ESSENTIAL OILS OF THE LEAVES OF GRAFTS OF *Citrus unshiu*

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The compositions of the essential oils obtained by steam distillation from the leaves of frost-resistant stocks of trifoliate orange (*P. trifoliata* (L.) Raf.) and Yunos-Yuzu citrus (*Citrus ionos* (Sieb.) and grafts of the mandarin orange *C. unshiu* Marc — *P. trifoliata* + *C. unshiu* and *C. ionos* + *C. unshiu* — have been investigated with the aid of vacuum distillation, GLC, chromatography on an alumina column, and IR spectroscopy. The leaves were collected in the period of the flowering of the plants. The essential oils of grafts of the unshiu mandarin on trifoliate orange and Yunos-Yuzu citrus differed from one another with respect to the amounts of the components.

Continuing an investigation of the essential oils of citruses growing in the Georgian SSR, we have made a comparative study of the essential oils of the leaves of the frost-resistant stocks Yunos-Yuzu citrus (*Citrus ionos* (Sieb.)) and trifoliate orange (*Poncirus trifoliata* (L.), Raf.), and Unshiu mandarin (*C. unshiu* Marc.) grafted onto them.

It is known [1] that the stock has a considerable influence on the physiological and biochemical processes of a citrus plant grafted onto it. Under the influence of the stocks, the amounts of essential oils and their components change in grafts of citrus fruits [2, 3]. There is no information in the literature on the influence of the stocks on the chemical composition of the essential oils of the leaves of Unshiu mandarin grafts.

We have published information on the composition of the essential oils of leaves collected in the period of forced dormancy and the beginning of vegetation from the Unshiu mandarin and Yunos-Yuzu citrus [4, 5]. In the present paper we give the results of an investigation of the essential oils of the leaves of stocks and grafts of mandarin orange collected in the flowering period.

The amounts of essential oils in the leaves of the stocks and grafts were as follows (% on the dry weight):

C. ionos	0.26
P. trifoliata	0.06
C. Yunos-Yuzu + *C. unshiu*	0.12
P. trifoliata + *C. unshiu*	0.14

A marked difference was observed in the amount of essential oils in the leaves of the stocks. There was considerably more of them in *C. ionos* than in *P. trifoliata*, but the leaves of a graft of mandarin orange on *C. ionos* contained a considerably smaller amount of essential oil than the leaves of a graft on *P. trifoliata*.

The quantitative and qualitative composition of the essential oils were studied by the GLC method. The essential oils of the stocks differed considerably from one another both in the quantitative and in their qualitative sets of components (Table 1).

A comparative investigation of the essential oils of the leaves of the grafts showed that they did not differ from one another with respect to the qualitative composition of the components, but they did differ with respect to the quantitative distribution of the individual substances. A comparatively high amount of α-thujene, α-pinene, and, particularly, β-pinene was characteristic of the essential oil of the leaves of a graft onto trifoliate orange. Such monoterpene hydrocarbons as limonene, γ-terpinene, and p-cymene were present at the same

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TABLE 1. Amounts of Identified Components in the Essential Oils of Stocks of the Unshiu Mandarin Orange (in % on the total oil)

Component	P. trifoliata	C. ionos	P. trifoliata + C. unshiu	C. ionos + C. unshiu
α-Thujene	—	—	1,2	0.4
α-Pinene	0,5	3,8	3,8	1,7
Camphene	—	0 1	0,1	0.2
β-Pinene	—	12,1	14 2	6,2
Sabinene	—	2 7	1.3	2,0
Myrcene	8,4	0 3	0,8	1,3
α-Phellandrene	—	0,3	0,1	0,6
α-Terpinene	—	0,2	0,3	1.2
Limonene	0,6	8,8	3,9	3.7
Ocimene	—	2.8	—	—
γ-Terpinene	65,8	38 6	35.4	35 2
p-Cymene	0,5	1,4	15,2	15.9
Citronellal	0,2	0,3	—	—
Decanal	—	0.6	—	—
Linalyl acetate		0.2	—	—
Linalool	—	7,6	6,2	3,2
Nonanol	10 6	—	—	—
Terpinen-4-ol	—	6,2	—	—
α-Terpineol	—	—	2,3	2.8
Neral	—	—	0.8	1,3
Geranial	0,5	0,2	—	—
Ylangene	—	2.2	—	—
β-Caryophyllene	—	1.2	0,8	3.8
Cubabene	—	0,1	—	—
Muurulene	—	0.4	5,3	8,1
β-Elemene	—	0.8	5.3	8,1
β-Selinene	—	0,8	0,2	1.6
Citronellol	—	—	0,1	0,6
Nerol	0,6	0.2	—	—
Geranyl acetate	0.2	—	—	—
Geraniol	—	—	0,2	0,8

level, their total amount in the oils being more than 54%. The essential oils of the leaves of a graft of trifoliate orange were richer in sesquiterpene hydrocarbons. The same oil contained α-terpineol and geraniol in comparatively large amounts. There was a more pronounced difference between the grafts in the amount of linalool.

The observed difference in the quantitative amounts of the individual substances in the essential oils of grafts of Unshiu mandarin orange on *C. ionos* and trifoliate orange stocks was due, in all probability, to an interaction of the stock and the graft.

EXPERIMENTAL

The essential oils were obtained by the steam distillation of the leaves of fruit-bearing plants from the experimental section of the Batumi Botanical Garden of the Academy of Sciences of the Georgian SSR.

In the investigation the methods of vacuum distillation, chromatographic separation on alumina and gas-liquid chromatography on a Varian Aerograph 1860 instrument with a flame-ionization detector were used.

The IR spectra were recorded on a UR-10 instrument.

The analytical GLC of the essential oils was carried out in two columns. The first column, with dimensions of 550 × 0.2 cm, contained 10% of the stationary phase FFAP on chromosorb W 80/100 mesh. The rate of flow of the carrier gas (helium) was 40 ml/min. The temperature of the column was raised from 80 to 230°C at the rate of 2°C/min. The second column, with dimensions of 450 × 0.2 cm, contained SE-30 on Chromosorb 80/100 mesh. Its temperature was raised from 90 to 220°C at the rate of 2°C/min. The rate of flow of helium was 35 ml/min.

The amounts of the components of the essential oils were determined by the method of internal standards and internal normalization.

The preparative isolation of the monoterpene hydrocarbons was carried out on a 600 × 0.9 cm aluminum column with 30% of the stationary phase FFAR on Chromosorb W 60/80 mesh at a temperature of 140°C with a rate of flow of helium of 150 ml/min.

The preparative GLC of the sesquiterpene hydrocarbons and of the oxygen-containing monoterpenes was carried out on a 600 × 0.9 cm column containing 30% of Carbowax 20M on Chromosorb W 60/80 mesh, the conditions being varied somewhat according to the fraction being investigated.

Fractionation of the Essential Oils. The essential oils were subjected to vacuum distillation in a column with an efficiency of 40 theoretical plates to give two fractions, the first of which contained the monoterpene hydrocarbons. Distillation was completed at 80°C (22 mm Hg). The distillation process was monitored by GLC.

Identification of the Monoterpenes. α-Thujene, camphene, α-phellandrene, α-terpinene, ocimene, and terpinolene were identified in the essential oils from their retention times and the increase in the size of the corresponding peaks on the addition of the pure substances to a sample of the fraction. The remaining components were isolated by preparative GLC, and their IR spectra were recorded; these spectra agreed with those given in the literature [6].

Identification of the Sesquiterpene Hydrocarbons. The residue from the separation of the monoterpene hydrocarbons, consisting of the sesquiterpene hydrocarbons and the oxygen-containing compounds, were chromatographed on a column with Al_2O_3 (Brockman activity grade II; 1:10). Petroleum ether eluted the sesquiterpene hydrocarbons, and ethyl alcohol the oxygen-containing compounds. β-Selinene, muurulene and cubabene were identified in the essential oils by analytical GLC (SE-30 column) from their relative retention times and the use of additives.

β-Elemene and β-caryophyllene were isolated from the sesquiterpene hydrocarbon fractions of the essential oils by preparative GLC (column temperature 170°C, rate of flow of helium 180 ml/min). They were identified by a comparison of their IR spectra with those given in the literature [6].

Identification of the Oxygen-Containing Monoterpenes. The fractions eluted by ethyl alcohol consisted of oxygen-containing monoterpenes.

Linalool, terpinen-4-ol, and α-terpineol were isolated by preparative GLC (temperature 180°C, rate of flow of carrier gas 190 ml/min). They were identified by comparing their IR spectra with those given in the literature. Citronellol, nerol, and geraniol were identified by the method of additives using the pure alcohols.

The fractions of oxygen-containing compounds were saponified with 0.5 N ethanolic caustic soda. A comparison of chromatograms of saponified and unsaponified samples of oils according to the disappearance or increase in the areas of the peaks showed the presence of esters of terpene alcohols in the oils. Geranyl acetate was identified in the essential oil of the trifoliate orange and linalyl acetate in the oil of the leaves of the Yunos-Yuzu citrus.

Decanal, citronellal, neral, and geranial were identified by the addition of the pure substances.

CONCLUSIONS

1. The compositions of the essential oils of the leaves of *P. trifoliata* and of *C. ionos* and of grafts of them on Unshiu mandarin orange have been investigated.

2. It has been estabished that the essential oils of grafts of Unshiu mandarin orange on *C. trifoliata* and *C. ionos* differ from one another with respect to the amounts of their individual components.

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