SUMMARY

A comparative investigation of the inhibiting influence on transport Na^+,K^+-ATPase of four flavone aglycones and eight of their glycosides has been performed on the microsomal fraction of the cells of the cerebral cortex.

It has been shown that in concentrations of $1 \cdot 10^{-4}$ to $5 \cdot 10^{-6}$ M myricetin, quercetin, luteolin, and myricetin 3'-glucoside possess an appreciable inhibiting effect. For kaempferol and its glycosides, as for the glycosides of quercetin and luteolin, the inhibiting effect is extremely feeble.

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DITERPENES OF *Lagochilus pubescens*

Z. I. Mavlyankulova, U. N. Zainutdinov,
and Kh. A. Aslanov

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The plant *Lagochilus pubescens* (family Labiatae) is widely grown but has been little studied [1, 2]. From this plant we have previously isolated the hydrocarbon nonacosane, β-sitosterol, the flavonoid 5-hydroxy-4',7-dimethoxyflavone, and diterpenes — lagochilin and 3,18-O-isopropylidenelagochilin [3, 4].

By chromatography on alumina and silica gel followed by elution with various solvents, from an ethereal fraction of the chloroform extract [4] we have isolated five individual diterpenes.

Diterpene (I), $C_{26}H_{44}O_5$, mp 118-119°C (from ether), $[\alpha]_D$ −50° (c 1; ethanol); M^+ 436.

The IR spectrum of (I) had bands at 1095 cm^{-1} characteristic for an ether bond. The mass spectrum showed the peak of the molecular ion with m/e 436 and peaks with m/e 238, 225, and 212, which shows that (I) belongs to the labdane group with a grindelane skeleton [5]. The PMR spectrum has signals in the 1.1-1.3 ppm regions due to the methyl groups of an isopropylidene part of the molecule. The signals of methyl groups at C_4, C_8, and C_{10} are located, just as in lagochilin, in the 0.65-0.85 ppm region. Protons located adjacent to oxygen ($-CH_2-O-$, $>CH-O-$) resonate in the 3.2-3.7 ppm region. The acid hydrolysis of (I) led to the formation of lagochilin (V).

On the basis of its IR, PMR, and mass spectra and chemical transformation, (I) was identified as di-O-isopropylidenelagochilin [4, 6].

Diterpene (II), $C_{27}H_{44}O_7$, mp 120-121°C (from ether), M^+ 480.

The IR spectrum of (II) has an absorption band at 1735 cm^{-1} which is characteristic for an ester group. In the PMR spectrum, the signals of the methyl groups of the main skeleton

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V. I. Lenin Tashkent State University. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 46-49, January-February, 1977. Original article submitted April 22, 1976.

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have positions similar to those for lagochilin [6]. Signals in the 1.2-1.4 ppm region are due to the methyl group of an isopropylidene part of the molecule. Singlets in the 1.8-2.0 ppm region belong to the protons of two acetyl groups. In the main spectrum of (II) there are the peaks of the molecular ion with m/e 480 (M^+) and peaks with m/e 282, 269, 256.

The alkaline hydrolysis of (II) formed 3,18-O-isopropylidenelagochilin (IV). Consequently, the diterpene (II) is di-O-acetyl-3,18-O-isopropylidenelagochilin [4, 6].

Diterpene (III), $C_{25}H_{42}O_6$, M^+ 438, proved to be new, not described in the literature.

It is a liquid substance readily soluble in chloroform, acetone, dioxane, and ethanol, sparingly soluble in petroleum ether, and insoluble in water.

The IR spectrum of (III) has absorption bands characteristic for ester (1735 cm^{-1}) and hydroxy (3200-3600 cm^{-1}) groups. The presence of a free hydroxy group was confirmed by the production of an O-acetyl derivative (II). The PMR spectrum of (III) showed a broad signal at 3.46 ppm belonging to an axial proton at C_3 and four lines in the 3.2-3.46 region belonging to the protons at C_{18}. The assignment of the remaining signals was done by analogy with the work of Chizhov et al [6]. The mass spectrum of (III) has the peaks of the molecular ion with m/e 438 (M^+) and peaks at m/e 240, 227, and 214.

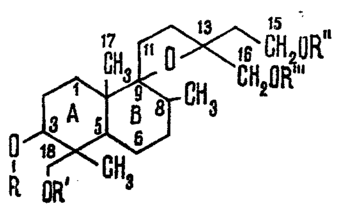
The alkaline hydrolysis of (III) formed 3,18-O-propylidenelagochilin (IV) and consequently, (III) is a mono-O-acetyl-3,18-O-isopropylidenelagochilin the acetyl group in which must be present at C_{15} or C_{16}.

The position of the acetyl group was established by a comparative study of the PMR spectra of (III) and its acetyl derivative (II).

In the PMR spectrum of (II) a signal at 4.22 ppm corresponds to the protons of a methylene group at C_{15} and is split into a triplet by the action of methylene group at C_{14}. The signals of the two protons at C_{16} form a AB system in the 3.9-4.2 ppm region (J = 12 Hz). The nonequivalence of these protons is due to the anisotropic influence of the two adjacent carbonyls of acetyl groups. In the PMR spectrum of (III) the signal of the resonance of the protons at C_{15} has the form of a triplet in a higher field (3.89 ppm) and the quartet of the proton at C_{16} is converted into a singlet resonating in the previous region. It is known that on acetylation the signals of protons located adjacent to hydroxy groups shift downfield by 1.2 ppm [7]. It follows from a comparison of the spectra of (II) and (III) that the acetyl group in (III) is present at C_{16} and the hydroxyl at C_{15}.

Diterpene (IV), $C_{23}H_{40}O_5$, mp 150-151°C (from ether), $[\alpha]_D$ −42° (cl; ethanol), M^+ 396. On the basis of its IR, PMR, and mass spectra and a series of chemical reactions, this diterpene was identified as 3,18-O-isopropylidenelagochilin [4, 6].

Diterpene (V), $C_{20}H_{36}O_5$, mp 167-168°C (from ether), $[\alpha]_D$ −9.43° (cl; ethanol), M^+ 356, was identified as lagochilin [3].



I. $R+R'=C(CH_3)_2$
$R''+R'''=C(CH_3)_2$
II. $R+R'=C(CH_3)_2$
$R''=R'''=-COCH_3$
III. $R+R'=C(CH_3)_2$
$R''=H$
$R'''=-COCH_3$
IV. $R+R'=C(CH_3)_2$
$R''=R'''=H$
V. $R=R'=R''=R'''=H$

EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrometer and the NMR spectra on a Varian XL-100 spectrometer in CCl_4 and in pyridine. The chemical shifts are given in the δ scale relative to HMDS. The mass spectra were taken on a MAT-311 instrument. The specific rotations were determined on a Carl Zeiss Polamat S instrument (GDR). The purity of the substances was checked on "Silufol" plates in systems 1) chloroform—acetone (9:1), 2) ether, and 3) ethyl acetate—methanol (9:1), with concentrated H_2SO_4 as the revealing agent.

Isolation of the Combined Diterpenes. The comminuted epigeal part (1.2 kg) of *Lagochilus pubescens* was extracted with chloroform. The residue after the distillation of the chloroform was diluted with water and was extracted with petroleum ether, hexane, benzene, and ether. The ethereal extract was evaporated to dryness. The residue (31 g) was chromatographed on alumina with elution by benzene–methanol (30:1). Fractions amounting to 300-500 ml were collected, their total number being 55.

When these fractions were rechromatographed on silica gel, the following compounds were isolated: di-O-isopropylidenelagochilin (I) with mp 118-119°C (from ether), R_f 0.84 (system 1); di-O-acetyl-3,18-O-isopropylidenelagochilin (II) with mp 120-121°C (from ether), R_f 0.8 (system 1); 16-O-acetyl-3,18-O-isopropylidenelagochilin (III) with R_f 0.48 (system 1); 3,18-O-isopropylidenelagochilin (IV), mp 150-151°C (from ether), R_f 0.25 (system 2); and lagochilin (V) with mp 167-168°C (from ether), R_f 0.3 (system 3).

The acid hydrolysis of di-O-isopropylidenelagochilin (I) was performed by a known method [6], giving lagochilin with mp 167-168°C, R_f 0.3 (system 3).

Alkaline Hydrolysis of Di-O-acetyl-3,18-O-isopropylidenelagochilin (II). A mixture of 40 mg of the substance and 2 ml of 10% methanolic KOH was heated on the water bath for 5 min and was then left at room temperature for 18 h. The solvent was evaporated off and the residue was treated with ether. The ether extract was evaporated, giving colorless crystals with mp 150-151°C (from ether), R_f 0.25 (system 2) — 3,18-O-isopropylidenelagochilin (IV).

Acetylation of 16-O-Acetyl-3,18-O-isopropylidenelagochilin (III). A mixture of 30 mg of the substance, 1 ml of absolute pyridine, and 0.4 ml of acetic anhydride was left at room temperature for a day. Then it was evaporated and treated with ether and the solvent was distilled off. This gave a crystalline substance with mp 120-121°C (from ether), R_f 0.8 (system 1) — di-O-acetyl-3,18-O-isopropylidenelagochilin (II).

Alkaline Hydrolysis of 16-O-Acetyl-3,18-O-isopropylidenelagochilin (III). A mixture of 50 mg of substance and 2 ml of 8% ethanolic KOH was heated for 5-10 min. Then the reaction mixture was treated with ether. This gave a white crystalline substance with mp 150-151°C (from ether), R_f 0.25 (system 2) — 3,18-O-isopropylidenelagochilin (IV).

SUMMARY

Compounds synthesized previously — di-O-isopropylidenelagochilin, di-O-acetyl-3,18-O-isopropylidenelagochilin, and 3,18-O-isopropylidenelagochilin — have been isolated for the first time from the plant *Lagochilus pubescens*, and some supplementations have been made to their physicochemical constants.

A new diterpene compound has been isolated for which, on the basis of IR, PMR, and mass spectra and chemical transformations, the structure of 16-O-acetyl-3,18-O-isopropylidenelagochilin is proposed.

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