USE OF CENTRIFUGAL COLUMN CHROMATOGRAPHY FOR THE ANALYTICAL SEPARATION OF THE CAULOSIDES FROM THE ROOTS OF *Caulophyllum robustum*

L. I. Berezhevskaya and L. I. Glebko

UDC 543.544.547.918

Analytical centrifugal chromatography (ACC) has been used repeatedly for the analysis of natural compounds [1-5]. We have used it to estimate caulosides in a technical preparation of them consisting, according to TLC, of three caulosides, A, B, and C [6] and three substances of undetermined nature (∿50% of the weight of the preparation).

A chromatographic column (a glass tube 3 × 100 mm with a 1.5-ml reservoir and a small aperture at its bottom end) was charged by the dry method with type KSK silica gel (two-hour fraction) activated at 120°C (2 h) to form a 90-mm layer (∿300 mg). The column in a Teflon bung was inserted into a centrifuge tube and the sorbent was compacted by centrifugation under the regime selected for analysis.

An aliquot part (20-30 µl) of a methanolic solution of the preparation (200-300 µg) was deposited on the column, the reservoir was filled with the eluent (1 ml), and centrifugation was carried out 1500 rpm for 4 min, the eluent being collected in separate tubes after each centrifugation step. The eluates were evaporated to dryness and the color reaction (7) was performed: To each tube was added 2 ml of a mixture of sulfuric acid and ethanol (1:07), the mixture was heated at 60°C for 30 min and cooled, and the optical density of the solution was measured at 530 nm, elution curves being plotted in the coordinates optical density versus fraction number (Fig. 1).

The quality of the separation in the selection of the chromatographic regime was checked by TLC, and in the working variant of the method it was checked from the elution curves. The recovery of caulosides from the column was not less than 96%, which was taken into account in calculating the results of the analyses. To separate the glycosides with a

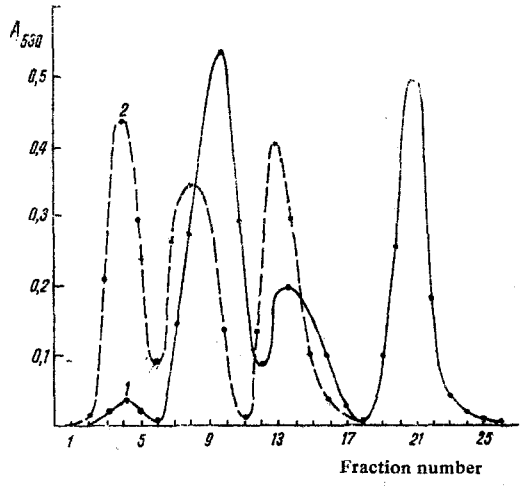

Fig. 1. Elution curves of a technical preparation of caulosides A, B, and C (1) and of an artificial mixture of them (2).

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 241-242, March-April, 1983. Original article submitted September 9, 1982.

0009-3130/83/1902-0239$07.50 © 1983 Plenum Publishing Corporation

reproducibility of not less than 5% rel. using gradient elution in the n-hexane (100%) → n-hexane—ethanol (85:15) system required 25 centrifugation steps. The results agreed with those obtained with the aid of TLC and the elution of the substances from the spots [7].

It must be mentioned that ACC, like any other chromatographic method requires a strict observance of the standardized conditions for a given mixture of substances. Thus, we used the column packing only once, since its re-use led to extreme compaction of the sorbent which changed the quality of the separation.

Hence, our experimental work has shown that in spite of the length of the analysis, ACC can be used for separating a simple mixture of glycosides at the microgram level and for the quantitative determination of them with the aid of a color reaction.

LITERATURE CITED

1. J. Meijer, M. Eid, and P. Verduin, Z. Anal. Chem., 236, No. 6, 426 (1968).
2. E. Ribi, C. Filz, G. Goode, S. Strain, K. Yamamoto, S. Harris, and J. Simmons, J. Chromatogr. Sci., 8, 577 (1970).
3. S. Sarkar, K. Jogi, and A. Mohimen, J. Chromatogr. Sci., 12, 206 (1977).
4. H. Pfander, F. Haller, F. Leuenberger, and H. Thommen, Chromatographia, 9, No. 12, 630 (1976).
5. L. L. Abramenko, L. I. Brutko, and E. A. Tukalo, Khim. Prir. Soedin., 333 (1981).
6. L. I. Strigina, N. S. Chetyrina, and G. B. Elyakov, Khim. Prir. Soedin., 552 (1970).
7. L. I. Berezhevskaya, Zh. I. Ul'kina, and L. I. Glebko, Khim. Prir. Soedin., 643 (1980).

ALKALOIDS OF *Eschscholtzia californica*

S. A. Parfeinikov and D. A. Murav'eva

UDC 547.943

The plant *Eschscholtzia californica* Cham. (family Papaveraceae), which is widely cultivated in the whole of the European territory of the USSR as a decorative plant and is in the catalog of the majority of botanical gardens of this region, has not yet previously been studied chemically in the Soviet Union. We have investigated the alkaloid composition of *E. californica* collected in the period of flowering and incipient fruit-bearing in the botanical garden of the Pyatigorsk Pharmaceutical Institute. Methanolic extraction of the epigeal part yielded 1.1% of total alkaloids on the weight of the dry plant. These were separated into phenolic and nonphenolic fractions. The nonphenolic material was treated with methanol, and allocryptopine and protopine were isolated [1]. The mother liquor after the evaporation of these alkaloids was chromatographed on a column of silica gel. Elution with chloroform and chloroform—ethanol in various proportions gave protopine, allocryptopine, and eschscholtzine [2].

The total phenolic alkaloids were separated similarly, and isocorydine and N-methyllaurotetanine, quantitatively the main alkaloid, were isolated [3, 4].

Californidine was isolated from the fourth fraction in the form of its iodide [5].

All the alkaloids isolated were identified on the basis of physicochemical properties (melting points, TLC), spectral characteristics, (UV, IR, NMR, and mass spectroscopy), and direct comparisons with authentic samples.

This is the first time that isocorydine has been isolated in plants of the genus *Eschscholtzia*.

Pyatigorsk Pharmaceutical Institute.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 242-243, March-April, 1983. Original article submitted May 25, 1982.

0009-3130/83/1902-0240$07.50

© 1983 Plenum Publishing Corporation