Naphthoquinone and Iridoid with NGF-potentiating Activity from *Verbena littoralis*

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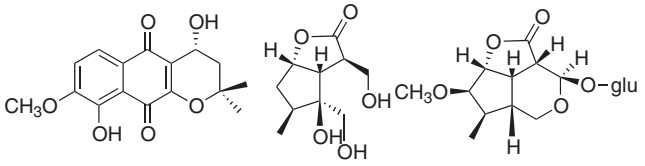
One new naphthoquinone, (4*R*)-4,9-dihydroxy-8-methoxy-α-lapachone (**1**), and two new iridoids, 7a-hydroxygelsemiol (**2**) and 6-methoxysemperoside (**3**) were isolated from *Verbena littoralis* H. B. K. 1 (3-30 μM) and **2** (3 μM) showed a markedly enhancing activity of nerve growth factor (NGF)-mediated neurite outgrowth from PC12D cells.

The aerial parts of *Verbena littoralis* H. B. K. (Verbenaceae) from Paraguay gave a methanol extract that showed an enhancing activity of NGF-mediated neurite outgrowth from PC12D cells. We isolated active components **1** and **2** as enhancers of NGF action from the extract by activity-guided fractionation and **3**. Compounds that possess this property may be useful in the treatment of neurodegenerative diseases including cerebrovascular dementia, Parkinson's disease, and Alzheimer's disease.[1,2]



1 **2** **3**

The MeOH extracts of *V. littoralis* (5 kg) were partitioned into EtOAc, *n*-BuOH and H_2O three fractions. The EtOAc and *n*-BuOH soluble materials were separated by a series of bioassay-directed chromatographic separations employing silica gel column, Sephadex LH-20 column and semi-preparative HPLC column chromatography (YMC-pack NH_2 A-624 and YMC-AM-324 ODS) to yield **1** (2.2 mg), **2** (22.9 mg), and **3** (13.4 mg).

Compound **1** was obtained as red amorphous powder, and its molecular formula was determined to be $C_{16}H_{16}O_6$ by HREIMS [m/z 304.0905, Δ-4.2 mmu]. The IR spectrum showed the presence of hydroxyl (3600-3300 cm^{-1}) and carbonyl (1640 and 1610 cm^{-1}) moieties. A detailed examination of the ^1H and ^{13}C NMR spectral data of **1**, aided by DEPT and HMQC experiments, disclosed the presence of two carbonyls, six sp^2 quaternary carbons (three of which were oxygen-bearing), one sp^3 oxygen-bearing quaternary carbon, one sp^3 oxygen-bearing methine, two sp^2 methine, one sp^3 methylene, one methoxyl, and two methyl groups.

In the ^1H NMR spectrum of **1**, a pair of *ortho*-coupled aromatic proton signals at δ 7.63 (1H, $J = 8.5$ Hz, H-6) and 7.06 (1H, $J = 8.5$ Hz, H-7) were positioned to the C-6 (δ_C 120.24) and C-7 (δ_C 115.53) by the HMBC correlations of H-6/C-5 (δ_C 184.43), H-6/C-8 (δ_C 153.88) and H-6/C-9a (δ_C 114.29), and H-7/C-5a (δ_C 125.47) and H-7/C-9 (δ_C 152.63), respectively (Figure 1). Two hydroxyl proton signals at δ 12.16 (9-OH) and 3.83 (4-OH) were assigned to the C-9 and C-4 (δ_C 60.12), correspondingly, because the proton of the 9-OH correlated with C-8, C-9 and C-9a, and H-4 (δ 4.93) correlated with C-2 (δ_C 79.83), C-5 and C-10a (δ_C 153.57) in the HMBC spectrum. A methoxyl signal at δ 3.97 (3H, s) was placed at the C-8 by the HMBC correlation of 8-OCH_3/C-8. A pair of gem-dimethyl signals at δ 1.43 (3H, s, H_3-11) and 1.54 (3H, s, H_3-12) were located at C-2 on the basis of the interaction peaks of H_3-11/C-2, H_3-12/C-2, H_3-11/C-12 (δ_C 27.08), and H_3-12/C-11 (δ_C 26.70) of the HMBC spectrum. The remaining signals of ^1H and ^{13}C NMR spectra can be entirely assigned by the analysis of DEPT, ^1H–^1H COSY, HMQC and HMBC spectral data. The absolute configuration remained undetermined by a negative optical rotation, because the absolute configuration of a similar compound 4-hydroxy-9-methoxyl-α-lapachone had been determined to be *S* which shown a positive optical rotation.[3] On the basis of the above evidences, the structure of **1** was elucidated as (4*R*)-4,9-dihydroxy-8-methoxy-α-lapachone.[4]

Compound **2** was obtained as a colorless amorphous solid and its molecular formula $C_{10}H_{16}O_5$ was determined by HRFABMS m/z 217.1088 $[M+H]^+$ (calcd, 217.1076). The IR spectrum indicated the presence of hydroxyl (3600-3100 cm^{-1}) and lactone (1745 cm^{-1}) moieties. Comprehensive analysis of NMR spectral data, especially ^1H–^1H COSY, HMQC and HMBC data, revealed the structure of **2**. In the ^1H NMR spectrum of **2**, two pairs of oxygen-bearing methylene proton signals at δ 3.64 (1H, d, $J = 12.0$ Hz, H-1a) and δ 3.61 (1H, d, $J = 12.0$ Hz, H-1b), and at δ 3.88 (1H, dd, $J = 10.8$, 4.2 Hz, H-3a) and δ 3.86 (1H, dd, $J = 10.8$, 4.8 Hz, H-3b) were positioned to the C-1 (δ_C 66.27) and C-3 (δ_C 63.88), respectively, by the HMBC correlations of H-1a (H-1b) with C-4a (δ_C 54.86), C-7 (δ_C 38.08) and C-7a (δ_C 85.32), H-3a (H-3b) with C-4 (δ_C 47.40), C-4a and C-9 (δ_C 181.41). One doublet signal at δ_C 0.94 (3H, d, $J = 6.6$ Hz, H_3-8) was assigned to the methyl carbon C-8 (δ_C 12.68) by the cross peak H_3-8/H-7 (δ 1.76) of the ^1H–^1H COSY spectrum and the HMBC correlations of C-8/H-6 (δ 1.92, 1.95), C-8/H-7, H_3-8/C-6 (δ_C 40.67), H_3-8/C-7 and H_3-8/C-7a. The presence of a five-membered lactone ring was deduced by the analysis of the downfield chemical shift signals at δ 4.99 (1H, ddd, $J = 7.2$, 5.4, 1.2 Hz, H-5), which revealed that the methine (δ_C 85.08, C-5) was linked to an acyloxy group, and this was confirmed by the HMBC correlations of C-9 with H-3, H-4 (δ 2.85), H-4a (δ 2.96) and H-5. The cyclopentane ring was determined by the analysis of ^1H–^1H COSY and HMBC spectral data (Figure 1). The relative stereochemistry of **2** was established by NOESY spectrum data (Figure 1). The correlations of H-3a/H-4a, H-4a/H-5, H-5/H-

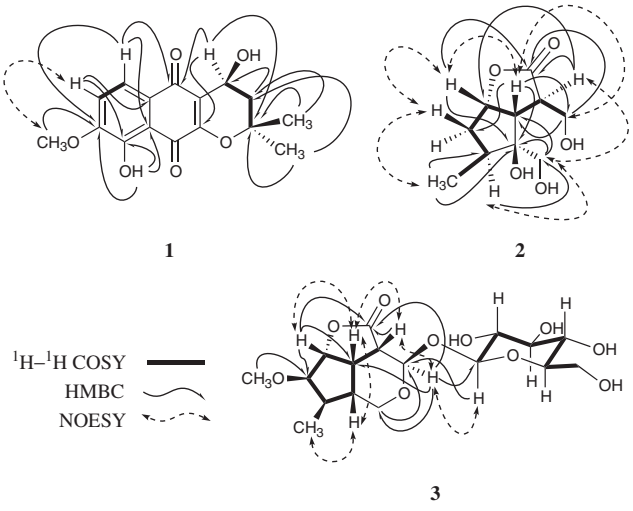


Figure 1. Selected correlations of HMBC, NOESY and ^{1}H–^{1}H COSY of **1-3**.

6β, and H-6β/H_3-8 suggested that the H-4a, H-5, H-6β, 4-CH_2OH, and 7-CH_3 groups oriented on the same side of the planar structure in the β-positions. The observed NOEs from H-1b to H-4 and H-7, from H-7 to H-6α suggested that the H-4, H-6α, H-7, and 7a-CH_2OH were in the β-position as well as 7a-OH was in the β-position. Thus, the structure of **2** was determined to be 7a-hydroxygelsemiol by the above analysis.[5]

The positive ion FABMS of **3** gave a quasimolecular ion peak $[M + Na]^+$ at m/z 413 and the molecular formula was determined to be $C_{17}H_{26}O_{10}$ by HRFABMS (m/z 413.1435, $\Delta + 1.2$ mmu, $[M + Na]^+$). ^{1}H and ^{13}C NMR spectral data of **3** revealed the presence of one ester carbonyl, twelve sp^3 methine (eight of which were oxygen-bearing), two sp^3 oxygen-bearing methylene, one methyl, and one methoxyl groups. The initial analysis of the NMR spectral data indicated that the molecule consist of a monoterpene iridoid and a sugar moiety. Comparing the ^{1}H and ^{13}C NMR spectral data with those of semperoside,[6] we saw almost complete coincidence for all the signals, except for one methoxyl group (δ_C 49.85; δ 3.29, 3H, s) which was assigned to the C-6 (δ_C 77.78) by the correlation of C-6/6-OCH_3 in the HMBC spectrum (Figure 1). Thus, the structure of **3** was determined to be 6-methoxysemperoside.[7] Stereochemistry of **3** was determined as shown by the NOESY spectrum and the analysis of coupling constants. H-4, H-4a, H-5, H-7a, and 7-CH_3 were in the β-position, because of the presence of cross-peaks of H-4/H-4a, H-4a/H-5, H-4a/H-7a, and H-7a/7-CH_3. The β-position of 6-OCH_3 was assigned by the correlation of H-6/H-7 in the NOESY spectrum. The configuration at C-3 had the oxygen in the β-position, with the proton α, like other iridoids including semperoside,[6] from the detailed analysis of the molecular model of **3** on the basis of following observation; 1) H-3 appeared as a singlet, so the angle between H-3 and H-4 should be near 90°, 2) there were NOEs between H-3 and H-4 and between H-1 and H-1′, but not NOEs of H-3/H-4a and H-3/H-1α (H-1β).

The ability of **1–3** to enhance NGF's effects for stimulating neurite outgrowth from PC12D cells was assessed utilizing methodology previously reported.[8] **1** (3, 10, and 30 μM) and **2** (3 μM) did not induced neurite outgrowth from PC12D cells in the absence of NGF, but increased the NGF-induced proportion (2 ng/mL, 25%) of neurite-bearing cells by 30.2%, 34.2%, 40.05 and 40.3%, respectively. Increasing the concentrations of **2** from 3 μM to 10 or 30 μM showed the cytotoxicity on PC12D cells.

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References and Notes

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3 H. Itokawa, K. Matsumoto, H. Morita, and K. Takeya, *Phytochemistry*, **31**, 1061 (1992).
4 **1**: mp 151-153 °C; $[\alpha]^{30}_D$ −40.9° ($c = 0.13$, $CHCl_3$); UV (MeOH) λ_{max} 211 ($\log \varepsilon$, 4.2), 227 (4.0), 262 (2.8), 301 (2.4), 433 (2.1) nm; IR (neat) 3600-3300 (br, OH), 1640, 1610, 1460 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.16 (s, 9-OH), 7.63 (1H, d, $J = 8.5$ Hz, H-6), 7.06 (1H, d, $J = 8.5$ Hz, H-7), 4.93 (1H, t, $J = 6.5$ Hz, H-4), 3.97 (3H, s, 8-OCH_3), 3.83 (s, 4-OH), 2.11 (1H, dd, $J = 14.5$, 6.0 Hz, H-3α), 2.01 (1H, dd, $J = 14.5$, 6.5 Hz, H-3β), 1.54 (3H, s, H_3-12), 1.43 (3H, s, H_3-11); ^{13}C NMR (150 MHz, $CDCl_3$) δ 185.40 (s, C-10), 184.43 (s, C-5), 153.88 (s, C-8), 153.57 (s, C-10a), 152.63 (s, C-9), 125.47 (s, C-5a), 123.58 (s, C-4a), 120.24 (d, C-6), 115.53 (d, C-7), 114.29 (s, C-9a), 79.83 (s, C-2), 60.12 (d, C-4), 56.40 (q, 8-OCH_3), 39.60 (t, C-3), 27.08 (q, C-12), 26.70 (q, C-11); EIMS m/z 304 $[M]^+$ (51.8), 271 (9.2), 248 (34.5), 220 (43.6), 202 (36.4), 192 (42.0), 151 (10.6), 83(100); HREIMS m/z 304.0905 $[M]^+$ (Calcd for $C_{16}H_{16}O_6$, 304.0945), 248.0297 $[M\text{-}C_4H_8]^+$ (Calcd for $C_{12}H_8O_6$, 248.0320).
5 **2**: mp 143-145 °C; $[\alpha]^{30}_D$ −8.7° ($c = 0.33$, CH_3OH); UV (MeOH) λ_{max} 214 ($\log \varepsilon$, 4.3), 281 (3.5), 310 (sh.) nm; IR (neat) 3600-3100 (br, OH), 1745 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 4.99 (1H, ddd, $J = 7.2$, 5.4, 1.2 Hz, H-5), 3.88 (1H, dd, $J = 10.8$, 4.2 Hz, H-3a), 3.86 (1H, dd, $J = 10.8$, 4.8 Hz, H-3b), 3.64 (1H, d, $J = 12.0$ Hz, H-1a), 3.61 (1H, d, $J = 12.0$ Hz, H-1b), 2.96 (1H, dd, $J = 7.2$, 6.0 Hz, H-4a), 2.85 (1H, ddd, $J = 6.0$, 4.8, 4.2 Hz, H-4), 1.98 (1H, m, H-7), 1.95 (1H, ddd, $J = 14.4$, 5.4, 1.2 Hz, H-6β), 1.92 (1H, dd, $J = 14.4$, 5.4 Hz, H-6α), 0.94 (3H, d, $J = 6.6$ Hz, H_3-8); ^{13}C NMR (150 MHz, CD_3OD) δ 181.41 (s, C-9), 85.32 (s, C-7a), 85.08 (d, C-5), 66.27 (t, C-1), 63.88 (t, C-3), 54.86 (d, C-4a), 47.40 (d, C-4), 40.67 (t, C-6), 38.08 (d, C-7), 12.68 (q, C-8); FABMS m/z 239 $[M+Na]^+$ (12), 217 $[M+H]^+$ (100), 185 (30), 181 (19), 93 (86); HRFABMS m/z 217.1088 $[M+H]^+$ (Calcd for $C_{10}H_{17}O_5$, 217.1076).
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7 **3**: mp 138-140 °C; $[\alpha]^{30}_D$ +29.1° ($c = 0.30$, CH_3OH); UV (MeOH) λ_{max} 206 ($\log \varepsilon$, 3.8), 277 (1.9) nm; IR (neat) 3600-3300 (br, OH), 1760 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 5.30 (1H, s, H-3), 4.68 (1H, d, $J = 6.5$ Hz, H-5), 4.46 (1H, d, $J = 8.0$ Hz, H-1′), 4.39 (1H, dd, $J = 12.5$, 3.5 Hz, H-1β), 3.93 (1H, d, $J = 3.0$ Hz, H-6), 3.80 (1H, dd, $J = 12.0$, 2.0 Hz, H-6′b), 3.65 (1H, dd, $J = 12.0$, 5.0 Hz, H-6′a), 3.44 (1H, d, $J = 12.5$ Hz, H-1α), 3.34 (1H, t, $J = 9.0$ Hz, H-3′), 3.30 (1H, ddd, $J = 9.0$, 5.0, 2.0 Hz, H-5′), 3.29 (3H, s, 6-OCH_3), 3.28 (1H, t, $J = 9.0$ Hz, H-4′), 3.25 (1H, dd, $J = 11.0$, 6.5 Hz, H-4a), 3.18 (1H, dd, $J = 9.0$, 8.0 Hz, H-2′), 2.97 (1H, d, $J = 11.0$ Hz, H-4), 1.82 (1H, m, H-7), 1.75 (1H, ddd, $J = 13.0$, 12.0, 4.5 Hz, H-7a), 0.98 (3H, d, $J = 6.5$ Hz, H_3-8); ^{13}C NMR (125 MHz, CD_3OD) δ 177.65 (s, C-9), 103.97 (d, C-1′), 98.66 (d, C-3), 88.28 (d, C-5), 78.33 (d, C-5′), 77.99 (d, C-3′), 77.78 (d, C-6), 75.35 (d, C-2′), 71.33 (d, C-4′), 62.62 (t, C-6′), 57.45 (t, C-1), 49.85 (q, 6-OCH_3), 41.97 (d, C-4), 38.57 (d, C-7a), 38.11 (d, C-7), 35.64 (d, C-4a), 11.02 (q, C-8); FABMS m/z 413 $[M+Na]^+$ (20), 391 $[M+H]^+$ (5), 304 (10), 282 (50), 256(13), 197 (32), 155 (73), 136 (78), 107 (47); HRFABMS m/z 413.1435 $[M+Na]^+$ (Calcd for $C_{17}H_{26}O_{10}Na$, 413.1424), 197.0842 $[C_{10}H_{13}O_4]^+$ (Calcd for 197.0814).
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