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A NEW DIARYLHEPTANOID AND A RARE DAMMARANE TRITERPENOID FROM *Alnus nepalensis*

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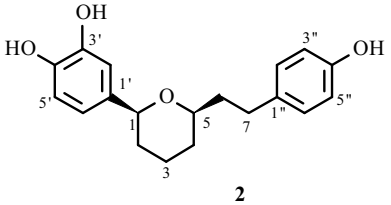
A new diarylheptanoid, a rare dammarane triterpenoid, and centrolobol were isolated from the leaves of Alnus nepalensis *D. Don (Betulaceae). Their structures were determined by using spectroscopic analysis.*

Keywords: *Alnus nepalensis*, Betulaceae, diarylheptanoid, dammarane.

Alnus nepalensis D. Don. (Betulaceae) is a woody medicinal plant of Vietnam. Continuing our previous phytochemical study of the leaves of *A. nepalensis* growing in northern Vietnam [1], we report on the isolation and structure determination of three compounds, **1**–**3**. Compound **1** is a rare dammarane triterpenoid and was only reported in a composition containing dammarane triterpene compounds for preventing and treating arterial sclerosis, dementia, cancer, and oxidative stress [2], but unfortunately without the inclusion of its physico-chemical and spectroscopic data; **2** is a new minor diarylheptanoid; and **3** was identified as centrolobol by comparing its spectroscopic data (EI-MS, ^{1}H and ^{13}C NMR) with literature values [3, 4].

Compound **1** was isolated as white crystals, mp 145–146°C, $[\alpha]_D^{26}$ +37.9° (*c* 0.09, $CHCl_3$). Compound **1** was found to have the molecular formula $C_{30}H_{46}O_3$ by positive-ion HR-ESI-MS and HR-APCI-MS. The IR spectrum showed the presence of an α,β-unsaturated carboxylic acid (2600–3300 and 1703 cm^{-1}) and an α,β-unsaturated double bond (1641 cm^{-1}). The ^{1}H NMR spectrum of **1** showed the presence of five tertiary methyl groups (all s) (δ 0.9, 0.97, 1.04, 1.06, and 1.11), a vinylic methyl [1.87 (s)], a terminal methylene group of a two-substituted double bond [4.75 (d, J = 1 Hz) and 4.82 (br.s)], and an α,β-unsaturated double bond [6.92 (br.t, J = 7 Hz)]. The ^{13}C NMR spectral data of **1** confirmed the presence of six methyl groups (all q) (δ 12.1, 15.4, 15.9, 16.1, 21.0, and 26.8), a terminal unsaturated methylene group [108.3 (t) and 151.4 (s)], an α,β-unsaturated carboxylic acid [127.1 (s), 144.7 (d), and 172.5 (s)], together with an isolated carbonyl group [218.1 (s)]. The NMR spectroscopic data of **1** were very similar to those of dammaradienone [5] in the ring system of the dammarane skeleton, but the side chain was oxidized to a carboxylic acid functional group at C-27. The 2D NMR spectra, including ^{1}H–^{1}H COSY, HSQC, and HMBC (Fig. 1), confirmed the structure of **1** as 3-oxodammara-20(21),24-dien-27-oic acid. The (*E*)-geometry of the C-24/C-25 double bond was determined by comparing the carbon-13 chemical shifts of C-24 (δ 144.7), C-25 (127.1), and C-26 (12.1) of **1** with those reported for ganoderic acid AP2 (24-(*E*) geometry): C-24 (144.7, d), C-25 (127.1, s), and C-26 (12.1, q) [6] and 24-(*Z*)-3-oxodammara-20(21),24-dien-27-oic acid (24-(*Z*) geometry): C-24 (146.2, d), C-25 (137.8, s), and C-26 (20.5, q)] [7]. Therefore, the structure of **1** was determined to be 24-(*E*)-3-oxodammara-20(21),24-dien-27-oic acid.



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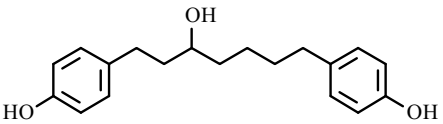

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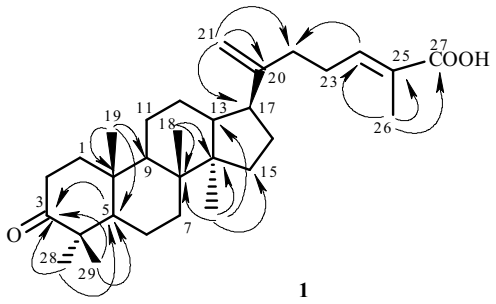

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Fig. 1. HMBC correlations of **1.**

Compound **2** was isolated as white needles, mp 210–212°C, $[\alpha]_D^{24}$ −216.8° (*c* 0.05, MeOH). Its molecular formula was determined to be $C_{19}H_{22}O_4$ by positive-ion HR-ESI-MS. The IR spectrum showed the presence of hydroxyl groups (3367 cm^{-1}) and aromatic rings (1603, 1512, and 1442 cm^{-1}). The ^1H NMR spectrum of **2** showed the presence of two aromatic rings, two oxygenated methine groups [δ 3.45 (1H, m) and 4.22 (1H, dd, J = 10 Hz, 2 Hz)], and methylene proton signals between 1.27–1.93 (8H). The two aromatic rings were identified as a 4-hydroxyphenyl group [δ 6.73 (2H, d, J = 8.5 Hz) and 7.02 (2H, d, J = 8.5 Hz)] and a 3,4-dihydroxyphenyl group [6.74 (1H, dd, J = 8 Hz, 2 Hz), 6.79 (1H, d, J = 8 Hz), and 6.89 (1H, d, J = 2 Hz)] on the basis of ^1H NMR analysis. The ^1H NMR data suggested that **2** had the structure of a cyclic diarylheptanoid [8–11], which was in good agreement with its molecular formula. The oxygenated heptane chain was cyclized between C-1 (δ 4.22) and C-5 (3.45) through an oxygen since the unique presence of a methylene group linked to an aromatic ring [2.59–2.7 (2H, m)] was observed. Since compound **2** was obtained in a minute amount, the positions of the aromatic moieties were determined by EI-MS. The base peak at *m/z* 107 corresponding to the formation of a *p*-hydroxybenzyl cation in the EI-MS spectrum of **2** indicated the location of the 4-hydroxyphenyl group at C-7. Except for the substitution pattern of the aromatic ring at C-1, the protons of **2** and (–)-centrolobine [11] were resonanced in similar chemical shifts. The relative configurations of C-1 and C-5 were determined on the basis of the axial orientations of H-1 (δ 4.22, $J_{1,2ax}$ = 10 Hz) and H-5 (3.45, $J_{4ax,5}$ = 10 Hz). Therefore, the structure of **2** was determined to be 1,5-epoxy-1-(3′,4′-dihydroxyphenyl)-7-(4′′-hydroxyphenyl)heptane.

EXPERIMENTAL

General Procedure. Optical rotations were measured on a Jasco P-1030 digital polarimeter. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. EI-MS spectra were measured on a Hewlett-Packard 5989 B mass spectrometer. HR-ESI-MS spectra were measured on a Thermo Fischer Scientific LTQ Orbitrap XL mass spectrometer. HR-APCI-MS spectra were measured on an AB (Applied Biosystems) QSTAR mass spectrometer. ^1H (500 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a Bruker Avance 500 NMR spectrometer with TMS as an internal standard. Silica gel 60 (0.063–0.100 and 0.063–0.200 mm) (Merck, Germany) was used for open-column (CC) and flash-column chromatography (FC). TLC was carried out on Merck TLC plates (silica gel 60 F_{254}) and detected by spraying with 1% vanillin/conc. H_2SO_4, followed by heating on a hot plate.

Plant Material. The leaves of *A. nepalensis* were collected in Dong Van District, Ha Giang Province, Vietnam by a botanist, Dr. Tran Ngoc Ninh of the Institute of Biological Resources and Ecology, Vietnam Academy of Science and Technology, Hanoi, Vietnam in June 2007. A voucher specimen of the plant (No. 10.999) was deposited at the same Institute.

Extraction and Isolation of 1–3. The dried powdered leaves of *A. nepalensis* (1.48 kg) were extracted separately with MeOH at room temperature (seven times, each time for three days). The combined MeOH extract was concentrated under reduced pressure, and the resultant MeOH extract was successively partitioned between water and organic solvents of increasing polarities. After removal of the organic solvents *n*-hexane (56.6 g), CH_2Cl_2 (20.5 g), and EtOAc (48.9 g), soluble fractions were obtained. Part of the CH_2Cl_2-soluble fraction (17.5 g) was chromatographed by silica gel CC using CH_2Cl_2–EtOAc (49:1, 29:1, 19:1, 9:1, 2:1, and 1:1) to afford eight fractions. Separation of fraction 4 (2.25 g) first by silica gel CC with *n*-hexane–EtOAc (15:1 and 4:1) and then by silica gel FC with *n*-hexane–acetone (9:1 and 2:1) afforded **1** (6.5 mg). Successive separation of fraction 5 by silica gel CC with CH_2Cl_2–EtOAc (29:1 and 4:1), silica gel FC with CH_2Cl_2–EtOAc (9:1 and 4:1), and silica gel FC with *n*-hexane–EtOAc (4:1 and 1:1) afforded **2** (2.8 mg) and **3** (166 mg).

24-(*E*)-3-Oxodammara-20(21),24-dien-27-oic Acid (1). White crystals, mp 145–146°C; $[\alpha]_D^{26}$ +37.9° (*c* 0.09, $CHCl_3$). IR (film, ν_{max}, cm^{-1}): 2600–3300, 1703, 1641, 1454, 1383, 1283, 1079. Negative-ion ESI-MS, *m/z* 453.5 $[M - H]^-$ ($C_{30}H_{45}O_3$); positive-ion HR-ESI-MS, *m/z* 477.33377 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.33392); negative-ion APCI-MS, *m/z* 453.5 $[M - H]^-$ ($C_{30}H_{45}O_3$); positive-ion HR-APCI-MS, *m/z* 455.35129 $[M + H]^+$ (calcd for $C_{30}H_{47}O_3$, 455.35197); EI-MS, *m/z* (I_{rel}, %): 454 ($[M]^+$, $C_{30}H_{46}O_3$) (11), 436 (18), 408 (11), 234 (32), 205 (93), 189 (39), 161 (29), 121 (100), 95 (91), 67 (71). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.9 (3H, s, CH_3-30), 0.97 (3H, s, CH_3-19), 1.04 (3H, s, CH_3-18), 1.06 (3H, s, CH_3-29), 1.11 (3H, s, CH_3-28), 1.11 (1H, m, H-12a), 1.16 (1H, ddd, J = 11.7, 9.5, 2.1, H-15a), 1.29 (1H, dd, J = 12.8, 4.4, H-11a), 1.37 (1H, m, H-7a), 1.42 (2H, m, H-5, H-16a), 1.45 (1H, m, H-9), 1.47 (1H, m, H-1a), 1.49 (1H, m, H-6a), 1.54 (1H, m, H-11b), 1.59 (1H, m, H-6b), 1.62 (2H, m, H-12b, H-15b), 1.64 (1H, m, H-7b), 1.7 (1H, ddd, J = 12, 11.5, 3.5, H-13), 1.87 (3H, s, CH_3-26), 1.95 (2H, m, H-1b, H-16b), 2.12 (2H, m, H-22a,b), 2.22 (1H, ddd, J = 15, 11, 7, H-17), 2.38 (2H, q, J = 7.5, H-23), 2.49 (1H, m, H-2a), 2.53 (1H, m, H-2b), 4.75 (1H, d, J = 1, H-21a), 4.82 (1H, br.s, H-21b), 6.92 (1H, br.t, J = 7, H-24). ^{13}C NMR ($CDCl_3$, δ, ppm): 12.1 (C-26), 15.4 (C-18), 15.9 (C-30), 16.1 (C-19), 19.7 (C-6), 21.0 (C-29), 21.9 (C-11), 24.9 (C-12), 26.8 (C-28), 27.7 (C-23), 28.9 (C-16), 31.4 (C-15), 32.7 (C-22), 34.1 (C-2), 34.8 (C-7), 36.9 (C-10), 39.9 (C-1), 40.4 (C-8), 45.6 (C-13), 47.4 (C-4), 47.7 (C-17), 49.5 (C-14), 50.3 (C-9), 55.4 (C-5), 108.3 (C-21), 127.1 (C-25), 144.7 (C-24), 151.4 (C-20), 172.5 (C-27), 218.1 (C-3).

1,5-Epoxy-1-(3′,4′-dihydroxyphenyl)-7-(4″-hydroxyphenyl)heptane (2). White needles, mp 210–212°C; $[\alpha]_D^{24}$ –216.8° (*c* 0.05, MeOH). IR (film, ν_{max}, cm^{-1}): 3367, 1603, 1535, 1512, 1442, 1375, 1220, 1076, 1024, 816. Positive-ion HR-ESI-MS, *m/z* 337.1412 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_4Na$, 337.1410). EI-MS, *m/z* (I_{rel}, %): 314 ($[M]^+$, $C_{19}H_{22}O_4$) (3), 296 (2), 190 (6), 176 (5), 149 (13), 137 (13), 123 (15), 107 (100), 91 (8), 77 (22). ^1H NMR ($CDCl_3$ + CD_3OD, δ, ppm, J/Hz): 1.3 (1H, qd, J = 10, 3.5, H-4ax), 1.53 (1H, qd, J = 11, 3.5, H-2ax), 1.63 (2H, m, H-3ax, H-4eq), 1.71 (1H, m, H-6a), 1.8 (1H, br.d, J = 13.5, H-2eq), 1.83–1.93 (2H, m, H-3eq, H-6b), 2.59–2.7 (2H, m, H-7a,b), 3.45 (1H, m, H-5), 4.22 (1H, dd, J = 10, 2, H-1), 6.73 (2H, d, J = 8.5, H-2″, H-6″), 6.74 (1H, dd, J = 8, 2, H-6′), 6.79 (1H, d, J = 8, H-5′), 6.89 (1H, d, J = 2, H-2′), 7.02 (2H, d, J = 8.5, H-3″, H-5″).

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