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SUPPLEMENTARY MATERIAL TO

# An isoflavane and saponins from *Astragalus depressus* L.

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## ISOLATION OF THE FRACTIONS

7 g of ethyl acetate extract were subjected to vacuum liquid chromatography (VLC) using silica gel as the stationary phase (5 cm×5 cm; fractions of 100 mL). The elution was performed with a gradient of solvents $CHCl_3$–MeOH (100:0, 99:1, 95:5, 90:10, 80:20 and 70:30 *V*/*V*) and then $CHCl_3$–MeOH–$H_2O$ (70:30:1, 70:30:3, 70:30:5, 70:30:7, 60:40:7 and 50:50:1 *V*/*V*/*V*). Fractions having similar TLC profiles were pooled to give 10 fractions (F1––F10). Fraction F4 (1.3 g) was applied to silica gel CC (70 cm×5 cm, fractions of 50 mL) and eluted with $CHCl_3$–MeOH (100:0, 99.5:0.5, 99:1, 95:5, 93:7, 90:10 and 80:20 *V*/*V*) to obtain 8 fractions. The fractions (175 mg) eluted with $CHCl_3$–MeOH (99:1 and 95:5 *V*/*V*) were purified on Sephadex LH-20 CC (40 cm×2.5 cm, fractions of 30 mL), eluted with $CHCl_3$ to provide two compounds **1** (15 mg) and **4** (5 mg). Fraction F5 (1.34 g) was chromatographed over silica gel column (70 cm×5 cm, fractions of 50 mL) using a $CHCl_3$–MeOH gradient system as for F4 providing 15 fractions. Fractions (130 mg) eluted with $CHCl_3$–MeOH (95:5 *V*/*V*) were purified on Sephadex LH-20 CC (40 cm×2.5 cm, fractions of 30 mL) using $CHCl_3$ as eluent to give 7 mg of **3**. The fraction (34 mg) eluted with $CHCl_3$–MeOH (95:5 *V*/*V*) was purified by silica gel column chromatography (30 cm×2.5 cm, fractions of 20 mL) eluted with $CHCl_3$–MeOH (100:0, 99:1, 95:5 and 93:7 *V*/*V*) to yield pure compound **6** (4 mg). Fraction (38 mg) eluted with $CHCl_3$–MeOH (95:5 *V*/*V*) was chromatographed through a silica gel $SiO_2$ column (30 cm×2.5 cm, fractions of 20 mL) using a gradient of $CHCl_3$–MeOH (97:3, 96:4, 95:5, 94:6 and 93:7 *V*/*V*) to give **2** (5.8 mg). Fraction F7 (614 mg) was subjected to silica gel CC (50 cm×3 cm, fractions of 30 mL) eluting with $CHCl_3$–MeOH (100:0, 99.5:0.5, 99:1, 95:5 and 90:10 *V*/*V*) to give 5 fractions. Fractions (25 mg) eluted with $CHCl_3$–MeOH (90:10 *V*/*V*) were purified by preparative TLC using $CH_2Cl_2$–MeOH (75:25) yielding 2 mg of **5**.

The *n*-butanol extract (8 g) was subjected to reversed phase RP-18 vacuum liquid chromatography (VLC) using a gradient of solvents water–methanol (80:20, 60:40, 20:80 and 0:100 *V*/*V*). Fractions having similar TLC profiles were pooled to afford 10 fractions (F1 to F10). Fraction F4 (545 mg) was subjected to silica gel CC (50 cm×3 cm, fractions of 30 mL)

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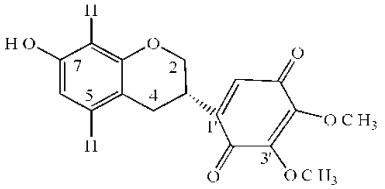
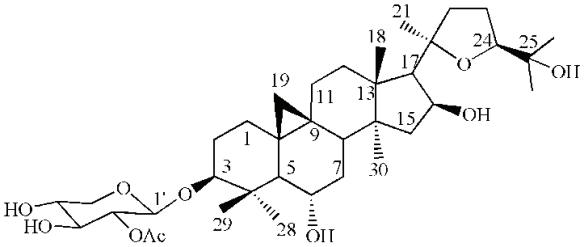
and eluted with $CHCl_3$–MeOH (100:0, 99:1, 98:2, 97:3, 95:5, 90:10, 80:20, 70:30 and 60:40 *V*/*V*) to provide 20 fractions. Fractions (50 mg) eluted with $CHCl_3$–MeOH (95:5 and 90:10 *V*/*V*) were precipitated in acetone to obtain 10 mg of **7**. The fraction (26.6 mg) eluted with $CHCl_3$–MeOH (60:40 *V*/*V*) was purified on TLC RP-18 using MeOH–$H_2O$ (80:20 *V*/*V*) as eluent, which enabled the isolation of **9** (5 mg). Fraction F5 (330 mg) was applied to silica gel CC (50 cm×3 cm, fractions of 30 mL) eluting with $CHCl_3$–MeOH–$H_2O$ (90:10:0.5, 90:10:1, 80:20:1, 80:20:2, 70:30:2 and 70:30:3 *V*/*V*/*V*) to give 3 mg of **8**.

CHARACTERIZATION DATA FOR COMPOUNDS **1** AND **2**



Structure of pendulone (**1**)



Structure of cyclogaleginoside A (**2**)

*Pendulone (**1**)*. Red powder; $^1$H-NMR (600 MHz, $CDCl_3$, $\delta$ / ppm): 2.72 (1H, *dd*, *J* = 16.0, 6.2 Hz, H-4b), 3.05 (1H, *dd*, *J* = 16.0, 6.2 Hz, H-4a), 3.45 (1H, *qd*, *J* = 6.2, 2.5 Hz, H-3 ), 4.04 (6H, *s*, $OCH_3$), 4.07 (1H, *dd*, *J* = 10.6; 6.2 Hz, H-2b), 4.27 (1H, *dd*, *J* = 10.6, 2.5 Hz, H-2a), 4.97 (1H, *br s*, 7-OH), 6.34 (1H, *d*, *J* = 2.5 Hz, H-8), 6.39 (1H, *s*, H-6′), 6.43 (1H, *dd*, *J* = 8.3, 2.5 Hz, H-6), 6.93 (1H, *d*, *J* = 8.3 Hz, H-5); $^{13}$C-NMR (150 MHz, $CDCl_3$, $\delta$ / ppm): 28.9 ($CH_2$, C-4), 30.8 (CH, C-3), 61.2 ($CH_3$, 4′-OMe), 61.4 ($CH_3$, 3′-OMe), 68.1 ($CH_2$, C-2), 103.4 (CH, C-8), 108.8 (CH, C-6), 112.3 (C, C-4a), 130.4 (CH, C-5), 131.0 (CH, C-6′), 144.6 (C, C-4′), 145.1 (C, C-3′), 146.6 (C, C-1′), 154.7 (C, C-8a), 155.2 (C, C-7), 183.5 (C, C-2′), 184.1 (C, C-5′); ESI-MS (*m/z* (relative abundance, %)): 339 ($(M+Na)^+$, 100); optical rotation, $\alpha$ (589 nm, 20 °C, 10 g $dm^{-3}$ in MeOH, 10 cm): –70.3°.

*Cyclogaleginoside A (**2**)*. Amorphous powder; $^1$H-NMR (600 MHz, $CD_3OD$, $\delta$ / ppm): 0.28 (1H, *d*, *J* = 4.3 Hz, H-19exo), 0.60 (1H, *d*, *J* = 4.3 Hz, H-19endo), 0.93 (3H, *s*, H-29), 1.02 (3H, *s*, H-30), 1.14 (3H, *s*, H-26), 1.22 (2H, *m*, H-1b, H-11b), 1.22 (3H, *s*, H-28), 1.23 (3H, *s*, H-21), 1.28 (3H, *s*, H-27), 1.29 (3H, *s*, H-18), 1.37 (2H, *m*, H-5, H-15b), 1.38 (1H, *m*, H-7b), 1.48 (1H, *dt*, *J* = 12.1, 4.1 Hz, H-7a), 1.56 (1H, *td*, *J* = 13.2, 2.4 Hz, H-1a), 1.65 (2H, *m*, H-2b, H-22b), 1.68 (2H, *m*, H-12a, H-12b), 1.82 (1H, *dd*, *J* = 12.1, 4.1 Hz, H-8), 1.96 (1H, *m*, H-2a), 1.97 (1H, *dd*, *J* = 12.9, 7.8 Hz, H-15a), 2.04 (1H, *m*, H-11a), 2.05 (2H, *m*, H-23a, H-23b), 2.38 (1H,*d*, *J* = 7.8 Hz, H-17), 2.63 (1H, *q*, *J* = 10.7 Hz, H-22a), 3.17 (1H, *dd*, *J* = 11.6, 4.5 Hz, H-3), 3.22 (1H, *t*, *J* = 10.7 Hz, H-5′b), 3.45 (1H, *td*, *J* = 9.6, 4.1 Hz, H-6), 3.46 (1H, *t*, *J* = 9.3 Hz, H-3′), 3.56 (1H, *ddd*, *J* = 10.7, 9.3, 5.4 Hz, H-4′), 3.78 (1H, *dd*, *J* = 8.3; 6.0 Hz, H-24), 3.86 (1H, *dd*, *J* = 10.7, 5.4

Hz, H-5′a), 4.42 (1H, *d*, *J* = 7.9 Hz, H-1′), 4.68 (1H, *q*, *J* = 7.8 Hz, H-16), 4.73 (1H, *dd*, *J* = 9.3, 7.9 Hz, H-2′), additional signal: 2.10 (3H, *s*, OAc); $^{13}$C-NMR (150 MHz, $CD_3OD$, $\delta$ / ppm): 15.0 ($CH_3$, C-29), 19.1 ($CH_3$, C-30), 20.4 (C, C-9), 20.6 ($CH_3$, C-18), 25.2 ($CH_3$, C-26), 25.4 ($CH_2$, C-11), 25.5 ($CH_2$, C-23), 26.2 ($CH_3$, C-27), 27.1 ($CH_3$, C-21), 27.2 ($CH_3$, C-28), 29.0 ($CH_2$, C-2), 29.2 (C, C-10), 30.8 ($CH_2$, C-19), 31.7 ($CH_2$, C-1), 32.5 ($CH_2$, C-12), 34.1 ($CH_2$, C-22), 37.6 ($CH_2$, C-7), 41.4 (C, C-4), 44.5 (C, C-13), 45.4 ($CH_2$, C-15), 45.6 (C, C-14), 47.3 (CH, C-8), 53.0 (CH, C-5), 57.7 (CH, C-17), 65.5 ($CH_2$, C-5′), 68.1 (CH, C-6), 69.9 (CH, C-4′), 71.0 (C, C-25), 73.2 (CH, C-16), 74.3 (CH, C-2′), 74.8 (CH, C-3′), 81.2 (CH, C-24), 86.9 (CH, C-20), 88.6 (CH, C-3), 103.8 (CH, C-1′), additional signals: 19.8 ($CH_3$, OAc), 170.4 (C, OAc); ESI-MS (*m/z* (relative abundance, %)): 687 ($(M+Na)^+$, 100); Optical rotation, $\alpha$ (589 nm, 20 °C, 10 g $dm^{-3}$ in MeOH, 10 cm): +36.1°.