Phytochemical Investigation of *Turnera diffusa*

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A phytochemical investigation of *Turnera diffusa* afforded 35 compounds, comprised of flavonoids, terpenoids, saccharides, phenolics, and cyanogenic derivatives, including five new compounds (**1**−**5**) and a new natural product (**6**). These compounds were characterized as luteolin 8-*C*-*E*-propenoic acid (**1**), luteolin 8-*C*-*β*-[6-deoxy-2-*O*-(α-L-rhamnopyranosyl)-*xylo*-hexopyranos-3-uloside] (**2**), apigenin 7-*O*-(6″-*O*-*p*-*Z*-coumaroyl-*β*-D-glucopyranoside) (**3**), apigenin 7-*O*-(4″-*O*-*p*-*Z*-coumaroylglucoside) (**4**), syringetin 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (**5**), and laricitin 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (**6**). Their structures were determined by spectroscopic and chemical methods.

Turnera diffusa Willd. ex Schult., a small shrub, belongs to the family Turneraceae. It grows in tropical and subtropical parts of America with the common name damiana. The ancient Maya used it for treatment of "giddiness and loss of balance".[1] Mexican Indians have traditionally used its leaves to make a beverage for its reputed aphrodisiac effects.[2] Damiana products were first marketed in the U.S. in 1874, with the claims of being a "powerful invigorant" and "powerful aphrodisiac, to improve the sexual ability of the enfeebled and aged".[3] From 1888 to 1947, damiana was adopted into the *National Formulary*. Currently, damiana products can still be found in the market, with most of these used in combination with other herbs, such as ginkgo, ginseng, and saw palmetto.

As part of a program to address the issues of authenticity of material source, safety, and efficacy of botanical products, we have undertaken a phytochemical investigation of the largely unexamined constituents of *T. diffusa*.[4] This paper describes the identification of six new compounds, luteolin 8-*C*-*E*-propenoic acid (**1**), luteolin 8-*C*-*β*-[6-deoxy-2-*O*-(α-L-rhamnopyranosyl)-*xylo*-hexopyranos-3-uloside] (**2**), apigenin 7-*O*-(6″-*O*-*p*-*Z*-coumaroyl-*β*-D-glucopyranoside) (**3**), apigenin 7-*O*-(4″-*O*-*p*-*Z*-coumaroyl-*β*-D-glucoside) (**4**), syringetin 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (**5**), and laricitin 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (**6**). These were isolated along with 29 known compounds from the leaves of *T. diffusa*.

Compound **1** was obtained as fine yellow crystals. Its ^{13}C NMR spectrum displayed 18 resonances, which, in conjunction with the HRESIMS, indicated the molecular formula $C_{18}H_{12}O_8$. The ^{1}H and ^{13}C NMR spectroscopic data of **1** resembled with those of demethyltorosaflavone D (luteolin 6-*C*-*E*-propenoic acid),[5] showing resonances at δ_H 13.47 (s, OH-5), 6.34 (s, H-6), 8.00 (H-1″, d, J = 16.0 Hz), and 6.80 (H-2″, d, J = 16.0 Hz) and at δ_C 162.8 (C-5), 99.3 (C-6), 164.7 (C-7), and 102.3 (C-8). The unambiguous HMBC correlations of the hydroxyl proton at C-5 (δ_H 13.47) with C-5 (δ_C 162.8), C-6 (δ_C 99.3), and C-10 (δ_C 104.4), of H-6 (δ_H 6.34) with C-5 (δ_C 162.8) and C-7 (δ_C 164.7), and of H-1″ (δ_H 8.00) with C-7 (δ_C 164.7) and C-9 (δ_C 156.3) revealed the connectivity of the propenoic acid moiety at C-8 rather than at C-6 as in demethyltorosaflavone D.[14] Thus, compound **1** was established as luteolin 8-*C*-*E*-propenoic acid.

Compound **2** was obtained as yellow needles. Its molecular formula, $C_{27}H_{28}O_{14}$, was determined by HRESIMS, which showed a $[M + H]^+$ ion at m/z 577.1578. The UV absorption pattern (λ_{max} 268, 279, 348 nm) suggested a flavone skeleton. The NMR spectroscopic data of compound **2** were found to correlate with those of cassiaoccidentalin B.[6] The HMBC NMR correlations of H-1″ (δ_H 5.20) with C-7 (δ_C 161.9), C-8 (δ_C 103.2), and C-9 (δ_C 156.3), of the hydroxyl proton at C-5 (δ_H 12.88) with C-5 (δ_C 162.7), C-6 (δ_C 98.7), and C-10 (δ_C 104.5), and of H-6 (δ_H 6.31) with C-5 (δ_C 162.7) and C-7 (δ_C 161.9) supported the linkage of the sugar residue at C-8 in compound **2**. The NOESY NMR spectroscopic correlations of H-2′ and H-6′ on the B ring with H-2″ and H-4″ (Figure 1) confirmed the position of the saccharide moiety at C-8. Compound **2** is thus luteolin 8-*C*-*β*-[6-deoxy-2-*O*-(α-L-rhamnopyranosyl)-*xylo*-hexopyranos-3-uloside].

Compound **3** was obtained as a pale yellow powder. The HRESIMS of **3** showed a $[M + H]^+$ ion at m/z 579.1511, corresponding to the molecular formula $C_{30}H_{26}O_{12}$, similar to that of echinacin.[7] When its ^{1}H and ^{13}C NMR spectroscopic data were compared with those of echinacin, the coupling constants (J = 16.0 Hz) of the *E*-olefinic protons of a coumaroyl moiety (H-2‴ and H-3‴) were absent in **3**, showing instead the coupling constants (J = 12.8 Hz) characteristic of *Z*-geometry. The NOESY correlation (Figure 1) of H-2‴ with H-3‴ was used to confirm the *Z*-geometry of the double bond. Accordingly, compound **3** was assigned as apigenin 7-*O*-(6″-*O*-*p*-*Z*-coumaroyl-*β*-D-glucopyranoside).

The HRESIMS, m/z 601.1319 $[M + Na]^+$, together with ^{13}C NMR spectroscopic data was used to deduce the molecular formula, $C_{30}H_{26}O_{12}$, of compound **4**, identical to echinaticin.[7] A comparative NMR spectroscopic data analysis of **4** with that of echinaticin revealed that **4** is its *Z*-isomer due to the observation of characteristic coupling constants (J = 12.8 Hz) of *cis*-olefinic protons of the coumaroyl moiety (H-2‴ and H-3‴). This assignment was confirmed by the NOESY correlations (Figure 1) of H-2‴ with H-3‴. Accordingly, compound **4** was identified as apigenin 7-*O*-(4″-*O*-*p*-*Z*-coumaroyl-*β*-D-glucoside).

Compound **5** was obtained as a yellow powder, and its molecular formula, $C_{29}H_{34}O_{18}$, was determined by HRESIMS, which showed ions at m/z 693.1656 $[M + Na]^+$ and 671.1833 $[M + H]^+$. Its UV absorption pattern (λ_{max} 252, 265, 360 nm), as well as the characteristic signal for a chelated hydroxyl group (δ_H 12.55) in the ^{1}H NMR spectrum, indicated a flavonoid skeleton with a hydroxyl group at C-5. The NMR spectroscopic data of **5** resembled those of quercetin 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside],[8] except for the missing resonances of an ABX coupling system in ring B in **5**, showing instead the resonances for two methines at $\delta_{H/C}$ 7.48 (H-2′ and H-6′)/107.3 (C-2′ and C-6′) and for two methoxyl groups at $\delta_{H/C}$ 3.84/56.8 (OMe at C-3′ and C-5′). The HMBC correlations of H-2′ and H-6′ (δ_H 7.48) with C-2 (δ_C 156.7), C-1′ (δ_C 120.2), C-3′ and C-5′ (δ_C 147.9), and C-4′ (δ_C

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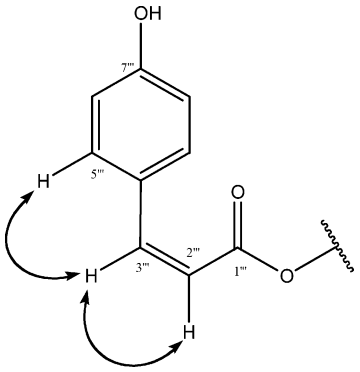


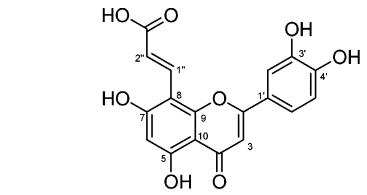
Figure 1. Selected NOE correlations of **3** and **4**.

139.1) and of methoxyl groups (δ_H 3.84) with C-3′ and C-5′ (δ_C 147.9) revealed a 3′,4′,5′-trisubstituted ring B with methoxyl groups at C-3′ and C-5′. Thus, compound **5** was established structurally as syringetin 3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside].

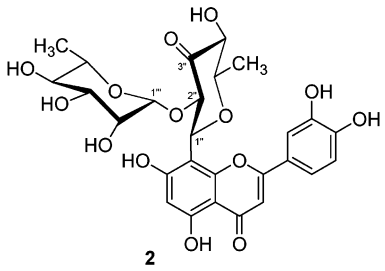
Compound **6** was obtained as a yellow powder. Its molecular formula, $C_{28}H_{32}O_{18}$, was established by HRESIMS *m*/*z* 657.1695 $[M + H]^+$. Similar to **5**, the ^1H and ^{13}C NMR spectroscopic data of **6** resembled those of quercetin 3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside],[8] showing resonances of an AX system [$\delta_{H/C}$ 7.56 (d, $J = 1.6$ Hz, H-2′)/106.1 (C-2′) and $\delta_{H/C}$ 7.13 (d, $J = 1.6$ Hz, H-6′)/109.9 (C-6′)] and a methoxyl group [$\delta_{H/C}$ 3.83/56.6 (OMe at C-3′), 145.8 (C-3′)] in **6**, instead of an ABX system in ring B. The NOESY correlation of H-2′ (δ_H 7.56) and methoxyl protons (δ_H 3.83) at C-3′ confirmed the position of the methoxyl at C-3′. Finally, the structure of **6** was identified as laricitin 3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside]. Compound **6** was discussed once in the literature,[9] without mentioning its source and NMR spectroscopic data. Thus, to the best of our knowledge, it is a new natural product.

The assignments of ^1H and ^{13}C NMR spectroscopic data for each compound were made as a result of extensive 2D NMR experiments, and the sugars were confirmed for compounds **2**, **5**, and **6** by acid hydrolysis and GC-MS analysis.

Moreover, 29 known compounds were isolated also from the leaves of *T. diffusa*. These were characterized as 2″-*O*-rhamnosylorientin,[4] 2″-*O*-rhamnosylvitexin,[4] luteolin 8-*C*-α-L-rhamnopyranosyl-(1→2)-quinovopyranoside,[4] echinacin,[7] echinaticin,[7] apigenin 7-*O*-β-D-glucoside,[4] chrysoeriol 7-*O*-β-D-glucoside,[10] tricin 7-*O*-β-D-glucoside,[11] acacetin,[12] acacetin 7-*O*-methyl ether,[13] velutin,[14] pinocembrin,[15] quercetin 3-*O*-[β-D-glucosyl-(1→6)-β-D-glucoside],[16] teuhetenone A,[17] 11-hydroxyeremophil-6,9-dien-8-one,[18] caryophyllene epoxide,[19] ficaprenol-11,[20] (*E*,*E*,*E*,*E*)-squalene,[21] *p*-arbutin,[4] 4-*O*-β-D-glucopyranosyl-*p*-coumaric acid,[22] maltol 3-*O*-glucoside,[23] tetraphyllin B,[24] methyl β-fructofuranoside, methyl α-fructofuranoside, glucose, fructose, rhamnose, sucrose, and sitosterol 3-*O*-β-D-glucoside. The structures of these compounds were identified on the basis of spectroscopic evidence and by comparison with literature values.



1



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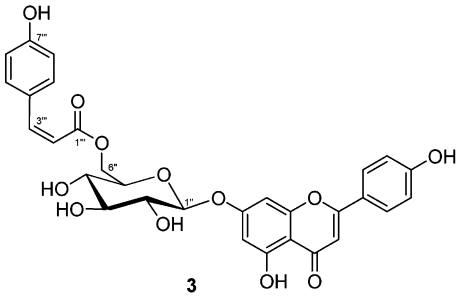

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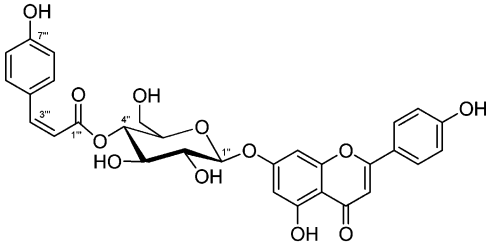

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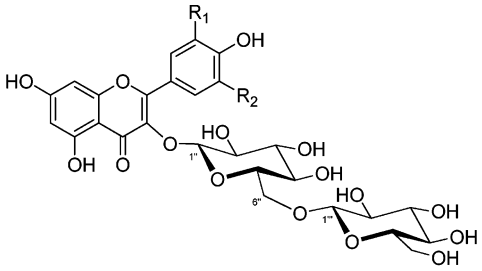

5 $R_1 = R_2 = OCH_3$

6 $R_1 = OCH_3$ $R_2 = OH$

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research AutoPol IV polarimeter. UV spectra were obtained on a Hewlett-Packard 8453 UV/vis spectrometer. IR spectra were recorded on Bruker Tensor 27 FT-IR and MIRacle ATR-FT-IR spectrometers. NMR spectra were recorded on a Bruker Avance DRX-400 NMR spectrometer. HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was performed using silica gel (J. T. Baker, 40 μm for flash chromatography), Sephadex LH-20 (Amersham Biosciences), and Biotage Horizon chromatography system with Flash cartridges (Biotage, Inc.). TLC was carried out on silica gel 60 GF_{254} plates (EM Science, Germany). GC-MS analysis was carried out on a HP 5890 II/5972 system [column: JW DB-5, 30 m × 0.25 mm, 0.25 μm; carrier gas: He; injection temperature: 280 °C; detection temperature: 280 °C; column temperature: 150 °C (1 min), 10 °C/min to 250 °C (20 min)]. Sugar standards were purchased from Sigma-Aldrich.

Plant Material. The leaves of *T. diffusa* (Lot #549.4105) were purchased from Frontier Natural Products Co., Norway, IA 52318. A voucher specimen (voucher #2433) has been deposited at the Herbarium of University of Mississippi.

Extraction and Isolation. The dried, cut, and sifted leaves of *T. diffusa* (910 g) were percolated at room temperature with MeOH (4 L × 3). The solvent was then evaporated under reduced pressure to yield 204 g of a MeOH extract. A $CHCl_3$-soluble portion (57 g) of the MeOH extract was subjected to column chromatography (VLC) over silica gel (880 g) and eluted with CH_2Cl_2, followed by EtOAc, EtOAc−MeOH (1:1), and MeOH to give four fractions (A−D). Caryophyllene

epoxide (30 mg), ficaprenol-11 (88 mg), and (*E*,*E*,*E*)-squalene (72 mg) were obtained from fraction A, initially by silica gel column chromatography (hexanes−EtOAc, 100:5 → 100:30), then using the Biotage chromatography system (CH_2Cl_2−EtOAc, 100:12). Fraction B (18.1 g) was treated in a manner similar to that of fraction A to afford acacetin (32 mg), acacetin 7-*O*-methyl ether (35 mg), velutin (23 mg), pinocembrin (60 mg), teuhetenone A (112 mg), and 11-hydroxyeremophil-6,9-dien-8-one (96 mg). The $CHCl_3$-insoluble portion of the MeOH extract (147 g) was dissolved with MeOH, filtered, and dried under vacuum to give a residue (108 g), which was then subjected to column chromatography over silica gel (1320 g), eluting with $CHCl_3$−MeOH and $CHCl_3$−MeOH−H_2O of increasing polarity to afford 14 fractions (1−14). Compounds **1** (47 mg), **4** (32 mg), echinaticin (380 mg), and sitosterol 3-*O*-*β*-D-glucoside (48 mg) were obtained from fraction 4 (5.5 g) by column chromatography over silica gel ($CHCl_3$−MeOH, 100:15), Sephadex LH-20, then the Biotage chromatography system, or by crystallization. Compounds **3** (54 mg) and echinacin (423 mg) were obtained from fraction 5 (4.8 g); **2** (73 mg), apigenin 7-*O*-*β*-D-glucoside (62 mg), chrysoeriol 7-*O*-*β*-D-glucoside (20 mg), tricin 7-*O*-*β*-D-glucoside (57 mg), and *p*-arbutin (376 mg) from fraction 6 (10.5 g); tetraphyllin B (620 mg), 4-*O*-*β*-D-glucopyranosyl-*p*-coumaric acid (20 mg), maltol 3-*O*-glucoside (76 mg), and L-rhamnose (63 mg) from fraction 7 (12.8 g); fructose (90 mg) from fraction 8 (6.2 g); luteolin 8-*C*-α-L-rhamnopyranosyl-(1→2)-quinovopyranoside (45 mg), glucose (150 mg), and sucrose (88 mg) from fraction 9 (8.6 g); 2″-*O*-rhamnosylvitexin (64 mg), **5** (41 mg), methyl *β*-fructofuranoside (72 mg), and methyl α-fructofuranoside (48 mg) from fraction 11 (6.2 g); and 2″-*O*-rhamnosylorientin (75 mg), quercetin 3-*O*-[*β*-D-glucosyl-(1→6)-*β*-D-glucoside] (15 mg), and **6** (25 mg) from fraction 12 (4.6 g), in a manner similar to the procedure described for fraction 1.

Luteolin 8-*C*-*E*-propenoic acid (1): fine yellow needles from MeOH−H_2O; mp 251−252 °C; UV (MeOH) λ_{max} (log ϵ) 249 (4.42), 284 (4.50), 345 (4.28) nm; IR (ATR) ν_{max} 3077, 1653, 1601, 1371, 1257, 1191, 839 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ_H 13.48 (1H, s, OH-5), 8.00 (1H, d, J = 16.0 Hz, H-1″), 7.38 (1H, d, J = 8.8 Hz, H-6′), 7.36 (1H, s, H-2′), 6.92 (1H, d, J = 8.8 Hz, H-5′), 6.80 (1H, d, J = 16.0 Hz, H-2″), 6.72 (1H, s, H-3), 6.34 (1H, s, H-6); ^{13}C NMR (MeOH-d_4, 100 MHz) δ_C 182.2 (C, C-4), 169.0 (C-3″), 165.0 (C, C-2), 164.7 (C, C-7), 162.8 (C, C-5), 156.3 (C, C-9), 150.3 (C, C-4′), 146.4 (C, C-3′), 133.4 (CH, C-1″), 122.3 (C, C-1′), 120.2 (CH, C-2″), 119.4 (CH, C-6′), 116.4 (CH, C-5′), 114.3 (CH, C-2′), 104.4 (C, C-10), 104.2 (CH, C-3), 102.3 (C, C-8), 99.3 (CH, C-6); HRESIMS *m*/*z* 357.0607 $[M + H]^+$ (calcd for $C_{18}H_{13}O_8$, 357.0610).

Luteolin 8-*C*-[6-deoxy-2-*O*-(α-L-rhamnopyranosyl)-*xylo*-hexopyranos-3-uloside] (2): yellow needles (MeOH−H_2O); mp 210 °C (dec); $[\alpha]^{25}_D$ −33.3 (*c* 0.072, MeOH); UV (CD_3OD) λ_{max} (log ϵ) 268 (4.28), 279 (4.27), 348 (4.32) nm; IR (ATR) ν_{max} 3470, 1651, 1609, 1571, 1260, 1091, 1056, 1017, 812 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) flavone aglycon δ_H 12.88 (1H, s, OH-5), 7.56 (1H, d, J = 2.4 Hz, H-2′), 7.51 (1H, dd, J = 8.4, 2.4 Hz, H-6′), 6.96 (1H, d, J = 8.4 Hz, H-5′), 6.63 (1H, s, H-3), 6.31 (1H, s, H-6), sugar moiety δ_H 5.20 (1H, d, J = 10.0 Hz, H-1″), 5.08 (1H, d, J = 10.0 Hz, H-2″), 4.79 (1H, br s, H-1‴), 4.25 (1H, d, J = 9.8 Hz, H-4″), 3.96 (1H, m, H-2‴), 3.62 (1H, m, H-5″), 3.30 (1H, dd, J = 9.6, 3.2 Hz, H-3‴), 3.15 (1H, dd, J = 9.6, 9.6 Hz, H-4‴), 2.37 (1H, m, H-5‴), 1.59 (1H, d, J = 7.0 Hz, H-6″), 0.70 (1H, d, J = 7.0 Hz, H-6‴); ^{13}C NMR (MeOH-d_4, 100 MHz) flavone aglycon δ_C 182.6 (C, C-4), 165.2 (C, C-2), 162.7 (C, C-5), 161.9 (C, C-7), 156.3 (C, C-9), 149.7 (C, C-4′), 146.0 (C, C-3′), 122.7 (C, C-1′), 119.1 (CH, C-6′), 115.3 (CH, C-5′), 113.6 (CH, C-2′), 104.5 (C, C-10), 103.2 (C, C-8), 102.7 (CH, C-3), 98.6 (CH, C-6), sugar moiety δ_C 205.3 (C-3″), 99.3 (CH, C-1‴), 79.4 (CH, C-5″), 78.7 (CH, C-4″), 77.0 (CH, C-2″), 74.3 (CH, C-1″), 71.7 (CH, C-4‴), 70.6 (CH, C-2‴), 70.3 (CH, C-3‴), 69.0 (CH, C-5‴), 18.2 (CH_3, C-6″), 16.3 (CH_3, C-6‴); HRESIMS *m*/*z* 577.1578 $[M + H]^+$ (calcd for $C_{27}H_{29}O_{14}$, 577.1557).

Apigenin 7-*O*-(6″-*O*-*p*-*Z*-coumaroyl-*β*-D-glucopyranoside) (3): pale yellow powder (MeOH−$CHCl_3$); $[\alpha]^{25}_D$ −164.5 (*c* 0.062, MeOH); UV (MeOH) λ_{max} (log ϵ) 269 (4.44), 318 (4.55) nm; IR (ATR) ν_{max} 3268, 1659,1594, 1494, 1450, 1242, 1176, 1069, 828 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) flavone aglycon δ_H 7.92 (2H, d, J = 8.4 Hz, H-2′, 4′), 6.94 (2H, d, J = 8.4 Hz, H-3′, 5′), 6.80 (1H, s, H-3), 6.77 (1H, d, J = 2.0 Hz, H-8), 6.42 (1H, d, J = 2.0 Hz, H-6), sugar moiety δ_H 5.13 (1H, d, J = 7.2 Hz, H-1″), 4.41 (1H, br d, J = 7.6 Hz, H-6″), 4.23 (1H, dd, J = 12.0, 7.6 Hz, H-6″), 3.79 (1H, m, H-5″), 3.34 (1H, m, H-3″), 3.33 (1H, m, H-2″), 3.23 (1H, m, H-4″), coumaroyl moiety δ_H 7.52 (2H, d, J = 8.4 Hz, H-5‴, 9‴), 6.65 (2H, d, J = 8.4 Hz, H-6‴, 8‴), 6.61 (1H, d, J = 12.8 Hz, H-3‴), 5.76 (1H, d, J = 12.8 Hz, H-2‴); ^{13}C NMR (DMSO-d_6, 100 MHz) flavone aglycon δ_C 182.4 (C, C-4), 164.7 (C, C-2), 163.0 (C, C-7), 161.8 (C, C-6′), 161.6 (C, C-5), 157.4 (C, C-9), 129.0 (2CH, C-2′, 4′), 121.6 (C, C-1′), 116.5 (2CH, C-3′, 5′), 105.9 (C, C-10), 103.6 (CH, C-3), 100.0 (CH, C-6), 95.1 (CH, C-8), sugar moiety δ_C 100.0 (CH, C-1″), 76.7 (CH, C-3″), 74.6 (CH, C-5″), 73.5 (CH, C-2″), 70.4 (CH, C-4″), 63.5 (CH_2, C-6″), coumaroyl moiety δ_C 166.2 (C═O, C-1‴), 159.3 (C, C-7‴), 144.0 (CH, C-3‴), 133.0 (2 × CH, C-5‴, 9‴), 125.6 (C, C-4‴), 115.2 (CH, C-2‴), 115.2 (2 × CH, C-6‴, 8‴); HRESIMS *m*/*z* 579.1511 $[M + H]^+$ (calcd for $C_{30}H_{27}O_{12}$, 579.1503).

Apigenin 7-*O*-(4″-*O*-*p*-*Z*-coumaroyl-*β*-D-glucopyranoside) (4): pale yellow powder (MeOH−$CHCl_3$); $[\alpha]^{25}_D$ −93.3 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 269 (4.41), 319 (4.57) nm; IR (ATR) ν_{max} 3288, 1658,1606, 1511, 1246, 1152, 1081, 1039, 832 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) flavone aglycon δ_H 7.95 (2H, d, J = 8.8 Hz, H-2′, 4′), 6.95 (2H, d, J = 8.8 Hz, H-3′, 5′), 6.86 (1H, s, H-3), 6.86 (1H, d, J = 2.0 Hz, H-8), 6.49 (1H, d, J = 2.0 Hz, H-6), sugar moiety δ_H 5.21 (1H, d, J = 7.6 Hz, H-1″), 4.80 (1H, dd, J = 10.0, 9.6 Hz, H-4″), 3.79 (1H, m, H-5″), 3.59 (1H, m, H-3″), 3.44 (1H, m, H-6″), 3.38 (1H, m, H-2″), 3.35 (1H, m, H-6″), coumaroyl moiety δ_H 7.71 (2H, d, J = 8.8 Hz, H-5‴, 9‴), 6.91 (1H, d, J = 12.8 Hz, H-3‴), 6.77 (2H, d, J = 8.8 Hz, H-6‴, 8‴), 5.80 (1H, d, J = 12.8 Hz, H-2‴); ^{13}C NMR (DMSO-d_6, 100 MHz) flavone aglycon δ_C 182.5 (C, C-4), 164.8 (C, C-2), 163.3 (C, C-7), 161.8 (C, C-6′), 161.6 (C, C-5), 157.4 (C, C-9), 129.1 (2CH, C-2′, 4′), 121.5 (C, C-1′), 116.5 (2CH, C-3′, 5′), 105.9 (C, C-10), 103.6 (CH, C-3), 100.1 (CH, C-6), 95.4 (CH, C-8), sugar moiety δ_C 100.0 (CH, C-1″), 75.2 (CH, C-5″), 74.2 (CH, C-3″), 73.8 (CH, C-2″), 71.1 (CH, C-4″), 60.9 (CH_2, C-6″), coumaroyl moiety δ_C 165.5 (C″O, C-1‴), 159.4 (C, C-7‴), 144.2 (CH, C-3‴), 133.2 (2 × CH, C-5‴, 9‴), 125.9 (C, C-4‴), 115.7 (CH, C-2‴), 115.4 (2 × CH, C-6‴, 8‴); HRESIMS *m*/*z* 579.1499 $[M + H]^+$ (calcd for $C_{30}H_{27}O_{12}$, 579.1503).

Syringetin 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (5): yellow powder (MeOH); $[\alpha]^{25}_D$ −9.7 (*c* 0.031, MeOH); UV (MeOH) λ_{max} (log ϵ) 252 (4.26), 265 (4.25), 360 (4.29) nm; IR (ATR) ν_{max} 3379, 1655,1609, 1566, 1494, 1457, 1359, 1191, 1053, 1031, 1009, 810, 775 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) flavone aglycon δ_H 7.48 (2H, s, H-2′, 6′), 6.49 (1H, d, J = 2.0 Hz, H-8), 6.21 (1H, d, J = 2.0 Hz, H-6), 3.84 (6H, s, OCH_3-3′ and OCH_3-5′), sugar moiety δ_H 5.53 (1H, d, J = 7.2 Hz, H-1″), 4.09 (1H, d, J = 7.6 Hz, H-1‴), 3.88 (1H, m, H-6″), 3.50 (1H, m, H-6″), 3.48 (1H, m, H-6‴), 3.27 (1H, m, H-6‴), 3.34 (1H, m, H-5‴), 3.32 (1H, m, H-3″), 3.22 (1H, m, H-2″), 3.17 (1H, m, H-4‴), 3.00 (1H, m, H-3‴), 2.93 (1H, m, H-4″), 2.83 (1H, m, H-2‴), 2.76 (1H, m, H-5‴); ^{13}C NMR (DMSO-d_6, 100 MHz) flavone aglycon δ_C 177.8 (C, C-4), 164.6 (C, C-7), 161.6 (C, C-5), 156.9 (C, C-9), 156.6 (C, C-2), 147.9 (2C, C-3′, 5′), 139.1 (C, C-4′), 133.7 (C, C-3), 120.2 (C, C-1′), 107.3 (2CH, C-2′, 6′), 104.6 (C, C-10), 99.2 (CH, C-6), 94.5 (CH, C-8), sugar moiety δ_C 103.4 (CH, C-1‴), 101.4 (CH, C-1″), 77.1 (CH, C-3‴), 76.9 (CH, C-5‴), 75.86 (CH, C-5″), 76.7 (CH, C-3″), 74.7 (CH, C-2″), 73.8 (CH, C-2‴), 70.16 (CH, C-4‴), 70.15 (CH, C-4″), 68.1 (CH_2, C-6″), 61.2 (CH_2, C-6‴), 56.8 (2×CH_3, OCH_3-3′ and OCH_3-5′); HRESIMS *m*/*z* 671.1833 $[M + H]^+$ (calcd for $C_{29}H_{35}O_{18}$, 671.1823).

Laricitin 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (6): yellow powder (MeOH); $[\alpha]^{25}_D$ −3.8 (*c* 0.052, MeOH); UV (MeOH) λ_{max} (log ϵ) 257 (4.28), 270 (4.27), 360 (4.28) nm; IR (ATR) ν_{max} 3321, 1655, 1616, 1561, 1451, 1367, 1263, 1192, 1043, 988, 811 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) flavone aglycon δ_H 7.56 (H, d, J = 1.6 Hz, H-2′), 7.13 (H, d, J = 1.6 Hz, H-6′), 6.41 (1H, d, J = 2.0 Hz, H-8), 6.20 (1H, d, J = 2.0 Hz, H-6), 3.83 (3H, s, OCH_3-3′), sugar moiety δ_H 5.53 (1H, d, J = 7.2 Hz, H-1″), 4.10 (1H, d, J = 7.6 Hz, H-1‴), 3.88 (1H, m, H-6‴), 3.51 (1H, m, H-6‴), 3.48 (1H, m, H-6‴), 3.26 (1H, m, H-6‴), 3.34 (1H, m, H-5‴), 3.33 (1H, m, H-3″), 3.22 (1H, m, H-2″), 3.15 (1H, m, H-4‴), 2.99 (1H, m, H-3‴), 2.93 (1H, m, H-4″), 2.83 (1H, m, H-2‴), 2.76 (1H, m, H-5″); ^{13}C NMR (DMSO-d_6, 100 MHz) flavone aglycon δ_C 177.8 (C, C-4), 164.6 (C, C-7), 161.7 (C, C-5), 156.8 (C, C-9), 156.8 (C, C-2), 148.1 (C, C-3′), 145.8 (C, C-5′), 137.9 (C, C-4′), 133.7 (C, C-3), 121.6 (C, C-1′), 109.9 (CH, C-6′), 106.1 (CH, C-2′), 104.5 (C, C-10), 99.2 (CH, C-6), 94.1 (CH, C-8), sugar moiety δ_C 103.6 (CH, C-1‴), 101.3 (CH, C-1″), 77.1 (CH, C-3‴), 77.0 (CH, C-5‴), 76.9 (CH, C-5″), 76.8 (CH, C-3″), 74.6 (CH, C-2″), 73.9 (CH, C-2‴), 70.18 (CH, C-4‴), 70.17 (CH, C-4″), 68.3

(CH_2, C-6″), 61.2 (CH_2, C-6‴), 56.6 (CH_3, OCH_3-3′); HRESIMS *m*/*z* 657.1695 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{18}$, 657.1667).

Acid Hydrolysis of Compounds 2, 5, and 6. A solution of each compound (2 mg) in 1 N HCl (1 mL) was heated at 80 °C in a stoppered reaction vial for 4 h. Each reaction mixture after drying under reduced pressure was loaded onto a small column packed with polyamide and eluted with water. The eluate was concentrated to dryness. It was then dissolved in pyridine (1 mL) and reacted with L-cysteine methyl ester hydrochloride (1−2 mg) at 60 °C for 1 h. An equal volume of acetic anhydride was added, and the mixture continued to react for a further 1 h. The acetylated thiazolidine derivatives were then applied to GC-MS to determine the absolute configuration of the sugars by comparing their retention times with those of acetylated thiazolidine derivatives of standard D-glucose (23.40 min) and L-rhamnose (17.02 min).[25]

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