PHENYLNAPHTHALENES AND DIHYDRONAPHTHALENE DERIVATIVES FROM THE ROOTS OF VITEX NEGUNDO

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Six phenylnaphthlene-type (1, 3~7) and a spirocyclohexadienone-type (8) neolignans, a dihydronaphthalene dione (2), and a sesquiterpenoid derivative (9) were isolated from the active fraction of the roots of Vitex negundo L (Verbenaceae). Their structures were elucidated by extensive spectroscopic analysis including 1D and 2D NMR and MS. Among them, compounds, 1 and 2, were identified as new isolates from natural products.

Key words: Vitex negundo, neolignans, phenylnaphthalenes, dihydronaphthalene dione, spirocyclohexadienone, sesquiterpenoid

Introduction

Vitex negundo L. (Verbenaceae) has been used as a folk medicine for treating rheumatoid arthritis, relieving cough and subduing asthma.¹ Different kinds of constituents such as flavonoids,²-⁴ iridoids,⁵ lignans,⁶,⁷ triterpenoids,⁸ and sesquiterpenoids⁹ have been isolated from this plant. In the course of our search for neuroprotective agents and immunomodulators from natural products, the ethanolic extract of V. negundo was investigated in vitro and found to exert significant bioactivities. We here reported that the isolation and structural elucidation of two new compounds, vitrofolal G (1) and vitroldienone (2), along with known compounds including five phenylnaphthalene-type and a spirocyclohexadienone-type neolignans, and a sesquiterpenoid were isolated from the active fractions of roots.

Materials and Methods

I. General experimental procedures

NMR spectra were recorded on a Varian unity INOVA-500 spectrometer (Varian, Palo Alto, CA, USA) using CD₃OD as a solvent. Chemical shifts are given in parts per million (ppm) and were referenced to the solvent signals at 3.30 ppm and 49.0 ppm for ¹H and ¹³C NMR, respectively. Mass spectra (EIMS and

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HREIMS) were run on a Finnigan MAT 95S (Thermoquest, San Jose, CA, USA) and JEOL SX-102A (JEOL, Tokyo, Japan) mass spectrometer, respectively. ESIMS was recorded on a Finnigan MAT LCQ ion trap mass spectrometer system.

IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer (Thermo Electron, Akron, OH, USA). Optical rotation was measured on a Jasco DIP 370 digital polarimeter (Hachioji, Tokyo, Japan). Preparative HPLC were performed on a Shimadzu LC-8A equipped with a RID-10A detector (Shimadzu, Tokyo, Japan), using a 250 × 10 mm. Lichrosorb® Si 60 (7 µM) column (Merck). Flow rate was 4 ml min⁻¹. Column chromatography (CC) used silica gel 60 (Merck) and Sephadex™ LH-20 (Amersham Biosciences, Uppsala, Sweden). Analytical thin layer chromatography (TLC) used silica gel 60 GF₂₅₄ (Merck). (Merck, Darmstadt, Germany).

II. Plant Materials

The roots of V. negundo were collected at northeastern coast of Taiwan in November 2008 and authenticated by Mr. Jun-Chih Ou, former associate research fellow, National Research Institute of Chinese Medicine, and by comparison with a voucher specimen (Lin.no.121191) which was deposited in the Herbarium of the Institute of Plant Biology, National Taiwan University, Taipei, Taiwan.

III. Extraction and Isolation

Roots of V. negundo (10 kg) were extracted at 60°C with ethanol (EtOH) overnight (3 × 60 l). The dried EtOH extract (342 g) was partitioned with EtOAc and n-BuOH, and separated into EtOAc- (72 g), n-BuOH- (125 g) and H₂O-soluble (145 g) fractions, respectively. The EtOAc-soluble fraction was subjected to silica gel CC using an increasing percentage of EtOAc in hexane. The fractions eluted with 35%~50% EtOAc/hexane was then passed over a Sephadex LH-20 column and eluted with EtOAc. The lignan-rich fraction was submitted to preparative HPLC on Lichrosorb® Si 60 using 30% EtOAc/hexane to yield 1 (12 mg) and 2 (18 mg), vitrofolal A (3, 15 mg), vitrofolal B (4, 21 mg), vitrofolal E (5, 12 mg), vitrofolal C (6, 15 mg), detetrahydroconidendrin (7, 6.9 mg), futoenone (8, 13 mg) and (−)-viteralone (9, 205 mg).

(1) Vitrofolal G (1)

Colorless amorphous; IR (KBr) νₘₐₓ 3285 (OH), 2740, 2740, 1728 (CHO), 1616, 1576, 1500 (C=C) cm⁻¹; ¹H NMR(CDCl₃, 500 MHz): δ 3.93, 3.99, and 4.11 (3H each, s, OCH₃-3′, 4′, 6); 7.01 (1H, d, J= 2.0 Hz, H-2′), 7.02 (1H, d, J = 8.0 Hz, H-5′), 7.03 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 7.35 and 7.44 (1H, each, s, H-8, -5], 7.78 and 8.21 (1H each, d, J = 2.0 Hz, H-2, -4), 10.14 (1H, s, CHO); ¹³C NMR(CDCl₃, 125 MHz): δ 56.0 (OCH₃), 56.0 (OCH₃), 56.1 (OCH₃), 107.5 (C-8), 108.7 (C-5), 111.2 (C-5′), 113.0 (C-2′), 122.1 (C-6′), 122.8 (C-2), 129.1 (C-8a), 131.9 (C-4a), 132.9 (C-4), 133.0 (C-3), 133.2 (C-1′), 140.5 (C-5), 148.0 (C-6), 148.5 (C-7), 148.8 (C-3′), 149.2 (C-4′), 192.2 (CHO); Key HMBC correlations: CHO/C-2, -4, -3; H-4/C-2, -3, -4a, -5, -8a, -CHO; H-2/C-3, -4, -1′; H-5/C-4a, C-6, -7,8a; H-8/C-4a, -6, -7; OCH₃-3′ (δ₁H 3.93)/C-3′; OCH₃-4′ (δ₁H 3.99)/C-4′; OCH₃-6( δ₁H 4.11); Key NOESY correlations: OCH₃-6/C-5; OCH₃-3′/H-2′; OCH₃-4′/H-5′; ESIMS m/z 339 (M+H)⁺. HREIMS: m/z 366.12564 (calcd. for C₂₀H₁₉O₅, 339.12327).

(II) Vitroldienone (2)

Colorless needle from EtOH, mp 126-128°C;
[α]D –52° (MeOH, c 1.0); IR (KBr) νmax 3400, 1756, 1640, 1512 cm⁻¹; ¹H NMR(CDCl₃, 500 MHz): δ 0.94 (3H, d, J = 7.0 Hz, H-11), 1.42 (3H, s, H-12), 2.81 (1H, m, H-4), 6.14 (1H, s, H-6), 6.22 (1H, d, J = 10.0 Hz, H-2), 6.85 (1H, s, H-9) and 7.39 (1H, dd, J = 8.0 Hz, H-5'), 7.59 and 8.23 (1H each, d, J = 2.0 Hz, H-2'), 7.25 and 7.47 (1H each, s, H-8), -10, 6.14 (1H, d, J = 3.0 Hz, OH-7), 5.85 (1H, d, J = 3.0 Hz, H-7), 6.52 (1H, br s, OH-2), 7.34 (1H, s, H-8), 7.15 and 7.65 (1H each, d, J = 9.0 Hz, H-6, -5), 8.08 (1H, s, H-4), 10.02 (1H, s, CHO), 10.69 (1H, br s, OH-2); ¹³C NMR(CDCl₃, 125 MHz): δ 55.8 (OCH₃-3'), 56.0 (OCH₃-4'), 61.5 (OCH₃-8, 109.9 (C-5'), 114.5 (C-2') 116.0 (C-6), 119.5 (C-1'), 119.8 (C-3), 123.1 (C-6'), 123.1 (C-1), 124.4 (C-4a), 128.5 (C-5), 131.4 (C-8a). 138.3 (C-4'), 139.9 (C-8), 147.8 (C-3'), 148.1 (C-4'), 151.3 (C-7), 154.4 (C-2), 196.0 (CHO); ESIMS m/z 377 [M+Na⁺].

(v) Vitrofolar E (5)¹⁰

Colorless amorphous; ¹H NMR(CD₂OD, 500 MHz): δ 3.87 and 4.01 (3H each, s, OCH₃-3', -6), 6.87 (1H, dd, J = 8.0, 2.0 Hz), 6.92 (1H, d, J = 8.0 Hz), 6.97 (1H, d, J = 2.0 Hz, H-2'), 7.25 and 7.47 (1H each, s, H-8), -5), 7.59 and 8.23 (1H each, d, J = 1.6 Hz, H-2, -4), 10.00 (1H, s, CHO); ¹³C NMR(CDCl₃, 125 MHz): δ 56.4 (OCH₃-3'), 56.5 (OCH₃-6), 109.4 (C-5), 110.0 (C-8), 114.5 (C-2'), 116.3 (C-3'), 122.6 (C-2), 123.6 (C-6'), 130.3 (C-8a), 133.1 (C-4a), 133.2 (C-4), 133.4 (C-3), 133.6 (C-1'), 140.9 (C-1), 147.4 (C-4'), 149.0 (C-3'), 150.9 (C-6), 151.4 (C-7), 194.6 (CHO); ESIMS m/z (%) 325 (M+H⁺).

(vi) Vitrofolar C (6)¹¹

Colorless amorphous; ¹H NMR(CDCl₃, 500 MHz): δ 3.70, 4.03 and 4.03 (3H each, s, OCH₃-9, -10, -1), 5.06 (1H, d, J = 3.0 Hz, OH-7), 5.85 (1H, d, J = 3.0 Hz, H-7), 6.52 (1H, br s, OH-2), 7.34 (1H, s, H-8), 7.40 and 7.82 (1H each, d, J = 8.5 Hz, H-3, -4), 8.20 and 8.31 (1H each, s, H-5, -11), 10.14 (1H, s, CHO); ¹³C NMR(CDCl₃, 125 MHz): δ 55.8 (OCH₃-9, 55.9 (OCH₃-10, 62.6 (OCH₃-1), 73.8 (C-7), 107.4 (C-11), 109.4 (C-8), 117.9 (C-3), 125.2 (C-11c), 128.6 (C-4), 128.7 (C-6), 130.1 (C-4a), 132.1 (C-11a), 135.2 (s, C-7a), 135.3 (C-7), 125.2 (C-11c), 128.6 (C-4), 128.7 (C-6), 130.1 (C-4a), 132.1 (C-11a), 135.2 (s, C-7a),
137.8 (C-5), 138.5 (C-11b), 140.2 (C-1), 144.0 (C-6a), 148.9 (C-9), 149.3 (C-10), 150.4 (C-2), 194.5 (CHO); ESIMS m/z 367 (M+H)+.

(Ⅵ) Detetrahydroconidendrin (7)\textsuperscript{7,11}

Colorless amorphous; \textsuperscript{1}H NMR(CD\textsubscript{3}OD, 500 MHz): δ 3.86 and 4.03 (3H each, s, OCH\textsubscript{3}-3', -6), 5.27 and 5.30 (1H each, d, J = 15.0 Hz, H-9), 6.84 (1H, dd, J = 7.8, 1.8 Hz, H-6'), 6.96 (1H, d, J = 1.8 Hz, H-2'), 6.97 (1H, dd, J = 7.8, H-5'), 7.15 and 7.50 (1H, s, H-8, -5), 8.30 (1H, s, H-4); \textsuperscript{13}C NMR(CD\textsubscript{3}OD, 125 MHz): δ 56.4 (OCH\textsubscript{3}-6), 56.6 (OCH\textsubscript{3}-3'), 71.2 (C-9), 108.9 (C-5), 109.0 (C-8), 114.0 (C-2'), 116.8 (C-5'), 121.5 (C-2), 123.4 (C-6'), 125.0 (C-4), 129.0 (C-1'), 131.2 (C-4a), 133.4 (C-1), 133.8 (C-8a), 139.1 (C-3), 147.8 (C-4'), 149.5 (C-3'), 151.0 (C-7), 151.4 (C-6), 174.3 (C-10); ESIMS m/z 353 [M+H]+.

(Ⅶ) Futooneone (8)\textsuperscript{12}

Colorless amorphous; [α]\textsubscript{D} +25° (MeOH, c 1.0); IR (KBr) \nu \textsubscript{max} 3200 (OH), 1660 (conjugated C=O), 1620, 1510 (C=C) cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CD\textsubscript{3}COCD\textsubscript{3}, 500 MHz): δ 0.53 (3H, d, H-14), 2.19 (1H, m, H-9b), 2.21 (1H, m, H-11), 2.25 (1H, m, H-7a), 2.49 (1H, m, H-7b), 2.55 (1H, m, H-10), 3.58 (3H, s, OCH\textsubscript{3}), 5.06 (1H, m, H-8), 5.54 (1H, s, H-6), 5.68 (1H, s, H-3), 5.95 (2H, s, -OCH\textsubscript{2}O-), 6.75 (1H, d, J = 8.0 Hz, H-17), 6.77 (1H, d, J = 8.0 Hz, H-18), 6.89 (1H, s, H-14); \textsuperscript{13}C NMR(CD\textsubscript{3}COCD\textsubscript{3}, 125 MHz): δ 14.7 (C-12), 38.5 (C-9), 43.8 (C-7), 45.3 (C-11), 47.1 (d, C-10), 51.0 (s, C-4), 55.2 (OCH\textsubscript{3}), 83.0 (C-8), 101.2 (C-6), 101.9 (-OCH\textsubscript{2}O-), 108.7 (C-17), 108.8 (C-14), 110.4 (C-3), 122.1 (C-18), 139.0 (C-13), 147.1 (C-15), 148.8 (C-16), 154.2 (C-2), 180.6 (C-5), 182.5 (C-1); EIMS m/z 340 (M\textsuperscript{+}).

(Ⅷ) (−)-Viteralone (9)\textsuperscript{8,13}

Colorless needles; \textsuperscript{1}H NMR(CDCl\textsubscript{3}, 500 MHz): δ 1.34 (3H, d, H-14), 2.08 (1H, ddt, J = 13.4, 5.4, 2.6 Hz, H-3), 2.25 (1H, tt, J = 13.4, 5.4 Hz, H-3), 2.59 (1H, ddd, J = 18.1, 9.9, 2.6 Hz, H-2), 2.84 (3H, s, H-15), 2.86 (1H, J = 18.1, 13.4, 5.4 Hz, H-2), 3.51 (1H, m), 8.07 (1H, s, H-9), 8.34 (1H, s, H-11), 10.09 (1H, s, H-13); \textsuperscript{13}C NMR(CDCl\textsubscript{3}, 125 MHz): δ 17.2 (C-15), 18.9 (C-14), 28.9 (C-3), 28.9 (C-4), 32.8 (C-2), 108.8 (C-8), 125.3 (C-12), 127.5 (C-7), 130.4 (C-10), 130.9 (C-6), 143.7 (C-5), 155.0 (C-8), 160.2 (C-11), 183.6 (C-13), 198.1 (C-1); EIMS m/z 242 (M\textsuperscript{+}).

Results and Discussion

An EtOAc-soluble fraction of the ethanolic extract from the roots of V. negundo was purified by silica gel and Sephadex LH-20 column chromatography to give a lignan-rich mixture with significantly immunomodulatory effect. The mixture was subjected to preparative HPLC to afford two new compounds, vitrofolal G (1) and vitroldienone (2), together with five known phenylnaphthalene-type norlignans, vitrofolal A (3),\textsuperscript{10} vitrofolal B (4),\textsuperscript{10} vitrofolal E (5),\textsuperscript{10} vitrofolal C (6),\textsuperscript{11} and detetrahydroconidendrin (7),\textsuperscript{7,11} a spiro-cyclohexadienone-type neolignan, futoenone (8),\textsuperscript{12} and a sesquiterpoid, (−)-viteralone (9).\textsuperscript{8,13}

Vitrofolal G (1) was obtained as amorphous powder. The HREIMS gave a molecular formula C\textsubscript{20}H\textsubscript{16}O\textsubscript{3} with 12 indices of hydrogen deficiency. The \textsuperscript{1}H NMR spectrum exhibited two meta-coupled aromatic protons [δ 7.78 and 8.21 (J = 2.0 Hz)], two isolated singlet aromatic protons [δ 7.35 and 7.44], an ABX-system phenyl protons [δ 7.01 (J = 2.0 Hz), 7.02 (J = 8.0 Hz), 7.03 (J = 8.0, 2.0 Hz)], three methoxy groups [δ 3.93, 3.98, 4.11 (3H each, s)], and an alde-
The hydride proton [δ 10.14]. \(^{13}\)C and DEPT NMR spectra of 1 suggested the presence of 7 aromatic protons, 9 quaternary aromatic (including 4 oxygenated) carbons, an aldehyde, and three methoxy groups. The HMQC spectrum established a direct connection between proton and their respective carbon. On the basis of the HMBC spectrum, the selected correlations of protons to carbons indicated the presence of a phenylnapthalene with an aldehyde group. Comparison of \(^1\)H and \(^{13}\)C spectra of 1 are similar to those of vitrofolal E (5) except for a methoxy group at 4'-position in place of a hydroxyl. Further confirmation by a NOESY experiment showed NOE correlations between δ 4.11 (OCH\(_2\)-6)/H-5 (δ 7.44), 3.99 (OCH\(_2\)-4')/H-5(δ 7.02), and δ 3.93 (OCH\(_2\)-3')/H-2(δ 7.01).

Vitroldienone (2) was obtained as colorless needles with [α] \(_D\) -52°. The HREIMS and \(^{13}\)C NMR of 3 gave a molecular formula of C\(_{12}\)H\(_{12}\)O\(_3\) with 7 indices of hydrogen deficiency. The presence of hydroxy, α,β-unsaturated carbonyl, aromatic groups were shown by its IR spectrum. The \(^1\)H and \(^{13}\)C NMR data displayed a secondary methyl [δ\(_H\) 0.94 (J = 7.0 Hz); δ\(_C\) 17.7], a primary methyl [δ\(_H\) 1.42 (s); δ\(_C\) 27.0], two isolated dienone olefines [δ\(_H\) 6.85, δ\(_C\) 129.3; δ\(_H\) 6.14, δ\(_C\) 126.5; δ\(_C\) 183.8], an α,β-unsaturated carbonyl [δ\(_H\) 6.22 (J = 10.0 Hz), δ\(_C\) 128.5; δ\(_H\) 7.39 (dd, J = 10.0, 6.5 Hz), δ\(_C\) 158.1; carbonyl (δ\(_C\) 188.2)]. The COSY spectrum showed the correlations of protons at δ 6.22 (H-2) with those at δ 7.39 (H-3) and δ 2.81 (m, H-4), suggested the linkage of –CH=CH-CH=CH\(_3\)–. Analysis of HMQC and HMBC spectra indicated the correlations of H-11 with C-3, C-4 and C-5; of H-12 with C-4, C-5, C-6 and
C-10; of H-6 with C-4, C-5,C-7, C-8, C-10 and C-12; and of H-9 with C-1 C-5, C-7, C-8 and C-10, indicated the structure of 3 to be 4,5-dihydro-7-hydroxy-4,5-dimethylnaphthalene-1,8-dione. NOESY correlation was observed between two methyl groups to confirm their relative configuration to be cis form.

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**References**

黃荊根部苯萜和二氫萘衍生物之研究

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自民間藥用觀葉科植物黃荊根部分離出七個新木脂素其中有六個化合物是苯萜類型之新木脂素 (1, 3-7) 和一個螺環己二烯酮類型之新木脂素 (8)、一個二氫萘二酮 (2) 及一個倍半萜衍生物 (9)，上述化合物均藉由一維、二維核磁共振光譜及質譜分析其結構，其中化合物1和2確認為新化合物。

關鍵字：黃荊、新木脂素、苯萜、二氫萘二酮、螺環己二烯酮、倍半萜衍生物