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ACCUMULATION DYNAMICS OF ECDYSTERONE AND CARBOHYDRATES IN CALLUS TISSUE OF *Ajuga turkestanica*

R. P. Zakirova and M. Kh. Malikova

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The accumulation dynamics of ecdysterone, water-soluble polysaccharides, and pectinic substances during growth of callus tissue of Ajuga turkestanica *were studied.*

Key words: callus tissue, ecdysterone, polysaccharides.

The specific content of secondary compounds increases gradually in most instances during the growth cycle under *in vitro* conditions [1]. We studied the accumulation dynamics of ecdysterone and carbohydrates in callus tissue of *Ajuga turkestanica*.

Tissue culture obtained from ovaries of plants [2] was grown for eight weeks. The yield of raw mass was determined and chemical analyses of samples were performed every seven days. The ecdysterone content was estimated by a semiquantitative TLC method that was simple and capable of obtaining information from sample with a low content and limited amount of raw material [3].

The growth dynamics and biosynthetic activity showed that raw mass continues to grow until the eighth week, when the culture loses vitality, becomes watery, and turns dark. Ecdysterone accumulation continues until the end of the sixth week of cultivation and then ceases (Table 1). Apparently this is due to depletion of the nutrient medium in components needed for biosynthesis of secondary metabolites [1].

Next, the dried callus tissue was used for successive isolation of carbohydrates. Ethanol-soluble sugars (ESS) were extracted by boiling ethanol (82°); water-soluble polysaccharides (WSPS), by water; pectinic substances (PS), by a mixture of oxalic acid and ammonium oxalate solutions (5%).

Ethanol extracts of all samples characteristically contain fructose, saccharose, and fructooligosaccharides with R_f 0.11 and 0.3 (relative to saccharose).

The WSPS and PS are cream-colored and light brown powders that are very soluble in water. Their aqueous solutions do not give a reaction with starch. Table 1 shows that WSPS and PS quantitatively accumulate as the mass of callus tissue increases. Their content is greatest in the eighth week. WSPS predominate quantitatively in all growth stages.

According to paper chromatography (PC), the monosaccharides of WSPS are neutral monosaccharides.

GLC indicates clearly that galactose predominates over other monosaccharides in hydrolysates of eight WSPS samples. Furthermore, the amount of galactose increases as the callus tissue grows (Table 2). The contents of glucose, arabinose, and xylose do not change. Rhamnose is observed in trace amounts.

Thus, it can be concluded from the data that the WSPS are based on galactans: arabinogalactans, xylogalactans, or their mixture.

The monosaccharides of PS consist of acidic and neutral sugars.

The hydrolysates of all samples contain galacturonic acid.

Galactose and arabinose dominate the neutral monosaccharides. Glucose and mannose are present in lesser amounts. Xylose and rhamnose are missing or present in insignificant amounts.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 226-227, May-June, 2001. Original article submitted July 9, 2001.

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TABLE 1. Accumulation of Ecdysterone and Carbohydrates

Cultivation time, weeks	Raw mass	Ecdysterone content, %	Content, %	
			WSPS	PS
1	1.61	Tr.	1.0	0.88
2	1.53	0.007	2.85	1.5
3	2.07	0.016	4.0	2.0
4	2.98	0.02	5.6	2.8
5	4.74	0.028	7.5	3.5
6	5.56	0.035	9.4	4.0
7	8.42	0.035	12.6	4.8
8	9.12	0.029	13.7	5.7

ESS composition: fructose, saccharose, fructooligosaccharides.

TABLE 2. Monosaccharide Composition of Carbohydrates

Monosaccharides	Weeks of growth															
	WSPS								PS							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Gal	10	5.2	8.4	9.0	10.8	13.5	14.7	15.2	3.2	3.9	3.5	6.2	6.0	7.0	8.0	8.3
GL	Tr.	Tr.	1.0	Tr.	1.0	2.0	1.5	2.4	2.8	2.5	1.0	1.0	1.0	1.0	1.0	1.0
Man	1.2	Tr.	2.3	1.4	Tr.	1.0	1.0	1.0	1.3	1.2	1.0	1.0	2.3	1.0	2.4	1.7
Ara	1.0	1.5	2.5	2.7	3.5	2.8	3.0	2.5	3.4	1.0	1.2	5.5	4.8	3.2	2.8	3.3
Xyl	1.0	1.0	3.2	1.0	1.8	3.0	2.4	1.0	1.0	-	-	-	1.0	1.0	-	-
Rha	-	-	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	-	-	-	-	-	-	-
GalUA	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 50°C.

PC of sugars was performed in descending mode using 1-butanol—acetic acid—water (4:1:5, v/v) and FN-12,13 paper (Germany). Developers were acidic anilinium phthalate for monosaccharides and alcoholic urea (5%) for fructose-containing sugars.

GLC was performed on a Chrom-5 instrument using a stainless-steel column (200×0.3 cm), 5% silicone XE-60 on chromaton NAW-0.200-0.255 mm, 210°C, He carrier gas at 60 mL/min. Acetates of monosaccharide aldonitriles were prepared for analysis [4].

Cultivation. Callus tissue was grown in the dark in 100-mL Erlenmeyer flasks containing medium (50 mL). Modified Murashige—Skoog medium was used for the cultivation [2]. The culture grew in the dark at 26±1°C at 70% relative humidity.

Ecdysterone Determination. The semiquantitative method is based on the measurement of spot areas [3]. Tissue was dried in a thermostatted apparatus at 60°C before being extracted three times with hot methanol. Silufol UV-254 plates (Czech Rep.) were used for TLC. The required amount of extract was applied using a microsyringe. Samples with a known amount of standard ecdysterone were also chromatographed. The sensitivity of the ecdysterone determination was 0.1 μg. A calibration curve was constructed using the amount of applied substance and the size of the spots. The chromatograms were visualized using vanillin—H_2SO_4 followed by heating in a drying oven at 120°C.

Isolation of Ethanol-Soluble Sugars. Ground samples of callus tissue were extracted twice with ethanol (82°, 1:5) on a water bath. The combined extracts were evaporated and chromatographed. Fructose, saccharose, and

fructooligosaccharides were found.

Preparation of WSPS. Raw material remaining after ethanol extraction was dried. Polysaccharides were extracted by water at room temperature (1:10, 1:5). The aqueous extracts were concentrated and treated with ethanol (1:5). The precipitate was separated by centrifugation, washed with ethanol, and dried. Hydrolysis was carried out in H_2SO_4 (1 N) for 12 h at 100ºC. The hydrolysates were neutralized by $BaCO_3$, deionized, and chromatographed. Tables 1 and 2 list the yields and monosaccharide compositions.

Preparation of PS. Raw material remaining after WSPS isolation was used to isolate PS by treatment with oxalic acid and ammonium oxalate solutions (0.5%, 1:10, 1:5) at 70ºC. The combined extracts were evaporated and treated with ethanol (1:5). The precipitate was washed and dried with ethanol.

Hydrolysis of PS was carried out in H_2SO_4 (2 N) for 24 h at 100ºC. The hydrolysates were worked up and chromatographed on paper. Aldonitrile acetates were prepared for GLC.

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