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TRITERPENE SAPONINS FROM SEEDS OF THE CHINESE PLANT *Nigella glandulifera*

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Nigella glandulifera is a traditional Uigur medicinal plant. The extract of its seeds is supposed to have diuretic, analgesic, spasmolytic, lactogenic, and bronchodilating properties and can be used to treat edema and bronchial asthma and release uriary bladder stones [1]. A total of 16 compounds have been isolated to date from this plant [2-5]. Herein we communicate the additional isolation of five known triterpene saponins **1**-**5**, of which **2** and **5** were isolated for the first time from plants of the genus *Nigella*. Compound **1** was obtained for the first time from *N. glandulifera*.

Ground seeds (10 kg) of *N. glandulifera* were defatted by petroluem ether (4 × 10 L). The defatted seeds were extracted with EtOH (50%, 4 × 10 L). The EtOH was evaporated in vacuo to afford a syrupy residue that was suspended in distilled water and successively fractionated by petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The condenssed *n*-BuOH fraction was dissolved in distilled water and fractionated over a column of ion-exchange resin (AB-8) using water, H_2O:EtOH (10:30 and 50%), and EtOH. This produced five fractions. Fractions 2, 3, and 5 were separated by chromatography over silica gel (200-300 mesh) with gradient elution by $CHCl_3$:MeOH (100:1 → 0:1) and over Sephadex LH-20 with elution by MeOH. Similar fractions were combined using TLC analysis to produce five triterpenes **1**-**5**.

Compound 1, $C_{30}H_{48}O_4$, colorless crystals ($CHCl_3$:MeOH), mp >300°C.

Spectral data for **1** agree with those reported for hederagenin [6, 7].

Compound 2, $C_{30}H_{48}O_5$, amorphous white powder, mp 226-228°C, positive reaction with Lieberman—Burchard reagent. IR spectrum (KBr, ν_{max}, cm^{-1}): 3418, 2946, 1706, 1050.

PMR spectrum (600 MHz, CD_3OD, δ, ppm, J/Hz): 0.72 (3H, s), 0.84 (3H, s), 0.92 (3H, s), 0.96 (3H, s), 0.99 (3H, s), 1.19 (3H, s), 2.87 (1H, dd, J = 9.0, 12.0), 3.28 (1H, H-3), 3.35 (1H, d, J = 11.4, H-23a), 3.53 (1H, H-23b), 5.25 (1H, br.s, H-12).

^{13}C NMR spectrum (150 MHz, CD_3OD, δ, ppm): 180.43 (C-28), 145.17 (C-13), 123.52 (C-12), 74.02 (C-3), 73.91 (C-21), 67.46 (C-23), 49.47 (C-17), 48.78 (C-9), 47.71 (C-5), 47.32 (C-19), 43.35 (C-4), 43.04 (C-14), 42.23 (C-18), 40.59 (C-22), 40.01 (C-8), 38.97 (C-1), 37.41 (C-10), 37.37 (C-20), 33.32 (C-7), 30.30 (C-29), 28.33 (C-15), 27.10 (C-2), 26.92 (C-16), 25.92 (C-27), 24.06 (C-11), 19.21 (C-6), 18.10 (C-26), 17.82 (C-30), 16.32 (C-25), 12.77 (C-24).

The spectral data agreed with those reported in the literature for 21β-hydroxyhederagenin [8].

Compound 3, amorphous brown powder, mp 222-224°C.

Spectral data for **3** agreed with those reported in the literature for 3-*O*-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin [3, 9].

Compound 4, $C_{64}H_{104}O_{30}$, amorphous white powder, positive reaction with Lieberman—Burchard reagent. Acid hydrolysis of **4** produced hederagenin, xylose, rhamnose, arabinose, and glucose.

Analysis of the HMBC spectrum revealed all types of intraglycosidic bonds in two trisaccharide fragments of **4** and the attachment site of these fragments to the aglycon. The spectrum of **4** gave correlations between glycosylated C-3 (C-81.15) of the aglycon and the anomeric arabinose proton (5.06 ppm), C-2 (C-74.09) of arabinose and the anomeric H-1 proton of rhamnose (6.35 ppm), C-3 (83.02) of rhamnose and the anomeric proton of a terminal xylose (5.38), and correlation peaks between glycosylated C-28 (C-176.6) of the aglycon and the anomeric H-1 proton (6.23 ppm) of an internal glucose, C-6

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(C-69.1) of the internal glucose and the anomeric proton of a second glucose unit (4.99 ppm), and C-4 (C-78.2) of the second glucose and the anomeric H-1 proton of a terminal rhamnose (5.89 ppm). All spectral data agreed with those reported for 3-*O*-[*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]-28-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-hederagenin [9, 10].

Mass spectrum (FAB-EI, *m*/*z*): 1375 [M + Na], 1243, 927, 905, 437. IR spectrum (KBr, ν_{max}, cm^{-1}): 3400 (OH), 1721 (ester C=O), 1246 (C–O).

PMR spectrum (600 MHz, C_5D_5N, δ, ppm, J/Hz): 0.85, 0.87, 0.97, 1.09, 1.14, 1.19 (3H each, s, CH_3), 5.35 (br.s, C-12), 6.35 (br.s, Rha-H), 6.23 (d, J = 8.5, Glu-H), 5.89 (br.s, Rha-H), 5.38 (d, J = 7.2, Xyl-H), 5.06 (d, J = 6.6, Ara-H), 4.99 (d, J = 7.8, Glu-H).

^{13}C NMR spectrum (150 MHz, C_5D_5N, δ, ppm): 176.6 (C-28), 144.58 (C-13), 123.02 (C-12), 107.70 (C-Xyl-1), 104.94 (C-Glu′-1), 104.85 (C-Ara-1), 102.83 (C-Rha′-1), 101.35 (C-Rha-1), 95.72 (C-Glu-1), 83.02 (C-Rha-3), 81.15 (C-3), 78.81 (C-Glu-3), 78.57 (C-Xyl-3), 78.30 (C-Glu′-4), 78.14 (C-Glu-5), 77.25 (C-Glu′-5), 76.59 (C-Glu′-3), 75.75 (C-Glu-2), 75.44 (C-Xyl-2), 75.01 (C-Glu′-2), 74.09 (C-Ara-2), 73.98 (C-Rha′-4), 73.11 (C-Ara-3), 72.85 (C-Rha-4), 72.66 (C-Rha′-3), 72.15 (C-Rha′-2), 71.21 (C-Rha-2), 70.93 (C-Xyl-4), 70.39 (C-Glu-4), 70.39 (C-Rha′-5), 69.92 (C-Ara-4), 69.68 (C-Rha-5), 69.27 (C-Glu-6), 67.52 (C-Xyl-5), 66.50 (C-Ara-5), 64.04 (C-23), 61.34 (C-Glu′-6), 48.30 (C-5), 47.70 (C-9), 47.10 (C-17), 46.24 (C-19), 43.70 (C-4), 42.20 (C-14), 41.73 (C-18), 40.00 (C-8), 39.17 (C-1), 36.98 (C-10), 34.06 (C-21), 33.19 (C-29), 32.83 (C-22), 32.63 (C-7), 30.82 (C-20), 28.40 (C-15), 26.48 (C-2), 26.15 (C-27), 23.92 (C-30), 23.77 (C-11), 23.42 (C-16), 18.63 (C-Rha-6), 18.52 (C-Rha′-6), 18.21 (C-6), 17.62 (C-26), 16.29 (C-25), 14.32 (C-24).

Compound 5, $C_{64}H_{104}O_{30}$, amorphous yellow powder.

Acid hydrolysis indicated a carbohydrate composition for **5** of L-arabinose, D-glucose, D-xylose, and L-rhamnose.

NMR spectra (1D and 2D) showed resonances of six anomeric protons at 4.99 (d, J = 6.6 Hz), 104.69; 6.28 (d, J = 1.5), 101.16; 6.12 (d, J = 8.0), 95.82; 4.94 (d, J = 8.0), 104.74; 5.75 (br.s), 102.56; and 5.38 (d, J = 7.4), 107.5.

Based on HMBC data, which exhibited cross peaks due to through-space coupling between C-3 (81.77) of the aglycon and anomeric proton H-1 (4.99 ppm) of *α*-L-arabinopyranosyl, C-2 (75.23) of *α*-L-arabinopyranosyl and anomeric proton H-1 (6.28 ppm) of the terminal rhamnopyranosyl, the structure of the 3-*O*-glycosyl chain was established as *α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl.

The bond type and sequence of monosaccharides in the tetrasaccharide fragment on C-28 were established analogously. The HMBC spectrum showed cross peaks between C-28 (176.37) of the aglycon and anomeric proton H-1 (6.12) of the ether-bonded *β*-glucopyranosyl, C-2 (80.80) of the ether-bonded *β*-glucopyranosyl and anomeric proton H-1 (5.38) of *β*-xylopyranosyl, C-6 (69.03) of the ether-bonded *β*-glucopyranosyl and proton H-1 (4.94) of a second *β*-glucopyranosyl, and C-4 (77.97) of the second *β*-glucopyranosyl and proton H-1 (5.75 ppm) of a terminal *α*-L-rhamnopyranosyl.

Thus, the terminal *β*-xylopyranosyl and the second *β*-glucopyranosyl are bonded to C-2 and C-6, respectively, of the ether-bonded *β*-glucopyranosyl whereas the other teriminal *α*-L-rhamnopyranosyl is bonded to C-4 of the second *β*-D-glucopyranosyl.

Mass spectrum (EI, *m*/*z*): 1375 [M + Na], 1243, 472, 437, 208.

PMR spectrum (600 MHz, CD_3OD, δ, ppm, J/Hz): 0.72, 0.82, 0.93, 0.96, 1.00, 1.19 (3H each, s, CH_3), 5.35 (br.s, C-12), 6.27 (1H, d, J = 1.5, Rha-H), 6.12 (d, J = 7.8, Glu-H), 5.38 (d, J = 7.2, Xyl-H), 5.75 (br.s, Rha-H), 4.99 (d, J = 6.6, Ara-H), 4.94 (d, J = 7.8, Glu-H).

^{13}C NMR spectrum (150 MHz, CD_3OD, δ, ppm): 176.37 (C-28), 143.93 (C-13), 122.84 (C-12), 106.5 (C-Xyl-1), 104.74 (C-Glu-1), 104.69 (C-Ara-1), 102.65 (C-Rha′-1), 101.16 (C-Rha-1), 93.82 (C-Glu′-1), 81.77 (C-3), 80.80 (C-Glu′-2), 78.59 (C-Glu-3), 78.59 (C-Xyl-3), 78.38 (C-Glu′-3), 77.97 (C-Glu′-5), 77.03 (C-Glu-5), 76.35 (C-Glu-4), 75.55 (C-Xyl-2), 75.38 (C-Glu-2), 75.23 (C-Ara-2), 74.70 (C-Ara-3), 73.88 (C-Rha′-4), 73.24 (C-Rha-4), 72.90 (C-Rha′-3), 72.64 (C-Rha-3), 72.46 (C-Rha′-2), 7.192 (C-Rha-2), 70.99 (C-Xyl-4), 70.66 (C-Glu′-4), 70.16 (C-Rha′-5), 69.77 (C-Rha-5), 69.45 (C-Ara-4), 69.03 (C-Glu′-6), 67.32 (C-Xyl-5), 65.40 (C-Ara-5), 63.77 (C-23), 62.08 (C-Glu-6), 48.10 (C-9), 47.59 (C-5), 46.98 (C-17), 46.12 (C-19), 43.60 (C-4), 42.09 (C-14), 41.62 (C-18), 39.88 (C-8), 39.07 (C-1), 36.86 (C-10), 33.95 (C-21), 33.08 (C-29), 32.73 (C-7), 32.51 (C-22), 30.71 (C-20), 28.29 (C-15), 26.42 (C-2), 26.05 (C-27), 23.82 (C-16), 23.66 (C-30), 23.32 (C-11), 18.55 (C-Rha-6), 18.42 (C-Rha′-6), 18.09 (C-6), 17.50 (C-26), 16.19 (C-25), 14.35 (C-24).

The spectral data agreed with those reported for 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]-hederagenin-28-*O*-{*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-[*β*-D-xylopyranosyl-(1→2)]-*β*-D-glucopyranosyl ether [11].

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