# 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→2)-*β*-D-quinovopyranosyl quinovic acid (1) and 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-quinovopyranosyl pyrocincholic acid 28-*O*-*β*-D-glucopyranosyl-(1→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→4)-*α*-L-rhamnopyranosyl quinovic acid,[4)] 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester,[5)] and 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→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 $C_{42}H_{66}O_{14}$. Acid hydrolysis of **1** afforded D-glucose and D-quinovose which were identified by GLC analysis of their thiazolizine derivatives.[6)] The $^1$H-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 $\delta$ 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 $^{13}$C-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. $^1$H-Detected heteronuclear multiple-bond connectivity (HMBC) correlations between H-1′ of quinovose and C-3 ($\delta$ 91.4), and between H-1″ of glucose and C-2′ of quinovose ($\delta$ 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 $^{13}$C-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→2)-*β*-D-quinovopyranosyl quinovic acid.

Saponin **2** was obtained as a white powder, $C_{53}H_{86}O_{21}$, $[\alpha]_D$ −43°. Acid hydrolysis of **2** gave D-glucose, D-quinovose, and L-rhamnose. Its $^1$H- and $^{13}$C-NMR spectra revealed the signals due to two *β*-glucopyranose, a *β*-quinovopyranose and an *α*-rhamnopyranose unit. Furthermore, its $^{13}$C-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 pyrocincholic acid 28-*O*-*β*-D-glucopyranosyl-(1→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 pyrocincholic acid and the ester linkage of the carboxyl group at C-28 of pyrocincholic 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→4)-*β*-D-quinovopyranosyl pyrocincholic acid 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

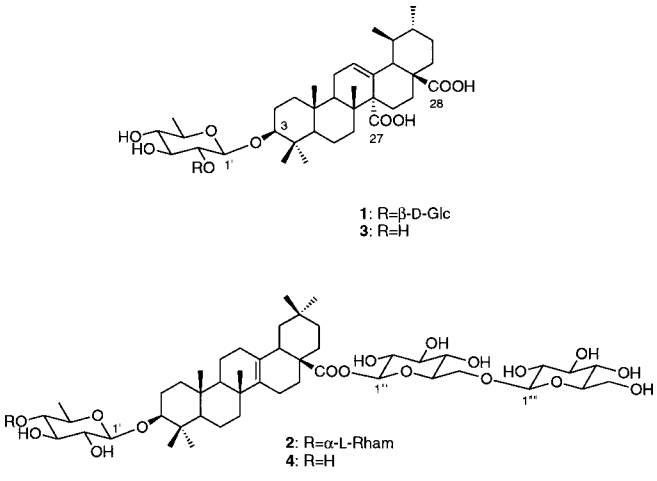


Chart 1

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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-*O*-Qui | 3-*O*-Qui | 3-*O*-Qui | 3-*O*-Qui | 3-*O*-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′-*O*-Glc | 4′-*O*-Rha | 4′-*O*-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-*O*-Glc | 28-*O*-Glc | | 28-*O*-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‴-*O*-Glc | 6‴-*O*-Glc | | 6‴-*O*-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. $^1$H- (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 60$F_{254}$ 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 MeOH–$CHCl_3$ (7 : 93—2 : 3), prep. HPLC ($\mu$Bondasphere 5 $\mu$ C18-100 Å, MeOH–$H_2O$, 3 : 1, 4 : 1, 3 : 2) and prep. TLC ($CHCl_3$–MeOH, 4 : 1, 7 : 3; AcOEt–$C_6H_6$–EtOH, 4 : 1 : 2) to afford 3-*O*-$\beta$-D-glucopyranosyl quinovic acid (13.5 mg), **3** (94.4 mg), 3-*O*-$\beta$-D-glucopyranosyl-(1→4)-$\alpha$-L-rhamnopyranosyl quinovic acid (9.2 mg), **1** (19.6 mg), **2** (8.4 mg), 3-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-(1→3)-6-*O*-methyl-$\beta$-D-glucuronopyranosyl oleanolic acid 28-*O*-$\beta$-D-glucopyranosyl ester (19.4 mg), and 3-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-$\beta$-D-glucopyranosyl-(1→3)-$\beta$-D-glucuronopyranosyl oleanolic acid 28-*O*-$\beta$-D-glucopyranosyl ester (65.8 mg).

3-*O*-$\beta$-D-Glucopyranosyl-(1→2)-$\beta$-D-quinovocyranosyl Quinovic Acid (**1**): Colorless crystalline solid. mp 220—222 °C ($H_2O$). $[\alpha]_D^{22}$ +43° ($c$=0.6, MeOH). IR (KBr) cm$^{-1}$: 3426, 2928, 1697, 1074. $^1$H-NMR ($CD_3OD$) $\delta$: 0.74 (1H, br d, $J$=11.5 Hz, H-5), 0.84 (3H, s, $H_3$-24), 0.89 (3H, s, $H_3$-26), 0.90 (3H, d, $J$=5.0 Hz, $H_3$-29), 0.92 (3H, d, $J$=5.0 Hz, $H_3$-30), 0.97 (3H, s, $H_3$-25), 1.04 (3H, s, $H_3$-23), 1.26 (3H, d, $J$=6.0 Hz, $H_3$-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 $(M-H)^-$, 587. HR-SI-MS $m/z$: 793.4386 (Calcd for $C_{42}H_{65}O_{14}$: 793.4377). HMBC: H-18 to C-28; H-1′ to C-3; H-1″ to C-2′.

3-*O*-$\alpha$-L-Rhamnopyranosyl-(1→4)-$\beta$-D-quinovopyranosyl Pyrocincholic Acid 28-*O*-$\beta$-D-Glucopyranosyl-(1→6)-$\beta$-D-glucopyranosyl Ester (**2**): Amorphous powder. $[\alpha]_D^{25}$ −43° ($c$=0.5, MeOH). IR (KBr) cm$^{-1}$: 3421, 2941, 1734, 1636, 1065. $^1$H-NMR (pyridine-$d_5$) $\delta$: 0.78 (1H, br d, $J$=12.0 Hz, H-5), 0.81 (3H, s, $H_3$-25), 0.90 (3H, s, $H_3$-30), 0.90 (3H, s, $H_3$-29), 0.95 (3H, s, $H_3$-24), 1.04 (1H, dd, $J$=12.0, 2.5 Hz, H-9), 1.14 (3H, s, $H_3$-26), 1.21 (1H, br t, $J$=13.0 Hz, H-19), 1.31 (3H, s, $H_3$-23), 1.41 (3H, d, $J$=5.5 Hz, $H_3$-6′), 1.71 (3H, d, $J$=6.0 Hz, $H_3$-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″, 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 $(M-H)^-$, 733. HR-SI-MS $m/z$: 1057.5571 (Calcd for $C_{53}H_{85}O_{21}$: 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; $H_3$-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; $H_2$-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% $H_2SO_4$ (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 $C_{18}$ cartridge with $H_2O$. 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 SPB™-1, 30 m×0.25 mm; column temperature, 230 °C; $N_2$ 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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