PHYTOECDYSONES OF *Serratula xeranthemoides*

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In a search for plants rich in ecdysones, we have investigated *Serratula xeranthemoides* Bieb. (family Compositae) [1] growing in the Askaniya-Nova reserve, Kherson oblast [2]. (According to the Flora of the USSR [3], the name *S. xeranthemoides* may be considered as a synonym of *S. erucifolia* (L.)). In the flower heads and leaves of this plant we have detected qualitatively ecdysones having the same composition, but the inflorescences contained more of the desired compounds than the leaf blades. According to TLC, the plant is characterized by at least five ecdysones, two of them in considerable amounts.

From a purified methanolic extract of the dry inflorescences by chromatogarphy on alumina with elution by the chloroform–methanol (10:1) system we have isolated compound (I), $C_{27}H_{44}O_7$, with mp 234-235°C. The fact the substance (I) belongs to the class of ecdysones was confirmed by its UV and IR spectra: $\lambda_{max}^{C_2H_5OH}$ 242 nm (log ε 3.98); ν_{max}^{KBr}: 3350-3450 (OH), 1615, 1660 cm^{-1} (cyclohexenone). By comparing the indices given above and the characteristics of the mass and PMR spectra and also by a direct comparison on TLC, compound (I) was identified as ecdysterone — the most widespread ecdysone in the vegetable kingdom [4-6].

By chromatographing an extract of the inflorescences on silica gel with elution by chloroform–methanol–water (60:32:6), in addition to ecdysteron (I), we isolated a substance (II), $C_{27}H_{44}O_8$, with mp 246-248°C, $\lambda_{max}^{C_2H_5OH}$ 245 nm (log ε 4.0). Fragments with m/e 349, 361, 343, and 325 in the mass spectrum of compound (II) show the presence of four hydroxy groups in the steroid nucleus. In the PMR spectrum, the methyl groups of steroid (II) have the following chemical shifts (ppm): 18-CH_3 (1.10), 26-CH_3 and 27-CH_3 (1.25), 19-CH_3 (1.29), and 21-CH_3 (1.45). The characteristics described and also the R_f value on TLC show the identity of ecdysone (II) with integristerone A, which has recently been isolated from *Rhaponticum integrifolium* [7]. This is the first time that integristerone A has been found in plants of the genus *Serratula*.

We have previously described the spectrophotometric characteristics of the products of the Chugaev reaction with ecdysterone and a number of other ecdysones and have considered the possibility of using this reaction for quantitative determinations [8]. It is characteristic that integristerone A, like ecdysterone, gives maxima at 380 and 470 nm in the Chugaev reaction, but with different molar absorption coefficients. The ratio of the intensities of the maxima at 380 and 470 nm is 0.98 for integristerone A, and 1.6 for ecdysterone.

The fairly high intensity of the absorption maxima mentioned above for compound (II) (380 nm, log ε 3.87; 470 nm, log ε 3.90) has permitted the Chugaev reaction to be used for the quantitative determination of integristerone A in raw material and for following the change in the amounts of the main ecdysones of *S. xeranthemoides* according to the phase development of the plant.

Below we give the results of a quantitative determination of integristerone A and ecdysterone in various vegetation periods of the plant in cultivation and under the conditions of natural growth (% on the weight of the air-dry raw material):

Phase of Development	Ecdysterone	Integristerone A
Budding and incipient flowering (flower heads)		
wild plant	0.19	0.13
cultivated plant	0.16	0.11

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Phase of Development	Ecdysterone	Integristerone A
Flowering	0.33	0.18
end of flowering and incipient fruit formation	0.28	0.21
Seeds	0.11	0.05

As can be seen from these figures, the cultivated plants contained somewhat less ecdysones than the wild plants. With the ripening of the plant the amount of ecdysones investigated increased. The maximum accumulation of ecdysterone took place in the flowering phase, but the content of integristerone A continued to increase as the plant ripened and reached a maximum at the end of the flowering phase and beginning of the formation of seeds. It is characteristic that in the budding phase of both the cultivated and the wild plants a considerable amount of an unidentified more polar ecdysone [R_f 0.11 on TLC, SiO_2, chloroform–methanol (4:1)] was observed. In the subsequent phases its amount fell sharply.

Of course, the figures obtained characterizing the amount of ecdysones as a function of the vegetation phase of the plant require further elaboration.

EXPERIMENTAL

For chromatography we used LS silica gel and alumina (activity grade IV). The ecdysones were revealed with vanillin–sulfuric acid. The UV spectra were obtained on a Specord or CF-4 spectrophotometer, the IR spectra on a UR-20 instrument (KBr), the PMR spectra on a JNM-4H-100 instrument (δ scale, 0 — HMDS), and the mass spectra on a MKh-1303 instrument fitted with a system for the direct introduction of the substances into the ion source at an ionizing voltage of 40 V and a temperature of 140-200°C.

For the quantitative determination of ecdysterone and integristerone A, the comminuted plant raw material (0.75 g) was steeped in methanol (50 ml) for two days, the extract was chromatographed by TLC in the chloroform–methanol (4:1) system, and the phytoecdysones were eluted with ethanol. Each sample contained 50-250 μg of ecdysones. The calibration curve for the phytoecdysones were linear at concentrations of 10-80 μg per ml of solution subjected to photometry in the measuring range from 340 to 580 nm. Analysis of a synthetic mixture of ecdysones of known compositions showed that the error of the determination does not exceed 2.5%.

Isolation of Ecdysterone. Air-dry inflorescences of *S. xeranthemoides* (0.5 kg) were extracted with methanol at room temperature. The methanolic extract was concentrated, diluted with two volumes of water, and treated repeatedly with hexane to extract hydrophobic compounds. After the additional elimination of methanol by distillation, the aqueous layer was carefully extracted with butanol. By chromatography on alumina with elution by mixtures of chloroform and methanol of increasing polarity (0-10% of methanol) the butanol extract yielded 1.3 g of ecdysterone [4, 9], $C_{27}H_{44}O_7$, mp 234-235°C (acetone), $[\alpha]_D^{20}$ +59.6° (c 0.64; methanol); $\lambda_{max}^{C_2H_5OH}$ 242 nm (log ε 3.98); ν_{max}^{KBr} (cm^{-1}): 3350-3450 (OH), 1615, 1660 (cyclohexenone). PMR spectrum (C_5D_5N, δ, ppm): 0.93 (3 H at C-19, s), 1.06 (3 H at C-18, s), 1.24 (6 H at C-26 and C-27, s), 1.44 (3 H at C-21, s), 6.00 (H at C-7, broadened singlet). Mass spectrum (m/e): 462, 444, 426, 411, 408, 363, 345, 344, 328, 327, 309, 301, 300, 125, 99, 81, 69. The yield of ecdysterone was 0.26 reckoned on the weight of the air-dry raw material.

Isolation of Integristerone A. The butanolic extract obtained by the method described above from 1.0 kg of air-dry inflorescences was chromatographed on a column of silica gel. On elution with chloroform–methanol–water (60:32:6), in addition to 2.5 g of ecdysterone, 1.5 g of integristeron A (II) [7] was isolated: $C_{27}H_{44}O_8$, mp 246-248°C (ethyl acetate), $[\alpha]_D^{20}$ +39.4° (c 1.20; methanol); $\lambda_{max}^{C_2H_5OH}$ 245 nm (log ε 4.09; ν_{max}^{KBr} (cm^{-1}): 3350-3480 (OH), 1670 (cyclohexenone).

PMR spectrum (C_5D_5N, δ, ppm): 1.10 (3 H at C-18, s), 1.25 (6 H at C-26 and C-27, s), 1.29 (3 H at C-19, s), 1.45 (3 H at C-21, s), 6.14 (H at C-7, broadened singlet). Mass spectrum (m/e): 478, 460, 442, 424, 409, 391, 379, 374, 368, 361, 343, 325, 316, 301, 143, 125, 99, 81, 69. The yield of integristerone A reckoned on the weight of the air-dry raw material was 0.15%.

SUMMARY

Ecdysterone and integristerone A have been isolated from the inflorescences of *Serratula xeranthemoides*.

The amount of these phytoecdysones in the flower heads increases as the plant ripens, reaching a maximum for ecdysterone in the full-flowering phase and for integristerone A at the end of the flowering phase and the beginning of fruit formation.

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TRITERPENE GLYCOSIDES OF *Cauliphyllum robustum*. THE STRUCTURES OF CAULOSIDES b AND c

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We have detected a series of triterpene glycosides in a methanolic extract from the stems, leaves, and flowers of *Cauliphyllum robustum* Maxim.

From a total methanolic extract of the leaves of this plant by column chromatography on silica gel we have isolated in the individual state two glycosides, which we have called caulosides b (I) and c (II). On acid hydrolysis, each of the caulosides gave hederagenin and a mixture of two monosaccharides. Chromatography on paper showed the presence of L-arabinose and L-rhamnose in both cases.

Caulosides b and c underwent methylation with diazomethane and their IR spectra contained in each case the absorption band of a free carboxy group; consequently, the carbohydrate chain consisting of the monosaccharides mentioned is attached to the hederagenin by a O-glycosidic bond.

Methanolysis of the completely methylated caulosides b (III) and c (IV), obtained by Hakomori's method [1] gave as aglycone the methyl ester of 23-O-methylhederagenin (V) and a mixture of partially methylated methyl glycosides of monosaccharides. The latter were acetylated and analyzed by the chromatographic–mass spectrometric (GLC-MS) method. The results of the analysis of the partially methylated monosaccharides and a determination of the configuration of the glycosidic bonds on the basis of Klyne's rule [2] and the chemical shifts of the carbon atoms of the monosaccharides in the ^{13}C NMR spectrum [3-5] showed for cauloside b the structure of hederagenenin 3-O-α-L-rhamnopyranosyl-1(1 → 2)-α-L-arabinopyranoside, identical with saponin P_D from *Akebia quinata* Decne [6], α-hederin from *Hedera helix* [7], and kalopanax saponin A from *Kalopanax septemlobum* [8].

On partial hydrolysis with 0.4 N sulfuric acid, cauloside c gave two progenins, one of which proved to be identical with cauloside b and the other with cauloside A (VI), which we have isolated previously from the roots of this plant and contains L-arabinose as the sole

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