Coumarins from the Fruits of *Seseli devenyense*

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Eight new coumarins were isolated from the fruits of *Seseli devenyense* Simonkai. Their structures were established from NMR and mass data and their absolute configurations from chemical degradation correlation reactions. The new structures are the decanoic and dodecanoic esters of (+)-lomatin (**3**, **4**), the decanoates of (+)-*cis*-khellactone at positions 4′ (**5**) and 3′ (**6**) as well as the 2′*S* epimer of 8-(2,3-dihydroxy-3-methylbutyl)-7-hydroxychromen-2-one (**7**) named devenyol, its two *O*-monoglucosides at positions 3′ and 7 named devenyosides A (**8**) and B (**9**), and the corresponding 3′- and 7-*O*-diglucoside named devenyoside C (**10**). This plant is an interesting example of stereochemical diversity based on biodiversity given that other members of the Apiaceae family produce exclusively the 2′*R* epimers of compounds **7**−**9**.

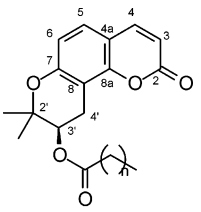
Coumarins represent a category of heterocyclic natural compounds characterized by extensive chemodiversity, with more than 1800 reported structures and a variety of pharmacological activities.[1] One very interesting subclass of this category is linear or angular pyranocoumarins that possess antiproliferative,[2] antiviral,[3] or antibacterial[4] activities.

The genus *Seseli* (Apiaceae) is a well-known source of the aforementioned subclass of coumarins, and it contains numerous species that have been used in folk medicine since ancient times.[5]

Having as target the investigation of the chemodiversity of the natural pyranocoumarins and their related metabolites, we studied the fruits of *Seseli devenyense* Simonkai, a plant widespread in Eastern and Central Europe[6] that has not been previously studied. The plants were cultivated in the Botanical Garden of the Department of Pharmacognosy of the Medical University of Lublin, Poland.

Results and Discussion

Fourteen coumarins were isolated by fractionation of the MeOH extract of the ripe fruits of *S.* devenyense. Four known coumarins were identified, (+)-*cis*-khellactone[7] and its derivatives laserpitine,[8] 3′-*O*-angeloyl-*cis*-khellactone,[9] and praeroside II.[10] The MeOH extract also contained a variety of medium-chain (C6−C12) aliphatic esters of (+)-lomatin and (+)-*cis*-khellactone. The hexanoate and octanoate of lomatin (**1**, **2**) had been previously reported as constituents of *S. gummiferum*,[11] while **2** had also been reported from *Libanotis lehmannae*.[12] The decanoate and dodecanoate of lomatin (**3**, **4**) as well as the decanoate of (+)-*cis*-khellactone at positions 4′ (**5**) and 3′ (**6**) are reported herein for the first time. Additionally, a new coumarin aglycone, the 2′*S* epimer of 8-(2,3-dihydroxy-3-methylbutyl)-7-hydroxychromen-2-one (**7**), and three new glucosides, the *O*-monoglucosides at positions 3′ (**8**) and 7 (**9**) and the corresponding 3′- and 7-*O*-diglucoside (**10**), were isolated and identified.



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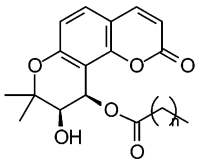
3: n = 8
4: n = 10



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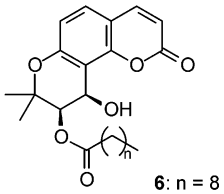

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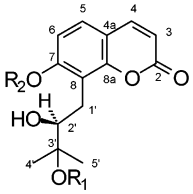

7: $R_1 = R_2 = H$
8: R_1 = glu, $R_2 = H$
9: $R_1 = H$, R_2 = glu
10: $R_1 = R_2$ = glu

The empirical formula of compound **3** was established by accurate mass measurement as $C_{24}H_{32}O_5$. The UV spectrum was suggestive of a 7-oxygenated coumarin chromophore.[13] The ^{1}H NMR spectrum displayed two pairs of doublets at 6.24 and 7.63 ppm (J = 9.5 Hz) and at 6.79 and 7.26 ppm (J = 8.6 Hz) typical for H-3, H-4, H-6, and H-5 of a 7,8-disubstituted coumarin.[2,13] Other high-field signals accounted for a $-CH_2CH-$ system (5.13, t, J = 5.0 Hz, H-3′, 3.19, dd, J = 15.1, 5.0 Hz, H-4′a, 2.97, dd, J = 15.1, 5.0 Hz, H-4′b), two methyls (1.37, 1.34 ppm), and a set of signals corresponding to a linear aliphatic ester. The NMR profile of **3** was very similar to that of previously reported lomatin esters.[11] Indeed, the carbonyl of the aliphatic ester showed a cross-peak in the HMBC spectrum with the proton at 5.13 ppm (H-3′), while this proton was

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correlated with the geminal methyls of the pyrano ring. The length of the aliphatic ester was deduced by the alkaline hydrolysis of **3** that afforded decanoic acid, which was identified by GC-MS. Thus, the structure of compound **3** was established as decanoyllomatin. Alkaline hydrolysis of **3** also afforded (+)-lomatin, which was identified by NMR, MS, and $[\alpha]_D$ data,[14,15] revealing that the configuration at C-3′ of **3** is *R*. The absolute configuration of the prepared lomatin was unequivocally confirmed using Mosher ester methodology as previously described.[16]

In a similar fashion, compound **4** was identified as dodecanoyllomatin. The UV, IR, and NMR spectra were almost identical with **3**, but the HRMS data showed that this compound contained an additional C_2H_4 group obviously corresponding to two more methylenes on the aliphatic side chain. Indeed hydrolysis of **4** afforded (+)-lomatin and dodecanoic acid and confirmed the 3′*R* configuration.

Accurate mass measurement of compound **5** indicated a molecular formula of $C_{24}H_{32}O_6$. Analysis of the NMR spectra indicated that it was an esterified derivative of *cis*-khellactone. When compared with the spectrum of *cis*-khellactone, the NMR spectra of **5** showed additional resonances for one long linear aliphatic ester (with 10 carbon atoms), while the chemical shift of H-4′ (6.41 ppm, $J = 4.9$ Hz) suggested esterification at this site. In addition, the HMBC spectrum showed that H-4′ (identified by its correlations with C-2′, C-8a, and C-7) was also correlated with the carbonyl of the aliphatic moiety. Acid hydrolysis in MeOH afforded decanoic acid, identified by GC-MS, (+)-*cis*-methylkhellactone (resolved using Mosher ester methodology), and (−)-*trans*-methylkhellactone as previously reported for 4′-esters of (+)-*cis*-khellactone[16] and confirming the absolute configuration as 3′*R*, 4′*R*.

Compound **6** had the same molecular formula as **5**, with their UV, IR, and NMR spectra being very similar. The only important difference in the ^{1}H NMR spectrum was the chemical shifts of H-3′ and H-4′, suggesting a change in the position of esterification in comparison with **5**. Indeed the HMBC spectrum showed that the carbonyl of the decanoyl moiety this time was correlated with H-3′. Alkaline hydrolysis of **6** gave decanoic acid, identified by GC-MS, and exclusively (+)-*cis*-khellactone (identified as previously) as expected for the case of homobenzylic esters, confirming the absolute configuration as 3′*R*, 4′*R*.

The molecular formula of compound **7** was found to be $C_{14}H_{16}O_5$ by HRMS. The IR and UV spectra were suggestive of a simple coumarin with a free hydroxyl at position 7 (bathochromic shift with NaOAc). The ^{1}H NMR and ^{13}C NMR spectra, apart from the typical set of signals corresponding to positions 3, 4, 5, and 6, revealed the presence of an isoprenyl chain dihydroxylated at positions 2′ and 3′. The NMR data were identical with those described for tortuoside aglycone[17] prepared by hydrolysis of tortuoside from *Seseli tortuosum*. The only difference between **7** and tortuoside aglycone was related to the sign of the specific rotation. The absolute configuration of that compound had been established as 2′*R* on the basis of chemical correlation reactions. The opposite sign of the optical rotation found for **7** suggests that it is the 2′ epimer of tortuoside aglycone. Consequently, compound **7** is 8-[(2*S*),3-dihydroxy-3-methylbutyl]-7-hydroxychromen-2-one, for which the name devenyol is proposed.

Compound **8** was obtained as an amorphous solid with molecular formula $C_{20}H_{26}O_{10}$ given by HRMS. The IR and UV spectra were similar to those of **7**, also suggesting a simple coumarin with a free hydroxyl at position 7 (bathochromic shift with NaOAc). The ^{1}H NMR and ^{13}C NMR spectra showed a clear resemblance to **7**, but in the case of **8** an additional set of peaks corresponding to a glucose moiety (anomeric proton at 4.58 ppm, $J = 7.7$ Hz) was observed. The identification of β-D-glucose was based on (1) the enzymatic hydrolysis of **8** by β-D-glucosidase and (2) the presence of characteristic coupling constants of H-1″, 2″, 3″, and 4″, which were more clearly observed in the peracetylated derivative **8a**. The enzymatic hydrolysis afforded as aglycone compound **7**, identified by NMR, MS, and specific rotation data, suggesting that **8** is a glucoside derivative of **7** with the same configuration (*S*) at position 2′. The site of attachment of glucose was revealed by the HMBC data, which showed a ^3J correlation of the anomeric proton to the quaternary oxygenated carbon (C-3′) of the isoprenyl side chain. Consequently, **8** is the 2′*S* epimer of tortuoside,[17] named devenyoside A.

The HRMS analysis of compound **9** gave the same molecular formula as **8.** An important difference with **8** was observed in the UV spectrum, where the addition of NaOAc did not induce a bathochromic shift, suggesting that the hydroxyl at position 7 was substituted. As in the case of **8** the ^{1}H NMR and ^{13}C NMR spectra showed the typical signals of a simple 7,8-bisubstituted coumarin and a glucose moiety. Some important differences in the ^{1}H NMR spectrum as compared with **8** were the downfield shift of the H-6 (+0.54 ppm) and of the anomeric proton (+0.38 ppm), suggesting the attachment of the sugar to the phenolic hydroxyl. Enzymatic hydrolysis of **9**, as in the case of **8**, afforded as aglycone compound **7**, identified by NMR, MS, and specific rotation data, suggesting that **9** was also a glucoside derivative of **7** with the same configuration (*S*) at position 2′. The site of attachment of glucose was identified from the HMBC spectrum, where a ^3J correlation of the anomeric proton with C-7 was observed, confirming the initial observation of the UV spectrum. Consequently, **9** is the (−)-7-*O*-β-D-glucopyranoside of 8-[(2*S*),3-dihydroxy-3-methylbutyl]-7-hydroxychromen-2-one, named devenyoside B. The 2′ epimer of devenyoside B had been previously reported from *Diplolophium buchananii* (Apiaceae).[18]

Finally, compound **10** was shown to have the molecular formula $C_{26}H_{36}O_{15}$, an increase of $C_6H_{10}O_5$ when compared with **8** or **9**, suggesting the presence of an additional hexosyl moiety The UV spectrum of **10**, as in the case of **9**, suggested that 7-OH was substituted. The NMR spectra of **10** were similar to those of **8** and **9**, but the observation of two anomeric protons indicated that it was a diglycoside. The enzymatic hydrolysis of **10**, as in the case of **8** and **9**, afforded compound **7**, previously identified, suggesting that **10** was a diglucoside derivative of **7** once again possessing the same configuration (*S*) at position 2′. The sites of attachment of the two glucosyl moieties were indicated by the HMBC spectrum, where the first anomeric proton was correlated with C-7 and the second anomeric proton with C-3′ of the isoprenyl side chain. Consequently, **10** is (−)-3′-*O*-β-D-glucopyranosyl-8-[(2*S*),3-dihydroxy-3-methylbutyl]-7-hydroxychromen-2-one 7-*O*-β-D-glucopyranoside, named devenyoside C.

The most interesting aspect of this work is the number of new coumarins isolated from a single plant. Considering the large number of coumarins already known,[1] this indeed represents a significant find. It should also be noted that this plant is an interesting example of stereochemical diversity based on biodiversity given that other members of the Apiaceae family produce exclusively the 2*R* epimers of compounds **7**−**9**.

Experimental Section

General Experimental Procedures. Specific rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu-160A spectrophotometer. The IR spectra were obtained on a Perkin-Elmer Paragon 500 instrument. NMR spectra were recorded at 25 °C on Bruker DRX 400 and Bruker AC 200 spectrometers [^{1}H (400 MHz) and ^{13}C (50 MHz)]; chemical shifts are expressed in ppm downfield to TMS, which was used as internal standard. The 2D NMR experiments (COSY, HMQC, and HMBC) were performed using standard Bruker microprograms with gradient pulse sequences. EIMS were determined on an HP-6890 spectrometer, FABMS were obtained using a ZAB HF instrument, HREIMS and HRFABMS were obtained on an AEI MS-902 mass spectrometer. Liquid chromatography was performed on columns containing Si gel 60 Merck (40−63 μm). The GC-MS analyses were carried out using a Hewlett-Packard 6890-5973 GC-MS system operating in EI mode (equipped with a HP 5MS 30 m × 0.25 mm, 0.25 μm film thickness capillary column). He (1 mL/min) was used as carrier gas. The initial temperature of the column was 60 °C, and then it was heated to 280 °C at a rate of of 3 °C/min. The identification of compounds by GC-MS was based on comparison of their retention indices (RI), obtained using *n*-alkanes (C_9−C_{25}), by comparison of their EI-mass spectra with the NIST/NBS, Wiley library spectra, and literature[19] as well as by co-injection with authentic samples. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing RP-18 Si gel 60 Merck (20−40 μm). Reversed-phase high-performance liquid chromatography (RP-HPLC) was performed with a THERMO Finnigan SPECTRA system.

Plant Material. Ripe fruits of *S. devenyense* were collected in October 2003, in the Botanical Garden of the Medical University of Lublin, Poland. A voucher sample (2080) is kept in Index Seminum−Anno 2003, Hortus Botanicus Universitatis Mariae Curie-Sklodowska, Lublin, Poland. The plant material was identified by M. Fransczak-Byc and K. Dabrowska.

Extraction and Isolation. The air-dried and powdered plant material (31 g) was extracted in a Soxhlet apparatus for 48 h with petroleum ether and then with MeOH. After evaporation of the solvents, the obtained extracts were 4.3 and 1.6 g, respectively. The MeOH extract was submitted to column chromatography (3.0 cm) on silica gel 60 Merck (65 g, 40−63 μm) with CH_2Cl_2/MeOH (from 100:0 to 50:50 gradient) as eluents to afford 350 fraction of 10 mL each: fractions A1−A34, CH_2Cl_2; A35−A46, CH_2Cl_2/MeOH (99.9:0.1); A47−A61, CH_2Cl_2/MeOH (99.8:0.2); A62−A74, CH_2Cl_2/MeOH (99.6:0.4); A75−A209, CH_2Cl_2/MeOH (99.2:0.8); A210−A222, CH_2Cl_2/MeOH (99:1); A223−A235, CH_2Cl_2/MeOH (98.8:1.2); A236−A248, CH_2Cl_2/MeOH (98.5:1.5); A249−A262, CH_2Cl_2/MeOH (98:2); A263−A332, CH_2Cl_2/MeOH (95:5); A333−A344, CH_2Cl_2/MeOH (92:8); A345−A352, CH_2Cl_2/MeOH (85:15); A353−A360, CH_2Cl_2/MeOH (50:50).

Fractions A35−A133 (38 mg) were rechromatographed in HPLC [Nucleosil C18, 100-7 (7 μm, 250 × 21 mm), H_2O/MeOH (60:40 to 40:60 in 60 min), flow rate 1 mL/min] to afford four lomatin esters: **1** (2 mg), **2** (13 mg), **3** (15 mg), and **4** (5 mg).

Fractions A134−A144 (96 mg) were rechromatographed by column chromatography (1.3 cm, 40−63 μm) on Merck silica gel 60 (4.0 g, 40−63 μm) with CH_2Cl_2/MeOH (from 100:0 to 99.5:0.5 gradient) as eluents to afford 60 fractions of 10 mL each: Fractions B10−B15 afforded (+)-4′-decanoyl-*cis*-khellactone (**5**) (15 mg), B17−B22 (+)-3′-decanoyl-*cis*-khellactone (**6**) (10 mg), B25−B28 laserpitin (2 mg), and B32−B42 3′-angeloyl-*cis*-khellactone (3 mg).

Fractions A176−A185 afforded (+)-*cis*-khellactone (3.6 mg). Fractions A276−A284 afforded devenyol (**7**) (7 mg).

Fractions A353−A360 (400 mg) were rechromatographed by MPLC on RP-18 silica gel 60 Merck (20−40 μm) with H_2O/MeOH (from 100:0 to 70:30 gradient) as eluents to afford 130 fractions of 20 mL each: fractions C1−C20, H_2O eluted; C21−C59, H_2O/MeOH (90:10); C60−C85, H_2O/MeOH (80:20) eluted; C86−C130, H_2O/MeOH (70:30) eluted. Fractions C60−C65 afforded devenyoside C (**10**) (10 mg), C78−C84 devenyoside B (**9**) (20 mg), C91−C100 devenyoside A (**8**) (80 mg), and C105−C115 praeroside II (5 mg).

(+)-Hexanoyllomatin (1): colorless oil; $[\alpha]_D^{25}$ +30.3° ($CHCl_3$, *c* 0.1); UV (MeOH) λ_{max} 326 (3.32), 289 (sh) nm; IR ($CHCl_3$) ν_{max} 2980, 1732, 1606, 1144 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, J = 9.5 Hz, H-4), 7.26 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 6.24 (1H, d, J = 9.5 Hz, H-3), 5.13 (1H, t, J = 5.0 Hz, H-3′), 3.19 (1H, dd, J = 15.1, 5.0 Hz, H-4′a), 2.97 (1H, dd, J = 15.1, 5.0 Hz, H-4′b), 2.30 (2H, t, J = 7.5 Hz, H-2″), 1.59 (2H, quint, J = 7.5 Hz, H-3″), 1.37 (3H, s, 2′b), 1.34 (3H, s, CH_3-2′a), 1.24 (4H, m, H-4″,5″), 0.86 (3H, t, J = 7 Hz, H-6″); EIMS *m/z* 344 (5), 228 (30), 213 (100), 187 (10), 176 (15); HREIMS *m/z* 344.1630 (cacld for $[C_{20}H_{24}O_5]^+$, 344.1624).

(+)-Octanoyllomatin (2): amorphous solid; $[\alpha]_D^{25}$ +30.1° ($CHCl_3$, *c* 0.1); UV (MeOH) λ_{max} 326 (3.32), 289 (sh) nm; IR ($CHCl_3$) ν_{max} 2980, 1731, 1606, 1144 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, J = 9.5 Hz, H-4), 7.26 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 6.24 (1H, d, J = 9.5 Hz, H-3), 5.13 (1H, t, J = 5.0 Hz, H-3′), 3.19 (1H, dd, J = 15.1, 5.0 Hz, H-4′a), 2.97 (1H, dd, J = 15.1, 5.0 Hz, H-4′b), 2.30 (2H, t, J = 7.5 Hz, H-2″), 1.59 (2H, quint, J = 7.5 Hz, H-3″), 1.37 (3H, s, CH_3-2′b), 1.34 (3H, s, CH_3-2′a), 1.24 (8H, m, H-4″-7″), 0.86 (3H, t, J = 7 Hz, H-8″); ^{13}C NMR ($CDCl_3$) δ 14.0 (C-8″), 22.6 (C-7″), 22.9 (C-2′a), 23.0 (C-4′), 24.6 (C-2′b), 24.9 (C-3″), 28.9 (C-4″), 29.0 (C-5″), 31.6 (C-6″), 34.3 (C-2″), 69.1 (C-3′), 76.8 (C-2′), 106.9 (C-8), 112.1 (C-4a), 112.5 (C-3), 114.3 (C-6), 126.7 (C-5), 143.8 (C-4), 153.3 (C-8a), 156.2 (C-7), 161.2 (C-2), 173.0 (C-1″); EIMS *m/z* 372 (5), 228 (30), 213 (100), 187 (10), 176 (15); HREIMS *m/z* 372.1931 (calcd for $[C_{22}H_{28}O_5]^+$, 372.1937).

(+)-Decanoyllomatin (3): amorphous solid; $[\alpha]_D^{25}$ +29.5° ($CHCl_3$, *c* 0.1); UV (MeOH) λ_{max} 326 (3.32), 289 (sh) nm; IR ($CHCl_3$) ν_{max} 2980, 1730, 1606, 1144 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, J = 9.5 Hz, H-4), 7.26 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 6.24 (1H, d, J = 9.5 Hz, H-3), 5.13 (1H, t, J = 5.0 Hz, H-3′), 3.19 (1H, dd, J = 15.1, 5.0 Hz, H-4′a), 2.97 (1H, dd, J = 15.1, 5.0 Hz, H-4′b), 2.30 (2H, t, J = 7.5 Hz, H-2″), 1.59 (2H, quint, J = 7.5 Hz, H-3″), 1.37 (3H, s, CH_3-2′b), 1.34 (3H, s, CH_3-2′a), 1.24 (12H, m, H-4″-9″), 0.86 (3H, t, J = 7 Hz, H-10″); ^{13}C NMR ($CDCl_3$) δ 14.1 (C-10″), 22.6 (C-9″), 22.9 (C-2′b), 23.0 (C-4′), 24.6 (C-2′a), 24.9 (C-3″), 29.0 (C-4″), 29.2, 29.4 (C-5″,6″,7″), 31.8 (C-8″), 34.3 (C-2″), 69.1 (C-3′), 76.8 (C-2′), 106.9 (C-8), 112.1 (C-4a), 112.5 (C-3), 114.3 (C-6), 126.7 (C-5), 143.9 (C-4), 153.3 (C-8a), 156.2 (C-7), 161.2 (C-2), 173.1 (C-1″); EIMS *m/z* 400 (5), 228 (30), 213 (100), 187 (10), 176 (15); HREIMS *m/z* 400.2255 (calcd for $[C_{24}H_{32}O_5]^+$, 400.2250).

(+)-Dodecanoyllomatin (4): amorphous solid; $[\alpha]_D^{25}$ +29.3° ($CHCl_3$, *c* 0.1); UV (MeOH) λ_{max} 326 (3.32), 289 (sh) nm; IR ($CHCl_3$) ν_{max} 2980, 1730, 1606, 1144 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, J = 9.5 Hz, H-4), 7.26 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 6.24 (1H, d, J = 9.5 Hz, H-3), 5.13 (1H, t, J = 5.0 Hz, H-3′), 3.19 (1H, dd, J = 15.1, 5.0 Hz, H-4′a), 2.97 (1H, dd, J = 15.1, 5.0 Hz, H-4′b), 2.30 (2H, t, J = 7.5 Hz, H-2″), 1.59 (2H, quint, J = 7.5 Hz, H-3″), 1.37 (3H, s, CH_3-2′b), 1.34 (3H, s, CH_3-2′a), 1.24 (16H, m, H-4″-11″), 0.86 (3H, t, J = 7 Hz, H-12″); ^{13}C NMR ($CDCl_3$) δ 14.1 (C-12″), 22.6 (C-11″), 22.9 (C-2′a), 23.0 (C-4′), 24.6 (C-2′b), 24.9 (C-3″), 29.0 (C-4″), 29.2−29.4 (C-5″-9″), 31.8 (C-10″), 34.3 (C-2″), 69.1 (C-3′), 76.7 (C-2′), 106.9 (C-8), 112.1 (C-4a), 112.5 (C-3), 114.3 (C-6), 126.7 (C-5), 143.8 (C-4), 153.3 (C-8a), 156.2 (C-7), 161.2 (C-2), 173.1 (C-1″); EIMS *m/z* 428 (5), 228 (30), 213 (100), 187 (10), 176 (15); HREIMS *m/z* 428.2558 (cacld for $[C_{26}H_{36}O_5]^+$, 428.2562).

(+)-4′-Decanoyl-*cis*-khellactone (5): amorphous solid; $[\alpha]_D^{25}$ +47.2° ($CHCl_3$, *c* 0.1); UV (MeOH) λ_{max} (log ϵ) 327 (3.17), 288 (sh) nm; IR (MeOH, CaF_2) ν_{max} 1725, 1607 cm^{-1}; ^{1}H NMR ($CDCl_3$ 400 MHz) δ 7.60 (1H, d, J = 9.5 Hz, H-4), 7.35 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 6.41 (1H, d, J = 4.9 Hz, H-4′), 6.23 (1H, d, J = 9.6 Hz, H-3), 4.02 (1H, d, J

= 4.9 Hz, H-3′), 2.43 (2H, m, H-2″), 1.67 (2H, m, H-3″), 1.46 (3H, s, CH_3-2′b), 1.43 (3H, s, CH_3-2′a), 1.25 (12H, br s, H-4″−H-9″), 0.86 (3H, t, J = 7 Hz, H-10″); ^{13}C NMR ($CDCl_3$) δ 14.1 (C-10″), 21.2 (C-2′b), 22.6 (C-9″), 24.8 (C-3″), 25.4 (C-2′a), 29.1 (C-4″), 29.2, 29.4 (C-5″,6″,7″), 31.8 (C-8″), 34.3 (C-2″), 63.6 (C-4′), 71.3 (C-3′), 78.6 (C-2′), 106.9 (C-8), 112.3 (C-4a), 113.0 (C-3), 114.5 (C-6), 129.3 (C-5), 143.4 (C-4), 154.1 (C-8a), 156.9 (C-7), 160.0 (C-2), 175.1 (C-1″); HREIMS *m/z* 416.2255 (cacld for $[C_{24}H_{32}O_6]^+$, 416.2250).

(+)-3′-Decanoyl-*cis*-khellactone (6): amorphous solid; $[\alpha]_D^{25}$ +45.2° ($CHCl_3$, *c* 0.1); UV (MeOH) λ_{max} (logϵ) 327 (3.17), 288 (sh) nm; IR (MeOH, CaF_2) ν_{max} 1725, 1607 cm^{-1}; ^{1}H NMR ($CDCl_3$ 400 MHz) δ 7.64 (1H, d, J = 9.5 Hz, H-4), 7.33 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 6.25 (1H, d, J = 9.6 Hz, H-3), 5.41 (1H, d, J = 4.9 Hz, H-4′), 5.17 (1H, d, J = 4.9 Hz, H-3′), 2.35 (2H, m, H-2″), 1.67 (2H, m, H-3″), 1.47 (3H, s, H-CH_3-2′a), 1.39 (3H, s, CH_3-2′b), 1.25 (12H, br s, H-4″−H-9″), 0.86 (3H, t, J = 7 Hz, H-10″); ^{13}C NMR ($CDCl_3$) δ 14.1 (C-10″), 22.5 (C-2′a), 22.6 (C-9″), 24.9 (C-3″), 25.4 (C-2′b), 29.1 (C-4″), 29.2, 29.4 (C-5″,6″,7″), 31.6 (C-8″), 34.2 (C-2″), 60.0 (C-4′), 72.1 (C-3′), 77.7 (C-2′), 110.7 (C-8), 112.3 (C-4a), 112.6 (C-3), 114.6 (C-6), 128.8 (C-5), 144.0 (C-4), 154.1 (C-8a), 156.0 (C-7), 160.7 (C-2), 173.2 (C-1″); HREIMS *m/z* 416.2258 (cacld for $[C_{24}H_{32}O_6]^+$, 416.2250).

Devenyol: (−)-8-[(2*S*),3-Dihydroxy-3-methylbutyl]-7-hydroxychromen-2-one (7): amorphous solid; $[\alpha]_D^{25}$ −44.6° (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ϵ) 326 (3.55), 256 (sh) nm; UV (MeOH+NaOAc) λ_{max} (log ϵ) 371 (3.35) nm; IR (MeOH, CaF_2) ν_{max} 1711, 1607, 1250 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.29 (3H, s, H-4′), 1.30 (3H, s, H-5′), 2.93 (1H, dd, J = 13.8, 10.1 Hz, H-1′a), 3.17 (1H, dd, J = 13.8, 1.5 Hz, H-1′b), 3.68 (1H, dd, J = 10.1, 1.5 Hz, H-2′), 6.19 (1H, d, J = 9.3 Hz, H-3), 6.84 (1H, d, J = 8.5 Hz, H-6), 7.35 (1H, d, J = 8.5 Hz, H-5), 7.86 (1H, d, J = 9.3 Hz, H-4); ^{13}C NMR (CD_3OD) δ 25.4 (C-4′), 25.6 (C-5′), 26.4 (C-1′), 74.0 (C-3′), 79.5 (C-2′), 111.9 (C-3), 113.4 (C-4a), 115.5 (C-8), 114.2 (C-6), 128.3 (C-5), 146.7 (C-4), 155.1 (C-8a), 163.8 (C-2), 161.3 (C-7); CI-MS (CH_4) *m/z* 265 (M + H)$^+$; HRFABMS *m/z* 265.1069 (cacld for $[C_{14}H_{16}O_5 + H]^+$, 265.1076).

Devenyoside A: 2′-*epi*-Tortuoside: (−)-3′-*O*-*β*-D-glucopyranoside of 8-[(2*S*),3-dihydroxy-3-methylbutyl]-7-hydroxychromen-2-one (8): amorphous solid; $[\alpha]_D^{25}$ −38.8° (MeOH, *c* 0.4); UV (MeOH) λ_{max} (log ϵ) 326 (3.63), 256 (3.26) nm; UV (MeOH+NaOAc) λ_{max} (log ϵ) 374 (4.09) nm; IR (MeOH, CaF_2) ν_{max} 1709, 1606, 1583, 1211 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.36 (3H, s, H-4′), 1.40 (3H, s, H-5′), 2.96 (1H, dd, J = 13.8, 10.1 Hz, H-1′a), 3.13 (1H, dd, J = 13.8, 1.5 Hz, H-1′b), 3.23 (1H, dd, J = 8.5, 7.7 Hz, H-2″), 3.29 (1H, m, H-5″), 3.32 (1H, t, J = 8.5 Hz, H-4″), 3.41 (1H, t, J = 8.5 Hz, H-3″), 3.62 (1H, dd, J = 12.0, 5.2 Hz, H-6b″), 3.79 (1H, dd, J = 10.1, 1.5 Hz, H-2′), 3.80 (1H, dd, J = 12.0, 2.0 Hz, H-6a″), 4.58 (1H, d, J = 7.7 Hz, H-1″), 6.02 (1H, d, J = 9.3 Hz, H-3), 6.72 (1H, d, J = 8.5 Hz, H-6), 7.23 (1H, d, J = 8.5 Hz, H-5), 7.76 (1H, d, J = 9.3 Hz, H-4); ^{13}C NMR (CD_3OD) δ 21.9 (C-4′), 23.9 (C-5′), 26.3 (C-1′), 62.5 (C-6″), 71.5 (C-4″), 75.1 (C-2″), 77.5 (C-5″), 77.9 (C-3″), 79.6 (C-2′), 81.9 (C-3′), 98.6 (C-1″), 109.0 (C-3), 111.6 (C-4a), 115.6 (C-8), 116.3 (C-6), 128.3 (C-5), 147.1 (C-4), 155.5 (C-8a), 164.9 (C-2), 167.3 (C-7); HRFABMS *m/z* 427.1610 (cacld for $[C_{20}H_{26}O_{10} + H]^+$, 427.1604).

Acetylation of Devenyoside A (2′-*epi*-Tortuoside) (8a). A solution of **8** (5 mg) and Ac_2O (1 mL) in dry pyridine (1 mL) was stirred for 12 h at room temperature. Evaporation of the reagents under reduced pressure afforded the hexaacetyl derivative **8a**: ^{1}H NMR ($CDCl_3$) δ 1.33 (3H, s, H-4′), 1.37 (3H, s, H-5′), 1.83 (3H, s, 2′-OAc), 2.00 (3H, s, OAc), 2.01 (3H, s, OAc), 2.03 (3H, s, OAc), 2.07 (3H, s, OAc), 2.42 (3H, s, 7-OAc), 3.04 (2H, m, H-1′), 3.73 (1H, m, H-5″), 4.11 (1H, dd, J = 12.0, 2.4 Hz, H-6b″), 4.22 (1H, dd, J = 12.0, 5.8 Hz, H-6a″), 4.81 (1H, d, J = 7.9 Hz, H-1″), 4.98 (1H, t, J = 9.6 Hz, H-2″), 5.03 (1H, t, J = 9.6 Hz, H-4″), 5.21 (1H, dd, J = 7.5, 5.5 Hz, H-2′), 5.26 (1H, t, J = 9.6 Hz, H-3″), 6.38 (1H, d, J = 9.6 Hz, H-3), 7.01 (1H, d, J = 8.4 Hz, H-6), 7.36 (1H, d, J = 8.4 Hz, H-5), 7.66 (1H, d, J = 9.6 Hz, H-4).

Devenyoside B: (−)-7-*O*-*β*-D-Glucopyranoside of 8-[(2*S*),3-dihydroxy-3-methylbutyl]-7-hydroxychromen-2-one (9): amorphous solid; $[\alpha]_D^{25}$ −169.5° (MeOH, *c* 0.4); UV (MeOH) λ_{max} (log ϵ) 312 (3.85), 256 (sh) nm, no shift with NaOAc; IR (MeOH, CaF_2) ν_{max} 1710, 1606, 1250 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.302 (3H, s, H-4′), 1.305 (3H, s, H-5′), 3.12 (2H, m, H-1′a, 1′b), 3.39 (1H, t, J = 9.0 Hz, H-4″), 3.47 (1H, t, J = 9.0 Hz, H-3″), 3.49 (1H, m, H-5″), 3.56 (1H, t, J = 9.0, 7.6 Hz, H-2″), 3.63 (1H, dd, J = 9.4, 4.1 Hz, H-2′), 3.71 (1H, dd, J = 12.0, 5.8 Hz, H-6b″), 3.93 (1H, dd, J = 12.0, 2.2 Hz, H-6a″), 4.96 (1H, d, J = 7.6 Hz, H-1″), 6.29 (1H, d, J = 9.5 Hz, H-3), 7.26 (1H, d, J = 8.6 Hz, H-6), 7.49 (1H, d, J = 8.6 Hz, H-5), 7.91 (1H, d, J = 9.5 Hz, H-4); ^{13}C NMR (CD_3OD) δ 25.1 (C-4′), 25.8 (C-1′), 25.9 (C-5′), 62.5 (C-6″), 71.2 (C-4″), 75.1 (C-2″), 77.9 (C-5″), 78.5 (C-3″), 79.2 (C-2′), 74.0 (C-3′), 103.2 (C-1″), 113.3 (C-6), 114.0 (C-3), 115.4 (C-4a), 118.2 (C-8), 128.4 (C-5), 146.2 (C-4), 154.4 (C-8a), 161.0 (C-7), 163.2 (C-2); HRFABMS *m/z* 427.1612 (cacld for $[C_{20}H_{26}O_{10} + H]^+$, 427.1604).

Devenyoside C: (−)-3′-*O*-*β*-D-Glucopyranosyl 8-[(2*S*),3-dihydroxy-3-methylbutyl]-7-hydroxychromen-2-one 7-*O*-*β*-D-glucopyranoside (10): amorphous solid; $[\alpha]_D^{25}$ −36° (MeOH, *c* 0.4); UV (MeOH) λ_{max} (log ϵ) 313 (3.67), 256 (sh) nm, no shift with NaOAc; IR (MeOH, CaF_2) ν_{max} 1710,1607, 1250 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.40 (3H, s, H-4′), 1.43 (3H, s, H-5′), 3.12 (2H, m, H-1′a,b), 3.21 (1H, dd, J = 9.0, 7.7 Hz, H-2″), 3.29 (1H, m, H-5″), 3.32 (1H, t, J = 9.0 Hz, H-4″), 3.39 (2H, t, J = 9.0 Hz, H-3″, 4‴), 3.48 (2H, t, J = 8.9 Hz, H-3‴), 3.49 (1H, m, H-5‴), 3.56 (1H, dd, J = 8.9, 7.6 Hz, H-2‴), 3.65 (1H, dd, J = 12.2, 5.4 Hz, H-6b″), 3.71 (1H, dd, J = 12.2, 5.8 Hz, H-6b‴), 3.79 (1H, dd, J = 12.0, 2.0 Hz, H-6a″), 3.81 (1H, dd, J = 9.0, 3.8 Hz, H-2′), 3.94 (1H, dd, J = 12.2, 2.2 Hz, H-6a‴), 4.59 (1H, d, J = 7.7 Hz, H-1″), 4.98 (1H, d, J = 7.6 Hz, H-1‴), 6.29 (1H, d, J = 9.6 Hz, H-3), 7.24 (1H, d, J = 8.7 Hz, H-6), 7.49 (1H, d, J = 8.7 Hz, H-5), 7.91 (1H, d, J = 9.6 Hz, H-4); ^{13}C NMR (CD_3OD) δ 21.8 (C-4′), 23.6 (C-5′), 25.2 (C-1′), 62.5 (C-6″, 6‴), 71.2 (C-4″, 4‴), 74.8 (C-2‴), 75.0 (C-2″), 77.0 (C-5‴), 77.5 (C-5″), 77.9 (C-3″), 78.0 (C-3‴), 78.2 (C-2′), 81.6 (C-3′), 98.2 (C-1″), 102.8 (C-1‴), 112.8 (C-6), 113.7 (C-3), 115.4 (C-4a), 118.1 (C-8), 128.1 (C-5), 146.0 (C-4), 154.3 (C-8a), 161.2 (C-7), 163.2 (C-2); HRFABMS *m/z* 589.2139 (cacld for $[C_{26}H_{36}O_{15} + H]^+$, 589.2132).

Alkaline Hydrolysis of Lomatin Esters (3, 4) and 3′-Decanoyl-*cis*-khellactone (6). To a solution of **3** (10 mg) or **4** (5 mg) or **6** (10 mg) in MeOH (2 mL) was added methanolic KOH (2 mL, 1 N), and the reaction mixture was refluxed for 1 h. The reaction mixture was diluted with H_2O, concentrated to remove organic solvent, acidified with H_2SO_4, and extracted with EtOAc. The EtOAc phase was washed with $NaHCO_3$ and submitted to preparative TLC (cyclohexane/EtOAc) to give either (+)-lomatin in the case of **3** and **4** or (+)-*cis*-khellactone in the case of **6**. The aqueous phase was acidified with H_2SO_4, extracted with Et_2O, and submitted to GC-MS analysis (for the identification of decanoic and dodecanoic acid).

Acid Hydrolysis of 4′-Decanoyl-*cis*-khellactone (5). To a solution of **5** (10 mg) in MeOH (3 mL) was added methanolic HCl (3 mL, 1 N), and the reaction mixture was refluxed for 24 h. The mixture was diluted with H_2O, concentrated to remove organic solvent, alkalized with $NaHCO_3$, and extracted with EtOAc. The EtOAc phase was washed with H_2O and subjected to preparative TLC (cyclohexane/EtOAc) to give (+)-*cis*-methylkhellactone and (−)-*trans*-methylkhellactone, identified by ^{1}H NMR and specific rotation, identical with previously reported data.[16] The aqueous $NaHCO_3$ phase was acidified with H_2SO_4, extracted with Et_2O, and subjected to GC-MS analysis (for the identification of decanoic acid).

Enzymatic Hydrolysis of Devenyosides A−C (8−10). To a solution of **8**, **9**, or **10** (10 mg) in H_2O (2 mL) was added *β*-D-glucosidase (15 mg), and the reaction mixture was stirred for 48 h at 37 °C. The mixture was then extracted with EtOAc, and the organic phase was evaporated to dryness. Purification of the residue with pTLC afforded in all cases devenyol (**7**), identified by ^{1}H NMR and specific rotation.

References and Notes

(1) Murray, R. D. H. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Eds.; Springer-Verlag: Wien, 2002; Vol. 83, pp 1−529.

(2) Magiatis, P.; Melliou, E.; Skaltsounis, A. L.; Mitaku, S.; Léonce, S.; Renard, P.; Pierré, A.; Atassi, G. *J. Nat. Prod.* **1998**, *61*, 982−986.
(3) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K.-H. *J. Med. Chem.* **1999**, *42*, 2662−2672.
(4) Melliou, E.; Magiatis, P, Mitaku, S.; Skaltsounis, A. L.; Chinou, E.; Chinou, I. *J. Nat. Prod.* **2005**, *68*, 78−82.
(5) Dioscurides. *De Materia Medica*, Codex Neapolitanus Graecus 1 of the National Library of Naples; Militos Press: Athens, 2002; pp 215−216.
(6) Tutin, T. G.; Heywood, V. H.; Burges, N. A.; Moorel, D. M.; Valentine, D. H.; Walters, S. M.; Webb, D. A. *Flora Europaea*; The University Press: Cambridge, 1968; Vol. 2, pp 334−338.
(7) Lemmich, J.; Shabana, M. *Phytochemistry* **1984**, *23*, 863−865.
(8) Swager, T. M.; Cardellina J. H. *Phytochemistry* **1985**, *24*, 805−813.
(9) Delgado, G.; Garduno J. *Phytochemistry* **1987**, *26*, 1139−1141.
(10) Takata, M.; Okuyama, T.; Shibata, S. *Planta Med.* **1987**, *53*, 323−327.
(11) Nielsen, B. E.; Larsen, P. K.; Lemmich, J. *Acta Chem. Scand.* **1970**, *23*, 2863−2867.
(12) Aminov, A. M. *Chem. Nat. Comp.* **1975**, *11*, 249.
(13) Murray, R. D. H.; Mendez, J.; Brown S. A. *The Natural Coumarins*; John Wiley & Sons Ltd.: Bristol, 1982; pp 27−32.
(14) Murray, R. D. H.; Ballantyne, M. M.; Mathai, K. P. *Tetrahedron* **1971**, *27*, 1247−1251.
(15) Skaltsounis, A. L.; Mitaku, S.; Gaudel, G.; Tillequin, F.; Koch, M. *Heterocycles* **1992**, *34*, 121−128.
(16) Valencia-Islas, N.; Abbas, H.; Bye, R.; Toscano, R.; Mata, R. *J. Nat. Prod.* **2002**, *65*, 828−834.
(17) Ceccherelli, P.; Curini, M.; Marcotullio, M. C.; Madruzza, G.; Menghini, A. *J. Nat. Prod.* **1990**, *53*, 536−538.
(18) Lemmich, J. *Phytochemistry* **1995**, *38*, 427−432.
(19) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectometry*; Allured Publishing Corp.: Carol Stream, IL, 2001.

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