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Phenolic Compounds from *Nymphaea odorata*

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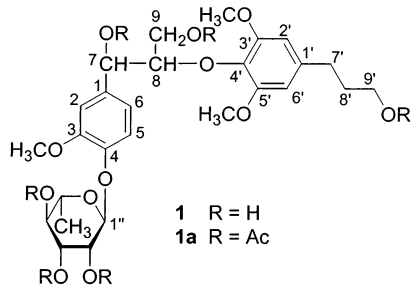
Assay-guided fractionation of the ethanol extract of *Nymphaea odorata* resulted in the identification of two lignans, one new (**1**) and one known (**2**), together with six known flavonol glycosides (**3**−**8**). The structures of **1**−**8** were established by spectroscopic analysis as nymphaeoside A (**1**), icariside E_4 (**2**), kaempferol 3-*O*-α-L-rhamnopyranoside (afzelin, **3**), quercetin 3-*O*-α-L-rhamnopyranoside (**4**), myricetin 3-*O*-α-L-rhamnopyranoside (myricitrin, **5**), quercetin 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside (**6**), myricetin 3-*O*-β-D-galactopyranoside (**7**), and myricetin 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside (**8**). Compounds **3**, **4**, and **7** showed marginal inhibitory effect against fatty acid synthase with IC_{50} values of 45, 50, and 25 μg/mL, respectively.

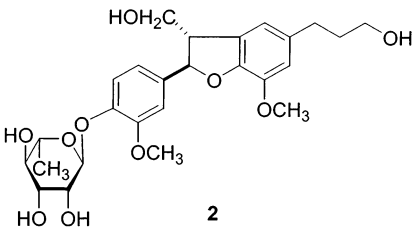
Fatty acid synthase (FAS) is an enzyme essential to the infective process in *Candida albicans*.[1,2] Early work on the structure−activity relationship among a series of structurally related FAS inhibitors showed that the fungal and human enzymes could be differentially inhibited.[3] This suggested that with appropriate inhibitors FAS could be used as a therapeutic target. In the course of our continued efforts of searching for fatty acid synthase (FAS) inhibitors from natural sources,[4] we investigated an active ethanolic extract of *Nymphaea odorata*.

Nymphaea odorata Ait. (Nymphaeaceae), or American white water lily, is a herbaceous hydrophyte native to the southeastern United States. Previous work on this plant resulted in the isolation of five compounds with plant growth-inhibitory properties.[5] Bioactivity-guided fractionation of the leaves of *N. odorata* resulted in the isolation of eight constituents (**1**−**8**). This work describes the isolation, structure elucidation, and biological activity of these compounds.

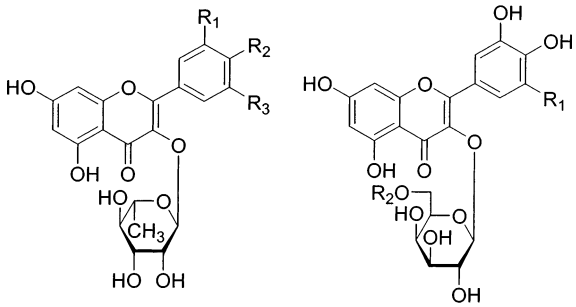
Compound **1** was obtained as a colorless amorphous solid. Its molecular formula of $C_{27}H_{38}O_{12}$ was determined by HRESIMS and indicated nine degrees of unsaturation. The ^{13}C NMR spectrum of **1** displayed 27 signals, of which 21 were assigned to the aglycone moiety including two aromatic rings, three methoxy groups, one hydroxypropyl group, and one 1,3-propanediol group. The remaining six signals corresponded to a deoxyhexose. The ^1H NMR spectrum of **1** displayed signals for a 1,3,4-trisubstituted aromatic ring [δ 7.11 (1H, br s, H-2), δ 7.08 (1H, d, J = 8.0 Hz, H-5), δ 6.93 (1H, d, J = 8.0 Hz, H-6), δ 3.85 (3H, s, OCH_3-3)], a 1′,3′,4′,5′-tetrasubstituted aromatic ring [δ 6.57 (2H, s, H-2′,6′), δ 3.83 (6H, s, OCH_3-3′,5′)], a 1,2,3-trioxygenated propyl function [δ 4.99 (1H, d, J = 5.2 Hz, H-7), δ 4.22 (1H, m, H-8), δ 3.95 (1H, m, H-9b), δ 3.57 (1H, m, H-9a)], and a hydroxypropyl group [δ 2.65 (2H, t, J = 8.0 Hz, H-7′), δ 1.82 (2H, m, H-8′), δ 3.57 (2H, m, H-9′)].



1 R = H
1a R = Ac



2



3 $R_1 = R_3 = H$, $R_2 = OH$
4 $R_1 = R_2 = OH$, $R_3 = H$
5 $R_1 = R_2 = R_3 = OH$
6 $R_1 = H$, $R_2 = Ac$
7 $R_1 = OH$, $R_2 = H$
8 $R_1 = OH$, $R_2 = Ac$

These data suggested that the aglycone of **1** is a lignan, identified as 1-[4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2,6-dimethoxyphenoxy]-1,3-propanediol,[6,7] and these inferences were confirmed by HMQC and HMBC correlations (Figure 1). Acid hydrolysis of **1** afforded rhamnose, which was identified by co-TLC with an authentic sample. The characteristic NMR signals of δ_H 5.38 (1H, br s, H-1″) and δ_H 1.26 (3H, d, J = 5.9 Hz, H-6″), as well as their protonated carbon signals at δ_C 101.4 (C-1″)

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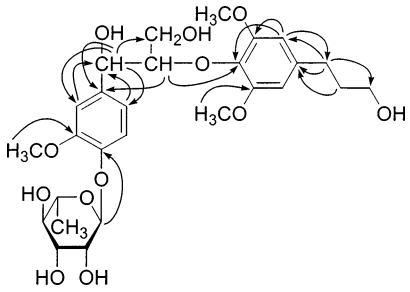

Figure 1. Key HMBC (H to C) correlations of **1**.

and δ_C 18.8 (C-6″) also indicated the presence of a rhamnose moiety. A HMBC correlation between H-1″ (δ 5.38) of rhamnose and C-4 (δ 146.0) of aglycone established that the rhamnose was attached to C-4 of aglycone. The α-pyranose form of rhamnose in **1** was confirmed[8,9] by a set of carbon signals at δ 101.4, 72.2, 72.0, 73.8, 70.7 and 18.8, which correlated with δ 5.38 (br s), 4.12 (br s), 3.95 (m), 3.48 (t, J = 9.5 Hz), 3.91 (m), and 1.26 (d, J = 5.9 Hz), respectively, in the HMQC spectrum. The stereochemistry for C-7 and C-8 was shown to be *erythro*[6] from the chemical shift value at δ 6.00 (1H, d) and the J coupling constant value (4.7 Hz) of H-7 in **1a**, the corresponding acetate derivative of **1**. HMQC and HMBC analyses allowed for the complete assignments of the ^{1}H and ^{13}C signals of **1** (1-[4-*O*-(α-L-rhamnopyranosyl)-3-methoxyphenyl)]-2-[4-(3-hydroxypropyl)-2,6-dimethoxyphenoxy]-1,3-propanediol), a new natural product named nymphaeoside A.

The known compounds were identified by comparison of their spectral data with reported values as icariside E_4 (**2**),[10] kaempferol 3-*O*-α-L-rhamnopyranoside (afzelin, **3**),[11,] quercetin 3-*O*-α-L-rhamnopyranoside (**4**),[12] myricetin 3-*O*-α-L-rhamnopyranoside (myricitrin, **5**),[13] quercetin 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside (**6**),[14] myricetin 3-*O*-β-D-galactopyranoside (**7**),[14] and myricetin 3-*O*-(6″-*O*-acetyl)-β-D-galactopyranoside (**8**).[14] This is the first report of the isolation of **2**−**8** from this plant.

Compounds **1**−**8** were evaluated in the fatty acid synthase (FAS) inhibition assay.[4] The results showed compounds **3**, **4**, and **7** had weak inhibitory effects against FAS with IC_{50} values of 45, 50, and 25 μg/mL, respectively. Cerulenin[15] was used as a positive control (IC_{50} of 0.19 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Hewlett-Packard 8435 spectrometer. IR spectra were obtained on an ATI Mattson Genesis Series FTIR spectrometer. The NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. 2D NMR spectra were measured on a Bruker Avance DRX-500 operating at 500 MHz using standard pulse programs and acquisition parameters. HRESIMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. Si gel (40 μm, J. T. Baker) and RP Si gel (RP-18, 40 μm, J. T. Baker) were used for low-pressure chromatography. HPLC was performed using an ODS column (Phenomenex, Prodigy ODS prep, 21.2 mm i.d. × 250 mm, 10 μm). TLC was performed on Si gel 60 F_{254} (EM Science) using $CHCl_3$/MeOH (4:1, solvent A), toluene/EtOAc/MeOH (4:1:1, solvent B), and $CHCl_3$/EtOAc (6:1, solvent C) or reversed-phase KC_{18} F Si gel 60 (Whatman) using MeOH/H_2O (70:30, solvent D). The detailed procedures for the bioassays are described in a previous paper.[4]

Plant Material. The plant material (leaves) was collected in Florida in July 1996 and identified by Dr. Charles Burandt. A voucher specimen is on deposit at the National Center for Natural Products Research, The University of Mississippi (voucher # BUR 190796 1A).

Extraction and Isolation. The dried and powdered (60 mesh) plant material (400 g) was percolated with 95% EtOH (3000 mL × 3). The ethanolic extract was evaporated to dryness (50.1 g, IC_{50} 25 μg/mL). Part of the EtOH extract (33.0 g) was chromatographed over a silica gel column (300 g) eluting with $CHCl_3$/MeOH (9:1, 4:1, 1:1, 1:4, and 0:1, each 1000 mL). On the basis of TLC analysis, nine combined fractions were obtained: A (5.19 g), B (0.70 g), C (0.70 g), D (2.17 g), E (8.09 g), F (3.68 g), G (5.28 g), H (4.10 g), and I (2.25 g). Part of fraction B (0.66 g) was rechromatographed over a silica gel column (120 g) using $CHCl_3$/MeOH (7:1 and 4:1, each 1500 mL) to afford five fractions: B_1 (235.2 mg), B_2 (250 mg), B_3 (9 mg), B_4 (50.0 mg), and B_5 (60 mg). Part of B_2 (100 mg) was further applied onto a ODS column (10 g) washing with MeOH/H_2O (60:40) and then MeOH. The aqueous MeOH fraction (20 mg) was purified by HPLC (MeOH/H_2O, 60:40, 3 mL/min, UV 276 nm) to yield **1** (10.6 mg, t_R 22.5 min). Part of B_4 (40 mg) was separated using an ODS column (10 g) and washing with MeOH/H_2O (0:100, 50:50, and 80:20, each 200 mL). The 50% MeOH fraction (22 mg) was refractionated using HPLC (MeOH/H_2O, 50:50) to afford **2** (9.5 mg, t_R 21 min), **3** (2.1 mg, t_R 29.4 min), and **6** (2.6 mg, t_R 26 min).

Part of the FAS inhibitory fraction E (6.0 g) was chromatographed over a ODS column (200 g) eluting with MeOH/H_2O mixtures (100:0, 50:50, and 0:100, each 1000 mL) to give fractions E_1 (2.50 g), E_2 (1.48 g), E_3 (1.51 g), and E_4 (0.50 g). Fraction E_3 was further purified using HPLC (MeOH/H_2O, 50:50, 3 mL/min, UV 276 nm) to afford **4** (2.6 mg, t_R 54.1 min), **5** (8.0 mg, t_R 36.5 min), **7** (3.0 mg, t_R 33.0 min), and **8** (6.0 mg, t_R 40.5 min). Fractions F−I, E_1, and E_2, rich in very polar compounds (possibly tannins), were not further investigated.

Nymphaeoside (1): colorless amorphous powder; $[\alpha]^{22}_D$ −23.1° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.05), 230 (sh), 276 (3.17) nm; IR (KBr) ν_{max} 3401, 1587, 1504, 1456, 1417, 1220, 1119, 1057, 1019, 973 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.26 (3H, d, J = 5.9 Hz, H-6″), 1.82 (2H, m, H-8′), 2.65 (2H, t, J = 8.0 Hz, H-7′), 3.48 (1H, t, J = 9.5 Hz, H-4″), 3.57 (4H, m, H-9, 9′), 3.83 (6H, s, OCH_3-3′, 5′), 3.85 (3H, s, OCH_3-3), 3.91 (1H, m, H-5″), 3.95 (1H, m, H-3″), 4.12 (1H, br s, H-2″), 4.22 (1H, m, H-8), 4.99 (1H, d, J = 5.2 Hz, H-7), 5.38 (1H, br s, H-1″), 6.57 (2H, br s, H-2′,6′), 6.93 (1H, d, J = 8.0 Hz, H-6), 7.08 (1H, d, J = 8.0 Hz, H-5), 7.11 (1H, br s, H-2); ^{13}C NMR (CD_3OD) δ 154.3 (C-3′,5′), 151.6 (C-3), 146.0 (C-4), 139.9 (C-1), 138.0 (C-1′), 134.6 (C-4′), 120.4 (C-6), 119.2 (C-5), 112.4 (C-2), 106.8 (C-2′,6′), 101.4 (C-1″), 87.2 (C-8), 73.8 (C-4″), 73.7 (C-7), 72.2 (C-2″), 72.0 (C-3″), 70.7 (C-5″), 62.1 (C-9′), 61.4 (C-9), 56.6 (OCH_3-3′,5′), 56.4 (OCH_3-3), 35.4 (C-8′), 33.4 (C-7′), 18.8 (C-6″); HRESIMS m/z 572.2698 $[M + NH_4]^+$, 577.2258 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_{12}$, 572.2701 $[M + NH_4]^+$, 577.2255 $[M + Na]^+$).

Acetylation of 1. Compound **1** (6 mg) was dissolved in Ac_2O/pyridine (1:2, 3 mL), and the mixture was allowed to stand at room temperature for 24 h. After addition of 10 mL of H_2O, the resulting mixture was applied onto a low-pressure ODS column (5 g) washing with H_2O (50 mL) and then MeOH (30 mL). The MeOH fraction was evaporated to give **1a** (5 mg).

1a: colorless amorphous powder; $[\alpha]^{22}_D$ −35.4° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.18), 230 (sh), 276 (3.14) nm; IR (KBr) ν_{max} 2940, 1744, 1589, 1508, 1460, 1369, 1220, 1124, 1039 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.16 (3H, d, J = 5.9 Hz, H-6″), 1.88 (2H, m, H-8′), 1.95 (3H, s, −COCH_3), 1.99 (3H, s, −COCH_3), 2.04 (6H, s, −COCH_3 × 2), 2.11 (3H, s, −COCH_3), 2.19 (3H, s, −COCH_3), 2.55 (2H, t, J = 7.1 Hz, H-7′), 3.70 (6H, s, OCH_3-3′,5′), 3.80 (3H, s, OCH_3-3), 4.05 (2H, m, H-9′), 4.12 (1H, m, H-5″), 4.20 (1H, m, H-9a), 4.39 (1H, m, H-9b), 4.55 (1H, br t, H-8), 5.07 (1H, t, J = 9.8 Hz, H-4″), 5.31 (1H, br s, H-1″), 5.49 (1H, br s, H-2″), 5.53 (1H, m, H-3″), 6.00 (1H, d, J = 4.7 Hz, H-7), 6.32 (2H, s, H-2′,6′), 6.82 (1H, d, J = 8.0 Hz, H-6), 6.92 (1H, br s, H-2), 6.98 (1H, d, J = 8.0 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 171.5 (C═O), 171.3 (C═O × 2), 170.4 (C═O ×

2), 170.1 (C═O), 153.4 (C-3′,5′), 150.7 (C-3), 145.1 (C-4), 137.8 (C-1′), 133.6 (C-1)[a], 133.5 (C-4′)[a], 119.8 (C-6), 118.7 (C-5), 112.0 (C-2), 105.6 (C-2′, 6′), 97.7 (C-1″), 81.1 (C-8), 74.5 (C-7), 71.4 (C-4″), 70.1 (C-2″)[b], 69.3 (C-3″)[b], 67.7 (C-5″), 64.1 (C-9′), 63.1 (C-9), 56.3 (OCH_3-3, 3′, 5′), 32.9 (C-7′), 30.5 (C-8′), 21.4 (−COCH_3), 21.3 (−COCH_3), 21.2 (−COCH_3 × 2), 21.1 (−COCH_3), 20.7 (−COCH_3), 17.7 (C-6″); assignments were based on COSY, HMQC, and HMBC spectra ([a,b]The signals may be interchangeable); HRESIMS *m*/*z* 829.2871 $[M + Na]^+$ (calcd for $C_{39}H_{50}O_{18}$, 829.2889 $[M + Na]^+$).

Acid Hydrolysis of 1. Compound **1** and authentic rhamnose were spotted on a silica gel TLC plate and hydrolyzed in situ by exposure to HCl vapor at 70 °C for 25 min. The TLC plate was then developed with $CHCl_3$/MeOH/AcOH/H_2O (14:6:2:1) and sprayed with 10% H_2SO_4 for detection. Rhamnose was detected with an R_f value of 0.2.

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Supporting Information Available: ^1H and ^{13}C NMR data of **2**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Zhao, X. J.; McElhaney-Feser, G. E.; Bowen, W. H.; Cole, M. F.; Broedel, S. E., Jr.; Cihlar, R. L. *Microbiology* **1996**, *142*, 2509−2514.
(2) Zhao, X. J.; McElhaney-Feser, G. E.; Sheridan, M. J.; Broedel, S. E., Jr.; Cihlar, R. L. *Infect. Immun.* **1997**, *65*, 829−832.
(3) Broedel, S. E., Jr. *Recent Res. Dev. Antimicrob. Agents Chemother.* **1996**, *1*, 25−33.
(4) Li, X.-C.; Joshi, A. S.; ElSohly, H. N.; Khan, S. I.; Jacob, M. R.; Zhang, Z. Z.; Khan, I. A.; Ferreira, D.; Walker, L. A.; Broedel, S. E.; Raulli, R. E.; Cihlar, R. L. *J. Nat. Prod.* **2002**, *65*, 1909−1914.
(5) Elakovich, S. D.; Spence, S.; Yang, J. Book of Abstracts, 214th ACS National Meeting, Las Vegas, NV, September 7−11, 1997; AGRO-036; American Chemical Society: Washington, DC.
(6) Hwang, B. H.; Sakakibara, A.; Miki, K. *Holzforschung* **1981**, *35*, 229−232.
(7) Hauteville, M.; Lundquist, K.; von Unge, S. *Acta Chem. Scand. B* **1986**, *40*, 31−35.
(8) Kasai, R.; Okihara, M.; Asakawa, J.; Mizutani, K.; Tanaka, O. *Tetrahedron* ***179***, *35*, 1427−1432.
(9) Alaoui, A.; Charrouf, Z.; Souflaoui, M.; Carbone, V.; Malorni, A.; Pizza, C.; Piacente, S. *J. Agric. Food Chem.* **2002**, *50*, 4600−4603.
(10) Miyase, T.; Ueno, A.; Takizawa, N.; Kobayashi, H.; Oguchi, H. *Phytochemistry* **1989**, *28*, 3483−3485.
(11) Matthes, H. W. D.; Luu, B.; Ourisson, G. *Phytochemistry* **1980**, *19*, 2643−2650.
(12) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389−1397.
(13) Addae-Mensah, I.; Achenbach, H. *Phytochemistry* **1985**, *24*, 1817−1819.
(14) Foo, L. Y.; Lu, Y.; Molan, A. L.; Woodfield, D. R.; McNabb, W. C. *Phytochemistry* **2000**, *54*, 539−548.
(15) Price, A. C.; Choi, K.-H.; Heath, R. J.; Li, Z.; White, S. W.; Rocks, C. O. *J. Biol. Chem.* **2001**, *276*, 6551−6559.

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