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Acylated farnesyl diglycosides from *Guioa crenulata*

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Abstract

Chemical investigation of the methanol extract of the leaves of Caledonian *Guioa crenulata* led to the isolation and characterisation of four farnesyl diglycosides, crenulatosides A, B, C and D, along with three known flavonol glycosides and one known trimeric proanthocyanidin possessing a doubly linked structure. The structures of these compounds were determined on the basis of spectroscopic studies and chemical evidence. The ethanol and ethyl acetate extracts of the leaves exhibited no cytotoxic activity and no inhibition of acetylcholinesterase.

Keywords: *Guioa crenulata*; Sapindaceae; Farnesyl glycoside; Sesquiterpene; Kaempferol; Quercetin; Proanthocyanidin trimers

1. Introduction

The Sapindaceae family, a tropical and subtropical woody family, is represented by 150 genera (about 2000 species) distributed into two subfamilies (Sapindoideae and Dodonaeoideae) (Delaude, 1993). Many genera of this family e.g *Pometia*, *Dodonaea* and *Sapindus* have attracted the attention of various authors for their phytochemical and biological properties. Plants of the Sapindaceae family generally contain saponins, flavonoids, phenolic acids, and tannins (proanthocyanidins) in their leaves (Umadevi and Daniel, 1991). The genus *Guioa* (Sapindoideae) is represented by 105 species which are distributed from Indomalaysia to Australia, among them, nine are located in New Caledonia (De Kok, 2002). No previous chemical work has been reported on this genus. In a continuation of our chemotaxonomic studies on the constituents of plants of the Sapindaceae family, we have examined the leaves of *Guioa crenulata* Radlk., a tree of moderate size (height of about 5 m), growing in New Caledonia.

This species was selected in a screening program for potential cytotoxic compounds from New Caledonian plants. The ethanolic and ethyl acetate extracts of the leaves were tested in a preliminary biological screening, but no in vitro cytotoxic activity was revealed against P 388 cells, and no activity was detected in the tubulin test and on topoisomerase I. In addition, no inhibition of acetylcholinesterase activity was observed. New Caledonian *Guioa gracillis* extracts are used in cosmetic and pharmaceutical preparations for the treatment of skin aging (Renimel et al., 1998).

This paper reports on the isolation and the structural determination of three known flavonoids glycosides (**1–3**), a known trimeric proanthocyanidin possessing a doubly linked structure (**4**) and four new acyclic sesquiterpene glycosides (**5–8**), crenulatosides A, B, C and D, from the leaves of *G. crenulata* Radlk.

2. Results and discussion

The leaves were defatted and then were extracted with 80% aq. methanol. After concentration of the hydromethan-

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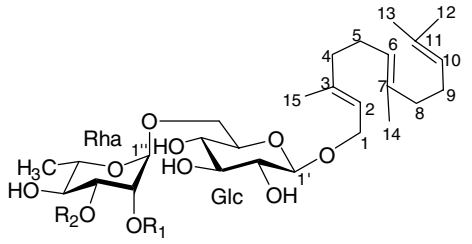
olic extract, the aqueous residue was extracted with ethyl acetate. The aqueous fraction was subjected to column chromatography over Sephadex LH-20. Fractions eluted with 50% aqueous methanol were further purified by silica gel column chromatography and finally by semi-preparative HPLC to afford three flavonoids glycosides **1**–**3** and one trimeric proanthocyanidin **4**. The ethyl acetate fraction was treated in similar fashion to purify four new acyclic sesquiterpene glycosides (**5**–**8**).



	R_1	R_2
5	H	H
6	H	CH_3CO-
7	CH_3CO-	H
8	CH_3CO-	CH_3CO-

The monosaccharides obtained by acid hydrolysis of the crude extract, were identified as L-rhamnose, D-glucose and D-galactose by TLC and by measurement of their optical rotation after purification. The three known flavonoids glycosides were isolated and identified by comparison of their spectral data (NMR, MS, UV and $[\alpha]_D$) with those described in the literature as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl] kaempferol (**1**) (Yasukawa and Takido, 1987), 3-*O*-[2,6-di-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosyl] kaempferol (mauritianin) (**2**) (Yasukawa and Takido, 1987; De Simone et al., 1990; Dini et al., 2004) and 3-*O*-[2,6-di-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosyl] quercetin (**3**) (Yasukawa et al., 1989; Dini et al., 2004).

The isolated proanthocyanidin (**4**) epicatechin-(4β → 8, 2β*O* → 7)-epicatechin-(4β → 8)-epicatechin was identified from its 1D and 2D NMR data and by comparison of its spectral data (NMR, MS, CD, UV and $[\alpha]_D$) with the literature values (Nonaka et al., 1983; Kohei et al., 2001).

Compound **5** was a diglycoside as shown by the presence in the ^{13}C NMR spectrum of two signals at δ_C 102.2 and 102.4 which were correlated in HSQC spectrum with two anomeric protons at δ_H 4.77 and 4.29, respectively (Table 1). The COSY and HSQC experiments allowed complete assignment of all the protons and carbons of each sugar unit. A β-D-glucose unit was identified starting from the anomeric proton at δ_H 4.29 (*d*, $J_{1-2} = 7.8$ Hz), on the basis of the large coupling constants between H-1, H-2, H-3, H-4 and H-5. The second unit with its anomeric proton at δ_H 4.77 (*d*, $J_{1-2} = 1.7$ Hz) was identified as L-rhamnose with its methyl group at δ_H 1.29 (*d*, $J_{5-6} = 6.3$ Hz) and δ_C 18.1 (Table 1). It is known that $^1J_{H-C} < 165$ Hz and $\delta_{C5} > 73.1$ ppm indicate a β configuration for L-rhamnose, and $^1J_{H-C} > 165$ Hz and $\delta_{C5} < 70.0$ ppm indicate an α configuration (Kasai et al., 1979; Backinowsky et al., 1980; Massiot and Lavaud, 1995). The chemical shift of carbon 5 observed at δ_C 69.8 and the coupling constant $^1J_{C-H}$ of 170 Hz, measured in the HMBC spectrum for the anomeric carbon, showed that its configuration was α. The positive electrospray ionization ESI-MS of **5** showed an ion peak at *m*/*z* 531 $[M + H]^+$ and the positive HR-MS showed a $[M + Na]^+$ ion at *m*/*z* 553.2986 in agreement with an Mr of 530 ($C_{27}H_{46}O_{10}$). When the elements of the sugars were removed, a formula of $C_{15}H_{25}O$ (222 *amu*) for the aglycon was found, suggesting a sesquiterpene moiety. Further analysis of the ^{13}C NMR spectrum revealed the presence of three olefinic methines at δ 121.3, 125.1 and 125.4 correlated in the HSQC spectrum with three protons at δ 5.39 (*ddq*, $J = 7.8, 6.4, 1.3$), 5.14 (*ddq*, $J = 8.3, 6.4, 1.3$ Hz) and 5.11 (*tm*, $J = 7$ Hz), respectively. The resonances of four methyl groups attached on three olefinic quaternary carbons were observed at δ 16.1, 16.6, 17.8 and 25.9 together with four methylenes and one oxymethylene (δ_C 66.0) (Table 1). Examination of the correlations of these signals on HSQC, COSY, and HMBC spectra led to the assignment of the aglycon as farnesol. The chemical shifts of methyl carbons, C-14 and C-15 at δ_C 16.1 and 16.6, and those of methylenes C-4 and C-8 at δ_C 40.7 and 40.9 are very similar to the values observed for (2*E*, 6*E*)-farnesol (Kasai et al., 1986; Tanaka, 1991). Only one methyl resonance, for the terminal methyl C-12, appeared above δ 20 in the ^{13}C NMR spectrum of **5**, this was particularly indicative for the *E* stereochemistry of the double bounds in the farnesyl chain (Numata et al., 1992). The absence of a signal in the region δ 30–33 in the ^{13}C NMR spectrum of **5** ruled out the other possible configurations (2*E*, 6*Z*), (2*Z*, 6*Z*), or (2*Z*, 6*E*) for the farnesyl moiety (Tanaka, 1991). The deshielding of C-1 (δ 66.0) of the farnesol unit and C-6′ (δ 68.0) of glucose suggested the points of linkage of sugar units. Cross peaks were observed in the HMBC experiment between H-1″ of rhamnose (δ 4.77) and C-6′ of glucose (δ 68.0) and between H-1′ of glucose (δ 4.29) and C-1 of farnesol (δ 66.0). These results led to the assignment of **5**, crenulatoside A, as 1-*O*-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]-(2*E*, 6*E*)-farnesol.

The positive HR-MS spectrum of compound **6** showed a pseudomolecular ion peak $[M + Na]^+$ at *m*/*z* 595.3104 ($C_{29}H_{48}O_{11}Na$) suggesting the presence of a supplementary acetyl group (42 *amu*) compared to **5**. The ^1H and ^{13}C NMR spectra of **6** showed signals for an acetyl group at δ_H 2.12 (3H, *s*) and δ_C 21.1 (CH_3) and 172.5 (CO). Two anomeric carbons were detected at 102.0 and 102.3 in the ^{13}C NMR spectrum. The first unit with its anomeric proton at δ_H 4.30 (*d*, $J = 7.8$ Hz) and with its methylene carbon C-6′ at δ_C 68.1 was identified as β-D-glucose (Table 1). The second glycosidic unit with its anomeric proton at δ_H 4.78 (*d*, $J = 1.8$) was identified as an α-L-rhamnose which possessed a deshielded H-3″ at δ_H 4.96 indicating the position of acetylation. The deshielding of C-6′ of glucose suggested the point of linkage of the rhamnose as in compound **5** (Table 1). The sequence of the glycosidic

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of crenulatosides A–D (**5–8**) in CD_3OD

	5		**6**		**7**		**8**	
	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C
Farnesol								
1	4.25 (*dd*, 11.9, 7.8)	66.0	4.26 (*dd*, 11.9, 7.9)	66.0	4.25 (*dd*, 11.8, 7.9)	66.1	4.26 (*dd*, 11.7, 7.6)	66.1
	4.31 (*dd*, 11.9, 6.4)	–	4.31 (*dd*, 11.9, 6.3)	–	4.30 (*dd*, 11.8, 7.3)	–	4.30 (*dd*, 11.7, 6)	–
2	5.39 (*ddq*, 7.8, 6.4, 1.3)	121.3	5.39 (*tq*, 6.4, 1.3)	121.2	5.39 (*tm*, 6.4)	121.4	5.39 (*tm*, 6.3)	121.3
3	–	142.4	–	142.6	–	142.4	–	142.5
4	2.09 (*m*)	40.7	2.08 (*tm*, 6.4)	40.7	2.09 (*m*)	40.7	2.09 (*m*)	40.7
5	2.16 (*tm*, 6.4)	27.4	2.15 (*tm*, 7.4)	27.4	2.15 (*tm*, 7.3)	27.4	2.15 (*tm*, 6.8)	27.4
6	5.14 (*ddq*, 8.3, 6.4, 1.3)	125.1	5.15 (*tq*, 6.9, 1.2)	125.2	5.15 (*tm*, 6.9)	125.1	5.15 (*tq*, 6.7, 1.1)	125.2
7	–	136.3	–	136.3	–	136.3	–	136.3
8	2.00 (*ddm*, 8.1, 7)	40.9	2.00 (*tm*, 8.1)	40.9	2.00 (*ddm*, 7.9, 7)	40.9	2.01 (*tm*, 7.3)	40.9
9	2.09 (*m*)	27.8	2.10 (*m*)	27.8	2.10 (*m*)	27.8	2.10 (*m*)	27.8
10	5.11 (*tm*, 7)	125.4	5.11 (*tm*, 7.1)	125.4	5.11 (*tm*, 7.1)	125.4	5.12 (*tm*, 7)	125.4
11	–	132.1	–	132.1	–	132.1	–	132.1
12	1.69 (*d*, 1.2)	25.9	1.69 (*d*, 1.1)	25.9	1.69 (*brs*)	25.9	1.69 (*brs*)	25.9
13	1.62 (*d*, 0.7)	17.8	1.62 (*brs*)	17.8	1.62 (*brs*)	17.8	1.62 (*brs*)	17.8
14	1.63 (*d*, 1.1)	16.1	1.63 (*d*, 1)	16.1	1.63 (*brs*)	16.1	1.63 (*brs*)	16.1
15	1.73 (*d*, 1.1)	16.6	1.73 (*d*, 1)	16.6	1.73 (*brs*)	16.6	1.73 (*brs*)	16.6
β-D-*Glucopyranose*								
1′	4.29 (*d*, 7.8)	102.4	4.30 (*d*, 7.8)	102.3	4.29 (*d*, 7.8)	102.5	4.31 (*d*, 7.8)	102.5
2′	3.19 (*dd*, 9, 7.8)	75.0	3.20 (*dd*, 9, 7.8)	75.0	3.19 (*dd*, 9, 7.9)	75.0	3.21 (*dd*, 8.9, 7.9)	75.0
3′	3.36 (*t*, 9)	78.1	3.36 (*t*, 9)	78.1	3.35 (*t*, 9)	78.1	3.36 (*t*, 8.9)	78.1
4′	3.29 (*dd*, 9.6, 8.9)	71.6	3.30 (*dd*, 9.4, 9)	71.7	3.29 (*dd*, 9.4, 9)	71.6	3.30 (*t*, 8.9)	71.7
5′	3.37 (*ddd*, 9.6, 6, 1.8)	76.8	3.40 (*ddd*, 9.6, 6.3, 1.8)	76.9	3.38 (*ddd*, 9.2, 6.2, 1.7)	77.0	3.37 (*ddd*, 9.6, 6.3, 1.7)	76.9
6′a	3.62 (*dd*, 11.1, 6)	68.0	3.63 (*dd*, 11.2, 6.3)	68.1	3.65 (*dd*, 11.3, 6.2)	67.8	3.67 (*dd*, 11.3, 6.4)	68.2
6′b	4.00 (*dd*, 11.1, 1.8)		4.03 (*dd*, 11.2, 1.8)	–	3.99 (*dd*, 11.3, 1.6)	–	4.03 (*dd*, 11.3, 1.7)	–
α-L-*Rhamnopyranose*								
1″	4.77 (*d*, 1.7)	102.2	4.78 (*d*, 1.8)	102.0	4.80 (*d*, 1.3)	99.2	4.82 (*d*, 1.6)	99.4
2″	3.86 (*dd*, 3.4, 1.7)	72.2	4.02 (*dd*, 3.4, 1.8)	69.8	5.07 (*dd*, 3.6, 1.6)	74.0	5.26 (*dd*, 3.4, 1.7)	71.2
3″	3.68 (*dd*, 9.5, 3.4)	72.3	4.96 (*dd*, 9.8, 3.4)	75.7	3.86 (*dd*, 9.6, 3.6)	70.5	5.09 (*dd*, 9.9, 3.4)	73.2
4″	3.39 (*t*, 9.5)	74.0	3.59 (*t*, 9.7)	71.3	3.36 (*t*, 9.6)	74.2	3.51 (*t*, 9.8)	71.4
5″	3.68 (*m*)	69.8	3.79 (*dq*, 9.6, 6.2)	69.9	3.72 (*dq*, 9.5, 6.2)	69.8	3.85 (*dq*, 9.5, 6.2)	69.8
6″	1.29 (*d*, 6.3)	18.1	1.31 (*d*, 6.2)	18.1	1.29 (*d*, 6.2)	18.1	1.32 (*d*, 6.2)	18.0
2″acetate								
CH_3^-					2.10 (*s*)	20.9	2.03 (*s*)	20.8
C=O					–	172.9	–	171.7
3″acetate								
CH_3^-			2.12 (*s*)	21.1			2.11 (*s*)	20.7
C=O			–	172.5			–	172.1

chain in **6** was determined by an HMBC experiment which showed cross peaks between C-1 (δ 66.0) of farnesol and H-1′ of glucose (δ 4.30), between C-6′ (δ 68.1) of glucose and H-1″ of esterified rhamnose (δ 4.78), and between H-3″ (δ 4.96) of rhamnose and the carbonyl (δ 172.5) of the acetyl group. Thus, compound **6**, crenulatoside B, is 1-*O*-[3-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]-(2*E*, 6*E*)-farnesol.

The spectral data of compound **7** obtained from the positive HR-MS (*m*/*z* 595.3087 $[M + Na]^+$) and NMR experiments were similar to those of **6** except for the position of the acetyl group on the rhamnose moiety. This suggested that **6** and **7** were two regioisomers with (2*E*, 6*E*)-farnesol as the aglycon. In the HMBC spectrum, the correlation of the deshielded rhamnose methine proton at δ_H 5.07 (H-2″) with the carbonyl of the acetyl group at δ_C 172.9 revealed that the acetate was attached to C-2″of rhamnose. The other correlations led to the assignment of compound **7**, crenulatoside C, as 1-*O*-[2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]-(2*E*, 6*E*)-farnesol.

The positive HR-MS of compound **8** showed a pseudomolecular ion peak $[M + Na]^+$ at *m*/*z* 637.3204 ($C_{31}H_{50}O_{12}Na$) suggesting the presence of a supplementary acetyl group compared with **6** and **7**. The ^{1}H and ^{13}C NMR data of **8** were closely comparable to those of **5**, except for the signals of the rhamnose moiety and the presence of two acetate methyls (δ_H 2.03 and 2.11 and δ_C 20.8 and 20.7) (Table 1). The α-L-rhamnose possessed two deshielded protons H-2″ and H-3″ at δ 5.26 and 5.09, indicating the positions of the two acetyl groups. Thus, compound **8**, crenulatoside D, was assigned the structure 1-*O*-[2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl]-(2*E*, 6*E*)-farnesol.

A similar glucoside of all-*trans*-farnesol, with a tetrasaccharide chain, has been reported from a Sapindaceae spe-

cies, *Lepisanthes rubiginosa* (Adesanya et al., 1999). Triglycosides of 12-hydroxy-all-*trans*-farnesol have been also reported from many species of the genus *Sapindus* (Sapindaceae), *S. mukurossi* (Kasai et al., 1986), *S. trifoliatus* (Kasai et al., 1988) and *S. delavayi* (Wong et al., 1991). Monoglucosides of 5,12-dihydroxyfarnesol have been reported from *Asteriscus pygmaeus* (Ahmed, 1992). Other farnesyl diglycosides, with arabinose as the sugar moiety, have been isolated from a soft coral, *Sinularia* sp. They enhance glucose absorption and may be used for the prevention and treatment of diabetic disorders (Sato et al., 1988; Shindo et al., 1992). To our knowledge, crenulatosides A–D are the first farnesyl diglycosides isolated from higher plants.

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 NMR spectrometer in CD_3OD (^{1}H at 500 MHz and ^{13}C at 125 MHz). 2D NMR experiments were performed using standard Bruker microprograms (XWIN-NMR version 2.6 software). ESI-MS experiments were performed using a Thermofinnigan MS-Q instrument. HR-MS experiments were performed using a Micromass Q-TOF.micro instrument (Manchester, UK) with an electrospray source (eV = 35 V, 80 °C, flow of injection 5 μl/min).

Optical rotations were measured on a Perkin–Elmer 241 polarimeter. UV spectra were obtained using a Philips PU 8720 spectrophotometer, and CD spectra were recorded with a Jasco J-810 spectropolarimeter. CC was carried out on Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), Kieselgel 60 (63–200 mesh) Merck or LiChroprep RP-18 (40–63 μm) Merck. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, a P580 pump, a diode array detector UVD 340S and a Chromeleon® software. A C-18 reversed phase column (201SP510, 250 × 10 mm, 5 μm, 90 Å, Dionex, vydac, France) was used for semi-preparative HPLC with a binary gradient elution (solvent A: H_2O, pH 2.4 with 0.0025% TFA, solvent B: MeCN) at 25 °C, and a flow rate of 3 ml min^{-1}, the chromatogram was monitored at 205 and 210 nm. TLC was performed on Kieselgel 60 F254 Merck®, with $CHCl_3$–MeOH–H_2O (70:30:5 and 8:2:0).

3.2. Plant material

The leaves of *G. crenulata* Radlk. were collected at Mont Mandjélia in New Caledonia in December 1999 and authenticated by M. Litaudon of the ICSN-CNRS, France. The voucher specimen (LIT 1002) has been deposited at the Herbarium of Botanical and Tropical Ecology Department of the IRD Centre, Noumea, New Caledonia.

3.3. Extraction and isolation

The powdered air-dried leaves (72 g) were defatted by extraction with hexane (500 ml) at room temperature and then macerated in 20% aq. methanol (700 ml) for 3 h and boiled under reflux for 3 h. The water–methanol extract was filtered and concentrated. This pasty residue was suspended in H_2O (150 ml) and partitioned between H_2O and ethyl acetate (3 × 150 ml) affording an ethyl acetate soluble fraction (7.3 g) and an aqueous soluble fraction (12 g).

The aqueous fraction (12 g) was dissolved in H_2O and applied to a Sephadex LH-20 column (30 × 750 mm) eluting successively with H_2O, 50% aq. MeOH and MeOH. Twenty-two fractions (I-XXII) of 250 ml were collected and monitored by silica TLC with the eluent $CHCl_3$–MeOH–H_2O (70:30:5). Fraction I (1.5 g) was subjected to silica gel CC (27 × 270 mm) using a gradient of $CHCl_3$–MeOH–H_2O (95:5:0 to 60:40:7), and fractions of 100 ml were collected. Frs. [22–27] eluted with $CHCl_3$–MeOH (7:3) and Frs. [28–32] eluted with $CHCl_3$–MeOH–H_2O (70:30:1) gave 214 mg of compound **4** in pure form (yield 0.30%). Fraction VI (1 g) was subjected to silica gel CC (25 × 250 mm) using a gradient of $CHCl_3$–MeOH–H_2O (8:2:0 to 60:40:7), fractions of 50 ml were collected. Frs. [16–32] eluted with $CHCl_3$–MeOH (7:3) gave 500 mg of pure compound **4** (yield 0.69 %). Frs. [52–55] eluted with $CHCl_3$–MeOH–H_2O (60:40:7) were further purified by semi-prep. HPLC, using an isocratic elution (21% B), to give compound **3** at R_t 7.8 min (8 mg, yield 0.01%) and compound **2** at *R*t 9.4 min (14 mg, yield 0.02%). Fractions VIII and IX (240 mg) were subjected to silica gel CC (15 × 160 mm) using a gradient of $CHCl_3$–MeOH–H_2O (8:2:0 to 70:30:5), fractions of 5 ml were collected. Frs. [13–39] eluted with $CHCl_3$–MeOH (75:25) were purified by semi-prep. HPLC, using an isocratic elution (21% B), to give 3 mg of compound **1** (yield 0.004%).

A part of the ethyl acetate fraction (5 g) was subjected to reversed phase RP-18 vacuum liquid chromatography (VLC) (50 × 50 mm) eluting successively with 40, 80% aqueous methanol and MeOH. Three fractions of 250 ml were collected and monitored by TLC in $CHCl_3$–MeOH (8:2). A part (700 mg) of the fraction eluted with MeOH–H_2O (40:60) was subjected to reversed phase RP-18 CC (22 × 180 mm) using a gradient of MeOH–H_2O (6:4–10:0), and fractions of 10 ml were collected. Frs. [26–33] eluted with MeOH–H_2O (7:3) were subjected to silica gel CC (15 × 130 mm) using a gradient of $CHCl_3$–MeOH (95:5–7:3), and fractions of 5 ml were collected. Then, fractions eluted with $CHCl_3$–MeOH (85:15) were purified by semi-prep. HPLC using a linear gradient (35–50% B) during 30 min to give compounds **5**, **6** and **7** at R_t 21.9 min (6 mg, yield 0.008%), 26.4 min (14 mg, yield 0.02%) and 27.3 min (6 mg, yield 0.008%). Frs. [37–49] eluted with MeOH–H_2O (8:2) were subjected to silica gel CC (10 × 100 mm) using a gradient of $CHCl_3$–MeOH (100:0–9:1), fractions of 5 ml were collected. Fractions eluted with $CHCl_3$ contain 4 mg of compound **8** in a pure form (yield 0.006%).

3.4. Acid hydrolysis

Two hundred milligrams of the crude water–methanol extract of the leaves were refluxed with 30 ml of 2 N HCl for 4 h 30 min. The mixture was extracted with EtOAc (3×15 ml). The acid aqueous layer was neutralised with 0.5 M NaOH and freeze-dried. Three sugars were identified as rhamnose, glucose and galactose, by comparison with authentic samples on TLC in MeCOEt/*iso*PrOH/Me_2CO/H_2O (20:10:7:6). After prep TLC of the sugar mixture (100 mg) in this solvent, the optical rotation of each purified sugar was measured.

3.5. Crenulatoside A (**5**)

$[\alpha]_D^{20}$ −44° (MeOH; *c* 1). ESI-MS (positive ion mode): *m/z* 531 $[M + H]^+$. ^1H and ^{13}C NMR data (CD_3OD): see Table 1. HR-MS: *m/z*. 553.2986 (calcd for $C_{27}H_{46}O_{10}Na$, 553.2989).

3.6. Crenulatoside B (**6**)

$[\alpha]_D^{20}$ −28° (MeOH; *c* 1), ESI-MS (positive ion mode): *m/z* 573 $[M + H]^+$, 595 $[M + Na]^+$. ^1H and ^{13}C NMR data (CD_3OD): see Table 1. HR-MS: *m/z* 595.3104 (calcd for $C_{29}H_{48}O_{11}Na$, 595.3094).

3.7. Crenulatoside C (**7**)

$[\alpha]_D^{20}$ −24° (MeOH; *c* 0.58), ESI-MS (positive ion mode): *m/z* 595 $[M + Na]^+$. ^1H and ^{13}C NMR data (CD_3OD): see Table 1. HR-MS: *m/z* 595.3087 (calcd for $C_{29}H_{48}O_{11}Na$, 595.3094).

3.8. Crenulatoside D (**8**)

$[\alpha]_D^{20}$ −31° (MeOH; *c* 0.33), ESI-MS (positive ion mode): *m/z* 661 $[M + 2Na]^+$. ^1H and ^{13}C NMR data (CD_3OD): see Table 1. HR-MS: *m/z* 637.3204 (calcd for $C_{31}H_{50}O_{12}Na$, 637.3200), 653.2916 (calcd for $C_{31}H_{51}O_{12}K$, 653.2939).

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