STEROLS OF GRAPEFRUIT AND ORANGE RINDS

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It is known that the formation of sterols in plants takes place through complex reaction stages [1]. It is assumed that in the biosynthesis of the main sterols, such as β-sitosterol, the precursors are 4,4-dimethyl steroid compounds, and also 24-methylene- or 24-ethylene-sterols [2]. Consequently, the detection of such compounds in plants is very important.

Investigating the sterol composition of citrus fruit cultivated in Georgia, we have studied the sterols of the rind of Citrus paradisi Macf. (grapefruit), of the variety Duncan and Citrus sinensis Osb. (orange) of variety Washington Navel.

Lipid extracts from the air-dry material were obtained by the method of Folch et al. [3]. The results of TLC analysis on silica gel in systems of solvents given in the literature [4, 5] showed the presence of free sterols (FSTs), sterol glycosides (STGs), and esterified sterols (ESTs) in the lipids of grapefruit and orange rinds. After saponification of the EST fraction and acid hydrolysis of the STG fraction, the sterols were isolated in the form of a complex with digitonin [6]. The digitonin complex was decomposed with dimethyl sulfoxide [7], and the sterols were isolated with n-hexane and purified with the aid of preparative TLC on silica gel.

As the result of GLC-MC analysis it was found that in their qualitative composition the total preparations of the three forms of sterols isolated from grapefruit and orange rinds were similar consisting mainly of the following sterols: β-sitosterol (I), stigmasterol (II), campesterol (III), cholesterol (IV), ergosterol (V), 24-methylenecholesterol (VI), 24-methylenelophenol (VII), 24-ethylcholesta-5,25-dienol (VIII), and 24-ethylidenelophenol (IX) (Table 1).

The main sterol components (I-IV) and ergosterol (V) were identified by a direct comparison of their mass spectra and retention times with the analogous indices of authentic sterols. A comparison of the retention times obtained for the sterols (VI-IX) with literature figures [8, 9] on the gas-chromatographic investigation of the sterol fractions of orange juice isolated by liquid chromatography with gradient elution, and also the nature of the fragmentation of their mass spectra, confirmed the presence of the structures.

In all the samples, the shape of the chromatographic peak for β-sitosterol was characterized by a slight asymmetry apparently due to the presence of a considerable number of spatial isomers and other sterol components appearing in the form of shoulders and apices on the main peak [8]. We observed the greatest number of sterol accompanying components of β-sitosterol for the esterified forms of the sterols.

GLC-MS analysis was carried out on a Finnigan-3200F chromato-mass spectrometer with an automatic data-processing system. Conditions of chromatography: glass capillary column with phase SE-30, 25 m long, 0.3 mm in internal diameter. Flow of helium 1.3 ml/min. Column temperature 290°C, injector temperature 280°C. Size of sample 1 μl. Solvent cyclohexane.

The IR and NMR spectra of the sterol preparations from all the samples showed the presence of methyl groups, a Δ^5-bond, a trans-Δ^{22}-bond, and OH groups showing mainly the presence of Δ^5-sterols, which agrees with the results of GLC analysis on a large number of Δ^5-sterol components (about 96%) in the combined sterols.

UV-Spectrometric analysis of the sterols [10] showed that grapefruit and orange rinds contain a larger amount of FSTs than of STGs and ESTs. The percentage of FSTs was 0.015-

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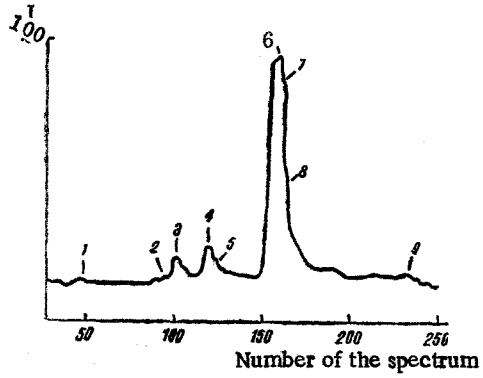

Fig. 1

TABLE 1

Compound	M	Retention time, min	Values of m/e and relative intensities of the characteristic ions
1. Cholesterol (IV)	386	20,37	386 (40)*, 371 (15), 368 (19), 353 (25), 301 (32), 275 (100), 247 (14), 273 (40), 255 (60), 231 (33), 213 (66).
2. Ergosterol (V)	396	22,49	396, 378, 363, 337, 271, 257, 253, 227, 211
3. Campesterol (III)	400	22,67	400 (73), 385 (36), 382 (44), 367 (43), 315 (82), 289 (88), 273 (66), 261 (21), 255 (100), 231 (78), 213 (82).
4. Stigmasterol (II)	412	23,48	412 (21), 397 (2), 394 (1), 379 (3), 327 (3), 300 (33), 273 (17), 271 (68), 255 (100), 231 (10), 213 (35)
5. 24-Methylenecholesterol (VI)	398	23,79	398, 383, 370, 355, 314, 273, 269, 255
6. β-Sitosterol (I)	414	25,54	414 (66), 399 (6), 396 (7), 381 (13), 329 (48), 303 (71), 275 (22), 273 (62), 255 (96), 231 (43), 213 (100)
7. 24-Methylenelophenol (VII)	412	25,88	412, 397, 394, 379, 370, 314, 287, 269, 245, 227
8. 24-Ethylcholesta-5,25-dienol (VIII)	412	26,48	412, 397, 371, 345, 314, 273, 255, 229, 211, 69
9. 24-Ethylidenelophenol (IX)	426	26,95	426, 408, 393, 327, 310, 273, 269, 267, 245, 227

*The intensities of the ions (in percentages of the maximum are given in parentheses in the range of m/e values above 210 (for the main sterol components).

0.02%, of STGs 0.005-0.01%, and of ESTs 0.001% on the air-dry material. The distribution of the individual sterols in the total preparations of all the samples varied.

LITERATURE CITED

1. E. Heftmann, Lloydia, 31, 293 (1968).
2. M. A. Palmer and B. N. Bowden, Phytochemistry, 14, 2049 (1975).
3. J. Folch, M. Lees, and G. H. S. Stanley, J. Biol. Chem., 226, 497 (1957).
4. M. Lepage, J. Lipid Res., 5, 587 (1964).
5. T. Miyazawa, S. Ito, and Y. Fujino, Cereal Chem., 5, 623 (1974).
6. R. L. Stedman and W. Rusaniwskyj, Tob. Sci., 3, 44 (1959).
7. C. H. Issidorides and J. Kitagawa, J. Org. Chem., 27, 4693 (1962).
8. S. Nagy and H. E. Nordby, Lipids, 6, 826 (1971).
9. H. E. Nordby and S. Nagy, J. Chromatogr., 79, 147 (1973).
10. P. S. Cowley, F. I. Evans, and E. A. Ginman, Planta Med., 19, 249 (1971).