New 2′-Oxygenated Flavonoids from *Andrographis affinis*

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Three new 2′-oxygenated flavonoids, (2*S*)-5,7,2′,3′,4′-pentamethoxyflavanone (**1**), 5-hydroxy-7,8,2′,5′-tetramethoxyflavone (**2**), and echioidinin 2′-*O*-β-D-(6″-*O*-acetyl) glucopyranoside (**3**), together with four known flavonoids, 7-*O*-methyldihydrowogonin, 7-*O*-methylwogonin, skullcapflavone I 2′-methyl ether, and skullcapflavone I, and two diterpenoids, andrograpanin and 14-deoxy-11,12-didehydroandrographolide, were isolated from the whole plant of *Andrographis affinis*. The structures were elucidated by spectral and chemical studies.

Andrographis affinis Nees (Acanthaceae) is a slender undershrub found widely in the Pulney and Nilgiri Hills of Tamilnadu, South India.[1] In a continuation of our investigations on flavonoid constituents of *Andrographis* species,[2−7] we investigated the whole plant of *A. affinis*, a plant hitherto not examined for its chemical constituents. In this study we report the isolation and characterization of seven flavonoids and two diterpenoids from this plant. Six known compounds, including four flavonoids and two diterpenoids, were identified as 7-*O*-methyldihydrowogonin, 7-*O*-methylwogonin, skullcapflavone I 2′-methyl ether, skullcapflavone I, andrograpanin, and 14-deoxy-11,12-didehydroandrographolide, respectively, by comparison of their physical and spectral data with literature values.[5,8−10]

Compound **1** was obtained as colorless needles. The HRESIMS of **1** showed a peak at *m*/*z* 375.1521 $[M + H]^+$ corresponding to the molecular formula $C_{20}H_{22}O_7$. The ^{13}C NMR spectrum of **1** showed resonances for all 20 carbons present in the molecule. The UV spectrum showed absorption maxima at 282 and 320 (sh) nm characteristic of a flavanone moiety.[11] Addition of $AlCl_3$ and NaOAc caused no shift in the UV absorption maxima, indicating the absence of free hydroxyl groups at the 5- and 7-positions, respectively.

The ^1H NMR spectrum of **1** showed signals for five aromatic methoxyl groups at δ 3.88, 3.87, 3.85, 3.84, and 3.78. Three sets of double doublets at δ 5.64 (1H, $J = 13.3$, 2.8 Hz), 3.03 (1H, $J = 16.6$, 13.3 Hz), and 2.71 (1H, $J = 16.6$, 2.8 Hz) were typical of H-2, $H\text{-}3_{ax}$, and $H\text{-}3_{eq}$, respectively, of a flavanone moiety.[12] Two of the five methoxyl groups at δ 3.87 and 3.78 were placed at C-5 and C-7, respectively, as indicated by ^3J correlations with carbons at δ 162.3 (C-5) and 165.5 (C-7) in the HMBC spectrum. These assignments were further supported by NOE correlations of C-5 methoxyl protons (δ 3.87) with H-6, and C-7 methoxyl protons (δ 3.78) with H-6 (δ 6.06) and H-8 (δ 6.10) in the NOESY spectrum. The chemical shift of C-2 in 2′-unsubstituted flavanones[13] usually appears at δ 79.0. However, in compound **1**, the C-2 signal appeared at an upfield position (δ 74.2), indicating the presence of oxygenation at C-2′. The methoxyl group at δ 3.88 was placed at C-2′ on the basis of HMBC correlation with C-2′ (δ 151.3). The ^1H NMR spectrum also showed a typical AB system for two *ortho*-coupled aromatic protons at δ 6.71 (d, $J = 8.7$ Hz) and 7.18 (d, $J = 8.7$ Hz), assigned to ring B. In the NOESY spectrum, one of the *ortho*-coupled aromatic protons at δ 7.18 showed a strong NOE correlation with $H\text{-}3_{ax}$ (δ 3.03), thus indicating its attachment to C-6′ and placing the other *ortho*-coupled aromatic proton at C-5′. The $^1H-^1H$ COSY correlations between the protons at δ 6.71 and 7.18 also supported their placement at C-5′ and C-6′, respectively. The remaining two methoxyl groups at δ 3.84 and 3.85 should therefore be placed at C-3′ and C-4′. The methoxyl group at δ 3.85 was placed on C-4′ on the basis of its correlation with H-5′ (δ 6.71) in the NOESY spectrum. The remaining methoxyl group (δ 3.84) was assigned to C-3′, as evidenced by the appearance of a ^{13}C NMR signal at δ 60.7, characteristic of a methoxyl group in a di-*ortho*-substituted environment.[8] The absolute configuration at C-2 was determined to be *S*[14] on the basis of the negative Cotton effect at 287 nm observed in the CD spectrum of **1**. Thus, the structure of **1** was established as (2*S*)-5,7,2′,3′,4′-pentamethoxyflavanone. Incidentally, **1** is the only known naturally occurring flavanone with 2′,3′,4′-trioxygenation in ring B.

Compound **2** was isolated as a yellow solid. HRESIMS showed a peak at *m*/*z* 359.1050 $[M + H]^+$ corresponding to the molecular formula $C_{19}H_{18}O_7$. The ^{13}C NMR spectrum of **2** showed resonances for all 19 carbons present in the molecule. The UV spectrum showed absorption maxima at 272 and 358 nm. A bathochromic shift of 25 nm in band I absorption maximum with $AlCl_3$/HCl suggested that compound **2** was a 5-hydroxyflavone.[15]

The ^1H NMR spectrum of **2** showed a D_2O exchangeable downfield signal at δ 12.62 assigned to a chelated hydroxyl group at C-5. In the HSQC spectrum, a sharp one-proton singlet at δ 7.06 correlating with C-3 (δ 110.5) was ascribed to H-3 of a 2′-oxygenated flavone.[16] In the ^1H NMR spectrum, a sharp one-proton singlet at δ 6.40 was attributed to H-6. This was further substantiated by a HMBC correlation observed with C-5 (δ 157.5) and a long-range NOESY correlation with 5-OH (δ 12.62). The ^1H NMR spectrum also showed a typical ABX system for three aromatic protons at δ 6.96 (d, $J = 9.04$ Hz), 7.01 (dd, $J = 3.08$, 9.04 Hz), and 7.51 (d, $J = 3.08$ Hz) characteristic of a 2′,5′-dioxygenated flavone.[17] In addition, the chemical shift values of the B-ring carbons of **2** appearing at δ 152.5 and 153.5 were very similar to those observed for the B-ring

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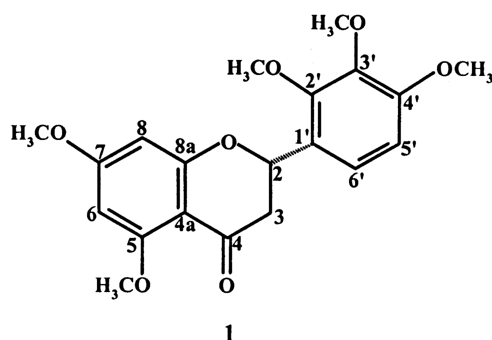

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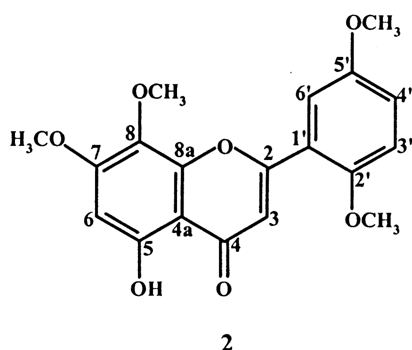

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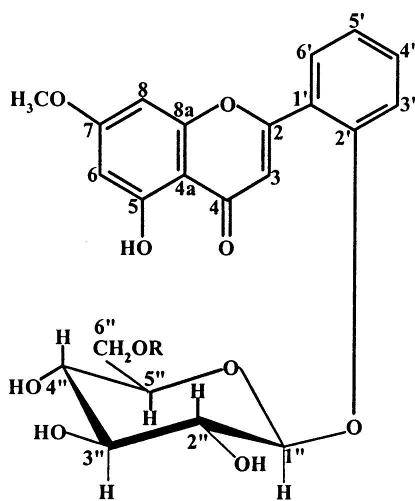

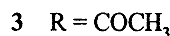

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carbons of 2′,5′-dioxygenated flavones.[17,18] The ^{1}H NMR spectrum also exhibited signals for four methoxyl groups at δ 3.91, 3.90, 3.88, and 3.83. The methoxyl group at δ 3.91 was placed at C-7 on the basis of HMBC correlation observed between the methoxyl protons and carbon 7 (δ 158.5) and NOE correlation with H-6 (δ 6.40) in the NOESY spectrum. The second methoxyl group at δ 3.90 was placed at C-8, as it showed long-range correlation with H-6′ (δ 7.51) in the NOESY spectrum. The methoxyl groups at δ 3.88 and 3.83 were placed at C-2′ and C-5′ on the basis of NOE correlations of C-2′ methoxyl protons (δ 3.88) with H-3 (δ 7.06) and H-3′ (δ 6.96), and C-5′ methoxyl protons (δ 3.83) with H-6′ (δ 7.51) and H-4′ (δ 7.01) in the NOESY spectrum. Thus, the structure of compound **2** was elucidated as 5-hydroxy-7,8,2′,5′-tetramethoxyflavone.

Compound **3** was isolated as a greenish-yellow crystalline solid. HRESIMS analysis established the molecular formula to be $C_{24}H_{24}O_{11}$. This was corroborated by a decoupled ^{13}C NMR spectrum which showed signals for all 24 carbons present in the molecule. The UV spectrum showed absorption maxima at 265 and 320 nm. This along with a positive Molisch's test indicated a flavone glycoside.[19] A bathochromic shift of 45 nm in band I absorption maximum with $AlCl_3$/HCl suggested it to be a 5-hydroxyflavone.[15] The addition of NaOAc caused no shift in the UV absorption maxima (265 nm), indicating the absence of a free hydroxyl group at the 7-position. The IR spectrum of **3**, apart from hydroxyl (3300 cm^{-1}) and flavone carbonyl (1650 cm^{-1}) absorption bands, showed an additional carbonyl absorption band at 1725 cm^{-1}, indicating the presence of an ester group in **3**.

The ^{1}H NMR spectrum of **3** was very similar to echioidin[6,20] (**3a**) except for an additional signal at δ 1.98 (s, 3H), which, in conjunction with two carbon resonances at δ 170.1 and 20.5 in its ^{13}C NMR spectrum, showed the presence of an acetyl moiety in **3**. Alkaline hydrolysis of **3** gave **3a**, indicating that the acetyl moiety was attached to a glucosyl residue. The acetyl moiety in **3** was found to be linked to the C-6″-hydroxyl of glucose,[21] as this carbon signal was shifted downfield by 2.7 ppm while the C-5″ signal was shifted upfield by 3.4 ppm compared to **3a**. The site of esterification in **3** was also revealed by downfield shifts of δ 0.59 and 0.52 observed for H-6″a (δ 4.05, dd, J = 11.9, 6.6 Hz) and H-6″b (δ 4.27, dd, J = 11.9, 1.9 Hz), respectively, compared with H-6″a (δ 3.46, dd, J = 11.7, 6.0 Hz) and H-6″b (δ 3.75, dd, J = 11.7, 1.6 Hz) of **3a**. An acetyl moiety at C-6″ in **3** was further supported by the presence of a cross-peak between the C-6″ methylene protons (δ 4.05 and 4.27) of the glucose residue and carbonyl carbon (δ 170.1) of the acetyl moiety in the HMBC spectrum. Thus, the structure of compound **3** was established as echioidinin 2′-*O*-β-D-(6″-*O*-acetyl)glucopyranoside.

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured in MeOH at 25 °C on a Perkin-Elmer 241 polarimeter. IR spectra were recorded in KBr disks on a Perkin-Elmer 283 double-beam spectrophotometer. UV spectra were obtained on a Shimadzu UV-240 spectrometer. The CD spectrum was recorded in MeOH at 25 °C on a JASCO J 715 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were determined on Bruker Avance 400 and Bruker AC 300 spectrometers using DMSO-d_6 and $CDCl_3$, with TMS as internal standard. HSQC, HMBC, and phase-sensitive NOESY (with 500 ms mixing time) spectra were recorded using the standard pulse sequence. HRESIMS and ESIMS/MS were recorded in positive ion mode on an API Q-STAR PULSA of Applied Bio-system. CC was performed on Acme silica gel finer than 200 mesh (0.08 mm).

Plant Material. The whole plant of *A. affinis* Nees was collected in January 2001 from the hills of Nilgiri, South India. A voucher specimen (DG 002) was deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

Extraction and Isolation. Air-dried and powdered whole plant (3 kg) was successively extracted with *n*-hexane, Me_2CO, and MeOH. The *n*-hexane extract was purified over a silica gel column using *n*-hexane and EtOAc step gradient as eluents. The *n*-hexane−EtOAc, 8:2 and 1:1, eluates yielded **1** (15 mg) and **2** (20 mg), respectively. The Me_2CO extract was defatted with *n*-hexane, and the residue obtained was purified over a silica gel column using an *n*-hexane−EtOAc step gradient. The *n*-hexane−EtOAc, 9:1, 8:2, 7:3, 1:1, and 1:9, eluates yielded 7-*O*-methyldihydrowogonin (25 mg), 7-*O*-methylwogonin (20 mg), skullcapflavone I 2′-methyl ether (30 mg), skullcapflavone I (18 mg), and **3** (35 mg), respectively.

The MeOH extract was triturated with *n*-BuOH. The BuOH-soluble part on further purification over a silica gel column using *n*-hexane−EtOAc mixtures, 3:7 and 2:8, afforded andrograpanin (1.7 g) and 14-deoxy-11,12-didehydroandrographolide (1.2 g), respectively.

5,7,2′,3′,4′-Pentamethoxyflavanone (1): colorless needles ($CHCl_3$); mp 166−168 °C; $[\alpha]_D^{25}$ −21.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (4.39), 320 (sh) (4.05), (MeOH + NaOAc) 283, 320 (sh) (MeOH + $AlCl_3$) 281, 320 (sh) nm; CD (MeOH) λ nm ($\Delta\epsilon$) 287 (−0.23), 320 (+0.07); IR (KBr) ν_{max} 1672 (C=O), 1609, 1576, 1499, 1461, 1359 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.18 (1H, d, J = 8.7 Hz, H-6′), 6.71 (1H, d, J = 8.7 Hz, H-5′), 6.10 (1H, d, J = 2.3 Hz, H-8), 6.06 (1H, d, J = 2.3 Hz, H-6), 5.64 (1H, dd, J = 13.3, 2.8 Hz, H-2), 3.88 (3H, s, 2′-OMe), 3.87 (3H, s, 5-OMe), 3.85 (3H, s, 4′-OMe), 3.84 (3H, s, 3′-OMe), 3.78 (3H, s, 7-OMe) 3.03 (1H, dd, J = 16.6, 13.3 Hz, H-3_{ax}), 2.71 (1H, dd, J = 16.6, 2.8 Hz, H-3_{eq}); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 189.8 (C-4), 165.5 (C-7), 165.3 (C-8a), 162.3 (C-5), 154.0 (C-4′), 151.3 (C-2′), 142.0 (C-3′), 124.7 (C-1′), 121.3 (C-6′), 107.3 (C-5′), 106.0 (C-4a), 93.4 (C-8), 93.0 (C-6), 74.2 (C-2), 61.3 (2′-OMe), 60.7 (3′-OMe), 56.1 (5-OMe), 56.0 (4′-OMe), 55.5 (7-OMe), 44.8 (C-3); ESIMS/MS (positive mode) *m*/*z* 375.1 $[M + H]^+$ (13), 221.0 ($^{0,4}B^+ - H_2O$) (18), 206.1 ($^{0,4}B^+ - H_2O - Me$) (10), 191.0 ($^{0,4}B^+ - H_2O - 2Me$) (18), 181.1 ($^{1,3}A^+$) (100), 166.0 ($^{1,3}A^+ - Me$) (24), 138.0 ($^{1,3}A^+ - Me - CO$) (35); HRESIMS *m*/*z* 375.1521 $[M + H]^+$ (calcd for $C_{20}H_{22}O_7$ + H, 375.1443).

5-Hydroxy-7,8,2′,5′-tetramethoxyflavone (2): yellow solid ($CHCl_3$); mp 195−197 °C; UV (MeOH) λ_{max} (log ϵ) 272 (3.65), 358 (3.25), (MeOH + NaOAc) 272, 358, (MeOH + $AlCl_3$/HCl) 280, 383 nm; IR (KBr) ν_{max} 3380 (OH), 2900 (OMe), 1650 (C=O), 1580, 1500, 1460, 1240, 1210 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.62 (5-OH), 7.51 (1H, d, J = 3.08 Hz, H-6′), 7.06 (1H, s, H-3), 7.01 (1H, dd, J = 9.0, 3.0 Hz, H-4′) 6.96 (1H, d, J = 9.0 Hz, H-3′), 6.40 (1H, s, H-6), 3.92 (3H, s, 7-OMe), 3.90 (3H, s, 8-OMe), 3.88 (3H, s, 2′-OMe), 3.83 (3H, s, 5′-OMe); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.1 (C-4), 161.0 (C-2), 158.5 (C-7), 157.5 (C-5), 153.5 (C-5′), 152.5 (C-2′), 149.5 (C-8a), 129.0 (C-8), 120.7 (C-1′), 118.3 (C-4′), 113.9 (C-6′), 113.0 (C-3′), 110.5 (C-3), 104.8 (C-4a), 95.4 (C-6), 61.5 (8-OMe), 56.3 (7-OMe), 56.1 (2′-OMe), 55.7 (5′-OMe); ESIMS/MS (positive mode) *m*/*z* 359.1 $[M + H]^+$ (2), 344.0 $[M + H - Me]^+$ (5), 331.0 $[M + H - CO]^+$ (5), 329.0 $[M + H - 2Me]^+$ (100), 314.0 $[M + H - 3Me]^+$ (18), 300.0 $[M + H - 4Me]^+$ (13), 286.0 $[M + H - 3Me - CO]^+$ (65), 197.0 ($^{1,3}A^+$) (2), 163.0 ($^{1,3}B^+$) (2); HRESIMS *m*/*z* 359.1050 $[M + H]^+$ (calcd for $C_{19}H_{18}O_7$ + H, 359.1130).

Echioidinin 2′-O-β-D-(6″-*O*-acetyl)glucopyranoside (3): greenish-yellow crystalline solid (MeOH); mp 278−280 °C; $[\alpha]_D^{25}$ −64.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 265 (3.90), 320 (3.66), (MeOH + NaOAc) 268, 320, (MeOH + $AlCl_3$/HCl), 272, 365 nm; IR (KBr) ν_{max} 3300 (OH), 2850 (OMe) 1725 (ester C=O), 1650 (C=O), 1610, 1600, 1450, 1100 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.87 (5-OH), 7.91 (1H, dd, J = 7.8, 1.6 Hz, H-6′), 7.56 (1H, ddd, J = 7.8, 7.8 1.6 Hz, H-4′), 7.32 (1H, dd, J = 7.8, 1.6 Hz, H-3′), 7.21 (1H, ddd, J = 7.8, 7.8 1.6 Hz, H-5′), 7.04 (1H, s, H-3), 6.74 (1H, d, J = 2.2, Hz, H-8), 6.38 (1H, d, J = 2.2, Hz, H-6), 5.15 (1H, d, J = 7.3 Hz, H-1″), 4.05 (1H, dd, J = 11.9, 6.6 Hz, H-6″a) 4.27 (1H, dd, J = 11.9, 1.8 Hz, H-6″b), 3.85 (3H, s, 7-OMe), 3.60 (1H, ddd, J = 9.5, 6.6, 1.9 Hz, H-5″), 3.21−3.32 (3H, m, H-2″,3″,4″), 1.98 (3H, s, OAc-6″); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 182.0 (C-4), 170.1 (6″-O*C*OCH$_3$), 165.2 (C-7), 161.1 (C-5), 161.0 (C-2), 157.5 (C-8a), 155.2 (C-2′), 132.9 (C-4′), 129.1 (C-6′), 122.1 (C-5′), 120.3 (C-1′), 115.7 (C-3′), 110.4 (C-3), 104.7 (C-4a), 100.1 (C-1″), 97.9 (C-6), 92.6 (C-8), 76.7 (C-3″), 73.7 (C-5″), 73.1 (C-2″), 69.6 (C-4″), 63.2 (C-6″), 56.0 (7-OMe), 20.5 (6″-OCO*C*H$_3$); ESIMS/MS (positive mode) *m*/*z* 489.2 $[M + H]^+$ (2), 285.0 [M + H − acetyl glucosyl]$^+$ (100), 167.0 ($^{1,3}A^+$) (5), 119.0 ($^{1,3}B^+$) (3); HRESIMS *m*/*z* 489.1317 $[M + H]^+$ (calcd for $C_{24}H_{24}O_{11}$ + H, 489.1396).

Alkaline Hydrolysis of 3. A solution of compound **3** (5 mg) in 1% KOH (5 mL) was refluxed for 2 h. The reaction mixture was acidified with 1 N HCl and extracted with Et_2O followed by *n*-BuOH. The residue obtained from the *n*-BuOH extract was crystallized from MeOH to yield **3a** (4 mg), mp 276−278 °C; UV, IR, ^{1}H NMR, and MS data were in good agreement with literature values;[20] ^{13}C NMR (DMSO-d_6, 75 MHz) δ 182.1 (C-4), 165.2 (C-7), 161.1 (C-5), 161.0 (C-2), 157.5 (C-8a), 155.5 (C-2′), 133.0 (C-4′), 129.9 (C-6′), 121.9 (C-5′), 120.0 (C-1′), 115.5 (C-3′), 110.4 (C-3), 104.7 (C-4a), 100.2 (C-1″), 97.9 (C-6), 92.6 (C-8), 77.1 (C-5″), 76.7 (C-3″), 73.3 (C-2″), 69.5 (C-4″), 60.5 (C-6″), 56.0 (7-OMe).

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