Lignan Derivatives and a Norditerpene from the Seeds of *Vitex negundo*

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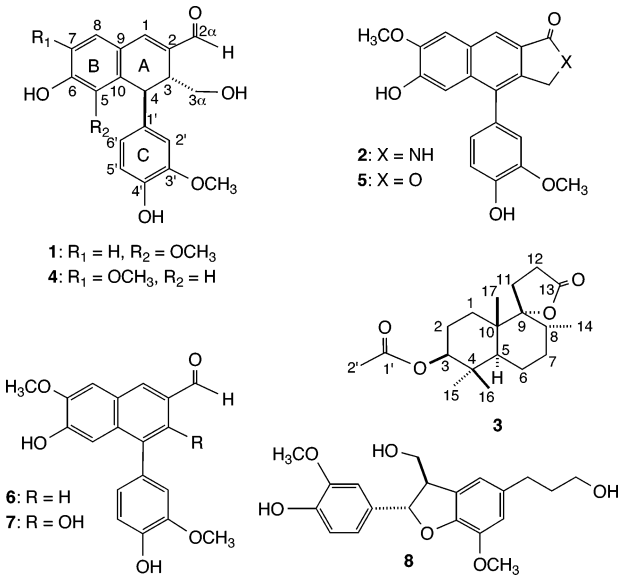
A new phenyldihydronaphthalene-type lignan, vitedoin A (**1**), a new phenylnaphthalene-type lignan alkaloid, vitedoamine A (**2**), and a new trinorlabdane-type diterpene, vitedoin B (**3**), were isolated from the seeds of *Vitex negundo* along with five known lignan derivatives (**4**−**8**). Their chemical structures were determined mainly on the basis of NMR and MS data. Compounds **1**, **2**, and **4**−**7** showed stronger antioxidative activity than α-tocopherol using the ferric thiocyanate method. Compounds **1**, **2**, and **4**−**7** showed higher radical-scavenging effect on the stable free radical, 1,1-diphenyl-2-picrylhydrazyl, than L-cysteine.

Vitex negundo L. (Verbenaceae) grows widely throughout South Asia, Japan, East Africa, and South America. The seeds of this plant were used as a folk medicine for analgesia and sedation.[1] Regarding the chemical constituents of the seeds, the presence of a phenyldihydronaphthalene-type lignan,[2] a diterpene,[3] a flavonoid,[3] and triterpenoids[4] has been previously reported. In the course of our studies on natural antioxidants,[5] the MeOH extract of the seeds showed stronger antioxidative activity than *tert*-butylhydroxyanisole (BHA), a synthetic antioxidant, using the ferric thiocyanate method.[6] The present paper describes the isolation and structure elucidation of a new phenyldihydronaphthalene-type lignan (**1**), a new phenylnaphthalene-type lignan alkaloid (**2**), and a new trinorlabdane-type diterpene (**3**) along with five known lignan derivatives (**4**−**8**) from the MeOH extract of the seeds of *Vitex negundo* L., and the antioxidative evaluation of **1**, **2**, and **4**−**7**.

The MeOH extract of the seeds of *V. negundo* L. was successively subjected to Diaion HP20, Sephadex LH20, silica gel, and Chromatorex ODS column chromatography as well as HPLC on ODS to afford **1**−**8**. Compounds **4**−**8** were identified as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde, [2] detetrahydroconidendrin,[7] vitrofolal E,[7] vitrofolal F,[7] and 2α,3*β*-7-*O*-methylcedrusin,[8] respectively, on the basis of their spectral data.

Compound **1**, trivially named vitedoin A, was obtained as a white powder. The HRFABMS indicated the molecular formula of **1** to be $C_{20}H_{20}O_6$. The ^{1}H NMR spectrum was closely analogous to that of **4**, except for the splitting patterns and chemical shifts of two aromatic protons [*δ* 7.17 (d, *J* = 8.5 Hz), 6.88 (d, *J* = 8.5 Hz) in **1**; 7.11 (s), 6.68 (s) in **4**]. The ^{13}C NMR spectrum was also similar to that of **4**, although the signals due to the B ring moiety were slightly shifted. Therefore, **1** was a positional isomer of **4** with a methoxyl group or a hydroxyl group on the B ring. This was confirmed by the HMBC and difference NOE spectra. In the difference NOE spectra, key correlations were observed between H-4 and OCH_3-5; H-3 and H-2′; H-3 and H-6′; and H-2′ and OCH_3-3′. The structure of **1** was therefore defined as 6-hydroxy-4*β*-(4-hydroxy-3-methoxyphenyl)-3α-hydroxylmethyl-5-methoxy-3,4-dihydro-2-naphthaldehyde.



Compound **2**, trivially named vitedoamine A, was obtained as a white powder. The EIMS showed an $[M]^+$ ion peak at *m/z* 351. The molecular formula of **2** was determined as $C_{20}H_{17}NO_5$ by HREIMS. The ^{1}H NMR spectrum of **2** was similar to that of **5**, apart from the appearance of the signal due to one amide proton (*δ* 8.45) and the chemical shifts of two methylene protons [*δ* 4.28 (d, *J* = 16.5 Hz), 4.18 (d, *J* = 16.5 Hz) in **2**; *δ* 5.37 (br d, *J* = 14.0 Hz), 5.26 (br d, *J* = 14.0 Hz) in **5**]. In comparing the chemical shifts of the ^{13}C NMR signals between **2** and **5**, the signals due to C-2 and C-3α in **2** were shifted by +8.7 and −24.9 ppm, respectively. From these data, **2** was considered to be a derivative of **5**, in which a lactone group in **5** was replaced by a lactam group. This was confirmed by the HMBC spectrum. Thus, **2** was concluded to be 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-nicoticmethyl-2-naphthoic acid-*γ*-lactam.

Compound **3**, trivially named vitedoin B, was obtained as colorless needles and analyzed for the molecular formula $C_{19}H_{30}O_4$ by HRFABMS. It did not show a parent ion peak in the EIMS but showed an intense fragment ion peak at *m/z* 262 $[M - CH_3COOH]^+$. The ^{1}H NMR spectrum of **3** indicated signals due to one oxygenated methine proton

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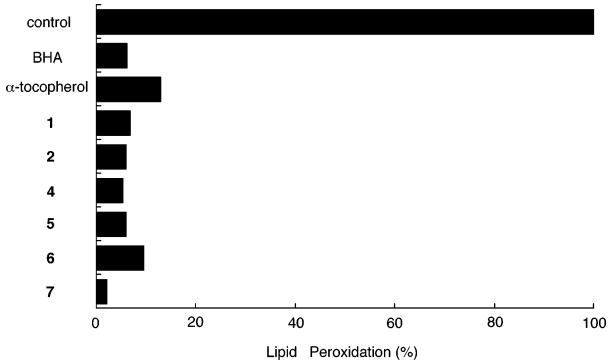

Figure 1. Antioxidative activities of compounds **1**, **2**, **4**−**7**, α-tocopherol, and BHA after 5 days of lipid peroxidation. The final concentration of the sample tested was 0.5 mM.

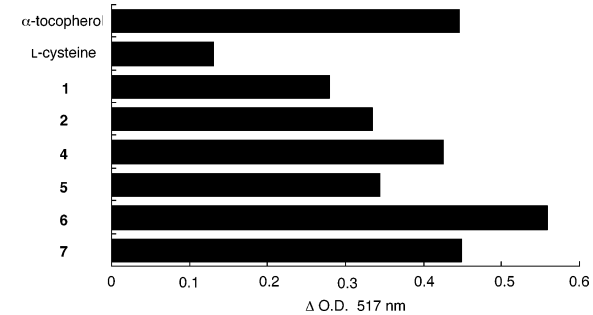

Figure 2. DPPH radical-scavenging effects of compounds **1**, **2**, **4**−**7**, L-cysteine, and α-tocopherol. The final concentration of each sample tested was 0.02 mM. ΔOD = OD of control at 517 nm (1.125) − OD of sample. DPPH, 0.1 mM.

[δ 4.31 (dd, J = 5.0, 11.5 Hz)], one acetyl group (δ 2.01), three tertiary methyl groups (δ 0.91, 0.84, 0.82), and one secondary methyl group [δ 0.77 (d, J = 6.5 Hz)]. The ^{13}C NMR spectrum of **3** showed 19 carbon signals including two carbonyl carbons (δ 176.8, 169.9), one oxygenated quaternary carbon (δ 92.3), and one oxygenated methine carbon (δ 79.4). These ^{1}H and ^{13}C NMR signals were assigned with the aid of ^{1}H−^{1}H COSY, HMQC, and HMBC techniques, and the planer structure of **3**, a trinorlabdane-type diterpene possessing one acetyl group and a γ-spirolactone group, was characterized as illustrated. The relative stereochemistry of **3** was determined by analysis of difference NOE spectra. The structure of **3** was therefore defined as (*rel*-3*S*,5*S*,8*R*,9*R*,10*S*)-3-acetoxy-14,15,16-trinor-13,9-labdanolide. The absolute configurations of **1** and **3** have not been confirmed.

The antioxidative activity of **1**, **2**, and **4**−**7** was evaluated using linoleic acid as the substrate by the ferric thiocyanate method,[6] and the activity was compared with that of α-tocopherol and BHA, each at a 0.5 mM concentration. Compounds **1**, **2**, and **4**−**7** showed stronger antioxidative activity than that of α-tocopherol. Compounds **2**, **4**, **5**, and **7** exhibited more potent antioxidative activity than that of BHA (Figure 1). The scavenging effect of **1**, **2**, and **4**−**7** on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was also examined.[9] All of these compounds exhibited stronger activity than that of L-cysteine, and **4**, **6**, and **7** showed activity similar to α-tocopherol (Figure 2). The strong antioxidative activity and DPPH radical-scavenging effect of **8** were previously reported.[10]

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting-point apparatus and are uncorrected. Optical rotations were measured with a JASCO DTP-1000 KUY digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL alpha 500 spectrometer at 500 and 125 MHz, respectively, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. EIMS and HRFABMS were obtained on a JEOL JMS-DX-303HF instrument. Column chromatography was carried out over silica gel 60 (Merck, Art. 9385), Diaion HP 20 (Mitsubishi Chemical Industries Co., Ltd.), Sephadex LH20 (Pharmacia Fine Chemicals), and Chromatorex ODS (Fuji Silysia Chemical Ltd.). HPLC separation was run on a Shimadzu LC-10AS micro pump with a Shimadzu RID-10A RI-detector. For HPLC column chromatography, COSMOSIL 5C_{18}-AR-II (Nacalai Tesque, 20 mm i.d. × 250 mm) was used.

Plant Material. Seeds of *Vitex negundo* L. were purchased in June 2000, from An-Guo City medical market, China, and identified by Prof. Toshihiro Nohara, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University. A voucher specimen is deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Kyushu Tokai University.

Extraction and Isolation. Powdered seeds of *V. negundo* L. (1026 g) were extracted with MeOH. The MeOH extract (58 g) was subjected to passage over Diaion HP 20 (40% MeOH, 70% MeOH, 90% MeOH, MeOH, acetone) to give fractions 1−5. Fraction 3 (16.1 g) was chromatographed over Sephadex LH20 (MeOH) to afford fractions 3.1−3.6. Chromatography of fraction 3.5 (234 mg) over silica gel [$CHCl_3$−MeOH−H_2O (1:0:0, 20:1:0, 15:1:0, 14:2:0.1)] furnished **5** (49 mg), **2** (20 mg), and fractions 3.5.1−3.5.3. Fraction 3.5.1 (46 mg) was subjected to Chromatorex ODS (60% MeOH, MeOH) to give fractions 3.5.1.1−3.5.1.5. Fraction 3.5.1.2 (9 mg) and fraction 3.5.1.3 (58 mg) were each subjected to HPLC (50% MeOH) to give **5** (3 mg) and **7** (4 mg) from fraction 3.5.1.2 and **6** (4 mg) from fraction 3.5.1.3. Fraction 3.2 (7010 mg) was chromatographed over silica gel [$CHCl_3$−MeOH−H_2O (1:0:0, 20:1:0, 15:1:0, 14:2:0.1)] to afford fractions 3.2.1−3.2.8. Fraction 3.2.5 (1372 mg) was subjected to Chromatorex ODS (60% MeOH, 70% MeOH, 80% MeOH) to give fractions 3.2.5.1−3.2.5.6. Fraction 3.2.5.6 (80 mg) was successively subjected to HPLC (80% MeOH) and silica gel [$CHCl_3$−MeOH (50:1, 25:1)] to afford **3** (10 mg). Chromatography of fraction 2 (9.59 g) over Sephadex LH20 with MeOH gave fractions 2.1−2.4. Fraction 2.2 (4281 mg) was subjected to silica gel [hexane−acetone (1:0, 5:1, 3:1, 2:1, 0:1), MeOH] to afford fractions 2.2.1−2.2.9. Fraction 2.2.8 (2738 mg) was chromatographed over silica gel [$CHCl_3$−MeOH (19:1, 10:1, 5:1, 3:1, 0:1)] to give fractions 2.2.8.1−2.2.8.7. Fraction 2.2.8.2 (831 mg) was successively subjected to Chromatorex ODS (40% MeOH, 60% MeOH, 80% MeOH, MeOH) and HPLC (40% MeOH) to afford **4** (226 mg), **1** (154 mg), and **8** (12 mg).

Vitedoin A (1): amorphous powder; $[\alpha]^{13}_{D}$ −79.4° (*c* 3.2, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.47 (1H, s, H-2α), 7.51 (1H, s, H-1), 7.17 (1H, d, J = 8.5 Hz, H-8), 6.88 (1H, d, J = 8.5 Hz, H-7), 6.62 (1H, d, J = 1.5 Hz, H-2′), 6.55 (1H, d, J = 8.5 Hz, H-5′), 6.19 (1H, dd, J = 1.5, 8.5 Hz, H-6′), 4.76 (1H, s, H-4), 3.67 (3H, s, OCH_3-3′), 3.48 (3H, s, OCH_3-5), 3.32 (1H, dd, J = 4.5, 10.5 Hz, H-3α), 3.05 (1H, dd, J = 4.5, 10.5 Hz, H-3), 2.84 (1H, dd, J = 10.5, 10.5 Hz, H-3α); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 192.5 (C, C-2α), 153.6 (C, C-6), 147.2 (C, C-3′), 146.7 (CH, C-1), 145.9 (C, C-5), 144.8 (C, C-4′), 135.1(C, C-1′), 134.4 (C, C-2), 132.8 (C, C-10), 126.1 (CH, C-8), 123.7 (C, C-9), 119.3 (CH, C-6′), 115.3 (CH, C-7), 115.1 (CH, C-5′), 111.9 (CH, C-2′), 60.8 (CH_2, C-3α), 59.6 (CH_3, OCH_3-5), 55.6 (CH_3, OCH_3-3′), 41.8 (CH, C-3), 36.4 (CH, C-4); EIMS *m/z* 356 $[M]^+$; HRFABMS *m/z* 357.1376 (calcd for $C_{20}H_{21}O_6$, 357.1338).

Vitedoamine A (2): amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.45 (1H, s, NH), 8.09 (1H, s, H-1), 7.55 (1H, s, H-8), 7.11 (1H, s, H-5), 6.98 (1H, d, J = 1.5 Hz, H-2′), 6.96 (1H, d, J = 8.0 Hz, H-5′), 6.82 (1H, dd, J = 1.5, 8.0 Hz, H-6′), 4.28 (1H, d, J = 16.5 Hz, H-3α), 4.18 (1H, d, J = 16.5 Hz, H-3α), 3.93 (3H, s, OCH_3-3′), 3.80 (3H, s, OCH_3-7); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 170.2 (C, C-2α), 149.0 (C, C-6), 148.6 (C, C-7), 147.7 (C, C-3′), 146.1 (C, C-4′), 135.6 (C, C-3), 132.1 (C, C-4), 130.1 (C, C-10), 128.5 (C, C-2), 127.8 (C, C-9), 127.7 (C, C-1′), 122.0 (CH, C-6′), 120.4 (CH, C-1), 115.7 (CH, C-5′), 113.6 (CH, C-2′), 108.2 (CH, C-8), 107.6 (CH, C-5), 55.8 (CH_3,

OCH$_3$-3′), 55.5 (CH$_3$, OCH$_3$-7), 44.3 (CH$_2$, C-3α); EIMS *m/z* 351 [M]$^+$; HREIMS *m/z* 351.1070 (calcd for $C_{20}H_{17}NO_5$ 351.1106).

Vitedoin B (3): colorless needles (hexane−EtOAc); mp 95−96°C; $[\alpha]^{29}_D$ +4.7° (*c* 0.9, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 125 MHz) δ 4.31 (1H, dd, *J* = 5.0, 11.5 Hz, H-3), ca. 2.54 (1H, H-12), ca. 2.47 (1H, H-12), 2.15 (1H, ddd, *J* = 8.0, 11.5, 13.0 Hz, H-11), 2.01 (3H, s, H$_3$-2′), 1.84 (1H, ddd, *J* = 5.0, 11.5, 11.5 Hz, H-11), 1.78 (1H, m, H-8), ca. 1.62 (1H, H-2), ca. 1.58 (1H, H-2), ca. 1.54 (1H, H-6), ca. 1.54 (1H, H-7), ca. 1.51 (1H, H-1), 1.43 (1H, dddd, *J* = 4.5, 12.5, 12.5, 12.5 Hz, H-6), 1.37 (1H, br d, *J* = 12.5 Hz, H-5), 1.22 (1H, ddd, *J* = 4.5, 12.0, 12.0 Hz, H-1), 1.19 (1H, dddd, *J* = 4.5, 12.5, 12.5, 12.5 Hz, H-7), 0.91 (3H, s, H$_3$-17), 0.84 (3H, s, H$_3$-16), 0.82 (3H, s, H$_3$-15), 0.77 (3H, d, *J* = 6.5 Hz, H$_3$-14); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.49 (1H, dd, *J* = 4.5, 11.5 Hz, H-3), 2.03 (3H, s, H$_3$-2′), 0.96 (3H, s, H$_3$-17), 0.88 (3H, s, H$_3$-15), 0.88 (3H, s, H$_3$-16), 0.86 (3H, d, *J* = 7.0 Hz, H$_3$-14); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 176.8 (C, C-13), 169.9 (C, C-1′), 92.3 (C, C-9), 79.4 (CH, C-3), 46.1 (CH, C-5), 41.2 (C, C-10), 37.2 (C, C-4), 35.6 (CH, C-8), 30.6 (CH$_2$, C-7), 28.8 (CH$_2$, C-12), 28.7 (CH$_2$, C-1), 27.7 (CH$_3$, C-15), 24.2 (CH$_2$, C-11), 22.7 (CH$_2$, C-2), 20.8 (CH$_3$, C-2′), 20.2 (CH$_2$, C-6), 16.2 (CH$_3$, C-16), 15.1 (CH$_3$, C-14), 15.1 (CH$_3$, C-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.2 (C, C-13), 170.6 (C, C-1′), 93.3 (C, C-9), 80.0 (CH, C-3), 46.1 (CH, C-5), 41.9 (C, C-10), 37.7 (C, C-4), 36.7 (CH, C-8), 30.8 (CH$_2$, C-7), 29.4 (CH$_2$, C-1, C-12), 27.9 (CH$_3$, C-15), 24.9 (CH$_2$, C-11), 23.2 (CH$_2$, C-2), 21.2 (CH$_3$, C-2′), 20.9 (CH$_2$, C-6), 16.6 (CH$_3$, C-16), 15.8 (CH$_3$, C-14 or C-17), 15.4 (CH$_3$, C-17 or C-14); EIMS *m/z* [M]$^+$ absent; 262 [M − CH$_3$-COOH] $^+$; HRFABMS *m/z* 345.2132 [M + Na]$^+$ (calcd for $C_{19}H_{30}O_4Na$, 345.2042).

Antioxidative Activity. A mixture of 2.51% linoleic acid EtOH solution (0.80 mL), 0.05 M phosphate buffer (pH 7.0, 1.60 mL), EtOH (0.60 mL), and H_2O (0.80 mL) was added to a 10 mM EtOH solution (0.20 mL) of each sample in a vial with a cap and placed in the darkness at 40 °C to accelerate oxidation. After the fifth day of incubation, this assay solution (0.05 mL) was diluted with 75% EtOH (4.85 mL), which was followed by adding 30% ammonium thiocyanate (0.05 mL). Precisely 3 min after the addition of 0.02 M ferrous chloride in 3.5% HCl (0.05 mL) to the reaction mixture, the absorbance was measured at 500 nm. The control sample was prepared in the same manner by mixing all the same chemicals and ingredients and by excluding the test compounds. α-Tocopherol and BHA were used as standard samples.

Scavenging Effect on DPPH. The method of Uchiyama et al.[9] was applied slightly modified. EtOH solution (1.00 mL) of each testing sample was added to a mixture of 0.1 M acetic acid buffer (pH 5.5, 1.00 mL) and 0.5 mM DPPH EtOH solution (0.50 mL) in a test tube and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. α-Tocopherol and L-cysteine were used as standard samples.

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