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CARBON DIOXIDE EXTRACT FROM WOODY VERDURE OF THE SCOTCH PINE. GROUP COMPOSITION AND ACIDS

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The group and individual compositions of the acids of an extract from the woody verdure of Scotch pine obtained on a pilot plant have been studied. The yield of extract amounted to 4.0% on the weight of the raw material, and 88% of the extract dissolved in petroleum ether. The petroleum-ether-soluble substances of the extract contained 32.8% of free acids and 55.4% of neutral substances. The compositions of the higher fatty acids, the diterpene acids, and the bicyclic acids have been determined. Pinifolic acid and its monomethyl ester, 18-acetoxy- and 18-hydroxyanticopalic acids, an acid of the 4-epiimbricatic series, and oxo and hydroxy acids of the abietic type have been isolated and identified.

In order to study the composition of the extractive substances of woody verdure, even in industry petroleum ether is the main extractant, sometimes being used with gasoline, trichloroethylene, isopropanol, and other solvents [1, 2], while steam is used for the isolation of the essential oil [3]. In these cases, the woody verdure is subjected to the action of high temperatures, which may lead to a change in the chemical composition of the extracts obtained. A promising extractant of the woody verdure that is free from these defects may

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TABLE 1. Group Composition of a Carbon Dioxide Extract from the Woody Verdure of the Scotch Pine

Substances	Amount	
	g	%
Extracted by petroleum ether, including:	50.5	88.75
free acids	18.5	4
neutral substances	31.2	55.38
Extracted by diethyl ether	1.1	1.93
Extracted by ethyl acetate	0.8	1.41
Extracted by butanol	1.0	1.76
Soluble in water (residue)	0.6	1.05
Obtained	54.0	94.90
Taken for analysis	56.9	100.0
Losses	2.9	5.10

be liquid carbon dioxide [4]. There is experience of the carbon dioxide method of extracting pine needles and buds [5], and of pine, fir, and cedar woody verdure [6]. The favorable effect of extraction with carbon dioxide has been shown, but the composition of the substances extracted has been studied inadequately. The presence of higher fatty and resin acids [5, 6], of phenolic acids, and of monoterpene hydrocarbons [5] in an extract has been shown.

We have studied the group composition of the acids of a carbon dioxide extract obtained from the woody verdure of the Scotch pine in a pilot plant of a factory for the production of dry ice (Sukhumi). The yield of extract was 4.0% on the weight of the dry raw material. The initial woody verdure contained (according to extraction with petroleum ether having a boiling range of 70-100°C in a Soxhlet apparatus) 12.5%, and the woody verdure after extraction with carbon dioxide 6.8%, of substances extractable by petroleum ether. The amounts of α-tocopherol and its acetate (0.32 and 0.24%, respectively), of carotene (21 mg), and of combined carotenoids (32 mg calculated to 100 g of dry matter of the extract) were determined in the extract obtained. The amount of chlorophyll derivatives in the extract (~90 mg/100 mg of extract) was smaller than in a gasoline extract. The extractive substances were separated by successive extraction with solvents into groups differing in polarity (Table 1).

The greatest amount of extractive substances in the carbon dioxide extract dissolved in petroleum ether. The proportion of more polar compounds was about 5%. The losses, also amounting to about 5%, were partially connected with the presence of water in the extract, the amount of which was not determined.

The substances of the carbon dioxide extract that were soluble in petroleum ether (bp 40-70°C) were separated into free acids (37.0%) and neutral substances (62.4%). As compared with the extraction of the woody verdure of the pine by gasoline, the carbon dioxide extract contained a somewhat larger amount of neutral substances and a smaller amount of free acids.

TABLE 2. Results of the Separation of the Free Acids by Column Chromatography

Fraction number	Eluent	Amount in the free acids		Observation
		g	%	
1	Petroleum ether 8 : 2	11,30	62,02	Sum of resin acids and higher fatty acids
2	« 7 : 3	3,26	17,89	Monomethyl ester of pinifolic acid
3	« 6 : 4	0,45	2,48	Acetoxy acid
4	« 1 : 1	0,28	1,50	Monomethyl ether of pinosylvin
5	« 4 : 6	0,40	2,19	Oxo acids
6	« 2 : 8	0,91	4.99	Hydroxy acids
7	Ethanol	1,62	8,94	Pinifolic acid and waxy substances
Total		18,22	100,0	
Taken for separation		18,50		
Losses		0,28		

Gasoline extracted a material consisting of 55% of neutral substances and 45% of free acids [7].

By column chromatography (CC) on silica gel, the free acids were separated into seven fractions differing in the polarity of their components (Table 2). The main fraction, including more than half the extracts, sonsisted of higher fatty acids and resin acids. Fraction 1 contained 34.2% of resin acids and 65.8% of fatty acids (without allowing for the losses on analysis, which amounted to 2.8%). The compositions of the higher fatty acids (methyl esters) and of the resin acids (after methylation with diazomethane) were determined by the GLC method:

Acids	Amount, %
Saturated	51.5
Lauric	0.4
Tridecanoic	4.1
Myristic	5.0
Margaric	1.5
Palmitic	21.3
Stearic	6.2
Arachidic	3.4
Behenic	6.0
Lignoceric	3.6
Unsaturated	42.7
Palmitoleic	3.7
Oleic	10.6
Linoleic	12.3
Linolenic	11.5
Isolinolenic	4.6
Unidentified	5.8

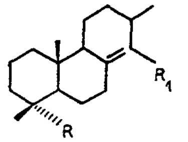
Acids	Amount, %
Resin acids	
Pimaric	4.0
Levopimaric and/ or palustric	4.0
Isopimaric	16.2
Abietic	37.8
Dehydroabietic	31.6
Neoabietic	4.4
Polar acids	
Monomethyl ester of pinifolic acid	43.7
18-Acetoxyanticopalic	2.9
15-Acetoxy-4-epiimbricatic	1.3
18-Hydroxyanticopalic	3.7
15-Hydroxy-4-epiimbricatic	1.0
Pinifolic	~0.3
7-Oxodehydroabietic	5.0
12-Hydroxydehydroabietic	2.9
15-Hydroxyabietic	1.8
15-Hydroxydehydroabietic	1.8

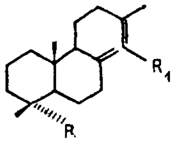
The higher fatty and resin acids had the compositions customary for the extractive substances of pine woody verdure: The main components of the fatty acids were linoleic, oleic, palmitic, stearic, and behenic acids, and those of the resin acids were abietic, dehydroabietic, and isopimaric acids.

The remainder of the free acids consisted of polar diterpene acids, fractions of which were methylated with diazomethane and were analyzed by the GLC method, or individual components were isolated by CC and their structures were determined by spectral methods.

According to TLC, fraction 2 consisted mainly of a single component. After methylation of the fraction with diazomethane and additional purification on a column of silica gel with the addition of 5% of silver nitrate, a compound was isolated in the form of an oil which was identified from its PMR spectrum as the dimethyl ester of pinifolic acid (I). Two other compounds eluted from the column after the dimethyl pinifolate had the same R_f value on a plate coated with silica gel impregnated with silver nitrate as standard methyl esters of 18-acetoxyanticopalic acid (II) and of 15-acetoxy-4-epiimbricatic acid (III). Their presence was also confirmed by GLC with the addition of the corresponding methyl esters.

The presence of a considerable amount of acids of the labdane type is characteristic for Scotch pine needles. Monomethyl pinifolate — the main component of the free acids of its needles — amounts to about 25% of the total acids [7] or 50% of the diterpene acids [8].





I. $R = R_1 = COOCH_3$
III. $R = COOCH_3$; $R_1 = CH_2OAc$
VIII. $R = R_1 = COOH$
IX. $R = COOCH_3$; $R_1 = COOH$
XI. $R = COOH$; $R_1 = CH_2OH$

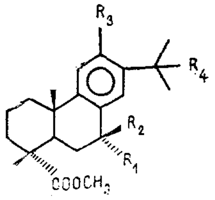
II. $R = CH_2OAc$; $R_1 = COOCH_3$
X. $R = CH_2OH$; $R_1 = COOH$

In the carbon dioxide extract, the monomethyl pinifolate was likewise one of the main components in the free acid group, but its amount was somewhat lower than in a needle extract [7] or in the corresponding group of compounds of a chlorophyll–carotene paste [8]. This is probably connected, on the one hand, with the presence of shoots in the raw material, in which only an insignificant amount of monomethyl pinifolate has been detected [9] and, on the other hand, with a somewhat lower extracting capability of liquid carbon dioxide with respect to the polar acids.

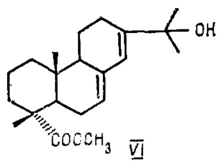
Fraction 3, after methylation with diazomethane and analysis by GLC, contained the same components as fraction 2, but in a different ratio. The main component of this fraction was methyl 18-acetoxyanticopalate. The ratio in the fraction of the dimethyl pinifolate, 18-acetoxyanticopalic acid, and 15-acetoxy-4-epiimbricatic acid was 3:5:2, respectively. In addition to the compounds identified, fraction 3 contained a small quantity (about 10% of the fraction) of another three, unidentified, components (GLC).

After its acetylation, fraction 4 yielded the acetate of the monomethyl ether of pinosylvin and an acid of undetermined structure.

The following fraction consisted of a single component which, after methylation, purification by CC, and recrystallization from hexane was isolated in the form of crystals with mp 64-66°C. It was identified from its PMR spectrum as methyl 7-ketodehydroabietate (IV).



IV, V, VII



VI

IV. $R_1 + R_2 = O$; $R_3 = R_4 = H$
V. $R_1 = R_2 = R_4 = H$; $R_3 = OH$
VII. $R_1 = R_2 = R_3 = H$; $R_4 = OH$

Fraction 6 included six components (GLC, after methylation of the fraction with diazomethane). The methyl esters were separated by column chromatography into three groups of substances. The fraction eluted first contained crystals which, after recrystallization from acetonitrile, had mp 160-162°C. We had previously isolated a compound with mp 161-163°C from Scotch pine shoots and identified it as methyl 12-hydroxydehydroabietate [10]. The IR and PMR spectra of the ester isolated from the carbon dioxide extract and of methyl 12-hydroxydehydroabietate (V) were identical.

The second group of substances of the fraction contained two compounds (GLC). After acetylation, analysis of the fraction by TLC on silica gel and on silica gel with the addition of 5% of silver nitrate, and by GLC with authentic samples of methyl 18-acetoxyanticopalate and 15-acetoxy-4-epiimbricatate showed that this group contained the compounds mentioned in a ratio close to 3:1.

The third group of esters contained three components in a ratio of 2:4:4. After attempted acetylation of the fraction, the R_f values of the compounds had not changes, which showed the presence of tertiary hydroxy groups in them. Attempts to separate the fraction into individual compounds by CC on silica gel with the addition of nitric acid were unsuccessful. According to the PMR spectrum, it contained equal amounts of methyl 15-hydroxyabietate (VI) and methyl 15-hydroxydehydroabietate (VII).

The fraction eluted from the column with ethanol (see Table 2) probably contained polymeric substances. After methylation of the fraction with diazomethane and TLC of the methyl esters on silica gel with added silver, pinifolic acid (VIII) in the form of its dimethyl ester was identified in trace amounts.

Thus, the results of study of the group composition of an extract obtained with liquid carbon dioxide from the woody verdure of the Scotch pine have shown the presence mainly of weakly polar compounds with a high proportion of neutral substances (more than 60%) and a low amount of chlorophyll derivatives. The composition of the components of the acids was practically identical with that of the acids of a chlorophyll—carotene paste (gasoline extract) and contained saturated and unsaturated higher fatty acids and resin acids (monobasic tricyclic diterpene acids), and also ester acids, hydroxy acids, and dibasic acids of the labdane type and oxo and hydroxy acids of the abietin and dehydroabietin series.

EXPERIMENTAL

PMR spectra (δ scale, HMDS, CCl_4) were recorded on a Varian A56/60 instrument and IR and UV spectra on UR-20 and Specord UVVIS instruments using solutions in CCl_4 and ethanol, respectively. Air-dry type L silica gel (100-160 μm) was used for chromatography with petroleum ether containing increasing (from 20 to 100%) amounts of diethyl ether as eluent. Analysis of the methyl esters was performed on a LKhM-8MD chromatograph with a flame-ionization detector under the following conditions. Higher fatty acids: steel column (3 mm × 2 m), liquid phase 10% of diethyleneglycol succinate deposited on Chromaton N-AW (silanized), with programming of the temperature of the thermostat from 180 to 205°C at the rate of 4°C per min; resin acids: steel column (3 mm × 2 m); liquid phase 5% of XE-60 on Inerton-super, with programming of the temperature from 160 to 210°C at the rate of 4°C per min; polar acids: as for resin acids but with programming of the temperature from 190 to 260°C at the rate of 6°C per min.

Isolation of the Total Acids. The woody verdure gathered at the end of February, 1986, in the Emel'yanovo Forestry Farm, Krasnoyarsk Krai, was extracted with liquid carbon dioxide on a pilot plant at a dry ice factory (Sukhumi) at the end of March. The extraction of 18.5 kg of ground woody verdure with a moisture content of 20% consisting of 65% of needles and 35% of shoots yielded 0.92 kg of extract. An average sample of the extract (56.9 g) was treated with 200 cm^3 of water and the mixture was extracted successively with various solvents [11]. The ground initial woody verdure and the treated material were extracted with petroleum ether (bp 70-100°C) in Soxhlet apparatuses for 10 h. The petroleum-ether-soluble fraction (50.0 g) was separated in the usual way into free acids and neutral substances [11]. This gave 18.5 g of free acids and 31.2 g of neutral substances.

Separation of the Combined Acids. The free acids obtained were chromatographed on a column of silica gel. The results are given in Table 2. Part of fraction 1 (1.0 g) was methylated in a mixture of methanol and sulfuric acid, and the resin acids (0.33 g) were separated from the fatty acid methyl esters (0.64 g) by the procedure of [12]. The remaining fractions were methylated with diazomethane. Fractions 4 and 6 were acetylated with acetic anhydride in pyridine at room temperature for 14 h.

Monomethyl pinifolate (IX): This was isolated and identified in the form of the dimethyl ester (I), and when fraction 3 was taken into account the yield of this component amounted to 16.57% (here and below, on the free acids); it was compared by TLC and GLC with an authentic sample of dimethyl pinifolate.

The methyl esters of 18-acetoxyanticopalic and of 15-acetoxy-4-epiimbricatic acids were identified by GLC with the addition of authentic samples of the corresponding esters. The amounts of the components were 1.09 and 0.46%, respectively.

Pinosylvin acetate monomethyl ester (0.25 g; 1.37%) gave a PMR spectrum identical with that of an authentic sample [13].

Methyl 7-ketodehydroabietate (IV): 0.35 g (1.92%), mp 64-66°C. PMR spectrum (ppm): 1.13 and 1.23 (6H, d, J = 7 Hz, isopropyl group), 1.18 (3H, s, CH_3-10), 1.23 (3H, s, CH_3-4), 3.57 (3H, s, $-COOCH_3$), 7.23 (2H, s, H-11 and H-12), 7.84, (1H, s, H-14). According to the literature [10]: mp 65-67°C.

Identification of Hydroxy Acid Methyl Esters. Fraction 6 was methylated with diazomethane. Chromatography on silica gel with, as eluent, petroleum ether (with the addition of diethyl ether in amounts increasing from 2 to 8%) separated the product into three groups of substances. The first fraction eluted from the column contained crystals (0.21 g; methyl 12-hydroxydehydroabietate), and the second (0.35 g) and third (0.26 g, methyl esters of 15-hydroxyabietic and 15-hydroxydehydroabietic acids) had a salve-like consistency. They were acetylated with acetic anhydride in pyridine. The acetates of the second fraction were identified by TLC on silica gel with the addition of silver nitrate and by GLC with authentic samples of methyl 18-acetoxyanticopalate and 15-acetoxy-4-epiimbricatate. The R_f values of the spots (TLC) were identical and the peaks on a chromatogram (GLC) of the fraction coincided with those of added authentic samples of the methyl esters of the acetoxy acids mentioned. The amount of 18-hydroxyanticopalic acid (X) in the free acids was 1.42% and that of 15-hydroxy-4-epiimbricatic acid (XI) 0.44%. The third fraction consisted of methyl 15-hydroxyabietate and methyl 15-hydroxydehydroabietate, these being identified by the GLC method with the addition of authentic samples of the methyl esters mentioned. Conditions of chromatography: LKhM-8MD chromatograph; flame-ionization detector; 3 mm × 3 m column containing 3% of SE-30 on Inerton-super, with programming of the temperature from 130 to 190°C at the rate of 4°C per min.

Methyl 12-hydroxydehydroabietate (V): crystals, 0.21 g, mp 160-162°C (acetonitrile). PMR spectrum, ppm: 1.12 (3H, s, CH_3-10); 1.21 (3H, s, CH_3-4), 1.18 (d, J = 7 Hz, 6H, isopropyl group), 3.61 (3H, s, $-COOCH_3$), 6.83 (1H, s, H-11), 6.60 (1 H, s, H-14). According to the literature: mp 161-163°C [10].

The tocopherol content was determined as in [14].

CONCLUSIONS

The group composition and the composition of the acids of the extractive substances from the woody verdure of the Scotch pine extracted by carbon dioxide have been studied. The carbon dioxide extracts contained 88% of substances soluble in petroleum ether. The composition of the acids of the carbon dioxide extracts was close to that of the acids extractable by gasoline; they consisted of higher fatty acids and resin acids and ester acids and hydroxy and oxo acids of the labdane and abietin types.

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