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DETERMINATION OF SOLASODINE IN CELL CULTURES OF Solanum laciniatum BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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UDC 581.198:547.926

A method is proposed for the quantitative determination of solasodine by HPLC on a Millikhrom domestic microcolumn chromatograph. The amounts of solasodine in various cell lines of Solanum laciniatum have been determined. It has been shown that the genetic transformation leads to a substantial increase in the amount of solasodine in the biomass. Amounts of solasodine in multishoot cultures are comparable with its amount in normal mature plants.

The steroid alkaloid solasodine is the starting material for obtaining a whole series of drugs [1]. In Solanum laciniatum plants it is found both in the pure form and in the form of the glycoalkaloids solasonine and solamargine [2]. In view of the practical significance of solasodine throughout the world, the possibility is being widely studied of obtaining it by the methods of biotechnology [3]. Solasodine is synthesized in cultures of cell tissues of S. laciniatum in amounts far smaller than in normal plants [4]. One of the methods of raising the level of synthesis of alkaloids in a tissue culture is genetic transformation [5].

We have previously obtained a tissue culture and have performed the genetic transformation of S. lacinatum cells by various strains of Agrobacterium tumefaciens. The transgenic lines obtained as the result of genetic transformation differed from ordinary callus cells by their capacity for growing in a hormone-free medium [6]. A culture of differentiated cells – a multishoot culture [7] – has been obtained. In the present communication we give an analysis of the transgenic lines obtained for their level of steroid alkaloids.

In world practice, a number of methods are used for the analysis of alkaloids, the most effective of which is high-pressure liquid chromatography [2].

We have investigated the following cell lines: (I) – calluses growing in a hormone-containing medium; (II) – tumor cells obtained by transformation by a strain of agrobacterium from pTi A6, growing in a hormone-free medium; (III) – a multishoot culture obtained by transformation by a mutant strain of agrobacterium from the pTi A6 tms, growing in a hormone-free medium. To analyze the levels of alkaloids in the lines investigated we used a Millikhrom domestic microcolumn liquid chromatograph.

M. A. Aitkhozhina Institute of Molecular Biology and Biochemistry, Academy of Sciences of the Kazakh SSR, Alma-Ata. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 538-541, July-August, 1991. Original article submitted October 16, 1990; revision submitted January 10, 1991.

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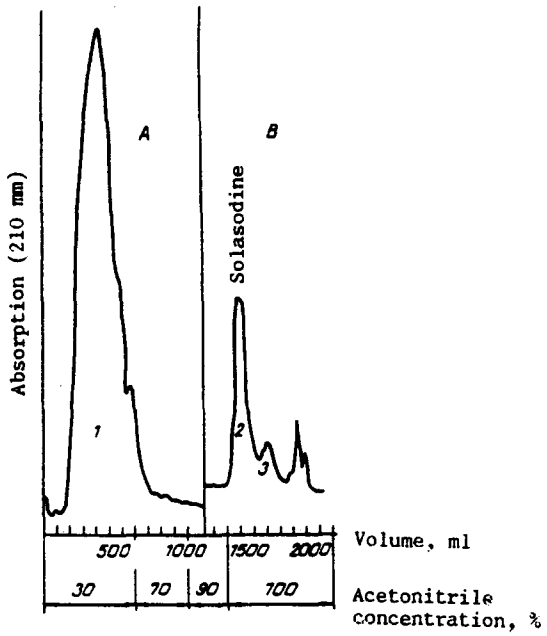

Fig. 1. Chromatography profile of an extract of a multishoot culture: A) sensitivity of the instrument 12.8 o.u.; B) 64 o.u. The substance of peak 1 has not been identified but it contains trace amounts of glycoalkaloids; the substance of peak 2 was solasodine, and the substance of peak 3 diosgenin.

To obtain a complete pattern of the alkaloid composition of the cell lines, we selected the mildest method of treating the cell mass. The biomass was dried and was treated with methanol in a Soxhlet apparatus, the methanol was driven off from the extract and the mixture was subjected to preliminary purification by filtration through a column with the support Silasorb C8 15 μm (Chemapol, Czechoslovakia). The samples obtained were chromatographed on a column containing LiChrosorb C18. Elution was performed in a stepwise concentration gradient of acetonitrile in the presence of 0.01 M tris-HCl, pH 7.2. Figure 1 shows the chromatography profile of line (III).

Analysis of the substances by the TLC method confirmed the presence of solasodine in the fractions of peak 2. The substances (corresponding to the fractions of peak 1) were not identified, but they contained glycoside derivatives of solasonine in trace amounts. The substance contained in fraction 3 corresponded to diosgenin. Below we give the characteristics of the compounds obtained on the use of the TLC method:

Substance	R_f	$SbCl_3$	Coloration of spots on treatment with Dragendorff reagent	I_2 vapor
Solasodine	0.87	Reddish violet	Orange-red	Yellow
Substance in peak 2 (Fig. 1)	0.87	Reddish violet	Orange-red	Yellow
Diosgenin	0.92	Pinkish lilac	–	Yellow
Substance in peak 3 (Fig. 1)	0.92	Pinkish lilac	–	Yellow

To determine solasodine in the cell lines we first chromatographed a solution of a commercial preparation with a known concentration. The area of the peak (P1) corresponding to 0.01 mg of solasodine was determined by a gravimetric method. Then in the chromatographic profile of the sample being determined we likewise measured the area of the corresponding peak (P2). The amount of solasodine in the cell lines was determined from the formula (P2/P1) × 0.01 mg. Below we give the amounts of solasodine present in the lines investigated.

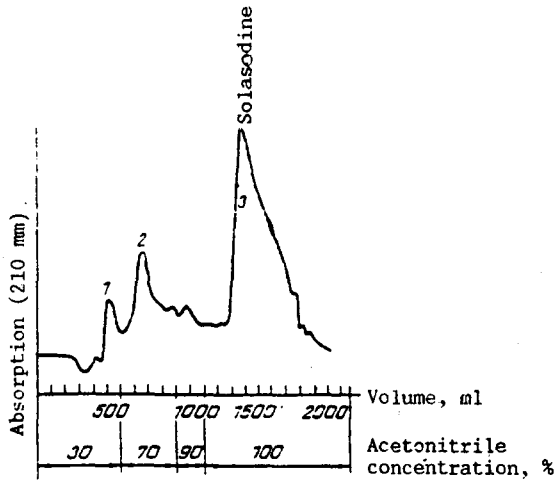

Fig. 2. Chromatographic profile of a commercial preparation of solasodine: the substances of peaks 1 and 2 were not identified; the substance of peak 3 was solasodine.

Line of S. laciniatum	Weight of dry material, g	Amount of solasodine % on dry weight
Normal calluses	5.6	0.055
Tumor cells	3.6	0.28
	4.0	0.36
Multiplet culture	10.4	1.73; 1.75
	8.2	1.75; 1.80

We had determined the amount of steroid alkaloids in the normal callus cells previously [6]. Tumor cells of S. laciniatum transformed by agrobacteria with the wild plasmid pTi A6 – line (II) – synthesized four times as much solasodine as the ordinary calluses [line (I)]. A multishoot culture obtained by transformation with an agrobacterial strain containing the mutant plasmid Ti A6 tms – line (III) – synthesized solasodine at the level of normal mature plants. The amount of alkaloid in this culture was approximately 30 times greater than in normal calluses. All the cultures synthesized as the main product the aglycon of the steroid alkaloids – solasoline – which is obtained industrially by the acid hydrolysis of its glycosides.

EXPERIMENTAL

Isolation of the Alkaloids from Cell Lines of S. laciniatum. The biomass was heated at 100°C for 1 h and was then dried in the air-dry state. It was then ground in a porcelain mortar to a finely-dispersed powder and was placed in a Soxhlet apparatus. The extraction of 1 g of the dry mass was carried out in 50 ml of methanol for 6 h. Then the extract was evaporated to dryness with the aid of vacuum rotary apparatus and the residue was dissolved in the minimum volume of methanol and deposited on a column containing 5 g of the support Silasorb C8 15 μm (Chemapol, Czechoslovakia) to eliminate resinification products. The column was then washed with 50 ml of 40% methanol and with pure methanol until alkaloids were totally absent from the washing solution. The eluates were evaporated in vacuum to dryness and the residues were dissolved in 10 ml of methanol. The samples prepared in this way were used for the quantitative determination of solasodine by the HPLC method.

Analysis of the Samples by the HPLC Method. A sample prepared as described above was deposited on a KAX-2 column (2 × 64 mm) with the support LiChromsorb C 18 (5.0 μm) and elution was carried out with a stepwise gradient of acetonitrile in the presence of Tris-HCl, pH 7.2. Alkaloids were detected at 210 nm. The fractions containing the compounds under investigation were combined, evaporated, and analyzed by the TLC method.

Analysis of the Samples by the TLC Method. Thin-layer chromatography was conducted on Kieselgel 60 plates (Merck) in the methanol–chloroform (1:4) system in the presence of com-

mercial solasodine as marker. The plates were dried and were sprayed with one of three revealing agents – antimony trichloride in chloroform, the Dragendorff reagent, and iodine vapor, as described in the literature [8].

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ALKALOIDS OF Peganum harmala.

UNUSUAL REACTION OF PEGANINE AND VASICINONE

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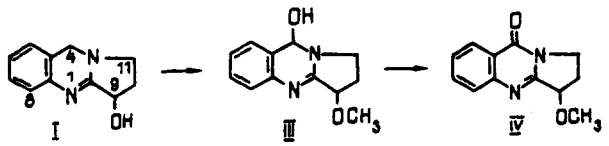
UDC 547.944/945

A number of derivatives of peganine and vasicinone have been obtained: 4-hydroxy-9-O-methylpeganine, isodihydrovasicinone, and 9-methoxy-, 9-phenoxy-, and 9,9-dichlorodeoxyvasicinones. An unusual ease of reduction of vasicinone and of 9-chlorodeoxyvasicinone has been found. An introduction of a hydroxy group into position 4 of a dihydroquinazoline alkaloid with the aid of sodium hydride has been detected.

Continuing a search for physiologically active compounds among the quinazoline alkaloids, we have obtained derivatives of peganine (I) and vasicinone (II) and have found an unusual behavior of them in some cases.



Scheme 1

When (I) was methylated with methyl iodide in the presence of NaH we isolated product (III) (Scheme 1), the molecular mass of which was 16 a.m.u. greater than for the expected O-methylpeganine, and which had the elementary composition $C_{12}H_{14}N_2O_2$. In the mass spectrum of (III) the most intense ion was $(M - 17)^+$, corresponding to the ejection of an OH group from the molecular ion. The PMR spectrum of (III), as compared with (I) had a signal from an OCH_3 group at 3.20 ppm, a one-proton singlet at 5.75 ppm, and a broadened one-proton signal at 7.60 ppm which disappeared on deuteration, showing the presence of a –CH–OH grouping. The ready ejection of the OH group under electron impact showed that it was possibly present at C-4 or C-11. The absence of a two-proton singlet in the 4.5 ppm region caused by C-4

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Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 541-545, July-August, 1991. Original article submitted October 29, 1990.