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ISOLATION AND ^{1}H AND ^{13}C NMR OF AMMIOL AND KHELLOL GLUCOSIDES

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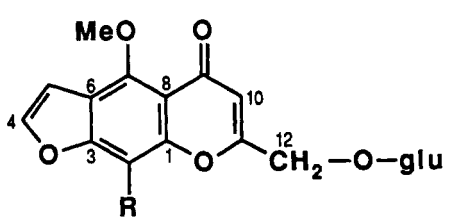
ABSTRACT.—The glucosides of the furanochromones ammiol and khellol were isolated from *Ammi visnaga* seeds and identified by their mass and nmr spectra. Although the aglycones are well-known compounds, this is the first report of ammiol glucoside and the first compilation of the ^{13}C-nmr spectra of the glucosides. ^{1}H nmr showed both glucosides to have β linkages.

Khellol and ammiol are well-known furanochromones that exhibit a wide variety of biological activities. Their antiatherosclerotic, lipid-altering, antipyretic, and spasmolytic properties have made them of considerable interest in medicine (1,2). Despite the attention given to these compounds, isolation of the parent glucosides from plant material has seldom been accomplished. In fact, ammiol glucoside has not been previously described. Here, we report the separation and isolation of the two glucosides **1** and **2** from *Ammi visnaga* L. (Umbelliferae) seeds by hplc and describe their ^{1}H- and ^{13}C-nmr spectra.

The crude glucoside mixture proved to be difficult to redissolve in aqueous MeOH or EtOH. Because of this property, hplc had to be carried out with dilute injections, and it took several of them to afford enough of the purified compounds for spectral studies. Solution of the purified compounds proved to be even more difficult; limited solubility was finally obtained with DMSO. Collection of nmr data was therefore time consuming.



1 R=H
2 R=OMe

The purified glucosides were viscous oils that did not yield crystals in our experiments. Cims verified their identification, and eims and nmr of the hydrolysis products confirmed that khellol and ammiol were the aglycones (1,3). The ^{1}H nmr (Table 1) of the glucosides showed typical shifts and coupling constants for the furanochromone moiety, but there is much overlap in the shifts for the glucosyl protons. Our assignments for the furanochromone protons are in agreement with those of Badawi and Fayez (3). The spectra clearly show the β-glucosyl configuration ($J = 6.7$ Hz) for the anomeric protons. The ^{13}C-nmr spectra also agree with the structures. To our knowledge, this is the first report of these spectra for either compound.

EXPERIMENTAL

PLANT MATERIAL AND EXTRACTION.—*A. visnaga* seeds were collected in Israel and identified by staff botanists at the Beltsville Agricultural Research Center, Beltsville, Maryland. A 400-g sample of finely ground seed was Soxhlet-extracted for 6 h in hexane and then for 24 h in MeOH. After the MeOH extract had been reduced to near dryness on a rotatory exaporator, it was partitioned between $CHCl_3$ and H_2O (pH 2). The H_2O layer was made alkaline (pH 10) with NH_4OH and re-extracted with $CHCl_3$. After neutralization and freeze-drying, about 6 g of ma-

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[2]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

TABLE 1. Nmr Spectral Assignments for Ammiol Glucoside [**2**] and Khellol Glucoside [**1**].[a]

^{1}H		Position Number	^{13}C	
2 (DMSO)	**1** (DMSO + CD_3OD)[b]		**2** (DMSO)	**1** (DMSO + CD_3OD)
—	—	1	147.79 (s)	154.95 (s)
—	7.43 (d,0.8)	2	129.87 (s)	94.73 (d)
—	—	3	147.15 (s)	152.86 (s)
7.62 (d,2.2)	7.94 (d,2.3)	4	146.86 (d)	145.50 (d)
6.99 (d,2.2)	7.23 (dd,2.3,0.8)	5	106.18 (d)	105.00 (d)
—	—	6	119.08 (s)	116.31 (s)
—	—	7	149.61 (s)	152.86 (s)
—	—	8	129.87 (s)	111.88 (s)
—	—	9	178.67 (s)	176.88 (s)
6.41 (s)	6.41 (s)	10	109.80 (d)	108.97 (d)
—	—	11	165.78 (s)	163.04 (s)
4.00 (d,14.7); 4.76 (d,14.7)	4.62 (d,15.1); 4.73 (15.1)	12	66.40 (t)	65.12 (t)
4.04 (s)	—	2-OMe	62.31 (q)	—
4.11 (s)	4.10 (s)	7-OMe	61.79 (q)	60.83 (q)
4.64 (d,6.7)	4.37 (d,6.7)	glucose 1	102.95 (d)	102.24 (d)
3.6–4.1 (m)	3.15–3.27 (m)	2	74.04 (d)	73.12 (d)
3.6–4.1 (m)	3.15–3.27 (m)	3	77.05 (d)	76.24 (d)
3.6–4.1 (m)	3.15–3.27 (m)	4	70.71 (d)	69.74 (d)
3.6–4.1 (m)	3.15–3.27 (m)	5	77.36 (d)	76.68 (d)
3.69 (dd, 11.9,1.6); 3.90 (dd,11.9,5.4)	3.55 (dd,10,5.4); 3.75 (dd,10,7)	6	62.40 (t)	60.87 (t)

[a]Parentheses in body of table enclose multiplicities and J values: s=singlet, d=doublet, t=triplet, q=quartet; J values are in Hz.

[b]Data from Badawi and Fayez (3).

terial containing the crude glucosides was collected.

CHROMATOGRAPHY AND ISOLATION.—The crude glucoside fraction was then dissolved in H_2O-MeOH (1:1) and separated by hplc on a Zorbax-ODS (Dupont) 9.4 × 250-mm column with an isocratic solvent consisting of EtOH-MeCN-H_2O (1:1:8) at a flow rate of 5 ml/min; peaks were detected by a differential refractometer. About 1 mg of amniol glucoside and 4 mg of khellol glucoside were collected per injection. The two glucosides appeared to represent about 70% of the material eluted from the column; we estimate that the crude glucoside fraction contained about 20 and 80 mg of ammiol and khellol glucosides, respectively.

Nmr spectra were obtained with a Bruker WM-300-WB instrument with the Aspect 2000 data system. The 16K FIDs were acquired at 300 MHz and 75.5 MHz for ^{1}H and ^{13}C spectra, respectively. DEPT experiments were used to determine the C-H multiplicities in the ^{13}C spectra. Solvents are given in Table 1, and shifts were measured relative to TMS as an internal reference.

Cims spectra were obtained on a Finnegan 4535/TSQ/MS/MS through the solid inlet probe with isobutane as the reagent gas. Khellol glucoside [**1**]: m/z (rel. int.) 410 (3), $[MH]^+$ 409 (15), 261 (5), $[aglycone + H]^+$ 247 (14), $[aglycone + H - O]^+$ 231 (100), 217 (16), 179 (5), 163 (45), 145 (30), 85 (15); ^{1}H- and ^{13}C-nmr see Table 1. Ammiol glucoside [**2**]: m/z 440 (2), $[MH]^+$ 439 (20), 277 (10), 261 (100), 247 (15), 163 (50), 145 (25); ^{1}H- and ^{13}C-nmr see Table 1.

Glucosides **1** and **2** were hydrolyzed by refluxing for 2 h in 10% HCl in MeOH. Khellol and ammiol were recovered by extraction with $CHCl_3$. They were identified by nmr (3) and gc-ms.

LITERATURE CITED

1. R.B. Gammill, *J. Org. Chem.*, **49**, 5035 (1984).
2. Y. Kondo and T. Takemoto, *Chem. Pharm. Bull.*, **20**, 1940 (1972).
3. M.M. Badawi and M.B.E. Fayez, *Indian J. Chem.*, **5**, 93 (1967).

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