and extracted with chloroform. The chloroform extracts were washed with water, dried over $Na_2SO_4$, and filtered, and the chloroform was distilled off. On standing, a solution of the residue in a mixture of diethyl ether and petroleum ether deposited a crystalline substance which, after recrystallization from the same mixture of solvents, had mp 169-171°C (decomp.). Yield 75%.

Acetylation of the Chlorohydrin. A solution of 0.03 g of badkhyzinin chlorohydrin in 0.3 ml of pyridine was treated with 0.3 ml of acetic anhydride, and the mixture was heated on the water bath (for 1 min) and was left at room temperature for 24 h. Then it was evaporated in a porcelain dish on the water bath, the residue was dissolved in diethyl ether, and the solution was filtered through a 1 cm layer (*d* 1 cm) of deactivated $Al_2O_3$. Crystals of $C_{22}H_{27}O_6Cl$ with mp 132-133°C were obtained from a mixture of ether and hexane. Yield 95%.


## SUMMARY

It has been established that in the formation of the chlorohydrin, the opening of the oxide ring of badkhyzinin takes place mainly from the side of the methylenic double bond.


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# THE STRUCTURES OF JUNIFERIN AND JUNIFERININ


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We have previously reported the isolation from the roots of *Ferul juniperina* Eug. Kor. of the new substances juniferin (I) and juniferinin (II), which are esters of the sesquiterpene alcohol juniferol with vanillic (I) and p-hydroxybenzoic and acetic (II) acids, respectively [1].

In the present paper we give a proof of the structures of these substances. Since juniferin (I) and juniferinin (II) are esters of juniferol (III), the determination of the structures of (I) and (II) amounted to the elucidation of the structure of (III) and a determination of the positions of the acyl residues in the esters.

Juniferol (III), with the composition $C_{15}H_{26}O_2$ ($M^+$ 238), gives an IR spectrum having absorption bands at 1665 $cm^{-1}$ (double bond) and 3200-3600 $cm^{-1}$ (hydroxy group), and a UV spectrum with a maximum at 211 nm (log ε 3.2), which is characteristic for a nonconjugated double bond. The presence in the PMR spectrum of (III) (Fig. 1a) of the signals from two olefinic and two vinylmethyl protons shows that juniferol contains two double bonds.

The composition, the presence of two double bonds, and the absence of carbonyl and epoxy groups, and also the nature of the signals of the methyl groups in the PMR spectrum of (III) permit the assumption that the monocylic skeleton of humulane (X) is the most probable for juniferol [2-6]. The positions of the hydroxy groups and of the double bonds were established on the basis of the following chemical transformations and spectral characteristics. Since


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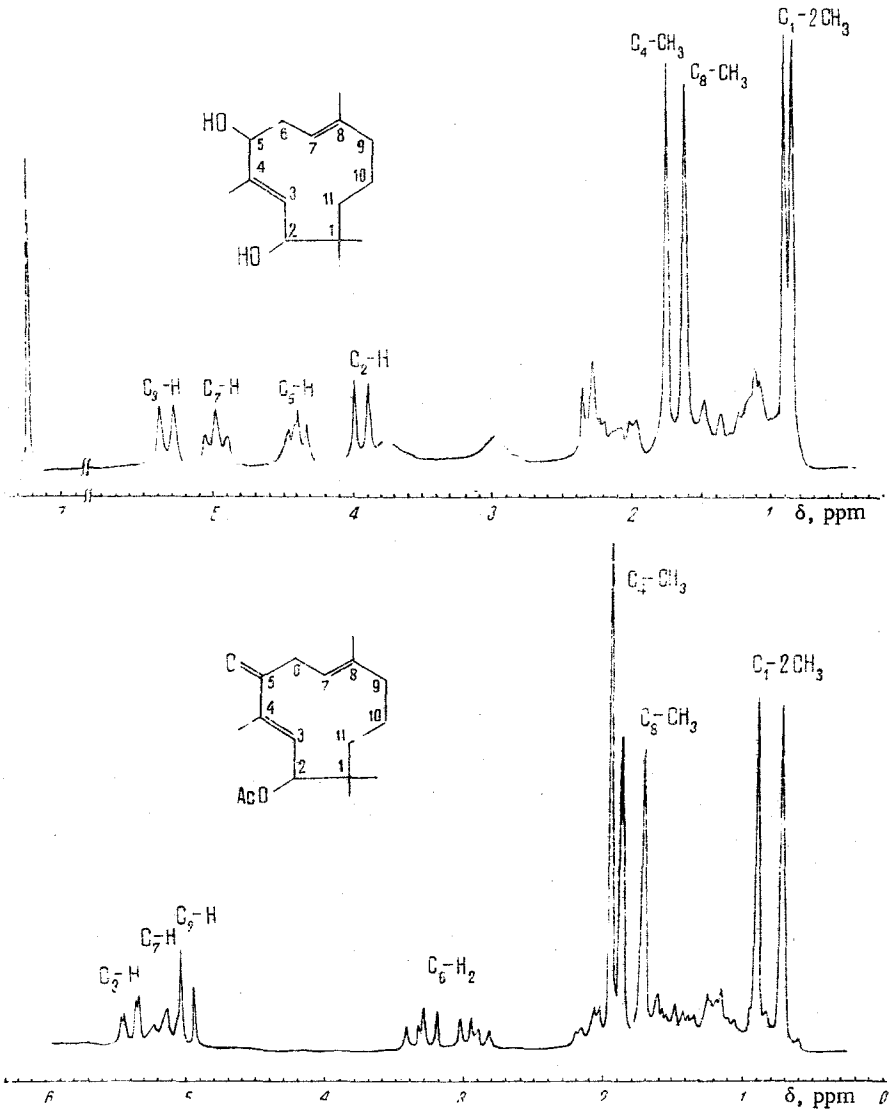



Fig. 1. PMR spectra of juniferol (a) and of the ketone of the monacetate of juniferol (b).

the PMR spectrum of (III) shows the signals from two vinylmethyl groups, the two double bonds are located at $C_4$—$C_5$ or $C_3$—$C_4$ and $C_8$—$C_9$ or $C_7$—$C_8$.

The choice between the two possible positions for the double bonds was made on the basis of the fact that in the PMR spectrum of juniferol the signals from one olefinic and one hemihydroxylic proton appear in the form of doublets with the same spin—spin coupling constant (SSCC) (Table 1), i.e., these protons form an AB system and interact only with one another, and the small widths of the signal from the olefinic protons can be ascribed to allyl splitting with the protons of the vinylmethyl group. To prove this hypothesis, we prepared the monoacetate (V) and diacetate (IV) of juniferol, juniferol epoxide (VIII), the monoacetate (VII) and the diacetate (VI) of the epoxide, the ketone of the monoacetate (IX), and the acetates of juniferin (XI) and of juniferinin (XII).

A comparison of the characteristics of the PMR spectra of (II, IV-VII, XI, and XII) (see Table 1) shows that the signals from one olefinic and one hemihydroxylic proton appear at 5.02-5.42 ppm in the form of a two-proton singlet. In the PMR spectra of (I, III, VIII, and IX), these protons appear in the form of a one-proton doublet at 3.95-5.01 and 5.35-5.52 ppm with the same SSCC. In view of the facts given, the $C_4$—$C_5$ position is excluded for one double bond and we suggest the $C_3$—$C_4$ position with the hydroxy group at $C_2$, which explains the multiplicity of the signals of these protons observed in the PMR spectra of substances (I-IX, XI).

TABLE 1. Chemical Shifts (ppm) and Spin–Spin Coupling Constants (Hz) of the Protons of Juniferol Derivatives

| Substance | $C_2$–H | $C_3$–H | $C_3$–$CH_3$ $C_4$–$CH_3$ | $C_6$–H | $C_7$–H | $C_4$–$2CH_3$ | $COOCH_3$ |
|---|---|---|---|---|---|---|---|
| *Juniferin (I) | 4,18d J=10 | 5,52d J=10 | 1,67s 6H | 5,50q $J_1$=10 $J_2$=5 | 5,05t $J_1=J_2$=7,5 | 0,88s 0,95s | — |
| *Juniferinin (II) | 5,42s | 5,42s | 1,73s 1,75s | 5,65q $J_1$=10 $J_2$=5 | 5,20t $J_1=J_2$=7,5 | 0,78s 0,93s | 2,1s |
| Juniferol (III) | 3,95d J=10 | 5,35d J=10 | 1,58s 1,71s | 4,40q $J_1$=10 $J_2$=5 | 4,96t $J_1=J_2$=7,5 | 0,81s 0,85s | — |
| Diacetate of (III) (IV) | 5,17s | 5,17s | 1,62s | 5,32q | 5,1t $J_1=J_2$=7,5 | 0,76s 0,89s | 1,98; 2,0s |
| Monoacetate of (III) (V) | 5,02s | 5,02s | 1,60s 1,70s | 4,71q $J_1$=6 $J_2$=3 | 5,04t $J_1=J_2$=8 | 0,91s 0,78s | 1,95s |
| Epoxide of juniferol (VIII) | 4,29d J=6 | 5,40d J=6 | 1,82s 1,31s | 4,54q $J_1$=6 $J_2$=3 | 2,55q $J_1$=6 $J_2$=3 | 0,88s 0,98s | — |
| Diacetate of (VIII) (VI) | 5,39s | 5,39s | 1,81s 1,43s | 5,72q $J_1$=8 $J_2$=3 | 2,60q $J_1$=8 $J_2$=3 | 0,81s 0,89s | 1,98s 2,0s |
| Monoacetate of (VIII) (VII) | 5,22s | 5,22s | 1,80s 1,34s | 4,80q $J_1$=8 $J_2$=3 | 2,61q $J_1$=8 $J_2$=3 | 0,86s 0,91s | 2.0s |
| *Acetate of (I) (XI) | 5,42s | 5,42s | 1,65s 6H | 5,85q $J_1$=6 $J_2$=3 | 5,07t $J_1=J_2$=8 | 0,78s 0,90s | 1,88s 2,12s |
| *Acetate of (II) (XII) | 5,40s | 5,32s | 1,75s 6H | 5,65q $J_1$=6 $J_2$=3 | 5,20t $J_1=J_2$=8 | 0,74s 0,85s | 1,9s 2,17s |
| Ketone of the monoacetate (VII) (IX) | 5,01d J=6 | 5,44q $J_1$=6 $J_2$=1,5 | 1,72s 1,89d J=1,5 | — | 5,18t $J_1=J_2$=7,5 | 0,71s 0,88s | 1,98s |

*Signals also appear from the protons of the residues of vanillic acid (I, XI) and p-hydroxybenzoic acid (II, XII).
s) singlet; d) doublet; t) triplet; q) quartet.

In the PMR spectrum of (III), the signals of two other protons — hemihydroxylic and olefinic — appear at 4.4 ppm (q, 1/2, Σ = 18 Hz) and 4.96 ppm (t, 1/2 Σ = 18 Hz), respectively. The identical values of the half-width of the signals give grounds for assuming that these protons interact with the protons of the same methylene group. A comparison of the PMR spectra of (I-VIII, XI, and XII) has shown that the half-width of the signals of olefinic (I-V, XI, and XII), epoxide (VI-VIII), and hemihydroxylic protons are identical.

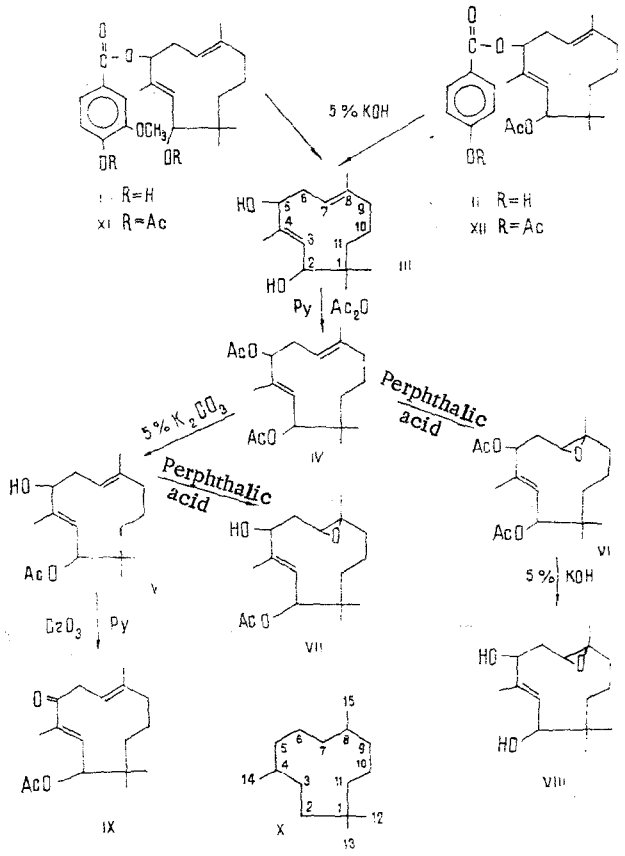
Such multiplicity of the signals in the spectra could be observed with two possible positions of the double bond and hydroxy group: double bond at $C_7$–$C_8$ and hydroxy group at $C_5$, or double bond at $C_8$–$C_9$ and hydroxy group at $C_{11}$. The choice of one of these variants was made on the basis of the spectral characteristics of the product of the oxidation of juniferol monoacetate by Sarett's method [7]. This gave the ketone of the monoacetate of juniferol (IX), $C_{17}H_{26}O_2$, the PMR spectrum of which (Fig. 1b) showed the disappearance of the signal from the hemihydroxylic proton and paramagnetic shifts of the signals of the olefinic proton (Δδ 0.42 ppm, $C_8$–H) and of the methylenic protons ($C_6$–$H_2$). The vicinal spin–spin coupling constants of $C_6$–$H_2$ and of the olefinic protons ($C_7$–H) are identical.

The UV spectrum of the ketone (IX) showed the characteristic maximum of an α,β-unsaturated carbonyl group.

On the basis of what has been said, the $C_7$–$C_8$ position for the double bond and $C_5$ for the hydroxy group have been established unambiguously. It must be observed that the acylation of the $C_2$-hydroxy group of juniferol leads to a paramagnetic shift of the signal of the proton at $C_5$ and diamagnetic shift of the signal of the $C_8$–H proton, while the acylation of

$C_5$ causes a downfield shift of the $C_3$—H and $C_2$—H signals in the PMR spectra of (I, II, IV-IX) as compared with the initial substance (III).

The positions of the acid residues in (I) and (II) were determined from the values of CS's and multiplicities of the signals of the hemiacyl protons in the PMR spectra of these substances and their acetyl derivatives (see Table 1). In the spectra of (I) and (XI), the signal from $C_5$—H appears at 5.50 and 5.85 ppm. This shows that the vanillic acid residue is located at $C_5$. In the PMR spectrum of (II), as in that of (I), the signal from $C_5$—H is found at 5.65 ppm. Judging from these facts, the p-hydroxybenzoic acid residue in (II) is located at $C_5$ and the acetic acid residue at $C_2$. The transformations of the esters (I and II) and of juniferol (III) that have been described can be represented in the following form:




It must be mentioned that this is the first time that humulane derivatives have been isolated from plants of the genus *Ferula*.

## EXPERIMENTAL

The UV spectra were taken on a Hitachi instrument in ethanolic solutions, the IR spectra on a UR-20 spectrophotometer (tablets with KBr), the PMR spectra on a JNM-4H-100 MHz instrument, 0 — HMDS, and the mass spectra on an MKh-1303 mass spectrometer fitted with a glass system for the introduction of the substance into the ion source. The $R_f$ values are given in the hexane—ethyl acetate (3:1) system on Silufol R plates, the spots being revealed with a 1% solution of vanillin in concentrated sulfuric acid.

Isolation and Separation of the Esters. Isolation of Juniferinin. The dried and comminuted roots (9 kg) were extracted with methanol (3 × 54 liters). The extract was concentrated, diluted with water (1:2), and extracted five times with diethyl ether. The ethereal extract was washed successively with 5% sodium carbonate solution and with 0.5% and 1% solutions of caustic potash. The last extracts were acidified and the phenolic compounds were extracted with ether. After the elimination of the solvent, 50 g of combined substances was obtained which was deposited on a column (7 × 120 cm) containing KSK silica gel and was eluted with chloroform, 50-ml fractions being collected. After the elimination of the eluent, frac-

tions V-XV yielded 12 g of a mixture of two substances with $R_f$ 0.35 and 0.5 (fraction A). Fractions XX-XXV yielded crystals of juniferinin (5 g) with the composition $C_{24}H_{32}O_5$, mp 164-165°C (from hexane), $[\alpha]_D^{20}$ + 33.4° (c 1.8; ethanol), $R_f$ 0.21. UV spectrum, $\lambda_{max}$: 211 nm (log ε 4.0), 260 nm (log ε 4.3). IR spectrum, $\nu_{max}$; 1520, 1615, 1695, 1690, 1710, 3200-3400 $cm^{-1}$.

Isolation of Juferin and Juniferin. Fraction A (12 g) was rechromatographed on a column (5 × 120 cm) of silica gel with elution by hexane–ethyl acetate (25:1) and the collection of 50-ml fractions. Fractions V-VIII yielded crystals of juferin (2 g) with the composition $C_{22}H_{30}O_4$, mp 90-91°C (from a mixture of hexane and ethyl acetate), $[\alpha]_D^{20}$ + 120.4° (c 0.77; ethanol), $R_f$ 0.5. UV spectrum, $\lambda_{max}$: 213.5 nm (log ε 4.3), 255.5 nm (log ε 4.5). IR spectrum, $\nu_{max}$: 1520, 1595, 1615, 1690, 3200-3600 $cm^{-1}$.

The subsequent fractions X-XVI yielded crystals of juniferin, $C_{23}H_{32}O_5$, (6 g) with mp 85-86°C (from a 3:1 mixture of hexane and ethyl acetate), $[\alpha]_D^{20}$ − 1.6 (c 5.8; ethanol), $R_f$ 0.35. UV spectrum, $\lambda_{max}$: 211 nm (log ε 4.0), 260 nm (log ε 4.3). IR spectrum, $\nu_{max}$: 1520, 1600, 1620, 1690, 3200-3600 $cm^{-1}$.

Hydrolysis of Juniferin. A solution of 0.5 g of (I) in 30 ml of 5% aqueous caustic potash was heated on the water bath for 1 h. Then the reaction mixture was diluted with water and extracted with ether. The ethereal extract was washed with water and dried over anhydrous sodium sulfate, and the solvent was distilled off. This gave 0.4 g of a crystalline substance with the composition $C_{15}H_{26}O_2$ ($M^+$ 238), mp 135-136°C (from ether) $[\alpha]_D^{20}$ − 85.4° (c 2; ethanol). The mother liquor was acidified with 5% sulfuric acid and treated with ether. Elimination of the solvent gave an acid with the composition $C_8H_8O_4$, mp 205-206°C, identical with vanillic acid [8].

Hydrolysis of Juniferinin. Substance (II) (0.3 g) was saponified by the method described above. This gave juniferol (III) and p-hydroxybenzoic acid $C_7H_6O_3$ (mp 210-212°C), and acetic acid was identified chromatographically ($R_f$ 0.13; system consisting of butanol saturated with 1.5 N ammonia).

Acetylation of Juniferol. A solution of 1 g of juniferol in 6 ml of anhydrous pyridine was treated with 4 ml of acetic anhydride. The mixture was heated on the water bath for 1 h. The acetyl derivative was isolated in the usual way, $C_{19}H_{30}O_4$ (IV), ($M^+$ 322) $R_f$ 0.85; $[\alpha]_D^{20}$ −70.3° (c 2.6; methanol). IR spectrum, $\nu_{max}$ 1740 $cm^{-1}$.

Preparation of the Monoacetate of Juniferol (V). A solution of 0.8 g of (IV) in 20 ml of methanol was made alkaline by the addition of a 5% aqueous solution of potassium carbonate and was then left at room temperature for four days. TLC showed the presence in the reaction mixture of two substances with $R_f$ 0.85 and 0.54. The mixture was diluted with water and the reaction products were extracted with ether. The residue after the elimination of the solvent (0.75 g) was transferred to a column (2 × 100 cm) of silica gel impregnated with 5% silver nitrate, and elution was carried out with chloroform. Fractions X-XII gave the monoacetate of juniferol (0.5 g), composition $C_{17}H_{28}O_3$ (V), $R_f$ 0.54, $[\alpha]_D^{25}$ −29.25° (c 1.14; methanol). IR spectrum, $\nu_{max}$: 1725, 3200-3600 $cm^{-1}$.

Epoxidation of Juniferol Diacetate. An ethereal solution of perphthalic was added to a solution of 0.5 g of (IV) in ether, and the mixture was left at room temperature for five days. Then it was diluted with water and extracted with ether, and the ethereal extract was washed with 5% sodium carbonate solution and with water. The solvent was distilled off and the residue was chromatographed on a column (1 × 20 cm) of silica gel, with elution by a mixture of hexane and ethyl acetate (6:1). This gave compound (VI) with the composition $C_{19}H_{30}O_5$ ($M^+$ 338), mp 87-88°C (from hexane), $R_f$ 0.81.

Epoxidation of Juniferol Monoacetate. By the method described above, compound (V) yielded an epoxide with the composition $C_{17}H_{28}O_4$ ($M^+$ 296) (VII), mp 51-53°C, $R_f$ 0.53.

Juniferol Epoxide (VIII). Juniferol epoxide diacetate (0.25 g) was hydrolyzed with a 5% aqueous methanolic solution of caustic potash. The neutral fraction of the hydrolyzate yielded juniferol epoxide with the composition $C_{15}H_{26}O_3$, mp 141-142°C, $R_f$ 0.27.

Oxidation of Juniferol Monoacetate. A solution of 0.6 g of chromium trioxide in 8 ml of

dry pyridine, and the mixture was stirred at 30-35°C for 24 h. Then it was poured into ice water and the mixture was diluted with 5% sulfuric acid and was extracted with petroleum ether—diethyl ether (1:1). The extract was washed with water and the solvent was distilled off, and 0.4 g of the oxidation product was transferred to a column (1 × 20 cm) of silica gel impregnated with 5% silver nitrate solution and was eluted with hexane—ethyl acetate (5:1). This gave substance (IX) with the composition $C_{17}H_{26}O_3$, $[\alpha]_D^{25}$ − 239.6° (c 2; methanol), $R_f$ 0.16 [hexane—ethyl acetate (4:1) system]. UV spectrum, $\lambda_{max}$: 225 nm (log ε 3.0), 205 nm (log ε 3.2). IR spectrum, $\nu_{max}$: 1700 $cm^{-1}$.

Acetylation of Juniferin. Compound (I) (0.1 g) was acetylated with acetic anhydride in pyridine, giving 0.1 g of (XI) with the composition $C_{27}H_{36}O_7$, $[\alpha]_D^{25}$ − 35.6° (c 2.5; methanol), $R_f$ 0.42.

Acetylation of Juniferinin. Compound (II) (0.1 g) was acetylated as described above, giving 0.1 g of (XII) with the composition $C_{26}H_{34}O_6$, $[\alpha]_D^{25}$ + 18.9° (c 3.3; methanol), $R_f$ 0.54. IR spectrum, $\nu_{max}$: 1700 $cm^{-1}$ (inflection), 1725 $cm^{-1}$.

## SUMMARY

Two new esters of the new sesquiterpene alcohol juniferol — juniferin and juniferinin — have been isolated from the roots of *Ferula juniperina*. On the basis of the products of chemical transformations and spectral characteristics the structure of 2,5-dihydroxy-1,1,4,8-tetramethylcycloundeca-3,7-diene has been proposed for juniferol.

It has been shown that juniferin is 5-O-vanilloyljuniferol, and juniferinin is 2-O-acetyl-5-O-p-hydroxybenoyljuniferol.

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