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COMPARATIVE CHROMATOGRAPHIC STUDY OF THE SESQUITERPENES OF SOME *Tanacetum* SPECIES

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A comparative qualitative and quantitative analysis has been made by the HPLC method of the sesquiterpene lactones of various fractions obtained from the epigeal parts of three species of tansy, Tanacetum *L., growing in Kazakhstan. Five of seven standard lactones have been identified and it has been shown that the amount of each lactone varies within wide limits both in individual fractions and in different* Tanacetum *species.*

Sesquiterpene lactones form a large group of biologically active natural compounds that are distributed mainly in plants of the aster (Compositae and Asteraceae) families. At the present time, more than 2000 representatives of this series have been isolated and studied [1].

A promising and widely distributed taxon of the aster family consists of plants of the tansy genus, *Tanacetum* L., which number 34 species growing only on the territory of the CIS, 15 of them being found in Kazakhstan [2]. An analysis of the literature shows that they contain 50 sequiterpene lactones (25 germacranolides, 12 eudesmanolides, and 13 guaian-olides) [1].

It must be mentioned that in spite of the enormous number of natural sequiterpene lactones found, there is no universal method for their isolation. Depending on the physicochemical properties of the sequiterpene lactones, various methods for their isolation, purification, and analysis have been described in the literature. At the present time, high-performance liquid chromatography (HPLC) has come into the widest use for the analysis of natural compounds and, in particular, sequiterpene lactones [3-6].

The aim of the present work was a chromatographic study of sequiterpene lactones from the epigeal parts of plants of the *Tanacetum* genus. We investigated three species of *Tanacetum* growing in Kazakhstan (*T. vulgare, T. santolina*, and *T. ulutavicum*). As standard specimens we used germacranolides and eudesmanolides that we had isolated previously from *T. vulgare* and *T. karelinii* — tamirin, hanphyllin, and tavurin [7, 8] — and crispolide, tatridin A, artemorin acetate, and tanacetol isolated from *T. vulgare* [9, 10] and kindly provided by Dr. G. Appendino.

The chromatographic analysis of the fractions isolated from the various species of *Tanacetum* was conducted on a Milikhrom microcolumn liquid chromatograph in the reversed-phase variant under isocratic conditions. By varying the composition of the mobile phase (methanol—water) between 70:30 and 50:50 and the wavelength between 210 and 250 nm it was possible to achieve a fairly satisfactory separation of the above-mentioned sequiterpene lactones in a short time of analysis.

Figure 1 shows chromatograms of individual fractions of sequiterpene lactones obtained from the epigeal parts of various *Tanacetum* species in the flowering phase. Table 1 gives the results of the quantitative analysis of three species of tansy. As can be seen from Fig. 1 and Table 1, of the seven standard specimens five lactones were identified. All the fractions of the various *Tanacetum* species contained tamirin, hanphyllin, and tavurin, which we had isolated from *T. vulgare* and *T. karelinii* growing in Kazakhstan, and artemorin acetate. Tanacetol was detected only in the *T. vulgare* fractions. Two lactones (crispolide and tatridin A) were not detected in the *Tanacetum* species from the Kazakhstan area that were investigated.

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TABLE 1. Levels of Sequiterpene Lactones (in %) in Various *Tanacetum* Species Growing in Kazakhstan

Sequiterpene lactone	*T. vulgare* fraction				*T. ulutavicum* fraction				*T. santolina* fraction		
	113–140	141–158	169–186	211–217	32–54	59–73	74–93	97–109	60–80	81–113	130–150
1. Crispolide	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
2. Tatridin A	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
3. Tamirin	1.73	6.71	9.67	23.65	2.80	3.26	12.55	15.04	3.10	15.29	2.92
4. Hanphyllin	13.80	9.68	7.72	3.83	9.94	10.35	11.63	14.18	4.65	4.85	1.55
5. Tavurin	6.10	5.72	4.63	2.43	2.80	3.22	5.46	6.05	5.38	2.60	1.35
6. Artemorin acetate	2.45	1.94	1.89	1.82	n/d	2.59	2.54	2.15	26.72	1.94	9.84
7. Tanacetol B	0.80	0.71	0.51	0.50	n/d	n/d	n/d	n/d	n/d	n/d	n/d

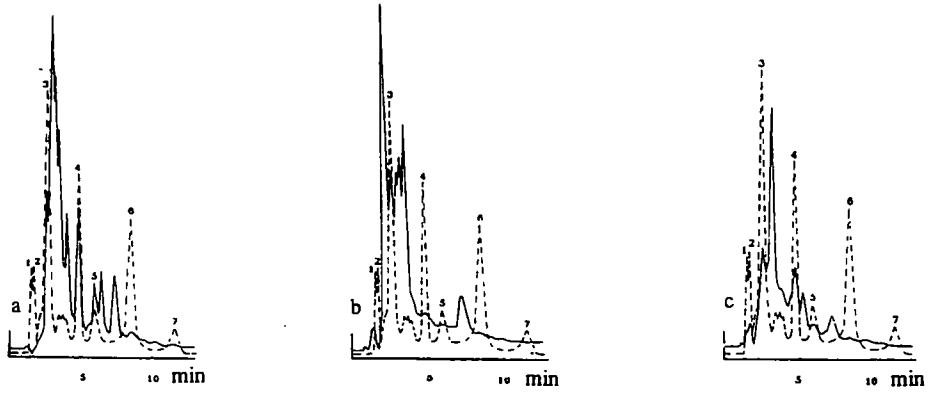

Fig. 1. Chromatograms of fractions of sequiterpene lactones isolated from *Tanacetum* species growing in Kazakhstan: *a*) *T. vulgare* 141-158; *b*) *T. santolina* 81-113; *c*) *T. ulutavicum* 97-109. Dotted line) mixture of standard specimens of sequiterpene lactones: *1*) cryspolide; *2*) tatridin A; *3*) tamyrin; *4*) hanphyllin; *5*) tavurin; *6*) artemorin acetate; *7*) tanacetol B.

The amounts of lactones from the epigeal parts of the *Tanacetum* species varied within wide limits, both in the individual fractions and in the different species. As can be seen from Table 1, the most promising species from the point of view of isolating sequiterpene lactones is *T. vulgare* (both in the number of lactones identified and in their amount it differs considerably from the other *Tanacetum* species shown here; the total amount of the lactones under consideration reaches 30%).

EXPERIMENTAL

We used a Milikhrom microcolumn liquid chromatograph (Nauchpribor Production Combine, Opel) with a 2 × 62 mm^2 stainless steel column filled with the sorbent LiChrosorb RP-18 (5 nm). The mobile phase was methanol—water (50:50 by volume), the rate of flow 100 μl/min, UV detector (250 nm).

The epigeal parts of the tansy plants were gathered in the flowering phase: *T. vulgare* and *T. santolina* in the nursery of the Karaganda Scientific Research Institute of Agriculture, and *T. ulutavicum* in the Dzhezkazganskaya oblast.

The comminuted and dried epigeal parts (leaves and flowers) of *T. vulgare* were extracted with hot water (T = 80°C), and the extract obtained was treated with chloroform. The epigeal parts of *T. santolina* and *T. ulutavicum* were extracted three times with hot chloroform, and the extracts obtained were treated with a 2:1 mixture of alcohol and water. Then the total extractive substances were chromatographed on a column of silica gel with various eluents (benzene, ether, ethyl acetate). As a result, 174 fractions were obtained from *T. vulgare*, 200 from *T. santolina*, and 155 from *T. ulutavicum*. A solution of 1 mg of the combined fractions in 1 ml of methanol was investigated by the HPLC method.

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