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Rubiginoside, a farnesyl glycoside from *Lepisanthes rubiginosa*

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Abstract

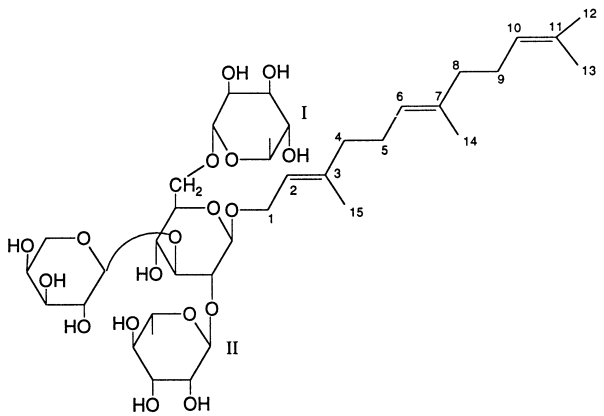
Chemical investigation of the methanolic fraction of *Lepisanthes rubiginosa* bark has led to the isolation and characterisation of a new tetrasaccharide derivative of farnesol named rubiginoside along with known triterpenoid saponins.

Keywords: Lepisanthes rubiginosa; Sapindaceae; Rubiginoside; Farnesyl glycoside; Farnesol; Hederagenin glycosides; Triterpenoid saponins

1. Introduction

There is no reported work or local use for *Lepisanthes rubiginosa* (Roxb.) Leenh., but its family Sapindaceae is known for its variety of saponins particularly hederagenin glycosides (Delaude, 1993).

In a systematic study of plants from Vietnam[2], the alcoholic extract of the bark of *L. rubiginosa* demonstrated nonspecific toxicity in vivo in mice bearing P388 murine leukemia at 40 mg/kg and in vitro cytotoxicity against A549 (human lung) tumor cells at 10–30 μg/ml. Fractionation and purification of the MeOH extract led to the isolation of the known compounds stigmasterol-3β-*O*-D-glucoside (Alam, Chopra, Ali, & Niwa, 1996), 3-*O*-α-L-arabinopyranosyl hederagenin (Li, Wang, Wu, & Yang, 1990), 3-*O*-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin (Saito et al., 1990), 3-*O*-β-D-xylopyranosyl(1-3)-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin (Kimata et al., 1983; Saito et al., 1990), 3-*O*-α-L-arabinopyranosyl(1-3)-α-L-rhamnopyranosyl(1-2)-α-arabinopyranosyl hederagenin (Saito et al., 1990), 3-*O*-β-D-glucopyranosyl(1-3)-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin (Saito et al., 1990), 3-*O*-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl ester (Kimata et al., 1983; Saito et al., 1990), 3-*O*-β-D-xylopyranosyl(1-3)-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl ester (Kimata et al., 1983; Saito et al., 1990) and a new farnesyl glycoside, rubiginoside (**1**).



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2. Results and discussion

Acid hydrolysis of compound **1** gave glucose, rhamnose, arabinose and an unstable aglycone. The ^{1}H and ^{13}C NMR spectra (Table 1) showed the presence of four monosaccharide units identified by their anomeric signals δ_H 4.38 (d, $J=8$ Hz), δ_C 102.0 (β-D-glucose), δ_H 4.75 (brs)

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[2] This work has been done in the framework of a collaborative program between CNRS France and NCST Vietnam.

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Table 1
^{13}C (62.5 MHz) in ^{1}H (800 MHz) NMR data[a] for rubiginoside (**1**) (CD_3OD)

Position	δ C	δ H (J Hz)[b]	HMBC	Position	δ C	δ H (J Hz)	HMBC
Aglycone				*Rhamnose-I*			
1	66.9	4.20 dd (7, 7); 4.30, dd (8, 8)	2, 3, 1′	1″	103.0	4.75 br s	6′, 2″, 5′
2	121.9	5.38, t (7)	4, 15	2″	73.0	3.85	1″, 3″, 5″
3	14.35			3″	73.2	3.68 dd (9, 4)	4″, 6″
4	41.6	2.10 m	2, 3, 5, 15	4″	74.9	3.40	6″
5	28.2	2.15 m	3, 4, 6, 7	5″	70.6	3.68	6″
6	126.0	5.05 t (7)	4, 5, 8, 14	6″	19.0	1.27, d (6)	4″, 5″
7	137.2						
8	41.8	2.00 m	7, 9, 10, 14	*Rhamnose-II*			
9	28.7	2.10 m	7, 8, 10, 11	1‴	103.8	5.15 br s	2′, 2‴, 5‴
10	126.3	5.09 t (7)	9, 12, 13	2‴	72.9	3.98	3‴, 4‴
11	133.0			3‴	74.0	3.65	2‴
12	26.9	1.67 s	10, 11, 13	4‴	74.7	3.40	6‴
13	18.7	1.60 s	10, 11, 12	5‴	72.7	3.98	2‴, 6‴
14	17.1	1.61 s	6, 7	6‴	18.9	1.21, d (6)	4‴, 5‴
15	17.5	1.71 s	2, 3, 4				
Glucose				*Arabinose*			
1′	102.0	4.38, d (8)	1, 5′	1⁗	106.3	4.36, d (7)	3′, 3⁗, 5⁗
2′	80.6	3.45, dd (8, 8)	1, 3′, 1‴	2⁗	74.3	3.65	1⁗
3′	88.3	3.65	2′, 4′	3⁗	75.2	3.55 dd (9, 3)	1⁗
4′	70.7	3.40	5′	4⁗	70.6	3.85	2⁗
5′	77.1	3.40	2′, 4′	5⁗	68.6	3.65	1⁗, 3⁗, 4⁗
						3.92 dd (11,2)	1⁗, 3⁗
6′	68.6	3.98	5′, 1″				

[a] Assignments based on 2D experiments.
[b] H assignments with the same chemical shifts and no splitting assignments indicate overlap of the signals.

and 5.15 (brs), δ_C 103.0 and 103.8 (two α-L-rhamnoses) and 4.36 (d, $J=7$ Hz), δ_C 106.3 (α-L-arabinose). The presence of two units of rhamnose was also observed on the spectra with δ_H 1.21 (3H, d, $J=6$ Hz) and 1.27 (3H, d, $J=6$ Hz) and δ_C 18.5 and 18.6 representing the C-6 methyl groups. The FABMS showed $[M+Li]^+$ peak at m/z 815 corresponding to the molecular formula of $C_{38}H_{64}O_{18}$. When the elements of the sugars were removed a formula $C_{15}H_{25}O$ for the aglycone was found suggesting a sesquiterpene moiety.

Further analysis of the ^{13}C NMR spectrum revealed the presence of three olefinic methines at δ 121.9, 126.0 and 126.3 corresponding to signals δ 5.38 (H-2), 5.05 (H-6) and 5.09 (H-10) in the ^{1}H NMR for the aglycone. The resonances of four methyl groups located on three olefinic quaternary carbons were observed, together with four methylene and one oxymethylene groups. Examination of the interaction of these groups on HMQC, $^{1}H/^{1}H$ COSY, HOHAHA and HMBC spectra led to the formulation of farnesol as the aglycone. The signals were also in close agreement with literature values (Crombie, King, & Whiting, 1975; Kasai et al., 1986; Inoue et al., 1994).

Onward from the anomeric proton signals, direct couplings of protons of the monosaccharides were observed on the COSY spectrum and further progressive couplings with the other protons within each sugar were shown by the HOHAHA spectrum. This led to the assignments of all protons, and further of the carbon resonances using the HMQC correlations Table 1. The observed HMBC cross peaks Table 1 confirmed the preceding assignments and permitted the establishment of the linkage sites of the sugar units. Particulary diagnostic were the cross peaks from the β-anomeric proton of D-glucose to the C-1 of the aglycone at δ 66.9, from α-L-rhamnose-I H-1 (δ 4.75) to glucose C-6 (δ 68.6), from α-L-rhamnose-II H-1 (δ 5.15) to glucose C-2 (δ 80.6), and from α-L-arabinose H-1 to glucose C-3 (δ 90.2).

These results identified rubiginoside (**1**) as 1-*O*-α-L-rhamnopyranosyl(1-6)-α-L-rhamnosyl(1-2)-α-L-arabinopyranosyl(1-3)-β-D-glucopyranosyl all-*trans*-farnes-1-ol. Similar sesquiterpene triglycosides of 12-hydroxy-all-*trans*-farnes-1-ol with two monosaccharide units (two rhamnoses, or one rhamnose and one xylose) linked to glucose at the C-2 and C-3 positions respectively have been reported in *Sapindus mukurossi* (Kasai et al., 1986) and *Sapindus trifoliatus* (Kasai et al., 1988).

The MeOH extract of *L. rubiginosa* lost its toxicity on

fractionation and none of the isolated compounds was found active. Earlier reports on Sapindaceae saponins indicate that isolated mono and bidesmosides show a poor solubility in water (Kimata et al., 1983; Kasai et al., 1986), which could explain their lack of activity.

3. Experimental

3.1. General

^{1}H NMR: 250 or 400 MHz; ^{13}C NMR: 62.5 MHz; 2-D experiments: 400 or 800 MHz; VLC and CC: Merck silica gel H 60.

3.2. Plant material

The leaves of *L. rubiginosa* were collected at Yen Chau (Son La) Vietnam, and authenticated by one of us (VD). A voucher specimen (VN 059) was deposited at the Herbarium of the Institute of Ecology, NCST, Hanoi, Vietnam.

3.3. Extraction and isolation

The powdered air-dried barks (4.0 kg) were percolated with MeOH at room temperature yielding a brown extract (350 g). The extract (100 g) was dissolved in H_2O and extracted with BuOH affording a BuOH soluble fraction (90 g). Vlc of this fraction (50 g) with CH_2Cl_2 containing increasing quantities of methanol and further with mixtures of CH_2Cl_2–MeOH–H_2O gave 14 bulked frs. Fr 4 (CH_2Cl_2–MeOH 9:1, 1.0 g) was suspended in MeOH and stigmaterol-3β-*O*-glucoside (100 mg) precipitated out.

CC of fr 5 (CH_2Cl_2–MeOH 8:2, 2g) with CH_2Cl_2 containing increasing concentration of MeOH in CH_2Cl_2 followed by PTLC (CH_2Cl_2–MeOH 8:2) yielded 3-*O*-α-L-arabinopyranosyl hederagenin (59 mg). Repeated CC of fr 6 (CH_2Cl_2–MeOH 8:2, 4.0 g) as above gave 3-*O*-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin (133 mg). Similar CC of a portion of fr 7 (CH_2Cl_2–MeOH 8:2, 4 g) gave a major compound which was repurified by CC with EtOAc/MeOH gradient to give 3-*O*-β-D-xylopyranosyl(1-3)-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin (150 mg). A portion of fr 9 (CH_2Cl_2–MeOH–H_2O 80:20:2.5, 4 g) was fractionated by CC using CH_2Cl_2–MeOH (17:3) yielding 3-*O*-α-L-arabinopyranosyl(1-3)-α-L-rhamnopyranosyl (1-2)-α-arabinopyranosyl hederagenin (55 mg) and rubiginoside (**1**) (149 mg) and after repeated CC 3-*O*-β-D-glucopyranosyl(1-3)-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin (69 mg). Similar CC of fr 11 (CH_2Cl_2-MeOH-H_2O 60:40:2.5, 4.0 g) gave 3-*O*-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl ester (10 mg), while spectral analysis of fr 12 (CH_2Cl_2-MeOH 60:40:2.5, 4.0 g) showed the constituent to be 3-*O*-β-D-xylopyranosyl(1-3)-α-L-rhamnopyranosyl(1-2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl ester (3.3 g).

3.4. Rubiginoside (*1*)

Transparent glassy material, $[\alpha]_D$ -59° (*c* 1, MeOH); ^{1}H NMR and ^{13}C NMR see Table 1; FABMS *m*/*z* 815 $[M+Li]^+$.

3.5. Acid hydrolysis

A solution of compound **1** (7 mg) in dioxane/5% H_2SO_4 60:40 was refluxed for 3 h. The mixture was evaporated under reduced pressure, dissolved in 5 ml of H_2O and partitioned with EtOAc. The aqueous layer was neutralised with $BaCO_3$ and filtered. The filtrate was analysed by 2-D TLC using CH_2Cl_2/EtOH/H_2O (16:8:1) for the first direction and EtOAc–MeOH–HOAc–H_2O (12:3:3:2) for the second one, by comparison with authentic samples.

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