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CHEMICAL COMPOSITION OF *Codonopsis clematidea* GROWN *in vitro*

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The amounts of neutral and polar lipids, chlorophylls, carotenoids and alkaloids have been determined in differentiated tissues of Codonopsis clematidea *Schrenk grown by the tissue culture method. The qualitative and fatty-acid compositions of the neutral and polar lipids have been established and the presence has been shown of the quantitatively main alkaloid of the wild-growing plant, codonopsine, and of the minor alkaloid codonopsinine.*

Clematis Asia bell *Codonopis clematidea* Schrenk (fam. Campanulaceae) is a perennial herbaceous medicinal plant found in the Western Tien-Shan at heights of 1100-2300 m above sea level [1]. The epigeal parts of some *Codonopsis* species are used in Chinese folk medicine as astringents and the roots as general strengthening, antirheumatic, and hemostatic agents and as a substitute for ginseng [2]. The epigeal part of *C. clematidea* contains vitamins of groups A, D, and C, flavonoids, essential oils with a sharp unpleasant smell [3], and alkaloids [4, 5]. The alkaloids of this plant — codonopsine and codonopsinine — and also the total flavonoids exhibit a bile-stimulating and hepatoprotective action [6]. There is no information in the literature on the lipids of the vegetative organs of plants of the Campanulaceae family.

At the present time, the natural reserves of this plant are becoming exhausted, and its introduction into valley conditions is difficult. In view of this, there is interest in a search for methods of obtaining the crude biomass, one of which may be the tissue culture method. The results of our recent investigation of the lipids, pigments, rutin [7], and alkaloids [8] of *Ruta graveolens* L. (fam. Rutaceae) grown *in vivo* and *in vitro* have shown that the *in vitro* culture retains the capacity for biosynthesizing the main primary and secondary metabolites characteristic for the soil-grown plant.

We have obtained a tissue culture of *C. clematidea* by callusogenesis and regeneration (*in vitro*). When it was grown in a known medium using phytohormones from the stimulators of the auxin, cytokinin, and gibberellin types that we tested, the most active for the stimulation of organogenesis proved to be gibberellin.

In the nutrient medium, cells of an explant of *C. clematidea* fairly rapidly entered into active cell division with the formation of shapeless calluses of dense consistency possessing a pronounced morphogenic capacity, which is probably characteristic for this genotype. The conditions of the *in vitro* culture were regulated with the aim of intensifying growth and the accumulation of green biomass (see Experimental).

The biomass of 30- to 40-day plants was investigated. We determined the amounts and compositions of the pigments, lipids, and alkaloids of differentiated tissues (leaves and shoots) separated from the callus tissue. The moisture content of the differentiated tissues was 87%.

The pigments were isolated from the fresh tissues with acetone and were studied by spectroscopy and TLC. They consisted mainly of a mixture of β-carotene (R_f 0.96; UV spectrum, nm: λ_{max} (acetone) 427, 454, 480); chlorophylls *a* (R_f 0.27; 664) and *b* (R_f 0.24; 644), and minor xanthophylls with R_f 0.20 (orange) and 0.11 (yellow). The results of the quantitative estimation of the pigments are given in Table 1.

The lipids were isolated from the crude tissues after the enzymes had been inactivated. They were fractionated by countercurrent distribution into neutral (NLs) and polar (PLs). The qualitative compositions of the NLs and PLs were deter-

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TABLE 1. Amounts of Lipids and Pigments in Differentiated Tissues of *C. clematidea*

Components	mg/g a.d.m.	% on the weight of lipids
Pigments:	8.75	14.11
chlorophyll *a*	4.37	7.05
chlorophyll *b*	2.18	3.51
β-carotene and xanthophylls	2.20	3.55
Lipids + pigments:	62.00	100.0
neutral lipids + native and modified pigments	24.19	39.00
polar lipids + modified pigments	37.81	61.00

TABLE 2. Fatty Acid Compositions of the Lipids of Differentiated Tissues of *C. clematidea* (GLC, %)

Acid	Sum of the lipids	Neutral lipids	Polar lipids
12:0	0.4	Tr.	0.5
14:0	1.3	1.7	0.6
16:0	18.6	26.4	17.4
16:1	Tr.	Tr.	0.5
16:1 (3, *trans*)	0.3	–	0.4
17:0	0.7	0.9	Tr.
18:0	6.9	10.3	2.6
18:1	6.9	15.0	2.0
18:2	22.9	20.5	21.5
18:3	40.3	23.0	54.5
20:0	1.7	2.2	Tr.
Σ_{sat}	29.6	41.5	21.1
Σ_{unsat}	70.4	58.5	78.9

mined with the aid of chromatographic methods (TLC, GLC), qualitative reactions, and the results of chemical transformations, and the fatty acid (FA) compositions of the total lipids and of the NLs and PLs were established. The results of analysis of the lipids are given in Tables 1 and 2.

The amount of lipids with pigments in the differentiated tissues of *C. clematidea* was low (6.2% a.d.m., Table 1) and was 1.5 times smaller than the amount in *R. graveolens* tissues grown by a similar method. Here the ratio of PLs and NLs was 1.5:1, while in *in vivo* and *in vitro* cultures of *R. graveolens* this ratio was lower (1.2:1 and 0.8:1, respectively [7]).

The chlorophyll and carotenoid pigments were concentrated in the NLS (39% of the total lipids by weight), their total amount in relation to the weight of the NLs exceeding 36% and being 14% on the total lipids.

By TLC we detected carbohydrates, esters of FAs with phytosterols and with fatty alcohols, triacylglycerols, free FAs, fatty alcohols, triterpenols, sterols, and minor unidentified compounds in the NLs.

The polar lipids — a mixture of glyco- and phospholipids — contained chlorophyll pigments modified in the process of isolating and fractionating the lipids. In the glyco- and phospholipids we determined, mainly, components characteristic for the photosynthetic tissues of plants [9], namely: glycolipids containing mono- and digalactosyldiacylglycerols, sulfoquinovosyldiacylglycerols, and steryl glycosides and their esters with FAs; and phospholipids containing phosphatidylglycerols, phosphatidylethanolamines, phosphatidylcholines, phosphatidylinositols, and, in minor amounts, phosphatidic acids.

The figures of Table 2 show that the polar lipids of *C. clematidea* tissues were enriched with unsaturated FAs, among which linolenic acid predominated. The 18:3 and hexadec-*trans*-3-enoic (16:1(3,*trans*)) acids, which are characteristic acyl components of the galactosyldiacylglycerols and phosphatidylglycerols of the photosynthetic tissues of higher plants [9], were concentrated in the PLs.

The proportion of oleic acid and saturated acids, especially palmitic, in the total acids of the NLs was higher than in the PLs. This is possibly due to the presence in the NLs of components of surface lipids such as waxes and esters of triterpenols and sterols, the acids of which are more saturated [10].

The total alkaloids were isolated from the differentiated tissues by a method used previously for wild *C. clematidea* [4]. Quantitative analysis showed the presence of 0.127% of alkaloids on the weight of the air-dry plant. The amount of alkaloids in the *in vitro* culture was almost twice the level of nitrogen bases found for the wild plant (0.02-0.08%).

The nutrient medium also contained small amounts of alkaloids, which was apparently due to the diffusion of the water-soluble alkaloids into the nutrient medium. This fact shows the capacity of *C. clematidea* tissues for retaining a secretory function under culture conditions [11].

In the total nitrogen bases obtained, TLC showed qualitatively the presence of the main alkaloid codonopsine [4] and the minor base codonopsinine [5] produced by the wild plant.

Thus, under the cultivation conditions found, the differentiated tissue of an *in vitro* culture of *C. clematidea* synthesizes the pigments, neutral lipids, and glyco- and phospholipids that are characteristic of photosynthetic tissues *in vivo* and is capable of a more intensive accumulation of the alkaloids that are characteristic of the wild plant. This gives grounds for assuming that an *in vitro* culture of this plant may in future serve as a source of physiologically active alkaloids.

EXPERIMENTAL

For the conditions of recording the UV spectra of the pigments, the GLC of the methyl esters of the FAs, and the TLC of the neutral and polar lipids, see [7].

The TLC of the pigments was conducted on Silufol in the n-$C_6H_{14}-(CH_3)_2CO-C_6H_6-iso$-$C_3H_7OH$ (69.5:25:4:1.5) solvent system.

The *in vitro* culture of *C. clematidea* was grown with regulation of the conditions. The optimum factors proved to be Murashiga–Skoog medium with the phytohormone gibberellin in medium concentrations, cultivation of the tissue in a factorostatic room with illumination of 3500 lux, a 16-hour photo period, and a temperature of $+26 \pm 2°C$ at a humidity of 70% [12].

The conditions for isolating the lipids by a mixture of $CHCl_3$ and CH_3OH with fixation of the enzymes, the separation of the NLs and PLs by countercurrent distribution, and the isolation of the FAs have been described in [13], and those for obtaining the total alkaloids in [14]. The components of the NLs, PLs, FAs, pigments, and alkaloids were identified on TLC plates by comparison with known compounds isolated previously [4, 5, 7].

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