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5,7,2′,5′-TETRAHYDROXYDIHYDROFLAVONOL 3-RHAMNOSIDE FROM *PLINIA PINNATA*

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Key Word Index—*Plinia pinnata*; Myrtaceae; aerial parts; flavonols; triterpenes; 5,7,2′,5′-tetrahydroxydihydroflavonol 3-*O*-rhamnoside.

Abstract—A new dihydroflavonol glycoside, 5,7,2′,5′-tetrahydroxydihydroflavonol 3-*O*-α-L-rhamnopyranoside, was isolated from the aerial parts of *Plinia pinnata*. Myricetin, myricitrin, oleanolic acid, α-amyrin, and β-amyrin were also identified in the plant. The structures of the compounds were determined by spectroscopic methods.

INTRODUCTION

Plinia pinnata L. (Myrtaceae) is a tree of up to 8 m, which grows in the tropical regions of America. The genus includes about 20 species distributed in Guyana, Antillas Minores, and the Amazonic regions. No phytochemical or biological studies have been reported on this species. However, *P. rivularis* and *P. trunciflora* are employed in the traditional treatment of gout, and polar extracts of these plants, containing complex flavonoid mixtures exhibited weak inhibitory activities towards the enzyme xanthine oxidase [1].

In the present investigation of *P. pinnata* six compounds were isolated and identified including the new glycoside 5,7,2′,5′-tetrahydroxydihydroflavonol-3-rhamnoside (**1**).

RESULTS AND DISCUSSION

Compound **1** was separated by Sephadex LH-20 gel filtration, RP-18 column chromatography and semipreparative TLC from the methanolic leaf extract of *P. pinnata*. It gave a mass spectrum with a $[M+H]^+$ peak at *m/z* 451 consistent with the formula $C_{21}H_{22}O_{11}$ (which was confirmed by ^{13}C NMR and DEPT spectra) and a peak at *m/z* 305 due to the loss of one rhamnose unit. The IR spectrum showed OH functions (3550–3280 cm^{-1}) and a chelated carbonyl group (1620 cm^{-1}). Bands of an aromatic ring (1570 and 1511 cm^{-1}) and of a glycosidic linkage (3240 and 1060 cm^{-1}) were also present. The UV spectrum showed absorption maxima at 283 (band II), and 326 nm (*sh*, band I) which indicated a dihydroflavonol skeleton. The bathochromic shift of band II with $AlCl_3$/HCl (30 nm) was a characteristic feature for a 5-hydroxy-3-*O*-substituted dihydroflavonol [2].

The ^1H NMR spectrum (see Experimental) confirmed many of the above features and, in addition, revealed the rhamnosyl moiety to be α-linked (J H_1–H_2 = 1.9 Hz) and in the pyranose form [3]. The aromatic region exhibited two signals at δ6.95 (1H, *br s*) and 6.82 (2H, *br s*) due to a 2,5-disubstitution of ring B and a typical *meta*-coupled pattern for H-6 and H-8 protons (δ5.86 and 5.87, *d*, J = 1.9 Hz) [4]. Furthermore two doublets of H-2 and H-3 at δ5.06 and 4.56, with a coupling constant of 10.6 Hz were observed.

The ^{13}C NMR spectrum led to the identification of the sugar moiety and its site of linkage by comparison of the data for **1** with those of other dihydroflavonol derivatives and with methyl α-L-rhamnopyranoside. The identity of the sugar moiety was also confirmed by acid hydrolysis of **1** which gave the aglycone 5,7,2′,5′-tetrahydroxydihydroflavonol and L-rhamnose (see Experimental). The resonances of ring C of **1** showed many analogies with those of 3-*O*-glycosyl derivatives of taxifolin and aromadendrin [5–6]. The upfield shifts of C-4 (2.0 ppm) and C-2 (1.8 ppm) and the downfield shifts of C-3 (4.4 ppm) which compared with the data for the aglycone (see Experimental) evidenced the linkage of the α-L-rhamnopyranoside at C-3.

Consequently, the above data for **1** are uniquely consistent with the structure of 5,7,2′,5′-tetrahydroxydihydroflavonol 3-*O*-α-L-rhamnopyranoside, which has not previously been isolated and characterized.

From the same extract five known compounds: myricetin, myricitrin, oleanolic acid, α-amyrin, and β-amyrin were also isolated and identified by means of IR, UV and NMR data.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at 200 MHz in CD_3OD with a Bruker AC 200 instrument; chemical shifts are given in δ values (ppm) with TMS as int. standard. FAB-MS were registered in positive ion mode in thioglycerol matrix, using a VG ZAB instrument. UV

spectra were recorded in MeOH and IR spectra as Nujol mulls. TLC was carried out on silica gel 60 F_{254} Merck plates.

Plant material. Leaves of *P. pinnata* L. were collected in July 1991 at Puerto Ayacucho (Amazonia, Venezuela). A voucher specimen has been deposited in the Herbarium of the Botanic Garden of the Universidad Central de Venezuela.

Extraction and isolation of flavonol glycosides. Dried leaves of *P. pinnata* (1.55 kg) were extracted in Soxhlet with MeOH to give 305 g of residue. A portion of the crude extract (8.0 g) was filtered on a Sephadex LH-20 column (100 × 5 cm). Five fractions (I–V) were eluted with MeOH. Fraction II was further chromatographed on a flash silica gel column and semiprep. TLC employing $CHCl_3$–MeOH (9:1) to give oleanolic acid (15 mg), α-amyrin (115 mg), and β-amyrin (45 mg). Fraction III was further purified by RP-8 CC using MeOH–H_2O (1:1) as eluent to give **1** (15 mg), myricitrin (68 mg) and myricetin (85 mg).

Compound **1** (*5,7,2′,5′-tetrahydroxydihydroflavonol 3-O-α-L-rhamnopyranoside*). UV λ_{max}^{MeOH} nm: 283, 326*sh*, + $AlCl_3$: 313, + $AlCl_3$/HCl: 312. FAB-MS (*m/z*): $[M+Na]^+$ 473, $[M+H]^+$ 451, $[M+H-\text{deoxyhexose}]^+$ 305. ^{1}H NMR: δ1.19 (3H, *d*, *J* = 6.2 Hz, H_3-6″), 3.40–3.70 (4H, *m*, sugar protons), 4.56 (1H, *d*, *J* = 10.6 Hz, H-3), 4.94 (1H, *d*, *J* = 1.9 Hz, H-1″), 5.06 (1H, *d*, *J* = 10.6 Hz, H-2), 5.86 (1H, *d*, *J* = 1.9 Hz, H-6), 5.87 (1H, *d*, *J* = 1.9 Hz, H-8), 6.82 (2H, *br s*, H-3′ and H-4′), 6.95 (1H, *br s*, H-6′). ^{13}C NMR: δ17.9 (C-6″), 70.5 (C-5″), 71.8 (C-2″), 72.2 (C-3″), 73.8 (C-4″), 78.6 (C-3), 83.9 (C-2), 96.5 (C-8), 97.2 (C-6), 102.1 (C-1″ and C-10), 115.4 (C-3′ or C-4′), 116.3 (C-4′ or C-3′), 120.5 (C-6′), 129.5 (C-1′), 145.2 (C-2′ and C-5′), 163.2 (C-9), 164.0 (C-5), 169.0 (C-7), 196.3 (C-4).

Acid hydrolysis of **1**. Compound **1** (10 mg) was hydrolysed with 5% aq. MeOH–HCl under reflux for 3 hr and worked up in the usual way. The residue yielded L-rhamnose identified as described previously [7] and the aglycone 5,7,2′,5′-tetrahydroxydihydroflavonol that was characterized from the following data: ^{1}H NMR: δ4.49 (1H, *d*, *J* = 11.1 Hz, H-3), 4.97 (1H, *d*, *J* = 11.1 Hz, H-2), 5.88 (1H, *d*, *J* = 2.0 Hz, H-6), 5.92 (1H, *d*, *J* = 2.0 Hz, H-8), 6.75 (2H, *br s*, H-3′ and H-4′), 6.89 (1H, *br s*, H-6′). ^{13}C NMR: δ74.2 (C-3), 85.7 (C-2), 95.9 (C-8), 97.9 (C-6), 101.8 (C-10), 115.7 (C-3′ or C-4′), 116.4 (C-4′ or C-3′), 120.8 (C-6′), 129.7 (C-1′), 145.3 (C-2′ and C-5′), 163.8 (C-9), 164.7 (C-5), 169.0 (C-7), 198.3 (C-4).

Known compounds. Oleanolic acid, α-amyrin, and β-amyrin were identified by comparison of their spectroscopic data with those of authentic samples, myricitrin and myricetin were identified by comparison of their spectroscopic data with those reported in the lit. [8–10].

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