INVESTIGATION OF THE COUMARINS AND FUROCOUMARINS OF HIPPOMARATHRUM CASPIUM (DC.) GROSSH.

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Hippomarathrum caspium (Caspian horse fennel) is a perennial herbaceous plant of the family Umbelliferae, endemic in Eastern Transcaucasia (near Baku) [1]. We collected the material for investigation from the sea shore dunes of Bil'gya Azerbaijanian SSR.

The chemical composition of Hippomarathrum caspium has not been studied previously. By paper chromatography of chloroform extracts of the roots, leaves, and stems, with the subsequent use of the qualitative reaction with diazotized sulfanilic acid we have established that the stems and leaves of this plant contain three compounds of the coumarin series and the roots contain seven such compounds.

From the roots of Caspian horse fennel we have obtained and identified osthol, oxypeucedanin, heraclenin, oxypeucedanin hydrate, and a substance with mp 149-150° which is a sitosterol; from the stems and leaves we isolated xanthotoxin.

These substances were identified with known coumarin and furocoumarin compounds by their IR and UV spectra, mixed melting points, and the R_f values of the substances on paper chromatography with markers.

In addition to the compounds isolated, others were found by chromatography: in the roots a small amount of isoimperatorin, imperatorin, and umbelliferone, and in the leaves and stems bergaptene and umbelliferone.

Experimental

The following systems were used as labile phases for chromatography: 1) petroleum ether (bp 60-70°) [2], and 2) water–methyl alcohol (9 : 1).

Isolation of coumarin compounds from the roots. The chloroform extraction of 4 kg of the ground air-dry roots of Caspian horse fennel (by steeping) gave 329 g of resin (8.24%). Two hundred grams of this resin was dissolved in 60 ml of chloroform and the solution was chromatographed on a column of neutral alumina (Brockman activity 3.5) with an alumina tc resin ratio of 10 : 1. The substances were eluted from the column with petroleum ether (bp 40-60°), a mixture of petroleum ether and chloroform (4 : 1), chloroform, and finally ethyl alcohol. Ninety-four 400-ml fractions were collected. The composition of the fractions was determined by thin-layer chromatography on alumina and by examination of the chromatograms in UV light.

The first petroleum-ether eluates (fractions 1-20) contained essential and fatty oils, and the subsequent ones (fractions 21-48), in addition to fatty oils, contained substances having a pale blue fluorescence in UV light. The mixture of petroleum ether and chloroform (fractions 49-65) eluted four compounds. Chloroform (fractions 66-83) eluted one substance. Ethyl alcohol (fractions 84-94) eluted a resinous mass which fluoresced in alcoholic solution.

Isolation of osthole. The residue resulting from the removal of the solvent from fractions 21-48 was rechromatographed on alumina (ratio of Al_2O_3 to crystalline residue 40 : 1). Elution with a mixture of benzene and petroleum ether (1 : 1) gave a substance with mp 83-84° (from a 5 : 1 mixture of petroleum ether and alcohol).

IR spectrum: ν 1721 (lactone carbonyl), 1607, 1563 cm^{-1} (aromatic ring of a coumarin compound substituted at C_8; UV spectrum: λ_{max} 224, 256, 320 mμ (log ε 3.30, 3.04, 4.20, respectively).

The IR and UV spectra correspond to the IR and UV spectra of osthole [3] and [3, 4], respectively. A mixture of the substance with mp 83-84° and osthole gave no depression of the melting point: the R_f value of the substance obtained on paper (system 1) was 0.82, while osthole under the same conditions gave R_f 0.83. Found, %: C 73.83, 73.93; H 6.75, 6.73. Calculated for $C_{15}H_{16}O_3$, %: C 73.75: H 6.60.

Isolation of oxypeucedanin. After two recrystallizations from alcohol and chloroform (4 : 1), the mixture of substances (from fractions 49-65) gave a substance with mp 142-143°; a mixture of this with oxypeucedanin gave no depression of the melting point.

IR spectrum: ν 1727 (lactone carbonyl), 1618, 1606, 1578, 1545 cm^{-1} (aromatic ring of a furocoumarin compound substituted at C_5); UV spectrum: λ_{max} 222, 250, 266, 308 mμ (lg ε 4.20, 4.10, 4.02, 4.00).

The IR and UV spectra of the substance were identical with the IR and UV spectra of oxypeucedanin [3] and [4, 5], respectively. On paper chromatography (system 1), the substance with mp 142-143° gave a spot with R_f 0.42; under

the same conditions the R_f of oxypeucedanin was 0.43. Found, %: C 67.03, 66.97; H 5.04, 5.02. Calculated for $C_{16}H_{14}O_5$, %: C 67.12; H 4.92.

Isolation of heraclenin. After the isolation of oxypeucedanin the mother liquor was evaporated to small bulk. When the concentrated solution was cooled, crystals deposited in the form of nodules; after three recrystallizations from alcohol they melted at 107-108°, $[\alpha]_D^{22}$ +25.5° (c 2.51; pyridine).

IR spectrum: ν 1729 (lactone carbonyl), 1630, 1594 cm^{-1} (aromatic ring of a furocoumarin compound substituted at C_8); 1247 (oxide ring in a side chain); UV spectrum: λ_{max} 220, 248, 262, 302 mμ (lg ε 4.24, 4.29, 4.03, 4.02).

The IR and UV spectra of the substance with mp 107-108° correspond to the spectra of heraclenin [8]. Found, %: C 66.90, 66.94; H 5.15, 5.10. Calculated for $C_{16}H_{14}O_5$, %: C 67.12; H 4.92.

The hydrate of heraclenin that we obtained had mp 114-115°.

IR spectrum: ν 3400 (2—OH); UV spectrum: λ_{max} 220, 250, 262, 300 mμ (lg ε 4.45, 4.40, 4.24, 4.19). The IR and UV spectra of the substance with mp 114-115° also coincide with the IR and UV spectra of heraclenin hydrate [7, 8]. Found, %: C 62.94, 62.89; H 5.25, 5.37. Calculated for $C_{16}H_{16}O_6$, %: C 63.15; H 5.30.

We detected two other substances in fractions 49-65 giving spots on the chromatogram which fluoresced in UV light, and were stained by diazotized sulfanilic acid. The R_f values of the spots (0.75 and 0.61) agree with the R_f values of isoimperatorin and imperatorin. However, in view of the small amount of these compounds we were unable to isolate them in the pure state.

Isolation of oxypeucedanin hydrate. The slightly yellowish substance obtained from the chloroform fractions (66-83) after two recrystallizations (from a 4 : 1 mixture of alcohol and petroleum ether) had mp 135-136°. IR spectrum: ν 1703 (lactone carbonyl), 1620, 1605, 1576, 1539 cm^{-1} (aromatic nucleus of a furocoumarin substituted at C_5); UV spectrum: λ_{max} 220, 250, 268, 308 mμ (lg ε 4.45, 4.38, 4.34, 4.20).

The IR and UV spectra of the substance were identical with the IR and UV spectra of oxypeucedanin hydrate [3] and [5, 8], respectively. Found, %: C 62.98, 62.85; H 5.32, 5.19. Calculated for $C_{16}H_{16}O_6$, %: C 63.15; H 5.30.

A mixture of the substance with mp 135-136° and oxypeucedanin hydrate gave no depression of the melting point. On paper chromatography (system 2), the substance isolated and oxypeucedanin hydrate gave spots with R_f 0.48 and 0.49.

Detection of umbelliferone. On paper chromatography (system 2), the resinous mass isolated from the column with ethyl alcohol (fractions 84-94) gave a spot with a blue fluorescence in UV light and R_f 0.46 (under the same conditions, umbelliferone gave a spot with R_f 0.46).

Substance with mp 149-150°. In addition to oxypeucedanin and heraclenin, a crystalline substance with mp 149-150° (from alcohol) not fluorescing in UV light was isolated from the column (fractions 49-65). It gave a positive Liebermann-Burchard reaction [9]. The IR spectrum of this substance was similar to that of β-sitosterol. It is possible that the compound obtained is one of the sitosterols.

Isolation of furocoumarin compounds from the stems and leaves. Ground leaves and stems of Caspian horse fennel were extracted with chloroform. The yield of resin was 5% of the air-dry weight. A solution of 5 g of the resin in 20 ml of chloroform was chromatographed on a column of alumina by the method described above for the extract from the roots.

100-ml fractions were collected. Petroleum ether (fractions 1-10) eluted pigments; a mixture of petroleum ether and chloroform (4 : 1) and chloroform alone (fractions 11-20) eluted a mixture of two substances. Fractions 21-24 (alcohol) gave a resinous mass. After the solvent had been distilled off from fractions 11-20, the residue was rechromatographed on alumina (eluant — chloroform), giving crystals with mp 145-146° (from alcohol). A mixture of this substance with authentic xanthotoxin gave no depression of the melting point.

IR spectrum: ν 1713 (lactone carbonyl), 1628, 1589, 1594 cm^{-1} (aromatic ring of a furocoumarin substituted at C_8). UV spectrum: λ_{max} 220, 248, 300 mμ (lg ε 4.28, 4.37, 4.11). Found, %: C 66.59, 66.69: H 3.87, 3.72. Calculated for $C_{12}H_8O_4$, %: C 66.66; H 3.70.

The IR and UV spectra of the substance with mp 145-146° coincided with the spectra of xanthotoxin [3, 6]. On paper chromatography (system 1), this substance and xanthotoxin gave spots with similar R_f values (0.19, 0.20).

Fractions 11-20 contained a small amount of a substance having a R_f value on paper chromatography (system 1) identical with that of a reference sample of bergaptene (0.26).

The resinous mass from the alcoholic fractions (21-24), like that from the roots, was found to contain traces of umbelliferone; R_f on paper (system 2) 0.47.

The species was determined by S. G. Tamamshyan from herbarium specimens of the samples of Caspian horse fennel collected by us.

Type "M" Leningrad paper treated before use with a 20% aqueous solution of ethylene glycol was used for the chromatography.

The IR spectra were taken on a IKS-14 instrument in liquid paraffin and the UV spectra on a SF-4 instrument in ethyl alcohol.

Summary

1. Osthole, oxypeucedanin, heraclenin, and oxypeucedanin hydrate have been isolated from the roots of Hippomarathrum caspium (DC) Grossh. and identified; the presence of traces of isoimperatorin, imperatorin, and umbelliferone has been established chromatographically. A substance with mp 149-150° apparently belonging to the sitosterol group has been obtained.

2. Xanthotoxin has been isolated from the leaves and stems; the presence of a small amount of bergaptene and umbelliferone has been detected chromatographically.

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