CHEMICAL INVESTIGATION OF *Ervatamia yunnanensis*

Jing-Ling Du, Yong-Sheng Jin*, Li-Ming Qiao, Li Jin, and Hai-Sheng Chen*

UDC 547.972

The study of *Ervatamia yunnanensis* Tsiang, growing in Yunnan and Guangxi provinces of China, has focused on the isolation of alkaloids [1–6], which are demonstrated to have significant anti-addictive, anti-malarial, anti-reproductive, and anticancer activities [7, 8]. About 30 indole alkaloids have been isolated from *E. yunnanensis* Tsiang. Continuing our investigations into the biologically active constituents of nonalkaloid parts of *Ervatamia yunnanensis* by chromatographic and spectroscopic methods, we have isolated two lignans (**1** and **2**), five flavonoids (**6**, **9**, **11**, **13**, and **14**), two triterpenoids (**7** and **8**), three organic or fatty acids and derivatives (**3**, **4**, and **12**), vanillin (**10**), and one aromatic ketone (**5**).

The plant material was collected in May 2003 at Xishuangbanna, Yunnan province and identified as the stems of *Ervatamia yunnanensis* Tsiang by Senior Engineer Wang Hong, Xishuanbanna Tropical Plant Garden of the Chinese Academy of Sciences. The air-dried and powdered stems and branches (5 kg) were extracted with 95% EtOH (10 L) by reflux. The percolate was evaporated in vacuum to yield the EtOH extract (425 g), which was suspended in 2% HCl solution. The precipitate in the lower layer after centrifugation, which was deplete of alkaloids, was dissolved in distilled water; then the solution was chromatographed over macroporous adsorptive resin AB-8, eluting with a gradient mixture of water and alcohol. The compositions of fractions were analyzed by TLC. Similar fractions were combined to afford fraction A, 60.0 g (30% alcohol); fraction B, 40.0 g (60% alcohol), and fraction C, 15.0 g (90% alcohol). Fractions A, B, and C were subjected to repeated chromatography on silica-gel columns eluting with a gradient mixture of petroleum ether–ethyl acetate or chloroform–methanol, Sephadex LH-20 eluting with chloroform–methanol, methanol, or a gradient mixture of methanol–water, and C-18 reverse silica-gel columns eluting with a gradient mixture of methanol–water. A total of fourteen compounds (**1–14**) was isolated and identified based on NMR and mass spectrum, and by direct comparison with authentic samples and the literature. All the identified compounds are known compounds and are reported for the first time from *Ervatamia yunnanensis.*

(+)-Isolariciresinol-9-*O*-β-D-glucopyranoside (1), white crystalline powder, $C_{26}H_{34}O_{11}$, ESI-MS: *m/z* 521 $[M - H]^-$, 545 $[M + Na]^+$. ^{1}H NMR (300 MHz, CD_3OH, δ, ppm, J/Hz): 3.81, 3.80 (each 3H, $OCH_3 \times 2$), 4.10 (1H, d, J = 7.8, H-Glu-1), 6.17 (1H, s, H-5′), 6.63 (1H, dd, J = 8.1, 1.8, H-6), 6.64 (1H, s, H-2′), 6.73 (1H, d, J = 8.1, H-5), 6.79 (1H, d, J = 1.8, H-2).

^{13}C NMR (75 MHz, CD_3OH, δ): 148.9 (C-3), 147.2 (C-3′), 145.9 (C-4), 145.2 (C-4′), 38.7 (C-1), 134.4 (C-6′), 129.2 (C-1′), 123.2 (C-6), 117.4 (C-5′), 116.1 (C-5), 114.4 (C-2), 112.4 (C-2′), 69.5 (C-9), 65.2 (C-9′), 48.4 (C-7), 45.9 (C-8), 39.6 (C-8′), 33.9 (C-7′), 105.2 (Glc-1), 78.2 (Glc-5), 77.9 (Glc-3), 75.2 (Glc-2), 71.7 (Glc-4), 62.8 (Glc-6) [9].

Isolariciresinol (2), white powder, $C_{17}H_{26}O_{10}$, ESI-MS: *m/z* 383 $[M + Na]^+$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 1.78 (1H, m, H-2), 2.00 (1H, m, H-3), 2.75 (2H, d, J = 8.0, H-4), 3.39 (1H, dd, J = 4.0, 12.0, H-9b), 3.63–3.84 (10H, m, H-1, 10, H-9a, $2 \times OCH_3$), 6.20 (1H, s, H-8), 6.61 (1H, dd, J = 9.0, 2.4, H-6′), 6.63 (1H, s, H-5), 6.68 (1H, d, J = 2.4, H-2′), 6.75 (1H, d, J = 9.0, H-5′).

^{13}C NMR (150 MHz, CD_3OD, δ): 112.2 (C-5), 128.7 (C-5α), 115.8 (C-5′), 148.7 (C-6), 123.1 (C-6′), 145.7 (C-7), 134.0 (C-1′), 117.3 (C-8), 138.5 (C-8α), 113.8 (C-2′), 147.2 (C-3′), 145.3 (C-4′), 65.6 (C-10), 61.8 (C-9), 56.2, 56.2 ($2 \times OCH_3$), 48.1 (C-2), 47.7 (C-1), 39.7 (C-3), 33.5 (C-4) [10].

Berchemolide (3), white powder, $C_{28}H_{32}O_{16}$, ESI-MS: *m/z* 647 $[M + Na]^+$, 1271 $[2M + Na]^+$. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.20–4.50 (H-Glu), 3.78 (6H, s, $OCH_3 \times 2$), 5.29 (2H, d, J = 7.2, H-1′, 1‴), 7.36 (2H, d, J = 8.4, H-5, 5″), 7.40 (2H, d, J = 1.8, H-2, 2″), 7.74 (2H, dd, J = 1.8, 8.4, H-6, 6″).

School of Pharmacy, Second Military Medical University, e-mail: chenhaishengsmmu@yahoo.com.cn; yshjin@yahoo.com.cn. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 388–389, May–June, 2010. Original article submitted November 24, 2008.

0009-3130/10/4603-0459

^{13}C NMR (150 MHz, DMSO-d_6, δ): 165.1 (C-7, 7″), 149.9 (C-4, 4″), 148.5 (C-3, 3″), 123.0 (C-1, 1″), 122.6 (C-6, 6″), 114.5 (C-5, 5″), 112.3 (C-2, 2″), 98.4 (C-1′, 1‴), 77.0 (C-3′, 3‴), 3.5 (C-5′, 5‴), 72.9 (C-2′, 2‴), 70.6 (C-4′, 4‴), 65.0 (C-6′, 6‴), 55.6 ($OCH_3 \times 2$) [11].

Hexatriacontanoic acid (4), white candle solid, $C_{36}H_{72}O_2$, ESI-MS: *m/z* 559 $[M + Na]^+$.

3-Hydroxy-1-(3-hydroxy-4-methoxyphenyl)propan-1-one (5), colorless solitary crystal, $C_{10}H_{12}O_4$, ESI-MS: *m/z* 219 $[M + Na]^+$, 415 $[2M + Na]^+$. ^{1}H NMR (600 MHz, DMSO, δ): 3.02 (2H, t, J = 6.6, H-8), 3.74 (2H, t, J = 6.6, H-9), 3.79 (3H, s, OCH_3), 6.84 (1H, d, J = 8.4, H-6), 7.40 (1H, d, J = 1.8, H-2), 7.47 (1H, dd, J = 1.8, 8.4, H-5).

^{13}C NMR (150 MHz, DMSO-d_6, δ): 197.1 (C-7), 153.6 (C-4), 147.9 (C-3), 127.8 (C-1), 123.5 (C-6), 115.2 (C-5), 111.1 (C-2), 55.6 (-OCH_3), 57.4 (C-9), 40.9 (C-8).

5,3′4′-Trimethoxyflavone-7-*O*-glucopyranoside (6), pale-yellow powder, $C_{24}H_{26}O_{11}$, ESI-MS: *m/z* 513 $[M + Na]^+$. ^{13}C NMR (75 MHz, DMSO-d_6, δ): 163.2 (C-2), 103.1 (C-3), 181.5 (C-4), 158.2 (C-5), 98.1 (C-6), 165.8 (C-7), 93.2 (C-8), 162.9 (C-9), 103.1 (C-10), 122.6 (C-1′), 110.3 (C-2′), 149.7 (C-3′), 152.4 (C-4′), 118.8 (C-5′), 120.3 (C-6′), 101.2 (C-Glu-1), 56.3, 57.1, 55.9 ($OCH_3 \times 3$) [12].

Cycloartenol (7), white needle crystals from petroleum ether, $C_{26}H_{34}O_{11}$, EI-MS (*m/z*, %): 426 (43.15), 408 (82.18), 393 (100), 339 (47.71), 315 (6.23), 297 (9.42), 286 (74.26), 271 (41.36), 175 (79.48), 111 (40.36), 69 (28.67). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.26 (1H, d, J = 4.2), 0.53 (1H, d, J = 4.2), 0.63 (3H, s, H-29), 0.87 (3H, s, H-30), 0.88 (3H, d, J = 6.5, H-21), 0.89 (3H, s, H-18), 0.96 (3H, s, H-28), 1.55 (3H, s, H-26), 1.65 (3H, s, H-27), 1.92 (2H, m, H-23), 3.28 (1H, dd, J = 4.3, 10.2, 3α-H), 5.08 (1H, t, J = 7.1, H-24).

^{13}C NMR (125 MHz, DMSO-d_6, δ): 13.9 (C-29); 17.1 (C-26); 17.4 (C-18); 17.8 (C-21) 18.7 (C-30); 19.1 (C-9); 20.3 (C-6); 24.1 (C-23); 25.0 (C-27); 25.3 (C-28); 25.6 (C-7); 25.7 (C-10); 27.3 (C-16); 28.8 (C-19); 29.9 (C-2); 30.0 (C-11); 31.2 (C-1); 32.3 (C-12); 34.8 (C-15); 35.0 (C-20); 35.6 (C-22); 38.9 (C-4); 44.6 (C-13); 46.4 (C-8); 46.9 (C-5); 48.1 (C-14); 51.4 (C-17); 76.5 (C-3); 124.7 (C-24) 129.9 (C-25) [13].

***β*-Amyrin acetate (8)**, white needle crystals from ethyl acetate, $C_{32}H_{52}O_2$, ESI-MS: *m/z* 491 $[M + Na]^+$, 507 $[M + K]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82 (3H, s, H-28), 0.86 (3H, s, H-23), 0.98 (3H, s, H-30), 0.99 (3H, s, H-29), 1.01 (3H, s, H-24), 1.06 (3H, s, H-25), 1.12 (3H, s, H-26), 1.15 (3H, s, H-27), 2.04 (3H, s, CH_3CO), 4.53 (1H, dt, J = 11.4, 1.8, H-3), 5.18 (1H, dt, J = 16.8, 4.2, H-12) [14].

7-Hydroxy-4′-methoxyisoflavone (9), pale-yellow powder, $C_{16}H_{12}O_4$, ESI-MS: *m/z* 291 $[M + Na]^+$. Identification of compound **9** was performed by ^{13}C NMR data compared with those reported in [15].

Vanillin (10), colorless crystal, $C_8H_8O_3$, ESI-MS: *m/z* 153 $[M + H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 3.84 (3H, s, OCH_3), 6.91 (1H, m, H-2), 7.36 (1H, m, H-6, 5), 9.70 (1H, s, CHO).

^{13}C NMR (150 MHz, $CDCl_3$, δ): 192.9 (CHO), 154.8 (C-4), 149.6 (C-3), 130.4 (C-1), 127.9 (C-5), 116.3 (C-2), 111.27 (C-6), 56.3 (OCH_3).

4′,7-Dihydroxy-3′-methoxyisoflavone (11), nankeen powder, $C_{16}H_{12}O_5$, ESI-MS: *m/z* 307 $[M + Na]^+$. Identification of compound **11** was performed by ^{13}C NMR data compared with those reported in [16].

Ethyl 4-hydroxy-3-methoxybenzoate (12), nankeen crystal, $C_{10}H_{12}O_4$, ESI-MS: *m/z* 197 $[M + H]^+$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 1.36 (3H, t, J = 7.2, CH_3), 3.89 (3H, s, OCH_3), 4.31 (2H, q, J = 7.2, OCH_2), 6.84 (1H, d, J = 9.0, H-2), 7.53 (1H, d, J = 1.8, H-5), 7.54 (1H, dd, J = 1.8, 9.0, H-6).

^{13}C NMR (150 MHz, CD_3OD, δ): 152.8 (C-4), 148.8 (C-3), 125.0 (C-1), 122.9 (C-6), 116.0 (C-2), 113.7 (C-6), 168.1 (COOR), 56.6 (OCH_3), 61.9, 14.7 (OCH_2CH_3).

5,3′-Dihydroxy-4′-methoxyflavanone-7-*O*-*β*-D-rhamnopyranosidyl(1→6)-*β*-D-glucopyranoside (13), nankeen amorphous powder, $C_{28}H_{34}O_{15}$, ESI-MS: *m/z* 633 $[M + Na]^+$, 1243 $[2M + Na]^+$. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.79 (1H, m, H-3), 3.22 (1H, m, H-3), 3.30 (1H, m, H-2″); 3.45(1H, m, H-3″); 3.41(1H, m, H-4″); 3.77 (3H, s, 4′-OCH_3), 3.92 (1H, m, H-5″); 4.22 (1H, m, H-6″b); 4.35 (1H, d, J = 6.0, H-1‴); 4.59 (1H, m, H-6″a); 5.22 (1H, d, J = 7.2, H-1″), 5.49 (1H, m, H-2), 6.12 (1H, d, J = 2.4, H-2′), 6.13 (1H, d, J = 2.4, H-6), 6.89 (1H, H-5′), 6.93 (1H, s, H-8), 6.93 (1H, s, H-6′), 7.82 (1H, d, J = 9.0, H-2‴); 9.29 (1H, s, 3′-OH), 11.98 (1H, s, 5-OH).

^{13}C NMR (150 MHz, DMSO-d_6, δ): 17.7 (C-6‴); 42.0 (C-3); 55.7 (4′-OCH_3); 66.0 (C-6″) 68.2 (C-3‴); 70.0 (C-5‴); 70.2 (C-2‴); 70.7 (C-4″); 72.0 (C-2″); 72.9 (C-4‴); 75.5 (C-5″); 76.2 (C-3″); 78.2 (C-2); 95.5 (C-8); 96.3 (C-6); 99.4 (C-1″); 100.5 (C-1‴); 103.3 (C-10); 112.1 (C-5′); 114.1 (C-2′); 117.8 (C-6′); 130.9 (C-1′); 147.9 (C-4′); 162.3 (C-9); 163.0 (C-5); 165.1 (C-7); 196.8 (C-4) [17].

Chrysoeriol-3′-*O*-β-D-apifuranosidyl(1→2)-glucopyranoside (14), white powder, $C_{20}H_{22}N_2O$, ESI-MS: *m/z* 309 $[M + H]^+$. ^1H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 0.88 (3H, t, J = 7.2, H-18); 1.48 (overlapped, H-15); 1.50 (overlapped, H-6); 1.50 (overlapped, H-20); 1.51 (overlapped, H-19); 2.07 (1H, s , H-14); 2.21 (1H, m, H-6b); 2.62 (1H, m, H-17b); 2.84 (1H, dd, J = 4.8, 10.8, H-7); 2.84 (1H, dd, J = 4.8, 10.8, H-16); 2.97 (1H, s, H-21); 3.07 (1H, m, H-5a); 3.19 (1H, m, H-17a); 3.54 (1H, dt, J = 3.0, 14.4, H-5); 3.82 (3H, s, OCH_3); 4.49 (1H, d, J = 3.0, H-3); 6.64 (1H, dd, J = 2.4, 8.4, H-11); 6.86 (1H, d, J = 2.4, H-10); 7.08 (1H, d, J = 9.0, H-12).

^{13}C NMR (150 MHz, CD_3OD,): 12.3 (C-18); 22.6 (C-17); 25.5 (C-15); 28.1 (C-19); 30.4 (C-14); 34.0 (C-6); 41.1 (C-16); 42.8 (C-20); 55.1 (C-5); 56.5 (C-OCH_3); 60.2 (C-21); 93.2 (C-3); 101.1 (C-9); 108.8 (C-7); 111.1 (C-11); 111.9 (C-12); 131.2 (C-8); 131.6 (C-13); 144.5 (C-2); 155.0 (C-10) [18].

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (No. 20272081 and 20872178) and Shanghai Leading Academic Project (No. B906).

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