THE NEUTRAL SUBSTANCES OF THE OLEORESIN OF ABIES SIBIRICA

I. Oxygen-Containing Diterpene Compounds

M. A. Chirkova, A. E. Gorbunova, A. I. Lisina, and V. A. Pentegova

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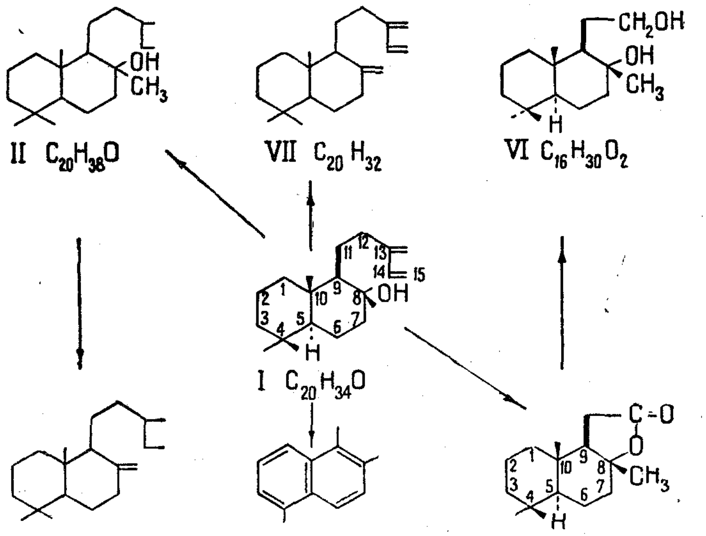
Continuing our investigation of the oleoresin of Abies sibirica Ldb. (Siberian fir) [1, 2] we have isolated from its high-boiling neutral fraction abietinal, abietinol, abienol, and previously unknown diterpene compounds: an alcohol $C_{20}H_{34}O$, a hydroxyketone $C_{18}H_{30}O_2$, and a polymer $(C_{20}H_{34}O)_4$.

When the diterpenes were chromatographed, an alcohol $C_{20}H_{34}O$ was isolated with n_D^{20} 1.5277, $[\alpha]_D^{20}$ + 29.09° C and λ_{max}^{EtOH} 234 mμ (log ε 4.3), which we have called isoabienol.

On dehydrogenation, isoabienol is converted to 1,2,5-trimethylnaphthalene. Consequently, this alcohol is a bicyclic diterpene compound [3]. The IR spectrum of isoabienol has absorption bands at 900, 990, 1280, 1655, and 3085 cm^{-1} which may be assigned to methylene $>C{=}CH_2$ and vinyl $CH{=}CH_2$ groups, and bands at 1085 and 3610 cm^{-1} which may be assigned to a tertiary hydroxyl group.

Isoabienol is readily dehydrated to a hydrocarbon $C_{20}H_{32}$; consequently, the hydroxyl group is tertiary. The amount of formaldehyde obtained when isoabienol is ozonized by Naves's method [4] corresponds to two methylene double bonds. The UV spectrum of isoabienol is characterized by an absorption maximum at 234 mμ, which indicates the presence of conjugated double bonds.

We have proposed for isoabienol the structure (I), which is shown by its chemical reactions.



The hydrogenation of isoabienol (I) gives a product (II) which is identical, from its IR spectrum, with tetrahydroabienol $C_{20}H_{38}O$. The saturated alcohol $C_{20}H_{38}O$ that we obtained is dehydrated by acetic anhydride to the hydrocarbon $C_{20}H_{36}$ (III), whose IR spectrum clearly shows the absorption bands of an exocyclic double bond (895, 1650, and 3085 cm^{-1}), as is also shown by ozonization by Naves's method. The hydrocarbon (III) is, from its n_D^{20} and IR spectrum, identical with the hydrocarbon obtained by the dehydration of tetrahydroabienol.

The ozonization of isoabienol with subsequent oxidation with potassium permanganate gave a neutral product with the composition $C_{16}H_{26}O_2$, whose constants are identical with those of the known lactone (V), [5, 6]. For confirmation, the lactone obtained was reduced to the diol $C_{16}H_{30}O_2$, the melting point and $[\alpha]_D^{20}$ of which also agreed with the corresponding characteristics of the known diol (VI) [5, 6].

Since the stereochemistry of the lactone (V) and the diol (VI) at C_8, C_9, C_5, and C_{10} is known, it may be concluded that it is also retained in isoabienol.

As can be seen from the data given, isoabienol differs from the diterpene alcohol abienol isolated previously [7, 8, 9] only by the position of one of the double bonds. Isoabienol, like abienol [8], has a high tendency to undergo polymerization.

The hydroxyketone $C_{18}H_{30}O_2$ has mp 126°C, $[\alpha]_D^{20} \pm 0°$ C, λ_{max}^{EtOH} 234 mμ (log ε 4.13) and 310 mμ (log ε 1.93). The polymer $(C_{20}H_{34}O)_4$ is converted by dehydrogenation with selenium into 1,2-trimethylnaphthalene. It is apparently a product of the polymerization of abienol and isoabienol.

Experimental

The oleoresin of Siberian fir (1600 g) was saponified with a 1% solution of caustic soda. The neutral unsaponifiables were extracted with diethyl ether. After removal of the ether, 850 g neutral unsaponifiables was obtained, and these were separated by vacuum distillation into three fractions: 1) monoterpenes, bp 25°–90° C (7 mm), 366 g (43%); 2) sesquiterpenes, bp 90°–130° C (4 mm), 263 g (30.9%); and 3) diterpenes (residue), 216 g (25.4%).

Chromatography of the Oxygen-Containing Diterpene Compounds

Fraction	Solvent	Yield, g	Main components
1	Petroleum ether	0.8	Diterpene hydrocarbons
2	Petroleum ether-benzene (1:1)	20.5	Isoabienol
3	Benzene	9.8	Abienol
4	Benzene-diethyl ether (1:1)	3.4	Abietinal Abietinol
5	Diethyl ether	5.7	Hydroxyketone $C_{18}H_{30}O_2$
6	Ethanol	6.2	Polymer

The diterpene compounds (residue) were subjected to chromatographic separation on alkaline alumina (activity grades I and II) at a ratio of 1:15. This gave 68.6 g of diterpene hydrocarbons and 116 g of oxygen-containing compounds, which we investigated.

The mixture of oxygen-containing compounds (70 g) was separated on neutral alumina (activity grade II) at a ratio of 1:40 (table).

A check on the content of material in the fractions was carried out by thin-layer chromatography on a supported layer of silica gel and on plates of alumina. The spots were revealed by means of $SbCl_5$ in chloroform.

Isoabienol $C_{20}H_{34}O$. Fraction 2 (see table) contained 20.5 g of crude isoabienol. This fraction (20.5 g) was rechromatographed on neutral alumina (activity grade II and III, ratio 1:50). The column was eluted with petroleum ether with a gradually increasing content of benzene (5, 10, 15, 25, 50%). The 50:50 mixture of petroleum ether and benzene eluted a substance (10.2 g) with n_D^{20} 1.5277 and $[\alpha]_D^{20}$ + 29.09°C; the UV spectrum of isoabienol taken on a SF-4 instrument (in ethanol) had an absorption maximum at 234 mμ (log ε 4.34) and the IR spectrum, taken on a UR-10 instrument, exhibited bands at 900, 990, 1085, 1280, 1600, 1655, 3010, 3085 and 3610 cm^{-1}.

Found, %: C 82.85; H 11.72; mol. wt. 290 (mass spectrometry on a MKh-1303 instrument). Calculated for $C_{20}H_{34}O$, %: C 82.69; H 11.80; mol. wt. 290.43.

The content of active hydrogen by Flaschentraeger's method was 0.34, 0.32%; 0.34% was calculated for one active hydrogen.

Dehydrogenation of isoabienol. A mixture of 1.5 g of isoabienol and 2.25 g of selenium was kept for 20 hr (without interruption) at 300°–310° C. The dehydrogenation product was purified by chromatography on alkaline alumina (activity grades I and II, 25 g). Petroleum ether eluted 0.65 g of a hydrocarbon with n_D^{20} 1.5804, the styphnate of which had mp 130°–131° C. The melting points of the substances were determined on a Kofler block and in sealed tubes. A mixture of the styphnate with an authentic sample of 1,2,5-trimethylnaphthalene styphnate gave no depression of the melting point (130° C). The UV spectrum of the hydrocarbon obtained had maxima at 230, 288, and 321 mμ (log ε 5.01, 3.84, and 3.04, respectively) agreeing with published data for 1,2,5-trimethylnaphthalene [10].

Hydrogenation of isoabienol. In the presence of platinic oxide (50 mg), 650 mg of isoabienol in 10 ml of absolute ethanol was hydrogenated for 8 hr. The consumption of hydrogen was 102.5 ml (0° C, 760 mm). The theoretical amount of hydrogen for two double bonds is 100.4 ml.

The yield of hydrogenated alcohol $C_{20}H_{38}O$ was 630 mg; n_D^{20} 1.5015, reaction with tetranitromethane negative, individuality of the hydrogenation product checked by thin-layer chromatography on silica gel with gypsum (KSK, 20% of gypsum).

Found, %: C 81.50; H 12.60. Calculated for $C_{20}H_{38}O$, %: C 81.61; H 12.96.

The hydrogenation of abienol under the same conditions gave tetrahydroabienol $C_{20}H_{38}O$ with the same refractive index. The IR spectra of the two hydrogenation products were identical.

Quantitative determination of methylene double bonds [4]. 2.0 mg of isoabienol $C_{20}H_{34}O$ in 3 ml of propionic acid was ozonized at −20° C for 30 sec (ozone content of oxygen current 5.88 mg/min). The amount of formaldehyde isolated was 0.35 mg (theoretical amount of formaldehyde for two methylene double bonds 0.41 mg).

Dehydration of tetrahydroabienol $C_{20}H_{38}O$ [11]. A mixture of 2.1 g of tetrahydroabienol, obtained from isoabienol, and 5 ml of a mixture of acetic anhydride and acetic acid (1:1) was heated on a boiling water bath for 4 hr. The dehydration product was extracted in the usual way and purified on a column of 10 g of silica gel (KSK, 20% H_2O, 140 μ). Petroleum ether eluted 2 g of a colorless substance with n_D^{20} 1.4980.

Found, %: C 87.08; H 13.04; mol. wt. 276 (mass spectrometry). Calculated for $C_{20}H_{36}$, %: C 86.88; H 13.12; mol. wt. 276.5.

Ozonization of the hydrocarbon $C_{20}H_{36}$ (2.5 mg) by Naves's method gave 0.27 mg of formaldehyde (theoretical amount of formaldehyde for one methylene double bond is 0.30 mg).

Dehydration of tetrahydroabienol (from abienol) gave a hydrocarbon $C_{20}H_{36}$ with n_D^{20} 1.4982; the IR spectra of the two hydrocarbons coincided.

Ozonolysis of isoabienol with subsequent oxidation by potassium permanganate [12]. At −25° C, 1.55 g of isoabienol in 30 ml of chloroform was ozonized until it was saturated with ozone (4 hr). The ozonide was decomposed by boiling with water. After the chloroform had been distilled off, the ozonolysis product was extracted by diethyl ether. With constant shaking and heating (25°-30° C) a solution of potassium permanganate (1.38 g in 20 ml of water) was added in portions over 5 min to a solution of the resulting product (1.38 g) in 15 ml of glacial acetic acid. After 48 hr, the excess of oxidizing agent and the manganese hydroxide that had separated, were decomposed with a saturated solution of sodium bisulfite and the mixture was extracted with ether.

The ethereal solution was treated with 1% aqueous caustic soda to separate the acidic and neutral products. This yielded 940 mg of a neutral product, the individuality of which was checked by thin-layer chromatography; mp 122°-123° C (from petroleum ether); $[\alpha]_D^{20}$ + 50° C. The IR spectrum had a strong band at 1795 cm^{-1}, which must be assigned to a lactone group.

Found, %: C 76.32; H 10.43; mol. wt. 250 (mass spectrometry). Calculated for $C_{16}H_{26}O_2$, %: C 76.75; H 10.47; mol. wt. 250.37.

A mixture with the lactone $C_{16}H_{26}O_2$ obtained from sclareol (mp 123° C, $[\alpha]_D^{20}$ +45° C) gave no depression of the melting point.

Reduction of the lactone $C_{16}H_{26}O_2$ [13]. A solution of 51 mg of the lactone in 5 ml of absolute ether was treated with 40 mg of lithium aluminum hydride in 3 ml of absolute ether. The mixture was kept on a water bath at 35°-36° C for 4 hr and at room temperature for 12 hr. After decomposition of the excess of lithium aluminum hydride, the organic fraction was extracted with ether, and the extract was washed with a 5% aqueous solution of caustic potash and with water. This yielded 50 mg of a product with mp 129°-130° C (after two recrystallizations from ethyl acetate). Published data for the diol (VI) $C_{16}H_{30}O_2$; mp 132° C. The IR spectrum of the diol (in KBr) had bands at 1065 and 1095 cm^{-1} belonging to primary and tertiary hydroxyl groups [6].

Found, %: C 75.34; H 11.99; mol. wt. 254 (mass spectrometry). Calculated for $C_{16}H_{30}O_2$, %: C 75.53; H 11.88; mol. wt. 254.4.

Abienol $C_{20}H_{34}O$. The main component of fraction 3 (see table) is abienol (5.3 g) with n_D^{20} 1.5330, mp 41° C, $[\alpha]_D^{20}$ +39.2° C (c 3.1; chloroform). UV spectrum 238 mμ (log ε 4.18). The constants and the UV and IR spectra of the abienol were identical with those given in the literature [7, 8, 9, 14]. The presence of a methylene double bond was confirmed by ozonization by Nave's method [4]. The abienol (2.3 mg) in propionic acid (3 ml) was ozonized at −20° C for 30 sec (ozone content of oxygen current 5.88 mg/min). The amount of formaldehyde produced (0.26 mg) corresponded to one methylene double bond (theoretical amount of formaldehyde for one methylene bond is 0.23 mg).

Abietinal $C_{20}H_{30}O$. The initial fractions eluted with a mixture of benzene and diethyl ether (1:1) (see table) contained 2.2 g of a colorless substance. After rechromatography on neutral alumina (activity grades II and III) at a ratio of 1:40, 1.3 g of abietinal was isolated with n_D^{20} 1.5280, $[\alpha]_D^{20}$ −86° C (c 3.0; chloroform); the UV spectrum (in C_2H_5OH) had a maximum at 240–241 mμ (log ε 4.01). The semicarbazone was prepared, mp 215°–216° C [15].

Found, %: N 12.34. Calculated for $C_{21}H_{33}ON_3$, %: N 12.70.

A mixture with the authentic semicarbazone of abietinal gave no depression of the melting point (215° C). The UV and IR spectra of the semicarbazones of the aldehyde from fir oleoresin and of abietinal obtained from abietic acid were identical.

Abietinol $C_{20}H_{32}O$. The last fractions eluted by benzene–diethyl ether (1:1) (see table) contained a compound (1.2 g) which crystallized after rechromatography and had a melting point of 83°–84° C. A mixture of this substance with a sample of abietinol gave no depression of the melting point (84° C). The UV spectrum had a maximum at 240–242 mμ (log ε 4.1). The IR spectrum of the alcohol isolated was identical with that of abietinol [16].

The polymer $(C_{20}H_{34}O)_4$. Ethanol (see table) eluted a fraction (6.2 g) which consisted of a bright yellow viscous mass readily soluble in diethyl ether and chloroform. The compound isolated was insoluble in ethanol and methanol; methanol precipitated it from a chloroform solution in the form of white flocs with mp 188° C, $[\alpha]_D^{20}$ +30° C (c 2.6; chloroform).

Found, %: C 82.19; H 11.65; mol. wt. 1130 (Rast). Calculated for $C_{20}H_{34}O)_4$, %: C 82.69; H 11.80; mol. wt. 1160.

The content of active hydrogen, by Flaschentraeger's method was 0.34%; 0.35% was calculated for four active hydrogen atoms.

Dehydrogenation of the polymer. The polymer (900 mg) was heated with selenium (1500 mg) at 300°–310° C for 18 hr. The dehydrogenation product was purified on alkaline alumina (activity grades I and II). An aromatic hydrocarbon was isolated with n_D^{20} 1.5820, whose UV spectrum had maxima at 230, 288, and 326 mμ (log ε 4.85, 3.97, and 2.85, respectively). A picrate with mp 141°–141.5° C and a styphnate with mp 130°–131° C were prepared. From the UV spectrum and the melting points of the picrate and styphnate the aromatic hydrocarbon was identical with 1, 2, 5-trimethylnaphthalene [10].

All the elementary analyses were performed in the analytical laboratory of NIOKh [Novosibirsk Institute of Organic Chemistry].

Summary

The high-boiling neutral fraction of the oleoresin of Abies sibirica Ldb. contains abientol $C_{20}H_{34}O$, abietinal $C_{20}H_{30}O$, abietinol $C_{20}H_{32}O$, a polymer $(C_{20}H_{34}O)_4$, and two previously unknown compounds: a diterpene alcohol $C_{20}H_{34}O$, which we have called isoabienol, and a hydroxyketone $C_{18}H_{30}O_2$. Structure (I) has been proposed for isoabienol.

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Novosibirsk Institute of Organic Chemistry, SO AS USSR