MASS-SPECTROMETRIC INVESTIGATION OF THE ABIENOLS – DITERPENE ALCOHOLS

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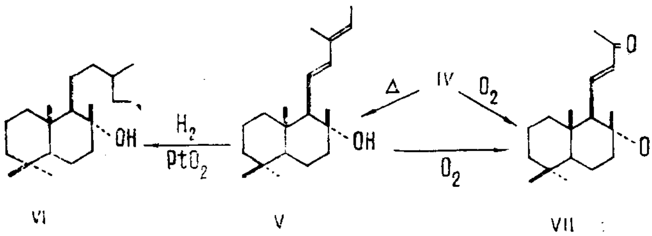
As is well known, a series of bicyclic diterpenoids of the labdane group with a conjugated system of double bonds in the side chain, such as the biformenes [1] and communic acids [2, 3], is present in some plants which produce them in the form of mixtures of three isomers; i.e., the dehydration of their biogenetic precursors (manool or its allyl isomer, or their oxidized forms) in vivo takes place in these plants nonselectively (the isomers with $\Delta^{11,13}$ ethylenic bonds are artefacts formed in the process of isolation [2, 4]). However, since there is still little factual information, it is unknown whether the dehydration of these precursors in vivo always gives mixtures of all three possible dienic products and, if they are obtained, in what ratio. Consequently, the study of the isomeric composition of the labdane diterpenoids with conjugated double bonds in the side chains isolated from plant materials is of biogenetic and, possibly, taxonomic interest.

Compounds of this type, widely distributed in the fir (Abies) genus the isomeric composition of which has not been studied, are the abienols [5]. Four abienols have been described in the literature: Δ^{12}-cis- (I) [6, 7], Δ^{12}-trans- (II) [8-10], iso- (III) [10, 11], and Δ^{13}-cis-neo- (IV) [4, 8] -abienols. We have also synthesized Δ^{13}-trans-neoabienol (V). These compounds are characterized by a high lability and similar properties, in consequence of which their isolation from natural sources, separation, and identification are associated with great difficulties.

In order to find a convenient and rapid method of identifying the abienols (I)-(V) we undertook a comparative study of their mass spectra.

The previously undescribed Δ^{13}-trans-neoabienol (V) was synthesized by the isomerization of cis-neoabienol (IV) with dimethyl sulfoxide on heating. The structure and stereochemistry of the substance (apart from the configuration of the Δ^{13} double bond) follow from the similarity of its IR, mass, and NMR spectra to the corresponding spectra of Δ^{13}-cis-neoabienol (IV) and from chemical transformations. The hydrogenation of compound (V) gave tetrahydroabienol (VI), and its autooxidation with air led to the known α, β-unsaturated hydroxy ketone (VII), which is also formed by the autooxidation of Δ^{13}-cis-neoabienol (IV) [12]. The stereochemistry of compounds (IV) and (V) with respect to the Δ^{13} double bond follows from their NMR spectra. Cardenas [13] has shown that the resonance signal of the proton at C_{13} in 1-alkyl- and 1,2-dialkylbutadiene derivatives shifts downfield by 20-40 Hz on passing from the trans to the cis isomers. In the diterpenoids (IV) and (V), the protons at C_{12} resonate in the form of doublets with



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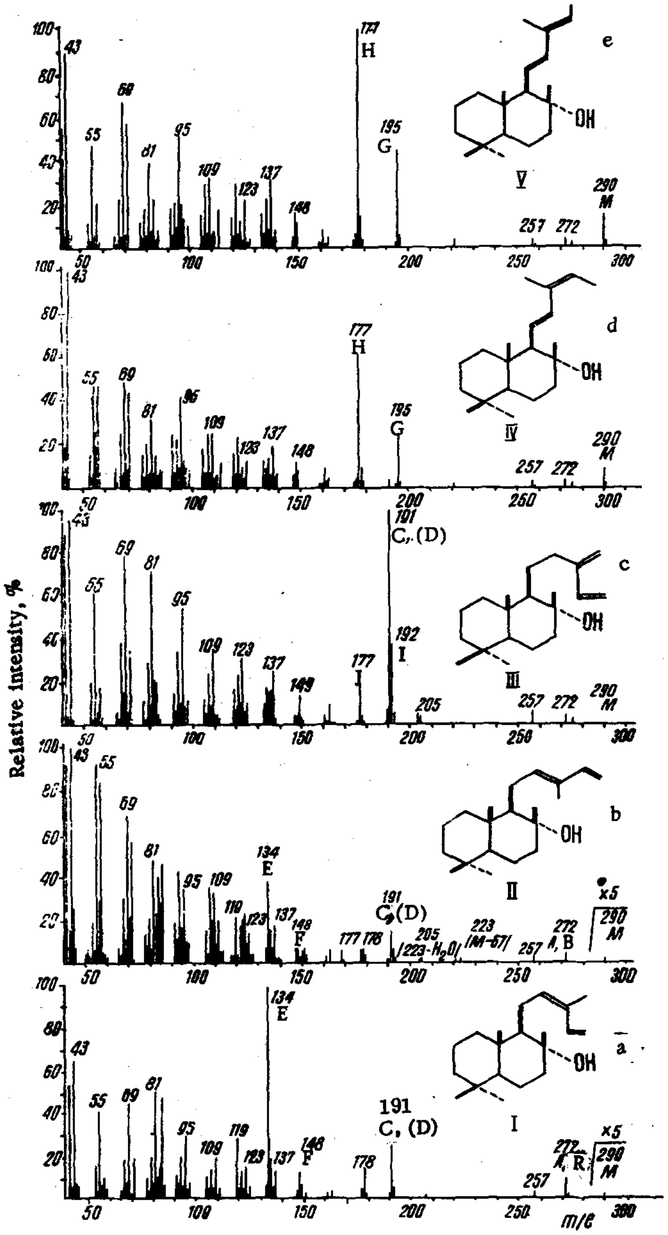


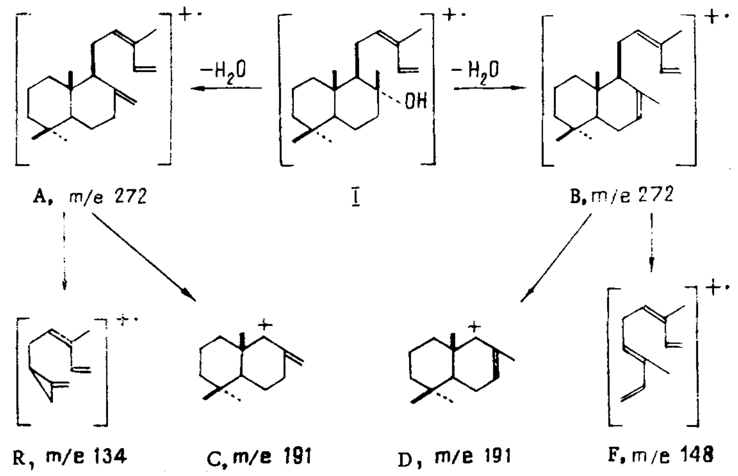
Fig. 1. Mass spectra of cis-abienol (I) (a), trans-abienol (II) (b), iso-abienol (III) (c), Δ^{13}-cis-neoabienol (IV) (d), and Δ^{13}-trans-neoabienol (V) (e).

C_{12} resonate in the form of doublets with centers at 6.40 and 6.02 ppm, respectively, i.e., neabienol (IV) has the cis configuration and its isomer (V), the trans configuration, with respect to the Δ^{13} double bond.

We did not set ourselves the task of a detailed investigation of the decomposition of the abienols under the action of electron impact, since the mass-spectrometric fragmentation of labdane diterpenoids has been discussed fairly fully by Enzell and Ryhage [14], but limited ourselves to a comparative study of the mass spectra of the abienols (I)-(V). Here we give only the possible fragmentation pathways leading to the formation of the characteristic fragmentary ions that enable these compounds to be distinguished from one another.

As can be seen from Fig. 1, in the mass spectra of the structurally isomeric abienols there are characteristic peaks by means of which they can be identified unambiguously. Thus, in the spectra of cis- and trans-abienols (I) and (II) the intense peak with m/e 134, in the spectra of isoabienol (III) the intense peak with m/e 191, and in the spectra of cis- and trans-neoabienols (IV) and (V) the two intense peaks with m/e 195 and 177 can be regarded as characteristic. So far as concerns the spectra of the steroisomeric cis- and trans-abienols (I) and (II), and also the cis- and trans-neoabienols (IV) and (V), they differ only by the intensity of a number of peaks, and this difference is retained with a decrease in the ionizing voltage.

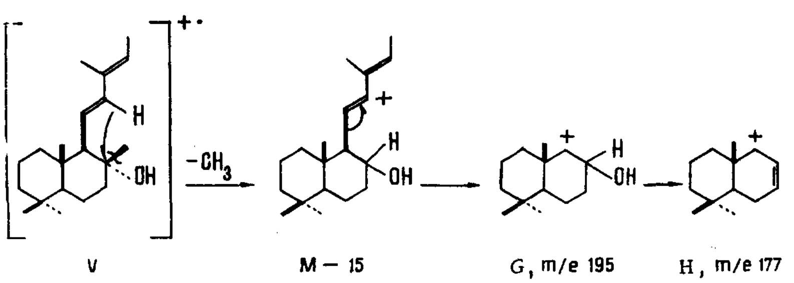
SCHEME 1



The results of a comparison of the mass spectra of the abienols (I)-(V) has shown that the structure of the side chain affects the stability of the molecular ion. In the mass spectra of the cis- and trans-abienols (I) and (II), as in other labdane compounds with an equatorial hydroxy group at C_8, the peaks of the molecular ions have a low intensity, and the fragmentation process may be considered to start from the dehydrated ions A and B [14] (Scheme 1). In the region of high mass numbers, the mass spectra of these abienols are practically identical: decomposition takes place mainly with the successive ejection of a water molecule and the side chain and leads to the fragmentary ions C and D (m/e 191). Their subsequent fragmentation gives ions with m/e 137, 123, 109, etc., that are characteristic for labdane interpenoids [14]. However, the mass spectra of the cis- and trans-abienols (I) and (II) differ with respect to the intensities of the peaks of the ions with m/e 272 (A and B), 191 (C and D), 148 (F), and 134 (E). The ion E is formed from the ion A with a semicyclic double bond as the result of the cleavage of the C_6-C_7 and C_9-C_{10} bonds and the localization of the charge on the aliphatic fragment, and the ion F is formed from the ion B with a trisubstituted double bond which decomposes mainly in the manner of a retro-Diels-Alder reaction [14] (see Scheme 1).

The mass spectra of the cis- and trans-neoabienols (IV) and (V) differ in the intensities of the peaks corresponding to the ions G with m/e 195 and H with m/e 177, the connection between which is confirmed by the presence of a metastable ion with m/e 160.5. In the spectrum of trans-neoabienol (V), the peak with m/e 177 is the maximum peak, and in the spectrum of cis-neoabienol (IV) it amounts to ~ 60% of the maximum peak (m/e 43). The peak with m/e 195 is also approximately twice as strong in the spectra of trans-neoabienol (V). In contrast to the other abienols, in the spectrum of each of the two neoabienols there is a peak with m/e 195 and a considerably less intense peak of the molecular ion with m/e 290. Thus, the presence of a Δ^{11} double bond stabilizes the molecular ion and is responsible for the fragmentation pathway shown in Scheme 2.

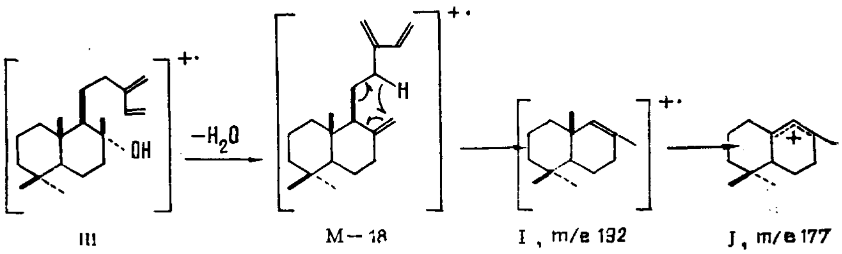
SCHEME 2



The considerably greater intensity of the peak with m/e 195 in the spectrum of trans-neoabienol (V) as compared with that of cis-neoabienol (IV) is apparently due to the greater ease of migration of a hydrogen atom from the side chain (from C_{12}) into the bicyclic part of the molecule (to C_8) in trans-neoabienol (V).

In the mass spectrum of isoabienol (III), the intensity of the molecular ion amounts to ~ 1.5%, i.e., it is greater than in cis- and trans-abienols (I) and (II) but smaller than in cis- and trans-neoabienols (IV) and (V). The maximum peak in its spectrum is that with m/e 191, which is also present (but is not the maximum peak) in the spectrum of the cis- and trans-abienols (I) and (II) and corresponds to the fragmentary ions C and D (see Scheme 1). In the mass spectrum of isoabienol there are also intense peaks of the ions I with m/e 192 and J with m/e 177 formed as the result of the McLafferty rearrangement of the dehydrated ion M−18 and the subsequent splitting out of a methyl group (Scheme 3). However, an alternative scheme of fragmentation may also be postulated if the splitting out of water takes place with the capture of a hydrogen atom from the side chain.

SCHEME 3



The different features in the mass spectra of the abienols (I)-(V) mentioned above enable them to be identified and give grounds for assuming that a combination of GLC and mass spectrometry may serve as a rapid and reliable method for their determination in plant material.

EXPERIMENTAL

The mass spectra were obtained on an MKh-1303 instrument fitted with a glass system for the direct introduction of the sample into the ion source at a temperature of the evaporator of 60-125°C and of the ionization chamber of 150°C, and an ionizing voltage of 70 V (for stereoisomeric compounds, also 50 and 30 V). The NMR spectra were recorded on a Varian 60 instrument in carbon tetrachloride (with hexamethyldisiloxane as internal standard). The hydrate of cis-abienol (I) and cis-neoabienol (IV) were isolated from dry Canada balsam by chromatographing the neutral fraction on a column of silica gel impregnated with silver nitrate, and the trans- and iso-abienols (II) and (III) were obtained by the elimination of acetic acid from the 13-monoacetate of sclareol by heating it with dimethyl sulfoxide [15]. The constants of the abienols (I)-(IV) corresponded to those given in the literature.

Preparation of Δ^{13}-trans-Neoabienol (V). A solution of 1 g of Δ^{13}-cis-neoabienol (IV) in 4 g of dimethyl sulfoxide was heated in a sealed tube at 124-126°C for 2 h. The solution was cooled, diluted with ether, washed with water, and dried with sodium sulfate, and the ether was evaporated off under reduced pressure in a current of nitrogen without heating. The residue (0.8 g) was chromatographed on a column containing 24 g of silica gel (impregnated with silver nitrate) which was washed with a mixture of benzene and light petroleum ether (78 : 22). This gave 90.4 mg of (IV), 196 mg of a mixture of the neoabienols (IV) and (V) and 88.8 mg of Δ^{13}-trans-neoabienol (V), mp 87-88°C (from acetonitrile), $[\alpha]_D^{24}$ + 10.7° (c 5; $CHCl_3$); $\lambda_{max}^{ethanol}$

236.5 nm (log ε 4.37), mol. wt. 290 (mass spectrometry). The results of the elementary analysis of this compound corresponded to the calculated figures (empirical formula $C_{20}H_{34}O$). IR spectrum (in CCl_4), cm^{-1}: 970 (trans-disubstituted double bond), 830, 1680 (trisubstituted double bond), 1925, 1645 (conjugated double bonds), 1130, 3590 (OH). NMR spectrum (δ, ppm); singlets at 0.75, 0.82, 0.84 (C_4 and $C_{10}-CH_3$), 1.68 ($C_{13}-CH_3$), and 2.10 (proton of an OH group), doublet with its center at 1.63 ($C_{14}-CH_3$), J = 7 Hz), one-proton quartet with its center at 5.34 ($C_{11}-H$, J_{H_9}, H_{11} = 10 Hz; $J_{H_{11}}$, H_{12} = 15 Hz) upon which is superposed the resonance signal of the proton at C_{14} with its center at 5.41, and a one-proton doublet with its center at 6.02 ($C_{12}-H$, J = 15 Hz).

Hydrogenation of Δ^{13}-trans-Neoabienol (V). Compound (V) (5 mg) was hydrogenated to complete saturation over the Pt from 5 mg of PtO_2 in 2 ml of methanol. According to TLC, the product was identical with a sample of tetrahydroabienol (VI).

Oxidation of Δ^{13}-trans-Neoabienol (V) with Air. A solution of 25 mg of Δ^{13}-trans-neoabienol (V) in 0.5 ml of acetonitrile was kept in the refrigerator for one month and at 20-23°C for three weeks. The product which consisted of the initial compound and a more polar substance was separated by preparative TLC on silica gel [solvent benzene–hexane (3:1)]. This yielded 6.8 mg of the initial Δ^{13}-trans-neoabienol and 5.1 mg of the hydroxy ketone (VII) with mp 120-122°C (from ether). This material gave no depression of the melting point in admixture with an authentic sample of the hydroxy ketone (VII) having mp 121-123°C. Their IR spectra taken in KBr tablets and their chromatographic properties were identical.

CONCLUSIONS

1. The synthesis of the previously undescribed Δ^{13}-trans-neoabienol has been performed.

2. A comparative mass-spectrometric investigation of five isomeric and stereoisomeric abienols has been performed.

3. It has been shown that all five abienols can be identified from their mass spectra.

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