Weakly Antimalarial Flavonol Arabinofuranosides from *Calycolpus warszewiczianus*

Daniel Torres-Mendoza,[†] José González,[‡] Eduardo Ortega-Barría,[‡] María V. Heller,[⊥] Todd L. Capson,[⊥] Kerry McPhail,[§] William H. Gerwick,[||] and Luis Cubilla-Rios*[,†]

Laboratory of Tropical Bioorganic Chemistry, Faculty of Natural, Exact Sciences and Technology, Apartado 0824-10835, University of Panama, Panama City, Republic of Panama, Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología, Secretaría Nacional de Ciencia, Tecnología e Innovación, Clayton, Ancon, Republic of Panama, Smithsonian Tropical Research Institute, Balboa, Ancon, Republic of Panama, and Oregon State University, Corvallis, Oregon 97331

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Three new flavonol arabinosides (**2**−**4**) were isolated from the young leaves of *Calycolpus warszewiczianus*. The structures were determined as myricetin-3-*O*-α-L-3″-acetylarabinofuranoside (**2**), myricetin-3-*O*-α-L-3″,5″-diacetylarabinofuranoside (**3**), and 5-galloylquercetin-3-*O*-α-L-arabinofuranoside (**4**). Molecular structures were elucidated using NMR spectroscopy in combination with IR and MS data. Two known compounds, myricetin-3-*O*-α-L-arabinofuranoside (**1**) and (−)-*epi*-catechin (**5**), were also isolated. The compounds were tested in vitro against a chloroquine-resistant strain of *Plasmodium falciparum*, *Leishmania mexicana*, and *Trypanosoma cruzi* parasites. Compound **4** demonstrated weak activity against a chloroquine-resistant strain of *P. falciparum* (14.5 μM), whereas none of the compounds demonstrated activity against *L. mexicana* and *T. cruzi* at the concentrations of 40 and 50 μg/mL, respectively, and no cytotoxicity was detected against mammalian cells below 100 μg/mL.

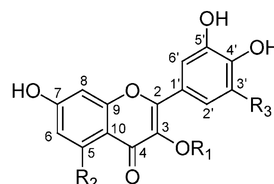
Collectively, malaria, Chagas' disease, and leishmaniasis affect 3 billion people.[1] Until effective and affordable preventive vaccines become available, drugs remain the mainstay of control for most parasitic diseases.[2] However, for many of these diseases the current repertoire of pharmaceutical agents is inadequate, a problem that is exacerbated by the emergence of drug-resistant parasites and the limited interest of the pharmaceutical industry in these diseases.[1] As a part of the Panama-ICBG Project, a program directed toward the discovery of new lead compounds for the treatments of parasitic diseases and cancer,[3] we report the isolation of new flavonol arabinofuranosides **2**, **3**, and **4** from the young leaves of *Calycolpus warzewiczianus* O. Berg (Myrtaceae).

There are no reported ethnopharmacologic uses for *C. warszewiczianus*. Previous chemical investigations were limited to isolation of a volatile oil from its leaves.[4] There are previous reports of flavonol glycosides with antimicrobial and antimalarial activity,[5] including a report that flavonol monoglycosides display slightly weaker antimalarial activity than the tri- and diglycosides.[5a]

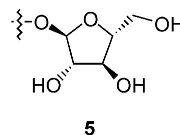
Bioassay-guided fractionation of young leaves from *C. warszewiczianus* resulted in isolation of three new and two known flavonol glycosides. Isolated compounds were tested for in vitro antiprotozoal and cytotoxic activities. Compounds **1** and **4** demonstrated weak activity against *Plasmodium falciparum* W2 (chloroquine-resistant).

The EtOAc/MeOH extract of young leaves of *C. warszewiczianus* showed activity against a chloroquine-resistant strain of *P. falciparum* (W2) and was fractionated by vacuum liquid chromatography using a polarity gradient with mixtures of *n*-hexane−EtOAc and MeOH into eight main fractions according to their TLC profiles. Compounds **2**−**5** and compound **1** were isolated from fractions 5 and 6, respectively.

Compound **1** was pale yellow at 254 nm using 2-aminoethyl diphenylborinate on a TLC plate, thus indicating it was a flavonoid. UV maxima occurred at 355 (band I) and 261 nm (band II), characteristic of a flavonol system. The IR spectrum suggested the presence of hydroxy and carbonyl groups.



1 R_1 = α-*L*-arabinofuranose, R_2 = R_3 = OH
2 R_1 = α-*L*-3"-acetylarabinofuranose, R_2 = R_3 = OH
3 R_1 = α-*L*-3", 5"-diacetylarabinofuranose, R_2 = R_3 = OH
4 R_1 = α-*L*-arabinofuranose, R_2 = galloyl, R_3 = H



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The ^{1}H NMR spectrum showed aromatics protons at δ 7.11 (2H, s), 6.36 (1H, d, J = 2.1 Hz), and 6.18 (1H, d, J = 2.1 Hz) due to the myricetin skeleton, together with an anomeric proton at δ 5.46 (1H, d, J = 1.6 Hz), indicating an α-configuration for a sugar moiety. Three other signals were present at δ 4.32 (1H, dd, J = 1.6/4.4 Hz) and 3.91 and 3.51, each representing two protons. The ^{13}C NMR spectrum showed 19 carbon peaks including one methylene, 11 quaternary, and seven methine carbons. The arabinofuranoside moiety **5** in compound **1** showed NOESY correlation between H_2-5″ and H-4″. Comparisons of the spectroscopic data with literature values[6,7] led to the identification of compound **1** as myricetin-3-*O*-α-L-arabinofuranoside.

Compound **2** gave a molecular formula of $C_{22}H_{20}O_{13}$ by HRESIMS. The ^{1}H NMR spectrum for **2** revealed signals very similar to those for compound **1**, the major difference being a resonance for a single acetate methyl singlet at δ 2.03. The ^{13}C NMR spectrum showed 22 resonances, including two carbons of an acetyl moiety (δ 171.4 and 21.3).

The presence of a single *O*-acetyl functionality in **2** was consistent with the difference in molecular weights of 42 amu between **1** and **2**. The position of the *O*-acetyl moiety in the arabinose moiety was assigned by an HMBC correlation between H-3″ (δ 4.85) and the acetate carbonyl at δ 171.4. Therefore, compound **2** was characterized as myricetin-3-*O*-α-L-3″-acetylarabinofuranoside.

* To whom correspondence should be addressed. Tel: (507) 681 5371. Fax: (507) 264 4450. E-mail: lucr@ancon.up.ac.pa.
[†] University of Panama.
[‡] Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología.
[⊥] Smithsonian Tropical Research Institute.
[§] Oregon State University.
[||] University of California at San Diego.

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Table 1. ^{1}H NMR Data (δ) for Compounds **2**−**4**[a]

H	2	3	4
flavonol			
6	6.22 d (2.1)	6.20 d (1.7)	6.20 d (2.1)
8	6.43 d (2.1)	6.39 d (1.7)	6.39 d (2.1)
2′	7.20 s	7.12 s	7.46 dd (2.1, 8.3)
3′			6.88 d (8.3)
6′	7.20 s	7.12 s	7.50 d (2.1)
arabinose			
1″	5.74 s	5.71 s	5.49 s
2″	4.41 d (1.3)	4.45 d (1.6)	3.94 d (1.6)
3″	4.85 dd (1.6, 4.2)	4.75 dd (1.6, 4.8)	4.37 dd (1.6, 2.8)
4″	3.94 q (3.4, 7.4)	3.85 m	3.95 m
5″	3.58 d (3.8)	3.93 dd (4.0, 18.0)	4.11 dd (3.2, 14)
		4.14 dd (4.0, 11.2)	
CH_3CO-3″	2.05 s	2.05 s	
CH_3CO-5″		1.98 s	
5-*O*-galloyl			
2‴			6.99 s
6‴			6.99 s

[a] Compounds **2** and **3** in acetone-d_6, **4** in MeOH-d_4.

The molecular formula of compound **3** was established as $C_{24}H_{22}O_{14}$ by HRESIMS and ^{13}C NMR data. The ^{1}H NMR spectrum for **3** exhibited a pair of *meta*-coupled aromatic protons at δ 6.20 and 6.39 (d, $J = 1.7$ Hz), two aromatic protons at δ 7.12, and a signal at δ 12.57 characteristic of a 5-OH group, which suggested that the flavonol moiety was myricetin.[8] Two acetate methyl singlets at δ 1.98 and 2.05 were evident, consistent with the increment of 84 amu for **3** compared to **1**. The sugar moiety showed proton signals at δ 3.85 (m), 3.93 (dd, $J = 4.0$, 18.0 Hz), 4.14 (dd, $J = 4.0$, 11.2 Hz), 4.45 (d, s, $J = 1.6$ Hz), and 4.75 (dd, $J = 1.6$, 4.8 Hz) and an anomeric proton singlet at δ 5.71. The ^{13}C spectrum showed 24 resonances, sorted by DEPT experiments into two CH_3, one CH_2, eight CH, and 12 quaternary carbons. Two of the quaternary signals were those of the carbonyl of two acetyl moieties (δ 171.3 and 170.6). The protons in the sugar moiety were assigned on the basis of COSY data. The presence of an arabinofuranosyl moiety having an α-configuration was suggested by the small coupling constants. The site of attachment of the arabinose moiety was determined by an HMBC correlation of the anomeric proton with C-3 of the flavonol moiety (δ 134.9). The positions of the *O*-acetyl moieties in the arabinosyl unit were assigned by HMBC correlations of one of H_2-5 (δ 3.93 and 4.14) of the arabinose and the acetate carbonyl at δ 170.6, and the other between H-3 (δ 4.75) of the arabinose and the carbonyl at δ 171.3. The combined data permitted the identification of compound **3** as myricetin-3-*O*-α-L-3″,5″-diacetylarabinofuranoside.

The molecular formula of compound **4** was established as $C_{27}H_{22}O_{15}$ from HRESIMS data. The ^{1}H NMR spectrum showed two aromatic doublets at δ 6.20 and 6.39 (each d, $J = 2.1$ Hz), similar to those observed in compounds **2** and **3**, and an ABM system at δ 6.88 (d, $J = 8.3$ Hz), 7.46 (dd, $J = 2.1$, 8.3 Hz), and 7.50 (d, $J = 2.1$ Hz), which allowed identification of the aglycone as quercetin.[9] A notable feature in the ^{1}H NMR spectrum of compound **4** was the appearance of a sharp low-field two-proton singlet at δ 6.99 for the magnetically equivalent 2- and 6-protons of a galloyl group. The assignment of the sugar protons was established from HSQC data and was consistent with an α-arabinofuranoside (Table 1). The ^{13}C spectrum showed 27 resonances, sorted by DEPT experiments into one CH_2, 11 CH, and 15 quaternary carbons. Placement of the sugar and galloyl moieties was determined by HMBC experiments, where the anomeric proton showed a correlation with C-3 (δ 134.9) of the aglycone. Hence, compound **4** was identified as 5-galloylquercetin-3-*O*-α-L-arabinofuranoside.

Flavonols are plant-derived polyphenolic compounds that are commonly consumed in the diet. There is experimental evidence that these compounds have effective vasodilatory and antioxidant activity that is able to prevent vascular reperfusion injury.[10] There is also information of their antibacterial and antifungal activities.[11] Interestingly, plants containing flavonols are used as a Chinese folk herb medicine, to treat malaria and scrofula.[12]

Table 2. ^{13}C NMR Data (δ) for Compounds **2**−**4**[a]

C	2	3	4
flavonol			
2	158.3	158.8	159.8
3	134.9	134.9	134.9
4	179.4	179.1	179.8
5	163.1	163.2	163.1
6	99.8	99.5	100.0
7	165.6	164.9	166.3
8	95.1	94.5	94.9
9	158.2	157.9	158.6
10	105.6	105.8	105.7
1′	122.5	122.1	123.0
2′	110.1	109.6	123.2
3′	146.6	146.3	116.5
4′	136.7	136.9	149.8
5′	146.6	146.3	146.3
6′	110.1	109.6	117.6
arabinofuranose			
1″	109.6	109.4	109.6
2″	81.9	81.2	79.2
3″	81.7	81.0	83.7
4″	87.1	83.4	84.6
5″	63.1	64.2	64.6
CH_3CO-3″	171.4	171.3	
	21.3	20.9	
CH_3CO-5″		170.6	
		20.6	
5-*O*-galloyl			
C=O			168.1
1‴			121.1
2‴			110.3
3‴			146.4
4‴			140.0
5‴			146.4
6‴			110.3

[a] Compounds **2** and **3** in acetone-d_6, **4** in MeOH-d_4.

Table 3. Compound Activities against Tropical Diseases[a]

compound	*P. falciparum*[b] IC_{50} (μM)	cytotoxicity[c] IC_{50} (μM)
1	27.8 ± 1.6	ND[d]
2	>50	ND
3	>50	456
4	14.5 ± 3.7	86.5
(−)-*epi*-catechin	>50	184.1
chloroquine	0.06	ND

[a] Results show the IC_{50} value ± the SD ($n = 3$). [b] Chloroquine-resistant strain of *Plasmodium falciparum*. [c] Experiments performed with Vero cells. [d] ND = not determined.

The antiparasitic activities of compounds **1**−**4** are presented in Table 3 along with their cytotoxic activities. Compounds **1** and **4** showed antiplasmodial activity when tested against a chloroquine-resistant strain of *P. falciparum* with IC_{50} values of 27.8 and 14.5 μM, respectively. It is likely that the galloyl moiety present in compound **4** is responsible for its greater antimalarial activity as compared to compound **1**, which lacks this modification. This is also supported by our previous investigations, which showed a similar antimalarial activity ($IC_{50} = 10$ μM) for the methyl ester of gallic acid.[13] The presence of the acetate group in the sugar moiety is probably responsible for decreasing the antiplasmodial activity in **2** ($IC_{50} > 50$ μM) and **3** ($IC_{50} > 50$ μM) with respect to **1** ($IC_{50} = 27.8$ μM). This antiplasmodial activity is consistent with the recent report that flavonol glycosides characteristically inhibit proliferation of *P. falciparum*.[5b] Interestingly, none of the flavonols tested demonstrated activity against *Leishmania* and *T. cruzi* parasites nor significant cytotoxic activity when tested against control mammalian cells.

Experimental Section

General Experimental Procedures. Optical rotations were measured with an Autopol III 6971 automatic polarimeter. IR spectra were taken on a Perkin-Elmer FT-IR Spectrum RXI spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C) with TMS as an internal standard. ESIMS were recorded on a Waters Micromass LCT Classic instrument. HPLC was carried out on a Waters LC system, including a 600 pump and a 996 photodiode array detector.

Plant Material. Young leaves of *C. warszewiczianus* O. Berg were collected from Barro Colorado Nature Monument in Gatún Lake in the Republic of Panama in February 2004. The material was identified by Professor Mireya Correa of the University of Panama and the Smithsonian Tropical Research Institute. Vouchers of the plant have been deposited in the herbarium of University of Panama (PMA 53401).

Extraction and Isolation. Upon collection, young leaves were transferred to sealed plastic bags, kept on ice, and processed within 6 h. After removal of the stems, 0.1 kg of fresh leaves was homogenized and concentrated to yield 16.5 g of crude extract (IC_{50} = 4 μg/mL).[14] Analysis by TLC on silica gel with detection using the NPR reagent (5% diphenylboric acid 2-aminoethyl ester in EtOH)[15] showed the presence of flavonoid-related compounds. Three aliquots of 5.5 g of the crude extract of young leaves were chromatographed by VLC on silica gel (7GF, J. T. Baker), eluting with a solvent gradient of hexane−EtOAc (10:0 to 0:10) and EtOAc−MeOH (10:0 to 0:10). The fractions were combined according to TLC profiles into eight main fractions (1−8); the flavonoids were present in fractions 5 and 6 (IC_{50} = 32 and 4 μg/mL, respectively). Fraction 5 was subjected to Sephadex LH-20 column chromatography and eluted with 80:20 EtOH−H_2O to yield six fractions (5a−5f). Fractions 5d, 5e, and 5f were fractionated by semipreparative reversed-phase HPLC (XTerra Prep RP_{18}, 10 μm, 10 × 250 mm) using isocratic elution (flow 1.5 mL/min, 50:50 MeOH−H_2O). Fraction 5d yielded (−)-*epi*-catechin (19 mg), identified by its spectral data and specific rotation ($[\alpha]^{25}_D$ −51),[16] and compound **2** (13 mg), while fraction 5e yielded compound **3** (4 mg) and fraction 5f yielded compound **4** (7 mg). Fraction 6 was subjected to Sephadex LH-20 column chromatography, under the same conditions as for fraction 5, yielding six fractions (6a−6f). Fraction 6c was fractionated by semipreparative reversed-phase HPLC (XTerra Prep-RP_{18}, 10 μm, 10 × 250 mm) using gradient elution (flow 1.5 mL/min, 40:60 MeOH−H_2O from 0 to 21 min and 49:51 MeOH−H_2O from 21 to 70 min), yielding compound **1** (20.1 mg).

Acid Hydrolysis. Compounds **1**−**4**, 10 mg each in a mixture of 2 M HCl (3 mL) and MeOH (3 mL), were separately refluxed for 9 h and then neutralized with NaOH. The neutralized product was subjected to an extraction with $CHCl_3$. The neutral H_2O layer was subjected to TLC analysis (eluent: MeOH−H_2O, 7:3), and L-arabinose was identified after comparison with an authentic sample. In each case the specific rotation of the sugar moiety was determined ($[\alpha]^{25}_D$ +103.5).[17]

Myricetin-3-*O*-α-L-3″-acetylarabinofuranoside (2): yellow solid; $[\alpha]^{25}_D$ −173.5 (*c* 0.48, MeOH); IR (film) ν_{max} 3322, 1652, 1606, 1370, 1200, 1040 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESMS 515.0 *m/z* $[M + Na]^+$ (100), 493.0 $[M + H]^+$ (24), 430.8 (13), 413.2 (25), 362.8 (17), 319.0 (13), 279.0 (27), 268.0 (22); TOF MS ES+ *m/z* 493.0997 $[M + H]^+$ (calcd for $C_{22}H_{21}O_{13}$, 493.0982).

Myricetin-3-*O*-α-L-3″,5″-diacetylarabinofuranoside (3): yellow-orange solid; $[\alpha]^{25}_D$ −156 (*c* 0.75, MeOH); IR (film) ν_{max} 3320, 1730, 1608, 1372, 1240, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESMS *m/z* 557.0 $[M + Na]^+$ (48), 535.0 $[M + H]^+$ (100), 319.0 (23), 279.0 (13), 217.0 (13); TOF MS ES+ *m/z* 535.1069 $[M + H]^+$ (calcd for $C_{24}H_{23}O_{14}$, 535.1088).

5-Galloylquercetin-3-*O*-α-L-arabinofuranoside (4): yellow solid; $[\alpha]^{25}_D$ −118.4 (*c* 0.25, MeOH); IR (film) ν_{max} 3322, 1650, 1606, 1502, 1358, 1234, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESMS *m/z* 609.0 $[M + Na]^+$ (75), 587 $[M + H]^+$ (100), 301.0 (16), 279.0 (30), 245.0 (35); TOF MS ES+ *m/z* 587.1042 $[M + H]^+$ (calcd for $C_{27}H_{23}O_{15}$, 587.1037).

Protozoal Inhibition Bioassays. The antiplasmodial activity was evaluated using a fluorometric method based on the detection of parasite DNA with the fluorochrome PicoGreen using a chloroquine-resistant strain (Indochina W2) of *P. falciparum*.[12] The parasites were maintained in vitro by a modification of the method of Trager and Jensen.[18]

For *Leishmania*, a promastigote colorimetric growth assay based upon the reduction of the tetrazolium salt (XTT) to a water-soluble formazan was used.[19] *L. mexicana* (WHO-MOHM/B2/82/BELZ) was cultured using established protocols.[20]

A β-galactosidase-expressing transgenic *T. cruzi* (Tulahuen strain, clone C4) was used to detect antitrypanosomal activity.[13] Growth of the intracellular stage of *T. cruzi* was determined from the cleavage of the colorimetric substrate chlorophenol red-β-D-galactoside.

Cytotoxicity Bioassay. Vero cells, derived from the kidney of the African green monkey, adhering to 96-well plates, were used to evaluate the toxicity of the compounds purified from *C. warszewiczianus* on the basis of reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma).[20] After treatment with the test compound and 4 h incubation at 37 °C, cell viability was evaluated in an ELISA reader at 570 nm.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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