# Isolation and Characterization of an Angular-Type Dihydropyranocoumaringlycoside from the Fruits of *Ammi visnaga* (L.) LAM. (Apiaceae)

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*Ammi visnaga*, Angular-Type Dihydropyranocoumarin-glycoside; *cis*-Khellactone-3′-β-D-glucopyranoside

An angular-type dihydropyranocoumaringlucoside, namely, *cis*-Khellactone-3′-β-D-glucopyranoside (**1**) was isolated from the fruits of *Ammi visnaga* (L.) LAM. (Apiaceae). The structure was established on the basis of acid hydrolysis and spectral data.

## Introduction

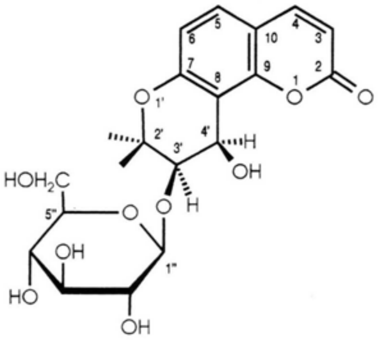
Our investigation of the MeOH extract. sicc. (Sonnenberg, 1994) of the fruits of *Ammi visnaga* (L.) LAM. (Apiaceae) resulted in the isolation of compound **1.**

This natural product has already been isolated from the roots of *Peucedanum praeruptorum* Dunn. (Apiaceae) and the structure determination has also been reported (Takata *et al.*, 1988).

## Results and Discussion

The isolation of compound **1** was successively achieved by extraction with EtOAc and MeOH–EtOAc on a silica gel column. The residue of the latter was separated on reversed phase material by LPLC and further by preparative HPLC. The structure was assigned on the basis of EIMS, FAB MS, HR MS, $^{1}$H NMR, $^{13}$C NMR and homonuclear COSY.

The IR spectrum indicated the presence of hydroxygroup (3405 $cm^{-1}$), α-pyrone ring (1715 $cm^{-1}$) and an aromatic ring (1603, 1488, 1405 $cm^{-1}$). EIMS of **1** showed a molecular ion peak $[M]^+$ at *m/z* 424, that was confirmed by negative FAB MS. From the HR MS the molecular formula could be deducted as $C_{20}H_{24}O_{10}$ . $^{1}$H NMR data for **1** revealed the presence of two pairs of doublets at δ (ppm) 8.00 (d, $J$ = 9.5 Hz, H-4), 6.30 (d, $J$ = 9.5 Hz, H-3) and at 7.55 (d, $J$ = 8.6 Hz, H-5) 6.79 (d, $J$ = 8.6 Hz, H-6). Characteristic signals at δ 3.90 and 5.18 (each 1H, d, $J$ = 4.1 Hz) corresponded to methine protons (H-3′ and H-4′). The relative *cis* configuration of **1** was assigned on the basis of the coupling constant $^{J}$H-3′, H-4′ = 4.1 Hz, which was characteristic between 4.1 and 5.0 for *cis* compounds (González *et al.*, 1979). The methyl signals of the dimethyl group at C-2′ appeared as two singlets at δ 1.40 (s, CH3) and δ 1.42 (s, CH3) close together as in *cis* compounds (González *et al.*, 1979; Macias *et al.*, 1989). Therefore the aglycon of **1** had to be an angular-type of dihydropyranocoumarine such as *cis*-Khellactone. The anomeric proton appeared at δ 4.55 (d, $J$ = 7.7 Hz, H-1″). Signals at δ 3.05 to 3.68 were referred to the other protons of the glucosyl moiety. All the signals have been unambiguously confirmed by means of the $^{13}$C NMR and COSY experiments. After acid hydrolysis (Amoros *et al.*, 1987) on tlc plate glucose was detected. Therefore compound **1** was determinated as *cis*-Khellactone-3′-β-D-glucopyranoside (see formula).



**1**

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This is the first report of the isolation of an angular-type dihydropyranocoumaringlycoside from these fruits.

## Experimental

### *General experimental procedures*

$^1$H NMR, $^{13}$C NMR and COSY experiments were performed on a Bruker AC-300 spectrometer operating at 300 and 75.47 MHz, respectively. NMR spectra were recorded in DMSO-$d_6$, and chemical shifts ($\delta$) were given in ppm with TMS as internal standard. EIMS, HR MS and negative FAB MS were obtained using MAT-711 and $CH_5$-DF Finnigan spectrometers. IR spectrum was recorded with a Perkin-Elmer 240 spectrophotometer. LPLC was performed with Lobar RP-8, 440×37mm, 40–63 μm of Merck, Darmstadt, elute MeOH–$H_2O$–HCOOH (50:50:0.01), flow rate 10 ml min$^{-1}$, UV detection at 254 nm. HPLC was performed with preparative and analytical devices of Knauer, Bad Homburg. Preparative parameters were column Euro-Prep 60–10, C-18, 10 μm, 250×32 mm, Knauer, Bad Homburg, elute MeOH–$H_2O$ (30:70), flow rate 20 ml min$^{-1}$, UV detection at 210 nm. Analytical parameters were column LiChrosorb RP-18, 5 μm, 250×4 mm, elute MeCN–$H_2O$ (20:80 to 50:50 v/v) in 30 min, flow rate 1 ml min$^{-1}$, UV detection at 225 nm.

### *Plant material*

Fruits of *Ammi visnaga* (L.) LAM. (charge Ch-B 00227090) of the provenience of Albania were obtained from Caesar & Loretz, Hilden, Germany. Extract. sicc. of A. visnaga fruits was prepared by exhaustive percolation at room temp. with 70% MeOH (1:12 G/V) by H. Finzelberg's Nachfolger, Andernach, Germany in 1988 (charge no. 1465701). Evaporation was done *in vacuo* at 40–60 °C, 150 mbar.

### *Extraction and isolation*

Extract. sicc. (20 g) from the fruits of *A. visnaga* was exhaustively percolated at room temp. with EtOAc (48 h) (5 liters), MeOH–EtOAc (30:70 v/v) (8 liters) on a silica gel column (600 g). The MeOH–EtOAc eluate was evaporated and a portion of this extract (1 g) was fractioned by LPLC with an elute (5 liters) of MeOH–$H_2O$–HCOOH (50 : 50 : 0.01 v/v/v) and three fractions were obtained (I–III). Fraction III (1 liter) was evaporated and separated by preparative HPLC to give pure compound **1** (16 mg) at $R_t$ = 97 min.

Detection of compound **1** was also possible in the *n*-BuOH-soluble fraction of the MeOH extract from the fruits of *Ammi visnaga* (L.) LAM. (Apiaceae). The n-butanol fraction was evaporated, purified on Sephadex LH 20 column by elution with MeOH and **1** (yield ca. 0.005%) identified using analytical HPLC ($R_t$ = 9.5 min).

### *cis-Khellactone-3'-β-*D*-glucopyranoside* (**1**)

Crystalline solid; $[\alpha]_D^{21}$ = −15 (C = 0.2; MeOH); UV (MeOH) $\lambda_{max}$ nm: 245, 325; IR (KBr) $\nu_{max}$ cm$^{-1}$: 3405 (OH),1715 (α-pyrone C=O), 1603, 1488, 1405 (aromatic C=C); Negative FAB MS (DMSO/glycerol): *m/z* $[M-H]^-$ 423, $[M-H-162]^-$ 261; EIMS: *m/z* $[M]^+$ 424, 406, 273, 262, 244, 229, 213,191,175,162; HR MS: *m/z* $[M]^+$ 424.1316 (calcd. for $C_{20}H_{24}O_{10}$, 424.1369), 406.1262 (calcd for $C_{20}H_{22}O_9$, 406.1264), 262.0839 (calcd for $C_{14}H_{14}O_5$, 262.0841), 191.0343 (calcd. for $C_{10}H_7O_4$, 191.0344); $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.40 (s, $CH_3$), 1.42 (s, CH3), 3.05–3.68 (6H, glucosyl protons), 3.90 (d, $J$ = 4.1 Hz, H-3′), 4.55 (d, $J$ = 7.7 Hz, anomeric proton, H-1″), 4.96 (br s, OH), 5.18 (d, $J$ = 4.1 Hz, H-4′), 5.80 (s, OH), 6.30 (d, $J$ = 9.5 Hz, H-3), 6.79 (d, $J$ = 8.6, H-6), 7.55 (d, $J$ = 8.6 Hz, H-5), 8.00 (d, $J$ = 9.5 Hz, H-4), 8.50 (s, OH); $^{13}$C NMR (see Table I).

### *Acid hydrolysis on TLC (Amoros et al., 1987)*

Compound **1** was applied on Si gel TLC and left in an HCL atmosphere at room temp. for 15 min. HCL vapour was eliminated under hot ventilation,

Table I. $^{13}$C NMR chemical shifts of compound **1** (DMSO-$d_6$, 75.47 MHz).

| Position | $\delta$ $^{13}$C | Position | $\delta$ $^{13}$C | Position (sugar moiety) | $\delta$ $^{13}$C |
|---|---|---|---|---|---|
| C-2 … | 160.2 | C-9 … | 154.0 | C-1″ … | 101.6 |
| C-3 … | 111.9 | C-10 … | 112.0 | C-2″ … | 73.8 |
| C-4 … | 144.6 | C-2′ … | 77.6 | C-3″ … | 76.4 |
| C-5 … | 129.1 | C-3′ … | 78.3 | C-4″ … | 69.9 |
| C-6 … | 113.8 | C-4′ | 58.1 | C-5″ … | 77.0 |
| C-7 … | 155.5 | 2′-$CH_3$ | 21.8* | C-6″ … | 61.1 |
| C-8 … | 110.6 | 2′-$CH_3$ | 27.0* | | |

* Assignments in the column may be interchanged.

afterwards authentic sugars were applied to the plate. The plate was developed with the solvent system EtOAc–MeOH–HAc–$H_2O$ (60:15:15:10) and spots detected by spraying with thymol–$H_2SO_4$ conc. reagent followed by heating. The hydrolysed sugar of **1** was detected as glucose.

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