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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF THE PHYTOECDYSTEROIDS OF *Melandrium nutans*

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With the aid of high-performance liquid chromatography, six phytoecdysteroids have been detected in the butanolic fraction of extractive substances from the epigeal part of *Melandrium nutans* L. Preparative separation has yielded ecdysterone and polypodine B.

The overwhelming majority of ecdysteroids — polyhydroxylated steroids of natural origin fulfilling the role of insect molting hormones — contain in their molecule a carbonyl group and α,β-unsaturation which give a characteristic absorption maximum in the UV spectrum. On the basis of these properties, Nigg et al. [1], using high-performance liquid chromatography (HPLC), separated a number of ecdysteroids: α-ecdysterone and its 5α isomer, 20-hydroxyecdysone and its 5α isomer, 26-hydroxyecdysone, and 22-deoxyecdysone, and also some of their synthetic analogues.

Later [2] HPLC was used for the analysis of a mixture of ecdysterone and inokosterone from *Achyranthes fauriei*. Separation was performed on a Permaphase ODS reversed-phase column using aqueous methanol as the mobile phase. Furthermore, using standard samples of ecdysterone and inokosterone, the optimum conditions for separation and then the percentages of the individual components in the plant extract were determined.

Using HPLC, Dinan et al. [3] found conditions for the differential determination of 3-dehydroecdysone, 20-hydroxy-3-dehydroecdysone, 3-epiecdysone, ecdysone, 20-hydroxy-3-epiecdysone, 2-deoxyecdysone, etc..

The selective effect of stationary and mobile phases has also been studied with several ecdysteroids as examples [4].

We have used HPLC on reversed-phase columns for the separation of a complex mixture of ecdysteroids from the plant *Melandrium nutans* L. (*Silene nutans* L. family Caryophyllaceae). First, on an analytical liquid chromatograph, we selected the conditions for separating the individual ecdysteroids isolated from the fairly well-studied plants *Rhaponticum integrifolium* C. Winkl. [5] and *Silene brahuica* Boiss [6].

Below we give the experimental results for the retention times of some ecdysteroids isolated from the plants mentioned. The substances that we extracted differ from one another by the nature and number of their oxygen-containing functions. The capacity factors of the ecdysteroids (column 25 × 0.46 cm, Zorbax C-8, eluent: 20% isopropanol; rate of flow 1 ml/min):

Integristerone	1.06
Sileneoside	1.10
Ecdysterone	2.67
2-Deoxyecdysone	2.85

In a methanolic extract of *M. nutans* with the aid of analytical HPLC we detected at least six phytoecdysteroids. Two compounds predominated: the universal molting hormone ecdysterone, which is widely distributed in animal and plant organisms, and polypodine B. The retention time of one of the other four phytoecdysteroids, according to our observations,

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coincided with that of integristerone A but the others have not been identified. Then the total ecdysteroid preparation was separated on a preparative liquid chromatograph. Ecdysterone and polypodine B were obtained in the crystalline form.

EXPERIMENTAL

The epigeal part of *Melandrium nutans* L., collected in July, 1981, in the environs of Ryazan' (6 kg, in the air-dry state) was exhaustively extracted with methanol. The extract was concentrated to 600 ml and was diluted with an equal amount of water, and the hydrophobic compounds were extracted with hexane. The aqueous methanolic fraction was extracted repeatedly with butanol. After the solvent had been driven off, 50.7 g of dry residue was obtained. The butanolic fraction of the extractive substances of *M. nutans* (7.0 g), after preliminary determination on an analytical chromatograph, was transferred in separate portions to a reversed-phase column. Each ecdysteroid was additionally purified by normal-phase chromatography on silica gel.

The following were obtained:

1) 300 mg (0.036%) of ecdysterone, $C_{27}H_{44}O_7$, mp 234-236°C (from ethyl acetate–methanol), $[\alpha]_D^{20}$ + 61.6 ± 2° (c 1.45; methanol, $\lambda_{max}^{C_2H_5OH}$ 244 nm, (log ε 4.05); and

2) 82 mg (0.010% of polypodine B, $C_{27}H_{44}O_8$, mp 258-259°C (from ethyl acetate–methanol) $[\alpha]_D^{20}$ + 85.2 ± 2° (c 0.81; methanol, $\lambda_{max}^{C_2H_5OH}$ 244 nm (log ε 4.09).

In an analytical determination from retention times, polypodine B was found between ecdysterone and sileneoside A.

The following chromatographs were used:

1) a Du Pont, model 850 analytical high-performance chromatograph (USA) fitted with a 25 × 0.46 cm Zorbax C-8 column using as the mobile phase 20% isopropanol at a rate flow of 1 ml/min and with a sample volume of 50 µl; and

2) a Du Pont preparative high-performance liquid chromatography with a 25 × 2.3 cm Zorbax C-8 column, 7 g of the material being dissolved in 50 ml of the mobile phase — 10% isopropanol. The samples were introduced through a storage loop with a volume of 2 ml directly adjacent to the separating column. The rate of flow was 25 ml/min, and the eluent was degassed by boiling.

Both chromatographs were fitted with UV detectors working at a fixed wavelength (254 nm) and a cell with a volume of 8 µl. Separation was performed at room temperature.

CONCLUSION

With the aid of high-performance liquid chromatography, six phytoecdysteroids have been detected in the butanolic fraction of the extractive substances from *Melandrium natans* L. (family Caryophyllaceae). Ecdysterone and polypodine B have been isolated by preparative separation.

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