Two New Constituents from the Leaves of *Magnolia coco*

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A new amide, magnolamide (**1**), and a new lignan, magnolone (**2**), whose structures were determined on the basis of spectral analysis, were found in the leaves of *Magnolia coco.* Eleven known compounds (episesamin, sesamin, magnolol, fargesin, aschantin, epieudesmin, syringaresinol, syringaresinol-*O*-*β*-D-glucopyroside, scoparone, oxoanolobine, and dicentrinone) were also isolated.

The stems and leaves of *Magnolia coco* (Lour.) DC. (Magnoliaceae) are used as an herbal remedy for the treatment of impaired liver function and cancer. Literature reports on the chemical constituents of *M. coco* collected in Taiwan have revealed the presence of aporphine alkaloids.[1−3] In the course of our study, focused on the investigation of the $CHCl_3$-soluble fraction, we isolated a new amide, magnolamide (**1**), and a new lignan, magnolone (**2**), together with eight lignans (episesamin, [4,6] sesamin, [4−6] magnolol,[7] fargesin,[4,8] aschantin,[4−6] epieudesmin,[4−6,8] syringaresinol,[9,10] syringaresinol-*O*-*β*-D-glucopyroside[11]), one coumarin (scoparone[12]), and two alkaloids (oxoanolobine[13] and dicentrinone[14]). We report herein the isolation and structure elucidation of these two novel compounds.

Magnolamide (**1**) was obtained as a pale yellow oil. The molecular formula of **1**, $C_{20}H_{24}N_2O_5$, was derived from HREIMS, ^{1}H NMR, and ^{13}C NMR data. The IR spectrum contained the absorption of an amide group (1651 cm^{-1}) and a benzene ring (1596 and 1515 cm^{-1}). The MS showed a molecular ion peak at *m*/*z* 372; an intense peak at *m*/*z* 343, indicating the loss of a CHO unit from the molecular ion; and a base peak at *m*/*z* 177, which corresponded to a feruloyl moiety.[15] The ^{1}H NMR spectrum confirmed the presence of a trans feruloyl moiety with two doublets at *δ* 6.41 and 7.42 (*J* = 15.7 Hz) as well as with signals of one methoxy group (*δ* 3.85) and three aromatic protons (*δ* 6.78, 7.03, and 7.08) in a typical ABX system. The ^{13}C NMR spectrum indicated the presence of 20 carbons, which included 10 carbons from a feruloyl moiety, four contiguous methylene carbons (*δ* 46.23, 39.98, 29.76, and 27.62), one CHO (*δ* 180.84), one CH_2OH (*δ* 56.45), and four carbon signals (*δ* 144.42, 133.40, 126.33, and 111.57) from a pyrrolylic ring. From the above data we suggested that this compound consists of a feruloyl moiety and a (2-formyl-5-hydroxymethyl)-pyrrolybutylamine segment.

The NOE experiments showed that the H-5 (*δ* 7.08) signal was enhanced upon irradiation of 6-OMe (*δ* 3.85), suggesting the placement of the aromatic methoxy group at C-6. Furthermore, irradiation of the signal at H-4′ (*δ* 4.35) enhanced CHO (*δ* 9.38), and irradiation of the signal at CHO (*δ* 9.38) enhanced H-6′ (*δ* 6.95), which suggested the placement of the pyrrolylic formyl group at C-5′. In addition, irradiation of the C*H*$_2$OH signal at *δ* 4.61 produced an NOE effect that was only observed at H-7′ (*δ* 6.23), indicating the placement of a pyrrolylic hydroxymethyl group at C-8′. From the above results the structure of **1** was assigned as an amide, namely, magnolamide. This is the first naturally occurring amide that has a (2-formyl-5-hydroxymethyl)-pyrrolybutylamine moiety as an amine unit.

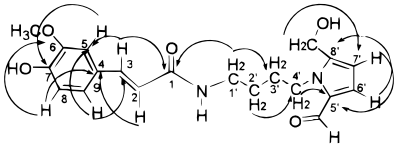

Figure 1. Correlation in HMBC spectrum of **1**.

The structure of **1** was also supported by its HMBC NMR spectrum (Figure 1), which showed the correlation of the amide carbon (*δ* 169.10) to H-3 (*δ* 7.42) and H-1′ (*δ* 3.30). Further analysis of this spectrum and the HETCOR spectrum also allowed the complete assignment of the ^{13}C NMR of **1.**

Magnolone (**2**) has a molecular ion peak at 386.1361 in its HREIMS, corresponding to the formula $C_{21}H_{22}O_7$. The EIMS shows characteristic fragmentation patterns at *m*/*z* 165 and 149 arising from benzoylic or tetrahydrofuran ring cleavage.[16] The IR spectrum gave the absorption of a hydroxyl group (3453 cm^{-1}), a carbonyl group (1668 cm^{-1}), and a benzene ring (1593 and 1514 cm^{-1}). The ^{1}H NMR spectrum displayed two aromatic methoxy groups at *δ* 3.87 and 3.89, one methylenedioxy group at *δ* 5.97, and two methylene groups with nonequivalent protons at *δ* 4.08−4.22 (2H, m, H-9) and 3.68 (2H, t, *J* = 1.5 Hz, H-9′), which exhibited a HETCOR correlation with the carbon signals at *δ* 71.37 and 61.03 at C-9 and C-9′, respectively. A pair of multiplets at *δ* 4.28 and 2.66 (each 1H, m), showing HETCOR correlations with the carbon signals at *δ* 50.01 and 54.33, were attributed to the methine protons at C-8 and C-8′, respectively. Additionally, the signal at *δ* 4.68 (d, *J* = 2.8 Hz, H-7′) was assigned to the oxymethine proton at C-7′, which indicated a HETCOR correlation with the carbon signal at *δ* 84.22. The remaining signals for six aromatic protons indicated the presence of both a piperonyl system [*δ* 6.78 (1H, d, *J* = 8.0 Hz, H-5′), 6.88 (1H, dd, *J* = 1.6, 8.0 Hz, H-6′), and 6.98 (1H, d, *J* = 1.6 Hz, H-2′)] and a veratryl system [*δ* 7.06 (1H, d, *J* = 8.4

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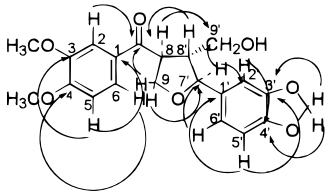

Figure 2. Correlation in HMBC spectrum of **2**.

Hz, H-5), 7.60 (1H, d, J = 2.1 Hz, H-2), and 7.70 (1H, dd, J = 2.1, 8.4 Hz, H-6)].

From the above data, the structure of **2** was proposed as an 8,8′-linked tetrahydrofuran lignan, namely, magnolone. ^{13}C NMR analysis of **2** provides further confirmation for the proposed structure. It clearly showed 12 aromatic carbons, two methoxy carbons, one methylenedioxy carbon, one carbonyl carbon, and five other carbon atoms, three of which bore oxygen atoms. The 3,4-dimethoxybenzoyl group at C-8 was verified by NOEs for 3-OCH_3/H-2 and 4-OCH_3/H-5 and by HMBC correlations for H-6/C-7, H-5/C-1, and H-9/C-7 (Figure 2). Thus, the structure of **2** can be assigned from the above evidence. Its relative configuration was determined as trans H-7′/H-8′ due to the chemical shift of H-7′ at 4.68 ppm.[17] The trans configuration of H-8 and H-8′ was supported by NOESY experiments, which provided the evidence for the *trans* relationship of H-7′/H-8′ and H-8/H-8′.

The structure of **2** was also supported by its HMBC NMR spectrum (Figure 2), which showed the correlation of the proton signals at H-8′ (δ 2.66) to C-8 (δ 50.01), C-7′ (δ 84.22), C-1′ (δ 137.05), and C-7 (δ 198.41), respectively. Further analysis of this spectrum and the HETCOR spectrum also allowed the complete assignment of the ^{13}C NMR of **2.**

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco micro-melting point apparatus and are uncorrected. The IR spectra were recorded on a BioRad FT-IR spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Hitachi model U-3200 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 200 (200 MHz) and Bruker AC-300 (300 MHz) FT-NMR spectrometers. FABMS and EIMS were obtained on a JEOL SX-102A and a JEOL JMS−HX100 spectrometer, respectively.

Plant Material. The leaves of *M. coco* (Lour.) DC. were collected in July 1995, at the garden of the National Research Institute of Chinese Medicine, Taipei, Taiwan. A voucher specimen is maintained in the herbarium of this institute.

Extraction and Isolation. The air-dried, powdered leaves of *M. coco* (8 kg) were extracted with 95% EtOH (3 × 80 L) at 50 °C. After filtration and evaporation of the solvent under vacuum, the residue was suspended in 5% HOAc aqueous solution and then extracted with $CHCl_3$. The $CHCl_3$-soluble fraction was chromatographed on a Si gel (70−230 mesh) column and successively eluted with *n*-hexane−EtOAc (4:1, 2:1, 1:1, 1:2) and EtOAc−MeOH (20:1, 10:1) to yield six fractions. Fraction 1 was rechromatographed on a Si gel (70−230 mesh) column using a gradient of *n*-hexane−EtOAc (8:1 to 2:1) as eluent to afford episesamin (2.7 g),[4,6] sesamin (24 g),[4−6] and magnolol (14.5 mg).[7] Fraction 2 was subjected to chromatography on a Si gel (70−230 mesh) column and eluted with a gradient of *n*-hexane−EtOAc (2:1 to 1:1) to obtain fargesin (3.3 g),[4,8] aschantin (0.24 g),[4−6] and magnolone (**2**, 16 mg). Fraction 3 (*n*-hexane−EtOAc, 1:1) contained scoparone (28 mg)[12] and epieudesmin (7.2 mg).[4−6,8] Fraction 4 (*n*-hexane−EtOAc, 1:2) contained syringaresinol (2.1 g).[9,10] Fraction 5 was chromatographed with Si gel (230−400 mesh) by using CH_2Cl_2−MeOH (10:1 to 8:1) as eluent to obtain magnolamide (**1**, 23.4 mg) and oxoanolobine (7.2 mg).[13] Fraction 6 was further chromatographed with Si gel (230−400 mesh) by using CH_2Cl_2−MeOH (7:1 to 6:1) as eluent to obtain syringaresinol-*O*-β-D-glucopyroside (106 mg)[11] and dicentrinone (5.5 mg).[14]

Magnolamide (1): isolated as a pale yellow oil; IR ν_{max} 3323 (br), 1651, 1596, 1515, 1457, 1372, 1277, 1124 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 202 (4.50), 236 (4.66), 294 (4.43) nm; ^{1}H NMR (CD_3OD) δ 1.57 (2H, m H-2′), 1.78 (2H, m, H-3′), 3.30 (2H, t, J = 7.2 Hz, H-1′), 3.85 (3H, s, OMe), 4.35 (2H, t, J = 7.2 Hz, H-4′), 4.61(2H, s, CH_2-OH), 6.23 (1H, d, J = 4.0 Hz, H-7′), 6.41 (1H, d, J = 15.7 Hz, H-2), 6.78 (1H, d, J = 8.2 Hz, H-8), 6.95 (1H, d, J = 4.0 Hz, H-6′), 7.03 (1H, dd, J = 8.2, 2.0 Hz, H-9), 7.08 (1H, d, J = 2.0 Hz, H-5), 7.42 (1H, d, J = 15.7 Hz, H-3), 9.38 (1H, s, CHO); ^{13}C NMR (CD_3OD) δ 180.84 (d, CHO), 169.10 (s, C-1), 149.74 (s, C-7), 149.20 (s, C-6), 144.42 (s, C-8′), 142.00 (d, C-3), 133.40 (s, C-5′), 128.23 (s, C-4), 126.33 (d, C-6′), 123.14 (d, C-9), 118.77 (d, C-2), 116.45 (d, C-8), 111.57 (d, C-7′), 111.43 (d, C-5), 56.45 (CH_2OH), 56.36 (q, 6-OMe), 46.23 (t, C-4′), 39.98 (t, C-1′), 29.76 (t, C-3′), 27.62 (t, C-2′); EIMS m/z [M]$^+$ 372 (28), 343 (39), 195 (19), 177 (100), 145 (36); HREIMS m/z 372.1700, calcd for $C_{20}H_{24}N_2O_5$ 372.1682.

Magnolone (2): viscous oil; $[\alpha]^{21}_D$ −11.25° (c 0.4, MeOH); IR (dry film) ν_{max} 3452 (br), 2878, 1668, 1593, 1514, 1419, 1264, 1161, 1036, 814 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 202 (3.34), 217 (3.84), 227 (3.84), 230 (3.70), 249 (2.16) nm; ^{1}H NMR (Me_2CO-d_6) δ 2.66 (1H, m, H-8′), 3.68 (2H, t, J = 1.5 Hz, H-9′), 3.87 (3H, s, 3-OMe), 3.89 (3H, s, 4-OMe), 4.08−4.22 (2H, m, H-9), 4.28 (1H, m, H-8), 4.68 (1H, d, J = 2.8 Hz, H-7′), 5.97 (2H, s, O−CH_2−O), 6.78 (1H, d, J = 8.0 Hz, H-5′), 6.88 (1H, dd, J = 1.6, 8.0 Hz, H-6′), 6.98 (1H, d, J = 1.6 Hz, H-2′), 7.06 (1H, d, J = 8.4 Hz, H-5), 7.60 (1H, d, J = 2.1 Hz, H-2), 7.70 (1H, dd, J = 2.1, 8.4 Hz, H-6); ^{13}C NMR (Me_2-CO-d_6) δ 50.01 (d, C-8), 54.33 (d, C-8′), 56.12, 56.22 (each q, 3-OMe and 4-OMe), 61.03 (t, C-9′), 71.37 (t, C-9), 84.22 (d, C-7′), 101.89 (t, O−CH_2−O), 107.77 (d, C-2′), 108.48 (d, C-5′), 111.58 (d, C-5), 111.93 (d, C-2), 120.90 (d, C-6′), 123.93 (d, C-6), 130.78, 137.05 (s, C-1 and C-1′), 147.94, 148.67, 150.27, and 154.73 (each s, C-4′, C-3′, C-3 and C-4), and 198.41 (s, C-7); EIMS m/z [M$^+$] 386 (28), 194 (29), 178 (68), 165 (100), 149 (63), 121 (24), 77 (33); HREIMS m/z 386.1361, calcd for $C_{21}H_{22}O_7$ 386.1366.

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