ACETYLLAGOCHILINS FROM Lagochilus pubescens AND THEIR INVESTIGATION BY PMR SPECTROSCOPY

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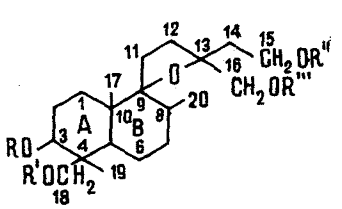
We have shown previously that Lagochilus pubescens Vved. contains diterpenoids, flavonoids, steroids, and high-molecular-weight hydrocarbons [1-3]. Continuing investigations of the plant we have isolated a number of new diterpenoid compounds of the 9,13-epoxylabdane group, which are found only rarely in nature [4].

The diterpenoids isolated proved to be various acetyl derivatives of lagochilin. The lagochilin molecule (I) contains four alcoholic hydroxyls, and therefore the existence of one tetra-, four tri-, six di-, and four monoacetyl derivatives of lagochilin is theoretically possible.

We have isolated eight acetyl derivatives of lagochilin (II-IX).



I. R=R'=R''=R'''=H
II. R=R'=R'''=H; R''=Ac
III. R=R'=R''=H; R'''=Ac
IV. R''=H; R=R'=R'''=Ac
V. R'''=H; R=R'=R''=Ac
VI. R=H; R'=R''=R'''=Ac
VII. R=R'=H; R''=R'''=Ac
VIII. R''=R'''=H; R=R'=Ac
IX. R=R'=R''=R'''=Ac

The IR spectrum of the diterpenoids isolated showed absorption bands in the regions of 1030 cm^{-1} (ether bond), 3200-3600 cm^{-1} (hydroxy groups), and 1735 cm^{-1} (ester group).

TABLE 1. Chemical Shifts of the Signals of Some Functional Groups of Acetylated Lagochilins (ppm relative to HMDS)

Substance	Solvent	C_3-H	$C_{15}-H_2$	$C_{16}-H_2$	$C_{18}-H_2$	C_4-CH_3	C_8-CH_3	$C_{10}-CH_3$	OAC
I	Py	3,95	4,03	3,64 3,78	3,46 3,88	0,94	0,80	0,72	—
II	Py	3,91	4,38	3,66	3,45 3,90	0,96	0,82	0,78	1,85
III	Py	3,92	3,90	4,16	3,48 3,91	0,92	0,80	0,79	1,82
IX	Py	4,81	4,22	3,94	3.72 3,88	0,70	0,68	0,68	2,06 1,96 1,84 1,84
IX	CCl_4	4,54	4,07	3,78 3,90	3,53 3,72	0,85	0,70	0,81	2,02 1,93 1,88 1,88
IV	CCl_4	4,58	3,60	3,86 4,10	3,53 3,74	0,89	0,72	0,9	2,03 1,97 1,92
V	CCl_4	4,50	4,12	3,32 3,46	3,56 3,71	0,88	0,73	0,83	1,94 1,92 1,91
VI	CCl_4	3,22	4,1	3,87	3,68 4,01	0,85	0,63	0,83	1,97 1,97 1,90
VII	CCl_4	3,40	4,06	3.88	3,2 3,4	0,84	0,68	0,80	1,98 1,89
VIII	CCl_4	4,50	3,65	3,35 3,57	3,65	0,86	0,71	0,84	1,94 1,92

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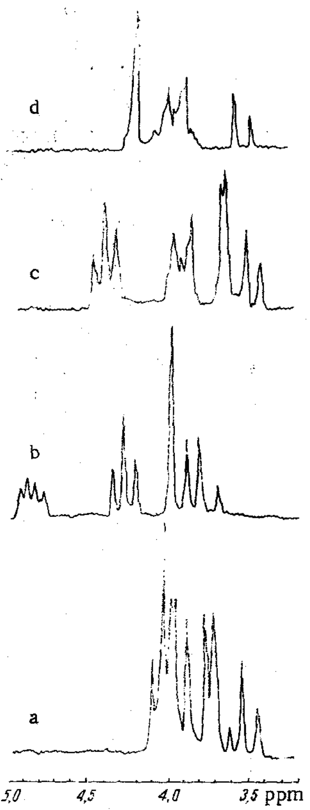

Fig. 1

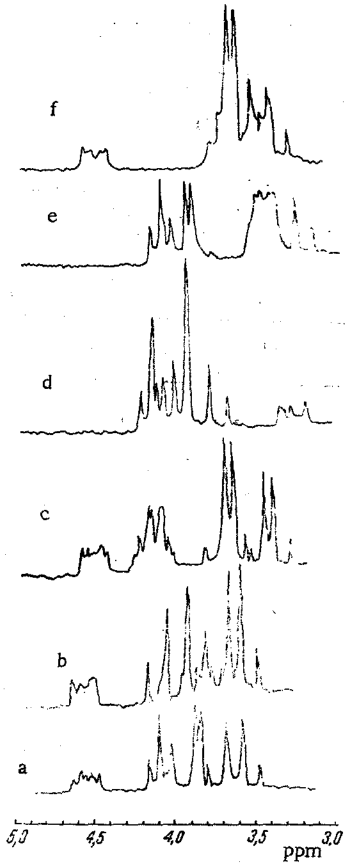

Fig. 2

The mass-spectrometric fragmentation of the diterpenoids isolated is characteristic for 9,13-epoxylabdanes [5] and shows strong peaks of molecular ions with m/e 398 in the case of compounds (II) and (III), 482 for (IV), (V), and (VI), and 440 for (VII) and (VIII), and characteristic peaks with m/e 214, 227, and 240 for (II-V), 256, 269, and 282 for (VI) and (VII) and 172, 185, and 198 for (VIII).

All the diterpenoids isolated gave tetraacetyllagochilin with acetic anhydride, and their hydrolysis led to the formation of lagochilin.

The number and positions of attachment of the acetyl groups in the individual derivatives were determined by PMR spectroscopy. The values of the chemical shifts of the signals of the protons of the main functional groups of the compounds investigated are given in Table 1.

The PMR spectra of lagochilin (in CF_3COOH) and its tetraacetate (IX) (in $CDCl_3$) are fairly similar to those considered by Chizhov et al. [6]. We recorded the PMR spectrum of lagochilin in pyridine (Fig. 1a) and performed the assignment of the signals by analogy with [6]. According to their integral intensity, the signals of six methylene and methine protons present in the α positions to hydroxy groups are located in the 3.4-4.1 ppm region. A triplet at 4.03 ppm is due to the two protons at C_{15} forming part of a hydroxyethylene grouping. Doublets of the methylene protons at C_{18} forming a characteristic spectrum of an AB spin system are located at 3.88 and 3.48 ppm. Similar doublets, but with a smaller difference in chemical shifts, are formed by the protons at C_{16}. The nonequivalence of the methylene protons at C_{16} and C_{18} is due to the different influence of the neighboring asymmetric centers. The signal of the methylene proton at C_3 is located in the 3.95 ppm region and is masked by other signals.

In the spectrum of lagochilin tetraacetate (Fig. 1b) there is a regular downfield shift of the signals of the protons at C_3, C_{15}, C_{16}, and C_{18} [7], and each group forms nonoverlapping and readily identified signals. Apart from the appearance of the four signals of acetyl-group protons, it is possible to observe an upfield shift of the signals of all the methyl groups (at C_4, C_{10}, and C_8) (see Table 1) as compared with their positions in the spectrum of lagochilin.

In the PMR spectrum of the diterpenoid (II) (Fig. 1c), the triplet of the methylene protons at C_{15} is located in the weak field, while the other signals in this region have practically the same chemical shifts as in the spectrum of lagochilin itself. Thus, a comparison of this spectrum with the spectra of lagochilin and its tetraacetate permits the statement that the acetyl group is located at C_{15}.

In the PMR spectrum of the diterpenoid (III) (Fig. 1d), the singlet of the two equivalent protons at C_{16} is located in the weak field which again determines the position of the acetyl group. The hydroxymethylene protons at C_{15} are nonequivalent and resonate in the 4.1-3.8 ppm region in the form of a broad signal together with the C_3 proton and one of the C_{18} protons.

Figure 2a shows the spectrum of lagochilin tetraacetate in CCl_4. An analysis of the positions of the signals (see Table 1) shows that on passing from pyridine to solutions in CCl_4 the $-OCH$ and $-OCH_2$ protons shift upfield, and the signals of the three C-methyls shift downfield, while the signals of the acetate groups are insensitive to the change in solvent.

In the spectrum of the diterpenoid (IV) (Fig. 2b), the triplet of the methylene protons at C_{15} is, because of their nonequivalence, converted into a more complex signal shifted upfield in comparison with its position in lagochilin tetraacetate. The signals of the C_3 and C_{18} protons are in similar positions to those in the spectrum of lagochilin tetraacetate, and the methylene protons at C_{16} are shifted still further downfield. Such a distribution of signals shows that the acetyl groups are located at C_3, C_{16}, and C_{18}, and the hydroxy group at C_{15}.

In the spectrum of the diterpenoid (V) (Fig. 2c), the signals of protons at C_3, C_{15}, and C_{18} have chemical shifts similar to those in the spectrum of lagochilin tetraacetate, while the signals of the AB spin system of the methylene proton at C_{16} are present in a higher field. Consequently, the acetyl groups are located at the C_3, C_{15}, and C_{18} carbon atoms.

In the 3.0-5.0 ppm region of the PMR spectrum of the diterpenoid (VI) (Fig. 2,d) the signal of the C_3 proton is present in the strongest field, which shows that the OAc groups are present at C_{15}, C_{16}, and C_{18}. Furthermore, in the spectrum of this compound the signal of the C_{10} methyl group is in the strongest field and the signals of the C_{18} protons in the weakest field as compared with their positions in the PMR spectra of the other acetyllagochilins (see Table 1). The somewhat unusual position of the signal of the C_3 proton at a high value of the field is probably due to the participation of the C_3 hydroxylic proton in the formation of a hydrogen bond with the carbonyl of the acetyl group at C_{18}, which changes the electronic properties of the hydroxy group. Such a tendency is observed in all cases where, in the molecule of a lagochilin derivative, there are sterically close hydroxy and acetyl groups.

In the PMR spectrum of the diterpenoid (VII) (Fig. 2e), the presence of the signals of the C_{15} and C_{16} methylene protons in the region of the weakest field shows that the acetyl groups are attached to precisely these carbon atoms. A complex signal in the 3.3-3.6 ppm region appears as the result of the superposition of the signals of the proton at C_3, one of the C_{18} protons, and the two hydroxy groups. In the spectrum of this compound taken in carefully dried pyridine solution there are the individual signals of the hydroxy groups at C_3 and C_{18} in the form of a doublet and a triplet, respectively, due to spin-spin coupling with the neighboring hydrogen atoms.

A comparison of the PMR spectra of lagochilin tetraacetate (Fig. 2a) and of the diterpenoid (VIII) (Fig. 2f) shows that the chemical shifts of the C_3 and C_{18} protons in them are similar in pairs, while the signals of the C_{15} and C_{16} protons in the spectrum of the diterpenoid (VIII) are located in a stronger field and, consequently, the acetyl groups are present in ring A at C_3 and C_{18}.

Analysis of the PMR spectra of the compounds considered and of the values of the chemical shifts (Table 1) shows that it is easy to determine the positions of the acetyl groups from the signals of the C_3, C_{15}, C_{16}, and C_{18} protons. The signals of the acetyl groups themselves, and also of the methyls of the basic skeleton, show no definite link between their chemical shifts and the positions of the acetyl groups. The width of the signal of the C_3 proton is ~15 Hz in all cases which shows its axial orientation, while the value of the geminal constant for the methylene protons at C_{16} and C_{18} is 11.2 Hz and changes insignificantly on acetylation. In all the spectra the methylene protons at C_{18} are nonequivalent, the difference in the chemical shifts varying from 0.15 to 0.45 ppm. The protons at C_{15} and C_{16} may be nonequivalent, but this is not shown at the given working frequency.

EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrophotometer and the PMR spectra on a Varian XL-100-15 spectrometer in carbon tetrachloride and pyridine solutions. The chemical shifts are given in the δ scale relative to HMDS as internal standard. The mass spectra were taken on a MAT-311 instrument. The specific rotations were determined on a Carl Zeiss Polamat S instrument (GDR).

For thin-layer chromatography (TLC) we used LS 5/40 μ silica gel, and also Silufol prepared plates in the following systems: 1) ether-hexane (4 : 1) and 2) carbon tetrachloride-acetone (7 : 5). The revealing agent was concentrated H_2SO_4 with 1% of vanillin.

The plant Lagochilus pubescens (4 kg) was extracted with methanol (5 × 15 liters), and the combined extracts were evaporated in vacuum to 0.7 liter, diluted with water (1 : 1), and extracted with benzene and chloroform. The benzene was distilled off from the benzene fraction and the viscous residue (110 g) was chromatographed on type LS 100/250 μ silica gel with elution by chloroform the polarity of which was gradually increased by the addition of methanol. Fractions with a volume of 200-250 ml were collected, the separation being monitored by TLC.

A total of 44 fractions was collected. Fractions 1-8 contained 40 g of hydrocarbons, flavonoids, sterols, and a very small amount of a mixture of diterpenoids.

Separation of Fractions 9-16. The combined material (9.1 g) was passed through a column of silica gel with elution by petroleum ether-ethyl acetate (10 : 1) and the solvents of gradually increasing polarity. From various fractions of the eluate we isolated: tetraacetyllagochilin, $C_{28}H_{44}O_9$ (IX) [oily liquid, $[\alpha]_D$ +17.8° (c 1; ethanol), R_f 0.47 (system 1)], 15, 16, 18-tri-O-acetyllagochilin, $C_{26}H_{42}O_8$ (VI) [mp 92-93°C (from ether), $[\alpha]_D$ + 55.58° (c 1, ethanol), R_f 0.28 (system 1)], 3, 16, 18-tri-O-acetyllagochilin (IV), $C_{26}H_{42}O_8$ [oily liquid, R_f 0.3 (system 1)], and 3, 5, 18-tri-O-acetyllagochilin, $C_{26}H_{42}O_8$ (V) [oily liquid, R_f 0.26 (system 1)].

Separation of Fractions 17-34. The combined material (10.4 g), after repeated chromatography with elution by benzene-methanol (9 : 1) and hexane-ethyl acetate (5 : 1) yielded 15, 16-di-O-acetyllagochilin (VII) [$C_{24}H_{40}O_7$, mp 86-87°C (from ether), $[\alpha]_D$ +7.4° (c 1; ethanol), R_f 0.45 (system 2)] and 3, 18-di-O-acetyllagochilin, $C_{24}H_{40}O_7$ (VIII) [mp 105-106°C (from ether) $[\alpha]_D$ +15.21° (c 1.2; ethanol), R_f 0.5 (system 2)].

Separation of Fractions 35-44. The mixture of diterpenoids (6.2 g) was treated with ether, which yielded 2.9 g of lagochilin. The mother liquor was passed through a column of type LS 100/160 μ silica gel and was eluted with hexane-ethyl acetate (5 : 1). This yielded 16-mono-O-acetyllagochilin, $C_{22}H_{38}O_6$ (III) [oily liquid, R_f 0.17 (system 2)] and 15-mono-O-acetyllagochilin, $C_{22}H_{38}O_6$ (II) [oily liquid, $[\alpha]_D$ −5.55° (c 1; ethanol), R_f 0.2 (system 2)].

Acetylation of the Diterpenoids (II-VIII). A mixture of 30-40 mg of the substance, 1 ml of absolute pyridine, and 0.5 ml of acetic anhydride was left for a day at room temperature. The reaction mixture was evaporated and treated with ether, and the solvent was distilled off. This gave an oily liquid with R_f 0.47 (system 1) – tetraacetyllagochilin (IX).

Alkaline Hydrolysis of (II-VIII). A mixture of 30-40 mg of the substance in 2 ml of 10% methanolic caustic potash was heated in the water bath for 5-10 min and was then left at room temperature for 10-12 h. The solvent was distilled off, the residue was treated with ether, and the ethereal extract was evaporated. This gave lagochilin, mp 167-168°C from ether, R_f 0.12 (system 2).

SUMMARY

The plant Lagochilus pubescens Vved. has yielded seven new diterpenoids, which have been characterized.

An investigation of chemical reactions and IR, mass, and PMR spectra has shows that they belong to the 9,13-epoxylabdane series and are acetylated derivatives of lagochilin.

On the basis of the results of a comparative study of PMR spectra of the diterpenoids isolated and of lagochilin the positions of attachment of the acetyl groups in these compounds have been determined.

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