

TWO DIHYDROPYRANOCOUMARINS FROM *PEUCEDANUM WAWRII*

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Abstract - Two dihydropyrano coumarins were isolated from the root of *Peucedanum wawrii* and the structures were established as 3'(*R*)-acetoxy-3',4'-dihydroxanthyletin (**1**) and 3'(*R*)-acetoxy-4'(*S*)-angeloyloxy-3',4'-dihydroseselin (**2**), respectively, by spectroscopic methods. The absolute configurations were deduced by chemical correlations with known compounds.

Peucedanum wawrii is a plant of Umbelliferae. In some areas of China such as Sichuan province and Yunnan province, besides *Peucedanum praeruptorum* and *P. decursivum*, the root of some other plants of *Peucedanum* genus including *P. wawrii* was used as Qianhu, a traditional Chinese medicine to cure some diseases such as cough due to pathogenic wind-heat, accumulation of phlegm and heat in the lung. So far there are only a few reports about the chemistry of the title plant.^{1,2} In order to discover the new active compounds from this plant, we studied further the chemical constituents. A new natural product and a new compound, along with fifteen known compounds were isolated and their structures were elucidated by chemical reactions and spectral analyses. This paper describes the isolation and structure elucidation of the new natural product and the new compound. By spectroscopic methods, the structures were established as 3'(*R*)-acetoxy-3',4'-dihydroxanthyletin (**1**) and 3'(*R*)-acetoxy-4'(*S*)-angeloyloxy-3',4'-dihydroseselin (**2**), respectively. The absolute configurations were deduced by chemical correlations with known compounds.

Structure of 1 Compound (**1**) was isolated as colorless needles. The molecular ion at m/z 288.0994 in the HRMS spectrum showed that the molecular formula was C₁₆H₁₆O₅. The signals at 1717, 1682 and 1627 cm⁻¹ in the IR spectrum were assigned to carbonyl and aromatic systems of a coumarin skeleton. The ¹HNMR spectrum in the aromatic proton

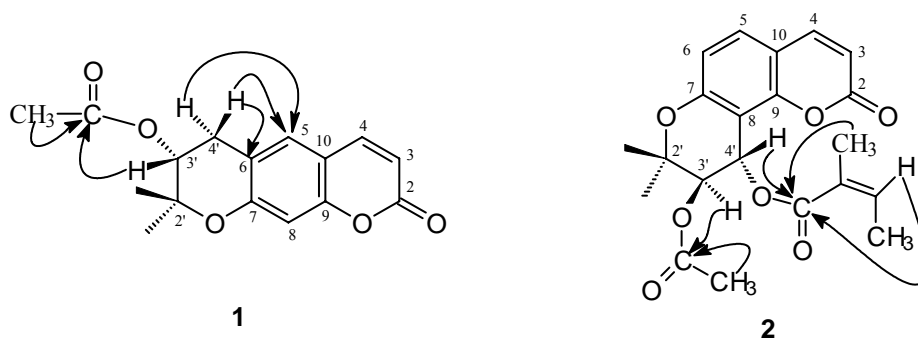


Figure 1 The structures and major correlations in HMBC spectra of **1** and **2**

region of **1** contained one pairs of doublets at δ 6.18 (1H, d, $J=9.4$ Hz), 7.92 (1H, d, $J=9.4$ Hz) and two singlets at δ 7.44 (1H, s), 6.73 (1H, s), which are identical with the C-3-H, C-4-H signals of the α -pyrone ring system and signals of C-5-H, C-8-H of the benzene ring, indicating **1** was a coumarin substituted at the C-6 and C-7 positions. In the ^1H NMR spectrum, the proton signals at δ 3.41 (1H, dd, $J=10.8, 3.4$ Hz) as well as the two proton signals at δ 2.34 (1H, dd, $J=14.2, 10.8$ Hz), 2.95 (1H, dd, $J=14.2, 3.4$ Hz) indicated that the substituted group between C-6 and C-7 formed a dihydropyran ring and also only C-3' were attached group by comparison with chemical shifts and the coupling pattern of the skeleton of smyrinol.³ In HMQC spectrum, the two proton signals at δ 2.34, 2.95 were correlated with the same carbon signal at δ 31.4, showing no substituted group at C-4', the same signals at δ 2.34, 2.95 were correlated with the carbon signals at δ 130.3 (C-5) and 125.1 (C-6) in HMBC spectrum, which confirmed the above conclusion. The signals at δ 2.43 (3H, s) in ^1H NMR and δ 28.8, 173.6 in ^{13}C NMR were due to an acetyl group. In HMBC spectrum, the carbon signal at δ 173.6 (acetoxy carbonyl) was correlated with the proton signal at δ 3.41 (C-3'-H), the evidence indicated that the acetoxy was attached to C-3' position.

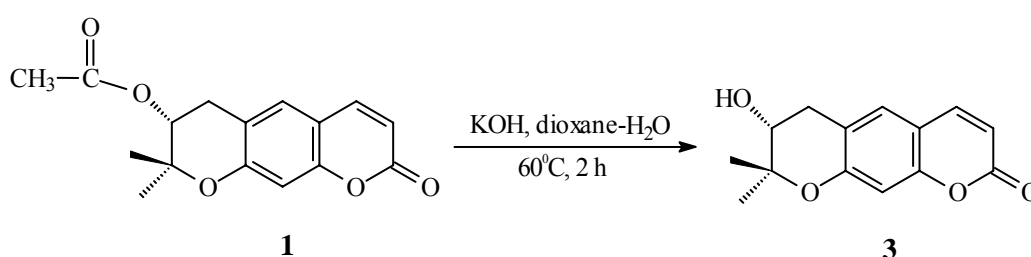


Figure 2 Alkaline hydrolysis of **1**

Stereochemistry of 1 There is a chiral carbon atom in the molecular structure of **1**. In order to determine the absolute configuration of C-3', a chemical correlation with a known compound was carried out. On the alkaline hydrolysis, **1**

gave a product, which was identified as 3'(*R*)-hydroxy-3',4'-dihydroxanthyletin (**3**) by spectral analysis and optical rotation ($[\alpha]_D -11.5^\circ$). The absolute configuration about C-3' of 3'(*R*)-hydroxy-3',4'-dihydroxanthyletin (**3**) ($[\alpha]_D -11.0^\circ$) was decided as *R* in the literature,⁴ accordingly, the absolute configuration about C-3' of **1** was also established as *R*. The chemical structure of **1** was finally elucidated as 3'(*R*)-acetoxy-3',4'-dihydroxanthyletin. Compound (**1**) has been synthesized from aegelinol,⁵ but it was isolated from natural resources for the first time.

HPLC was used to analyze the CHCl₃ extract of the roots of title plant, eluted with cyclohexane:CHCl₃ (90:10), the result showing the existence of **1** in the CHCl₃ extract, which indicated that **1** is a natural product, other than an artefact.

Structure of 2 Compound (**2**) was isolated as colorless needles, the molecular ion at m/z 386.1369 in the high resolution mass spectrum showed the molecular formula to be C₂₁H₂₂O₇. The signals at 1718, 1653 and 1607 cm⁻¹ in the IR spectrum were assigned to carbonyl and aromatic system of a coumarin skeleton. The ¹HNMR spectrum in the aromatic proton region of **2** contained two pairs of doublets at δ 6.25 (1H, d, $J=9.5$ Hz), 7.61(1H, d, $J=9.5$ Hz) and 7.37 (1H, d, $J=8.5$ Hz), 6.81 (1H, d, $J=8.5$ Hz), which are identical with the C-3-H, C-4-H signals of the α -pyrone ring system and signals of C-5-H, C-6-H of the benzene ring, indicating **2** was a kind of coumarin substituted at C-7 and C-8 positions. A pair of doublets at δ 5.35 (1H, d, $J=3.6$ Hz) and 6.30 (1H, d, $J=3.6$ Hz) were assigned to the methine protons at C-3'-H and C-4'-H linked to two ester groups, showing that C-7 and C-8 of **2** formed a dihydropyran ring by comparison with chemical shifts and the coupling pattern of Pd-Ia.⁶ The chemical shift value of C-4'-H' (δ 6.30) was larger than that of C-3'-H (δ 5.35) because of benzene ring effect. In ¹HNMR spectrum, the signals at δ 2.10 (3H, s) was due to an acetyl group and at δ 6.05 (1H, m), 2.02 (3H, br d), 1.88 (3H, br s) showed the existence of group of -CO(Me)C=CHMe. No NOE enhancement between δ 2.02 and δ 1.88 was observed in NOE difference spectrum, which indicated the group was angeloyl. In HMBC spectrum, the methyl proton signal at δ 2.10 in acetyl was correlated with the carbonyl signal at δ 169.4, accordingly the signal at δ 169.4 was assigned to the carbonyl signal of acetyl, which was correlated with proton signal at δ 5.35 (C-3'-H), indicating that the acetoxy group was attached to C-3'. In HMBC spectrum, the methyl proton signal at δ 1.88 and the olefinic proton signal at δ 6.05 in angeloyl were correlated with the carbonyl signal at δ 166.2, accordingly the signal at δ 166.2 was assigned to the carbonyl signal of angeloyl, which was correlated with proton signal at δ 6.30 (C-4'-H), indicating that the angeloyloxy group was attached to C-4'. The conclusion was further confirmed by the result of partial hydrolysis of **2**. A major product (**4**) was obtained from the partial alkaline hydrolysis of **2** and the structure of **4** was elucidated as 3'(*R*)-acetoxy-4'(*R*)-hydroxy-3',4'-dihydroxanthyletin by ¹HNMR and EIMS spectra.

Stereochemistry of 2 There are two chiral carbon atoms in the molecular structure of **2**, it was reported that the relative configuration of the type of the compounds can be determined by ¹HNMR spectrum. The relative configuration

at C-3' and C-4' was considered to be *trans* on the basis of the coupling constant of C-3'-H and C-4'-H being 3.6 Hz and the difference in the methyl proton signals at δ 1.47 and 1.39 of the 2'-gem-dimethyl group being 0.08.⁷⁻⁹ Compound (**2**) was a levo-compound ($[\alpha]_D -6.6^\circ$) and its absolute configurations were studied further by chemical correlation with known compounds. The enantiomer of **2**, named peucedanocoumarin II ($[\alpha]_D +7.0^\circ$) was isolated from the root of the same genus, *Peucedanum decursivum*,¹² and its total alkaline hydrolysis gave (+)-*trans*-khellactone and (-)-*cis*-khellactone. In the report, the complete hydrolysis of **2** under basic conditions gave a mixture of two products, isolated by HPLC. The compounds were identified by spectral analysis and optical rotation as (-)-*trans*-khellactone (**5**) as a main

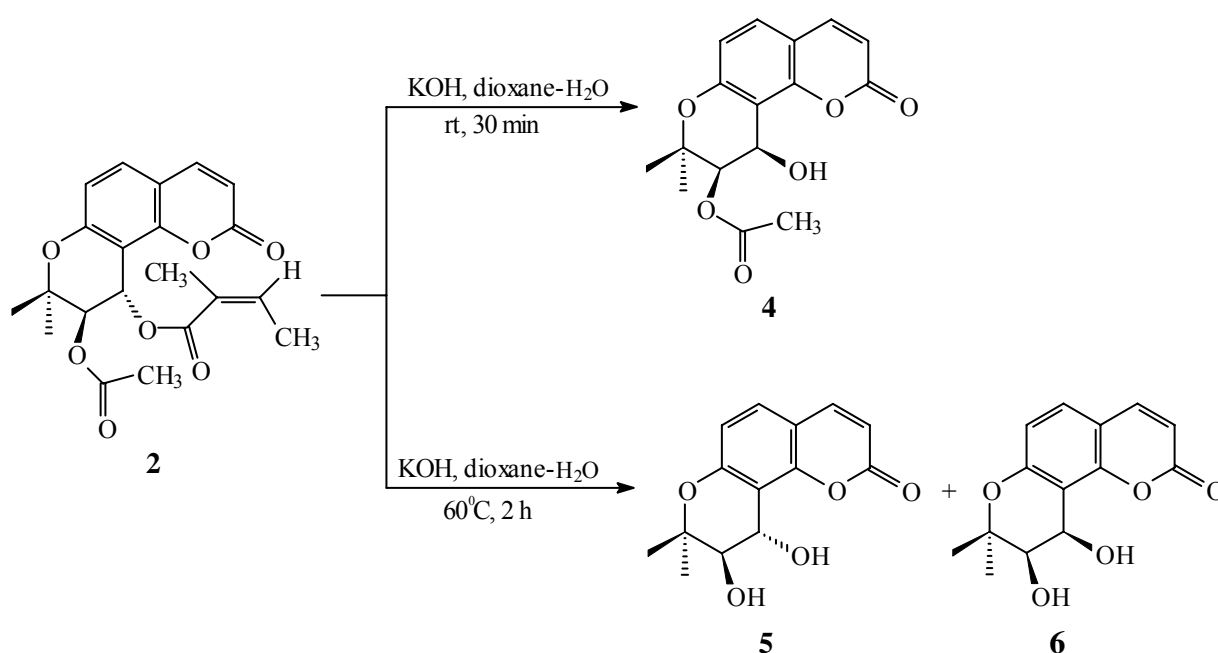


Figure 3 Partial and total alkaline hydrolysis of **2**

product and (+)-*cis*-khellactone (**6**) as a minor artefact arising from epimerization at C-4' because of the benzyl effect and S_N2 reaction mechanism. The absolute configurations of **5** and **6** were undoubtedly described previously as 3'*R*, 4'*S* and 3'*R*, 4'*R* by chemical methods and X- Ray diffraction analysis,^{10,11} accordingly, the absolute configuration of **2** was established as 3'*R*, 4'*S*. The chemical structure of **2** was finally elucidated as 3'(*R*)-acetoxy-4'(*S*)-angeloyloxy- 3', 4'-dihydroseselin.

EXPERIMENTAL

Mps were determined on a X4 micro melting-point apparatus, the thermometer was uncorrect. UV spectra were recorded on a Shimadzu UV-2501 PC spectrophotometer in MeOH solution. IR spectra were obtained on a Nicolet Impact-410 spectrophotometer. 1D NMR and 2D NMR spectra were recorded on a Bruker-DRX-400 spectrometer using TMS as an

internal standard. EIMS were measured on a JEOL-DX-300 mass spectrometer. $[\alpha]_D$ values were determined on a Perkin-Elmer 241 automatic polarimeter at 20°C.

Silica gel H (10-40 μm) was used for Column chromatography. Petrol refers that fraction having bp 60-90°C. Preparative HPLC was carried out on a Shimadzu Liquid Chromatograph LC-8A equipped with a UV detector, using a Shim-Packed PREP-SIL column (10 mm \times 250 mm, Shimadzu), detector wavelength: 320 nm, flow rate: 5.0 mL/min, mobile phase: CHCl_3 : MeOH (48:2).

Plant material Roots of *Peucedanum wawrii* were collected in Laoshan, Jingsu Province, China, in July, 1998 and identified by Prof. Menglan She, Botanical Institute of Jiangsu Province, China. A voucher specimen was deposited in Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Isolation The root material (1.03 kg) was extracted with 95% EtOH (4 \times 3000 mL) for 2 h at 70 °C, the concentrated extracts (85 g) were partitioned between water (2 L) and petrol (3 \times 1.5 L). The petrol solution was concentrated *in vacuo* to yield the residue (21 g). The residue was subjected to column chromatography on silica gel H (350 g) and eluted with a mixture of petrol: EtOAc gradually in creasing polarity. The fraction (2.0 g) of petrol: EtOAc (85:15) was subjected to column chromatography on silica gel H (50 g) again eluted with petrol: EtOAc (88:12), and was finally purified with preparative TLC developed with petrol: EtOAc (70:30) to give compound (**1**) (25 mg). The fraction (1.1 g) of petrol: EtOAc (80:20) was subjected to column chromatography on silica gel H (30 g) again eluted with petrol: EtOAc (85:15), and was finally purified with preparative TLC developed with petrol: EtOAc (60:40) to give compound (**2**) (42 mg).

Characterization

3'(R)-Acetoxy-3',4'-dihydroxanthyletin (1): Colorless needles, mp 174.0-176.0°C, $[\alpha]_D$ -4.1° (c=0.5, CHCl_3). UV (MeOH), λ 332.0 nm (log ϵ 4.18), 255.2 (3.49). IR ν_{max} cm^{-1} : 3388, 2977, 1717, 1696, 1682, 1627, 1386, 1200, 1146, 813. ^1H NMR (400 MHz, CDCl_3) δ : 6.18 (1H, d, $J=9.4$ Hz, H-3), 7.92 (1H, d, $J=9.4$ Hz, H-4), 7.44 (1H, s, H-5), 6.73 (1H, s, H-8), 3.41 (1H, dd, $J=10.8, 3.4$ Hz, H-3'), 2.34 (1H, dd, $J=14.2, 10.8$ Hz, H-4'), 2.95 (1H, dd, $J=14.2, 3.4$ Hz, H-4'), 1.12 (3H, s, C-2'- CH_3), 1.09 (3H, s, C-2'- CH_3), 2.43 (3H, s, COCH_3). ^{13}C NMR (100 MHz, CDCl_3) δ : 160.7 (C-2), 111.2 (C-3), 144.7 (C-4), 130.3 (C-5), 125.1 (C-6), 159.5 (C-7), 101.6 (C-8), 153.7 (C-9), 110.9 (C-10), 71.8 (C-2'), 76.8 (C-3'), 31.4 (C-4'), 26.2 (C-2'- CH_3), 24.7 (C-2'- CH_3), 173.6 (C=O), 28.8 (COCH_3). HREIMS m/z : 288.0994 (calcd 288.0998 for $\text{C}_{16}\text{H}_{16}\text{O}_5$). EIMS (70 eV) m/z (%): 288 (M^+ , 1.1), 264 (20.6), 246 (16.3), 206 (17.2), 176 (96.9), 175 (100), 163 (30.5), 84 (58.3), 66 (66.2).

3'(R)-Acetoxy-4'(S)-angeloyloxy-3',4'-dihydroseselin (2): Colorless needles, mp 125.0-126.5°C, $[\alpha]_D$ -9.8° (c=0.5, CHCl_3). UV (MeOH), λ 322.0 nm (log ϵ 4.08), 255.2 (3.86). IR ν_{max} cm^{-1} : 3328, 2917, 2847, 1737, 1718, 1704, 1653,

1607, 1489, 1372, 1230, 1149, 1113, 1035, 844. ¹HNMR (400 MHz, CDCl₃) δ: 6.25 (1H, d, *J*=9.5 Hz, H-3), 7.61 (1H, d, *J*=9.5 Hz, H-4), 7.37 (1H, d, *J*=8.5 Hz, H-5), 6.81 (1H, d, *J*=8.5 Hz, H-6), 5.35 (1H, d, *J*=3.6 Hz, H-3'), 6.30 (1H, d, *J*=3.6 Hz, H-4'), 1.47(3H, s, C-2'-CH₃), 1.39 (3H, s, C-2'-CH₃), 2.10 (3H, s, H-2''), 6.05 (1H, m, H-3'''), 2.02 (3H, br d, H-4'''), 1.88 (3H, br s, H-5'''). ¹³CNMR (100 MHz, CDCl₃) δ: 159.9 (C-2), 113.3 (C-3), 143.3 (C-4), 129.1 (C-5), 114.5 (C-6), 156.6 (C-7), 106.7 (C-8), 154.2 (C-9), 112.5 (C-10), 77.2 (C-2'), 71.7 (C-3'), 63.0 (C-4'), 23.6 (C-2'-CH₃), 23.8 (C-2'-CH₃), 169.4 (C-1'''), 20.8 (C-2'''), 166.2 (C-1'''), 139.0 (C-2'''), 127.1 (C-3'''), 20.4 (C-4'''), 15.8 (C-5'''). HREIMS *m/z*: 386.1369 (calcd 386.1366 for C₂₁H₂₂O₇). EIMS (70 eV) *m/z* (%): 386 (M⁺, 1.4), 326 (4.9), 311 (13.2), 287 (40.9), 245 (48.5), 229 (90.9), 213 (11.9), 83 (100), 55 (38.9), 43 (39.6).

Fifteen known compounds: The other compounds isolated from title plant in this investigation were identified as bocconin,¹³ Pd-Ib,⁶ *d*-laserpitin,¹⁴ (+)-praeruptorin A,¹⁵ (+)-anomalin,⁶ Pd-III,⁶ columbinandin,¹⁶ selinidin,¹⁷ (+)-deltoin,¹⁸ bergapten,¹⁸ umbelliferone, *d*-mannitol, hexacosanoic acid, β-sitosterol and daucosterol, respectively, by spectral analyses.

Reaction

Alkaline hydrolysis of 1. Compound (**1**) (10 mg) dissolved in dioxane (2.0 mL) was added to 0.5 mol/L KOH (2.0 mL) and the reaction mixture was stirred at 60°C for 2 h. The solution was neutralized with 10% H₂SO₄, extracted with CHCl₃, and the extract was washed with saturated NaHCO₃, dried with Na₂SO₄, and evaporated, the residue was purified with preparative TLC, developed with petrol: EtOAc (3:1), to give 3'(*R*)-hydroxy-3',4'-dihydroxanthyletin (**3**, 6 mg).

*3'(*R*)-Hydroxy-3',4'-dihydroxanthyletin (3)*: White cubic crystals, mp 176.5-178.0°C, [α]_D -11.5° (c=0.05, CHCl₃). IR ν_{max} cm⁻¹: 3384, 2974, 1706, 1627, 1574, 1386, 1145, 815. ¹HMR (400 MHz, CDCl₃) δ: 6.18 (1H, d, *J*=9.4 Hz, H-3), 7.92 (1H, d, *J*=9.4 Hz, H-4), 7.44 (1H, s, H-5), 6.73 (1H, s, H-8), 3.46 (1H, dd, *J*=10.2, 2.0 Hz, H-3'), 2.96 (1H, dd, *J*=13.7, 2.0 Hz, H-4'), 2.35 (1H, dd, *J*=13.7, 10.2 Hz, H-4'). ¹³CNMR (100 MHz, CDCl₃) δ: 160.7 (C-2), 111.2 (C-3), 144.7 (C-4), 130.4 (C-5), 125.1 (C-6), 159.5 (C-7), 101.5 (C-8), 153.7 (C-9), 110.9 (C-10), 76.7 (C-2'), 71.7 (C-3'), 31.3 (C-4'), 26.3 (C-2'-CH₃), 24.7 (C-2'-CH₃).

Partial alkaline hydrolysis of 2. Compound (**2**) (10 mg) dissolved in dioxane (1.5 mL) was added to 0.5 mol/L KOH (1.0 mL) and the reaction mixture was stirred at rt for 30 min. The solution was neutralized with 10% H₂SO₄, extracted with CHCl₃, and the extract was washed with saturated NaHCO₃, dried with Na₂SO₄, and evaporated, the residue was purified with HPLC to give 3'(*R*)-acetoxy-4'(*R*)-hydroxy-3',4'-dihydroseselin (**4**, 4 mg).

*3'(*R*)-Acetoxy-4'(*R*)-hydroxy-3',4'-dihydroseselin (4)*: White solid, [α]_D -6.5° (c=0.02, CHCl₃). ¹HNMR (400 MHz, CDCl₃) δ: 6.24 (1H, d, *J*=9.5 Hz, H-3), 7.64 (1H, d, *J*=9.5 Hz, H-4), 7.32 (1H, d, *J*=8.6 Hz, H-5), 6.78 (1H, d, *J*=8.6 Hz,

H-6), 5.43 (1H, d, $J=4.9$ Hz, H-3'), 5.19 (1H, d, $J=4.9$ Hz, H-4'), 1.48 (3H, s, C-2'-CH₃), 1.41 (3H, s, C-2'-CH₃), 2.16 (3H, s, H-2''). EIMS (70 eV) m/z (%): 304 (M⁺, 10.2), 244 (18.4), 191 (30.5), 167 (14.8), 149 (100), 119 (30.6), 117 (32.1), 55 (32.9), 43 (54.5).

Total alkaline hydrolysis of 2. Compound (**2**) (20mg) dissolved in dioxane (3.0 mL) was added to 0.5 mol/L KOH (3.0 mL) and the reaction mixture was stirred at 60°C for 2 h. The solution was neutralized with 10% H₂SO₄, extracted with CHCl₃, and the extract was washed with saturated NaHCO₃, dried with Na₂SO₄, and evaporated, the residue was purified with HPLC to yield two products. The first eluant (5 mg), which contained the epimerization artefact at C-4', gave (+)-*cis*-khellactone (**6**), the second eluant (8 mg) gave (-)-*trans*-khellactone (**5**).

(-)-trans-Khellactone (5): Colorless needles, mp 184.5-186.0°C, $[\alpha]_D -23.6^\circ$ (c=0.05, CHCl₃). IR ν_{\max} cm⁻¹: 3400, 2975, 2920, 1726, 1605, 1490, 1400, 1368, 1288, 1245, 1179, 1120, 1057, 912, 831. ¹HNMR (400 MHz, CDCl₃) δ : 6.24 (1H, d, $J=9.5$ Hz, H-3), 7.65 (1H, d, $J=9.5$ Hz, H-4), 7.31 (1H, d, $J=8.6$ Hz, H-5), 6.78 (1H, d, $J=8.6$ Hz, H-6), 3.84 (1H, d, $J=6.7$ Hz, H-3'), 4.99 (1H, d, $J=6.7$ Hz, H-4'), 1.52 (3H, s, C-2'-CH₃), 1.30 (3H, s, C-2'-CH₃), 1.25 (2H, s, 2OH). EIMS (70 eV) m/z (%): 262 (M⁺, 26.5), 191 (100), 162 (21.6), 134 (14.8), 107 (7.2), 77 (9.4), 72 (12.9), 57 (6.3).

(+)-cis-Khellactone (6): Colorless needles, mp 170.0-172.0°C, $[\alpha]_D +74.6^\circ$ (c=0.05, CHCl₃). IR ν_{\max} cm⁻¹: 3400, 2985, 2935, 1728, 1715, 1605, 1490, 1395, 1350, 1288, 1241, 1225, 1110, 1017, 992, 842. ¹HNMR (400 MHz, CDCl₃) δ : 6.25 (1H, d, $J=9.5$ Hz, H-3), 7.88 (1H, d, $J=9.5$ Hz, H-4), 7.46 (1H, d, $J=8.6$ Hz, H-5), 6.77 (1H, d, $J=8.6$ Hz, H-6), 3.76 (1H, d, $J=4.9$ Hz, H-3'), 5.10 (1H, d, $J=4.9$ Hz, H-4'), 1.43 (3H, s, C-2'-CH₃), 1.42 (3H, s, C-2'-CH₃), 3.35 (1H, s, OH), 2.15 (1H, s, OH). EIMS (70 eV) m/z (%): 262 (M⁺, 31.4), 213 (3.7), 191 (100), 162 (18.4), 134 (16.5), 107 (5.8), 89 (4.1), 77 (4.5), 72 (9.8), 57 (5.1), 37 (3.9).

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