FATTY-ACID COMPOSITION OF THE LIPIDS OF SOME SPECIES OF *Trifolium*

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We have continued a phytochemical investigation of some species of *Trifolium* exhibiting interesting biological activity [1, 3]. We give the results of an investigation of the fatty-acid composition of the lipids of the seeds of *Trifolium pratense* L. (red clover), *Trifolium trichocephalum* Bieb. and the seeds and roots of *Trifolium arvense* L. (rabbitfoot clover).

The raw material (ripe seeds) was collected in 1980 and 1981 in the environs of Tbilisi and Tetri-Tskaro (GSSR).

To obtain the total lipids, the air-dry comminuted raw material was extracted with petroleum ether (bp 40-50°) and, after the solvent had been distilled off, the total lipids (yield 3-6%) were obtained in the form of a greenish liquid with a specific odor and taste. When the composition of the lipids was investigated by thin-layer chromatography [TLC on type KSK silica gel in the petroleum ether–diethyl ether–acetic acid (84:15:1) system] zones were obtained which corresponded to sterol esters, triacylglycerols (TAGs), free fatty acids, diacylglycerols, and free sterols. The main components of all the lipids were the TAGs.

The total fatty acids (TFAs) were obtained from the lipids by alkaline hydrolysis at room temperature [4] with preparative separation of the substances [5]. The TFAs were methylated with diazomethane and purified by preparative TLC [6]. The fatty acid methyl esters were analyzed on a Pye-105 chromatograph. The fatty acids were identified from their C_{sp} values relative to the 18:0 acid [7]. The results are given in Table 1.

As can be seen, palmitic, oleic, and linolenic acids predominated in the fatty acids of the lipids of the seeds of rabbitfoot clover and palmitic and linolenic acids in the seeds of red clover and of *T. trichocephalum* and in the roots of rabbitfoot clover. All the samples had a high content of saturated acids.

TABLE 1. Fatty-Acid Composition of the Lipids of Some Species of *Trifolium* (GLC, %)

Acid	Seeds		Roots	
	T. arvense	T. pratense	T. trichocephalum	T. arvense
9:0	—	—	—	Tr.
10:0	—	—	—	2,14
11:0	—	—	—	0,41
12:0	Tr.	Tr.	0,60	3,90
13:0	—	—	—	Tr.
14:0	Tr.	0,14	1,71	1,02
15:0	Tr.	—	2,21	0,92
16:0	20,72	26,98	35,61	30,84
16:1	2,81	0,96	4,63	Tr.
17:0	—	—	—	1,83
18:0	—	1,59	4,01	3,64
18:1	32,73	12,17	1 60	2,08
18:2	40,71	55,72	47,01	43,92
18:3	3,03	Tr.	—	9,15
20:0	—	2,41	1,62	Tr.
Unidentified acids	—	—	1,09	0,15
$\Sigma_{saturated}$	20,72	31,15	45,76	44,70
$\Sigma_{unsaturated}$	79,28	68,85	54,24	55,30

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ANTHRAQUINONES OF *Galium fagetorum*

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In a chromatographic study on paper and in a thin layer of sorbent in various solvent systems of a butanolic extract from the epigeal organs of the bedstraw species *Galium fagetorum* Klok., family Rubiaceae, we detected no less than 15 substances of anthraquinone nature.

The raw material for investigation (0.8 kg) was gathered in the phase of full flowering of the plant in the Crimea at the Angarskii pass and was preserved in butanol at the site of collection.

The anthraquinones were extracted from the raw material with butanol. The extract was evaporated in vacuum and the residue was dissolved in 96% ethanol and mixed with KSK silica gel (100 g), and this was washed with acid [1] and was left under a weight at room temperature to dry out. The dried mixture was ground to a powder and extracted in a Soxhlet apparatus successively with petroleum ether (40-70°C), benzene, and methanol. The extract obtained with petroleum ether was evaporated to dryness, dissolved in 400 ml of ether, and extracted repeatedly with 10% Na_2CO_3 solution. The alkaline solution was made acid with dilute hydrochloric acid, and the anthraquinones were extracted with ether.

Part of the ethereal extract (0.75 g), containing four substances of anthraquinone nature with R_f 0.31 (I), 0.37 (II), 0.42 (III), and 0.61 (IV) (toluene—acetone—50% acetic acid (4:1:0.5) system; Silufol plates), was dissolved in chloroform, and the solution was mixed with silica gel (8.0 g) and deposited on a column of silica gel (100.0 g).

The column was eluted with petroleum ether (40-70°C) and mixtures of it with chloroform, which yielded fractions 1-4 containing a mixture of anthraquinones.

Rechromatography of the individual fractions gave substances (I-III) in crystalline form: I — $C_{15}H_{10}O_4$ (253^+), mp 179-180°C (ethanol), R_f 0.31; (II) — $C_{15}H_{10}O_4$ (253^+), mp 300-301°C (ethanol), R_f 0.37; (III) $C_{14}H_8O_4$ (239^+), mp 289-290°C (ethanol), R_f 0.42.

The demethylation of (I) [2] yielded compound (III). On the basis of their physical and chemical properties, results of UV spectroscopy, and a comparison with authentic samples, substances (I), (II), and (III) were identified as 2-hydroxy-1-methoxyanthraquinone (I), rubiadin (II), and alizarin (III).

The study of the other components from this raw material is continuing.

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