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Comparative phytochemical analysis of four Mexican *Nymphaea* species

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Abstract

Four Mexican *Nymphaea* species, *N. ampla*, *N. pulchella*, *N. gracilis* and *N. elegans* belonging to subgenera *Brachyceras* were analyzed. In this work two 5-glycosyl isoflavones, 7,3′,4′-trihydroxy-5-*O*-β-D-(2″-acetyl)-xylopyranosylisoflavone (**1**) and 7,3′,4′-trihydroxy-5-*O*-α-L-rhamnopyranosylisoflavone (**2**), were isolated from *N. ampla* and *N. pulchella*, respectively, together with other known 3-glycosyl flavones and triterpene saponins from the same four species. The structures were elucidated by 1D and 2D NMR, FABMS, and other spectroscopic analyses. These results confirmed that the four species were different from each other and established that *N. pulchella* represents a different taxa than *N. ampla*. In addition, the 5-glycosyl isoflavones could be considered as a taxonomic character of this group of plants.

Keywords: *Nymphaea ampla*; *N. elegans*; *N. gracilis*; *N. pulchella*; Nymphaeaceae; 5-glycosyl isoflavones; Taxonomic character

1. Introduction

Water Lily is a common name for a family of aquatic plants (Nymphaeaceae Salisb.), which is constituted by 70 species of cosmopolitan distribution (Cook, 1990; Gunn et al., 1992). The representative genus *Nymphaea* was established by Linneo (1753), and comprises about 40–45 species widespread in tropical and temperate regions. All are aquatics with perennial or annual rhizomes, floating or submerged leaves and solitary showy flowers. Many species are prized as ornamentals and have been introduced and naturalized outside of their active habitats (Wiersema and Hellquist, 1997). Conard (1905) subdivided *Nymphaea* into five subgenera (*Anecphya* (Casp.) Conard, *Brachyceras* (Casp.) Conard, *Hydrocallis* (Planch.) Conard, *Lotos* (DC.) Conard, and *Nymphaea* L.). During the last few decades, the interest in the study of these plants has increased as they have not been completely studied and their taxonomic relationships are unclear (Les et al., 1999). With regard to chemical constituents, only six species have been previously investigated, and these studies describe the presence of two lignans from *N. odorata* (Zhang et al., 2003), one hydrolysable tannin from *N. tetragona* (Kurihara et al., 1993); several glycosyl flavonols from *N.* x *marliacea* (Fossen et al., 1998a), *N. caerulea* (Fossen et al., 1999), *N. lotus* (Elegami et al., 2003), and *N. odorata* (Zhang et al., 2003), and various acylated anthocyanins from *N. candida* (Bendz and Jönsson, 1971), *N. x marliacea* (Fossen et al., 1998b; Fossen and Andersen, 1997), and

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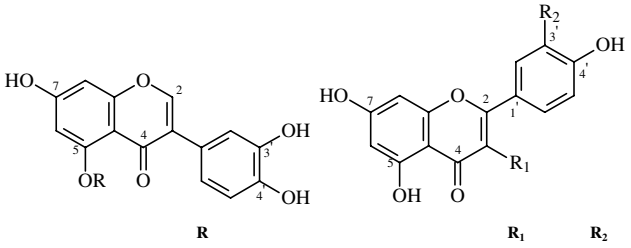
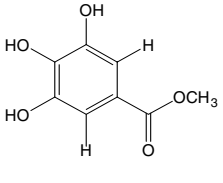
N. caerulea (Fossen and Andersen, 1999), as well as two rare macrocyclic flavonoids from *N. lotus* (Elegami et al., 2003). Recently a systematic study using phenetic relationships, based on general morphology, seed micromorphology, and chromosome number, identified twelve species occurring in México (Bonilla-Barbosa et al., 2000). In this study, four species belonging to the subgenera *Brachyceras* (*N. ampla*, *N. elegans*, *N. gracilis*, and *N. pulchella*) were found to have many general morphological variations, implicating that those are from different taxa (during decades *N. pulchella* has been considered as a variety of *N. ampla*). To the best of our knowledge, none have been chemically investigated, and we have now undertaken the comparative phytochemical study of the four Mexican species in the subgenera *Brachyceras*.



In this paper, we report the isolation and structure elucidation of nine compounds (**1**–**9**), including: 7,3′,4′-trihydroxy-5-*O*-β-D-(2″-acetyl)-xylopyranosyl-isoflavone (**1**), quercetin 3-rhamnoside (**4**), quercetin 3-xylopyranoside (**5**), quercetin 3-glucopyranoside (**6**), and methyl gallate (**7**) from *N. ampla*; 7,3′,4-trihydroxy-5-*O*-α-L-rhamnopyranosyl-isoflavone (**2**), and kaempferol 3- rhamnopyranoside (**3**) from *N. pulchella*; methyl-oleanolate-3-*O*-β-D-glucopyrnoside (**8**), and 28-*O*-β-D-glucopyranosyl-oleanolate (**9**) from *N. gracilis*; and quercetin 3-rhamnoside (**4**) from *N. elegans*. β-sitosterol and β-sitoteryl-3-*O*-β-D-glucopyranoside were common to *N. pulchella*, *N. gracilis* and *N. elegans*. Compounds **3–9** have been described previously in the literature, while compounds **1** and **2** are new natural products. Compounds **1** and **2** were characterized by using a combination of one-(1-D) and two-dimensional (2-D) NMR spectroscopy, including COSY, TOCSY, HMQC, HMBC and NOESY experiments, in conjunction with FAB-mass spectrometry, while compounds **3–9** were identified by comparison of their physical and spectral data with literature values.

1	Xyl-(2′′-OAc)	**3**	Rha	H
1a	H	**4**	Rha	OH
2	Rha	**5**	Xyl	OH
		6	Glc	OH



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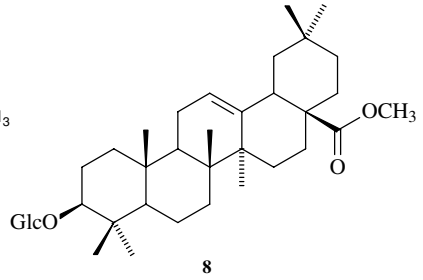

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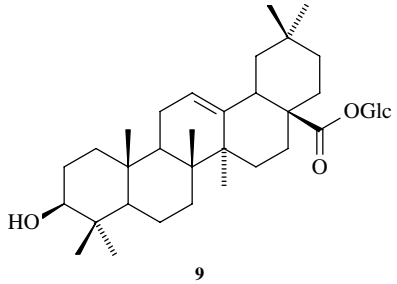

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2. Results and discussion

Compound **1** was obtained from the methanol extract of *Nymphaea ampla* as a yellow amorphous powder with $[\alpha]_D^{20}$ –8.0° (MeOH; *c* 0.8). Its IR spectrum indicated the presence of hydroxyl (3398 cm^{-1}) and carbonyl (1674 and 1724 cm^{-1}) groups, as well as aromatic rings (1499 cm^{-1}). The molecular formula, $C_{22}H_{20}O_{11}$, was inferred from the $[M + H]^+$ peak at *m*/*z* 461.3868 in the HRFAB-MS, and was supported by analysis of the ^{13}C NMR and DEPT spectra. The ^1H and ^1H–^1H COSY spectra of **1** (Table 1) revealed that this compound was an isoflavone with glycosidic and acetate functionalities present. The ^1H NMR spectrum showed a singlet at δ 7.7 assigned to H-2 of the isoflavone nucleus (Bohm, 1998). In the aromatic proton region, the signals due to an AMX system at δ 7.5 (1H, *d*, *J* = 2.4 Hz), 7.3 (1H, *dd*, *J* = 8.4, 2.4 Hz) and 6.8 (1H, *d*, *J* = 8.4 Hz), clearly showed the presence of a 1,3,4 trisubstituted aromatic ring upon examination of the chemical shifts and coupling patterns. The AB system at δ 6.3 and 6.1 (each 1H, *d*, *J* = 2.2 Hz) also indicated the presence of a 1,2,3,5-tetrasubstituted aromatic ring. Examination of its homonuclear correlation spectra led to identification of a β-xylopyranose moiety (Table 1), and the β configuration of the anomeric carbon being evident from the coupling constant of H-1″ (*J* = 7.6 Hz) as observed in the ^1H NMR spectrum. These data and the UV absorption maxima at 248 and 300 nm (only the band at 300 nm was shifted +15 nm by NaOAc–H_3BO_3) suggest that compound **1** was a glycosyl isoflavone with an *ortho*-dihydroxyl group in the B ring, but no free hydroxyl group at C-5 (Markham, 1982). In

Table 1
NMR spectroscopic data of **1, 1a** and **2** in CD_3OD

	1			**1a**		**2**	
	δ_C	δ_H	HMBC	δ_C	δ_H	δ_C	δ_H
2	156.21	7.61 *(s)*	C-3, C-4, C-1′	155.92	8.12*(s)*	155.73	7.58 *(s)*
3	132.84			132.71		133.11	
4	177.06			178.05		177.25	
5	161.22			162.11	12.32(s)	161.28	
6	99.23	6.12 (*d*, 2.4)	C-5, C-10, C-8	98.92	6.23 (*d*, 2.2)	93.41	6.18 (*d*, 2.4)
7	163.97			162.91		164.28	
8	94.04	6.27 (*d*, 2.4)	C-7, C-6, C-10	94.23	6.42 (*d*, 2.2)	98.64	6.42 (*d*, 2.0)
9	156.11			156.32		156.23	
10	103.99			102.90		103.79	
1′	121.43			122.25		121.32	
2′	115.68	7.52 (*d*, 2.4)		116.14	7.61 (*d*, 2.2)	116.06	7.34 (*d*, 2.4)
3′	144.29			144.22		144.77	
4′	148.11			148.31		148.50	
5′	115.64	6.79 (*d*, 8.4)		115.94	6.92 (*d*, 8.4)	115.17	6.90 (*d*, 8.0)
6′	127.05	7.45 (*dd*, 2.4,8.4)		126.92	7.43 (*d* d, 2.2,8.4)	126.89	7.61 (*dd*, 2.2, 8.4)
Xyl-1″	100.23	5.33 (*d*, 7.6)	C-5, C-2″, C-3″				
2″	74.15	4.92 (*t*, 7.6, 8.4)	C-1″, C-4″, –CO_2–				
3″	74.21	3.4 4 (*t*, 8.8, 9.2)					
4″	70.06	3.61 (*ddd*, 4.4, 8.8, 9.6)					
5″A	66.43	3.72 (*dd*, 5.2,11.6)					
5″B	66.43	3.11 (*dd*, 9.6, 11.6)	C-2″, –COO–				
CH_3CO_2–	21.54						
–COO–	169.48	2.20 (*s*)					
Rha-1″						103.58	5.31 (*d*, 1.5)
2″						72.12	4.21 (*dd*, 1.5, 3.5)
3″						72.31	3.74 (*dd*, 3.5, 9.5)
4″						73.34	4.42 (*dd*, 7.5)
5″						74.91	3.41 (*m*)
6″						17.65	0.95 (*d*, 6.5)

^1H and ^{13}C are 400 and 100 MHz, respectively.

agreement with this, the ^{13}C NMR spectrum (Table 1) exhibited signals for two carbonyl carbons (δ 177.1 and 169.4), along with 12 aromatic carbons, five of which (δ 161.2, 163.9, 156.1, 144.3 and 148.1) had oxygen functionalities and two vinylic carbons C-2 (δ 156.2) and C-3 (δ 132.8), together with four aliphatic oxygen-bearing methines and one oxymethylene corresponding to a pentose moiety at δ 100.2, 74.2, 74.1, 70.1, and 66.4, respectively. Observation of MS fragment ion peaks at *m/z* 326 and 134 derived from retro-Diels-Alder type cleavage, suggested the location of two hydroxyl groups on the B ring, and a hydroxyl group and an acetyl pentose in the A ring (Dewick, 1988). In addition, ^1H and ^{13}C NMR spectra revealed the presence of an acetate group in the molecule at δ_H 2.2 (s) and δ_C 169.4 and 21.5. Lower field shifts of H-2″ ($\delta\Delta$ 0.325) and C-2″ ($\delta\Delta$ 0.903) signals indicted the bonding site of the acetate function (Agrawal, 1992). All protonated carbons were assigned by CH correlation spectroscopy (HMQC). In the HMBC spectrum, the proton at C-2 (δ 7.6) showed C–H three bond correlations with the carbonyl carbon signal at δ 177.1 and C-9 (δ 156.1), which was further correlated with the doublet at δ 6.3 assigned to H-8. Comparable couplings from H-6 (δ 6.1) identified C-5 (δ 161.2) and C-10 (δ 103.9). All ^1H and ^{13}C NMR spectroscopic signals of the xylosyl moiety could be assigned based on 2D NMR spectroscopic data analysis. A TOCSY experiment revealed a correlation between the H-5″ signals at δ_H 3.1 and 3.7 and the anomeric proton at δ_H 5.3, demonstrating that they belong to the same spin system. The xylose moiety could then be assigned to C-5 because of a ^3J coupling between the anomeric proton (δ 5.3) and C-5 (δ 161.2); similarly the acetate group was assigned to C-2 of the xylose moiety because of the ^2J correlation between the methyl protons (δ 2.2) and the carbonyl carbon signal at δ 169.4, and further ^3J correlation of this carbon with H-2 (δ 7.6) of xylose. Characteristic correlations observed in the HMBC, COSY and TOCSY experiments are shown in Fig. 1. Finally, acid hydrolysis of compound **1** established unequivocally the position of the sugar moiety. Analysis of the NMR spectroscopic data of the aglycone (**1a**) and comparison with those of **1**, showed that **1a** differs only in the absence of all the ^1H and ^{13}C resonances of the xylose unit linked at C-5, and the appearance, instead, of a chelated

hydroxyl group (δ 12.8). On the basis of all this evidence, the natural product was identified as 7,3′,4′-trihydroxy-5-*O*-β-D-(2″-acetyl)-xylopyranosyl-isoflavone (**1**).

Compound **2** was isolated from the methanol extract of *N. pulchella* as a yellow amorphous powder with $[\alpha]_D$-19° (MeOH; *c* 0.2), whose molecular formula was confirmed to be $C_{21}H_{20}O_{10}$ by HRFAB-MS. The UV and IR spectra were very similar to **1**, and the signals at δ_H 7.5 and δ_C 155.7 in the ^1H and ^{13}C NMR spectra (Table 1), suggested the presence of an isoflavone type skeleton, as well as of chemical shifts indicative of a 5,7,3′,4′-pattern in the isoflavone nucleus. The ^1H and ^{13}C resonances in the one- and two-dimensional NMR spectra of (**2**) were assigned in a similar way as those of (**1**). Examination of its homonuclear correlation spectra led to the identification of a α-rhamnospyranose moiety (Table 1), which was placed at C-5 because of the cross-peak observed between H-1″ (δ_H 5.3) and C-5 (δ_C 161.2) in the HMBC spectrum. Thus, (**2**) was characterized as 7,3′,4′-trihydroxy-5-*O*-α-L-rhamnopyranosyl-isoflavone.

According to the results of this phytochemical study, *Nymphaea ampla*, *N. elegans*, *N. gracilis*, and *N. pulchella* synthesize different compounds, which makes these plants quite distinct, confirming the morphological variations observed between these species by Bonilla, and supporting the work on the taxonomic limits of this genus (Bonilla-Barbosa et al., 2000), in which *N. pulchella* is recognized as a valid, different, species from *N. ampla*. For this reason, the correct name for this taxa is *N. pulchella*, described by Candolle from Perú in 1821. By the other hand, 5-glycosyl isoflavones have for instance been identified only in five plant species, namely, *Dolichos biflorus* (Mitra et al., 1983), *Prunus avium* and *P. cerasus* (Khalid et al., 1989; Geibel et al., 1990), *Belamcanda chinensis* (Dewick, 1994), and *Ageratum conyzoides* (Yadava and Kumar, 1999), in addition to the isoflavones identified in *N. ampla* and *N. pulchella* in this paper, and may thus have chemotaxonomic significance as a taxonomic character of this group of plants.

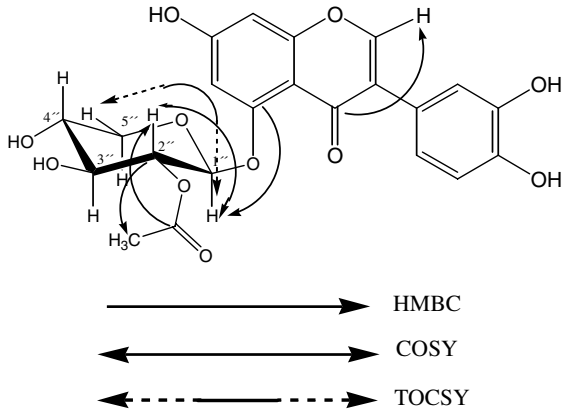

Fig. 1. Structure of 1 and characteristic correlations observed in HMBC (C → H), COSY, and TOCSY NMR experiments.

3. Experimental

3.1. General experimental procedures

Melting points were obtained on a Fisher–Johns melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin–Elmer 241 digital polarimeter at 20 °C. UV spectra were obtained on a Hewlett-Packard 8453 spectrometer. IR spectra (KBr) were recorded on a Bruker Vector 22 FTIR spectrophotometer. ^1H NMR, ^{13}C NMR and 2D NMR spectra were recorded on a Varian INOVA-400 spectrometer (^1H 400 MHz and ^{13}C 100 MHz) in CD_3OD, with TMS as internal standard. FAB-MS and HRFAB-MS were performed using a Hewlett Packard 5985-B and a JEOL Mstation JMS-700 mass spectrometer, respectively. Known compounds were identified by comparison of their spectral data with those in the literature. Column chromatography (CC) was performed on Merck (7734) silica gel (0.063–0.2 mm); and analytical TLC and HPTLC utilized Silica gel 60 F_{254} eluted with CH_2Cl_2/MeOH (85:15), and reversed-phase RP-18 F254s using H_2O–MeOH–CH_3CN (3:2:1) and compounds of interest were visualized by spraying with $[(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O]$–$H_2SO_4$.

3.2. Plant material

Nymphaea pulchella was collected in Axochiapan Morelos, on October 1999; *N. ampla* was collected by the side of the road of Coyuca de Benitez, Zihuatanejo, Guerrero, on May 2001; *N. gracilis* was collected in Jalisco, on May, 2002; and *N. elegans* was collected in Michoacán, on May 2002. All specimens were identified by Dr. Jaime Bonilla Barbosa, Departamento de Botánica, UAEMor.-México (CIB). Voucher specimens (voucher number 2743, 2172, 2606, and 2610, respectively) were deposited in the Herbaria of Universidad Autónoma del Estado de Morelos (HUMO) and Universidad Nacional Autónoma de México (MEXU; herbarium abbreviations according to Holmgren et al., 1990).

3.3. Extraction and isolation

Dried and powdered whole plants of *N. pulchella* (1.03 kg), *N. ampla* (1.1 kg), *N. gracilis* (1.2 kg) and *N. elegans* (2.8 kg) were extracted with MeOH (8 L × 3)

by maceration at room temperature separately. The combined extracts were individually concentrated in vacuo to dryness, to yield 60.9, 89.5, 237.8 and 304.6 g of individual MeOH extracts of *N. pulchella*, *N. ampla*, *N. gracilis* and *N. elegans*, respectively.

The MeOH extract (60.9 g) of *Nymphaea pulchella* was fractionated on a vacuum liquid chromatography column (VLC, 400 g) eluting with a gradient of *n*-hexane–EtOAc–MeOH (80:20:00–00:00:100) to afford 78 fractions. These fractions were pooled into 7 fractions (NP-1 to NP-7) according to their similar TLC behavior. Fraction NP-5 (1.2 g) was subjected to silica gel CC (36 g) using a gradient mixture of CH_2Cl_2–MeOH (95:05–50:50) as eluent to give five fractions (NP-5A to NP-5E). Fraction NP-5C (24 mg), eluted with CH_2Cl_2–MeOH (80:20) was purified by preparative RP_{18} TLC using a mixture of H_2O–CH_3CN–MeOH (3:2:1) as eluent to yield 7,3′,4,-trihydroxy-5-*O*-α-L-rhamnopyranosylisoflavone (**2**, 8 mg, 0.0008%, m.p. 186–187 °C) and kaempferol 3-rhamnoside (**3**, 14 mg, 0.002%, m.p. 168–171 °C) (Matthes et al., 1980).

The MeOH extract (89.5 g) of *N. ampla* was fractionated by percolation on silica gel obtaining four fractions: *n*-hexane (10.8 g), CH_2Cl_2 (15.3 g), CH_2Cl_2–MeOH (1:1, 39.6 g) and MeOH (10.1 g).The fraction soluble in CH_2Cl_2:MeOH (1:1) was subjected to silica gel CC (600 g) eluted with a gradient of *n*–hexane–CH_2Cl_2–MeOH (80:20:00–00:50:50) to afford 78 fractions. These fractions were pooled into seven fractions (NA-1 to NA-7) according to their similar TLC behavior. CC purification of NA-1 (10.3 g), performed with gradient of *n*-hexano–CH_2Cl_2 afforded β-sitosterol (4.8 g). Fraction NA-5 (8.4 g) was purified by CC using CH_2Cl_2–MeOH (95:05) as isocratic eluent to afford methyl gallate (**7,** 923.5 mg, 0.09%, m.p. 147–149 °C) (Fiuza et al., 2004). Fraction NA-6 (6.8 g) was purified by CC using gradient elution CH_2Cl_2–MeOH (95:5 → 0:100) to give 48 fractions. According to differences in composition monitored by TLC, four fractions (NA-6A to NA-6D) were obtained. Fraction NA-6C (0.253 g) was subjected to Si gel CC, eluting with a gradient of CH_2Cl_2–MeOH (98:02 → 50:50) to yield 15 fractions (NA-$6C_1$ to NA-$6C_{15}$). Fractions 8–12, eluted with CH_2Cl_2–MeOH (8:2) were purified by reversed-phase preparative TLC using a mixture of H_2O–CH_3CN–MeOH (3:2:1) to yield 7,3′,4′-trihydroxy-5-*O*-β-D-(2″-acetyl)-xylopyrnosylisoflavone (**1**, 0.001%, 12 mg, m.p. 176–178 °C) and quercetin 3-rhamnoside (**4**, 0.002%, 15 mg, m.p. 164–166 °C) (Markham et al., 1978). Fractions 13–15, eluted with CH_2Cl_2–MeOH (3:2) were purified by reversed-phase RP_{18} column chromatography using a gradient of H_2O–CH_3CN–MeOH (80:15:05 → 60:30:20) to yield quercetin 3-xylopyranoside (**5**, 14 mg, 0.002%, m.p. 183–185 °C) (Mabry et al., 1970) and quercetin 3-glucoside (**6**, 320 mg 0.004%, m.p. 195–198 °C) (Mabry et al., 1970).

The MeOH extract of *N. elegans* (237.8 g) was subjected to silica gel chromatography, eluted with *n*-hexane–EtOAc–MeOH (gradient from 90:10:00 to 00:20:80). According to the differences in composition monitored by TLC, 16 fractions (NG-1 to NG-16) were obtained. Fraction NG-4 (16.3 g) was fractionated on a silica gel column eluting with *n*-hexano–EtOAc gradient (90:10–50:50) to afford 7 fractions (NG-4A to NG-4G). CC of NG-4E with *n*-hexano–EtOAc (70:30) afforded β-sitosterol (2.8 g). Fraction NG-7 (5.3 g) was subjected to silica gel CC with EtOAc–MeOH (70:30) as isocratic eluent, to afford five fractions (NG-7A to NG-7E). Fraction NG-7C gave 3-*O*-β-D-sitosterol glycoside (700 mg). Fraction NG-12 (3.8 g) was applied to a silica gel column, eluted with a gradient of EtOAc–MeOH (70:30–00:100). According to differences in composition monitored by TLC, 12 fractions (NG-12A to NG-12L) were obtained. Fraction NG-12E gave methyl-oleanolate-3-*O*-β-D-glucopyranoside (**8**, 267.6 mg, 0.009%, m.p. 223–225 °C). Fraction NG-14 was also fractionated on silica gel column eluting with EtOAc–MeOH (3:7) to yield 28-*O*-β-D-glucopyranosyl-oleanolate (**9**, 387.4 mg, 0.014%, m.p. 287–289 °C) (Sakai et al., 1994).

The MeOH extract of *N. gracilis* (304.6 g) was fractionated on a vacuum liquid chromatography column (VLC- 430 g silica gel) eluting with *n*-hexane–EtOAc–MeOH mixtures of increasing polarity to yield nine fractions (NE-1 to NE-9). Fraction NE-3 (18.7 g), eluted with *n*-hexane–EtOAc (85:15), was applied to a silica gel CC (400 g) eluted with a gradient mixture of *n*-hexane–EtOAc to give five fractions (NE-3A to NE-3E). Fraction NE-3B, eluted with *n*-hexane–EtOAc (7:3), afforded 7.6 g of a mixture of stigmasterol and β-sitosterol. Fraction NE-5 (3.8 g), eluted with *n*-hexane–EtOAc (1:1) was subjected to silica gel CC, eluted with CH_2Cl_2–MeOH (gradient from 95:05 to 50:50). four fractions (NE-5A to NE-5D) were obtained. Fraction NE-5C, 1.26 g, (7:3) was purified on a silica gel column eluted with CH_2Cl_2–MeOH (8:2) to give 3-*O*-β-D-sitosteryl glycoside (347 mg) (Iribarren and Pomilo, 1984) and quercetin 3-rhamnoside (**4**).

*3.4. 7,3′,4′,Trihydroxy-5-O-β-D-(2″-acetyl) xylopyranosylisoflavone (**1**)*

Yellow amorphous powder, $[\alpha]_D^{25}$ –8.0° (MeOH, *c* 0.8); UV (MeOH) λ_{max} nm (log ε): 300 (4.2), 248 (3.3), (+NaOAc–H_3BO_3) 315, 248; IR (KBr) ν_{max} cm^{-1} 3320, 1724, 1674, 1065; For ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; positive FAB-MS: *m/z* 483 $[M + Na]^+$, 461 $[M + H]^+$

HRFAB-MS *m/z* 461.3868 ($[M + H]^+$, calcd. for $C_{22}H_{20}O_{11}$: 461.3876).

3.5. Acid hydrolysis of compound 1

Compound **1** (7 mg) in 5% HCl (5 ml) was heated until reflux began, this being maintained for 2 h. After cooling, the non-polar product was extracted with EtOAc. The EtOAc fraction was concentrated and the nonpolar reaction product was separated by precipitation with ice to afford **1a** (3 mg) m.p. 138–140 °C; For NMR spectral data, see Table 1. The aqueous layer was neutralized with 5% NaOH (5 ml) and concentrated until dryness for identification. The sugars were analyzed by silica gel TLC [EtOAc–MeOH–H_2O–AcOH (11:2:2:2)] by comparison with standard sugars.

3.6. 7,3′,4′-Trihydroxy-5-O-α-L-rhamnopyranosylisoflavone (2)

Yellow amorphous powder; $[\alpha]_D^{20}$ –19° (MeOH, *c* 0.2); UV (MeOH) λ_{max} (MeOH) nm (log ε): 312 (4.6), 272 (3.8), (+NaOAc–H_3BO_3) 325, 273; IR (KBr) ν_{max} cm^{-1} 3340, 1668; For ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHZ, CD_3OD), see Table 1; positive FAB-MS: *m/z* 455 $[M + Na]^+$, 433 $[M + H]^+$ HRFAB-MS *m/z* 433.4885 ($[M + H]^+$, calcd. for $C_{21}H_{20}O_{10}$: 433.4882).

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