CONSTITUENTS OF ANATOLIAN MEDICINAL PLANTS; FLAVONOIDS OF *HELICHRYSUM ARMENIUM*

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ABSTRACT.—The capitulums and leafy stems of two subspecies of *H. armenium* (*Compositae*), an Anatolian folklore drug, were examined for their flavonoids. Galangin 3-methyl ether, which is present only in the capitulums and leafy stems of subsp. *araxinum*, was found in the *Helichrysum* species for the first time.

The other compounds isolated are: apigenin, luteolin, naringenin, apigenin 4'-glucoside, apigenin 7-glucoside, astragalin, quercetin 3-glucoside, helichrysin A, helichrysin B and isosalipurposide in the capitulums; kaempferol, quercetin, astragalin, and quercetin 3-glucoside in the leafy stems.

There are about 300 *Helichrysum* species in the world (1), and 16 of them grow in Anatolia (2). Today in Europe, people use infusions prepared from the capitulums of *H. arenarium* and *H. italicum* because they are diuretic and they regulate the bile secretion. At present, the capitulums of *H. arenarium* are recorded in the Swiss, Polish and USSR pharmacopoeia.

In addition to investigating the constituents of the Anatolian medicinal plants, we studied the flavonoids in the capitulums and leafy stems of *H. armenium*. Like some other species (3, 4, 5, 6) *H. armenium* is used in the form of infusions as a diuretic and as a cure for kidney stones.

Two subspecies of *H. armenium* DC. grow in eastern Anatolia. These are subsp. *armenium* and subsp. *araxinum* (Kirp.) Takht. (2). There has been no chemical investigation on *H. armenium* other than the work on the quantitative and chromatographic examinations of the flavonoids of the subspecies of this plant growing and cultivated in the USSR (7).

This investigation showed us that the flavonoids are the same in the two subspecies of *H. armenium* except for galangin 3-methyl ether, which is found only in the subsp. *araxinum* and which has been identified in the *Helichrysum* species for the first time.

Apart from this compound, 12 flavonoids were isolated from the 2 subspecies, 6 being aglycones and 6 glycosides (table 1). The main flavonoids of the two subspecies of *H. armenium* are the same as those found in *H. arenarium*, which is used medically. These flavonoids are the derivatives of apigenin, luteolin, kaempferol, quercetin and naringenin. Moreover, the major flavonoids in the

TABLE 1. The flavonoids in *H. armenium*.

Flavonoids	subsp. *armenium*		subsp. *araxinum*	
	capit.	leafy st.	capit.	leafy st.
Galangin 3-methyl ether	−	−	+	+
Apigenin	+	−	+	−
Luteolin	+	−	+	−
Kaempferol	−	+	−	+
Quercetin	−	+	−	+
Naringenin	+	−	+	−
Apigenin 4'-glucoside	+	−	+	−
Apigenin 7-glucoside	+	−	+	−
Astragalin	+	×	+	×
Quercetin 3-glucoside	+	+	+	+
Helichrysin A	+	−	+	−
Helichrysin B	+	−	+	−
Isosalipurposide	+	−	+	−

(×): Identified chromatographically.

capitulums of *H. armenium* are the derivatives of naringenin (helichrysin A and B), just as they are in the capitulums of *H. arenarium* (8, 9, 10, 11, 12).

The qualitative and quantitative results (13) of our analysis show that the capitulums of the two subspecies of *H. armenium* growing in Anatolia can be a good substitute for the capitulums of *H. arenarium*.

EXPERIMENTAL

PLANT MATERIAL.—The capitulums and leafy stems of subsp. *armenium* were collected in August 1976 from Coruh-Kars. Those of subsp. *araxinum* were collected in July 1974 from Bingöl-Tunceli. The specimens are kept in the herbarium of Faculty of Pharmacy, University of Istanbul (ISTE 35 712, 30 424).

EXTRACTION PROCEDURE.—The dried material was extracted according to the previously used methods (3, 4, 5, 6).

SEPARATION AND IDENTIFICATION OF THE FLAVONOIDS.[1]—A total of 13 flavonoids from the petroleum ether-chloroform, ethanol-chloroform and ethanol-ethyl acetate extracts of the capitulums and leafy stems were purified by silica gel (a: Merck 0.2–0.5 mm; b: Merck 0.063–0.125 mm) and Polyamid (BASF Divergan S2 9010) column chromatography and also by preparative paper and thin layer chromatography when necessary.

I.—*Petroleum ether-chloroform extract:* A_1 from the capitulums and leafy stems of subsp. *araxinum*.

Yield: subsp. *armenium*—capitulums 0.8%; leafy stems 1.2%.

Yield: subsp. *araxinum*—capitulums 0.5%; leafy stems 0.8%.

A_1 (Galangin 3-methyl ether): The petroleum ether extract was chromatographed over two successive silica gel columns (a and b) with benzene-acetone. Fractions which contained A_1 yielded it upon concentration (yield: 0.008%). Tlc, uv (14) and ir spectral comparisons with an authentic sample proved that the compound A_1 was galangin 3-methyl ether.

II.—*Ethanol-chloroform extract:* A_2, A_3, A_4 from the capitulums; A_5, A_6 from the leafy stems of both subspecies.

Yield: subsp. *armenium*—capitulums 1.2%; leafy stems 1.8%.

Yield: subsp. *araxinum*—capitulums 2.6%; leafy stems 0.8%.

A_2 (Naringenin); A_3 (Apigenin); A_4 (Luteolin); A_5 (Kaempferol); and A_6 (Quercetin): The ethanol-chloroform extract was chromatographed on silica gel (a) with benzene-ethanol. The residue of the fractions were purified by tlc or preparative paper chromatography and A_2, A_3, A_4, A_5 and A_6 were identified respectively as naringenin, apigenin, luteolin, kaempferol and quercetin by direct comparison of physical properties (tlc, uv and ir) with authentic samples.

III.—*Ethanol-ethyl acetate extract:* G_1, G_2, G_3, G_4, G_5, G_6 and G_7 from the capitulums; G_3 and G_4 from the leafy stems too.

Yield: subsp. *armenium*—capitulums 1.9%; leafy stems 0.4%.

Yield: subsp. *araxinum*—capitulums 1.8%; leafy stems 0.5%.

The precipitate (10.74 g) of the ethanol-ethyl acetate extract of the capitulums was chromatographed on silica gel (a) with benzene-ethanol (2/1). The residue (5.6 g) of fractions 1–14 was rechromatographed on silica gel (b) with benzene-ethanol (2/1) as the eluent.

G_1 (Helichrysin B [(±)-naringenin]-5-glucoside): The precipitate of fractions 1–45 was crystallized from methanol, mp 228° (yield: 0.09%). Acid hydrolysis yielded glucose and naringenin. G_1 showed physical properties (tlc, uv and ir) identical with those of a known sample (5, 6).

G_2 (Helichrysin A [(−)-naringenin]-5-glucoside): After the removal of Helichrysin B, the methanolic solution was concentrated to dryness, and the residue was crystallized from methanol to give G_2, mp 162°, (yield: 0.02%). Acid hydrolysis yielded glucose and naringenin. The product was identical (mp, tlc, uv and ir) with helichrysin A (5, 6).

G_3 (Astragalin (kaempferol 3-glucoside): The residue (0.85 g) from the supernatant of fractions 1–45 was chromatographed on polyamid with benzene-ethanol as eluent. Fractions 40–49 (80/20) were purified by paper chromatography (BAW 4/1/5; Rf 0.45) (yield: 0.02%). Acid hydrolysis yielded glucose and kaempferol. The product was identical (tlc, uv and ir) with kaempferol 3-glucoside (4).

G_4 (Quercetin 3-glucoside) and G_5 (Isosalipurposide): The residue of fractions 73–128 of the polyamid column (benzene-ethanol 80/20, 60/40) was purified by preparative paper chromatography (BAW 4/1/5). The band Rf 0.37 gave quercetin 3-glucoside (yield: 0.002%). The product yielded glucose and quercetin by acid hydrolysis and showed physical properties (tlc, uv and ir) identical with a known sample (15).

Finally, the band Rf 0.20 gave isosalipurposide (yield: 0.001%). The product was identical (tlc, uv and ir) with isosalipurposide (5, 6).

G_6 (Apigenin 7-glucoside): The residue (0.80 g) from the supernatant of fractions 1–45 (silica gel column b), was chromatographed on polyamid with water and water-MeOH as eluents. Fractions 64–74 (40/60) were purified by preparative paper chromatography (BAW 4/1/5); the band Rf 0.23 yielded a yellow compound (yield: 0.01%).

Acid hydrolysis yielded glucose and an aglycon which showed physical properties (tlc and uv) identical with a known sample of apigenin. The uv shifts are in agreement with the literature values of apigenin 7-glucoside (14).

[1]Numerical values in the experimental part are based on the results obtained from subsp. *araxinum*.

G_7 (Apigenin 4'-glucoside): Fractions 60–143 of silica gel column (b) yielded a yellow compound (yield: 0.01%); uv λ max (MeOH), 322, 290(sh), 270; NaOMe, 363, 292(sh), 275; $AlCl_3$, 382, 340, 292(sh), 278; $AlCl_3$/HCl, 380, 335, 297(sh), 278; NaOAc, 362, 300(sh), 277; NaOAc/H_3BO_3, 327, 300(sh), 277.

Acid hydrolysis yielded glucose and apigenin. The uv spectra of the glycoside indicated that 5 and 7 OH groups were present. Uv shifts (with some small differences due to the solvent used) are in agreement with the literature values of apigenin 4'-glucoside (16).

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