# Isolation and Structure Elucidation of Two New Flavonoid Glycosides from the Infusion of *Maytenus aquifolium* Leaves. Evaluation of the Antiulcer Activity of the Infusion

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Droplet countercurrent chromatography and high-performance liquid chromatography fractionation of the aqueous infusion from *Maytenus aquifolium* Martius leaves afforded two flavonoid tetrasaccharides: quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-*O*-[β-D-glucopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→2)-*O*-β-D-galactopyranoside and kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-*O*-[β-D-glucopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→2)-*O*-β-D-galactopyranoside. All structures were elucidated by spectroscopic methods. Pharmacological essays of the infusion showed antiulcer activity in rats.

**Keywords:** *Maytenus aquifolium Martius; Celastraceae; espinheira santa; infusion; antiulcer acitivity; flavonoid glycosides; 1D and 2D NMR*

## INTRODUCTION

*Maytenus aquifolium* Martius (Celastraceae) is a plant widely used in Brazil either as a beverage or as a medicinal plant. This plant is popularly known in Brazil as "espinheira-santa", and the aqueous infusion of the leaves is traditionally utilized against stomach diseases. Several Celastraceae species also have folk indications of medicinal activities (Bernal and Correa, 1990). *M. aquifolium* was also reported as being an adulterant of the "yerba-mate" (*Ilex paraguariensis* St. Hil., Aquifoliaceae) in Paraguay (Gonzalez, 1986). This beverage is also largely employed in southern Brazil. The presence of cytotoxic triterpenes was reported in the roots of several *Maytenus* species, including *Maytenus ilicifolia* Martius, another species also called "espinheira -santa" (Shirota et al., 1994). However, the chemical composition of the *Maytenus* leaves is still underexplored (Sannomiya et al., 1997). Previous studies using GC/FID and GC/MS indicated triterpenes friedelan-3-ol and friedelin as being the main compounds from low-polarity extracts of *M. aquifolium*. These triterpenes are useful markers for differentiating *M. aquifolium* from *Sorocea bomplandii* Baill. (Moraceae), a species with closely related morphology but different chemical composition (Vilegas et al., 1994). To investigate the chemical constituents of the polar fraction of *M. aquifolium*, we isolated, from the infusion of the leaves, two new flavonoid tetraglycosides. The isolation of these polyglycosylated flavonoids will be a useful for the validation of these compounds as markers for the assessment of Brazilian *Maytenus* infusion. We also report the pharmacological antiulcer essay of the infusion of the leaves of *M. aquifolium*.

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## EXPERIMENTAL PROCEDURES

**Materials.** The leaves of *M. aquifolium* Martius were furnished by Ana Maria Soares Pereira, UNAERP, Ribeirao Preto, SP, Brazil. A voucher sample is deposited at the Herbario of the Universidade Estadual Paulista (UNESP).

A commercial sample was also purchased in the local market and submitted to the same experimental procedure.

**Apparatus.** The EI-MS spectra were performed in a Fisons Platform spectrometer both in the positive (90 V) and in the negative (100 V) mode. The sample was dissolved in MeOH and injected directely.

UV spectra were performed in an HP 8472-A spectrometer (MeOH, $c = 1$), and the IR spectrum was obtained via Nicolet impact 400, KBr.

A Bruker DRX-600 spectrometer, operating at 599.19 MHz for $^1$H and at 150.858 for $^{13}$C, using the UXNMR software package was used for NMR experiments in $CD_3OD$. The distortionless enhancement by polarization transfer (DEPT) experiments were performed using a transfer pulse of 135° to obtain positive signals for CH and $CH_3$ and negative ones for $CH_2$. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. $^1$H−$^1$H DQF−COSY (double quantum filtered COSY) (Bodenhausen and Ruben, 1986; Homans, 1990), $^1$H−$^{13}$C HSQC, and HMBC (Martin and Crouch, 1991) experiments were performed using the conventional pulse sequences as described in the literature.

Droplet countercurrent chromatography (DCCC) separation was performed in ascending mode on a Tokyo Rikakikai Co. instrument with 300 columns of 40 cm × 2 mm i.d. (solvent = $CHCl_3$/MeOH/$H_2O$ 43:37:20).

HPLC separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector, a Waters μ-Bondapak C-18 column, and a U6K injector.

GC spectra were run using a Hewlett-Packard 5890 gas chromatograph equipped with mass-selective detector MSD 5970 MS and a fused-silica column HP-5 (25 m × 0.2 mm i.d.; 0.33 μm film).

**Extraction and Isolation.** Leaves of *M. aquifolium* were air-dried and milled. Two hundred grams of the powdered plant was boiled for 8−9 min with water (1 L). The mixture was allowed to cool, filtered over filter paper, and evaporated

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**Table 1. Effect of Oral Treatment with Infusion of *M. aquifolium* on Stress-Induced Gastric Ulcers on Rats[a]**

| type of ulcer | control | indomethacin | 200 mg/kg | 500 mg/kg | 800 mg/kg |
|---|---|---|---|---|---|
| mild (I) | 40 ± 2.44 | 35 ± 2.87 | 35 ± 2.30 | 34 ± 1.63 | 25* ± 2.30 |
| intermediary (II) | 19 ± 1.63 | 23 ± 0.81 | 16 ± 2.32 | 15 ± 0.81 | 13* ± 1.91 |
| severe (III) | 17 ± 1.91 | 22 ± 4.89 | 10* ± 1.15 | 9* ± 0.81 | 8* ± 0.81 |

[a] Numbering indicates total counting of ulcers; results are mean ± SD. Statistical significance, Student *t* test: *, $p < 0.05$ ($n = 4$).

until dryness, affording 20 g of crude extract. An aliquot of this aqueous extract was submitted to the pharmacological essays. Another aliquot (1.0 g) was dissolved in 20 mL of a 1:1 mixture of mobile phase and stationary phase of the eluent described above. The solution was loop injected and fractionated by DCCC. A mixture of compounds **1** and **2**, checked by TLC [silica gel plates, *n*-BuOH/HOAc/$H_2O$ (60:15:25)], was further purified on reversed-phase HPLC on a C-18 $\mu$-Bondapak column (30 cm × 7.8 mm, flow rate = 2.5 mL/min) using MeOH/$H_2O$ (40:60) as the eluent to yield pure compounds **1** (35.2 mg, $t_R$ = 18 min) and **2** (30.0 mg, $t_R$ = 20 min).

**Acid Hydrolysis of Compounds 1 and 2.** A solution of each compound (3 mg) in 6% HCl (3.5 mL) was refluxed for 2 h. The reaction mixture was diluted with $H_2O$ and then extracted with EtOAc. The resulting aglycons were identified by their $^1$H NMR spectra.

**Methanolysis of Compounds 1 and 2.** Each compound (1.0 mg) was heated in a vial for 24 h at 80 °C in MeOH/2% HCl (2 mL). After MeOH and HCl distillation in a $N_2$ stream, $Ag_2CO_3$ and MeOH were added until $CO_2$ production stopped. The centrifugate was dried over $P_2O_5$. The resulting monosaccharides were treated with Trisil-Z (Pierce) and analyzed by GC/MS. Retention times were identical to those of the authentic Trisil sugars.

*Compound* ***1****:* $C_{39}H_{50}O_{25}$; UV $\lambda_{max}$ (MeOH) 266, 350; +KOH 273, 329, 395; +$AlCl_3$ 269, 303 sh, 351; +$AlCl_3$ + HCl 274, 303 sh, 349, 395; +NaOAc 273, 365; +NaOAc + $H_3BO_3$ 266, 354; IR (KBr) 3379 (OH), 1653 (C=O) cm$^{-1}$; EI-MS, *m/z* (rel intensity) (100 V, negative mode) 917 $[M - H]^-$ (100), 771 $[M - H - rha]^-$ (2), 755 $[M - H - glu]^-$ (2), 301 $[M - H - glu - 2\ rha - gal]^- = [A - H]^-$ (73); (90 V, positive mode) 957 $[M + K]^+$ (31), 941 $[M + Na]^+$ (50), 919 $[M + H]^+$ (9), 611 $[M + H - glu - rha]^+$ (13), 465 $[M + H - glu - 2\ rha]^+$ (11), 303 $[A + H]^+ = [M + H - glu - 2\ rha - gal]^+$ (100); $^1$H and $^{13}$C NMR data, see Tables 2 and 3.

*Compound* ***2****:* $C_{39}H_{50}O_{24}$; UV $\lambda_{max}$ (MeOH) 266, 350; +KOH 273, 329, 395; +$AlCl_3$ 269, 303 sh, 351; +$AlCl_3$ + HCl 274, 303 sh, 349, 395; +NaOAc 273, 365; +NaOAc + $H_3BO_3$ 266, 354; IR (KBr) 3379 (OH), 1653 (C=O) cm$^{-1}$; EI-MS, *m/z* (rel intensity) (100 V, negative mode) 901 $[M - H]^-$ (100), 755 $[M - H - rha]^-$ (3), 739 $[M - H - glu]^-$ (3), 285 $[M - H - glu - 2\ rha - gal]^- = [A - H]^-$ (75); (90 V, positive mode) 941 $[M + K]^+$ (30), 925 $[M + Na]^+$ (52), 903 $[M + H]^+$ (10), 595 $[M + H - glu - rha]^+$ (15), 449 $[M + H - glu - 2\ rha]^+$ (13), 287 $[A + H]^+ = [M + H - glu - 2\ rha - gal]^+$ (100); $^1$H and $^{13}$C NMR data, see Tables 2 and 3.

**Antiulcer Tests.** Pharmacological tests were done by Dr. José Carlos T. Carvalho, Departamento de Principios Ativos Naturais e Toxicologia, Faculdade de Ciencias Farmaceuticas, UNESP−Araraquara. Evaluation of antiulcer activity of the infusion was done on male Wistar rats, 3 months old (25−30 g weight), furnished by Central Biotery−UNESP, Botucatu, following a stress-induced gastric lesions method of Takagi et al. (1964). Groups (four rats each group) were maintained without any food during 24 h and afterward were treated orally, as following: 1, control (aqueous Tween 5%); 2, infusion in Tween 5%, at doses of 200, 500, and 800 mg/kg/os; 3, indomethacin in Tween 5%, 10 mg/kg/os. Thirty minutes after the treatment, each rat was immobilized in an individual compartment, according to the method described by Basile et al. (1989). In sequence, the rats were immersed in a water bath (25 °C) and maintained for 17 h. After this procedure, rats were killed by cervical traction, and their stomachs were removed, opened longitudinally, and examined by using a monocular stereomicroscopy (10× amplitude). The number and strength of stress-induced lesions was counted and classified as mild (I, presence of oedema, hyperemy, and petechiae); intermediary (II, presence of small hemorrhagic submucosae lesions), and severe (III, presence of a hemorrhagic zone, delimitated by strong erosion and invasive lesions). Data were analyzed using unpaired Student's *t* test, and $p < 0.05$ was taken as significant. Results are presented in Table 1.

## RESULTS AND DISCUSSION

The infusion from the leaves of *M. aquifolium* was prepared as described under Experimental Procedures and pharmacologically essayed. The doses assayed are those expected to be ingested in usual intakes of the infusion according to folk information. As shown in Table 1, in all cases increasing the dose led to the decrease of the number of ulcers. The most effective dose was 800 mg/kg, which reduced by ∼30−50% the number of ulcers. The major activity was verified in ulcers of type III, which are the most severe. We observed a dose-dependent effect of the infusion and a protective activity with the three tested doses. Carlini (1988) in a previous investigation did not detect any toxic effect of the infusion from *M. aquifolium*.

The infusion was fractionated by DCCC to investigate its chemical constituents as described above. A mixture of compounds **1** and **2** was further purified on reversed-phase HPLC to yield pure compounds **1** and **2**.

Acid hydrolisis of **1** and **2** released, respectively, quercetin and kaempferol identified by $^1$H and $^{13}$C NMR spectra. The gas chromatographic analysis of the methanolysis products showed the presence, for both compounds, of glucose, rhamnose, and galactose, in the ratio 1:2:1.

The ES-MS (100 V, negative ion) mass spectrum of **1** gave as base peak the $[M - H]^-$ ion at *m/z* 917. The fragment at *m/z* 301 corresponds to the deprotonated aglycon $[A - H]^-$. Fragment ions occurred at *m/z* 771 $[(M - H) - 146]^-$ and at *m/z* 755 $[(M - H) - 162]^-$, which were interpreted as independent losses of terminal deoxyhexose and hexose units. In the ES-MS spectrum in the positive mode (90 V) we observed the pseudo-molecular ion $[M + NH]^+$ at *m/z* 919. The adducts $[M + Na]^+$ at *m/z* 941 and $[M + K]^+$ at *m/z* 957 were also observed. The fragment at *m/z* 611 $[M + H - 146 - 162]^+$ refers to the loss of the two terminal sugars. The fragments at *m/z* 465 $[M + H - 146 - 162 - 146]^+$ and the base peak at at *m/z* 303 $[M + H - 146 - 162 - 146 - 162]^+ = [A + H]^+$ correspond to the subsequent losses of deoxyhexose and hexose moieties.

Compound **2** showed an $[M - H]^-$ ion in the ES-MS negative ion mass spectrum at *m/z* 901, which was 16 mass units lower than that of **1**, and prominent fragments at *m/z* 755 $[M - H - 146]^-$, 739 $[M - H - 162]^-$, and 285 $[M - H - 162 - 146 - 146 - 162]^-$ corresponding to the deprotonated aglycon. In the ES-MS spectrum in the positive mode **2** showed fragments at *m/z* 941 $[M + K]^+$, 925 $[M + Na]^+$, and 903 $[M + H]^+$ and fragments at 941, 595 $[M + H - 162 - 146]^+$, 449 $[M + H - 162 - 146 - 146]^+$, 287 $[M + H - 162 - 146 - 146 - 162]^+$, which were interpreted as the losses of the saccharidic units.

**Table 2. $^1$H NMR Assignments ($\delta_H$ in $CD_3OD$) of Compounds 1 and 2[a]**

| proton | 1 | 2 |
|---|---|---|
| 6 | 6.22 (d, 1.5) | 6.23 (d, 1.5) |
| 8 | 6.41 (d, 1.5) | 6.42 (d, 1.5) |
| 2′ | 7.75 (d, 1.5) | 8.07 (d, 8.4) |
| 3′ | | 6.93 (d, 8.4) |
| 5′ | 6.94 (d, 8.5) | 6.93 (d, 8.4) |
| 6′ | 7.61 (dd, 1.5 and 8.5) | 8.07 (d, 8.4) |
| 3-Gal | | |
| 1 | 5.64 (d, 7.5) | 5.55 (d, 7.5) |
| 2 | 3.97 (dd, 7.5 and 9.7) | 3.96 (dd, 7.5 and 9.7) |
| 3 | 3.74 (dd, 7.5 and 3.5) | 3.72 (dd, 7.5 and 3.5) |
| 4 | 3.49 (dd, 3.5 and 1.5) | 3.48 (dd, 3.5 and 1.5) |
| 5 | 3.44 (ddd, 1.5, 5.0, and 7.0) | 3.42 (ddd, 1.5, 5.0, and 7.0) |
| 6a | 3.48 (dd, 12.0 and 7.0) | 3.47 (dd, 12.0 and 7.0) |
| 6b | 3.72 (dd, 12.0 and 5.0) | 3.72 (dd, 12.0 and 5.0) |
| (6−1) Rha | | |
| 1 | 4.59 (d, 1.5) | 4.54 (d, 1.5) |
| 2 | 3.56 (dd, 3.5 and 1.5) | 3.54 (dd, 3.5 and 1.5) |
| 3 | 3.81 (dd, 9.5 and 3.5) | 3.79 (dd, 9.5 and 3.5) |
| 4 | 3.32 (t, 9.5 and 9.5) | 3.30 (t, 9.5 and 9.5) |
| 5 | 3.43 (dq, 9.5 and 6.0) | 3.41 (dq, 9.5 and 6.0) |
| 6 | 1.18 (d, 6.0) | 1.18 (d, 6.0) |
| (3−1) Glc | | |
| 1 | 4.59 (d, 7.5) | 4.59 (d, 7.5) |
| 2 | 3.36 (dd, 7.5 and 9.5) | 3.36 (dd, 7.5 and 9.5) |
| 3 | 3.43 (t, 9.5 and 9.5) | 3.42 (t, 9.5 and 9.5) |
| 4 | 3.64 (t, 9.5 and 9.5) | 3.64 (t, 9.5 and 9.5) |
| 5 | 3.39 (ddd, 9.5, 5.0, and 3.5) | 3.38 (ddd, 9.5, 5.0, and 3.5) |
| 6a | 3.76 (dd, 12.0 and 3.5) | 3.76 (dd, 12.0 and 3.5) |
| 6b | 3.90 (dd, 12.0 and 5.0) | 3.89 (dd, 12.0 and 5.0) |
| (2−1) Rha | | |
| 1 | 5.29 (d, 1.5) | 5.26 (d, 1.5) |
| 2 | 4.32 (dd, 3.5 and 1.5) | 4.31 (dd, 3.5 and 1.5) |
| 3 | 3.98 (dd, 9.5 and 3.5) | 3.96 (dd, 9.5 and 3.5) |
| 4 | 3.57 (t, 9.5 and 9.5) | 3.56 (t, 9.5 and 9.5) |
| 5 | 4.15 (dq, 9.5 and 6.0) | 4.15 (dq, 9.5 and 6.0) |
| 6 | 1.04 (d, 6.0) | 1.03 (d, 6.0) |

[a] Chemical shift values are in ppm and *J* values in Hz presented in parentheses. All signals were assigned by 2D-HOHAHA, DQF−COSY, HSQC, and HMBC studies. Gal, *β*-D-galactopyranosyl; Rha, α-L-rhamnopyranosyl; Glc, *β*-D-glucopyranosyl.

**Table 3. $^{13}$C NMR Assignments ($\delta_C$ in $CD_3OD$) of Compounds 1 and 2[a]**

| carbon | DEPT | 1 | 2 |
|---|---|---|---|
| 2 | C | 158.3 | 158.7 |
| 3 | C | 134.5 | 134.5 |
| 4 | C | 179.3 | 179.4 |
| 5 | C | 163.0 | 163.1 |
| 6 | CH | 99.7 | 99.8 |
| 7 | C | 165.6 | 165.8 |
| 8 | CH | 94.6 | 94.7 |
| 9 | C | 158.5 | 158.3 |
| 10 | C | 105.8 | 105.8 |
| 1′ | C | 123.2 | 122.9 |
| 2′ | CH | 117.4 | 132.2 |
| 3′ | C | 145.8 | 116.2 |
| 4′ | C | 149.6 | 161.2 |
| 5′ | CH | 116.1 | 116.2 |
| 6′ | CH | 123.0 | 132.2 |
| 3-Gal | | | |
| 1 | CH | 101.1 | 101.3 |
| 2 | CH | 77.2 | 77.6 |
| 3 | CH | 75.6 | 75.4 |
| 4 | CH | 71.0 | 71.0 |
| 5 | CH | 75.4 | 75.3 |
| 6 | $CH_2$ | 67.0 | 67.2 |
| (6−1) Rha | | | |
| 1 | CH | 102.2 | 102.3 |
| 2 | CH | 72.2 | 72.3 |
| 3 | CH | 71.6 | 71.6 |
| 4 | CH | 73.8 | 74.0 |
| 5 | CH | 69.6 | 69.7 |
| 6 | $CH_3$ | 17.9 | 17.9 |
| (3−1) Glc | | | |
| 1 | CH | 105.7 | 105.8 |
| 2 | CH | 75.3 | 75.4 |
| 3 | CH | 77.3 | 77.5 |
| 4 | CH | 69.7 | 69.8 |
| 5 | CH | 77.2 | 77.2 |
| 6 | $CH_2$ | 62.2 | 62.4 |
| (2−1) Rha | | | |
| 1 | CH | 101.8 | 101.9 |
| 2 | CH | 72.0 | 72.1 |
| 3 | CH | 83.0 | 83.0 |
| 4 | CH | 72.7 | 72.8 |
| 5 | CH | 70.7 | 70.7 |
| 6 | $CH_3$ | 17.6 | 17.8 |

[a] All signals were assigned by 2D-HOHAHA, DQF−COSY, HSQC, and HMBC studies. Gal, *β*-D-galactopyranosyl; Rha, α-L-rhamnopyranosyl; Glc, *β*-D-glucopyranosyl.

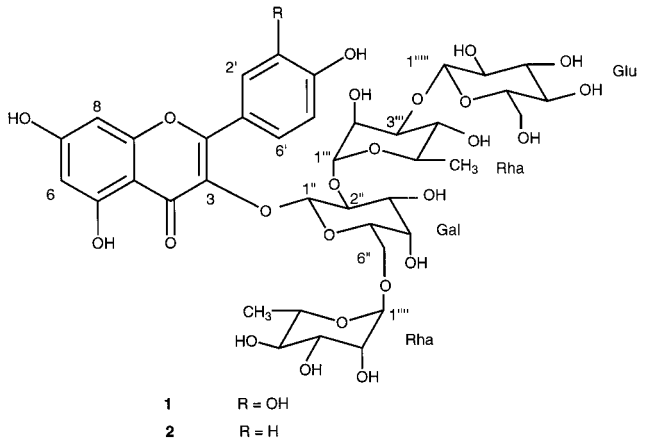
From the mass and $^{13}$C NMR and $^{13}$C DEPT NMR data, the molecular formulas $C_{39}H_{50}O_{25}$ and $C_{39}H_{50}O_{24}$ were deduced for compounds **1** and **2**, respectively.

The complete structures of **1** and **2** were elucidated by 1D and 2D NMR experiments at 600 MHz. The $^1$H NMR spectrum of **1** showed, for the aromatic side, two signals at $\delta$ 6.22 and 6.41 (both d, $J = 1.5$ Hz) could be assigned to H-6 and H-8, respectively, and signals at $\delta$ 6.94 (d, $J = 8.5$ Hz, H-5′), 7.61 (dd, $J = 8.5$, 1.5 Hz, H-6′), and 7.75 (d, $J = 1.5$ Hz, H-2′) could be ascribed to H-2′, H-5′, and H-6, respectively.

The $^{13}$C NMR shifts of the aglycon part of **1** corresponded well with the shifts for quercetin, the only significant difference being those referred to C-2 and C-3. These shifts are analogous to those reported when the 3-hydroxy group is glycosylated in a flavonol glycoside (Agrawal, 1989). Four anomeric protons were easily identified in the spectra of **1**. They resonated at $\delta$ 5.64 (d, $J = 7.5$ Hz), 5.29 (d, $J = 1.5$ Hz), 4.61 (d, $J = 7.5$ Hz), and 4.59 (d, $J = 1.5$ Hz) and correlated to carbons at $\delta$ 101.1, 102.2, 105.7, and 101.8, respectively. From the assigned aglycon and sugar values (Tables 2 and 3), it was apparent that a tetrasaccharide unit was attached to C-3 of the aglycon. The structure of the tetrasaccharide chain has been assigned by a combination of 2D-HOHAHA, 2D DFQ−COSY, HSQC, and HMBC experiments. Starting from the anomeric protons of each sugar unit, all of the hydrogens within each spin system could be identified using a combination of HOHAHA and COSY experiments. The assignments of all proton resonances for the sugar moieties (Table 2) immediately allowed us to assign the resonances of the linked carbon atoms by HSQC experiment (Table 3).

Information about the sequence of the tetrasaccharide chain was deduced from HMBC experiments. Key correlation peaks were observed between the anomeric proton of the galactose ($\delta$ 5.64) and the C-3 of the quercetin ($\delta$ 134.5), between the anomeric proton signal of the inner rhamnose ($\delta$ 5.29) and the C-6 of galactose ($\delta$ 67.0), between the anomeric proton of the glucose ($\delta$ 4.61) and the C-3 of the inner rhamnose ($\delta$ 83.2), and between the anomeric proton of the outer rhamnose ($\delta$ 4.59) and the C-2 of the galactose ($\delta$ 77.2).

The *β*-configurations at the anomeric positions for the galactopyranosyl and the glucopyranosyl units ($J_{H1-H2} = 7.5$ Hz) were easily seen from their relatively large $^3J_{H1-H2}$ coupling constants (7−8 Hz). The α-configuration in the rhamnose residues was clear from their H-1 nonsplitting pattern and their distinct C-3 and C-5




**1** R = OH
**2** R = H

**Figure 1.** Flavonoid glycosides isolated from *M. aquifolium* leaves: (**1**) quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-*O*-[β-D-glucopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→2)]-*O*-β-D-galactopyranoside and (**2**) kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-[β-D-glucopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-galactopyranoside. Gal, β-D-galactopyranosyl; Rha, α-L-rhamnopyranosyl; Glc, β-D-glucopyranosyl.

chemical shift differences from that of methyl β-L-rhamnopyranoside (Agrawal, 1989).

From these considerations the structure quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-*O*-[β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl(1→2)]-*O*-β-D-galactopyranoside (Figure 1) was assigned to **1**.

The $^1$H NMR spectrum of **2** displayed signals for two meta-coupled protons at $\delta$ 6.23 (d, $J$ = 1.5 Hz, H-6) and 6.42 (d, $J$ = 1.5 Hz, H-8) and also for an ortho-coupled system at $\delta$ 8.07 (d, $J$ = 8.4 Hz, H-2′ and H-6′) and 6.93 (d, $J$ = 8.4 Hz, H-3′ and H-5′), indicating a kaempferol derivative. The other signals in the $^1$H, $^{13}$C, and $^{13}$C DEPT NMR data were superimposable on those of **1**. These data suggested that the structure of **2** is kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-*O*-[β-D-glucopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→2)]-*O*-β-D-galactopyranoside (Sannomyia et al., 1997).

Flavonoids are widely distributed in several fruits and vegetables and then are common components of the human diet. As flavonols occur also in the leaves, the dietary intake of flavonols from leaves can be significative. The 3-*O*-glycosides of quercetin and kaempferol are the most important group of flavonoids. The interest in their chemistry is increasingly growing due to the relationship of these compounds to pharmacological activities, in particular their capillary strenghtening effects (Harborne, 1988). Recently, Vinson et al. (1995) described powerful antioxidant activities for several polyphenols containing *o*-dihydroxy groups. Hertog et al. (1992) observed an epidemiological relationship between the consumption of some products containing flavonoids and the incidence of coronary heart disease. Hertog et al. (1993) also reported the potentially anticarcinogenic properties of some flavonoids present in foods. Flavonoids are known as inhibitors of prostaglandin cyclo-oxygenase and lipoxygenase. In general, prostaglandines are powerful inhibitors of gastric acid secretion, and they prevent peptic ulcers. Flavonol glucosylrhamnogalactosides were described in some tea samples, but no information about their biological activities was mentioned (Finger et al., 1991). The isolation of polyglycosylated flavonoids from the infusion of *M. aquifolium* will be useful for the validation of these compounds as chemical markers for the assessment of the Brazilian *Maytenus* infusion.

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