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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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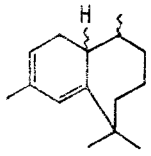
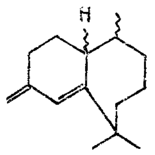
spectrum differed from that for podocephalol (VI) [2] only by the values of the chemical shifts of the signals of the protons of the aromatic ring and of the secondary methyl group. Together with the alcohol (V), products of the isomerization of the diene (III) were isolated from the reaction mixture, and among them longifolene (VII), α-longipinene (VIII), longicyclene (IX) and ar-himachalene (X) were determined in a ratio of 3:3:1:4. The formation of ar-himachalene confirmed the correctness of the proposed himachalene structure, and the isolation from the products of the isomerization of the diene (III) of (+)-longifolene and (+) α-longipinene with known absolute configurations showed the α-orientation of the H-1 atom.


III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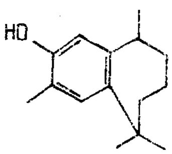
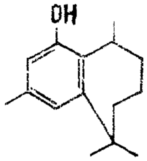
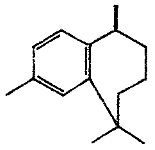
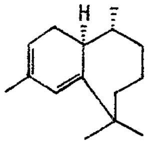
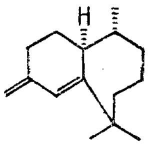

V

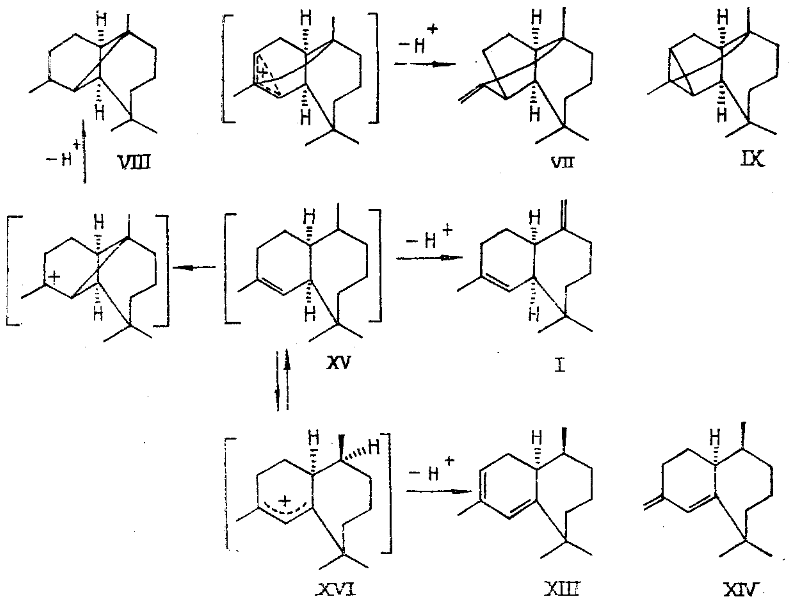

VI


X

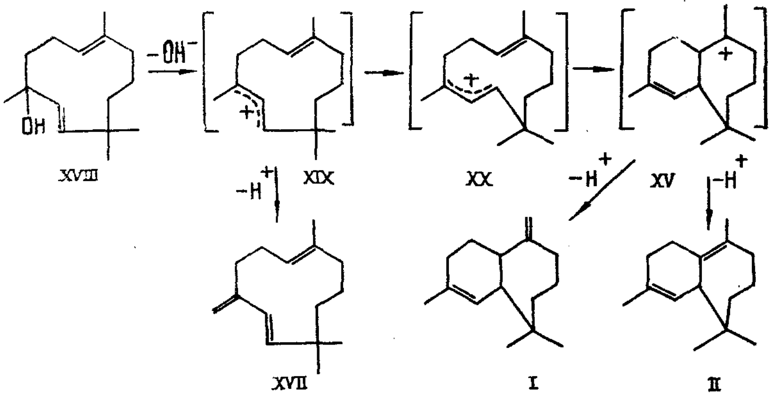

XI


XII

When the diene (IV) was heated in formic acid solution for 1 h, compound (III) was formed, which then isomerized into a mixture of compounds (VII)-(X). Earlier, compounds (XI) and (XII) had been obtained by the acid isomerization of the himachalenes (I) and (II) [3]. A comparison of the spectral characteristics of the dienes that we had isolated with the literature figures for (XI) and (XII) showed appreciable differences, which can be explained only by different orientations of the secondary methyl groups in these hydrocarbons. Thus, the compounds that we had isolated were (10S, 11S)-himachala-2,4-diene (XIII) and (10S, 11S)-himachala-3(12)-4-dienes (XIV), respectively. The formation of these dienes can be explained by the ease of a 1,3-hydride shift in the himachalene cations (XV) and (XVI). The same process apparently takes place in the isomerization of compounds (XIII) and (XIV) into hydrocarbons of the longifolane series (VII-IX).

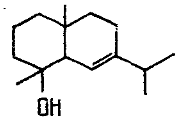


From the fraction of oxygen-containing mono- and sesquiterpenoids a crystalline sesquiterpene alcohol was isolated (mp 67-68°C; yield 4%), the PMR spectrum of which proved to be close to that of γ-humulene (XVII) [4]. In the weak-field region of the spectrum the signals of the protons of a trans-disubstituted double bond were observed (AB system; 5.22 and 5.32 ppm, doublets, J = 16 Hz) and that of a proton at a trisubstituted double bond (4.94 ppm, triplet, J = 8 Hz); in the strong-field region there were the signals of the protons of two geminal methyl groups (0.93 and 0.94 ppm), of a methyl group attached to a carbonyl carbon atom (1.25 ppm), and of a methyl group at a double bond (1.57 ppm). The main difference from the spectrum of γ-humulene was the absence of the signals of the protons of an exomethylene group and the appearance of the signal of a methyl group at 1.25 ppm. In the basis of these facts, and also the results of ^{13}C NMR spectroscopy and mass spectrometry, structure (XVIII) is suggested for the alcohol isolated.

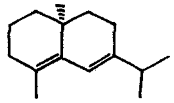


When the alcohol was dehydrated, according to the GLC two main products wre formed, in a ratio of 1:1. The PMR spectrum of the mixture obtained showed that these products were α- and β-himachalanes (I and II), while the expected γ-humulene (XVII) was formed in insignificant amounts. Apparently, the allyl cation (XIX) formed in the first stage readily isomerizes into the cation (XX), which then cyclizes with the formation of the himachalane structure (XV). The formation of α-himachalene (I) from γ-humulene (XVII) has been reported previously [4]. The results obtained indicate an interrelationship of the main biogenetic groups of sesquiterpenoids of Abies alba [1] and confirm the correctness of the proposed structure as humula-4,9-dien-8-ol (XVIII). The absolute configuration of this alcohol remains unelucidated.

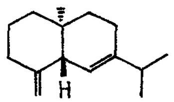
The new sesquiterpene alcohol isolated from the fraction of oxygen-containing mono- and sesquiterpenoids with a yield of 9% had mp 89-90°C, and according to the results of PMR and the color of the spot on TLC it corresponded of the selinane series. The presence of the signals of the methyl radicals of an isopropyl group in the form of a doublet at 0.99 ppm and the appearance of the signal of an isopropyl proton at 2.19 ppm (in the region of resonance of allyl protons) in the form of a sextet, together with the nature of the signal at 5.52 ppm of a proton at a trisubstituted double bond showed that the double bond was located at C-6—C-7. The presence of a signal of a methyl group at 1.09 ppm indicated that it was located at a carbinol carbon atom and, consequently, the chemical structure of the alcohol can be represented by formula (XXI). However, for such a structure the existence of right stereoisomers differing by the configurations of the C-4, C-5, and C-10 atoms is possible. Structure (XXI) for the alcohol isolated was confirmed by the fact that on the dehydration of this compound, of the two hydrocarbons formed the minor component was (−) δ-selinene (XXII). The molecule of the alcohol (XXI) must have the trans-linkage of the rings, since according to spectral characteristics, the main dehydration product (XXIII) differs from sibirene (XXIV). Furthermore, the alcohol (XXV) obtained as the main product of the oxymercuration-demercuration of sibirene likewise differed from that isolated from the oleoresin in its chromatographic and spectral characteristics. The isomerization of (+)-sibirene on activated silica gel formed (+)-δ-selinene (XXVI) with a known absolute configuration [5], which permits the conclusion that the C-10 atom in the molecule of the alcohol (XXI) has the S-configuration.



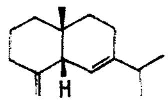
XXI



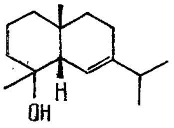
XXII



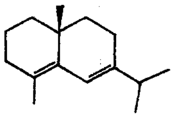
XXIII



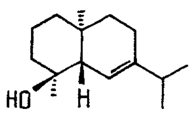
XXIV



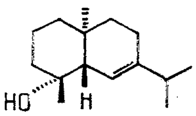
XXV



XXVI

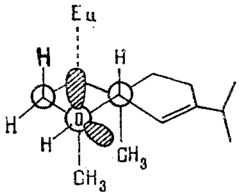


XXVII

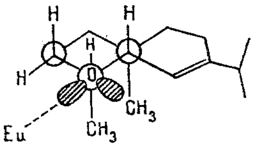


XXVIII

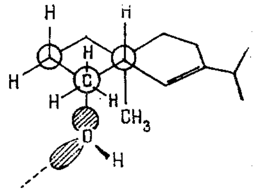
It was possible to elucidate stereochemistry of the carbinol carbon atom on the basis of PMR experiments with additions of paramagnetic shift reagents. It may be assumed that a different orientation of the hydroxy group will lead to appreciable differences in the induced chemical shifts of the protons of the methyl group at the C-10 atom and of the H-5 and H-06 atoms. We performed a calculation by the method of molecular mechanics for the two structures (XXVII) and (XXVIII), which differ by the configuration of the hydroxy group, and the refined coordinates obtained in this way were used to calculate the induced chemical shifts. The calculation was made on the assumption that the alcohol group can be present only in hindered conformations relative to the C—O bond and, therefore, there are fixed directions of the unshared pairs of electrons through which the coordination of the lanthanoid takes place, and it was borne in mind here that coordination is possible not in every direction but only in that where the formation of the complex of the substrate and the shift reagent does not encounter steric hindrances. Making use of this circumstance, the calculation was made for two variants of coordination at a β-oriented OH group (structures XXIX and XXX) and for one variant of coordination of an α-oriented OH group (structure XXXI).



XXIX



XXX



XXXI

Below, we give the values of the chemical shifts of the signals in the PMR spectrum induced by the addition of $Eu(fod)_3$ found experimentally for the alcohol (XXI) that had been isolated and those calculated for structures (XXIX-XXXI) ($\Delta\delta_i$, ppm):

Atom	XXIX	XXX	XXXI	Experiment
H-5	1.34	1,17	0.61	1,18
H-6	1.10	1.06	0.79	0.99
H-11	1,01	0.96	1.00	0.88
H-15	0.21	0.30	0.71	0.35

As we see, the calculated figures agree well with the experimental results for the coordination variant (XXX) with a β-oriented OH group. Thus, the alcohol isolated was (4S, 5S, 10S)-selina-6-en-4-ol (XXVII).

EXPERIMENTAL

The isolation of the sesquiterpenoids from the oleoresin of Abies alba, and also the instruments and methods used, have been given in [1].

(+)-Sibirene was isolated from the oleoresin of Pinus sibirica Du Tour by chromatography, $[\alpha]_D^{20}$ +118° (c 2.0).

The PMR spectrum of compounds (III), (IV), (XVIII), and (XXVII) are given in the discussion.

Calculations by the method of molecular mechanics were performed by the MM2 program [6] on a ES-1035 computer. The values of the chemical shifts induced by additions of the lanthanoid were calculated by means of the McConnell—Robertson equation [7].

(10S, 11S)-Himachala-2,4-diene (XIII). $[\alpha]_D^{20}$ +42.5° (c 3.0). Mass-spectrum (m/z, %): 204 (M^+, 68), 189(23), 161(48), 133(100), 119(87), 105(78). UV spectrum: λ_{max}^{EtOH} 256 nm (lg ε 3.68).

IR spectrum, cm^{-1}: 1660, 1600, 1465, 1385, 1379, 1202, 1110.

^{13}C NMR spectrum: quartets at 21.10, 22.30, 26.60, and 32.55 ppm; triplets at 23.04, 27.99, 39.76, and 40.91 ppm; doublets at 34.21, 37.57, 115.96, and 119.69 ppm; singlets at 38.47, 132.59, and 153.45 ppm.

Oxidation of Himachala-2,4-diene (XIII). At 0°C, 0.1 g of the diene (XIII) was oxidized with 1.5 ml of a solution of monoperphthalic acid (0.4 mmole/ml) in diethyl ether in the presence of $NaHCO_3$ for 18 h. After the reaction mixture had been worked up, 0.11 g of total products was obtained, the chromatography of which on silica gel (10 g) led to the isolation of 0.04 g of a mixture of hydrocarbons containing, according to GLC and PMR, longifolone (VII), α-longipinene (VIII), longicyclene (IX), and ar-himachalene (X) in a ratio of 3:3:1:4, and 0.04 g of the alcohol (V).

Mass spectrum (m/z, %): 218 (M^+, 26), 203(100), 161(35), 147(18).

PMR spectrum of (V): ppm: 1.29 (3H, s), 1.29 (3H, d, J=6.5 Hz), 1.38 (3H, s), 2.12 (3H, s), 3.21 (1H, m), 6.65 and 7.09 (1H each, s).

Isomerization of Himachala-2,4-diene (XIII). To 5 ml of a 15% solution of formic acid in methanol was added 0.05 g of the diene (XIII), and the mixture was heated at 60°C for 5 h. Then it was diluted with water and extracted with hexane. After the solvent had been distilled off, 0.3 g of a mixture of the hydrocarbons (VII-X), identified by GLC, was obtained. Preparative GLC led to the isolation of longifolene (VII), $[\alpha]_D^{20}$ +27° (c 3.0), α-longipinene (VIII), $[\alpha]_D^{20}$ +20° (c 3.0), and ar-himachalene (X), $[\alpha]_D^{20}$ $\sim$ 0° (c 2.0).

(10S, 11S)-Himachala-3(12), 4-diene (XIV). $[\alpha]_D^{20}$ +2.7° (c 22.0).

Mass-spectrum: 204 (M^+, 98%), 189(78), 161(100), 147(45), 133(64), 119(40), 105(63), 91(60).

IR spectrum, cm^{-1}: ν_{max}: 3080, 1632, 1601, 1460, 1385, 1370, 1110, 896, 874.

UV spectrum, λ_{max}^{EtOH}: 244 nm (lg ε 3.79).

Isomerization of Himachala-3(12), 4-diene (XIV). The diene (XIV) (0.05 g) was heated in 3 ml of a 15% solution of formic acid in methanol at 50°C for 2 h. The reaction mixture was diluted with water and extracted with hexane. After the solvent has been distilled off, 0.04 g of compound (XIII) was obtained.

Humula-4,9-diene-8-ol (XVIII). mp 67-68°C (acetonitrile).

Mass spectrum: (m/z, %): 222 (M^+, 1.5%), 204(47), 189(22), 161(28), 121(23), 119(22), 109(100).

Mass spectrum: PMR ^{13}C NMR spectrum: quartets at 18.48, 27.99, 27.99, and 28.89 ppm: triplets at 22.28, 23.78, 39.46, 40.54, and 44.27 ppm; doublets at 126.58, 134.28, 136.26 ppm; and singlets at 34.97, 72.77, and 132.92 ppm.

Dehydration of Humula-4,9-diene-8-ol (XVIII). A solution of 8 mg of the alcohol (XVIII) in 0.05 ml of pyridine was treated with 0.1 ml of a 30% solution of $POCl_3$ in pyridine, and the mixture was left at 0°C for 14 h. The excess of $POCl_3$ was decomposed with moist ether and, after the addition of 1 ml of water, the mixture was extracted with hexane. The solvent was distilled off, the resulting mixture was chromatographed on silica gel (1 g), when petroleum ether eluted 5 mg of a mixture of hydrocarbons containing, according to GLC and PMR, the himachalenes (I) and (II) (1:1), and traces of γ-humulene (XVII).

(4S, 5S, 10S)-Selina-5-en-4-ol (XXVII). mp 89-90°C (acetonitrile). $[\alpha]_D^{20}$ +15.5° (c 4.3).

IR spectrum, cm^{-1}: 3600, 1460, 1382, 1110, 1095, 920.

Mass spectrum (m/z, %): 222 (M^+, 17%), 204(92), 189(43), 161(87), 137(40), 135(42), 121(48), 105(52), 81(100).

^{13}C NMR spectrum: quartets at 17.26, 21.29, 21.48, and 21.94 ppm; triplets at 20.12, 23.08, 39.08, 40.33, and 42.84 ppm; doublets at 34.64, 54.04, and 116.80 ppm; and singlets at 33.48, 76.08, and 143.46 ppm.

Dehydration of Selina-6-en-4-ol (XXVII). A solution of 50 mg of the alcohol (XXVII) in 0.2 ml of pyridine was treated with 0.5 ml of a 30% solution of $POCl_3$ in pyridine, and the mixture was left at 0°C for 4 h. After the usual working up treatment, 48 mg of a mixture of products was isolated the chromatography of which on $SiO_2 + AgNO_3$ (2 g) yielded 9.4 mg of (−)-δ-selinene (XXII), $[\alpha]_D^{20}$ −240° (c 1.3) and 12.3 mg of selina-4(12), 6-diene (XXIII), $[\alpha]_D^{20}$ −62° (c 2.0). PMR spectrum of (XXIII) (ppm): 0.61 (3H, s), 1.00 (6H, d, J = 7 Hz), 2.50 (1H, W = 8 Hz) 4.54 (1H, W = 5 Hz), 4.73 (1H, W = 5 Hz), 5.39 m. d (1H, W = 6 Hz).

Oxymercuration-Demercuration of Sibirene (XXIV). Over 0.5 h, a solution of 61 mg of sibirene in 0.5 ml of THF was added dropwise to a solution of 95 mg of mercury(II) acetate in a mixture of 1 ml of water and 0.5 ml of THF. Then the mixture was stirred for another 1 h, after which 1 ml of a 12% aqueous solution of NaOH and 0.05 g of $NaBH_4$ were added. After vigorous stirring for 0.5 h, the mixture was diluted with 10 ml of water and was extracted with diethyl ether. The ethereal extract was evaporated and the residue was chromatographed on silica gel (1 g). Petroleum ether eluted 25 mg of the initial sibirene, and petroleum ether with the addition of 10% of diethyl ether eluted 15 mg of the alcohol (XXII); PMR spectrum: 0.98 (3H, s), 0.99 (6H, doublet J = 7 Hz, 1.15 (3H, s), 5.43 (1H, W = 11 Hz).

Isomerization of Sibirene (XXIV). Sibirene (0.5 g) was passed through activated (140°C, 4 h) silica gel. Petroleum ether eluted 0.45 g of a mixture consisting of 5 components in a ratio of 1:15:3:12:3 (GLC). The main components were isolated by GLC − (+)-δ-selinene $[\alpha]_D^{20}$ +210° (c 1.5) and 10-episelina-3,6-diene $[\alpha]_D^{20}$ −5° (c 2.0).

PMR spectrum of 1-episelina-3,6-diene, ppm: 0.86 (3H, s), 0.97 (6H, d, J = 7 Hz), 1.71 (3H, d, J = 2 Hz), 5.30 and 5.36 m. d (1 H each). ^{13}C NMR spectrum: quartets at 21.15, 21.52, 22.38, and 25.87 ppm; triplets at 22.31, 22.31, 28.68, 34.76 ppm; doublets at 35.04, 46.76, 119.63, and 120.07 ppm; singlets at 30.42, 135.35, and 140.75 ppm.

SUMMARY

1. The structures of two new sesquiterpene hydrocarbons from Abies alba have been established as (10S, 11S)-himachala-2,4-diene and (10S, 11S)-himachala-3(12), 4-diene.

2. The structure of humula-4,9-dien-8-ol has been proposed for a monocyclic alcohol on the basis of spectral characteristics and dehydration products.

3. The structure of a bicyclic alcohol has been established as (4S, 5S, 10S)-selina-6-en-4-ol by its conversion into (−)-δ-selinene. The configuration of the hydroxy group was determined by comparing the experimental values in the PMR spectra with a shift reagent with those calculated by the method of molecular mechanics.

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