CHEMICAL INVESTIGATION OF THE BIOMASS OF A CULTURE OF GINSENG CELLS. IV. QUANTITATIVE ANALYSIS OF THE GINSENOSIDES OF THE TOTAL GLYCOSIDIC FRACTION BY THE HPLC METHOD

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The total glycosidic fractions of four samples of ginseng cell cultures have been analyzed by reversed phase high-performance liquid chromatography, and it has been shown that only the BIO-2 samples contained ginsenosides – protopanaxadiol derivatives. The concentrations of these ginsenosides in the biomass of the cell culture was considerably lower than in the natural root. Samples of the biomass of a culture of cells of the IFRZh-1 strains contained no ginsenosides.

Previously, in a study of the chemical composition of the total glycosidic fraction (TGF) of a methanolic extract of a culture of ginseng cells by the TLC and GLC methods we succeeded in isolating and identifying only glycosides of β-sitosterol and of oleanolic acid [1, 2]. In the present paper we give the results of further investigations of the TGF of a number of strains of ginseng cell cultures (BIO-2, IFRZh-1, Omutninsk Chemical Factory).

In the TGF of Bio-2 we succeeded in detecting the presence of substances of triterpene nature the R_f values of which in TLC coincided with those of a number of ginsenosides. At the same time, the TLC and GLC methods did not permit a real assessment of the amount of the ginsenosides detected in the samples.

As Soldati and Sticher [3] have shown, the method of reversed-phase high-performance liquid chromatography (RFHPLC) proposed by them has permitted the amounts of the major and minor components of a glycosidic fraction to be determined. In turn, we have considered the possibility of using a Milikhrom domestic microcolumn chromatograph for the quantitative analysis of glycosides of the dammarane series in various mixture by the RFHPLC method [4]. Preliminary treatment of the sample was carried out on Polikhrom [5]. As a result it was possible to determine the presence and concentration of three ginsenosides (Rbl, Rc, and Rb2), which are protopanaxadiol derivatives, only in the TGF of the BIO-2 strain (suspension lines OBA-4 and BA-4 (Table 1 and Fig. 1)). At the same time we did not detect any ginsenosides in the THF of the IFRZh-1 strain (IFRZh-1 suspension, IFRZh-1 surface).

On the basis of the results obtained, it can be stated that with respect to their chemical compositions the TGFs of a culture of ginseng tissue and the natural root differ considerably.

EXPERIMENTAL

BIO-2 and IFRZh-1 callus cultures of ginseng (provided by the Omutninsk Chemical Factory, the K. A. Timiryazev Institute of Plant Physiology of the USSR Academy of Sciences, and the All-Union Scientific-Research Institute of Biotechnology) and suspension lines that we had obtained from these strains were investigated. For all the cultures we used the Murashige–Skoog medium as modified by N. F. Pisetskaya [6], with the elimination of agar for the suspension lines. The medium contained 2 mg/liter of α-naphthylacetic acid, 1 mg/liter of kinetin, and 30 mg/liter of sucrose. The growth cycle for the callus cultures was 30 days and for the suspension cultures 14 days. The biomass for the analysis of the gin-

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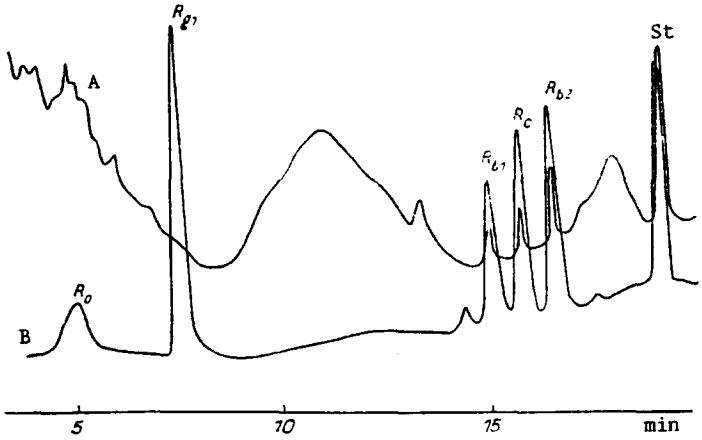

Fig. 1. Chromatograms of a sample of BA-4 (A) and of a mixture of panaxosides with a standard substance – St. (B).

TABLE 1. Amounts of Ginsenosides in Alcoholic Extracts of Ginseng Cell Cultures and a Commercial Tincture of the Natural Root

Sample	Amounts of ginsenosides, mg/ml						
	Rg1+Re	*Rf*	*Rb1*	*Rc*	*Rb2*	*Rd*	Σ
BA-4 (BIO-2)	—	—	0,016	0,044	0,1	—	0,16
OBA-4 (BIO-2)	—	—	0,049	0,01	0,02	—	0,035
Tincture Khabarovsk Pharmaceutical chemicals factory	0,92	0,25	0,88	0,44	0,36	0,2	2,99

senosides was taken at the end of the phase of exponential growth of the culture: on the 30th and 14th days for the callus and suspension cultures, respectively.

Milikhrom chromatograph, steel column (64 × 2 mm) filled with the reversed-phase sorbent Silasorb C18, 4.5 μm (KAKh-2). Polikhrom-1, 0.5-1.1 mm fraction (Biolar Scientific Production Combine, Olaine Chemical Reagents Factory). Acetonitrile, kh.ch. ["chemically pure"] for liquid chromatography (Khar'kov Chemical Reagents Factory). RFHPLC was carried out at room temperature in a gradient regime created by taking definite volumes of acetonitrile–water (20:80) → acetonitrile–water (60:40) solutions successively. The detection of the substances in the eluate was performed at 204 nm, the rate of feed of the solutions being 100 μl/min.

Purification of the Extracts of Ginseng Cell Cultures on Polikhrom-1. To 50 mg of a dry alcoholic extract of ginseng cell culture was added 1 ml of water, and the resulting suspension was deposited on a column filled with Polikhrom (5 g) that had previously been washed with water. Column 15 × 5 cm, height of the layer of sorbent 5.5 cm. The column was washed with water (30 ml) and then elution was carried out with ethanol (20 ml). The rate of elution was 1 ml/min. The ethanolic fractions obtained were evaporated under reduced pressure to constant weight, and then each residue was dissolved in a definite volume of methanol or in 40% aqueous acetonitrile and the solutions were used for HPLC.

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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Silene.

XVII. ECDYSTERONE 22,25-DI-O-BENZOATE FROM Silene scabrifolia

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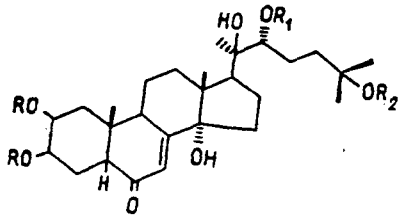
The new phytoecdysteroid ecdysterone 22,25-di-O-benzoate (IV) has been isolated from the epigeal organs of Silene scabrifolia Kom. The alkaline hydrolysis of (IV) gave ecdysterone 25-O-benzoate and ecdysterone (I). Details of the IR, UV, mass, PMR, and ^{13}C NMR spectra of compound (IV) are given.

Continuing a study of the phytoecdysteroids of the plant Silene scabrifolia, family Caryopyllaceae [1-3], from the mother liquors enriched with weakly polar derivatives we have isolated phytoecdysteroids (II), (III), and (IV).

Compound (II) was identified from its physicochemical constants and spectral characteristics as ecdysterone 2,3-monoacetonide 22-O-benzoate, which has been obtained by partial synthesis from ecdysterone 22-O-benzoate (VI) [1]. This is the first time that phytoecdysteroid (II) has been isolated from plant sources.

Ecdysteroid (III) proved to be identical with ecdysterone 2,3-monoacetonide, which has been isolated previously from Rhaponticum carthamoides (Willd) Iljin [4].

The spectral characteristics of ecdysteroid (IV) (absorption in the UV spectrum at 1720, 1295, 1610, 1590, and 730 cm^{-1} and intense peaks of ions with m/z 122 ($C_7H_6O_2$), 105 (C_7H_5O), and 77 (C_6H_5) in the mass spectrum) indicated the presence of a benzoate grouping. Signals in the PMR spectrum relating to ten aromatic protons at 7.39 ppm (6 H) and 8.25 ppm (4 H) showed the presence of two benzoic acid residues (Table 1).



I. R=R$_1$=R$_2$=H
II. R=>CMe$_2$; R$_1$=COC$_6$H$_5$; R$_2$=H
III. R=>CMe$_2$; R$_1$=R$_2$=H
IV. R=H; R$_1$=R$_2$=COC$_6$H$_5$
V. R=R$_1$=H; R$_2$=COC$_6$H$_5$
VI. R=R$_2$=H; R$_1$=COC$_6$H$_5$

The alkaline saponification of compound (IV) gave ecdysterone (I) and substance (V), which, according to its IR and mass spectra, still contained a benzoate grouping. The signals of five aromatic protons in the PMR spectrum of substance of (V) indicated the presence of only one benzoic acid residue.

It can be seen from Table 1 that, in comparison with ecdysterone, in ecdysteroid (V) the signals of the 26/27-methyl groups had undergone a considerable downfield shift. This fact permitted the assumption that in compound (V) the benzoic acid esterified the hydroxy group at C-25 and, consequently, it was ecdysterone 25-O-benzoate. It also follows from

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