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A FLAVONOID GLYCOSIDE FROM *MAYTENUS AQUIFOLIUM*

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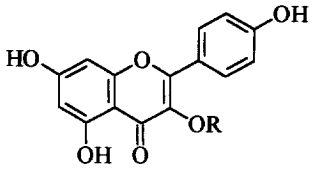
Key Word Index—*Maytenus aquifolium*; Celastraceae; flavonol glycoside; 'espinheira-santa'.

Abstract—The flavonol glycoside kaempferol-3-O-[2-glucopyranosyl(1–3)rhamnopyranosyl-6-rhamnopyranosyl]β-D-galactopyranoside was identified in the infusion of the leaves from *Maytenus aquifolium*.

INTRODUCTION

Two *Maytenus* species (*M. aquifolium* and *M. ilicifolia*) (Celastraceae) are extensively used in Brazilian folk medicine as anti-ulcer agents [1]. The literature reports the presence of friedelan derivatives as the apolar constituents of the leaves from both plants [2]. However, the infusion itself has not yet been chemically investigated. Furthermore, several adulterations have already been found, most from plants of the genus *Sorocea* due to their closely related morphology [3]. In this paper we report the isolation and structure elucidation of a new tetrasaccharide flavonol from the leaves of *M. aquifolium*. There are no reports in the literature related to the chemical investigation of this plant.



R = Gal(1→2)Rha(1→3)Glc
|
6
Rha

1

RESULTS AND DISCUSSION

The infusion from the leaves of *M. aquifolium* was prepared as described in the EXPERIMENTAL and it was first partitioned between ethyl acetate and water. The water-soluble layer was then partitioned between *n*-BuOH and water. The *n*-BuOH layer was evaporated and fractionated by DCCC, affording almost pure **1**. Substance **1** was purified by CC on cellulose eluted with mixture of BAW 6:5:1 (upper layer). Compound **1** was obtained as a gummy solid. It shows a bright yellow spot on TLC observed in UV light after being revealed with NP/PEG reagent, thus indicating a kaempferol derivative [4].

Acid hydrolysis of **1** released kaempferol, D-glucose, L-rhamnose and D-galactose, identified by TLC on cellulose plates compared to authentic samples. The IR spectrum showed a strong absorption band at 1653 cm^{-1} for a chelated carbonyl group and an intense broad band centered at 3379 from the ν_{OH} [5].

The bathochromic shift of the band I with $AlCl_3$/HCl (45 nm) in the UV spectrum indicates a 5-hydroxy-3-O-substituted flavonol. The strong bathochromic shift of band I with alcoholic KOH (45 nm) is characteristic of a free 4′-OH group in ring B. The small bathochromic shift of band II with NaOAc (7 nm) revealed the presence of free 7-OH group in ring A [6].

The ES-MS (100 V, negative ion) mass spectrum gave as base peak the $[M-H]^-$ ion at *m/z* 901, corresponding to a molecular formula $C_{39}H_{50}O_{24}$. The fragment at *m/z* 285 corresponds to the deprotonated aglycone $[A-H]^-$, thus indicating a kaempferol derivative. Fragment ions occurred at *m/z* 755 $[M-H-146]^-$ and at *m/z* 739 $[M-H-162]^-$, corresponding to independent losses of terminal deoxyhexose and hexose units, indicating a branched oli-

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gosaccharide. In the ES-MS spectrum in the positive mode (90 V) we observed the pseudomolecular ion $[M+H]^+$ at *m/z* 903. The adducts $[M+Na]^+$ at *m/z* 925 and $[M+K]^+$ at *m/z* 941 were also observed. The fragment at *m/z* 595 $[M+H-146-162]^+$ refers to the loss of the two terminal sugars. The fragments at *m/z* 449 $[M+H-146-162-146]^+$ and the base peak at *m/z* 287 $[M+H-146-162-146-162]^+ = [A+H]^+$ correspond to the subsequent losses of deoxyhexose and hexose moieties.

The complete structure of **1** was elucidated by 1D- and 2D-NMR experiments at 600 MHz. The ^{1}H NMR spectra (Table 1) displayed signals for two *meta*-coupled protons at δ 6.22 (*d*, $J = 1.5$ Hz, 1H) and δ 6.41 (*d*, $J = 1.5$ Hz, 1H) and also for an ortho-coupled system at δ 8.07 (*d*, $J = 8.4$ Hz, 2H) and δ 6.93 (*d*, $J = 8.4$ Hz, 2H), confirming a kaempferol derivative. The presence of signals for four anomeric protons in the ^{1}H NMR spectra and four anomeric carbons in the ^{13}C NMR spectra suggested **1** to be a tetrasaccharide (Table 1). The connecting positions of the sugars were established using the HMBC technique. A correlation was observed between the anomeric signal of the galactose (δ 5.55, *d*, $J = 7.5$ Hz) and the C-3 of the kaempferol (δ 134.5), the anomeric proton signal of the inner rhamnose (δ5.26, *d*, $J = 1.5$ Hz) and the C-2 of galactose (δ 77.6), the anomeric proton of the glucose (δ 4.59, *d*, $J = 7.5$ Hz) and the C-3 of the inner rhamnose (δ 83.0) and the anomeric proton of the outer rhamnose (δ 4.54, *d*, $J = 1.5$ Hz) and the C-6 of the galactose (δ 67.2) [7]. A careful analysis of the ^{1}H-^{1}H COSY spectrum combined with the HOHAHA data secured the assignments of the spin systems for each sugar belonging to the tetraglycoside moiety of **1** as presented in Table 1. The HMQC spectrum correlated the ^{1}H resonances with those from the corresponding carbons. The DEPT spectrum furnished the multiplicities of the carbon signals. The configurations of the glycosidic linkages were established from the splitting patterns of the anomeric proton signals in the ^{1}H NMR spectrum. The small coupling (1.5 Hz) of the anomeric proton of the rhamnose moieties is typical of the equatorial orientation between H-1 and H-2, indicating the α-configuration. The larger H-1/H-2 coupling (7.5 Hz) of the anomeric protons of glucose and galactose unities is typical of the axial orientation, indicating the β-configuration. Thus the structure of **1** was determined to be kaempferol-3-O-[2-glucopyranosyl(1–3)rhamnopyranosyl-6-rhamnopyranosyl]β-D-galactopyranoside, a new plant glycoside.

Table 1. ^{13}C NMR and ^{1}H NMR spectral data for **1** (in CD_3OD)

Position	^{13}C NMR δ	^{1}H NMR δ	J (Hz)
2	158.7		
3	134.5		
4	179.4		
5	163.1		
6	99.8	6.22 *d*	1.5
7	165.8		
8	94.7	6.41 *d*	1.5
9	158.3		
10	105.8		
1′	122.9		
2′, 6′	132.2	8.07 *d*	8.4
3′, 5′	116.2	6.93 *d*	8.4
4′	161.2		
3-Gal			
1	101.1	5.55 *d*	7.5
2	77.6	3.96 *dd*	7.5, 9.7
3	75.6	3.72 *dd*	7.5, 3.5
4	71.0	3.48 *dd*	3.5, 1.5
5	75.4	3.42 *ddd*	1.5, 5.0, 7.0
6	67.2	3.47 *dd*	12.0, 7.0
		3.72 *dd*	12.0, 5.0
(6–1)Rha			
1	102.2	4.54 *d*	1.5
2	72.2	3.54 *dd*	3.5, 1.5
3	71.6	3.79 *dd*	9.5, 3.5
4	73.8	3.30 *t*	9.5, 9.5
5	69.6	3.41 *dq*	9.5, 6.0
6	17.9	1.18 *d*	6.0
(3–1)glc			
1	105.7	4.59 *d*	7.5
2	75.3	3.36 *dd*	7.5, 9.5
3	77.3	3.42 t	9.5, 9.5
4	69.7	3.64 t	9.5, 9.5
5	77.2	3.38 *ddd*	9.5, 3.5, 5.0
6	62.2	3.76 *dd*	12.0, 3.5
		3.89 *dd*	12.0, 5.0
(2–1)Rha			
1	101.8	5.26 *d*	1.5
2	72.0	4.31 *dd*	3.5, 1.5
3	83.0	3.96 *dd*	9.5, 3.5
4	72.7	3.56 *t*	9.5, 9.5
5	70.7	4.15 *dq*	9.5, 6.0
6	17.6	1.03 *d*	6.0

EXPERIMENTAL

Plant material

Authentic sample of leaves of *Maytenus aquifolium* Martius were furnished by Ana Maria Soares Pereira, UNAERP, SP, Brazil.

General

TLC: cellulose, solvent: pyridine/EtOAc/HAc/H_2O 36:36:7:21. The plates were sprayed with NP/PEG reagent and the spots were further visualized under UV light (365 nm). CC: cellulose, solvent: BAW 6:1:5 (upper layer). DCCC: Tokyo Rikakikai Co. with 300 columns of 40 cm × 2 mm i.d.; solvent: $CHCl_3$/MeOH/H_2O 43:37:20, ascending. UV spectra: HP 8472-A, MeOH. IR spectrum: Nicolet Impact 400, KBr. ES-MS were performed on a Fisons Platform spectrometer both in the positive (90 V) and negative

mode (100 V). The sample was dissolved in MeOH and injected directly. RMN: ^{1}H-NMR, ^{13}C-NMR, DEPT and various 2D-NMR (^{1}H-^{1}H COSY, HOHAHA, NOESY, HMQC, HMBC) spectra were obtained in CD_3OD using a Brücker DRX-600 Spectrometer operating at 599.19 MHz for ^{1}H and 150.858 MHz for ^{13}C.

Preparation of the infusion

Leaves of *M. aquifolium* were air dried and milled. 200 g of the powder were boiled with 1 l water. The mixture was allowed to cool, filtered over filter paper and partitioned first with EtOAc and then with *n*-BuOH. The *n*-BuOH layer was evaporated and fractionated by DCCC. Fractions containing **1** were further purified on cellulose CC eluted with BAW yielding 30 mg of **1**.

Hydrolysis

10 mg of **1** were dissolved in 20 ml 1 M HCl and refluxed for 3 h. The solution was allowed to cool and extracted with $CHCl_3$. Both phases were evaporated and analysed by TLC with authentic samples of kaempferol, glucose, rhamnose and galactose. Kaempferol-3 - O - [2 - glucopyranosyl(1–3)rhamnopyranosyl - 6-rhamnopyranosyl]β-D-galactopyranoside **1**: Brown gummy solid. UV^{λ}_{max} (nm) MeOH: 266, 350; +KOH: 273, 329, 395; +$AlCl_3$: 269, 303sh, 351; +$AlCl_3$+HCl: 274; 303sh, 349, 395; +NaOAc: 273, 365; +NaOAc+H_3BO_3: 266, 354. ES-MS *m/z* (rel. int.) (100 V, NI): 901 $[M-H]^-$ (100), 755 $[M-H\text{-}Rha]^-$ (2), 739 $[M-H\text{-}Glc]^-$ (2), 285 $[A-H]^- = [M-H\text{-}Glc\text{-}2Rha\text{-}Gal]^-$ (73); (90 V, PI): 941 $[M+K]^+$ (31), 925 $[M+Na]^+$ (50), 903 $[M+H]^+$ (9), 595 $[M+H\text{-}Glc\text{-}Rha]^+$ (13), 449 $[M+H\text{-}Glc\text{-}2Rha]^+$ (11), 287 $[A+H]^+ = [M+H\text{-}Glc\text{-}2Rha\text{-}Gal]^+$ (100). ^{1}H and ^{13}C NMR: Table 1.

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