**2**), and mahuannin[7] B (**3**), two flavonoids, reynoutrin[8] (**4**) and hyperin[9] (**5**), as well as methyl gallate[10] (**6**) and 3-β-caffeoyl-12-oleanen-28-oic acid[11] (**7**). Compounds **1** and **2** are new natural products and were given the trivial names of geranin A and B, respectively. The structures of the known compounds were ascertained by comparison of their physical and spectroscopic properties with those reported in the literature.

Compound **1** was isolated as a red powder and responded positively to the vainillin−sulfuric acid reagent. The molecular formula was determined as $C_{30}H_{24}O_{10}$ on the basis of the ion peak at *m*/*z* 545 $[M + 1]^+$ in the positive FABMS (NBA), NMR (Tables 1 and 2), and elemental analysis data. Upon methylation with dimethyl sulfate compound **1** afforded the pentamethyl derivative, **1a**. The NMR spectra of **1** were similar to those of other A-type proanthocyanidins.[12−14] The ^{1}H NMR spectrum (Table 1) displayed the characteristic signals for the dihydropyran rings of the upper (U) and terminal (T) flavan-3-ol units. The resonances for upper dihydropyran ring appeared as an AB system [δ 4.08 (H-3U) and 4.26 (H-4U), each 1H, each d, J = 3.5 Hz], and those of the terminal unit were observed at δ 4.80, 1H, d, J = 8.0 Hz (H-2 T); 4.17, 1 H, ddd, J = 8.5, 8.0, and 5.5 Hz (H-3T); 2.93, 1H, dd, J = 16.5, 5.5 Hz (H-4Ta), and 2.58, 1H, dd, J = 16.5, 8.5 Hz (H-4Tb). The aromatic region of this spectrum exhibited two A_2B_2 and one AB system for two *p*-disubstituted and one tetrasubstituted benzene rings, respectively. Finally, a singlet, consistent with the presence of a pentasubstituted benzene, was observed at δ 6.10 (H-6T). The ^{13}C NMR data (Table 2) and HMQC correlations supported the above

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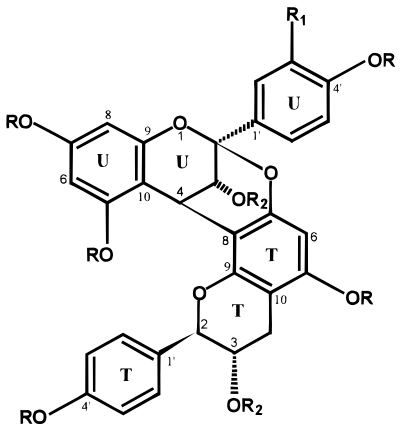

	R	R_1	R_2
1	H	H	H
1a	Me	H	H
1b	Me	H	(*S*)-MTPA
1c	Me	H	(*R*)-MTPA
2	H	OH	H
2a	Me	OMe	H
2b	Me	OMe	(*S*)-MTPA
2c	Me	OMe	(*R*)-MTPA

assignments. The two flavonoid moieties can be linked to build up the basic skeleton of an A-type proanthocyanidin through three type of linkages, namely (4→8,2→*O*→7); (4→6,2→*O*→5); and (4→6,2→*O*→7). A NOESY experiment allowed us to discriminate between these possibilities; thus, the correlations H-4U/H-2T, H-3U and H-6U/H-2′T, H-6′T defined the (4→8,2→*O*→7) linkage. It is important to point out that the correlations H-2T/H-3U and H-4U were also observed in the COSY spectrum of **1**. Additional evidence for the proposed linkage was deduced from the correlations OMe-5U/H-6U, H-4U, H-2′T, H-6′T, and H-2T observed in the NOESY spectrum of **1a**. An HMBC experiment correlated the proton spin systems and the carbon skeleton. The next step was then to assign the stereochemistry at the chiral centers. In the case of carbons 2U and 4U, the absolute stereochemistry was determined by CD measurements.[15] The CD spectrum of geranin A (**1**) showed a strong positive Cotton effect at 220 nm ($[\theta] = 3.26 \times 10^3$); this observation indicated *R* configuration at C-4U. The absolute stereochemistry at C-3U and C-3T was established using Mosher ester methodology.[13,16,17] The *R*-(+)- and *S*-(−)-MTPA esters **1c** and **1b**, respectively, were prepared from the methyl derivative **1a** using standard procedures.[18] The analysis of the $\Delta\delta_H(R\text{-}S)$ data (Table 3) of the *R*-(+)- and *S*-(−)-MTPA esters **1c** and **1b** showed positive differences for H-2′, 6′U [$\Delta\delta_H(R\text{-}S) = +0.13$] and H-3′,5′U [$\Delta\delta_H(R\text{-}S) = +0.19$] and a negative difference for H-6U [$\Delta\delta_H(R\text{-}S) = -0.12$] indicated that the absolute stereochemistry of the chiral center at C-3U was *R*. Thereafter, the absolute configuration at C-2U was automatically assigned as *S*. The positive difference found for H-4T*ax* [$\Delta\delta_H(R\text{-}S) = +0.13$] and the negative differences for H-2T [$\Delta\delta_H(R\text{-}S) = -0.10$] and H-2′,6′T [$\Delta\delta_H(R\text{-}S) = -0.10$] revealed that the absolute stereochemistry of the chiral center at C-3T was *S*. Thus, the absolute configuration at C-2T was determined as *R* because of the trans relationship between H-3T and H-2T. Accordingly, the structure for this new proanthocyanidin, designated as geranin A, was proposed to be *epi*-afzelechin-(4*β*→8,2*β*→*O*→7)-afzelechin (**1**).

Molecular mechanic calculations using the PCMODEL program were carried out to obtain additional stereostructural features of compound **1**. The minimum energy

Table 1. ^{1}H NMR (500 MHz) Chemical Shifts for Compounds **1** and **2** in MeOH-d_4[a]

ring/protons	**1**	**2**
U 3	4.08 d (3.5)	4.08 d (3.5)
4	4.26 d (3.5)	4.25 d (3.5)
6	5.96 d (2.5)	5.94 d (2.5)
8	6.08 d (2.5)	6.06 d (2.5)
2′	7.50 d (9.0)	7.12 d (1.85)
3′	6.82 d (9.0)	—
5′	6.82 d (9.0)	6.79 d (8.25)
6′	7.50 d (9.0)	7.0 dd (8.25, 1.85)
T 2	4.80 d (8.0)	4.80 d (8.0)
3	4.17 ddd (5.5, 8.0, 8.5)	4.16 ddd (5.6, 8.0, 8.4)
4a	2.58 dd (8.5, 16.5)	2.57 dd (8.4, 16.4)
4b	2.93 dd (5.5, 16.5)	2.93 dd (5.6, 16.4)
6	6.10 s	6.08 s
2′	7.29 d (8.4)	7.29 d (8.4)
3′	6.83 d (8.4)	6.81 d (8.4)
5′	6.83 d (8.4)	6.81 d (8.4)
6′	7.29 d (8.4)	7.29 d (8.4)

[a] Values in parentheses are *J* in Hz.

Table 2. ^{13}C NMR (125 MHz) Chemical Shifts for Compounds **1** and **2** in MeOH-d_4

ring/carbon	**1**	**2**
U 2	100.45	100.33
3	67.70	67.82
4	29.08	29.22
5	156.70	156.77
6	98.16	98.11
7	159.05	158.89
8	96.56	96.58
9	154.25	154.24
10	104.02	104.02
1′	131.64	132.28
2′	129.98	115.52
3′	115.54	145.63
4′	158.79	146.77
5′	115.54	116.29
6′	129.98	115.52
T 2	84.21	84.30
3	68.05	68.10
4	29.20	29.22
5	156.04	156.12
6	96.64	96.58
7	152.13	152.18
8	106.82	106.82
9	151.43	151.44
10	103.21	103.19
1′	129.81	129.91
2′	130.11	130.13
3′	116.33	116.29
4′	158.69	158.79
5′	130.11	130.13
6′	116.33	116.29

conformation of **1** represented by **1-*dieq*** in Figure 1 had E_{MMX}= 45.8 kcal mol^{-1}. A second local minimum was found for this compound at E_{MMX} = 47.1 kcal mol^{-1} corresponding to the conformation depicted in **1-*diax***, where the phenyl and hydroxyl group of the T-pyran ring are pseudoaxial. If it is assumed that $\Delta E_{MMX} \cong \Delta G° = 1.3$ kcal mol^{-1} and using the $\Delta G° = -RT(\ln K)$ equation, it can be calculated that $K = 9.0$ at 25 °C and, therefore, conformer **1-*diax*** only contributes with ca. 10% to the conformational equilibrium. However, in the pentamethyl derivative **1a**, conformer **1a-*diax*** becomes more significant being present in ca. 30%, as calculated from E_{MMX} = 63.1 kcal mol^{-1} for **1a-*dieq*** E_{MMX} = 63.6 kcal mol^{-1} for **1a-*diax*** ($\Delta E_{MMX} \cong \Delta G° = 0.5$ kcal mol^{-1}; $K = 2.3$). This phenomenum can be experimentally observed, since the coupling constant $J_{2T,3T}$ changes from 8.0 Hz in **1** to 6.5 Hz in **1a**. The calculated $J_{2T,3T}$ values for conformers **1a-*dieq*** and **1a-*diax*** using the PCMODEL program are 9.0 and 1.7 Hz, respectively.

Table 3. Partial ^{1}H NMR Data of the (*S*)- and (*R*)-Mosher Esters of **1** and **2**

	1				**2**			
protons	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta_{R-S}$	carbinol config	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta_{R-S}$	carbinol config
4U	5.02	5.03	+0.01	*R*	4.98	4.99	+0.01	*R*
6U	6.08	5.96	−0.12	*R*	6.09	5.97	−0.12	*R*
8U	6.26	6.22	−0.04	*R*	6.28	6.24	−0.04	*R*
OMe-5U	3.26	3.23	−0.03	*R*	3.35	3.34	−0.01	*R*
2′, 6′U	7.45	7.58	+0.13	*R*	*a*	*a*	−	−
3′U	6.71	6.90	+0.19	*R*	−	−	−	−
-5′U	6.71	6.90	+0.19	*R*	6.80	6.89	+0.09	*R*
3′5′T	6.85	6.77	−0.08	*S*	6.93	6.84	−0.09	*S*
2′,6′T	7.25	7.15	−0.10	*S*	7.26	7.15	−0.11	*S*
2T	5.26	5.16	−0.10	*S*	5.05	5.03	−0.02	*S*
4Tax	2.71	2.84	+0.13	*S*	2.83	2.97	+0.15	*S*

[a] Overlapped with ester signals.

Table 4. Antiprotozoal Activity of the MeOH−$CHCl_3$ (1:1) Extract and Compounds from *G. niveum*

	IC$_{50}$ μg/mL (CI)[a]	
compound	*G. lamblia*	*E. histolytica*
extract	20.6 (20.7−20.5)	8.7 (8.9−8.5)
1	2.4 (2.6−2.1)	184.7 (186.1−183.4)
2	6.0 (7.0−5.9)	13.6 (14.0−13.0)
5	85.1 (97.2−80.1)	108.9 (109.1−105.6)
6	49.2 (49.3−49.1)	143.6 (185.9−110.9)
7	31.2 (31.3−31.0)	22.6 (23.6−22.5)
8	ND	19.25 (19.5−18.7)
metronidazole[b]	0.21	0.04

[a] CI = 95% confidence intervals. [b] Positive control.

Therefore, the calculated value for the averaged coupling constant in the conformational equilibrium between 70% **1a-*dieq*** and 30% **1a-*diax*** is $J_{2T,3T} = 6.8$ Hz, which is very close to the experimental value of 6.5 Hz. To explain why the conformations of **1a** are closer in energy than the corresponding conformations of **1**, the interatomic distances between the carbon atom of the methoxyl group at C-5U and those of the phenyl ring at C-2T were calculated. Surprisingly, as exemplified in Figure 1, it was found that both groups were closer in **1a-*dieq*** than in **1a-*diax*.** Thus, although **1a-*dieq*** is the more stable conformer, **1a-*diax*** is present in important amounts in the dynamic equilibrium because of some steric hindrance being released when **1a-*dieq*** interconverts to **1a-*diax***. These characteristic features in **1** and **1a** can be useful for the stereochemical analysis of similar substances as it has been pointed out in a related work.[19]

The minimum energy structures of the Mosher diesters **1b** and **1c** were also calculated. As expected, the α-methoxy-α-(trifluoromethyl)phenylacetic acid moieties closely resembled the characteristic conformations found in the Mosher models,[16] allowing an easy interpretation of the results listed in Table 3.

Geranin B (**2**) was isolated as a chestnut powder. The positive FAB spectrum of compound **2** exhibited the $[M + H]^+$ peak at *m/z* 561. Thus, the *quasi*-molecular ion of **2** was 16 units larger than that of compound **1**; this difference suggested the presence of one additional hydroxyl group in **2**. The CD spectrum of **2** also revealed a positive cotton effect near 220 nm, indicating that the absolute configuration at C-4U was the same as in **1**. The 1D NMR (Tables 1 and 2) as well as the NOESY and HMBC spectra were almost identical to those of **1** and therefore, consistent with an A-type proanthocyanidins possessing a (4→8,2→*O*→7) interflavanyl linkage. The main differences between the NMR spectra of compound **1** and those of **2** were the signals attributable to the aromatic ring at C-2U. In the case of compound **2**, the proton signals for the benzene ring at C-2U were not observed as an A_2B_2 pattern but as an ABX spin system at δ 7.12 [d, J = 1.85 Hz (H-2′U)], 7.0 [dd, J = 8.25 and 1.85 Hz (H-6′U)] and 6.79 [d, J = 8.25 Hz (H-5′U)], consistent with a trisubstituted benzene ring. Moreover, the HMBC correlations observed for the aromatic nuclei of the rings at C-2U provided additional support for the substitution pattern proposed for such a ring in compound **2**. The absolute stereochemistry of the stereogenic centers C-3U and C-3T was also determined by analysis of the ^{1}H NMR (Table 3) of the di-(*S*)- (**2b**) and di-(*R*)- (**2c**) Mosher esters of the permethyl derivative **2a**. The positive value ($\Delta\delta_{R-S}$) obtained for H5′U [$\Delta\delta_H(R\text{-}S)$ = +0.09] as well as the negative value ($\Delta\delta_{R-S}$) calculated for H-6U [$\Delta\delta_H(R\text{-}S) = -0.12$] indicated an *R* stereochemistry at C-3U. In the case of C-3T the stereochemistry was established as *S* considering the positive difference found for H-4T*ax* [$\Delta\delta_H(R\text{-}S) = +0.13$] and the negative differences for H-2T [$\Delta\delta_H(R\text{-}S) = -0.02$] and H-2′,6′T. [$\Delta\delta_H(R\text{-}S) = -0.11$]. The stereochemistry at C-2U and C-2T was automatically assigned as *S* and *R*, respectively, following the same rationality as for compound **1**. On the basis of the above evidence, the structure for proanthocyanidin **2** was proposed as *epi*-catechin-(4β→8,2β→*O*→7)-afzelechin.

Table 3 summarizes the antiprotozoal activity data for some of the isolated compounds, of which the most active were the proanthocyanidins **1** and **2**. In both cases, *G. lamblia* was the most sensitive protozou. To our knowledge, this is the first report of antiprotozoal properties for this type of compound. It is important to point out that none of the geranins displays cytotoxic activity against three different cell lines (MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, and A-549 lung carcinoma). Finally, our findings could provide some scientific support for the ethnomedical use of the roots of this species.

Experimental Section

General Experimental Procedures. IR spectra were obtained in KBr disk on a Perkin-Elmer 599 B spectrophotometer. NMR spectra were recorded on a Varian VXR-500 S spectrometer. HMBC and HMQC spectra were obtained at 500/125 MHz. Melting points were determined using a Fisher Johns apparatus and are uncorrected. CD spectra were taken on a JASCO 720 spectropolarimeter at 25 °C in MeOH. Optical rotations were taken on a JASCO DIP-360 polarimeter. UV spectra were registered on spectrophotometer Perkin-Elmer 202. The FABMS spectra (positive mode) were recorded in a JEOL DX300 with a JMA system, using an NBA matrix. The target was bombarded with Xe atoms (10 keV). Semi prep. HPLC was performed on a Spherisorb S5ODS2 column (250 × 10 mm i.d., Waters) at a flow rate of 3.2 mL min $^{-1}$. The eluants were 5% formic acid (A) and acetonitrile (B). The isocratic profile used was 73% A.

Plant Material. The roots of *G. niveum* were obtained from plants at the end of the growing season in the municipality of

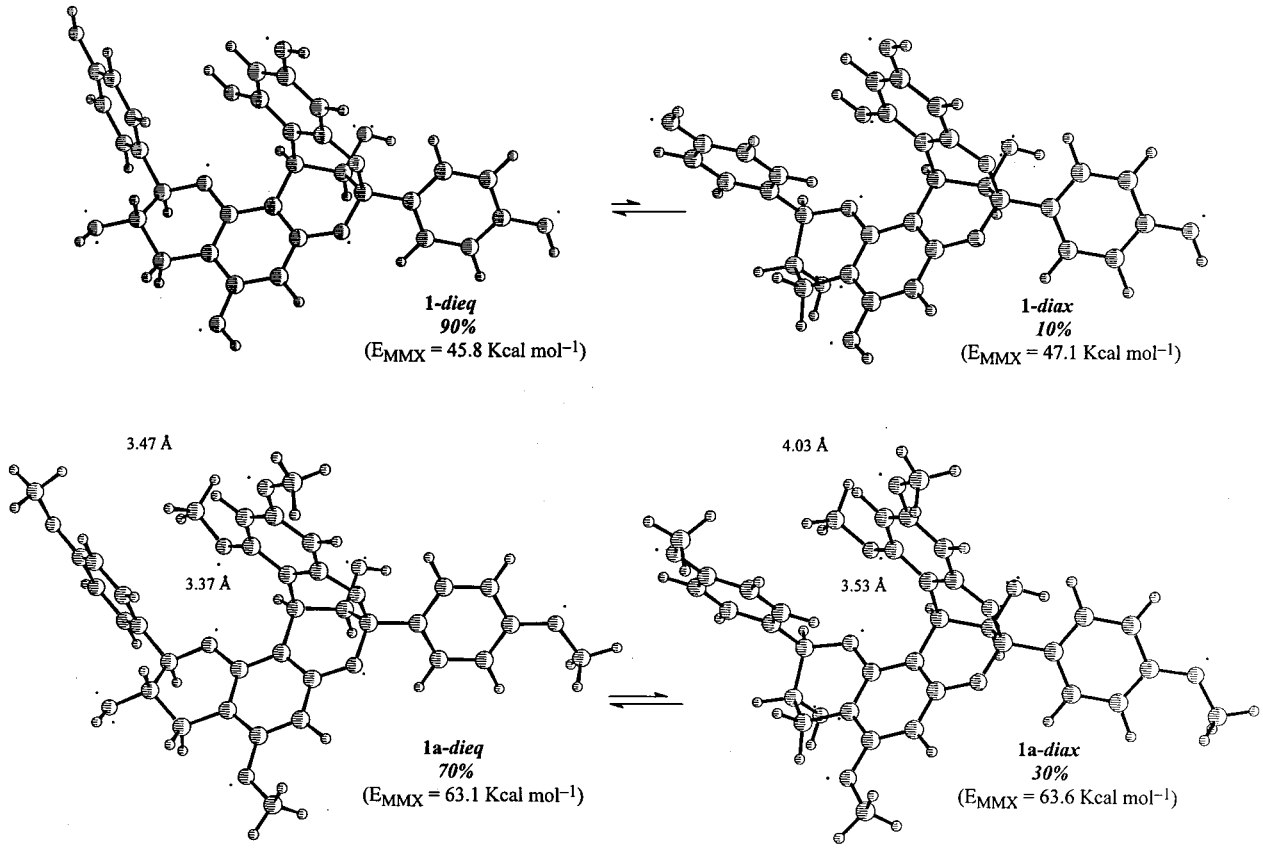

Figure 1. Conformational equilibria of **1** and **1a** obtained by MMX calculations.

Bocoyna, Chihuahua, and were air-dried in the shade. Voucher specimens (R. Bye 18054, 18265) are deposited in the Ethnobotanical Collection of the National Herbarium of Mexico (MEXU).

Extraction and Isolation. The air-dried plant material (5.3 kg) was ground and extracted exhaustively by maceration at room temperature with MeOH−$CHCl_3$ (1:1, 14.5 L × 3). After filtration, the extract was concentrated in vacuo to yield 173 g of a syrupy residue. The active extract (100 g) was suspended in H_2O (500 mL) and partitioned with $CHCl_3$ [F1, 2 g, 500 mL × 3, *E. histolytica*, IC_{50} (CI) 92.7 (93.4−92.4) μg/mL, *G. lamblia*, IC_{50} (CI) 138.1 (140.2−137.3) μg/mL]. The aqueous layer [F2, *E. histolytica*, IC_{50} (CI) 35.0 (37.4−34.3) μg/mL, *G. lamblia*, IC_{50} (CI) 34.8 (36.2−33.4) μg/mL] was dried and redissolved in H_2O (500 mL), and partitioned with EtOAc [F3, 94.2 g, 500 mL × 3, *E. histolytica*, IC_{50} (CI) 6.6 (6.8−6.3) μg/mL, *G. lamblia*, IC_{50} (CI) 18.7 (19.1−18.5) μg/mL]. The second aqueous layer [F4, 3.5 g, *E. histolytica*, IC_{50} (CI) 113.0 (114.2−112.8) μg/mL, *G. lamblia*, IC_{50} (CI) 127.9 (129.5−126.7) μg/mL] was concentrated in vacuo. The most active fraction F3 (94 g) was subjected to column chromatography over Si gel (950 g) and eluted with a gradient of $CHCl_3$/EtOAc [100, 50: 50, 100], EtOAc/acetone [50:50], and acetone/MeOH [50:50]. One hundred and twenty five fractions (500 mL each) were collected and pooled on basis of their TLC profiles to yield eight major fractions (F3-1−F3-8); bioactivities in the antiprotozoal assays showed one active pool [F3-5, 8.4 g, *E. histolytica*, IC_{50} (CI) 4.9 (5.1−4.5) μg/mL, *G. lamblia*, IC_{50} (CI) 3.6 (4.3−3.5) μg/mL]. F3-5 (2.2 g), eluted with EtOAc (100%), was further chromatographed on a Sephadex LH-20 (Pharmacia) column (50 g) eluted with EtOH to afford a mixture of compounds **1**−**3**, **4** (15 mg), **5** (276 mg), **6** (25 mg), and **7** (23 mg). The mixture **1**−**3** was resolved by HPLC (see general) with solvents A:B (73:27) to yield **1** (485 mg) and **2** (16 mg), **3** (10 mg).

Geranin A (1). Red powder; mp 245−248 °C; $[\alpha]_D$ +40° (*c* 0.3, MeOH); CD (MeOH) $\Delta\epsilon$ (nm) -1.35×10^3 (271.5), 2.21×10^3 (238), 3.26×10^3 (220), -4.82×10^3 (204); UV (ϵ) (MeOH) λ_{max} 204.5, 258, 274 nm; IR (KBr) ν_{max} 3396, 1614, 1516, 1454, 1234, 962 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); FABMS *m/z* $[M + H]^+$ 545 (10), 409 (12), 271 (27), 154 (100); *anal.* C 66.21%, H 4.46%, calcd for $C_{30}H_{24}O_{10}$, C 66.17%, H 4.44%.

Geranin B (2). Chestnut powder; mp 230−232 °C; $[\alpha]_D$ +18° (*c* 0.3, MeOH); CD (MeOH) $\Delta\epsilon$(nm): -2.3×10^3 (273), 4.74×10^3 (238), 1.70×10^3 (221); UV (MeOH) λ_{max} 205, 258, 274 nm; IR (KBr) ν_{max} 3394, 1613, 1516, 1455, 1230, 963 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); FABMS *m/z* $[M + H]^+$ 545 (41), 409 (34), 307 (100), 271 (98), 154 (84); *anal.* C 66.23%, H 4.44%, calcd for $C_{30}H_{24}O_{11}$, C 66.17%, H 4.44%.

Other Compounds (3−7). The identification of the known compounds **3**−**7** was accomplished by comparisons of their spectral data (UV, MS, ^1H and ^{13}C NMR) with those previously described for mahuanin B (**3**),[7] reynoutrin[8] (**4**) and hyperin[9] (**5**), methyl gallate[9] (**6**), and 3-β-caffeoyl-12-oleanen-28-oic acid[11] (**7**).

Methyl Ethers 1a and 2a. Compounds **1** (100 mg) and **2** (11 mg) were methylated with dimethyl sulfate and potassium carbonate in acetone to afford 75 mg of **1a** and 10 mg of **2a**. **1a**: mp 143−145 °C; IR (KBr) ν_{max} 3426, 1614, 1514, 1464, 1254, 1176, 1124, 1034, 832 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.61 (dd, J = 6.8, 16.7 Hz, H-4T), 2.75 (dd, J = 5.25, 16.7 Hz, H-4T), 3.35 (s, OMe-5U), 3.70 (s, OMe-5T), 3.73 (s, OMe-7U), 3.78 (s, OMe-4′T), 3.81 (s, OMe-4′U), 4.12 (m, H-3T), 4.14 (d, J = 3.65 Hz, H-3U), 4.83 (d, J = 3.65 Hz, H-4U), 4.89 (d, J = 6.5 Hz, H-2T), 6.02 (d, J = 2.3 Hz, H-6U), 6.17 (s, H-6T), 6.26 (d, J = 2.3 Hz, H-8U), 6.84 (d, J = 8.7 Hz, H-3′T and H-5′T), 6.94 (d, J = 8.9 Hz, H-3′U and H-5′U), 7.23 (d, J = 8.7 Hz, H-2′T and H-6′T), 7.62 (d, J = 8.9 Hz, H-2′U and H-6′U); FAB-MS *m/z* $[M + H]^+$ 615 (85), 465 (58), 313 (100). **2a**: IR (KBr) ν_{max} 3406, 2833, 1610, 1511, 1462, 1250, 1176, 1122, 1054, 832 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (dd, J = 7.2, 16.7 Hz, H-4T), 2.81 (dd, J = 5.1, 16.7 Hz, H-4T), 3.39 (s, OMe-5U), 3.74 (s, OMe-5T), 3.77 (s, OMe-7U), 3.81 (s, OMe-4′T), 3.91 (s, OMe-4′U), 3.93 (s, OMe-3′U), 4.17 (m, H-3T), 4.24 (d, J = 3.9 Hz, H-3U), 4.88 (d, J = 3.9 Hz, H-4U), 4.91 (d, J = 6.6 Hz, H-2T), 6.05 (d, J = 2.7 Hz, H-6U), 6.21 (s, H-6T), 6.30 (d, J = 2.7 Hz, H-8U), 6.80−7.4 (m, H-3′T, H-5′T, H-2′T, H-6′T, H-2′U, H-5′U, and H-6′U); FAB-MS *m/z* $[M + H]^+$ 645 (15), 614 (58), 154 (100), 135 (85).

Mosher Esters of Compounds 1 and 2. 1a (1.5 mg) or

2a (1.5 mg) was dissolved in CH_2Cl_2 (0.5 mL) and treated with (*S*)- or (*R*)- MTPA (5.9 mg), DCC (5.2 mg), and 4-DMAP (1.9 mg). The whole mixture was stirred at room temperature (25 °C) during 1 h and then poured into ice−water.

The resulting mixture was then extracted with $CHCl_3$; the organic phase was successively washed with 5% aq. HCl, saturated $NaHCO_3$, and brine, and then dried over Na_2SO_4 and filtered. Evaporation of the solvent from the filtrate under reduced pressure afforded a residue which was purified by column chromatography on Si gel (10 g, *n*-hexane−AcOEt 1:1) to give the corresponding (*S*)- and (*R*)-MTPA esters.

Molecular Modeling. Minimum energy structures were generated using the MMX force-field calculations, derived from the MM2 version,[20] as implemented in the PCMODEL program V 6.00. Conformational searches for the phenyl rings at C-2U and C-2T, for the hydroxyl hydrogens, for the methoxyl groups, and for the Mosher esters groups was carried out by the analysis of the rotational energy barrier plots in combination with the E_{MMX} convergence criteria employing the dihedral driver option. The π-system calculations were set for the restricted Hartree−Fock and full self-consistent field options.

Antiprotozoal Assay. The strains of microorganisms used in the antiprotozoal assays were *E. histolytica* HM1-IMSS and *G. lamblia* IMSS:0989:1. *E. histolytica* was maintained in TYI-S-33 medium, supplemented with 10% bovine serum, and *G. lamblia* was cultured in TYI-S-33 modified medium, supplemented with 10% calf serum. Both strains were axenically maintained and for the assays were employed in log phase of growth. In vitro testing against *E. histolytica* and *G. lamblia* was assessed using a method previously described.[4−6] Each test material (extract, primary fractions, and pure compounds) was dissolved in 1 mL of DMSO and 19 mL of culture medium and incorporated in disposable tubes with 4 mL of medium to obtain the required range of concentration: 2.5−200 μg/mL The tubes containing the test material-incorporated medium were inoculated with *E. histolytica* and *G. lamblia* to achive an inoculum of 6×10^3 and 5×10^4 trophozoites/mL, respectively. Each test included metronidazole (Sigma) as standard amoebicidal and giardicidal drug, a control (culture medium plus trophozites and DMSO), and a blank (culture medium). After incubation for 48 h at 37 °C, trophozoites were detached by chilling and 50 mL of each culture tube was subcultured in fresh medium before counting. The final number of parasites was determined with a haemocytometer and the percentage of trophozoite growth inhibition was calculated by comparison with the control culture. The results were confirmed by a colorimetric method: thus the trophozoites were washed by centrifugation and incubated for 45 min at 37 °C in phosphate-buffered saline (1.5 mL) containing MTT (3-[4,5-dimethylthiazol-2-il]-2,5-diphenyl tetrazolium bromide) and 250 μg of phenazine methosulfate (PMS). The final concentration of MTT in the buffer was 0.075%. The dye produced (formazan) was extracted with HCl/*i*-PrOH and the absorbance was determined at 570 nm. In both cases the percentage of inhibition calculated for each concentration was transformed into probit units. The plot of probit against log concentration was made; the best straight line was determined by regression analysis and the 50% inhibitory concentration (IC_{50}) values were calculated. The experiments were done by duplicate and repeated at least three times.

Cytotoxicity Assays. Cytotoxicity against human solid tumor cells was measured at the Purdue Cell Culture Laboratory, Purdue Cancer Center, in a seven-day MTT assay for MCF-7 breast carcinoma,[21] HT-29 colon adenocarcinoma,[22] and A-549 lung carcinoma,[23] with adriamycin as the positive control. Criteria of activity: ED_{50} values of <4 μg/mL.

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