LAGOCHILIN DIACETATES FROM *Lagochilus inebrians*

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The combined acetates of lagochilin obtained by chloroform extraction of the plant *L. inebrians*, with subsequent purification from wax-like substances, have been benzoylated and the benzoate derivatives have been subjected to separation. As the result of the study of their IR, PMR, and mass spectra and a number of chemical transformations of the compounds isolated it has been established that the initial mixture of lagochilin acetates consists of the 3,16-, 15,18-, and 3,15-diacetates.

Lagochilin, lagochilin tetraacetate [1, 2], and lagochilin 3-monoacetate [3] have been isolated from the plant *L. inebrians* previously, and the presence of three flavonoids has also been shown chromatographically [4]. By a further study of this plant we have shown that the alkaline hydrolysis of the combined diterpenoids forms lagochilin, and acetylation forms lagochilin tetraacetate. Consequently the total diterpenoids contain various acetates of lagochilin, but their separation has proved to be impossible. The combined material was then benzoylated and the reaction products were chromatographed on a column of SiO_2 with the isolation of an oily product (I) and of a crystalline substance (II) with mp 103-107°C.

Products (I) and (II) are soluble in the usual organic solvents and insoluble in water. The alkaline hydrolysis of (I) and (II) forms lagochilin.

Their IR spectra have absorption bands at 1720-1745 cm^{-1} and at 1580 and 1600 cm^{-1}, which are characteristic for ester groups and an aromatic nucleus. There are no absorption bands of —OH groups. In the mass spectrum of (II) there is the peak of the molecular ion with m/e 648 (M^+) and the peaks of fragments with m/e 588 ($M^+ - 60$), 528 ($M^+ - 60 - 60$), 331, and 344. The first two fragments appear as the result of the splitting out of one and two molecules of acetic acid, and the last two through the cleavage of ring B of the (II) molecule; such cleavage is characteristic for diterpenoids of the 9, 13-epoxylabdane group [5, 6], and it simultaneously shows that one of the benzoyl gruops in (II) is present at C-3 or C-18 and the other at C-15 or C-16.

In the PMR spectrum of (II) taken in CCl_4, the signals of the aromatic protons appeared in the 7.16-7.44 and 7.86-8.04 ppm region. At 1.74 and 1.94 ppm there were the signals of two acetyl groups. The signal of the H-3 proton was detected in the form of a poorly resolved quartet (4.65 ppm). The triplet of the 2H-15 protons resonated at 3.92 ppm in the form of a singlet and the signal of the 2H-18 protons was converted into a singlet, shifted downfield, and superimposed on the signal of the protons at C-16. In the PMR spectrum of (II) taken in $CHCl_3$, the overlapping of the signals at 3.92 ppm disappeared and in place of one singlet two appear (at 3.98 and 3.92 ppm), which corresponded in area to two protons each. The remaining signals were unchanged.

On comparing these results with the PMR spectrum of lagochilin tetraacetate (III) and with the literature [6, 7], it was found that the signals of the H-3 and 2H-16 protons in the PMR spectra of (II) and (III) coincide, while the signals of the 2H-15 and 2H-18 protons in (II) are shifted considerably downfield in comparison with the same signals in the PMR spectrum of (III).

On the basis of the facts given above, the structure of lagochilin 3,16-diacetate 15,18-dibenzoate is proposed for substance (II).

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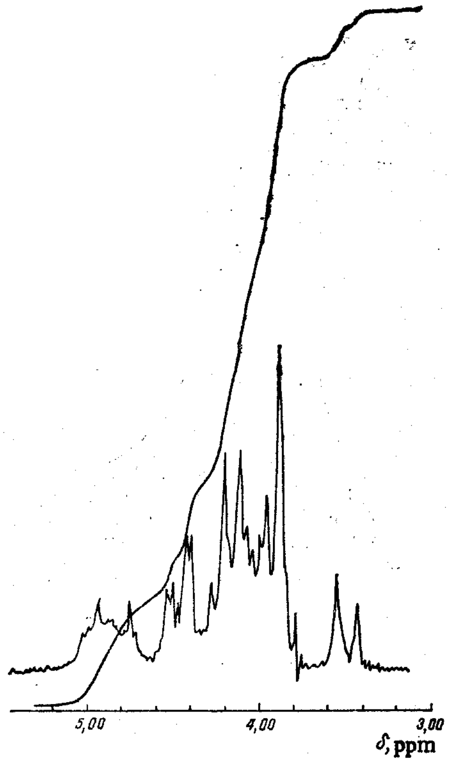
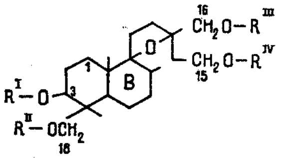


Fig. 1. Part of the PMR spectrum of I.



I a $R^{II} = R^{IV} = Ac$, $R^{I} = R^{III} = C_6H_5CO$
I b $R^{I} = R^{IV} = Ac$, $R^{II} = R^{III} = C_6H_5CO$
I c $R^{II} = R^{III} = Ac$, $R^{I} = R^{IV} = C_6H_5CO$
II. $R^{I} = R^{III} = Ac$, $R^{II} = R^{IV} = C_6H_5CO$
III. $R^{I} = R^{II} = R^{III} = R^{IV} = Ac$

The mass spectrum of (I) also has the peaks of fragments with m/e 331, and 344 due to the cleavage of ring B, and consequently in the case of (I), as well, one benzoyl group must be located at C-3 or C-18 and the other at C-15 or C-16. It must be mentioned that six diacetate-dibenzolates of lagochilin can exist theoretically, four of which (Ia, Ib, Ic, and II) on mass-spectrometric fragmentation according to the literature [4, 6] should give the above-mentioned fragments with m/e 331 and 344. Consequently, (I) consists of one of substances (Ia, Ib, or Ic), or a mixture of them.

In the PMR spectrum of (I) (solvent CCl_4), the signals of the aromatic protons appear in the 7.15-7.43 and 7.81-8.20 ppm region. There are four signals at 1.75, 1.83, 1.86, and 1.91 ppm (s, $4CH_3{-}C{=}O$), revealing the presence of four acetyl groups, which shows that (I) is a mixture of two isomers in the combination (Ia-Ib) or (Ia-Ic) or (Ib-Ic). At 3.51 ppm and 4.04 ppm (the presence of a signal at 4.04 ppm was established by recording the INDOR spectrum of (I), since it is masked by other signals) there are two one-proton doublets from the geminal protons at C-18 (^2J = 11 Hz). In the 3.80-4.64 ppm region there is a group of signals relating to the protons at C-15, C-16, and C-18, and at 3.91 ppm there is a three-proton signal (broadened singlet, 2H-16, part of a doublet with its center at 4.04 ppm and a contribution of part of the signal from the proton at C-15 or at C-16 of the other isomer). At 4.86 ppm there is a two-proton broadened signal as the result of the partial superposition of the signals of the protons from two isomers, 2(H-3).

On considering the theoretical spectra of (Ia, Ib, and Ic) and comparing them with the observed PMR spectrum of (I), and also bearing in mind the features of the PMR spectra of (II) and (III), the conclusion was reached that (I) is a mixture of (Ia) and (Ib). In the case of a mixture of (Ia) and (Ic), in the observed spectrum of (I) the signal appearing at 3.91 ppm should correspond in area to five protons but it actually corresponds to three protons. In the other case (Ib-Ic), the signal at 3.51 ppm in the PMR spectrum of (I) should be a two-proton signal, but its area actually corresponds to a single proton. The above-described PMR spectrum of (I), part of which is given in Fig. 1, corresponds to the PMR spectrum precisely of a mixture of (Ia) and (Ib). Consequently, the initial combined diterpenoids contain lagochilin 15,18- and 3,15-diacetates, in addition to the 3,16-diacetate.

EXPERIMENTAL

The individuality of the substances was checked by chromatography in a thin fixed layer of SiO_2 in the chloroform—acetone (4:1) (A) and benzene—ether (3:1) (B) systems. IR spectra were taken on a UR-10 instrument, and PMR spectra on a Varian XL-100-15 spectrometer (δ scale, HMDS).

Mass spectra were recorded on a Varian MAT-311 instrument.

Isolation of the Total Diterpenoids. The air-dry epigeal part of the plant *L. inebrians* (8 kg) was extracted with chloroform, and the total extractive substances so obtained (530 g) were mixed with 1 kg of Al_2O_3, and the wax-like substances (120 g) were extracted with hexane. The residual part of the combined material still adsorbed on the Al_2O_3 was placed in a column (6 × 81 cm) and was eluted first with hexane (fractions 1-60) and then with the hexane—CCl_4 (100:5-30) system, 500-ml fractions being collected. Fractions 61-105 were combined, the solvent was evaporated, and the total product (28 g) was placed in a column (3.4 × 96 cm) containing SiO_2 and was eluted with the hexane—ether (100:1-35) system, 500-ml fractions being collected. Fractions 6-40 were combined, and after the solvent had been distilled off the total product (11 g) contained substances with R_f 0.13 and 0.28 (A).

Hydrolysis and Acetylation of the Total Material from Fractions 6-40. A solution of 300 mg of the material in ethanol was treated with a 5% solution of KOH and the mixture was boiled under reflux for 5 min. The lagochilin that deposited was recrystallized from acetone, mp 168-169°C. Another 350 mg of the same combined material was dissolved in 3 ml of pyridine and treated with 2 ml of acetic anhydride. After two days, the usual work-up gave lagochilin tetraacetate with R_f 0.23 (B).

Preparation of the Benzoate Derivative of the Lagochilin Acetates. A solution of 9 g of the combined lagochilin acetates in 7 ml of pyridine was treated with 5 ml of benzoyl chloride. After three days the mixture was diluted with water and extracted with chloroform. The chloroform layer was treated repeatedly with a 15% solution of Na_2CO_3 until benzoic acid had been eliminated completely (the acid was revealed with a weakly alkaline solution of bromothymol blue). After the chloroform layer had been washed with distilled water and dried over Na_2SO_4, the solvent was distilled off.

Separation of the Benzoate Derivatives of Lagochilin Acetates. The combined benzoate derivatives of the lagochilin acetates (7 g) were placed in a column (2 × 205 cm) and were eluted with benzene and the benzene—ether (100:1-10) system, 1-liter fractions being collected. After the evaporation and combination of fractions 4-30, compound (I), $C_{38}H_{48}O_9$, R_f 0.57 (B), was obtained. By combining fractions 33-44 and evaporating the solvent, compound (II), $C_{38}H_{48}O_9$, R_f 0.49 (B) was obtained.

After recrystallization from the hexane—ether (10:3) system, compound (II) formed rhombohedral crystals.

Saponification of (I) and (II). A solution of 50 mg of (I) or (II) in ethanol was treated with 5% KOH solution and the mixture was boiled under reflux for 10 min. The lagochilin that deposited was recrystallized from acetone, mp 167-168°C.

SUMMARY

By benzoylating the total diterpenoids of the plant *L. inebrians* and the subsequent study of the benzoate derivatives by PMR and mass spectroscopy, and also a series of chemical transformations, it has been shown that the total diterpenoids of the plant *L. inebrians* contained, in addition to compounds isolated previously, lagochilin 3,16-, 15,18-, and 3,15-diacetates.

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