Two Triterpenoid Saponins from *Neonauclea sessilifolia*

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From the dried roots of *Neonauclea sessilifolia* (Rubiaceae), two new triterpenoid saponins, 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-quinovopyranosyl quinovic acid (**1**) and 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-quinovopyranosyl pyrocinnolic acid 28-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester (**2**), were isolated, together with five known saponins. The structures of the new saponins were determined by spectroscopic and chemical means.

Key words *Neonauclea sessilifolia*; Rubiaceae; roots; structure elucidation; triterpenoid saponin

In the course of our phytochemical studies on glycosidic constituents of the plants belonging to Rubiaceae, we recently examined the roots of *Neonauclea sessilifolia* (ROXB.) MERR. and isolated several unique chromone-secoiridoid glycosides and indole alkaloid glycosides.¹⁾ Further investigation of this plant material led us to isolate two new triterpenoid saponins, **1** and **2**, along with five known triterpenoid saponins, 3-*O*- β -D-glucopyranosyl quinovic acid,²⁾ 3-*O*- β -D-quinovopyranosyl quinovic acid (**3**),³⁾ 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl quinovic acid,⁴⁾ 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl oleanolic acid 28-*O*- β -D-glucopyranosyl ester,⁵⁾ and 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-*O*-methyl- β -D-glucuronopyranosyl oleanolic acid 28-*O*- β -D-glucopyranosyl ester.⁵⁾

Saponin **1** was isolated as a colorless crystalline solid, mp 220–222 °C. Its high resolution secondary ion mass spectrum (HR-SI-MS) showed a pseudomolecular ion at *m/z* 793.4386, indicating a molecular formula C₄₂H₆₆O₁₄. Acid hydrolysis of **1** afforded D-glucose and D-quinovose which were identified by GLC analysis of their thiazolizine derivatives.⁶⁾ The ¹H-NMR spectral features of **1** showed analogy to those of 3-*O*- β -D-quinovopyranosyl quinovic acid (**3**), a major saponin of this plant, except that **1** showed the signals due to an additional glucose moiety. Two anomeric proton signals at δ 4.39 (d, *J* = 7.5 Hz) and 4.66 (d, *J* = 7.5 Hz) indicated β -linkage of quinovopyranose and glucopyranose units in **1**. The ¹³C-NMR spectrum of **1** showed, besides 12 signals due to two hexose moieties, 30 carbon signals which were observed in the nearly identical frequencies of those of 3-*O*- β -D-quinovopyranosyl quinovic acid (**3**), indicating a quinovic acid as an aglycone unit and the glycosidation at C-3. ¹H-Detected heteronuclear multiple-bond connectivity (HMBC) correlations between H-1' of quinovose and C-3 (δ 91.4), and between H-1'' of glucose and C-2' of quinovose (δ 81.3) showed the attachment of 2-*O*- β -D-glucopyranosyl- β -D-quinovopyranose to C-3 of quinovic acid. The linkage of a terminal glucose to C-2' of quinovosyl moiety was supported by a comparative study of the ¹³C-NMR spectra of **1** and **3** which showed the downfield shift of C-2' of quinovose moiety (+5.4 ppm) and the upfield shift of C-1' and C-2' (–1.3 and –0.3 ppm, respectively). Consequently, glycoside **1** was deduced to be 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-quinovopyranosyl quinovic acid.

Saponin **2** was obtained as a white powder, C₅₃H₈₆O₂₁, [α]_D –43°. Acid hydrolysis of **2** gave D-glucose, D-

quinovose, and L-rhamnose. Its ¹H- and ¹³C-NMR spectra revealed the signals due to two β -glucopyranose, a β -quinovopyranose and an α -rhamnopyranose unit. Furthermore, its ¹³C-NMR showed 29 carbon signals as an aglycone moiety involving six tertiary methyl groups, a carbonyl group and two quaternary olefinic carbon signals. The NMR spectral features were closely similar to those of 3-*O*- β -D-quinovopyranosyl pyrocinnolic acid 28-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester (**4**),⁷⁾ except for the signals arising from an additional α -rhamnose moiety. The downfield shift of C-4' and upfield shift of C-3' and C-5' of **2**, relative to **4**, were ascribed to the glycosidation of 4'-hydroxy group in the quinovose moiety. The attachment of 4-*O*- α -L-rhamnopyranosyl- β -D-quinovopyranose unit to C-3 of pyrocinnolic acid and the ester linkage of the carboxyl group at C-28 of pyrocinnolic acid with 6-*O*- β -D-glucopyranosyl- β -D-glucopyranose unit were further confirmed by detailed two dimensional (2D)-NMR experiments, which showed HMBC interactions between H-3 and C-1' of quinovose, between H-1' of quinovose and C-3, between H-4' of quinovose and C-1'' of rhamnose, between H-1'' of rhamnose and C-4' of quinovose, between H-1''' of inner glucose and C-28, between H-6''' of inner glucose and C-1''' of terminal glucose, and between H-1''' of terminal glucose and C-6''' of inner glucose. Accordingly, glycoside **2** was assigned to 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-quinovopyranosyl pyrocinnolic acid 28-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester.

Chart 1

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Table 1. ^{13}C -NMR Spectral Data of **1**—**4**

C	1 ^{a)}	2 ^{a)}	2 ^{b)}	3 ^{c)}	4 ^{d)}	C	1 ^{a)}	2 ^{a)}	2 ^{b)}	3 ^{c)}	4 ^{d)}
1	39.9	39.5	38.4	39.9	38.5		3- <i>O</i> -Qui	3- <i>O</i> -Qui	3- <i>O</i> -Qui	3- <i>O</i> -Qui	3- <i>O</i> -Qui
2	27.2	27.2	26.7	27.1	26.9	1'	105.2	106.4	106.4	106.5	106.8
3	91.4	90.8	89.1	90.7	89.0	2'	81.3	76.0	76.0	75.9	76.0
4	40.4	40.3	39.6	40.1	39.6	3'	78.2	76.7	76.5	77.9	78.0
5	56.9	57.1	55.7	56.9	55.8	4'	76.9	85.8	84.9	77.0	77.0
6	19.3	18.9	18.7	19.3	18.8	5'	72.9	71.9	71.4	73.0	72.8
7	38.0	40.7	39.7	38.0	39.7	6'	18.2	18.5	18.6	18.2	18.9
8	40.7	38.2	38.0	40.7	38.1		2'- <i>O</i> -Glc	4'- <i>O</i> -Rha	4'- <i>O</i> -Rha		
9	48.0	57.8	56.5	48.0	56.5	1''	104.5	103.2	103.0		
10	37.8	38.9	37.2	37.8	37.2	2''	76.3	72.4	72.5		
11	23.9	19.5	18.1	23.8	18.1	3''	77.9	72.2	72.7		
12	130.4	32.1	32.0	130.4	32.1	4''	71.9	73.7	73.9		
13	133.9	131.2	130.3	133.9	130.3	5''	78.4	70.7	70.5		
14	57.3	137.9	136.9	57.3	137.0	6''	63.1	17.8	18.5		
15	26.5	21.6	21.0	26.5	21.0			28- <i>O</i> -Glc	28- <i>O</i> -Glc		28- <i>O</i> -Glc
16	25.7	24.4	24.1	25.7	24.8	1'''		95.7	95.7		95.7
17	*	46.6	45.7	*	45.7	2'''		74.0	74.0		75.2
18	55.5	40.2	39.5	55.5	39.5	3'''		78.2	78.8 ^{e)}		78.4
19	40.4	42.4	41.5	40.4	41.5	4'''		71.0	71.0		71.6
20	38.3	31.4	30.6	38.3	30.9	5'''		77.8	78.0		78.4
21	31.2	35.1	34.3	31.2	34.4	6'''		69.6	69.5		69.5
22	37.6	32.8	31.2	37.6	32.1			6'''- <i>O</i> -Glc	6'''- <i>O</i> -Glc		6'''- <i>O</i> -Glc
23	28.4	28.4	28.1	28.5	28.2	1''''		104.7	105.3		105.3
24	16.8	16.7	16.7	17.0	16.7	2''''		75.2	75.2		74.1
25	17.0	17.2	16.7	16.9	16.7	3''''		78.0	78.4 ^{e)}		78.8
26	18.2	21.2	20.9	18.2	20.9	4''''		71.6	71.6		71.0
27	179.0	—	—	179.0	—	5''''		78.0	78.5 ^{e)}		78.5
28	181.6	178.2	176.7	181.6	176.8	6''''		62.8	62.7		62.7
29	19.1	33.0	32.3	19.1	32.4						
30	21.5	25.2	25.0	21.5	25.0						

a) Measured in CD_3OD at 125 MHz. b) Measured in pyridine- d_5 at 125 MHz. c) Measured in CD_3OD at 75 MHz. d) Measured in pyridine- d_5 at 75 MHz. Data taken from ref. 7. e) Values are interchangeable. * Overlapped with solvent signal.

While triterpenoid saponins have so far been isolated from the plant species of the genera *Nauclea* and *Adina* (Rubiaceae), the present work gave the first example of isolation of triterpenoid saponins **1** and **2** from the *Neonauclea* species of the same family.

Experimental

IR spectra were recorded on a Shimadzu FTIR-8200 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. ^1H - (500 MHz) and ^{13}C - (125 MHz) NMR spectra were recorded on a Varian VXR-500 spectrometer with TMS as an internal standard. MS and HR-MS were obtained with a Hitachi M-4100 mass spectrometer. Glycerol was used for SI-MS and HR-SI-MS as the matrix. Medium pressure liquid chromatography (MPLC) was carried out with Wakogel 40C18. TLC was performed on precoated Kieselgel 60F₂₅₄ plates (Merck). HPLC was performed using a Waters system (600E System Controller, 486 Tunable Absorbance Detector). GLC was carried out on a Shimadzu GC-18A equipped with FID.

Isolation of Saponins The roots of *Neonauclea sessilifolia* were collected at Surat Thani, Thailand. A voucher specimen (NST-592) is deposited in the laboratory of Nippon Shinyaku Institute for Botanical Research. Dried roots (1.58 kg) of *N. sessilifolia* were extracted with hot MeOH and the extract was fractionated as previously reported in ref. 1. Fraction 9 in ref. 1 was purified by a combination of SiO_2 CC with $\text{MeOH}-\text{CHCl}_3$ (7:93—2:3), prep. HPLC ($\mu\text{Bondasphere } 5 \mu\text{ C18-100 } \text{\AA}$, $\text{MeOH}-\text{H}_2\text{O}$, 3:1, 4:1, 3:2) and prep. TLC ($\text{CHCl}_3-\text{MeOH}$, 4:1, 7:3; $\text{AcOEt}-\text{C}_6\text{H}_6-\text{EtOH}$, 4:1:1:2) to afford 3-*O*- β -D-glucopyranosyl quinovic acid (13.5 mg), **3** (94.4 mg), 3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl quinovic acid (9.2 mg), **1** (19.6 mg), **2** (8.4 mg), 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-*O*-methyl- β -D-glucuronopyranosyl oleanolic acid 28-*O*- β -D-glucopyranosyl ester (19.4 mg), and 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl oleanolic acid 28-*O*- β -D-glucopyranosyl ester (65.8 mg).

3-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl Quinovic Acid (**1**): Colorless crystalline solid. mp 220—222 °C (H_2O). $[\alpha]_{\text{D}}^{22} +43^\circ$ ($c=0.6$,

MeOH). IR (KBr) cm^{-1} : 3426, 2928, 1697, 1074. ^1H -NMR (CD_3OD) δ : 0.74 (1H, br d, $J=11.5$ Hz, H-5), 0.84 (3H, s, H₃-24), 0.89 (3H, s, H₃-26), 0.90 (3H, d, $J=5.0$ Hz, H₃-29), 0.92 (3H, d, $J=5.0$ Hz, H₃-30), 0.97 (3H, s, H₃-25), 1.04 (3H, s, H₃-23), 1.26 (3H, d, $J=6.0$ Hz, H₃-6'), 2.23 (1H, dd, $J=11.0$, 5.0 Hz, H-9), 2.25 (1H, br d, $J=11.0$ Hz, H-18), 2.99 (1H, t, $J=9.0$ Hz, H-4'), 3.12 (1H, dd, $J=11.5$, 4.5 Hz, H-3), 3.18 (1H, t, $J=9.0$ Hz, H-4''), 3.20 (1H, dd, $J=9.0$, 7.5 Hz, H-2''), 3.24 (1H, ddd, $J=9.0$, 6.0, 2.0 Hz, H-5''), 3.29 (1H, dq, $J=9.0$, 6.0 Hz, H-5'), 3.34 (1H, t, $J=9.0$ Hz, H-3'), 3.49 (1H, t, $J=9.0$ Hz, H-3'), 3.56 (1H, dd, $J=9.0$, 7.5 Hz, H-2'), 3.60 (1H, dd, $J=11.5$, 6.0 Hz, H-6''), 3.81 (1H, dd, $J=11.5$, 2.0 Hz, H-6'), 4.39 (1H, d, $J=7.5$ Hz, H-1'), 4.66 (1H, d, $J=7.5$ Hz, H-1''), 5.60 (1H, dd, $J=5.0$, 2.0 Hz, H-12). ^{13}C -NMR: Table 1. Negative ion SI-MS m/z : 793 ($\text{M}-\text{H}^-$), 587. HR-SI-MS m/z : 793.4386 (Calcd for $\text{C}_{42}\text{H}_{65}\text{O}_{14}$: 793.4377). HMBC: H-18 to C-28; H-1' to C-3; H-1'' to C-2'.

3-*O*- α -L-Rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-quinovopyranosyl Pyrocinnolic Acid 28-*O*- β -D-Glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl Ester (**2**): Amorphous powder. $[\alpha]_{\text{D}}^{25} -43^\circ$ ($c=0.5$, MeOH). IR (KBr) cm^{-1} : 3421, 2941, 1734, 1636, 1065. ^1H -NMR (pyridine- d_5) δ : 0.78 (1H, br d, $J=12.0$ Hz, H-5), 0.81 (3H, s, H₃-25), 0.90 (3H, s, H₃-30), 0.90 (3H, s, H₃-29), 0.95 (3H, s, H₃-24), 1.04 (1H, dd, $J=12.0$, 2.5 Hz, H-9), 1.14 (3H, s, H₃-26), 1.21 (1H, br t, $J=13.0$ Hz, H-19), 1.31 (3H, s, H₃-23), 1.41 (3H, d, $J=5.5$ Hz, H₃-6'), 1.71 (3H, d, $J=6.0$ Hz, H₃-6''), 1.89 (1H, br q, $J=12.0$ Hz, H-2), 2.77 (1H, dd, $J=12.0$, 4.0 Hz, H-18), 3.34 (1H, dd, $J=12.0$, 4.0 Hz, H-3), 3.67 (1H, dq, $J=9.0$, 5.5 Hz, H-5'), 3.70 (1H, t, $J=9.0$ Hz, H-4'), 3.90 (1H, ddd, $J=9.0$, 5.0, 2.0 Hz, H-5'''), 3.98 (1H, br t, $J=8.0$ Hz, H-2'), 4.02 (1H, br t, $J=8.5$ Hz, H-2'''), 4.08 (1H, br t, $J=8.5$ Hz, H-3'), 4.10 (1H, ddd, $J=9.5$, 4.5, 2.0 Hz, H-5''), 4.14 (1H, br t, $J=8.0$ Hz, H-2''), 4.20 (1H, br t, $J=8.0$ Hz, H-3''), 4.22 (1H, br t, $J=8.0$ Hz, H-3''), 4.24 (1H, br t, $J=8.5$ Hz, H-4'''), 4.35 (2H, br t, $J=9.0$ Hz, H-4''), 4.37 (2H, m, H-6'', H-6'''), 4.50 (1H, dd, $J=11.5$, 2.0 Hz, H-6'''), 4.54 (1H, dd, $J=9.0$, 3.0 Hz, H-3''), 4.64 (1H, br s, H-2''), 4.73 (1H, dd, $J=11.0$, 2.0 Hz, H-6'''), 4.78 (1H, d, $J=8.0$ Hz, H-1'), 4.90 (1H, dq, $J=9.5$, 6.0 Hz, H-5'), 5.05 (1H, d, $J=8.0$ Hz, H-1''), 5.59 (1H, d, $J=1.0$ Hz, H-1''), 6.27 (1H, d, $J=8.0$ Hz, H-1''). ^{13}C -NMR: Table 1. Negative ion SI-MS m/z : 1057 ($\text{M}-\text{H}^-$), 733. HR-SI-MS m/z : 1057.5571 (Calcd for $\text{C}_{53}\text{H}_{85}\text{O}_{21}$: 1057.5587). ROESY: H-3/H-1'; H-

4'/H-1"; H-6'''/H-1'''. HMBC: H-18 to C-13, 14, 17, 19; H-16 to C-28; H₃-26 to C-7, 8, 9, 14; H-3 to C-1'; H-1' to C-3; H-4' to C-1"; H-1" to C-4'; H-1''' to C-28; H₂-6''' to C-1'''; H-1'''' to C-6'''.

Acid Hydrolysis of Saponins 1 and 2 Each saponin (1 mg) was heated at 95 °C with dioxane (0.5 ml) and 5% H₂SO₄ (0.5 ml) for 1 h. After neutralization with Amberlite IRA-400 (OH⁻ form), the reaction mixture was concentrated and the residue was passed through a Sep-Pak C₁₈ cartridge with H₂O. The eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.125 ml) at 60 °C for 1 h. The solution was then treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.05 ml) at 60 °C for 1 h. The supernatant was applied to GLC; GLC conditions: column, Supelco SPBTM-1, 30 m×0.25 mm; column temperature, 230 °C; N₂ flow rate, 0.8 ml/min; *t*_R of derivatives, D-glucose 13.1 min, L-glucose 13.6 min, D-quinovose 8.7 min, L-rhamnose 9.0 min. D-Glucose and D-quinovose were detected from **1** and **2**, and L-rhamnose was additionally detected from **2**.

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