CHEMICAL COMPOSITION OF A CULTURE OF TISSUE OF Scorzonera hispanica

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Cultures of plant tissues and cells are new biological materials created by man. They have great scientific and practical importance in studies on genetic engineering and the biotechnology of plants.

In this communication, we give the first results of a study of the chemical composition of an extract of the tissue of the crown gall Scorzonera hispanica L., cultivated since 1988 in the Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the USSR Academy of Sciences in the form of a suspension [1]. The fresh biomass freed from nutrient medium by filtration (moisture content ~96-97%), was exhaustively extracted with water–methanol (1:1). The extract was concentrated in vacuum to eliminate the alcohol and the residue was extracted successively with hexane, chloroform, ether and ethyl acetate. The yields of extractive substances were: soluble in hexane, ~2.8%; in chloroform ~0.7; in ether, ~0.1%; in ethyl acetate, ~0.4%; in water, ~50% (% on the weight of the absolutely dry raw material).

From the hexane and chloroform extracts, compounds (I)-(III) and free sterols were isolated by the method of column chromatography on silica gel using hexane, benzene, acetone and mixtures of them as eluents.

Compound (I) – $C_{10}C_{10}O_4$, M^+ 194 mp 159-160°C (ethanol); ^1H NMR (CD_3OD, δ, ppm): 3.76 (3H, s, OCH_3); 6.24 (1H, d, J = 16 Hz, H-8), 6.83 (1H, d, J = 8 Hz, H-5); 6.92 (1H, d, J = 2 Hz, H-2); 7.05 (1H, dd, J = 2 and 8 Hz, H-6) 7.55 (1H, d, J = 16 Hz, H-7).

From its physicochemical constants and its IR, UV, PMR, ^{13}C NMR, and mass spectra, (I) was identified as methyl caffeate [2, 3].

Compound (II) – $C_{30}H_{48}O_3$, M^+ 456; mass spectrum, m/z (%): 438 (8), 410 (7), 395 (14), 248 (100) – was identified as oleanolic acid [4]. Melting point of methyl oleanolate 197-199°C (ethanol).

Compound (III) – $C_{35}H_{60}O_6$, mp 266-268°C (methanol–chloroform). The ^1H and ^{13}C NMR spectra and physicochemical constants (mixed melting point) enabled (III) to be identified as β-sitosterol β-D-glucopyranoside [5].

The composition of the components of the sterol fraction was investigated by chromatomass spectrometry on a LKB-2091 instrument. They were separated by means of a capillary column with the phase SE-30 in the isothermal regime (280°C). β-Sitosterol (M^+ 414), campesterol (M^+ 400), and stigmasterol (M^+ 412) were identified. The mass spectra of all the sterols contained the diagnostic peaks of ions with m/z $[M - 85]^+$ and $[M - 111]^+$, which are characteristic for C-5 unsaturated sterols [6]. The main component of the mixture was β-sitosterol.

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