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Rospiglioside, a New Totarane Diterpene from the Leaves of *Retrophyllum rospigliosii*

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Dedicated to Professor Pedro Joseph-Nathan on the occasion of his 65Th birthday

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Abstract. A new glucosyl totarane diterpene named rospiglioside, together with seven known abietane and totarane diterpenes identified as ferruginol, sugiol, sugiol acetate, totarol, totarol acetate, 4β-carboxy-19-nortotarol and 16-hydroxy-4β-carboxy-19-nortotarol have been isolated from the leaves of *Retrophyllum rospigliosii* (Pilger) C. N. Page (Podocarpaceae). The structure of rospiglioside was established on the basis of spectroscopic methods, mainly 1D- and 2D-NMR experiments.
Keywords: *Retrophyllum rospigliosii*, Podocarpaceae, diterpenes, abietane, totarane

Resumen. De las hojas del *Retrophyllum rospigliosii* (Pilger) C. N. Page (Podocarpaceae) fue aislado un nuevo glucosil diterpeno de la serie del totarano denominado rospigliósido, y siete diterpenos conocidos de las series del abietano y del totarano identificados como ferruginol, sugiol, acetato de sugiol, totarol, acetato de totarol, 4β-carboxi-19-nortotarol y 16-hidroxi-4β-carboxi-19-nortotarol. La estructura del rospigliósido fue establecida sobre la base de métodos espectroscópicos, principalmente experimentos de RMN uni- y bi-dimensionales
Palabras clave: *Retrophyllum rospigliosii*, Podocarpaceae, diterpenos, abietano, totarano

Introduction

Retrophyllum rospigliosii (Pilger) C. N. Page, also known as *Decussocarpus rospigliosii* (Pilger) De Laubenfels is a large tree growing in the Andean Region of Colombia, Ecuador and Venezuela. This tree, popularly known in Venezuela as "Pino Laso", has a notable ecological interest, since it is one of the few native conifers of the Andean rain forest. Its wood, valued for its durable heartwood, has potential in cabinetmaking and decorative works [1].

In previous communications we reported the isolation and characterization of several phenolic diterpenes of abietane and totarane groups and also some norditerpene dilactones from bark of *Retrophyllum rospigliosii* (at that time named *Decussocarpus rospigliosii*) [2, 3]. In a continuation of our studies, we now report the isolation and structure elucidation of a new glucosyl totarane diterpene, rospiglioside (**1**), together with seven known diterpenes, four of which (**2-5**) possess a totarane skeleton, and the other three ones (**6-8**) an abietane skeleton.

Results and discussion

The acetone extract of leaves of *R. rospigliosii* was preadsorbed on silica gel and sequentially extracted with hexane, CH_2Cl_2, EtOAc and MeOH in a soxhlet. The concentrate of CH_2Cl_2 extract was fractioned on a vacuum liquid chromatography column (VLCC) and the obtained fractions were rechromatographed on silica gel and Sephadex LH-20 (see experimental). The separation of the processed fractions was guided by the *Artemia salina* test [4], to give eight pure compounds. The seven known diterpenes, whose identity was established by 1D- and 2D-NMR studies and comparison of their spectral data with those reported in the literature, were shown to be totarol (**2)** [5], totarol acetate (**3**) [6], 4b-carboxy-19-nortotarol (**4**) [5,7], 16-hydroxy-4b-carboxy-19-nortotarol (**5**) [8], ferruginol (**6**) [9], sugiol (**7**) [10, 11] and sugiol acetate (**8**) [12].

Rospiglioside (**1**) was obtained as an oil, $[a]_D$: -34.0º (MeOH), and its HR-EIMS established the molecular formula $C_{26}H_{38}O_8$ (*m/z*: 478.2523 [M^+]). Its IR spectrum showed absorption bands of hydroxyl groups (3418 cm^{-1}), a carbonyl group (1722 cm^{-1}) and an aromatic ring (1636, 814 cm^{-1}). The ^{1}H NMR of **1** indicated the presence of two aromatic *ortho*-coupled protons [doublets at δ 6.62 and δ 6,94 (*J* = 8.6 Hz)], two tertiary methyl groups (singlets at δ 1.09 y δ 1.32) and an isopropyl group, attached to an aromatic ring (doublet at δ 1,33 (6H) and septet at δ 3.30 (1H, *J* = 7.2 Hz)); this last group was also characterized in the ^{1}H,^{1}H-COSY spectrum, which showed a cross-peak between the H-15 benzylic methine septet and the doublet of both H_3-16 and H_3-17 methyl groups.

A sharp doublet of an anomeric proton at δ 5.54 (*J* = 8.1 Hz) and several overlapped multiplets in the range 3.30-3.74 ppm were also observed in the ^{1}H NMR spectrum, indicating the presence of a glucopyranosyl moiety in the molecule. The large value for the splitting (*J* = 8.1 Hz) of the glucosyl anomeric proton doublet, due to a diaxial coupling between H-1' and H-2', suggested a β-configuration [13]. This was also confirmed in the ^{13}C NMR spectrum by the position of C-1' (δ 94.1), which agreed with that expected for a β-D-gluco-pyranosyl residue [14].

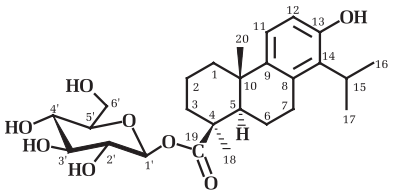
Apart from six signals typical of hexose, the ^{13}C NMR spectrum of **1** shows twenty additional carbon signals; the DEPT spectra revealed that there were four methyls, five methylenes, four methines including two aromatic sp^2 carbons, and seven quaternary carbons including a carboxyl and four aromatic carbons. On the basis of the analysis of $^{1}H,^{1}H$-COSY, HMQC and HMBC spectra, all proton and carbon signals of **1** were assigned (Table 1).

The HMBC spectrum provided information about the location of functional groups in the molecule, establishing a totarane structure for **1**. In effect, C-16 and C-17 methyl protons (δ 1.33) correlate with a methine carbon (δ 27.1; C-15) and a quaternary aromatic carbon (δ 130.1; C-14), which at the same time shows connection with an aromatic (δ 6.62; H-12) and two benzyls (δ 2.93 and δ 2.61; H-7) protons; the H-12 aromatic proton also correlates with an oxygenated aromatic carbon (δ 153.3; C-13) and this one has a ^{3}J long-range coupling with a second aromatic hydrogen (δ 6.94; H-11), thus confirming the partial structure of ring C for a totarane skeleton. This spectrum also shows the location, in the A/B decalin system, of an angular methyl group whose protons (δ 1.09; H-20) have long-range correlation with a methylene carbon (δ 40.2; C-1), an aromatic quaternary carbon (δ 139.8; C-9) and a methine carbon (δ 52.3; C-5). The hydrogen of this methine (δ 1.46; H-5) correlates with a tetrasubstituted sp^3 carbon (δ 43.7; C-4), a methyl carbon (δ 27.7 C-18), a carboxyl carbon (δ 175.6; C-19) and three methylene carbons, [δ 37.3 (C-3); δ 21.2 (C-6) and δ 29.5 (C-7)], thus establishing a spin system composed of a methine (C-5) and two adjacent methylene groups (C-6/C-7), located between a quaternary aromatic carbon (C-8) and a fully substituted sp^3 carbon (C-4) which bears both, a methyl (C-18) and a carboxyl group (C-19). The observation in the HMBC spectrum of a ^{3}J long-range correlation between C-19 (δ 175.6) and the anomeric proton (δ 5.54; H-1') clearly established that the glucosylation site was on C-19-carboxyl group. Finally the configuration of this glucosyl-carboxy group must be β-axial on the basis of the C-18 methyl and C-19 carbonyl carbons chemical shift values, which are in agreement with literature data [15]. From these results, the structure of compound **1** was concluded to be 4β-

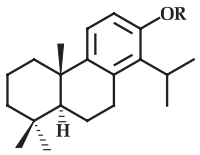
Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data and C-H correlations in HMBC spectrum of **1**.

Nº	^{1}H NMR	^{13}C NMR	HMBC(C → H)
1	α 1.27 (*m*) β 2.34 (*dt*, J= 12.5 and 3.5)	40.2 (*t*)	H-20
2	α 2.10 (*m*) β 1.55 (*dtt*, J= 12.5, 12.8 and 3.5)	19.9 (*t*)	H-1α
3	α 1.12 (*m*) β 2.23 (*dt*, J= 12.5 and 3.1)	37.3 (*t*)	H-1β, H-5, H-18
4	-	43.7 (*s*)	H-3α, H-5, H-18
5	1.46 (δ, J = 12.0)	52.3 (*d*)	H-3β, H-6α, H-6β, H-7β, H-18, H-20
6	α 2.29 (*m*) β 2.04 (*m*)	21.2 (*t*)	H-5, H-7α, H-7β
7	α 2.61 (*m*) β 2.93 (*dd*, J= 16.6 and 4.5)	29.5 (*t*)	H-5, H-6α, H-6β
8	-	133.4 (*s*)	H-6α, H-7α, H-7β, H-11
9	-	139.8 (*s*)	H-7α, H-7β, H-12, H-20
10	-	38.3 (*s*)	H-1α, H-6β, H-11, H-20
11	6.94 (*d*, J= 8.6)	123.8 (*d*)	NC
12	6.62 (*d*, J = 8.6)	114.2 (*d*)	NC
13	-	153.3 (*s*)	H-11, H-12
14	-	130.1 (*s*)	H-7α, H-7β, H-12, H-16, H-17
15	3.30 (*spt*, J = 7.2)	27.1 (*d*)	H-16, H-17
16	1.33 (*d*, J = 7.2)	19.7 (*q*)	H-17
17	1.33 (*d*, J = 7.2)	19.5 (*q*)	H-16
18	1.32 (*s*)	27.7 (*q*)	H-3α, H-5
19	-	175.6 (*s*)	H-3α, H-5, H-18, H-1'
20	1.09 (*s*)	23.1 (*q*)	H-1α, H-5,
1'	5.54 (*d*, J = 8.1)	94.1 (*d*)	H-2', H-3', H-5'
2'	3.45 (*m*)	72.9 (*d*)	H-3', H-4'
3'	3.35 (*m*)	77.2 (*d*)	H-1', H-2', H-3', H-5'
4'	3.40 (*m*)	70.3 (*d*)	H-2', H-3', H-5', H-6'
5'	3.35 (*m*)	77.2 (*d*)	H-1', H-3', H-4', H-6'
6'	3.65 and 3.81 (*m*)	61.8 (*t*)	H-4', H-5'

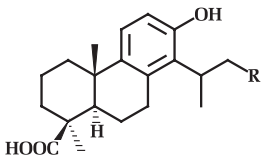
* Spectra recorded in $(CD_3)_2CO$, ppm from TMS



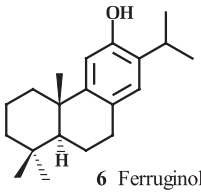
1 Rospiglioside



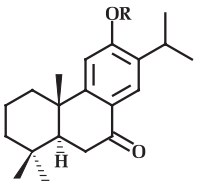
2 R = H Totarol
3 R = Ac Totarol Acetate



4 R = H 4β-Carboxi-19-Nortotarol
5 R = OH 16-Hydroxy-4b-Carboxi--19-Nortotarol



6 Ferruginol



7 R = H Sugiol
8 R = Ac Sugiol Acetate

carboxy-*O*-β-glucopyranosyl-19-nortotarol. Since this is a new diterpenes glucoside, it has been assigned the trivial name rospiglioside.

In preliminary biological tests, rospiglioside showed significant cytotoxic activity against *Artemia salina.* Currently, we are continuing studies directed to evaluate its potential effects on seed germination and root and shoot growth of lettuce (*Lactuca sativa*).

Experimental

General Experimental Procedures. Melting points were determined with a Fisher-Johns apparatus and they have not been corrected. Optical activities were measured in $CHCl_3$ on a Rudolph Research Autopol III polarimeter. IR spectra were recorded on a Perkin-Elmer FT-1725X spectrophotometer as film or KBr pellets. ^{1}H-, ^{13}C- and two-dimensional NMR spectra were acquired with a Bruker-Avance DRX-400 instrument, using $CDCl_3$ as solvent. EI-MS and HREI-MS were run on a Hewlett-Packard 5930A and on an Autospec VG spectrometer, respectively; direct inlet, 70 eV. TLC was carried out on 0.25 mm layers of silica gel PF 254 (Merck). VCC was performated with silica gel 60 (70-230 mesh.).

Plant material. The leaves of *Retrophyllum rospigliosii* (Pilger) C. N. Page were collected in La Carbonera, Municipio Autónomo Andrés Bello, Estado Mérida, Venezuela, in May 2000. A *voucher specimen* (J. M. Amaro-Luis, Nº 1630) was deposited at Herbarium MERF of Pharmacy Faculty, University of Los Andes.

Extraction and separation. Dried and pulverized leaves of *R. rospigliosii* (ca. 3.6 Kg) were extracted at room temperature with acetone and then with MeOH in a soxhlet to give, respectively, 548 and 144 g of crude extracts. The acetone extract was preadsorbed on silica gel and sequentially extracted with hexane, CH_2Cl_2, EtOAc and MeOH in a soxhlet. The CH_2Cl_2 fraction, after evaporation under reduced pressure, give 42 g of residue, which was chromatographed (VLC) over silica gel 60, eluting with hexane and EtOAc in mixtures of increasing polarity. Fractions of 500 mL were collected and combined for TLC similarity into eight major fractions (A-H). Fractions B was rechromatographed on a silica gel column using hexane-EtOAc (9:1) to furnish compounds **2** (320 mg), **3** (33 mg) and **8** (164 mg) (45 mg). Fraction C was applied to repeated silica gel (hexane-EtOAc 4:1 y 7:3) and Sephadex LH-20 (hexane-CH_2Cl_2-MeOH 1:1:1) column chromatography to yield the compounds **4** (120 mg), **6** (42 mg) and **7** (182 mg). Fraction D was filtered through a Sephadex LH-20 (hexane-CH_2Cl_2-MeOH 1:2:1) and several subfractions were further separated by PTLC on silica gel (eluted with hexane-EtOAc 7:3, developed 2x) giving **4** (83 mg) and **5** (45 mg). Fraction E was subjected to a column of Sephadex LH-20 eluted with Hexane-CH_2Cl_2-MeOH (1:2:2); subfractions 12-15 were combined and purified by PTLC (silica gel, hexane-EtOAc 1:1) to give compound **1** (35 mg).

Identification of known compounds. Compounds **2**-**8** were identified by comparison of their physical constants and their ^{1}H- and ^{13}C NMR data with those reported in literature for totarol (**2**) (m.p. 130-132 ºC; $[\alpha]_D$ +43º), totarol acetate (**3**) (m.p. 120-122 ºC), 4β-carboxy-19-nortotarol (**4**) (m.p. 180-182 ºC; $[\alpha]_D$ + 118º), 16-hydroxy-4β-carboxy-19-nortotarol (**5**) (m.p. 108-110 ºC; $[\alpha]_D$ + 77º), ferruginol (**6**) (m.p. 163-165 ºC; $[\alpha]_D$ +55º), sugiol (**7**) (m.p. 294-296 ºC; $[\alpha]_D$ + 23º) and sugiol acetate (**8**) (m.p. 165-166 ºC; $[\alpha]_D$ + 27º).

Rospiglioside (1). Colorless oil; $[\alpha]_D$ -34.0º (MeOH, *c* 0.45); HR-EIMS $[M^+]$ *m/z* 478.2523 (requires for $C_{26}H_{38}O_8$, 478.2567); UV (MeOH) λ_{max} 283 nm (log ε 3.55); IR (film) ν_{max} 3418, 2870, 2930, 1722 1600, 1636, 1074, 1028, 814; ^{1}H and ^{13}C NMR data, see Table 1; LR-EIMS, *m/z* (rel. int.): 478 $[M]^+$ (54), 316 [M-Gluc.]$^+$ (23), 301 [M-Gluc.-CH_3]$^+$ (100), 271

[M- Gluc.-COOH]$^+$ (5), 255 [M-Gluc.-CH_3-COOH]$^+$ (26), 213(24), 201 (5), 289 (6), 175 (7), 173 (5), 157 (10), 149 (7), 109(7), 95 (8), 91 (9), 81 (11), 73 (17), 60 (26).

Bioassay results. *Artemia salina* test was performed according to Meyer *et al.* methodology [4].

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