HETEROCYCLES, Vol. 60, No. 8, 2003, pp. 1915 - 1919
Received, 15th May, 2003, Accepted, 8th July, 2003, Published online, 15th July, 2003

A NEW PYRANOCOUMARIN FROM *PEUCEDANUM PRAERUPTORUM*

Ling-Yi Kong,[a]* Yi Li,[b] and Masatake Niwa [c]

[a]*Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China*

[b]*Analysis & Testing Center, Nanjing Normal University, Nanjing 210097, China*

[c]*Faculty of Pharmacy, Meijo University, Tempaka, Nagoya 468-8503, Japan*

Abstract - A new dihydropyranocoumarin was isolated from the root of *Peucedanum pareruptorum* and the structure were established as 3'(*R*)-isobutyryloxy-4'(*R*)-acetoxy-3',4'-dihydroseselin (**1**) by spectroscopic methods. The absolute configuration was deduced by chemical correlations with known compounds.

Peucedanum praeruptorum is a plant of Umbelliferae, the roots of *P. praeruptorum* and *P. decursivum* are 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. We have described the isolation and structural elucidation of two new dihydropyranocoumarins in the previous papers.[1, 2] In our continuous research work, a new dihydropyranocoumarin was further isolated from the root of *P. praeruptorum*. This paper describes the isolation and structure elucidation of the new compound. By spectroscopic methods, the structure was established as 3'(*R*)-isobutyryloxy-4'(*R*)-acetoxy-3',4'-dihydroseselin (**1**) and the absolute configuration was deduced by chemical correlation with known compounds.

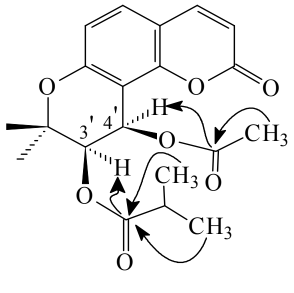

Figure 1 The structure and key HMBC correlations of **1**

Structure of 1 Compound (**1**) was isolated as white powder, the molecular ion at *m/z* 374.1372 in the high resolution mass spectrum showed the molecular formula to be $C_{20}H_{22}O_7$. The absorptions at 1721, 1653 and 1607 cm^{-1} in the IR spectrum were assigned to carbonyl and aromatic system of a coumarin skeleton. The ^{1}HNMR spectrum in the aromatic proton region of **1** contained two pairs of doublets at δ 6.24 (1H, d, *J*=9.5 Hz), 7.59(1H, d, *J*=9.5 Hz) and 7.36 (1H, d, *J*=8.4 Hz), 6.80 (1H, d, *J*=8.4 Hz), which are identical with the C-3-H and C-4-H signals of the α-pyrone ring system and signals of C-5-H and C-6-H of the benzene ring, indicating **1** was a kind of coumarin substituted at C-7 and C-8 positions. A pair of doublets at δ5.31 (1H, d, *J*=4.9 Hz) and 6.52 (1H, d, *J*=4.9 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 **1** formed a dihydropyran ring by comparison with chemical shifts and the coupling pattern of Pd-Ia.[3] The chemical shift value of C-4-H' (δ 6.52) was larger than that of C-3'-H (δ 5.31) because of benzene ring effect. In ^{1}HNMR spectrum, the signals at δ 2.60 (1H, m), 1.22 (3H, d, *J*=4.3 Hz), 1.20 (3H, d, *J*=4.3 Hz) showed the existence of isobutyryl group and δ 2.09 (3H, s) was due to an acetyl group. In HMBC spectrum, the proton signal at δ 2.60 in isobutyryl was correlated with the carbonyl signal at δ 175.84, accordingly the signal at δ 175.84 was assigned to the carbonyl signal of isobutyryl, which was correlated with proton signal at δ 5.31 (C-3'-H), indicating that the isobutyryloxy group was attached to C-3'. In HMBC spectrum, the methyl proton signal at δ 2.09 was correlated with the carbonyl signal at δ 169.87, accordingly the signal at δ 169.87 was assigned to the carbonyl signal of acetoxy group, which was correlated with proton signal at δ 6.52 (C-4'-H), indicating that the acetoxy group was attached to C-4'.

Stereochemistry of 1 There are two chiral carbon atoms in the molecular structure of **1**, it was reported that the relative configuration of the type of the compounds can be determined by ^{1}HNMR spectrum. The relative configuration at C-3' and C-4' was considered to be *cis* on the basis of the coupling constant of C-3'-H and C-4'-H being 4.9 Hz and the difference in the methyl proton signals at δ 1.45 and 1.41 of the 2'-gem-dimethyl group being 0.04.[4-6] Compound (**1**) was a levo-compound ($[\alpha]_D$ -19.8°) and its absolute configuration was studied further by chemical correlation with known compounds. On total alkaline hydrolysis, **1** gave a mixture of two products, isolated by HPLC. The compounds were identified by spectral analysis and optical rotation as (+)-*cis*-khellactone (**2**) and (-)-*trans*-khellactone (**3**) as a minor artifact arising from epimerization at C-4' because of the benzyl effect and SN_2 reaction mechanism. The absolute configurations of **2** and **3** were undoubtedly described previously as 3'*R*, 4'*R* and 3'*R*, 4'*S* by chemical methods and X- Ray diffraction analysis,[7, 8] accordingly, the absolute configuration of **1** was established as 3'*R*, 4'*R*. The chemical structure of **1** was finally elucidated as 3'(*R*)-isobutyryloxy-4'(*R*)-acetoxy-3',4'-dihydroseselin.

In the structures of dihydropyranocoumarins isolated from nature, there are generally acyl groups containing five carbon atoms such as isovaleryl, angeloyl, tigloyl, the isobutyl group has seldom been found in the plants. There is a only same plane formulation containing isobutyl isolated from *Seseli bocconi* subsp. *bocconi* and subsp. *praecox Gamisans*,

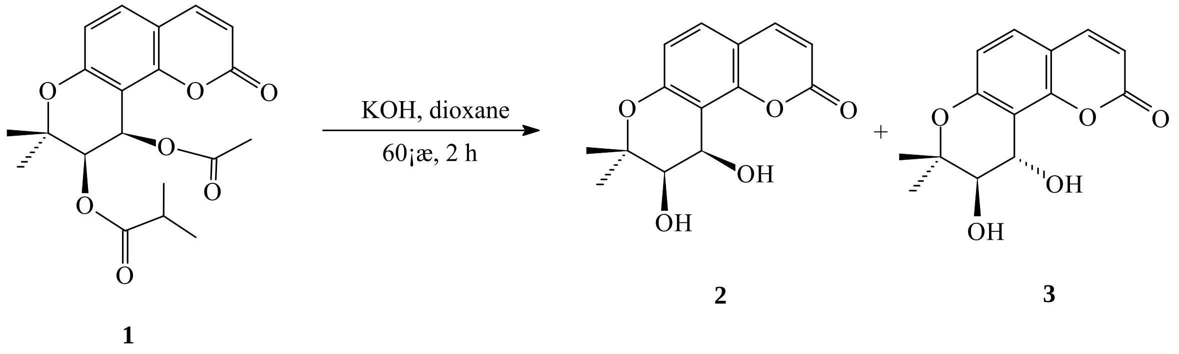

Figure 2 The total alkaline hydrolysis of **1**

named bocconin,[9] but its absolute configuration was not determined in the literature.[9] It is a dextro-compound ($[\alpha]_D$ +7.81°), its absolute configuration should be 3'*S*, 4'*S* from the result of our present research.

EXPERIMENTAL

Mps were determined on a X-4 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 Bio-Rad FTS-135 spectrophotometer. 1D NMR and 2D NMR spectra were recorded on a Bruker-DRX-500 spectrometer using TMS as an internal standard. EIMS were measured on a JEOL-DX-300 mass spectrometer. $[\alpha]_D$ values were determined on a JASCO-20C 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 × 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 praeruptorum* were collected in Wuyi county, Zhejiang Province, China, in September, 1999 and identified by Dr. Minjian Qin, China Pharmaceutical University. A voucher specimen was deposited in Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Isolation The root material (1.50 kg) was extracted with 95% EtOH (4×4000 mL) for 2 h at 80°C, the concentrated extracts (105 g) were partitioned between water (3 L) and petrol (3×2.0 L). The petrol solution was concentrated *in vacuo* to yield the residue (26 g). The residue was subjected to column chromatography on silica gel H (380 g) and eluted with a mixture of petrol: EtOAc gradually in creasing polarity. The fraction (0.6 g) of petrol: EtOAc (82:18) was subjected to column chromatography on silica gel H (20 g) again eluted with petrol: EtOAc (85:15), and was finally purified with preparative TLC developed with petrol: EtOAc (70:30) to give compound (**1**) (28 mg).

Characterization

*3'(R)-isobutyryloxy-4'(R)-acetoxy-3',4'-dihydroseselin (**1**)*: White powder, $[\alpha]_D$ –19.8° (c=0.1, $CHCl_3$). UV (MeOH), λ 322.0 nm (log ε 4.02), 256.0 (3.62), 244.8 (3.89). IR ν_{max} cm^{-1}: 3381, 2979, 2837, 1744, 1721, 1653, 1607, 1489, 1371, 1228, 1146, 1109, 1057, 841. ^{1}HNMR (500 MHz, $CDCl_3$) δ: 6.24 (1H, d, *J*=9.5 Hz, H-3), 7.59 (1H, d, *J*=9.5 Hz, H-4), 7.36 (1H, d, *J*=8.4 Hz, H-5), 6.80 (1H, d, *J*=8.4 Hz, H-6), 5.31 (1H, d, *J*=4.9 Hz, H-3'), 6.52 (1H, d, *J*=4.9 Hz, H-4'), 1.45 (3H, s, C-2'-CH_3), 1.41 (3H, s, C-2'-CH_3), 2.60 (1H, m, H-2"), 1.22 (3H, d, *J*=4.3Hz, H-3"), 1.20 (3H, d, *J*=4.3Hz, H-4"), 2.09 (3H, s, H-2'"). ^{13}CNMR (100 MHz, $CDCl_3$) δ: 159.73 (C-2), 113.32 (C-3), 143.17 (C-4), 129.19 (C-5), 114.43 (C-6), 154.09 (C-7), 107.26 (C-8), 156.65 (C-9), 112.58 (C-10), 77.22 (C-2'), 70.33 (C-3'), 60.57 (C-4'), 25.08 (C-2'-CH_3), 22.34 (C-2'-CH_3), 175.84 (C-1"), 34.15 (C-2"), 18.95 (C-3"), 18.84 (C-4"), 169.87 (C-1'"), 20.68(C-2'"). HREIMS *m/z*: 374.1372 (calcd 374.1366 for $C_{20}H_{22}O_7$). EIMS (70 *eV*) *m/z* (%): 374 (M^+, 1.1), 314 (9.8), 299 (18.6), 261 (10.5), 244 (20.4), 229 (100), 191 (9.7), 71 (14.5), 43 (25.5).

Reaction

*Total alkaline hydrolysis of **1***. Compound (**1**) (24 mg) dissolved in dioxane (3.5 mL) was added to 0.5 mol/L KOH (3.5 mL) and the reaction mixture was stirred at 60°C for 2 h. The solution was neutralized with 10% H_2SO_4, extracted with $CHCl_3$, and the extract was washed with saturated $NaHCO_3$, dried with Na_2SO_4, and evaporated, the residue was purified with preparative HPLC to yield two products. The first eluant (9 mg) gave (+)-*cis*-khellactone (**2**), the second eluant (5 mg), which contained the epimerization artefact at C-4', gave (-)-*trans*-khellactone (**3**).

*(+)-cis-Khellactone (**2**)*: Colorless needles, mp 169.0-171.0°C, $[\alpha]_D$ +71.8° (c =0.05, $CHCl_3$). IR ν_{max} cm^{-1}: 3402, 2985, 2934, 1729, 1715, 1608, 1490, 1392, 1347, 1290, 1241, 1228, 1112, 1017, 990, 841. ^{1}HNMR (500 MHz, $CDCl_3$) δ: 6.23 (1H, d, *J*=9.4 Hz, H-3), 7.89 (1H, d, *J*=9.4 Hz, H-4), 7.46 (1H, d, *J*=8.6 Hz, H-5), 6.78 (1H, d, *J*=8.6 Hz, H-6), 3.77(1H, d, *J*=4.9 Hz, H-3'), 5.11 (1H, d, *J*=4.9 Hz, H-4'), 1.43 (3H, s, C-2'-CH_3), 1.42 (3H, s, C-2'-CH_3), 2.15 (2H, s, 2OH). EIMS (70 *eV*) *m/z* (%): 262 (M^+, 35.4), 213 (3.1), 191 (100), 162 (20.8), 134 (17.5), 107 (3.8), 77 (4.9), 72 (9.2), 57 (5.1), 37 (4.8).

*(-)-trans-Khellactone (**3**)*: Colorless needles, mp 183.5-185.0°C , $[\alpha]_D$ -21.4° (c=0.05，$CHCl_3$). IR ν_{max} cm^{-1}: 3400, 2978, 2925, 1724, 1605, 1488, 1401, 1366, 1289, 1245, 1178, 1124, 1059, 912, 832. ^{1}HNMR (500 MHz, $CDCl_3$) δ: 6.25 (1H, d, *J*=9.4 Hz, H-3), 7.64 (1H, d, *J*=9.4 Hz, H-4), 7.33 (1H, d, *J*=8.6 Hz, H-5), 6.80 (1H, d, *J*=8.6 Hz, H-6), 3.86 (1H, d, *J*=6.9 Hz, H-3'), 5.01 (1H, d, *J*=6.9 Hz, H-4'), 1.50 (3H, s, C-2'-CH_3), 1.30 (3H, s, C-2'-CH_3), 1.55 (2H, s, 2OH). EIMS (70 *eV*) *m/z* (%): 262 (M^+, 28.9), 191 (100), 162 (24.2), 134 (12.6), 107 (9.1), 77 (10.5), 72 (11.7), 57 (8.2).

ACKNOWLEDGMENT

This research work was supported by the National Natural Science Foundation of China and the Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions of MOE, P. R. China (to L.-Y. K.).

REFERENCES AND NOTES

1. L. Y. Kong, Y. Li, Z. D. Min, X. Li, and T. R. Zhu, *Phytochemistry*, 1996, **41**, 1423.
2. L. Y. Kong, Z. D. Min, Y. Li, X. Li, and Y. H. Pei, *Phytochemistry*, 1996, **42**, 1689.
3. T. Okuyama and S. Shibata, *Planta Medica*, 1981, **42**, 89.
4. F. Bohlmann, V. S. B. Rao, and M. Grenz, *Tetrahedron Lett.*, 1968, 3947.
5. A. G. Gonzalez, J. T. Barroso, J. G. Luis, and L. F. Rodriguez, *Phytochemistry*, 1979, **18**, 1021.
6. B. E. Nielsen, P. K. Larsen, and J. Lemmich, *Acta. Chem. Scand.*, 1971, **25**, 529.
7. J. Lemmich, P. A. Pedersen, and B. E. Nielsen, *Tetrahedron Lett.*, 1969, 3363.
8. H. Bernotat, A. Niggli, L. Ulrich, and H. Schmid, *Helv. Chim. Acta*, 1969, **52**, 1165.
9. A. Bellino, P. Venturella, M. L. Marino, O. Servettaz, and G. Venturella, *Phytochemistry*, 1986, **25**, 1195.