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Article

Acylated Flavonol Glycosides and Terpenoids from the Leaves of *Alibertia sessilis*

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Dois novos flavonóis glicosilados, juntamente com iridóides, triterpenóides, esteróides e tocoferolquinona, foram isolados das folhas de *Alibertia sessilis* (Rubiaceae). As estruturas dos flavonóides foram determinadas através de métodos espectroscópicos, principalmente da RMN de ^{13}C e de ^{1}H.

Two novel acylated flavonol glycosides, along with iridoids, triterpenes, steroids and α-tocopherolquinone, were isolated from the leaves of *Alibertia sessilis* (Rubiaceae). The determination of the structures of the new compounds was based mainly on ^{1}H- and ^{13}C-NMR.

Keywords: Alibertia sessilis, *Rubiaceae, acylated flavonol glycosides, iridoids, terpenoids,* α-tocopherolquinone

Introduction

In the course of our continuing search for new active antifungal compounds from *Alibertia* (Rubiaceae) found in "Cerrado" region of S. Paulo, we studied *Alibertia sessilis* (Vell.) K. Schum. collected in Itirapina, in the state of São Paulo, Brazil. Previous investigations of the leaves of *A. macrophylla* resulted in the isolation of fungitoxic non-glycosidic iridoids 1β and 1α-hydroxydihydrocornin aglycones, and the caffeic acid esters: 2-phenylethyl caffeoate and 2-methyl-4-hydroxybutyl caffeoate[1]. *A. edulis*, an other species collected in the same region, was also investigated, and no active compound was detected[2]. Relatively few chemical studies of the *Alibertia* genus have been reported, despite evidence of its rich terpenoidic constitution[3].

In the present study, from the leaves of *A. sessilis* we obtained large amounts of the triterpenes oleanolic, ursolic, and *epi*-betulinic acid, and small amounts of the iridoids gardenoside, deacetylasperuloside, and 10-dehydrogardenoside, together with the fungitoxic α- and β-gardiol. Besides iridoids and triterpenes, the flavonols quercetin-3-O-rutinoside, quercetin-3-O-β-D-(2"-O-*trans*-*p*-coumaroyl) -rutinoside (**1**) and kaempferol-3-O- β-D-(2"-O-*trans*-*p*-coumaroyl)-rutinoside (**2**) were isolated. These last two glycosides are being reported for the first time from a natural source.

Results and Discussion

The hexanic extract of the leaves of *Alibertia sessilis* was submitted to a chromatographic process to afforded sitosterol and α-tocopherolquinone, identified by comparison with authentic samples. After methylation, the CH_2Cl_2 extract of the leaves gave ursolic, oleanolic, and *epi*-betulinic acid methyl esters[2,4]. After chromatographic separations, the butanolic soluble part of the hydroalcoholic extract afforded a mixture of the isomeric iridoids α and β gardiol[5], in addition to gardenoside and deacetylasperulosidic acid methyl ester[6,7]. These compounds were identified by comparing the spectroscopic data with the literature[5-7].

Gel permeation chromatography of the EtOAc soluble part of the hydroalcoholic extract gave quercetin-3-O-rutinoside[8] (rutin) and a mixture of the new acylflavonol glycosides 1 and 2. These were purified by subsequent preparative TLC. The ^{1}H-NMR of 1 and 2 showed signals that suggested the structure of quercetin and kaempferol, respectively, for the aglycones of the compounds. In both spectra signals corresponding to the *trans* *p*-coumaroyl group and a rhamnose sugar portion were also found (Table 1). The anomeric proton of the rhamnose ap-

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Table 1. ^{1}H-NMR spectral data for compounds 1 and 2 in DMSO-d_6*.

H	1	2
6	6.14, d (1.8)	5.90, d (1.8)
8	6.33, d (1.8)	6.07, d (1.8)
2'	7.48, d (1.8)	7.88, d (8.8)
3'		6.84, d (8.8)
5'	6.83, d (9.0)	6.84, d (8.8)
6'	7.51, dd (9.0 and 1.8)	7.88, d (8.8)
1"	5.56, d (8.4)	5.54, d (8.3)
2"	4.87, t (8.4)	4.80, t (8.3)
3"	*ca* 3.5	ca 3.5
4"	*ca* 3.4	ca 3.3
5"	*ca* 3.4	ca 3.3
6"	*ca* 3.7	ca 3.7
1'''	4.36, s	4.35, s
2'''	*ca* 3.4	ca 3.2
3'''	*ca* 3.5	ca 3.5
4'''	*ca* 3.1	ca 3.1
5'''	*ca* 3.3	ca 3.3
6'''	0.98, d (6.0)	0.99, d (6.0)
2'''', 6''''	7.52, d (8.0)	7.51, d (8.5)
3'''', 5''''	6.78, d (8.0)	6.77, d (8.5)
7''''	7.56, d (15.6)	7.58, d (15.6)
8''''	6.37, d (15.6)	6.36, d (15.6)

peared as a singlet (δ 4.36) in accordance with the α configuration. Another signal, which could be assigned to an anomeric proton of another sugar appeared at δ ~ 5.5 (d, J = 8 Hz) in both spectra.

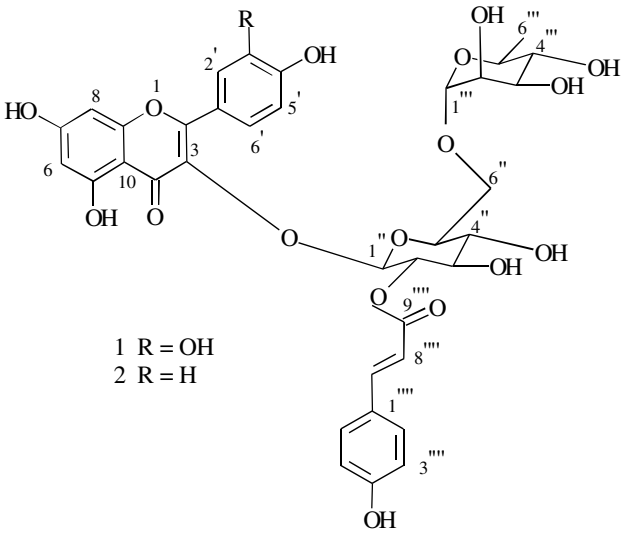
The ^{13}C-NMR spectra (Table 2) confirmed the structure of quercetin and kaempferol aglycones to **1** and **2**, respectively. The presence of rhamnose and coumaroyl moieties was also confirmed. The localization of the carbohydrates in the flavonols at C-3 was deduced by the chemical shifts of C-3, C-2, and C-4, the first being shielded and the latter two deshielded in relation to the free aglycones[8]. The rhamnose signals are unchanged, so the acyl group should be located on the other sugar moiety. The methylenic carbon of that sugar is deshielded by 6 ppm when compared with that of glucose. A COSY ^{1}H-^{1}H spectrum of **1** showed correlations between the anomeric proton signal at δ 5.56 and a signal at δ 4.87 (t, J = 8.4 Hz). These values suggested an acylation of the hydroxyl at C-2 and a diaxial hydrogen coupling between H-2/H-1 and H-2/H-3. These data are in agreement with an acylated C-2 glucose.

Comparative analyses of the ^{13}C-NMR carbohydrate data of **1** and **2** with those of the glucose in rutin[8,9,10] revealed that the

Table 2. ^{13}C-NMR spectral data for compounds 1 and 2 in DMSO-d_6.

C	1	2
2	156.6	157.4
3	133.0	132.6
4	177.2	176.4
5	161.4	161.4
6	98.9	101.2
7	164.7	166.4
8	93.9	95.2
9	156.9	156.1
10	104.1	102.1
1'	121.2	121.3
2'	116.4	130.9
3'	145.0	115.7
4'	148.8	160.4
5'	115.5	115.7
6'	122.0	130.9
1"	98.9	99.2
2"	74.2	74.4
3"	74.1	74.4
4"	70.8	71.0
5"	76.2	76.1
6"	67.4	67.3
1'''	101.1	101.3
2'''	70.8	70.8
3'''	70.6	70.8
4'''	72.0	72.2
5'''	68.6	68.8
6'''	18.0	18.2
1''''	125.4	125.4
2'''', 6''''	130.5	130.7
3'''', 5''''	116.0	116.4
4''''	160.0	160.6
7''''	145.1	146.3
8''''	114.6	114.6
9''''	166.0	168.2

C-2 of the glucose in **1** and **2** was deshielded by +2 ppm, and that C-1 and C-3 are shielded by -2 ppm. These observations are in agreement with the location of the coumaroyl group at C-2 of the glucose moiety. Thus, the structures of the two new acylated flavonol glycoside isolated from *Alibertia sessilis* were established as quercetin-3-O-β-D-(2"-O-*trans*-*p*-coumaroyl) -rutinoside (**1**) and kaempherol-3-O-β-D-(2"-O-*trans*-p-coumaroyl)-rutinoside (**2**).



Scheme 1.

The occurrence of iridoids and triterpenes in *Alibertia* is in agreement with chemosystematic correlations and botanical positioning of this genus in Gardenieae[3]. However, the isolation of this type of flavonol is unusual if we consider the chemical composition of other *Alibertia* already described in the literature.

Experimental

General experimental procedures

NMR spectra were measured at 200 MHz for ^1H, with TMS as the internal standard and 50 MHz for ^{13}C, using the solvent signal as reference.

Plant material

Alibertia sessilis (Vell.) K. Shum. (Rubiaceae) was collected in the Itirapina Botanic Reserve, in the Cerrado region of São Paulo, Brazil. A voucher specimen is deposited at the Botanical Institute of São Paulo (number S.P. 110683).

Extraction and isolation of the constituents

Dried and powdered leaves (515 g) of *A. Sessilis* were successively extracted with hexane, methylene chloride and ethanol/water (6:4). The crude hexane extract (10.4 g) was fractionated on silica gel column chromatography. Further purification of the fractions by preparative TLC, eluted with C_6H_6:EtOAc (9:1) yielded sitosterol (86 mg), α-tocopherolquinone (29 mg), and fatty material.

The $CHCl_3$ insoluble fraction (1.2 g) of the crude dichloromethanic extract (9.5 g) was methylated with CH_2N_2. Preparative TLC eluted with $CHCl_3$:MeOH (97:3) of this material gave three fractions: the first was a mixture of ursolic acid and oleanolic acid methyl esters (300 mg), the second was the methyl ester of ursolic acid (800 mg), and the third was the methyl ester of *epi*-betulinic acid (30 mg).

The crude ethanol/water (6:4) extract (48 g) was partitioned into ethyl acetate and then into n-butanol. The soluble part of *n*-Butanol (2.5 g) was submitted to column chromatography on silica gel, and eluted with $CHCl_3$ with increasing amounts of MeOH. After analysis by TLC some fractions were combined. Further preparative TLC eluted with $CHCl_3$:MeOH (85:15) of the less polar fractions gave a mixture (5 mg) of the isomeric iridoids -gardiol and β-gardiol. Another fraction of that column was submitted to reversed-phase HPLC [column C-8 (25 x 0.4 cm) and elution with a H_2O - MeOH gradient, at a flow rate of 8 mL/min, and detector UV (240 nm)], affording the iridoids gardenoside (17 mg) and deacetylasperulosidic acid methyl ester (4 mg).

The soluble part of ethyl acetate (200 mg) was precipitated with $CHCl_3$. The precipitate was dissolved in MeOH and submitted to column chromatography on Sephadex LH-20, and eluted with MeOH. After TLC, some fractions were combined and one of them was identified as quercetin-3-O-rutinoside (50 mg). Another fraction was submitted to preparative TLC, and eluted with $CHCl_3$:MeOH: *n*-BuOH:H_2O (25:5:10:1) to afford 12 mg of quercetin-3-O-β-D-(2"-*trans-p*-coumaroyl)-rutinoside (**1**) and 25 mg of kaempferol-3-O-β-D-(2"-*trans-p*-coumaroyl)-rutinoside (**2**).

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