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STRUCTURE AND STEREOCHEMISTRY OF LAPIFERIN*

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Lapiferin, $C_{22}H_{34}O_6$, mp. 137-138°C, $[\alpha]_D^{21}$ +63 (c 1.3; chloroform), a diester of the new carotane alcohol lapiferol with angelic and acetic acids, has been isolated from the roots of Ferula lapidosa Eug. Korov. On the basis of chemical transformations and spectral characteristics, the structure of 10α-acetoxy-6α-angeloyloxy-8α,9α-epoxy-trans-carotan-4β-ol has been proposed for it.

Continuing the separation of the neutral components of the roots of Ferula lapidosa Eur. Korov. [1], we have isolated a new compound with the composition $C_{22}H_{34}O_6$ (mass-spectrometrically), which we have called lapiferin (I).

The IR spectrum of lapiferin is typical for sesquiterpene esters, containing strong absorption bands of ester carbonyl groups of saturated (1740 cm^{-1}) and unsaturated (1715 cm^{-1}) acids and bands of a trisubstituted double bond (1655, 970 cm^{-1}) [2], of a hydroxy group (3540 cm^{-1}), and of ester–C–O bonds (1230-1260 cm^{-1}) [1].

A maximum in the UV spectrum with λ_{max} 218 nm (log ε 3.96) confirms the presence of an α,β-unsaturated ester carbonyl in the molecule of (I) [2].

The results of the fragmentation of lapiferin under the action of electron impact show that it belongs to the group of esters of sesquiterpene alcohols. In the mass spectrum there are the peaks of ions corresponding to a fragment of the molecule without an isopropyl group having m/z 351 $(M - C_3H_7)^+$, the residue of a sesquiterpene alcohol with m/z 234, and also peaks due to the ejection of residues of angelic and acetic acids with m/z 294 $(M - C_5H_8O_2)^+$ and 251 $(M - C_5H_8O_2 - CH_3CO)^+$ and of a molecule of water with m/z $(M - C_5H_8O_2 - 17)^+$.

In the PMR spectrum of (I) ($CDCl_3$) in the strong-field region the signals of the methyl groups of an isopropyl residue appear in the form of a doublet (6 H) at 0.85 ppm with ^3J = 7 Hz, of an angular methyl group in the form of a singlet at 1.26 ppm (3 H), and of a methyl attached to a carbon atom bearing oxygen at 1.42 ppm (s, 3 H). In addition, there are the signals of an epoxide proton at 2.86 ppm (d, ^3J = 5.5 Hz) [1], of two gem-acyl protons at 4.83 ppm (d, ^3J = 5.5 Hz) and 5.18 ppm (sx, ^3J = 10.0; 10.0; 3.0 Hz), and of acetic and angelic acid residues.

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A comparison of the spectral characteristics with those given in the literature [1-5] led to the conclusion that lapiferin was an ester of the carotane series, as was confirmed by the production of daucalene when (I) was dehydrogenated in the presence of palladium.

The alkaline hydrolysis of lapiferin yielded angelic acid and the sesquiterpene alcohol lapiferol (II) with the composition $C_{15}H_{26}O_4$.

In the PMR spectrum of lapiferol (pyridine-d_5), three-proton singlets at 1.2 and 1.26 ppm and doublets at 0.93 and 1.01 ppm with ^3J = 7 Hz relate to methyls located at an angular carbon atom, at an epoxide ring, and in an isopropyl group, respectively. Also standing out clearly are a one-proton doublet at 4.0 ppm with ^3J = 5.4 Hz and a sextet at 4.25 ppm with ^3J = 9.9, 9.9, and 3.7 Hz, corresponding to protons located geminally to secondary hydroxy groups. Characteristic are doublets at 2.71 ppm (^3J = 9.9 Hz) and 2.80 ppm (^3J = 5.4 Hz) showing a closeness of the values of the chemical shifts but differing by the magnitude of the constants of vicinal interaction with the neighboring protons.

These facts, together with the elementary composition, permit the conclusion that lapiferol is a bicyclic alcohol of the carotane series containing an epoxide ring and two secondary and one tertiary groups.

The positions of the functional groups in the molecules of lapiferol and, consequently, its diester with acetic and angelic acids, lapiferin, were determined in the following way.

The PMR spectra of (II) taken under double-resonance conditions showed that when an additional radio-frequency field with ν = 425 Hz was imposed, the doublet at 2.71 ppm was converted into a singlet. In addition, changes were observed among multiplets located in the region of the resonance of methylene protons. These changes taking place in the spectrum, and also the sextet nature of the signal at 4.25 ppm showed that one secondary hydroxy group must be in the equatorial position at C_6 of the carotane skeleton of the alcohol (II), since in these circumstances only H-5 (2.71 ppm) can exhibit doublet splitting with a constant ^3J = 9.9 Hz, which is characteristic of trans-diaxial interaction with H-6.

The sextet nature of the splitting of the signal at 4.25 ppm unambiguously shows that H-6 undergoes vicinal interaction not only with H-5 but also with the two protons of a neighboring methylene group; consequently, the C_7 position is free from substituents, as in the case of the derivatives of lapidol [1], of ferutinol [3, 4], and of laserol [5] studied previously [3].

In the PMR spectrum of (II) obtained with the superposition of an additional radio frequency field with ν = 400 Hz, equal to the resonance frequency of the protons at the second hydroxy group, the doublet at 2.8 ppm was converted into a singlet, which is characteristic for an epoxide proton. The existence of a spin–spin interrelationship between these two protons gives grounds for concluding that the other secondary hydroxy group and the epoxy function in the molecule of (II) are adjacent to one another. In principle, considering the absence of substituents at C_7, the C_3-C_4 and C_8-C_9 positions are equiprobable for the formation of the epoxide ring. However, as follows from an analysis of the PMR spectra of known compounds [6], an epoxide proton present in a five-membered ring appears in the form of a broadened singlet, and not a distinct doublet, as in the spectra of (I) and (II). This fact gave us grounds for considering that the epoxide ring in the molecule of (II) was formed at C_8-C_9 and, consequently, the second hydroxy group was located at C_{10}.

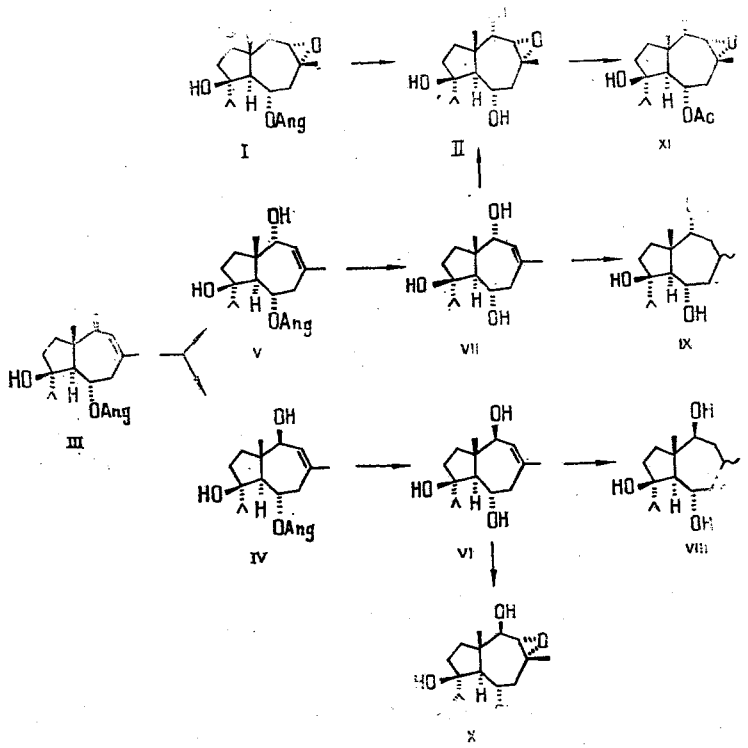
In the final account, the only possible position remaining for the tertiary hydroxy group is C_4, as is also confirmed by the presence in the mass spectra of (I) and (II) of a strong peak with m/z $(M-43)^+$ [3, 4].

Thus, for the molecule of the bicyclic alcohol lapiferol the structure (II) has been established in which the secondary hydroxy groups are present at C_6 and C_{10}, the tertiary hydroxy group at C_4, and the epoxide ring involves the C_8 and C_9 carbon atoms.

Structural formula (II) for lapiferol is identical with that of the epoxydihydrolapidol (X), obtained previously from lapidin in the determination of the structure of the latter [1]. The spectral characteristics of these two compounds are close. However, their physicochemical characteristics differ substantially. In all probability they are stereoisomers. For a chemical confirmation of the structure of lapiferol and to determine the configuration of its asymmetric centers, we effected a passage to it from lapidin (III) which has a known absolute configuration (see Scheme on following page).

When lapidin (III) was reduced with an excess of sodium tetrahydroborate and the reaction products were separated, two isomeric dihydrolapidins with the same composition $C_{20}H_{32}O_4$ (IV and V) were isolated.

A comparative study of the PMR spectra of compounds (IV) and (V) showed that while their general patterns were close they differed by the values of the chemical shifts and, particularly, by the natures of the split-



ting of the H-10 signals. Thus, in the spectrum of (IV) the H-10 atom appeared in the form of a broadened singlet with a half-width of 7.5 Hz at 3.96 ppm, while in the case of (V) it had the form of a distinct doublet with ^3J = 7.5 Hz at 3.74 ppm. These facts permit us to consider (IV) and (V) as stereoisomers at C_{10}. The use of the double-resonance procedure showed that in the case of compound (IV) H-10 interacted vicinally with the H-9 olefinic proton with ^3J = 3.5 Hz and with the protons of the methyl group at C-8 in homoallyl fashion with ^5J = 1.5 Hz, which also explained the nature of the broadening of the above-mentioned signal at 3.96 ppm.

When the PMR spectra of compound (V) was studied under similar conditions, no such homoallyl interaction between the H-CH_3 and H-10 protons was detected.

It follows from a consideration of the structure of compounds (IV) and (V) on molecular models that only if the α-axial orientation of H-10 is realized does the dihedral angle θ formed between the plane of the double bond ($C_8=C_9$) and the C_{10}–H bond amount to ~90°, which corresponds to the condition for the appearance of the highest value of the homoallyl coupling constant [7].

Furthermore, it is known [8] that when cyclic unhindered ketones are reduced with sodium tetrahydroborate, alcohols with equatorial hydroxy groups are formed in predominating yield. When lapidin was reduced, as the main product we isolated the dihydrolapidin (IV). On the basis of these facts, we concluded that in the molecule of dihydrolapidin (IV) the hydroxy group at C_{10} has the β-equatorial orientation, while in the case of the dihydrolapidin (V) it is α-axial. This conclusion finds additional confirmation in the following facts. The alkaline hydrolysis of compounds (IV) and (V) formed the dihydrolapidols (VI) and (VIII), the catalytic hydrogenation of which at the double bonds led to the corresponding tetrahydro derivatives (VIII) and (IX). In the PMR spectrum of (VIII), the signal of the proton geminal to the hydroxy group at C_{10} appeared at 3.54 ppm in the form of a quartet with ^3H = 10.2 and 5.0 Hz, which is due to the a,a and a,e interactions of H-10 with the neighboring protons at C_9. In the PMR spectrum of (IX) the analogous signal is located at 3.6 ppm and has the form of a broadened singlet with a half-width of 9.5 Hz, which is characteristic for e,a and e,e interactions with neighboring methylene protons.

Epoxidation with perphthalic acid of the dihydrolapidols (VI) and (VII) having, according to what has been said above, β-equatorial and α-axial hydroxy groups at C_{10}, respectively, led to the formation of the epoxy products (X) and (II). The latter, from its melting point and IR spectrum, proved to be identical with the lapiferol obtained from lapiferin by hydrolysis.

A comparative study of the PMR spectra of (X) and (II), which are compounds stereoisomeric at C_{10}, revealed one characteristic feature, namely the practical equality of the values of the spin–spin coupling constants of the H-9 proton with the H-10 proton (^3J = 5.3 and 5.4 Hz), regardless of the orientation of the latter. A consideration of the structural formulas of (X) and (II) using molecular models showed that, depending on

the configuration of the epoxide ring and of the hydroxy group at C_{10}, two sets of dihedral angles θ in the H–C_{10}–C_9–H fragment can be realized which permit a closeness of the values of the vicinal coupling constants of the protons in this fragment. If it is assumed that the epoxide ring has the α orientation, then for (X) and (II) the angle θ will be ~140° and ~30°, respectively while in the case of its β orientation these angles will be ~60°. In the second case, the coupling constant between the protons under consideration could be expected to be of the order of 2-3 Hz, but its values found experimentally are 5.3 and 5.4 Hz. On this basis, it was established that in compounds (X) and (II) the epoxide ring is present in the α orientation.

The trans linkage of the cyclopentane with the cycloheptane ring and the β-orientation of the hydroxy group at C_4 in lapiferol were unambiguously determined by the formation of the latter from lapidin.

It follows from the above fact that lapiferol is 8α,9α-epoxy-trans-carotane-4β,6α,10α-triol.

The positions of the acetic and angelic acid residues in lapiferin were determined by comparing the PMR spectra of lapiferin and of lapiferol diacetate (XI) obtained by acetylating lapiferol in the presence of sodium acetate. As is well known [9], the nature of the acid residue affects the chemical shift of a proton located geminally to the ester group. In the case of α,β-unsaturated acids this proton appears in a weaker field than in esters of saturated acids.

The signal of the gem-acyl proton at C_6 in lapiferin undergoes a paramagnetic shift in comparison with that in the spectrum of (XI) (by ~0.2 ppm), while the chemical shifts of the signals of the protons at C_{10} in the two compounds are practically the same (4.83 and 4.80 ppm). It follows from this that in lapiferin the angelic acid esterifies the hydroxy group at C_6 and acetic acid that at C_{10}.

Thus, the fact of the chemical passage from lapidin (III) to lapiferol (II), forming the basis of the ester lapiferin (I) under investigation, and the results of a subsequent investigation of the PMR spectra of these compounds and their derivatives, taken all together, have enabled us to establish that lapiferin has the structure of 10α-acetoxy-6α-angeloyloxy-8α,9α-epoxy-trans-carotan-4β-ol, as shown in formula (I) [10].

EXPERIMENTAL

The conditions for recording the spectra and also for the isolation of the total esters have been described previously [1]. The separation of the esters was carried out with the aid of column chromatography on silica gel. The substances were eluted with mixtures of hexane and ethyl acetate with increasing concentrations of the latter.

Isolation of Lapiferin (I). Fractions 26-29 (hexane–ethyl acetate (4 : 1)), on concentration, yielded 2.23 g (0.6% on the raw material) of a crystalline substance, $C_{22}H_{34}O_6$, mp 137-138°C (from hexane–ether); $[\alpha]_D^{21}$ +41.6° (c 1.2; chloroform).

The dehydrogenation of lapiferin was carried out by a procedure described previously [1]. It gave dauca-lene with mp 59-70°C; picrate with mp 89-90°C.

The hydrolysis of lapiferin was carried out with a 2% aqueous ethanolic solution of KOH with heating on the water bath for 1 h. The reaction product was isolated in the usual way. From 110 mg of lapiferin was obtained 75 mg of lapiferol (II), $C_{15}H_{26}O_4$, mp 112-114°C (hexane–ether); $[\alpha]_D^{21}$ +15.4° (c 1.3; chloroform). From the acid fraction of the hydrolysate was isolated an acid $C_5H_8O_2$, mp 43-44°C, identified as angelic acid.

Reduction of Lapidin. With cooling and stirring, 3.4 g of $NaBH_4$ was added in portions to a solution of 1.7 g of lapidin in 30 ml of methanol. Then part of the methanol was distilled off in vacuum and the residue was diluted with water. The reaction product was extracted with ethyl acetate. The residue after the evaporation of the solvent was chromatographed on a column of silica gel (60 × 1.8 cm).

The substances were eluted with the hexane–ethyl acetate (7 : 1) system. Two amorphous substances of the same composition $C_{20}H_{32}O_4$ were obtained – the dihydrolapidin (IV), 0.9 g, R_f 0.3, and the dihydrolapidin (V) 0.12 g, R_f 0.23 (hexane-ethyl acetate (2:1)).

Dihydrolapidol (XVI). With heating for 2 h, 300 mg of the dihydrolapidin (IV) was hydrolyzed with a 1% aqueous ethanolic solution of KOH. After the usual working up of the reaction mixture, a crystalline substance was isolated, $C_{15}H_{26}O_3$, mp 125-126°C (from chloroform), $[\alpha]_D^{20}$ +35.7° (c 1.4; ethanol).

Dihydrolapidol (VII). With heating for 1 h, 120 mg of the dihydrolapidin (V) was hydrolyzed with a 1% aqueous ethanolic solution of KOH. This gave 80 mg of a crystalline product, $C_{15}H_{26}O_3$, mp 182-183°C (hexane–ether), $[\alpha]_D^{20}$ +44° (c 0.9; ethanol).

Tetrahydrolapidol (VIII). A solution of 80 mg of dihydrolapidol (VI) in 15 ml of ethanol was treated with 20 mg of PtO_2 and hydrogenation was carried out for 30 min with shaking. After the end of the reaction, the catalyst was filtered off, and the solvent was distilled off. This gave a compound with the composition $C_{15}H_{28}O_3$ (70 mg) which melted with decomposition at 188-189°C (from ethyl acetate), $[\alpha]_D^{20}$ +80° (c 1.0; ethanol).

Tetrahydrolapidol (IX). The hydrogenation of 60 mg of the dihydrolapidol (VII) was carried out with 15 mg of PtO_2 in 15 ml of ethanol for 45 min. This gave 55 mg of a crystalline product, $C_{15}H_{20}O_3$, mp 207-208°C (from ethyl acetate).

Epoxydihydrolapidol (X). In the form of a solution in ether, 45 mg of the dihydrolapidol (VI) was epoxidated with an ethereal solution of perphthalic acid (1 ml). The reaction mixture was diluted and the ethereal layer was separated off. The aqueous mother liquor was treated with ether several times. The combined ethereal extracts were washed with sodium carbonate solution and with water. After the solvent had been distilled off, a compound was obtained with the composition $C_{15}H_{26}O_4$, mp 163-164°C (hexane–ethyl acetate), $[\alpha]_D^{21}$ +71° (c 1.1; ethanol).

Epoxydihydrolapidol (II). The epoxidation of 35 mg of the dihydrolapidol (VII) was carried out as described above. This gave a substance $C_{15}H_{26}O_4$ with mp 112-114°C (hexane–ether), $[\alpha]_D^{21}$ +16.2° (c 1.1; chloroform), which was identical with lapiferol according to its IR spectrum.

Lapiferol Diacetate (XI). With heating for 3 h, 120 g of lapiferol was acetylated with 1 ml of acetic anhydride in 5 ml of pyridine in the presence of fused sodium acetate. The reaction product was isolated by the usual method and was purified by chromatography on silica gel. This gave 100 mg of an oil product, $C_{19}H_{30}O_6$, $[\alpha]_D^{20}$ +27.7° (c 1.3; chloroform).

SUMMARY

The roots of Ferula lapidosa have yielded a new ester of the carotane series – lapiferin – and its structure and configuration have been established on the basis of chemical transformations and the analysis of spectral characteristics.

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