Antiprotozoal Activity of the Constituents of *Conyza filaginoides*[1]

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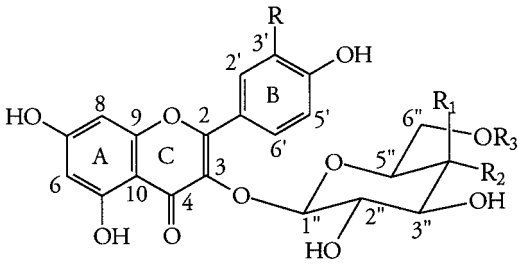
Bioassay-guided fractionation of the antiprotozoal extract of *Conyza filaginoides* led to the isolation of three new flavonol caffeoyl glycosides, namely, kaempferol 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-galactopyranoside (**1**), isorhamnetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-galactopyranoside (**2**), and quercetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-glucopyranoside (**3**). In addition, seven known compounds, erythrodiol (**4**), *β*-caryophyllene-4,5-α-oxide (**5**), astragalin (**6**), isoquercitrin (**7**), nicotiflorin (**8**), narcissin (**9**), and rutin (**10**), were obtained. The structures of the new isolates were elucidated by spectroscopic and chemical methods. Compounds were also assessed for antiamoebic and antigiardial activities, but none was significantly active compared to the standard drugs evaluated.

In a previous communication, we reported the isolation and structure elucidation of the spasmolytic compounds from a chloroform−methanol extract of *Conyza filaginoides* (D.C.) Hieron (Asteraceae).[2] Subsequently, we described the antiprotozoal properties of an extract prepared from the aerial parts of this species,[3] which is widely used in Mexico for the treatment of gastrointestinal ailments.[2] Accordingly, the present study describes the isolation and structure elucidation of the antiprotozoal constituents of this plant, including three novel flavonol caffeoyl glycosides, kaempferol 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-galactopyranoside (**1**), isorhamnetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-galactopyranoside (**2**), and quercetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-glucopyranoside (**3**), and several known compounds (**4**−**10**). Compounds **4**−**10** were identified as erythrodiol,[2] *β*-caryophyllene-4,5-α-oxide,[2] astragalin,[4] isoquercitrin,[2] nicotiflorin,[5] narcissin,[5] and rutin,[2] respectively, by comparison of their physical and spectroscopic data with those previously described in the literature.

Compound **1** was isolated as a yellow powder. The molecular formula was determined as $C_{30}H_{26}O_{14}$ on the basis of the ion peak at *m*/*z* 611 $[M + 1]^+$ in the positive FABMS, NMR (Table 1), and elemental analysis data. The NMR spectra indicated that **1** has a structure similar to those of tiliroside and related flavonols.[6−9] In particular the spectra showed the existence of a kaempferol core, a *trans*-caffeoyl moiety, and a galactopyranosyl unit. The linkage of the caffeoyl moiety with the galactopyranose unit and the linkage of the sugar portion with the flavonol core were confirmed by analysis of the HMBC spectrum. Thus, the caffeoyl carbonyl group (*δ* 167.4) correlated not only with the double bond protons but also with the 6″-methylene protons, suggesting that the caffeoyl moiety is connected to the 6″-methylene carbon. Moreover, the cross-peak observed between C-3 (*δ* 133.7) and the anomeric proton (*δ* 5.17, d, *J* = 7.5 Hz) showed that the site of glycosidation was at the C-3 position of kaempferol. Therefore, the structure was determined as kaempferol 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-galactopyranoside.

Compound **2** had the composition $C_{31}H_{28}O_{15}$ as determined by elemental analysis, and positive FABMS, differing from **1** by 30 mass units. This observation as well as the NMR data (Table 1) suggested that **2** was the 3′-methoxy derivative of **1**. The most obvious difference between the NMR spectra resulted from the presence of a signal for a methoxy group in **2** instead of the aromatic resonances attributed to H-3′/C-3 in **1**. As in the case of compound **1**, the order of the sugar and acyl units in the glycoside was determined by an HMBC experiment. Thus,



1: R= R_2= H, R_1= OH, R_3= CA
2: R= OMe, R_1= OH, R_2= H, R_3= TCA
3: R= R_2= OH, R_1= H, R_3= TCA
7: R= R_2= OH, R_1= R_3= H
8: R= R_1= H, R_2= OH, R_3= Rha
9: R= OMe, R_1= H, R_2= OH, R_3= Rha

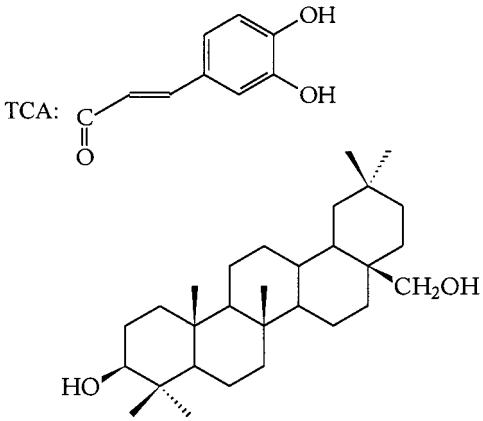


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Table 1. ^{1}H and ^{13}C NMR Assignments for Compounds **1**−**3** in MeOH-d_4[a]

	1		**2**		**3**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	157.1		157.3		159.1	
3	133.7		133.7		135.2	
4	177.7		177.7		179.3	
5	161.2		161.4		162.9	
6	99.2	6.12 (d, 2)	99.2	6.12 (brs)	100.5	6.11 (brs)
7	166.3		166.3		167.4	
8	93.8	6.28 (d, 2)	93.9	6.27 (brs)	95.2	6.28 (brs)
9	157.0		157.0		158.5	
10	104.1		103.7		105.2	
1′	121.4		121.5		123.3	
2′	130.7	7.98 (d, 9)	113.0	7.82 (d, 1.5)	114.6	7.59 (d, 2)
3′	114.6	6.79 (d, 9)	146.9		149.8	
4′	149.3		149.4		149.6	
5′	114.6	6.79 (d, 9)	112.8	6.80 (m)	117.3	6.79 (d, 8.5)
6′	130.7	7.98 (d, 9)	122.5	7.54 (dd, 8.5, 1.5)	123.2	7.55 (dd, 8.5, 2)
1″	102.8	5.17 (d, 7.5)	102.7	5.24 (d, 7)	104.0	5.19 (d, 8)
2″	74.3	3.44 (m)	74.4	3.47 (m)	75.8	3.51 (t, 8)
3″	70.2	3.5 (m)	74.4	3.5 (m)	78.1	3.5 (m)
4″	70.3	3.33 (m)	70.4	3.33 (m)	71.7	3.33 (m)
5″	76.6	3.44 (m)	76.6	3.47 (m)	75.7	3.43 (t, 8)
6″	62.9	4.28 (dd, 2, 11.8)	62.8	4.27 (dd, 2, 11.8)	64.3	4.29 (dd, 2, 11.8)
6″	62.9	4.16 (dd, 7, 11.8)	62.8	4.22 (dd, 7, 11.8)	64.3	4.18 (dd, 7, 11.8)
1‴	167.4		167.5		168.9	
2‴	113.7	6.01 (d, 16)	113.7	5.98 (d, 16)	115.9	6.03 (d, 15.5)
3‴	145.6	7.34 (d, 16)	145.7	7.30 (d, 16)	146.9	7.33 (d, 15.5)
4‴	126.2		126.2		127.7	
5‴	113.2	6.95 (d, 1.5)	113.0	6.94 (brs)	115.1	6.96 (brs)
6‴	145.1		145.3		145.9	
7‴	148.3		148.2		146.7	
8‴	115.0	6.78 (m)	115.1	6.78 (m)	116.5	6.78 (m)
9″	121.7	6.78 (m)	121.8	6.78 (m)	123.3	6.78 (m)
OMe			55.2	3.89 (s)		

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

Table 2. Antiprotozoal Activity of the Constituents of *C. filaginoides*[a]

	IC_{50} μg/mL (CI)[b]	
compound	*E. histolytica*	*G. lamblia*
1	30.0 (30.5−29.4)	47.0 (47.5−46.9)
2	47.7 (45.2−44.3)	15.3 (15.4−15.2)
3	14.0 (14.2−13.8)	104.9 (105.0−104.8)
4	12.7 (12.9−12.4)	29.9 (30.0−29.5)
5	71.3 (88.3−57.6)	53.8 (63.5−45.6)
6	61.2 (61.5−61.0)	47.5 (47.6−47.4)
7	14.7 (14.9−14.5)	87.3 (87.4−87.2)
8	30.9 (31.0−30.8)	22.5 (22.6−22.4)
9	17.2 (17.4−17.0)	94.7 (94.8−94.5)
10	119.7 (121.1−118.5)	178.7 (179.3−177.8)
metronidazole[c]	0.04 (0.10−0.03)	0.21 (0.27−0.14)
emetine[c]	1.05 (1.06−1.03)	0.41 (0.42−0.40)

[a] Results are expressed as mean ($n = 6$). [b] CI = 95% confidence intervals. [c] Positive control.

the structure of **2** was determined as isorhamnetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-galactopyranoside.

Compound **3**, a quercetin caffeoyl glucoside, was closely related to compound **2**. The NMR spectra (Table 1) differed from those of **2** in the sugar moiety and in the flavonol portion. The NMR spectra and acid hydrolysis revealed that the sugar and flavonol moieties were *β*-D-glucose and quercetin, respectively.[6−12] The order of the aglycon, sugar, and acyl units was also supported by an HMBC experiment as described for **1** and **2**. The structure of **3** was therefore assigned as quercetin 3-*O*-(6″-*O*-*E*-caffeoyl)-*β*-D-glucopyranoside.

Isolates **1**−**10** were evaluated for their antiprotozoal potential against *E. histolytica* and *G. lamblia* (Table 2). Compounds **4** and **8** were found to be active against both organisms. Compound **2** was the most active compound against *G. lamblia* (IC_{50} = 15.3 μg/mL). Compounds **3**, **4**, and **7** were the most active against the trophozoites of *E. histolytica* with IC_{50} values ranging from 12.0 to 14.7 μg/mL. To our knowledge, this is the first report of antiamoebic and antigiardial properties of compounds **1**−**9**. The results of the present study provide additional scientific evidence to support the use of *C. filaginoides* for the treatment of gastrointestinal ailments in Mexican traditional medicine.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher Johns apparatus and are uncorrected. Optical rotations were taken on a Jasco DIP-360 polarimeter. UV spectra were registered on a Perkin-Elmer 202 spectrophotometer. IR spectra were obtained in KBr disks on a Perkin-Elmer 599 B spectrophotometer. NMR spectra were recorded on a Bruker DMX500 spectrometer. HMBC and HMBC spectra were obtained at 500/125 MHz. The FABMS (positive mode) were recorded in a JEOL DX300 with a JMA system, using a NBA (nitrobenzyl alcohol) matrix. The target was bombarded with Xe atoms (10 keV). Preparative and analytical TLC were performed on precoated Si gel (Si gel 60, Merck F_{254}).

Plant Material. The aerial parts of *C. filaginoides* were collected in Ozumba, State of Mexico, Mexico. A voucher specimen (Calzada, 13593) was deposited in the Ethnomedical Collection at the Herbarium IMSSM of the Instituto Mexicano del Seguro Social (IMSS).

Extraction and Isolation. The air-dried plant material (4.6 kg) was ground and extracted exhaustively by maceration at room temperature with MeOH−$CHCl_3$ (1:1, 16 L × 2). After filtration, the extract was concentrated in vacuo to yield 686 g of a green residue. The active extract (20 g, *E. histolytica*, IC_{50} 141.0 μg/mL, *G. lamblia*, IC_{50} 79.1 μg/mL) was suspended in 10% MeOH−H_2O (200 mL) and partitioned with $CHCl_3$ (F1,

5.6 g, 200 mL × 2, *E. histolytica*, IC_{50} 64.9 μg/mL, *G. lamblia*, IC_{50} 101.8 μg/mL). The MeOH−H_2O layer (F2, *E. histolytica*, IC_{50} 83.7 μg/mL, *G. lamblia*, IC_{50} 60.9 μg/mL) was dried and redissolved in H_2O (200 mL) and partitioned with EtOAc (F3, 0.9 g, 200 mL × 2, *E. histolytica*, IC_{50} 82.8 μg/mL, *G. lamblia*, IC_{50} 117.1 μg/mL). The second MeOH−H_2O layer (F4, 12.7 g, *E. histolytica*, IC_{50} 103.1 μg/mL, *G. lamblia*, IC_{50} 15.9 μg/mL) was concentrated in vacuo. Fraction F1 (5 mg each plate) was subjected to preparative TLC on Si gel [EtOAc−MeOH−H_2O (400:16.5:13.5)] to yield **4** (8.1 mg, $R_f = 0.89$) and **5** (17.5 mg, $R_f = 0.92$). Fraction F3 (5 mg each plate) was also resolved by preparative TLC using the same conditions, to give **1** (4.6 mg, $R_f = 0.84$), **2** (15.8 mg, $R_f = 0.64$), **3** (11.2 mg, $R_f = 0.56$), **6** (10.9 mg, $R_f = 0.52$), and **7** (11.0 mg, $R_f = 0.50$). Finally, fraction F4 (5 mg each plate) was also subjected to preparative TLC [EtOAc−MeOH−H_2O (100:16.5:13.5)] to afford **8** (15.0 mg, $R_f = 0.40$), **9** (12.4 mg, $R_f = 0.36$), and **10** (8.0 mg, $R_f = 0.32$).

Kaempferol 3-*O*-(6″-*O*-*E*-caffeoyl)-β-D-galactopyranoside (**1**): yellow powder; mp 233−235 °C; $[\alpha]_D$ −26.7° (*c* 0.11 mg/mL, MeOH); UV (MeOH) λ_{max} 310, 380 nm; IR (KBr) ν_{max} 3414, 1650, 1600, 1500, 1451, 1359, 1260, 1196, 1167, 1085, 814 cm^{-1}; ^{13}C NMR (Table 1); FABMS *m/z* $[M + H]^+$ 611 (3); *anal.* C 59.021%, H 4.301%, calcd for $C_{30}H_{26}0_{14}$, C 59.019%, H 4.292%.

Isorhamnetin 3-*O*-(6″-*O*-*E*-caffeoyl)-β-D-galactopyranoside (**2**): yellow powder; mp 196−197 °C; $[\alpha]_D$ −79.1° (*c* 0.31 mg/mL, MeOH); UV (MeOH) λ_{max} 307, 375 nm; IR (KBr) ν_{max} 3402, 1652, 1602, 1507, 1453, 1356, 1258, 1199, 1169, 1086, 813 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* $[M + H]^+$ 641 (6); *anal.* C 58.130%, H 4.409%, calcd for $C_{31}H_{28}O_{15}$, C 58.127%, H 4.406%.

Quercetin 3-*O*-(6″-*O*-*E*-caffeoyl)-β-D-glucopyranoside (3): yellow powder; mp 237−239 °C; $[\alpha]_D$ −44.7° (*c* 0.15 mg/mL, MeOH); UV (MeOH) λ_{max} 300, 385 nm; IR (KBr) ν_{max} 3426, 1651, 1603, 1495, 1450, 1362, 1264, 1195, 1168, 1083, 815 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* $[M + H]^+$ 627 (7); *anal.* C 57.516%, H 4.120%, calcd for $C_{30}H_{26}O_{15}$, C 57.512%, H 4.183%.

Acid Hydrolysis of Compound 3. Compound **3** (5 mg) was dissolved in 1 mL MeOH and refluxed for 1 h with 6 N HCl (5 mL). The solution was extracted with EtOAc, and the residues from the organic phase were identified as quercetin and caffeic acid by comparative TLC [Si gel, $CHCl_3$−MeOH (8:2)] with authentic samples available in our laboratory. The aqueous phase was concentrated, and the sugar was identified as β-D-glucose by TLC [Si gel, BuOH−AcOH−H_2O (3:1:1)] with a standard sample (Merck).

Antiprotozoal Assays. In vitro antiprotozoal tests against *Entamoeba histolytica* (HM1-IMSS) and *Giardia lamblia* (IMSS: 0989:1) were performed according to standard protocols.[3,13,14]

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