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FLAVONOIDS FROM *Croton laevigatus*[a]

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Eight diterpenoids, one with the *trans*-clerodane and seven with cembrane skeletons, including one phenylpropylbenzoate, one lignanoid, two megastigmane glycosides, and two steroids, were isolated previously from leaves of *Croton laevigatus* Vahl. (Euphorbiaceae) [1–3]. In continuation of these studies, we communicate the isolation and identification of 11 flavonoids.

Air-dried ground leaves of *C. laevigatus* (20 kg) were extracted with MeOH (3 × 80 L). The obtained extract was condensed by evaporation *in vacuo* (2120 g). The residue was mixed with H_2O (8 L) and extracted successively with petroleum ether, CH_2Cl_2, and *n*-BuOH (3 × 8.0 L). The BuOH fraction was evaporated *in vacuo*. The solid (250 g) was dissolved in H_2O and chromatographed over a column of macroporous resin HPD-400 with elution successively by H_2O and EtOH (10, 30, 60, and 95%) to afford five fractions (fractions 1–5). The fraction obtained by elution with EtOH (95%) (fraction 5, 12.1 g) was passed over a chromatographic column packed with silica gel and eluted by $CHCl_3$:MeOH (50:1→0:1) to give isorhamnetin (**1**, 15 mg). The fraction obtained by extraction with EtOH (60%) (fraction 4, 50 g) was chromatographed over silica gel ($CHCl_3$:MeOH, 9:1→0:1) to isolate seven subfractions (fractions 4a–4g). Fraction 4c was again separated over a column of silica gel using $CHCl_3$:MeOH (9:1→4:1) and $CHCl_3$:MeOH:H_2O (6:1:0.05) to afford kaempferol 3-*O*-α-L-arabinoside (**4**, 8 mg) and isorhamnetin-3-*O*-(6″-*O*-*E*-caffeoyl)-β-D-galactopyranoside (**5**, 12 mg). Fraction 4d (10 g) was eluted from the silica gel column by $CHCl_3$:MeOH (20:1→0:1) and $CHCl_3$:Me_2CO (9:1) and was purified over Sephadex LH-20 with elution by MeOH to afford kaempferol (**2**, 2 mg), quercetin (**3**, 10 mg), and isorhamnetin-3-*O*-rutinoside (**6**, 20 mg). Repeated chromatography of fraction 4e (10 g) over a silica gel column ($CHCl_3$:MeOH, 4:1→0:1) and purification over Sephadex LH-20 isolated kaempferol-3-*O*-β-D-galactoside (**7**, 20 mg), kaempferol-3-*O*-rutinoside (**8**, 5 mg), kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside (**9**, 20 mg), and kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (mauritianin) (**10**, 1.5 g). Separation of fraction 4f (5.4 g) over a silica gel column using $CHCl_3$:MeOH:H_2O (3:1:0.025→0:1:0.025) isolated kaempferol-3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (**11**, 2 g).

Compounds **1**, **4**–**7**, and **9**–**11** were identified using PMR and ^{13}C NMR spectral data. Compounds **2**, **3**, and **8** when combined with authentic samples did not depress melting points and were identified as kaempferol, quercetin, and kaempferol-3-*O*-rutinoside, respectively.

Isorhamnetin (1), yellow amorphous powder, $C_{16}H_{12}O_7$, mp 303–305°C. The PMR and ^{13}C NMR spectra agreed with those in the literature [4].

Kaempferol-3-*O*-α-L-arabinoside (4), yellow amorphous powder, $C_{20}H_{18}O_{10}$. ESI-MS *m*/*z* 417 $[M - H]^-$. The PMR and ^{13}C NMR spectra agreed with those in the literature [5].

Isorhamnetin-3-*O*-(6″-*O*-*E*-caffeoyl)-β-D-galactopyranoside (5), yellow amorphous powder, $C_{31}H_{28}O_{15}$, mp 196–197°C. ESI-MS *m*/*z* 639 $[M - H]^-$. The PMR and ^{13}C NMR spectra agreed with those published [6].

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Isorhamnetin-3-*O*-rutinoside (6), yellow amorphous powder, $C_{28}H_{32}O_{16}$, mp 230–232°C. ESI-MS *m*/*z*: 623 $[M - H]^-$, 477 [M – H – rhamnosyl]$^-$, 315 [M – H – rhamnosyl – glycosyl]$^-$. The PMR and ^{13}C NMR spectra agreed with those in the literature [7].

Kaempferol-3-*O*-β-D-galactoside (7), yellow amorphous powder, $C_{21}H_{20}O_{11}$, mp 225–227°C. ESI-MS *m*/*z*: 447 $[M - H]^-$, 285 [M – H – galactose]$^-$. The PMR and ^{13}C NMR spectra agreed with those in the literature [8].

Kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside (9), yellow amorphous powder, $C_{27}H_{30}O_{15}$, mp 193–195°C. ESI-MS *m*/*z*: 593 $[M - H]^-$, 447 [M – H – rhamnosyl]$^-$, 285 [M – H – rhamnosyl – galactosyl]$^-$. The PMR and ^{13}C NMR spectra agreed with those published [9].

Kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (mauritianin) (10), yellow amorphous powder, $C_{33}H_{40}O_{19}$, mp 196–198°C. ESI-MS *m*/*z*: 739 $[M - H]^-$, 575 [M – H – rhamnosyl – H_2O]$^-$. The PMR and ^{13}C NMR spectra agreed with those published [10].

Kaempferol-3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (11), yellow amorphous powder, $C_{39}H_{50}O_{24}$, mp 216–218°C. ESI-MS *m*/*z*: 901 $[M - H]^-$, 755 [M – H – rhamnosyl]$^-$, 739 [M – H – glycosyl]$^-$, 575 [M – H – glycosyl – rhamnosyl – H_2O]$^-$. PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.66 (1H, s, OH-5), 8.04 (2H, d, J = 9.0, H-2′, 6′), 6.85 (2H, d, J = 9.0, H-3′, 5′), 6.41 (1H, d, J = 2.0, H-8), 6.19 (1H, d, J = 2.0, H-6), 5.58 (1H, d, J = 7.5, H-1 of 3-*O*-Gal), 5.05 (1H, s, H-1 of 2″-*O*-Ram), 4.39 (1H, d, J = 7.5, H-1 of 3″-*O*-Glc), 4.34 (1H, s, H-1 of 6″-*O*-Ram), 1.04 (3H, d, J = 6.0, CH_3 of 6″-*O*-Ram), 0.76 (3H, d, J = 6.0, CH_3 of 2″-*O*-Ram). ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 156.4 (C-2), 132.5 (C-3), 177.2 (C-4), 161.2 (C-5), 98.7 (C-6), 164.0 (C-7), 93.6 (C-8), 156.3 (C-9), 103.9 (C-10), 120.8 (C-1′), 130.7 (C-2′, 6′), 115.0 (C-3′, 5′), 159.8 (C-4′), 98.7 (C-1 of 3-*O*-Gal), 73.4 (C-2 of 3-*O*-Gal), 82.2 (C-3 of 3-*O*-Gal), 67.6 (C-4 of 3-*O*-Gal), 73.2 (C-5 of 3-*O*-Gal), 65.5 (C-6 of 3-*O*-Gal), 100.9 (C-1 of 2″-*O*-Ram), 70.5 (C-2 of 2″-*O*-Ram), 70.5 (C-3 of 2″-*O*-Ram), 71.9 (C-4 of 2″-*O*-Ram), 68.2 (C-5 of 2″-*O*-Ram), 17.2 (C-6 of 2″- *O*-Ram), 100.2 (C-1 of 6″-*O*-Ram), 70.3 (C-2 of 6″-*O*-Ram), 70.5 (C-3 of 6″-*O*-Ram), 71.9 (C-4 of 6″-*O*-Ram), 68.2 (C-5 of 6″-*O*-Ram), 17.8 (C-6 of 6″-*O*-Ram), 103.7 (C-1 of 3″-*O*-Glc), 74.1 (C-2 of 3″-*O*-Glc), 76.8 (C-3 of 3″-*O*-Glc), 69.8 (C-4 of 3″-*O*-Glc), 76.7 (C-5 of 3″-*O*-Glc), 61.0 (C-6 of 3″-*O*-Glc). The structure of **11** was determined conclusively by an analysis and comparison of spectral data with those of **10**.

All mentioned compounds were isolated from *C. laevigatus* for the first time. Among these, **4–7** and **9–11** were isolated for the first time from the genus *Croton*.

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