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CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Nelumbo nucifera* Gaertn. cv. *Rosa-plena*

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Nelumbo nucifera Gaertn. cv. *Rosa-plena* is a perennial aquatic crop grown and consumed throughout Asia. All parts of *N. nucifera* have been used for various medicinal purposes in oriental medicine. In particular, the leaves are known for diuretic and astringent properties, and are used to treat fever, sweating, and strangury and as a styptic [1]. To further understand the chemotaxonomy of the *Nelumbo* species [1, 2], *Nelumbo nucifera* Gaertn. cv. *Rosa-plena* was chosen for phytochemical investigation. There are no publications reported concerning the chemical components of this plant yet. The compounds derived from the leaves include seven aporphines: (–)-nuciferine (**1**) [2], (–)-nornuciferine (**2**) [4], (–)-asimilobine (**3**) [3], (–)-*N*-methylasimilobine (**4**) [3], (–)-caaverine (**5**) [5], (–)-anonaine (**6**) [4], and (–)-roemerine (**7**) [2]; two oxoaporphines: lysicamine (**8**) [4] and liriodenine (**9**) [4]; one dioxoaporphine – cepharadione B (**10**) [6]; one dehydroaporphine – 7-hydroxydehydronuciferine (**11**) [7]; two steroids: β-sitostenone (**12**) [3] and stigmasta-4,22-dien-3-one (**13**) [3]; two chlorophyll: pheophytin-a (**14**) [8] and aristophyll-C (**15**) [8]. Some of the isolated components from *Nelumbo* not yet published include (–)-caaverine (**5**), cepharadione B (**10**), and 7-hydroxydehydronuciferine (**11**).

The leaves of *Nelumbo nucifera* Gaertn. cv. *Rosa-plena* were collected from Tainan County, Taiwan, November 2008. The plant material was identified by Dr. Fu-Yuan Lu (Department of Forestry and Natural Resources, College of Agriculture, National Chiayi University). A voucher specimen (*Nelumbo nucifera* Gaertn. cv. *Rosa-plena*) was deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung County, Taiwan. The air-dried leaves of *Nelumbo nucifera* Gaertn. cv. *Rosa-plena* (1.5 kg) were extracted with MeOH (50 L × 5) at room temperature, and a MeOH extract (108.7 g) was obtained upon concentration under reduced pressure. The MeOH extract, suspended in H_2O (1 L), was partitioned with $CHCl_3$ (3 L × 4) to give fractions soluble in $CHCl_3$ (57.2 g) and H_2O (43.6 g). The $CHCl_3$-soluble fraction was chromatographed over silica gel (1700 g, 70–230 mesh) using *n*-hexane–EtOAc–MeOH mixtures as eluents to produce six fractions. Part of fraction 3 (7.83 g) was subjected to silica gel chromatography by eluting with *n*-hexane–acetone (7:1) and enriched gradually with acetone to furnish two fractions (3-1–3-2). Fraction 3-1 (4.73 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain β-sitostenone (9 mg) and stigmasta-4,22-dien-3-one (8 mg) and pheophytin-a (7 mg). Fraction 3-2 (2.13 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain (–)-caaverine (3.7 mg). Part of fraction 4 (10.27 g) was subjected to silica gel chromatography by eluting with *n*-hexane–acetone (5:1) and enriched with acetone to furnish three further fractions (4-1–4-3). Fraction 4-1 (4.37 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain aristophyll-C (9 mg) and lysicamine (15 mg) and 7-hydroxydehydronuciferine (12 mg) and (–)-nornuciferine (17 mg). Fraction 4-2 (3.05 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain (–)-roemerine (6 mg) and (–)-nuciferine (18 mg) and (–)-anonaine (5 mg) and cepharadione B (15 mg). Fraction 4-3 (2.51 g) was further purified on a silica gel column using *n*-hexane–acetone mixtures to obtain (–)-asimilobine (4 mg) and (–)-*N*-methylasmilobine (16 mg). Part of fraction 5 (5.34 g) was subjected to silica gel chromatography by eluting with CH_2Cl_2–MeOH (40:1) and enriched with MeOH to furnish two fractions (5-1–5-2). Fraction 5-1 (2.53 g) eluted with CH_2Cl_2–MeOH (30:1) was further separated using silica gel column chromatography and preparative TLC (CH_2Cl_2–MeOH (40:1)) and gave liriodenine (3.6 mg).

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(–)-Nuciferine (1) as in [2], $C_{19}H_{21}NO_2$, brown powder (MeOH), mp 165°C, UV (λ_{max}, nm): 230, 274, 312. IR (ν_{max}, cm^{-1}): 1605, 1500, 1250, 1425, 1375. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.65 (3H, s, 1-OCH_3), 3.88 (3H, s, 2-OCH_3), 6.63 (1H, s, H-3), 7.22–7.26 (3H, m, H-8, 9, 10), 8.36 (1H, d, J = 7.6, H-11). EI-MS *m/z* 295 $[M]^+$.

(–)-Nornuciferine (2) as in [4], $C_{18}H_{19}NO_2$, brown powder (MeOH), mp 127–129°C. UV (λ_{max}, nm): 230, 272, 310. IR (ν_{max}, cm^{-1}): 2900, 1590, 1440. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.65 (3H, *s*, 1-OCH_3), 3.89 (3H, s, 2-OCH_3), 6.64 (1H, s, H-3), 7.22–7.30 (3H, m, H-8, 9, 10), 8.36 (1H, d, J = 7.6, H-11). EI-MS *m/z* 281 $[M]^+$.

(–)-Asimilobine (3) as in [3], $C_{17}H_{17}NO_2$, brown powder (MeOH), mp 121–123°C. UV (λ_{max}, nm): 274, 308. IR (ν_{max}, cm^{-1}): 3500. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.57 (3H, s, 2-OCH_3), 6.70 (1H, s, H-3), 7.24–7.28 (3H, m, H-8, 9, 10), 8.26 (1H, d, J = 7.6, H-11). EI-MS *m/z* 267 $[M]^+$.

(–)-*N*-Methylasimilobine (4) as in [3], $C_{18}H_{19}NO_2$, white needles ($CHCl_3$), mp 173–174°C. UV (λ_{max}, nm): 372. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.55 (3H, s, N-CH_3), 3.58 (3H, s, 1-OCH_3), 6.70 (1H, s, H-3), 7.24–7.28 (3H, m, H-8, 9, 10), 8.27 (1H, d, J = 8.0, H-11), EI-MS *m/z* 281 $[M]^+$.

(–)-Caaverine (5) as in [5], $C_{17}H_{17}NO_2$, brown powder (MeOH), mp 204°C. UV (λ_{max}, nm): 231, 271, 310. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.90 (3H, s, 2-OCH_3), 6.66 (1H, s, H-3), 7.24–7.27 (3H, m, H-8, 9, 10), 8.37 (1H, d, J = 8.0, H-11), EI-MS *m/z* 267 $[M]^+$.

(–)-Anonaine (6) as in [4], $C_{17}H_{15}NO_2$, pale yellow powder (MeOH), mp 121–123°C. UV (λ_{max}, nm): 230, 272, 310. IR (ν_{max}, cm^{-1}): 1040, 950. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.65 (1H, t, J = 13.4, H-7a), 2.85 (1H, dd, J = 13.4, 5.2, H-7b), 3.11–3.29 (3H, m, H-4a, 4b, 5a), 3.53 (1H, m, H-5b), 3.98 (1H, dd, J = 13.4, 5.2, H-6a), 5.92 and 6.06 (each 1H, d, J = 1.6, OCH_2O), 6.55 (1H, s, H-3), 7.21–7.30 (3H, m, H-8, 9, 10), 8.06 (1H, d, J = 7.6, H-11), EI-MS *m/z* 265 $[M]^+$.

(–)-Roemerine (7) as in [2], $C_{18}H_{17}NO_2$, pale brown powder (MeOH), mp 98–99°C. UV (λ_{max}, nm): 234, 272, 312. IR (ν_{max}, cm^{-1}): 3420, 1045, 942. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.57 (3H, s, N-CH_3), 5.94, 6.09 (each 1H, d, J = 1.6, OCH_2O), 6.56 (1H, s, H-3), 7.23–7.27 (3H, m, H-8, 9, 10), 8.07 (1H, d, J = 8.0, H-11), EI-MS *m/z* 279 $[M]^+$.

Lysicamine (8) as in [4], $C_{18}H_{13}NO_3$, yellow needles ($CHCl_3$), mp 185–187°C. UV (λ_{max}, nm): 255, 283, 335. IR (ν_{max}, cm^{-1}): 1650. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.04 (3H, s, 1-OCH_3), 4.13 (3H, s, 2-OCH_3), 7.21 (1H, s, H-3), 7.60 (1H, t, J = 8.0, H-9), 7.79 (1H, t, J = 8.0, H-10), 7.84 (1H, d, J = 6.5, H-4), 8.61 (1H, d, J = 8.0, H-8), 8.95 (1H, d, J = 6.5, H-5), 9.21 (1H, d, J = 8.0, H-11), EI-MS *m/z* 291 $[M]^+$.

Liriodenine (9) as in [4], $C_{17}H_9NO_3$, yellow needles ($CHCl_3$), mp 280–282°C. UV (λ_{max}, nm): 248, 265, 308. IR (ν_{max}, cm^{-1}): 1625, 1022, 933. ^1H NMR (400 MHz, $CDCl_3$, δ, J/Hz): 6.37 (2H, s, OCH_2O), 7.16 (1H, s, H-3), 7.55 (1H, td, J = 8.2, 1.6, H-9), 7.74 (1H, td, J = 8.2, 1.6, H-10), 7.76 (1H, d, J = 5.4, H-4), 8.61 (1H, dd, J = 8.2, 1.6, H-8), 8.64 (1H, dd, J = 8.2, 1.6, H-11), 8.89 (1H, d, J = 5.4, H-5), EI-MS *m/z* 275 $[M]^+$.

Cepharadione B (10) as in [6], $C_{20}H_{18}NO_4$, orange prisms (EtOAc), mp 264–266°C. UV (λ_{max}, nm): 213, 240, 301, 315, 440. IR (ν_{max}, cm^{-1}): 1665, 1647. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.90 (3H, s, N-CH_3), 4.12, 4.15 (each 3H, s, OCH_3-2, 3), 7.55 (1H, s, H-7), 7.67 (2H, m, H-9, 10), 7.90 (1H, m, H-8), 8.31 (1H, s, H-3), 9.55 (1H, m, H-11), EI-MS *m/z* 321 $[M]^+$.

7-Hydroxydehydronuciferine (11) as in [7], $C_{19}H_{19}NO_3$, brown powder (MeOH), mp 258–260°C. UV (λ_{max}, nm): 254, 366. IR (ν_{max}, cm^{-1}): 3430, 1625. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.09 (3H, s, N-CH_3), 3.29 (2H, t, J = 6.0, H-4), 3.37 (2H, t, J = 6.0, H-5), 3.89 (3H, s, 1-OCH_3), 4.02 (3H, s, 2-OCH_3), 6.65 (1H, br.s, 7-OH), 7.03 (1H, s, H-3), 7.34 (1H, td, J = 8.4, 1.6, H-9), 7.45 (1H, td, J = 8.4, 1.6, H-10), 7.65 (1H, dd, J = 8.4, 1.6, H-8), 9.46 (1H, dd, J = 8.4, 1.6, H-11). EI-MS *m/z* 309 $[M]^+$.

β-Sitostenone (12) and Stigmasta-4,22-dien-3-one (13) as in [3], $C_{29}H_{48}O$ and $C_{29}H_{46}O$, white needles ($CHCl_3$), mp 85–86°C. IR (ν_{max}, cm^{-1}): 1675, 1620, 1460, 1385, 1375. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, H-18), 0.81 (3H, d, J = 6.7, H-26), 0.84 (3H, s, H-27), 0.86 (3H, t, J = 7.1, H-29), 0.92 (3H, d, J = 6.0, H-21), 1.02 (3H, s, H-19), 5.02 (1H, dd, J = 16.1, 8.3, H-22), 5.12 (1H, dd, J = 16.1, 8.3, H-23), 5.72 (1H, d, J = 1.4, H-3), EI-MS *m/z* 412 $[M]^+$.

Pheophytin-a (14) as in [8], $C_{55}H_{74}N_4O_5$, deep green needles ($CHCl_3$), mp 113–114°C. UV (λ_{max}, nm): 229, 274, 330, 372, 406, 508, 540, 610, 665. IR (ν_{max}, cm^{-1}): 3400, 1740, 1699, 1620. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): –1.62 (1H, br.s, NH, D_2O exchangeable), 0.81, 0.83 (each 3H, d, J = 6.6, H-38, 39), 0.87 (6H, d, J = 6.6, H-36, 37), 1.60 (3H, s, H-40), 1.01–1.10 (21H, m, H-24–35), 1.64 (3H, t, J = 7.6, H-8^2), 2.22 (1H, m), 2.32 (1H, m), 2.54 (1H, m), 2.65 (1H, m), 3.22, 3.42 (each 3H, s, H-7′, 2′), 3.54 (2H, q, J = 7.6, H-8′), 3.72, 3.88 (each 3H, s, H-12′, OCH_3), 4.22 (1H, m, H-17), 4.48 (2H, m, H-18), 4.49 (1H, d, J = 7.2, H-21), 5.16 (1H, t, J = 7.4, H-22), 6.19 (1H, d, J = 11.6, H-3^2), 6.30 (1H, d, J = 17.8, H-3^2), 6.28 (1H, s, H-13^2), 8.00 (1H, dd, J = 17.8, 11.4, H-3′), 8.57, 9.39, 9.52 (each 1H, s, H-20, 5, 10), EI-MS *m/z* 871 $[M]^+$.

Aristophyll-C (15) as in [8], $C_{53}H_{70}N_4O_5$, deep green needles ($CHCl_3$), mp 247–248°C. UV (λ_{max}, nm): 282, 360, 412, 480, 512, 550, 642, 702. IR (ν_{max}, cm^{-1}): 1740, 1725. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): –0.18, 0.12 (each 1H, br.s, NH, D_2O exchangeable), 0.78, 0.80 (each 3H, d, J = 7.0, H-38, 39), 0.83 (6H, d, J = 6.8, H-36, 37), 1.01–1.62 (21H, m), 1.62 (3H, s, H-40), 1.66 (3H, t, J = 7.6, H-8^2), 1.75 (3H, d, J = 7.0, H-18′), 2.04 (1H, m, H-17′), 2.45 (2H, m, H-17′, 17^2), 2.73 (1H, m, H-17^2), 3.15 (3H, s, CH_3-7), 3.37 (3H, s, CH_3-2), 3.62 (2H, q, J = 7.6, H-8′), 3.75 (3H, s, CH_3-12), 4.36 (1H, q, J = 7.0, H-18), 4.52 (2H, m, H-21), 5.22 (2H, m, H-17, 22), 6.22 (1H, d, J = 11.4, H-3^2), 6.30 (1H, d, J = 18.0, H-3^2), 7.89 (1H, dd, J = 18.0, 11.5, H-3′), 8.56, 9.40, 9.56 (each 1H, s, H-20, 5, 10), EI-MS *m/z* 842 $[M]^+$.

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