STEROLS OF GRAPEFRUIT, ORANGE, AND MANDARIN PULPS

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The composition and percentage distribution of the sterols in preparations of the free (I), glycosylated (II), and esterified (III) sterols in the pulp of citrus fruits have been studied. In the sterol preparations, cholesterol, campesterol, stigmasterol, and β-sitosterol have been identified and detected, and three other sterols have been detected but not identified. It has been established that the sequences of the relative amounts of sterols in preparations (I), (II), and (III) of the grapefruit and the orange are similar and differ from that of mandarin pulp.

We have previously [1] reported on the complex composition of the sterols of grapefruit and orange rind.

We have now studied the sterol compositions of the pulp of three species of citrus fruits *Citrus paradisi* Macf. (grapefruit), variety Duncan, *Citrus sinensis* Osb. (orange), variety Washington Navel, and *Citrus unshiu* Marc (mandarin), variety Unshiu, cultivated in the Georgian SSR.

The lipid extracts from a freeze-dried homogenate of the pulp was obtained by the method of Folch et al. [2]. In all the samples, free, glycosylated, and esterified sterols (FSTs, GSTs, and ESTs, respectively) were detected. The sterols were isolated by Grunwald's method [3].

The qualitative compositions of the sterols in the FST, GST, and EST preparations of the pulps of three citrus species were represented by cholesterol, campesterol, stigmasterol, and β-sitosterol. Three unidentified sterols were also detected in all the samples. Table 1 shows the distribution of the relative amounts of the main sterols in the FST, GST, and EST preparations.

The orange pulp is characterized by a relatively large amount of β-sitosterol in the FST preparations, while the maximum amount of cholesterol is observed in the mandarin pulp. There is three and six times as much cholesterol in the mandarin pulp than in the grapefruit and orange pulps, respectively (see Table 1). The quantitative sequence of the distribution of the sterols in the mandarin is: β-sitosterol >> campesterol > cholesterol > stigmasterol. In the grapefruit and the orange the sequence of concentrations of the sterols in the FSTs is the same and differs somewhat from that of the FSTs of the mandarin: β-sitosterol >> campesterol > stigmasterol > cholesterol.

In the GST and EST preparations, the mandarin pulp was characterized by the largest amount of cholesterol. The percentage content of β-sitosterol was greatest in the GSTs and the ESTs of the orange and the grapefruit (see Table 1). The quantitative sequences of the distribution of the sterols in the GSTs, ESTs, and FSTs of the mandarin were the same. In the GSTs of the orange and the grapefruit the sequences of concentrations of the sterols were the same as in the FSTs of the citruses, and for the ESTs the distribution of the sterols was similar to that in the mandarin.

Stigmasterol made up 1.03-3.96% of the total sterols in the preparations from all the samples. The level of campesterol was high in the grapefruit FSTs. β-Sitosterol dominated among the sterols.

It is known that the chemical compositions of the lipids, particularly the fatty-acid compositions (including the fatty acids of the esterified sterols) have great value in the

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TABLE 1

Sterol	Ratio of the individual sterols in the combined preparations, %				
	mandarin			grapefruit	
	FSTs	GSTs	ESTs	FSTs	GSTs
Cholesterol	10,38±0,31	3,15±0,18	4.0 ±0.12	3,43±0,27	1,68±0,04
Campesterol	13,25±0,42	12,34±0,32	10,24±0,29	15, 0±0,43	10,07±0,28
Stigmasterol	1,23±0.03	1,91±0,19	1,55±0,11	3,96±0,53	2, 0±0,21
β-Sitosterol	65,37±0,56	77,45±0,25	79,9 ±0.17	73,58±0,43	80,50±0,62
Other sterols	9,76±0,85	5,15±0,57	4,30±0,09	4,02±0,13	5,75±0,20

Sterol	Ratio of the individual sterols in the combined preparations, %			
	grapefruit	orange		
	ESTs	FSTs	GSTs	ESTs
Cholesterol	1,72±0,47	1,75±0,05	0,89±0,12	2,22±0,048
Campesterol	11 48±0,15	12,77±0,26	10, 5±0,33	9,54±0,23
Stigmasterol	1,38±0,30	2,11±0.19	1,03±0,15	1,89±0,05
β-Sitosterol	80,12±0,40	82, 0±0,43	84,76±0,5	31 58±0,37
Other sterols	5,4±0,29	1,37±0,38	2,82±0,22	4,77±0,4

investigation of the chemotaxonomy of the citruses [4, 5]. It has been shown previously [6] that the varieties of citrus fruits differ from one another with respect to their ratio of the individual sterols. Knights and Berrie [7] have considered the composition of the sterols with the aim of using it in the chemosystematics of the Cruciferae at the species level. We have established that the sequence of relative amounts of sterol in the FST, GST, and EST preparations of grapefruit and orange pulp are similar and differ from that of the mandarin pulp. The FST preparations of the pulps of the species of citrus studied are characterized by a considerable difference in the percentage contents of cholesterol and β-sitosterol. A relatively high amount of cholesterol is reported in the FST preparations of mandarin pulp as compared with grapefruit and orange pulps (see Table 1). The facts given, together with other characteristics, can be used in the solution of the problem of the selection of citruses to be cultivated in Georgia.

With respect to the amount of sterol forms, the pulps of the different species of citrus differ only slightly: 0.06-0.01 mg% for the mandarin, 0.065-0.01 mg% for the grapefruit and 0.05-0.08 mg% for the orange. It must be mentioned that the amount of sterols in the pulp is greater than in the rind [1].

EXPERIMENTAL

The sterols were identified by GLC analysis on a Chrom-41 instrument with a flame-ionization detector at a temperature of 280°C with the stationary phases SE-30 (3%) on Chromaton (0.25-0.315 mm) and OV-1 (3%) on Chromaton (0.20-0.25 mm), with columns 0.2 cm in internal diameter and 2.5 and 2 m, respectively, in length. The rate of flow of helium was 25 ml/min. The sample input was 1 μl, the solvent being chloroform.

The qualitative analysis of the sterols was carried on the basis of a direct comparison of their retention times with the analogous indices of authentic samples. Table 1 gives the figures obtained by the statistical treatment of the results of the calculation of the chromatographic areas of the individual sterols.

SUMMARY

The quantitative compositions and the percentage distributions of the sterols of the pulp of the grapefruit, the orange, and the mandarin cultivated in Georgia have been studied. Cholesterol, campesterol, stigmasterol, and β-sitosterol, and three unidentified sterols, have been detected.

It has been established that the sequences of their relative amount in preparations of the free, glycosylated, and esterified sterols of the grapefruit and the orange are similar and differ from that for the mandarin.

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8,13-EPOXYLABD-14-EN-19-OIC ACID — A COMPONENT OF THE NEEDLES OF *Pinus sylvestris*

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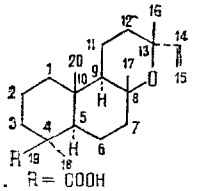
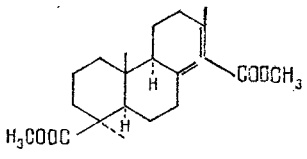
8,13-Epoxylabd-14-en-19-oic [(mannoyl oxide)-19-oic] acid, mp 64-66°C, $[\alpha]_D$ −39.2° (c 1.0; ethanol) has been isolated from the needles of *Pinus sylvestris*. The following derivatives have been obtained: methyl 8,13-epoxylabd-14-en-19-oate, with mp 83-85°C, $[\alpha]_D$ −43.2° (c 1.2; ethanol); 8,13-epoxylabd-14-en-19-ol, an oil, $[\alpha]_D$ −10.9° (c 1.0; ethanol), n_D^{25} 1.5025, cyclohexylammonium salt with mp 113-115°C, $[\alpha]_D$ −29.3° (c 1.0; ethanol); and 8,13-epoxydihydrolabd-14-en-19-oic acid with mp 61-63°C, $[\alpha]_D$ −23.1° (c 1.0; ethanol). The structures of the compounds were established by IR, mass, PMR, and ^{13}C NMR spectroscopy.

In a previous paper [1] devoted to a study of quantitative composition of the mixture of diterpene acids present in the needles of *Pinus sylvestris* L., we showed that in addition to known diterpene acids this mixture contained an acid of undetermined structure.

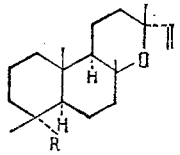
In the present investigation it has been shown that this acid is 8,13-epoxylabd-14-en-19-oic [(mannoyloxide)-19-oic] acid (I).



I. R = COOH
Ia. R = $COOCH_3$
Ib. R = CH_2OH
Ic. R = $COO^-\overset{+}{N}H_3C_6H_{11}$
Id. R = COOH; C_2H_5 at C_{13}



III



II. R = CH_3
IV. R = CH_2OAc

Table 1 gives the chemical shifts of the ^{13}C nuclei of methyl 8,13-epoxylabd-14-en-19-oate (Ia) and of related compounds: 8,13-epoxylabd-14-ene (mannoyl oxide) (II), dimethyl labda-8(17),13-diene-15,19-dioate (dimethyl agathate) (III), and 8,13-epoxylabd-14-en-18-ol acetate (yanol acetate) (IV).

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