LIPOSOLUBLE PIGMENTS OF EDIBLE ROOTS OF Beta vulgaris

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The qualitative and quantitative comopsition of the chlorophylls and carotenoids of the edible roots of table beets of the varieties Bordeaux and Nosovskaya Ploskaya have been determined by chromatographic and spectral methods.

In contrast to the water-soluble betalaines, the water-soluble pigments of table beets have scarcely been studied. In connection with the development of new types of preserved products based on table beets which possess valuable dietetic and medicinal properties [1] and the necessity for considering the composition of the liposoluble pigments in the technology of processing the beet raw material, we have investigated the presence of various forms of chlorophylls and carotenoids in roots crops of Beta vulgaris of two industrially important varieties – Bordeaux and Nosovskaya Ploskaya.

The total liposoluble pigments were isolated from the finely ground roots by the Bligh–Dyer method as components of the total lipids [2]. Chloroform extracts were carefully freed from betalain pigments by washing them with a 0.5 aqueous solution of $CaCl_2$ until the wash-waters were colorless. An aliquot part of the extract intended for the isolation of the carotenoids was freed from chlorophylls and lipids by saponification and was fractionated into carotenes and xanthophylls by the use of a column of sucrose [3]. Part of the extracts was freed from lipids and carotenoids by column chromatography on silica gel [4] and was used for determining chlorophylls.

The pigments were separated into individual compounds by GLC, using system 1 for chlorophylls and xanthophylls, and system 2 for carotenes. The pigments were protected from degradation by the addition of a stabilizer [5] to the solutions and by performing the operations in the absence of bright light. The pigments were identified on the basis of the results of chromatography in the presence of markers and from their absorption in the visible and ultraviolet regions [6]. In the detection of colorless and minor representatives of the carotenoids, the chromatograms were stained with iodine vapor. For quantitative determination, individual pigments were eluted from the adsorbent and their amounts were estimated by a colorimetric method based on absorption in characteristic regions of the spectrum and the corresponding molar extinction coefficients (% on the total mass):

Pigment	Bordeaux	Nosovskaya Ploskaya
Chlorophylls		
chlorophyll a	17.9	25.7
chlorophyll b	15.9	16.4
pheophytin a	13.3	9.3
pheophytin b	4.7	6.0
pheophorbide a	31.4	23.7
pheophorbibe b	16.8	18.9
Total amount, mg/kg	4.3	2.1
Carotenoids:		
phytoin	4.9	1.7
phytofluene	4.3	1.2
α-carotene	5.7	6.9
β-carotene	3.9	2.4

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Pigment	Bordeaux	Nosovskaya Ploskaya
lycopene	2.4	5.0
lutein	54.8	57.3
violaxanthin	10.4	9.8
neoxanthin	13.6	15.7
Total amount, mg/kg	3.6	1.9

As follows from the experimental results, the varieties of the root crop Beta vulgaris studied are characterized by comparatively low levels of chlorophylls and carotenoids. The roots of the variety Bordeaux contained approximately twice as much of total liposoluble pigments as those of Nosovskaya Ploskaya. In both varieties the amount of pigments of the chlorophyll group was somewhat higher than that of the carotenoids. The qualitative compositions of the pigments of the two varieties were identical, and the quantitative ratios of the individual compounds were similar.

In the green pigments, in addition to chlorophylls a and b, their derivatives – pheophytins and pheophorbides – were detected, the amounts of the pheophorbides being higher than that of the chlorophylls, and the ratio of the a- and b-forms of chlorophyll and its structural analogs being somewhat lower than in the photosynthesizing parts of the plants. This is apparently due both to the activity of chlorophyllase and to features of the occurrence of the biosynthesis of chlorophyll under the conditions of inadequate access of air. The presence of small amounts of pigments of the chlorophyll group in the edible roots Beta vulgaris is probably a widespread phenomenon, as may be indicated by their detection, for example, in the edible roots of the potato [7]. So far as concerns carotenoids, their amount in beets is substantially lower than in carrots, swedes, turnips, and other food root crops [8]. Eight representatives of carotenoids were detected among which xanthophylls predominated (>80 %). The amount of lutein was particularly great – more than 50% of the sum of all the carotenoids.

In the Beta vulgaris roots, vitamin-active pigments (α- and β-carotenes) amounted to 10% of the mass of the carotenoid complex.

EXPERIMENTAL

The Beta vulgaris roots were collected at the stage of technical ripeness from an experimental plot of the Konservpromkomoleks VINPKI, Odessa, Odessa province, UkrSSR). The pigments were extracted from a homogenate of high-quality roots freshly gathered and carefully freed from tops and dirt.

Column chromatography was performed on silica gel L 100/160 (Czechoslovakia), and thin-layer chromatography on cellulose for TLC (FRG) in the solvent systems: 1) heptane–methyl ethyl ketone (5:3); and 2) hexane–acetone (96:4). The bands of pigments separated by the TLC method were detected in the visible and UV light, and the pigments, after their removal from the plates together with the adsorbent and elution with acetone, were transferred into a suitable solvent, and their optical absorptions were determined at wavelengths corresponding to the maximum absorption. The absorption spectra were recorded on a Specord spectrophotometer.

SUMMARY

The chemical compositions and amounts of carotenoids and chlorophylls and their structural analogs in the edible roots of Beta vulgaris of the varieties Bordeaux and Nosovskaya Ploskaya have been investigated for the first time.

The total amount of liposoluble pigments was low – 2-8 mg/kg. Six pigments of the chlorophyll group and eight carotenoids were identified. The amounts of pigments of the chlorophyll groups were greater than the amount of carotenoids. The predominant compounds were lutein, neoxanthin, violaxanthin, chlorophylls a and b, and pheophorbides a and b.

LITERATURE CITED

1. S. A. Bogdanov and N. N. Pereverzeva, The Food Industry. Series 18. The Preserving, Vegetable-Drying, and Food-Concentrate Industry [in Russian], TsNIITEIPishcheprom [Central Scientific-Research Institute of Information and Technical and Economic Investigations of the Food Industry] No. 4 (1985), p. 2.

2. E. G. Bligh and W. I. Dyer, Can. J. Biochem. Physiol., 37, 911 (1959).
3. H. H. Strain and W. A. Svec, in: The Chlorophylls L. P. Vernnon and G. R. Seely, eds., Academic Press, New York (1966), p. 63.
4. M. Kates, Techniques of Lipidology, American Elsevier, New York (1972), [Russian translation, Moscow (1975), p. 122].
5. Yu. L. Zherebin and A. A. Kolesnik, Khim. Prir. Soedin., 111 (1984).
6. Pigments of the Plastids of Green Plants and a Procedure for Their Investigation [in Russian], Moscow (1964), p. 121; F. L. Kalinin and V. I. Zhidkov, Handbook on Biochemistry [in Russian], Kiev (1971), p. 1014.
7. T. Galliard, Phytochemistry, 7, 1909 (1968).
8. The Chemical Composition of Food Products [in Russian], Moscow, Book 2 (1987), p. 149.

NEW SESQUITERPENOIDS FROM THE OLEORESIN OF *Abies alba*

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The structures of four new sesquiterpenoids from the oleoresin of *Abies alba* have been studied. On the basis of various spectral characteristics, the structures of (10S, 11S)-himachala-2,4-diene, (10S, 11S)-himachala-3(12), 4-diene, humula-4,9-dien-8-ol, and (4S, 5S, 10S)-selina-6-en-4-ol have been proposed for the compounds isolated. The stereochemistry of the asymmetric centers was determined by the conversion of these compounds into known sesquiterpenes and also by analysis of PMR spectra with a shift reagent.

In a study of the composition of the volatile components of the oleoresin of *Abies alba* Mill.[1], four sesquiterpene compounds have been isolated the spectral characteristics of which proved to be different from those given in the literature. In the present paper we consider the structures of these terpenoids.

Two new hydrocarbons have been found in the sesquiterpene fraction of the oleoresin of *Abies alba* the amounts of which, according to GLC, were 23 and 16%. However, in isolation on silica gel impregnated with silver nitrate, the yields of these components proved to be considerably lower (~12 and ~7%, respectively). According to PMR and ^{13}C NMR spectroscopy, mass spectrometry, and UV spectroscopy, both compounds were bicyclic conjugated dienes.

The first compound, eluted by petroleum ether, consisted of a colorless liquid and possessed a homoannular dienic system (λ_{max} 256 nm). The PMR spectrum of this diene showed the signals of two protons at trisubstituted double bonds (5.23 and 5.54 ppm) two singlets of geminal methyl groups (1.03 and 1.09 ppm), the signal of a secondary methyl group (0.83 ppm, doublet, J = 6.5 Hz) and that of a methyl group at a double bond (1.72 ppm).

The second compound, eluted by petroleum ether containing 2% of diethyl ether, had in its PMR spectrum the signals of the protons of an exomethylene double bond (4.65 and 4.70 ppm) of the proton at a trisubstituted double bond (5.94 ppm), of two geminal methyl groups (0.96 and 1.13 ppm), and of a secondary methyl group (0.94 ppm, doublet, J = 6.5 Hz).

On the basis of the spectral characteristics obtained, and also in view of the high level of compounds of the longifolane series and the presence of α- and β-himachalenes (I and II) [1] in the oleoresin of *Abies alba* structures (III) and (IV), respectively, are suggested for the hydrocarbons isolated. The products of the oxidation of the diene (III) with monoperphthalic acid the aromatic alcohol (V) was isolated by chromatography on silica gel, and its PMR

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