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PLANT COUMARINS.
3. (+)-PTERYXIN FROM *Peucedanum terebinthaceum*

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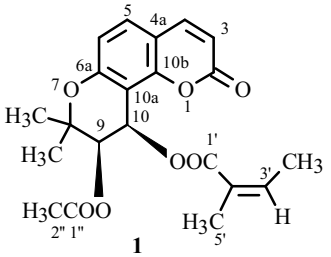
The potentially medically valuable pyranocoumarin (+)-pteryxin was isolated for the first time from Peucedanum terebinthaceum *Fischer et Turcz. The structure of (+)-pteryxin was rigorously proved using mass spectrometry, NMR, IR, and UV spectroscopy and comparison of the spectral characteristics of this compound and its basic hydrolysis products (+)-*cis- *and (-)-*trans-*khellactone and angelic and acetic acids.*

Key words: pyranocoumarin, (+)-pteryxin, *Peucedanum terebinthaceum* Fischer et Turcz., (+)-*cis*-khellactone, (-)-*trans*-khellactone, angelic acid, NMR spectroscopy.

In continuation of our work on plant coumarins [1], we investigated [2] the coumarin composition of *Peucedanum terebinthaceum* Fischer et Turcz., syn. *P. deltoideum* (Makino), (Umbelliferae, syn. Apiaceae) from the Mongolian flora [3]. The isolation from roots of this species of the furocoumarins deltoin [4] and peucedanin [5] has been reported. The dipyranocoumarin decursin has been isolated from fruit of this plant [6].

We isolated from the acetone extract of dry roots by chromatography a pure product as a viscous oil with $[\alpha]_{578}^{20}$ +10.7° (*c* 5.4, EtOH). The PMR and ^{13}C NMR spectra and high-resolution mass spectrum (*m/z* of the molecular ion and relative intensities of its fragments) of this product were similar to the corresponding spectra of (+)-pteryxin (**1**) {(±)-3′-*O*-angeloyl-4′-acetyl-*cis*-khellactone or (9*R*,10*R*)-9-(acetyloxy)-9,10-dihydro-8,8-dimethyl-2-oxo-2H,8H-benzo[1,2-b:3,4-b′]-dipyran-10-yl ester of 2-methyl-2*Z*-butenoic acid} [7-9]. The IR and UV absorption bands of our sample were also similar to the literature values [8, 9].



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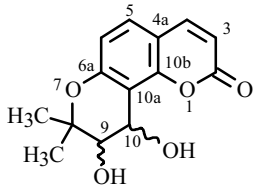

2a,b

2a: (+)-*cis*-9β,10β-OH, (-)-*cis*-9α,10α-OH
2b: (+)-*trans*-9α,10β-OH, (-)-*trans*-9β,10α-OH

The small difference of the chemical shifts for the geminal dimethyl groups in the PMR spectrum (0.02 ppm) and the small vicinal SSCC of H-9—H-10 (J = 5.0 Hz) [10] also argued in favor of the *cis*-configuration of the angeloyl and acetyl groups. We note for comparison that the difference in chemical shifts for the geminal dimethyl groups of model (-)-*cis*- (**2a**) and (+)-*trans*-khellactones (**2b**) were 0.01 and 0.22 ppm whereas the vicinal constants were 4.9 and 6.7 Hz, respectively [11].

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The presence of the angeloyl group in (+)-pteryxin was confirmed by the similar chemical shifts of the olefin proton (6.01 ppm) and the corresponding value in the methyl ester of angelic acid (5.97 ppm) [12]. The corresponding values for the isomeric methyl ester of tiglic acid were 6.72 ppm [12].

The structure of **1** isolated by us was confirmed by 2D ^{13}C—^{1}H COLOC NMR spectra. The presence of cross peaks for resonances of the C atom of the CO group bonded to the OAc group (δ 168.8) and atoms H-9 (δ 5.34) and C$\underline{H}_3$CO (δ 2.07) in addition to cross peaks for resonances of the C atom of the CO group bonded to the angeloyl fragment (δ 165.9) and atoms H-10 (δ 6.61) and C(5′)$\underline{H}_3$ (δ 1.85) argued in favor of the acetyl and angeloyl groups in the 9- and 10-positions, respectively. The assignments of atoms C-4a (δ 111.5, coupled with H-6) and C-10a (δ 106.3, coupled with H-6,9,10) could also be refined.

The compound (+)-pteryxin has been reported to be a crystalline compound with mp 79.5-81.0°C and $[\alpha]_D^{24}$ +14.3° (*c* 1.0, EtOH) [8]; mp 81.5-82.5°C (skellysolve B) and $[\alpha]_D^{22}$ +10° (*c* 0.65, EtOH) [13]; and mp 86.0-86.5°C and $[\alpha]_D^{23}$ +12.5° (*c* 2.0, EtOH) [14]; or as an oil with $[\alpha]_D^{25}$ +5.5° (*c* 0.13, EtOH) [9]. We were unable to produce a crystalline sample of **1**, which was apparently due to the presence of interfering impurities.

The structure of **1** was also confirmed by hydrolysis in basic medium. Angelic acid and (+)-*cis*- and (−)-*trans*-khellactones could be isolated. In addition, the PMR spectrum showed acetic acid. Obviously the formation of (−)-*trans*-khellactone was the result of epimerization of the chiral benzyl atom C-10 [11].

The content of **1** in dry roots of *P. terebinthaceum* found by us was 0.98%. It is known that this coumarin possesses anticoagulant [9] and antispasmolytic [13] activity. (±)-Praeruptorin [(+)-3′-*O*-acetyl-4′-*O*-angeloyl-*cis*-khellactone], which is isomeric to (+)-pteryxin, was isolated from roots of *P. praeruptorum* Dunn. and induces apoptosis of certain tumor cells [10]. Therefore, it was necessary to investigate the antitumor activity of (+)-pteryxin. Furthermore, (+)-pteryxin provides a source of (+)-*cis*-khellactone, on the basis of which several antiviral (anti-HIV) agents were synthesized [15, 16].

EXPERIMENTAL

We used freshly distilled solvents and chemically pure and analytically pure reagents. Analytical TLC used Sorbfil UV 254 plates (ZAO Sorbpolimer, RF, Krasnodar, 5-17 μm fixed with silica ash, 110 μm thick) with elution by AcOEt:hexane (1:3 v/v). Preparative TLC used silica gel (60-200 μm). The sorbent was mixed with luminophor K-35 (1 mass %, TU 6-09-458-76, USSR) in order to increase the sensitivity of visual monitoring in UV light.

IR spectra were recorded on a Vector 22 spectrometer in KBr disks; UV spectra, on a Specord UV-Vis spectrophotometer in ethanol ($c = 10^{-4}$ M). Molecular weights and elemental compositions of new compounds were determined using a high-resolution mass spectrometer (Finnigan MAT, Model 8200, EI, 70 eV). Optical rotation angles were measured with a Polamat A polarimeter (Carl Zeiss, λ 578 nm). Specific rotation was expressed in (deg×mL)/(g×dm); solution concentration, g/(100 mL). Melting points were determined on a Kofler apparatus.

NMR spectra were recorded on Bruker AC 200 (^{1}H, 200.13 MHz; ^{13}C, 50.32 MHz), Bruker AC-400 (^{1}H, 400.13 MHz; ^{13}C, 100.61 MHz); and Bruker DRX-500 (^{1}H, 500.13 MHz; ^{13}C, 125.76 MHz) for 10% solutions at 25°C with stabilization on the deuterium resonance of the solvent $CDCl_3$. Chemical shifts (ppm) were measured relative to resonances of internal standards of $CHCl_3$ (δ_H 7.24 ppm and δ_C 76.90 ppm). Multiplicity of resonances in the ^{13}C NMR spectrum were determined by standard methods using J-modulation (JMOD) and with off-resonance decoupling of protons. Resonances in ^{13}C NMR spectra were assigned and the location of the acetyl and angeloyl groups in (+)-pteryxin were found using 2D ^{13}C—^{1}H correlation spectra (COLOC 7-10 Hz) recorded on the Bruker DRX-500 instrument using standard Bruker programs.

Isolation of (+)-Pteryxin (1). Dry ground roots of *P. terebinthaceum* Fischer et Turcz. (1 kg) that were collected near Ulan-Bator in September 2004 were extracted exhaustively with acetone by soaking. The extract was concentrated in vacuo to completely remove acetone and give a heterogeneous resin that was extracted with benzene (3 × 20 mL). The solvent was removed in vacuo to afford a resin (36.3 g). This residue (2 g) was dissolved in ether (3 mL) and subjected to preparative TLC on a loose layer of silica gel (60-200 μm) containing K-35 luminophor (1%). We used plates (30 × 60 cm) with a sorbent layer (2 mm) and elution by hexane:AcOEt (3:1 v/v) at an angle of ~25° and eluent rise 27 cm. The sorbent band with R_f = 0.6 that absorbed strongly in UV light was collected. The product was eluted from the sorbent by the aforementioned mixture using a quartz column in order to observe the course of the elution by periodically irradiating the column with UV light.

Removal of solvent in vacuo produced a viscous oil (0.5 g), $[\alpha]_{578}^{20}$ +10.7° (*c* 5.4, EtOH). PMR spectrum (400.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.42 and 1.44 [3H each, s, gem. $(CH_3)_2$], 1.85 (3H, quintet, J = 1.4, CH_3-5′), 1.97 (3H,

dq, J = 7.3, 1.5, CH_3-4′), 2.07 (3H, s, CH_3-2″), 6.01 (1H, qq, J = 7.3, 1.5, CH-3′), three AB systems at 5.34 and 6.61 (1H each, d, J = 5.0, H-9 and H-10, respectively), 6.19 and 7.56 (1H each, d, J = 9.6, H-3 and H-4, respectively), and 6.78 and 7.33 (1H, each, d, J = 8.4, H-6 and H-5, respectively). ^{13}C NMR spectrum (100.61 MHz, δ_C, ppm): 14.6 C(4′), 19.4 C(5′), 19.7 C(2″), 21.2 and 24.3 gem. $(Me)_2$, 59.1 C(10), 6.95 C(9), 77.1 C(8), 106.3 C(10a), 111.5 C(4a), 112.3 C(3), 113.4 C(6), 126.5 C(2′), 128.2 C(5), 136.9 C(3′), 142.2 C(4), 152.8 C(10b), 155.6 C(6a), 158.7 C(2), 165.9 C(1′), 168.8 C(1″). Mass spectrum (*m*/*z*): found 386.13559, $C_{21}H_{22}O_7$; calc. (*m*/*z*) 386.13654. Mass spectrum (*m*/*z*, I_{rel}, %): 386 (4) $[M]^+$, 326 (25), 311 (46), 287 (64), 261 (26), 246 (11), 245 (69), 244 (21), 243 (21), 229 (82), 227 (30). IR spectrum (ν, cm^{-1}): 1146, 1191, 1608, 1741 (lactone C=O), 2856 and 2929. UV spectrum (EtOH, λ_{max}, nm, log ε): 206 (4.38), 256 (3.48), 323 (3.94).

Hydrolysis of (+)-Pteryxin in Basic Medium. A solution of **1** (531 mg, 1.38 mmol) in dioxane (40 mL) was treated with stirring with KOH solution (80 mL, 0.5 N, 40.0 mmol), heated at 60°C for 2 h, cooled, treated with stirring with H_2SO_4 (27 mL, 10%) until the pH was 4, and extracted with $CHCl_3$ (4 × 10 mL). The extract was dried over $MgSO_4$. The solvent was removed in vacuo to produce an oily residue that, according to analytical TLC (eluent hexane:AcOEt, 1:1 v/v), lacked starting (+)-pteryxin and contained a mixture of angelic acid and *cis*- and *trans*-khellactones (R_f 0.8, 0.6, and 0.5, respectively). According to the PMR spectrum, a resonance for protons of the methyl group of acetic acid (δ 2.1 ppm) was observed in addition to the resonances of the aforementioned products. This residue was dissolved in $CHCl_3$ (5 mL) and subjected to preparative TLC on a loose layer of silica gel (60-200 μm) containing K-35 luminophor (1%). We used plates (30 × 30 cm) with a sorbent layer (2 mm) and elution by hexane:AcOEt (1:2 v/v). The upper band that absorbed weakly in UV light and contained angelic acid and the lower band that absorbed strongly in UV light and contained a mixture of *cis*- and *trans*-khellactones were collected. The products were eluted from the sorbents by EtOAc. Solvent was removed from the effluents in vacuo to produce angelic acid (102 mg, 74%) and a mixture of *cis*- and *trans*-khellactones (292 mg, 81%) (the *cis*/*trans* ratio according of PMR spectra was 1:1.3). A solution (50 mg) of the aforementioned lactone mixture in $CHCl_3$ (0.5 mL) was subjected to preparative TLC on Sorbfil UV 254 plates (10 × 20 cm) with elution by hexane:AcOEt (1:1 v/v) and eluent rise 9 cm. Two bands that absorbed in UV light with R_f 0.5 and 0.6 and corresponded to *trans*- and *cis*-khellactone, respectively, were collected by elution from the sorbent with EtOAc. Solvent was removed in vacuo to produce the crystalline compounds.

Angelic acid: mp 42-44°C (sublimation at 100°C/1 torr). PMR spectrum (200.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.88 (3H, quintet, J = 1.4, CH_3-2), 2.01 (3H, dq, J = 7.4, 1.4, CH_3-4), 6.20 (1H, qq, J = 7.4, 1.4, H-3), 12.25 (1H, br.s, OH). Lit. [12] mp 42-44°C. The PMR spectrum agreed with that published [12].

(–)-*trans*-Khellactone: mp 185-186°C (benzene), $[\alpha]_{578}^{20}$ −18.3° (*c* 2.4, $CHCl_3$). PMR spectrum (200.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.29 and 1.50 [3H each, s, gem. $(Me)_2$], three AB systems at 3.83 and 4.98 (1H each, d, J = 6.8, H-9 and H-10, respectively), 6.23 and 7.63 (1H each, d, J = 9.6, H-3 and H-4, respectively), and 6.76 and 7.29 (1H each, d, J = 8.6, H-6 and H-5, respectively).

(+)-*cis*-Khellactone: mp 174-175°C (benzene), $[\alpha]_{578}^{20}$ +81.2° (*c* 2.0, $CHCl_3$). PMR spectrum (200.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.39 and 1.44 [3H each, s, gem. $(Me)_2$], three AB systems at 3.84 and 5.19 (1H each, d, J = 5.2, H-9 and H-10, respectively), 6.23 and 7.64 (1H each, d, J = 9.6, H-3 and H-4, respectively), and 6.77 and 7.30 (1H each, d, J = 8.6, H-6 and H-5, respectively).

Literature data [17]: (–)-*trans*-khellactone, mp 185-186°C (benzene), $[\alpha]_D^{21}$ −18.0 ± 1.0° (*c* 0.90, $CHCl_3$); (+)-*cis*-khellactone, mp 174-175°C (benzene), $[\alpha]_D^{20}$ +80.9 ± 1.0° (*c* 1.04, $CHCl_3$). PMR spectra agreed with those for (+)-*trans*- and (–)-*cis*-khellactone, respectively [18].

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