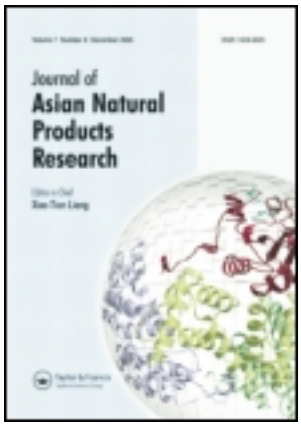

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TRITERPENOID SAPONINS FROM *NEONAUCLEA SESSILIFOLIA* MERR.

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Two new triterpenoid saponins, quinovic acid 3-*O*-6-deoxy-β-D-glucopyranoside, 28-β-D-glucopyranosyl ester and quinovic acid 3-*O*-α-L-rhamnopyranosyl-(4 → 1)-β-D-glucopyranoside, along with three known saponins, have been isolated from the EtOAc extracts of the dried stems of *N. sessilifolia* Merr. Structure elucidation of **1**–**3** was based on NMR, MS, IR and chemical analysis.

Keywords: *Neonauclea sessilifolia* Merr.; Triterpenoid saponins; Structure elucidation

INTRODUCTION

Many plants of the family Rubiaceae are known to be of medicinal value [1–3], and many interesting glycosides of quinovic acid have been obtained from this family [4]. One member of this family is the genus *Neonauclea*, which consists of many species that are employed in various countries of the world to treat different diseases, such as gastritis, arthritis, cancer and flammatory conditions [5–7].

Neonauclea sessilifolia is distributed in tropical Asia and the Peace Oceanic Islands [8]. A literature search revealed that no previous phytochemical and pharmacological study has been undertaken on this species. As part of ongoing phytochemical and pharmacological investigations into the genus *Neonauclea*, we collected the stems of *N. sessilifolia* Merr. from Xishuang Banna in Yunnan province [9]; the ethyl acetate extract had antibiotic and antitumor effects, from which two new saponins together with three known saponins: quinovic acid 3-*O*-6-deoxy-β-D-glucopyranoside (**3**) [10], quinovic acid 3-*O*-β-D-glucopyranoside (**4**) [11], cincholic acid 3-*O*-6-deoxy-β-D-glucopyranoside (**5**) [12] were isolated (Fig. 1). Among the above-mentioned saponins, the three known ones were isolated from this plant for the first time; compound **4** was for the first time isolated from this genus. All the structures were elucidated by spectroscopic methods. The assignments of the NMR data of compounds **1**–**3** were established by 2D NMR experiments.

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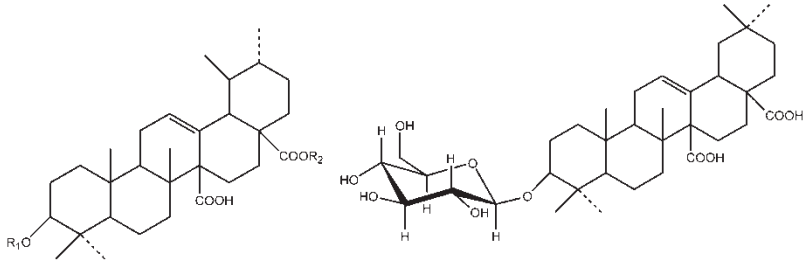

R_1	R_2 (5)
(1) 6-deoxy-D-glucose	D-glucose
(2)L-rhamnose(4→1) D-glucopyranoside	H
(3) 6-deoxy-D-glucose	H
(4) D-glucose	H

FIGURE 1 Chemical structures of compounds **1**–**5**.

RESULTS AND DISCUSSION

Compound **3**, a white amorphous powder, showed a peak at *m/z* 631 $[M - H]^+$ and a base peak at *m/z* 587 in negative its FABMS spectrum. The high-resolution negative FABMS exhibited the molecular ion peak at *m/z* 631.3878, corresponding to the molecular formula of $C_{36}H_{56}O_9$, which also was confirmed by the ^{13}C NMR and DEPT spectral data. The ^{13}C NMR showed signals for seven methyls, nine methylenes, twelve methines and eight quaternary carbons together with two carboxyl groups at δ 180.3 and 178.2 (Table II below). The chemical shifts of olefinic carbons at δ 134.2 and 129.0 were useful to distinguish between urs-12-ene and olean-12-ene analogues [13]; the ^1H NMR spectrum showed seven methyl signals at δ 0.76 (d, $J = 6.1$ Hz, CH_3), 1.18 (d, $J = 5.7$ Hz, CH_3), 1.61 (d, $J = 5.3$ Hz, CH_3), 0.84, 0.90, 1.05, 1.09 (s, $CH_3 \times 4$), an olefinic proton at δ 5.96 (1H, brs) and a signal at 3.14 (1H, dd, $J = 11.4$, 3.9 Hz). Thus, the genin of compound **3** was identified as quinovic acid.

The location of the sugar unit at the C-3 of the genin was determined using HMQC and HMBC. The HMQC sequence established the connectivity between C-3 (δ 88.6) and H-3 (δ 3.14), the anomeric carbon (δ 106.7) and proton (δ 4.63, d, $J = 7.9$ Hz). In the HMBC spectrum long-range connectivity was observed from the correlation peak between the anomeric carbon and H-3. The exact sequence of the sugar moiety was solved by ^1H–^1H COSY, which allowed the sequential assignment of the proton resonances, using the easily distinguished anomeric protons as the starting point of analysis. With respect to the stereochemistry, the NOESY correlations observed between H-1′ and H-3′, H-5′, indicated that the sugar was 6-deoxy-D-glucose. Accordingly, the structure of **3** was concluded to be quinovic acid 3-*O*-6-deoxy-β-D-glucopyranoside.

Compound **1**, a white amorphous powder, afforded a peak at *m/z* 793.4403 $[M - H]^+$ in the high-resolution negative FABMS, which was appropriate for a molecular formula of $C_{42}H_{66}O_{14}$, as confirmed by its ^{13}C NMR spectral data. The IR spectrum of **1** indicated the presence of hydroxyl (ν_{max} 3435, 1070 cm^{-1}) and carboxyl (ν_{max} 1717 cm^{-1}) groups. A comparison of the ^1H (Table I) and ^{13}C NMR (Table II) spectra of **1** and **3** showed the presence of six additional signals corresponding to a terminal β-D-glucopyranosyl group, which was confirmed by a HMQC-TOCSY experiment and acid hydrolysis of **1** and **3** (detected by TLC). The location of the glucose unit at the C-28 carboxy group of the genin was determined using HMQC and HMBC experiments. The HMQC experiment established the connectivity between C-18 (δ 54.7) and H-18 (δ 2.67, d, $J = 11.2$ Hz). In the HMBC spectrum

TABLE I ^{1}H NMR data of compounds **1**–**3** (500 MHz, J in Hz, δ in ppm; pyridine-d_5)

Proton	***1***	***2***	***3***
H-3	3.26 (1H, dd, $J = 11.0$,4.8 Hz)	2.95 (1H, dd, $J = 11.0$,4.3 Hz)	3.14 (1H, dd, $J = 11.4$, 4.0 Hz)
H-12	5.98 (1H, brs)	6.00 (1H, brs)	5.96 (1H, brs)
H-18	2.68 (1H, d, $J = 11.2$)	2.79 (1H, d, $J = 11.3$)	2.74 (1H, d, $J = 11.4$)
Me-23	0.92 (3H, s)	0.69 (3H, s)	0.90 (3H, s)
Me-24	1.12 (3H, s)	0.85 (3H, s)	1.09 (3H, s)
Me-25	0.88 (3H, s)	0.72 (3H, s)	0.84 (3H, s)
Me-26	1.20 (3H, s)	1.08 (3H, s)	1.05 (3H, s)
Me-29	1.15 (3H, d, $J = 5.9$ Hz)	1.20 (3H, d, $J = 5.8$ Hz)	1.18 (3H, d, $J = 5.9$ Hz)
Me-30	0.73 (3H, d, $J = 6.0$ Hz)	0.79 (3H, d, $J = 6.4$ Hz)	0.76 (3H, d, $J = 6.0$ Hz)
	3-*O*-Deoxy-D-glu	3-*O*-Rha	3-*O*-Deoxy-D-glu
1′	4.67 (1H, d, $J = 7.7$ Hz)	5.13 (1H, brs)	4.63 (1H, d, $J = 7.8$ Hz)
2′	3.96 (1H, t, $J = 8.3$ Hz)	4.38 (1H, t, $J = 7.6$ Hz)	3.91 (1H, t, $J = 7.8$ Hz)
3′	4.09 (1H, m)	4.15 (1H, overlap)	4.05 (1H, t, $J = 8.7$ Hz)
4′	3.69 (1H, t, $J = 8.7$ Hz)	4.35 (1H, m)	3.64 (1H, t, $J = 8.7$ Hz)
5′	3.78 (1H, m)	4.15 (1H, overlap)	3.73 (1H, m)
6′	1.66 (3H, d, $J = 5.9$ Hz)	1.68 (3H, d, $J = 6.0$ Hz)	1.61 (3H, d, $J = 5.9$ Hz)
	28-*O*-Glu	4′-*O*-Glu	
1″	6.35 (1H, d, $J = 8.0$ Hz)	5.23 (1H, d, $J = 6.9$ Hz)	
2″	4.15 (1H, t, $J = 7.8$ Hz)	4.12 (1H, t, $J = 7.8$ Hz)	
3″	4.24 (1H, t, $J = 8.3$ Hz)	4.23 (1H, t, $J = 7.8$ Hz)	
4″	4.40 (1H, t, $J = 7.8$ Hz)	4.45 (1H, m)	
5″	3.96 (1H, m)	3.80 (1H, m)	
6″	4.44, 4.42 (2H, AB system, $J = 11.9$, 1.5 Hz)	4.54, 4.52 (2H, AB system, $J = 10.0$, 3.3 Hz)	

TABLE II ^{13}C NMR data of compounds **1**–**3** (125 MHz, δ in ppm; pyridine-d_5)

Carbon	***1***		***2***		***3***	
1	39.5	6-Deoxy-glu	39.4	Rha	39.4	6-Deoxy-glu
2	26.9	1′106.7	26.5	1′103.7	26.9	1′106.7
3	88.2	2′75.9	88.2	2′72.7	88.6	2′75.9
4	39.5	3′78.4	40.0	3′71.9	40.1	3′78.4
5	55.8	4′76.9	55.5	4′85.1	55.9	4′76.9
6	18.6	5′72.7	18.7	5′68.2	18.7	5′72.7
7	37.5	6′18.1	37.8	6′18.3	37.6	6′18.3
8	40.2		39.0		39.7	
9	47.3	1″95.7	47.2	1″106.7	47.3	
10	36.4	2″74.2	38.8	2″76.5	37.1	
11	23.4	3″78.9	23.4	3″78.5	23.4	
12	129.6	4″71.2	129.0	4″71.5	129.0	
13	133.3	5″79.3	134.2	5″78.6	134.2	
14	56.8	6″62.3	56.9	6″62.7	56.9	
15	26.2		26.4		26.5	
16	25.5		25.6		25.5	
17	49.0		48.8		48.8	
18	54.7		55.0		55.0	
19	39.0		39.4		39.6	
20	37.5		37.5		37.8	
21	30.3		30.6		30.7	
22	37.2		37.2		37.4	
23	28.0		28.1		28.1	
24	17.1		18.9		19.0	
25	16.7		16.8		16.7	
26	18.8		18.9		18.8	
27	178.1		178.1		178.2	
28	176.5		180.5		180.3	
29	19.3		18.4		17.2	
30	21.2		21.4		21.4	

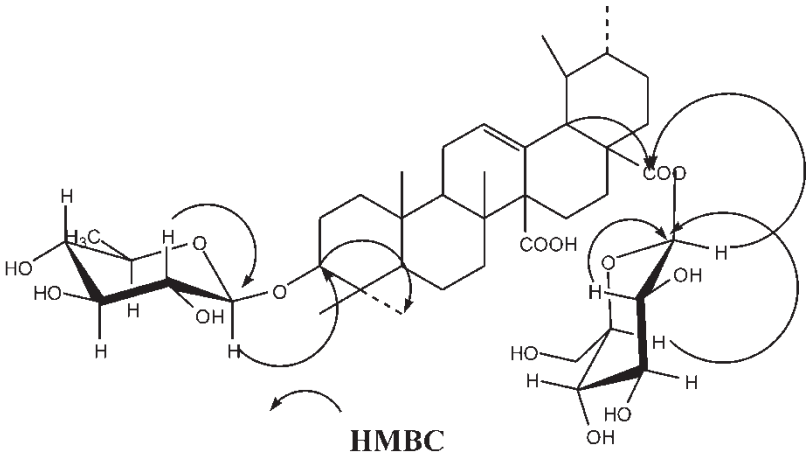

FIGURE 2 HMBC correlations for compound **1**.

long-range coupling was observed between H-18 and the carboxylic resonance of the genin C-28 (δ 178.1), which in turn showed correlation with the anomeric proton H-1 (δ 6.35, d, $J = 8.04$ Hz) (Fig. 2). These results indicated esterification of the carboxyl group (C-28) with a β-D-glucose moiety; consequently, the structure of **1** was determined as quinovic acid 3-*O*-6-deoxy-β-D-glucopyranosyl(28→1)β-D-glucopyranosyl ester.

Compound **2**, was obtained as a white powder. Its molecular formula was also determined as $C_{42}H_{66}O_{14}$ by the negative FABMS and ^{13}C NMR spectral data. Its IR spectrum showed absorption bands due to hydroxyl (ν_{max} 3427, 1068 cm^{-1}) and carboxyl (ν_{max} 1698 cm^{-1}) groups. Comparison of the ^{13}C NMR spectrum of **2** with that of **1** showed the location of the sugar unit at the C-3 (δ 88.2) of the genin, and the chemical shift of C-28 downshifted 4 ppm, which indicated that C-28 was free. Acid hydrolysis of **2** showed that the sugar moiety was composed of rhamnose and glucose, and the assignment of the proton and carbon of the sugar moiety was determined using $^1H-^1H$ COSY, HMQC, HMBC and HMQC-TOCSY experiments; the sequences of the protons and carbons of the rhamnose and glucose were determined. In the HMBC spectrum, long-range connectivity was observed in the correlation peaks between C-3 and the anomeric proton of the rhamnose (Table I), the C-4′ (δ 85.1) (Table II) of the rhamnose and the anomeric proton of glucose (Table I). By comparing the chemical shifts and coupling constants of the anomeric proton of rhamnose and glucose with literature data, compound **2** was determined to be quinovic acid 3-*O*-α-L-rhamnopyranosyl-(4→1)-β-D-glucopyranoside.

Compounds **4** and **5** were characterized by comparing their spectral data ^1H, ^{13}C NMR, MS, and IR from the literature.

EXPERIMENTAL

General Experimental Procedures

Optical rotations were measured on a HORIBA SEPA-300 high sensitivity spectropolarimeter. IR spectra were recorded on a BIO-RAD FTS-135 spectrometer with KBr pellets. MS and HRMS were taken on a VG AUTO.SPCE-3000. 1D and 2D NMR experiments were performed on a BRUKER AM-400 or DRX-500 spectrometer. Unless otherwise specified, chemical shifts are expressed in ppm with reference to the solvent signals. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical

Inc, China), silica gel H (60 μm; Qingdao Marine Chemical Inc, China), Lichroprep RP_{18} gel (40–63 μm, Merck, Darmstadt, Germany), and Sephadex-LH-20 (25–100 μm), respectively. TLC spots were detected by spraying with 5% H_2SO_4 followed by heating.

Plant Material

Dried stems of *N. sessilifolia* Merr. were collected at Xishaung Banna, Yunnan, China in May 2000. A voucher specimen (No. 0355188) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences. The plant was identified by Professor Hua Peng.

Extraction and Isolation

Dried stems of *N sessilifolia* (5.6 kg) were extracted (× 3) with ethanol (95%). After evaporation of ethanol *in vacuo*, the concentrated extract was suspended in water and extracted with EtOAc, n-BuOH. The EtOAc extract (18 g) was subjected to column chromatography over silica gel (200–300 mesh), developing with $CHCl_3$–MeOH (95:5, 9:1), and divided into four parts. Fraction 4 was chromatographed on Sephadex-LH-20 (methanol) to give two fractions, B and C. Fraction B was isolated by RP_{18} silica gel column to give compounds **3** (170 mg) and **4** (78 mg). Fraction C was repeatedly chromatographed over silica gel H, eluting with $CHCl_3$–MeOH (9:1), and purified by RP_{18} gel column chromatography using MeOH–H_2O (7:3) to yield compounds **1** (60 mg), **2** (48 mg), **5** (25 mg).

Compound **1** $[\alpha]_D^{25}$ +50.0 (*c* 0.002, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3435, 1714, 1699, 1070; Negative FAB-MS *m/z*: 793 [M − H], 631 [M − H − 162], 587 [M − H − 162 − 44], 441 [M − H − 162 − 44 − 146].

Compound **2** $[\alpha]_D^{25}$ −20.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3427, 1698, 1646, 1068; Negative FAB-MS *m/z*: 794 [M], 750 [M − 44], 588 [M − 162 − 44], 604 [M − 146 − 44].

Compound **3** $[\alpha]_D^{25}$ +25.0 (*c* 0.2, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3438, 1697, 1455, 1386, 1068; Negative FAB-MS *m/z*: 631 [M − H], 588 [M − H − 44], 469 [M − H − 162].

Acid Hydrolysis of Compounds 1–3

Each saponin (20 mg) in MeOH (1 ml) was refluxed in 5% aq. HCl (5 ml) for 8 h, and then extracted with EtOAc. The aqueous layer was adjusted to pH 7 with $NaHCO_3$. After evaporating to dryness, the sugars were analysed by comparison with authentic sugar on silica gel ($CHCl_3$–MeOH–H_2O [8:2:0.2]) using 5% H_2SO_4 as spraying reagent; D-glucose and 6-deoxy-D-glucose were identified.

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