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CHEMICAL CONSTITUENTS FROM THE STEMS OF *Michelia alba*

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We have continued the study of constituents from *Michelia alba* [1]. To further understand the chemotaxonomy and to continue searching for novel agents from Magnoliaceous plants, the stems of *M. alba* were chosen for the first time for phytochemical investigation. In this paper, we report the isolation of 20 pure substances. The compounds, including six aporphines, (–)-anonaine (**1**), (–)-norushinsunine (**2**), (–)-ushinsunine (**3**), (–)-*N*-formylanonaine (**4**) [2], (–)-romerine (**5**) [3], and (–)-asimilobine (**6**) [4]; two oxoaporphines, liriodenine (**7**) and oxoxylopine (**8**); one lignan, (+)-syringaresinol (**9**); one amide, *N-trans*-feruloyltyramine (**10**); seven benzenoids, 4-hydroxybenzaldehyde (**11**), *p*-anisaldehyde (**12**) [5], veratraldehyde (**13**) [5], 3,4,5-trimethoxybenzoic acid (**14**) [6], 3,4-dimethoxybenzoic acid (**15**) [7], eugenol (**16**) [8], and methyl isoeugenol (**17**) [8]; one triterpenoid, ficaprenol (**18**) [9]; two steroids, β-sitosterol (**19**) [10] and stigmasterol (**20**) [10], are isolated from the stems of *Michelia alba*. Compounds **4**, **5**, and **6** and **12**–**18** were isolated for the first time from this species [2].

The MeOH extract (156.7 g) was obtained from *M. alba* according to the literature method [1] (89.2 g) and H_2O (60.8 g). The $CHCl_3$-soluble fraction was chromatographed over silica gel (800 g, 70–230 mesh) using *n*-hexane–EtOAc–$CHCl_3$–MeOH mixtures as eluents to produce five fractions. Part of fraction 1 (12.86 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (40:1) and enriched gradually with EtOAc to furnish three fractions (1-1–1-3). Fraction 1-1 (5.32 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain eugenol (**16**) (23 mg), methylisoeugenol (**17**) (18 mg), and ficaprenol (**18**) (20 mg). Fraction 1-2 (4.63 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain *p*-anisaldehyde (**12**) (19 mg), veratraldehyde (**13**) (22 mg), 3,4,5-trimethoxybenzoic acid (**14**) (26 mg), and 3,4-dimethoxybenzoic acid (**15**) (25 mg). Fraction 1-3 (3.28 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain β-sitosterol (**19**) (45 mg) and stigmasterol (**20**) (38 mg). Part of fraction 2 (9.33 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (30:1) and enriched with EtOAc to furnish two further fractions (2-1–2-2). Fraction 2-1 (5.22 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain liriodenine (**7**) (62 mg). Part of fraction 2-2 (3.62 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain (+)-syringaresinol (**9**) (30 mg). Part of fraction 3 (11.36 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (10:1) and enriched with EtOAc to furnish three further fractions (3-1–3-3). Fraction 3-1 (5.59 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain oxoxylopine (**8**) (18 mg). Fraction 3-2 (5.45 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain 4-hydroxybenzaldehyde (**11**) (28 mg). Part of fraction 4 (25.63 g) was subjected to silica gel chromatography by eluting with $CHCl_3$–MeOH (100:1) and enriched with MeOH to furnish three fractions (4-1–4-3). Fraction 4-1 (11.62 g) was further purified on a silica gel column using $CHCl_3$–MeOH mixtures to obtain (–)-anonaine (**1**) (68 mg) and (–)-ushinsunine (**3**) (32 mg). Fraction 4-2 (5.32 g), eluted with $CHCl_3$–MeOH (60:1), was further separated using silica gel column chromatography and preparative TLC ($CHCl_3$–MeOH (100:1) and gave (–)-*N*-formylanonaine (**4**) (18 mg) and (–)-romerine (**5**) (15 mg). Fraction 4-3 (4.58 g), eluted with $CHCl_3$–MeOH (50:1), was further separated using silica gel column chromatography and preparative TLC ($CHCl_3$–MeOH (80:1) and gave *N-trans*-feruloyltyramine (**10**) (22 mg). Part of fraction 5 (10.82 g) was subjected to silica gel chromatography by eluting with $CHCl_3$–MeOH (30:1) and enriched with MeOH to furnish two fractions (5-1–5-2).

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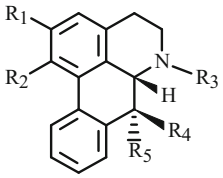

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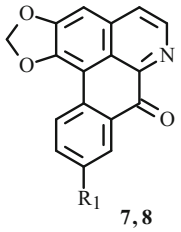

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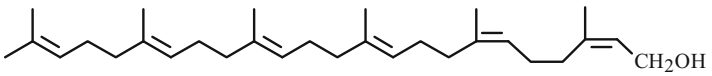

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1: $R_1R_2 = OCH_2O$, $R_3 = R_4 = R_5 = H$; **2:** $R_1R_2 = OCH_2O$, $R_3 = R_4 = H$, $R_5 = OH$
3: $R_1R_2 = OCH_2O$, $R_3 = CH_3$, $R_4 = H$, $R_5 = OH$; **4:** $R_1R_2 = OCH_2O$, $R_3 = CHO$, $R_4 = R_5 = H$
5: $R_1R_2 = OCH_2O$, $R_3 = CH_3$, $R_4 = R_5 = H$; **6:** $R_1 = OH$, $R_2 = OCH_3$, $R_3 = R_4 = R_5 = H$
7: $R_1 = H$; **8:** $R_1 = OCH_3$

Fraction 5-1 (6.32 g) was further purified on a silica gel column using $CHCl_3$–MeOH mixtures to obtain (–)-norushinsunine (**2**) (33 mg). Fraction 5-2 (2.88 g), eluted with $CHCl_3$–MeOH (20:1), was further separated using silica gel column chromatography and preparative TLC ($CHCl_3$–MeOH (60:1) and gave (–)-asimilobine (**6**) (16 mg).

(–)-*N*-Formylanonaine (4) as in [5], brownish needles (MeOH), UV (λ_{max}, nm): 211, 270, 316, IR (ν_{max}, cm^{-1}): 1655 (C=O), 1044, 936 (OCH_2O). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.73 (2H, m, H-4), 2.85 (1H, m, H-7α), 3.26 (1H, dd, J = 14.0, 4.4, H-7β), 3.16 and 3.42 (total 1H, m and td, J = 11.8, 2.8, H-5β), 3.85 and 4.50 (total 1H, each ddd, J = 12.8, 4.8, 3.6, H-5α), 4.65 and 5.07 (total 1H, each dd, J = 14.4, 4.4, H-6a), 5.99 and 6.12 (each 1H, d, J = 1.4, OCH_2O), 6.59 and 6.62 (total 1H, s, H-3), 7.20–7.30 (3H, m, H-8, 9, 10), 8.11 (1H, d, J = 7.6, H-11), 8.27 and 8.40 (total 1H, s, CHO), EI-MS *m/z*: 293 $[M]^+$.

(–)-Romerine (5) as in [6], colorless needles ($CHCl_3$), 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): 2.60 (3H, s, N-CH_3), 5.94 and 6.09 (each 1H, d, J = 1.2, OCH_2O), 6.56 (1H, s, H-3), 7.23–7.32 (3H, m, H-8, 9, 10), 8.08 (1H, d, J = 6.0, H-11), EI-MS *m/z*: 279 $[M]^+$.

(–)-Asimilobine (6) as in [6], brown powder ($CHCl_3$), UV (λ_{max}, nm): 216, 246, 275, 308. IR (ν_{max}, cm^{-1}): 3500, 1560, 1452, 1059, 960. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.60 (3H, s, 1-OCH_3), 6.73 (1H, s, H-3), 7.25–7.27 (3H, m, H-8, 9, 10), 8.30 (1H, d, J = 8.8, H-11), EI-MS *m/z*: 267 $[M]^+$.

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