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DITERPENE LACTONES AND IRIDOID GLYCOSIDES OF PLANTS OF THE GENUS *Lagochilus*

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The structure of the diterpenoid lagohirsin, found not only in the plant Lagochilus hirsutissimus but also in L. setulosus *and* L. olgae, *has been confirmed by independent synthesis. In L. hirsutissimus new mono- and diacetyllagohirsins have been found together with lagohirsin itself. Iridoid glycosides — harpagide and its 8-O acetate — have been found for the first time in plants of the genus Lagochilus.*

Plants of the genus *Lagochilus* family Labiatae contain lagochilin and its derivatives [1-4]. These plants may also serve as a source of iridoid glycosides.

Lagohirsin (I) has been isolated from the epigeal parts of *L. hirsutissimus, L. setulosus*, and *L. olgae*, and a probable structure for it has been proposed previously [5]. Lagohirsin possesses a neutral character and is readily soluble in methanol, chloroform, ethanol, and benzene and sparingly soluble in ether and petroleum ether, and insoluble in water. Under the action of an aqueous solution of caustic soda, (I) passes into solution, and on subsequent acidification is isolated in unchanged form, which shows that it is a lactone. The presence of a lactone group is also indicated by an absorption band at 1800 cm^{-1} in the IR spectrum of (I). There are also absorption bands in the 3600-3200 cm^{-1} region (hydroxy group), as has been confirmed by the formation of a diacetyl derivative (II).

The mass spectrum of (I) contained the peak of the molecular ion with *m/z* 352, having a low intensity, and peaks with *m/z* 168, 181, and 194, showing the presence of lactone and tetrahydrofuran groups. Similar fragmentation is characteristic for diterpenoids of the 9,13-epoxylabdane series [6].

The PMR spectrum of (I) contained the signals of three methyl groups, at C-4, C-10, and C-8. Two doublets at 2.87 and 2.2 ppm related to protons at C-14. The other pair of protons of the lactone ring, $-O-CH_2-$, produced signals in the form of two doublets at 4.00-4.22 ppm. The signals of the protons at C-18 and C-3 were located in the 3.3-3.6 ppm region. The assignment of the chemical shifts of the protons in the PMR spectrum is given in Table 1. These facts show that the diterpene isolated had the structure (I), as was confirmed by the formation of (III) when (I) was reduced with lithium tetrahydroaluminate.

The structure (I) was shown definitively by synthesis from lagochilin. The interaction of acetone and lagochilin in the presence of anhydrous copper sulfate gave 3,18-O-isopropylidenelagochilin (IV), which, on oxidation with $KMnO_4$ in acetone, formed the oxo products (V). After acid hydrolysis, the latter was converted into (I).

Another series of diterpenoids, (II), (VI), and (VII), was detected in the total extractive substances of *Lagochilus hirsutissimus*. The spectrum of (II) contained absorption bands characteristic for an ester group (1745 cm^{-1} and of the carbonyl of a five-membered lactone (1800 cm^{-1}). The acid hydrolysis of (II) formed lagohirsin. The PMR spectrum of (II, unlike that of (I) contained two signals in the form of singlets at 1.95 and 1.98 ppm, relating to the methyls of two acetyl groups, while the signals of the proton at C-3 was located at 4.35 ppm and the signals of the protons at C-18 at 3.63-3.81 ppm. Consequently, (II) was di-O-acetyllagohirsin.

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TABLE 1. Details of the PMR Spectra of Lagohirsin (I), Di-O-acetyllagohirsin (II), 3-Mono-O-acetyllagohirsin (VI), and 18-Mono-O-acetyllagohirsin (VII), Taken in $CDCl_3$ (0 — HMDS, δ, ppm)

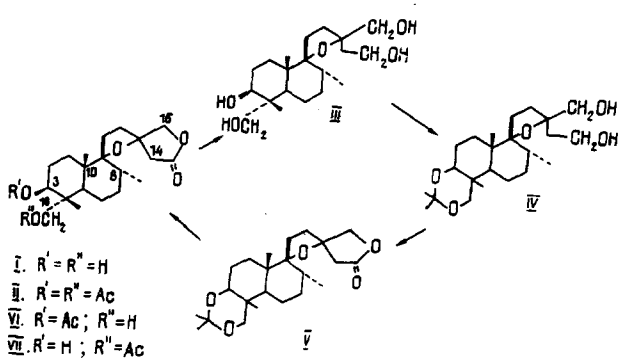
Protons	Chemical shifts, J, Hz.			
	I	II	VI	VII
CH_3-4	0.88 (3H, s)	0.76 (3H, s)	0.62 (3H, s)	0.73 (3H, s)
CH_3-8	0.76 (3H, d)	0.78 (3H, d)	0.78 (3H, d)	0.80 (3H, s)
	J=6.5	J=6.5	J=6.5	J=6.5
CH_3-10	0.82 (3H, s)	0.90 (3H, s)	0.88 (3H, s)	0.87 (3H, s)
H-3	3.47 (1H, m)	4.65 (1H, d.d)	4.72 (1H, d.d)	3.31 (1H, m)
		J_1=2.5; J_2=5.4	J_1=2.5; J_2=5.5	
CH_2-14	2.22 (1H, d)	2.43 (1H, d)	2.41 (1H, d)	2.24 (1H, d)
	2.87 (1H, d)	2.88 (1H, d)	2.88 (1H, d)	2.83 (1H, d)
	J=16.8	J=16.8	J=16.8	J=16.8
CH_2-16	4.00 (1H, d)	4.05 (1H, d)	3.98 (1H, d)	4.01 (1H, d)
	4.22 (1H, d)	4.39 (1H, d)	4.25 (1H, d)	4.21 (1H, d)
	J=7.9	J=7.9	J=7.9	J=7.9
CH_2-18	3.32 (1H, s)	3.63 (1H, d)	2.82 (1H, d)	3.68 (1H, d)
	3.57 (1H, d)	3.81 (1H, d)	3.29 (1H, d)	4.13 (1H, d)
	J=9.8	J=11.8	J=11.9	J=12.0

On acetylation, (VI) and (VII) were converted into di-O-acetyllagohirsin. Consequently, they were isomeric mono-O-acetyl derivatives of lagohirsin. Their structures were established from their mass and PMR spectra.

The signal of the methyl of the acetyl group in the form of a singlet at 1. 99 ppm and the signal of a proton at C-3 at 4.72 ppm in the form of a doublet of doublets in the PMR spectrum of (VI) showed the location of the acetyl group at C-3. In the PMR spectrum of (VII), the signal of the methyl of the acetyl group in the form of a singlet at 2.0 ppm and the signals of the protons at C-18 in the 3.68-4.13 ppm region showed the position of the acetyl group at C-18.

The mass spectra of (VI) and (VII) each showed the peak of the molecular ion with *m/z* 394 and the peaks characteristic for these compounds with *m/z* 194, 181, 168.

Two individual substances have also been isolated from *Lagochilus inebrians*: (VIII), $C_{17}H_{26}O_{11}$, mp 154-156°C, $[\alpha]_D^{24}$ −126° (*c* 1; methanol), soluble in methanol, ethanol, and water and sparingly soluble in ethyl acetate and chloroform; and (IX), $C_{15}H_{24}O_{10}$ amorphous, hygroscopic, yellowish-brown, readily soluble in water, methanol, and ethanol, and insoluble in chloroform and ethyl acetate, $[\alpha]_D^{24}$ −152° (*c* 1; water).



On the alkaline saponification of (VII) under mild conditions a saponification product was obtained that was identical with compound (IX) according to its chromatographic mobility and spectral characteristics. The facts given and also an analysis of the PMR and IR spectroscopic parameters showed that compound (IX) was the known iridoid glycoside harpagide, and compound (VIII) was its 8-acetyl derivative, which has been isolated previously from a series of plants of the Labiatae family [7, 8].

These iridoids have been isolated from *Lagochilus platycalyx* and identified similarly.

EXPERIMENTAL

IR spectra were taken on a Specord UR-75 spectrometer, PMR spectra on a Varian XL-100-15 instrument, and mass spectra on a MAT-311 instrument. We used silica gel LS5/40 μ for thin-layer chromatography, the revealing agaent being conc. H_2SO_4 with 1% vanillin.

The iridoid glycosides were revealed with a 3% alcoholic solution of vanillin in the presence of 0. 5% sulfuric acid and the Trim-Hill reagent [9].

Isolation and Separation of the Diterpenoids. The air-dry epigel part (2 kg) of *Lagochilus hirsulissimus*, *Lagochilus setulosus*, and *Lagochilus olgae* (separately) were extracted six times with chloroform, and each extract was concentrated, diluted with water (1:1) and extracted with petroleum ether and then with benzene. After the benzene had been distilled off, the residue (70 g) was chromatographed on a column (No. 1) with alumina (activity grade III, neutral) in a ratio of material to sorbent of 1:10 and was eluted with benzene to which methanol was gradually added to increase the polarity of the eluent. Fractions of 200-250 ml were collected.

Fractions 45-61 were rechromatographed on a column of type LS 100/160 μm silica gel in a ratio of material and sorbent of 1:40. Elution was performed with benzene–methanol (100:1). Fractions 12-21 yielded 0.35 g of lagohirsin, $C_{20}H_{32}O_5$ (M^+ 352), mp 141-143°C (from ether), $[\alpha]_D^{21}$ −2.1° (*c* 1.1; alcohol). Yield 0.3% on the dry raw material of *Lagochilus hirsutissimus*, 0.1% for *Lagochilus setulosus*, and traces for *L. olgae*. Fractions 33-38 from column were rechromatographed on a column of type LS 100/160 μm silica gel in a ratio of material to sorbent of 1:50, with elution by benzene. Fractions 5-12 yielded di-O-acetyllagohirsin, $C_{24}H_{36}O_7$ (M^+ 436). mp 126-127°C (from ether), $[\alpha]_D^{20}$ (M^+ +44° (*c* 1; alcohol).

Fractions 39-44 from column No. 1 were rechromatographed on a column of type LS 100/160 μm silica gel in a ratio of material to sorbent of 1:80, with elution by benzene. Fractions 4-13 yielded an oily substance — 3-mono-O-acetyllagohirsin, $C_{22}H_{34}O_6$ (M^+ 394).

Fractions 15-21 yielded an oily substance — 18-mono-O-acetyllagohirsin, $C_{22}H_{34}O_6$ (M^+ 394).

Reduction of Lagohirsin (I). A solution of 0.15 g of substance (I) and 30 ml of anhydrous tetrahydrofuran was added to a suspension of 0.3 g of lithium tetrahydroaluminate in 50 ml of tetrahydrofuran. The mixture was boiled for 1 h. After the usual working up, lagochilin (III), $C_{20}H_{36}O_5$ mp 167-168°C (from ether) was obtained.

Acetylation of Lagohirsin (I). A mixture of 0.1 mg of (I) and 5 ml of acetic anhydride was left for a day. After the usual working up, di-O-acetyllagohirsin was obtained: $C_{24}H_{36}O_7$, mp 126-127°C (from ether), $[\alpha]_D^{24}$ −44° (*c* 1; alcohol).

Synthesis of Lagohirsin. Preparation of 3,18-O-Isopropylidenelagochilin (IV). A solution of 4 g of III in 50 ml of anhydrous acetone was treated with 1.5 g of anhydrous copper sulfate, and the mixture was stirred for 8 h. Then it was filtered, the solvent ws distilled off, and the residue was transferred to a column of silica gel (type LS 100/250 μm) in a ratio of material to sorbent of 1:50. On elution with benzene–ether with gradually increasing proportions of the latter, fractions 20-31 yielded (IV) (1.2 g), which was crystallized from ether, mp 150-151°C, $[\alpha]_D^{20}$ −42° (*c* 0.5; alcohol).

Oxidation of 3,18-O-Isopropylidenelagochilin (IV). A solution of 1 g of (IV) in 40 ml of acetone was treated with 5 g of potassium permanganate, and the mixture was stirred for 15 h. The excess of potassium permanganatae was decomposed with oxalic acid. The solution was filtered and, after evaporation of the solvent, the residue was transferred to a column of silica gel (type LS 100/160 μm) in a ratio of material to sorbent of 1:40. Elution with hexane–ether (95:5) yielded 0.5 g of (V), mp 107-108°C (hexane–ether (1:1)), $[\alpha]_D^{22}$ −18.5° (*c* 1; alcohol).

Hydrolysis of 3,18-O-Isopropylidenelagohirsin (V). A solution of 0.5 g of (V) in 5 ml of ethanol was treated with 5 ml of a 0.05 M solution of sulfuric acid. The resulting mixture was heated to 60°C over 20 min. Then it was diluted with 5 ml of water and extracted with ether, and the extract was washed with 5 ml of a 0.1 M solution of sodium hydrogen carbonate and with distilled water. After the solvent had been distilled off, lagohirsin (I) was obtained with mp 142-143°C (from ether), $[\alpha]_D^{22}$ −2.0° (*c* 1; alcohol).

Hydrolysis of Di-O-Acetyllagohirsin (II). A solution of 0.05 g of (II) in 6 ml of ethyl alcohol was treated with 6 ml of a 0.2 M solution of sulfuric acid, and the mixture was heated on the water bath at 60°C for 10 min After the working up procedure described above, lagohirsin (I) was obtained with mp 142-143°C (from ether).

Acetylation of 3- and 18-Mono-O-acetyllagohirsins. Compounds (IV) and (V) (0.05) were treated with 3 ml of acetic acid and the mixture was left for a day. After the usual working up, di-O-acetyllagohirsin was obtained , $C_{24}H_{36}O_7$, mp 126-127°C (from ether).

Isolation of the Total Iridoid Glycosides. The air-dry comminuted herbage of *Lagochilus inebrians* (0.5 kg) was exhaustively extracted with methanol with heating under reflux in the water bath. The extract was concentrated in vacuum, the residue was dissolved in 10 ml of water, and the solution was extracted successively with hexane, ethyl acetate, and *n*-butanol. This gave 8.0 g of concentrated *n*-butanol extract and 19 g of aqueous residue containing iridoid glycosides with R_f 0.28 and 0.14 in a chloroform–methanol–water (70:24:4) system (system 1).

Isolation of 8-O-Acetylharpagide (VIII). The *n*-butanolic extrct (8.0 g was subjected to chromatographic separation on a column of silica gel in a ratio of material to sorbent of 130 in system 1. Fractions with a volume of 30-50 ml were collected. Fractions 9-18 yielded 630 mg of a white crystalline substance with R_f 0.28 which, after recrystallization from methanol with added chloroform, had mp 154-156°C, $[\alpha]_D^{21}$ −126° (*c* 1, methanol).

Isolation of Harpagide (IX). The aqueous residue (19 g) was chromatographed similarly on a column of silica gel. Fractions 67-75 gave 704 mg of an amorphous yellowish-brown substance with R_f 0.14 $[\alpha]_D^{24}$ −152° (*c* 1; water).

Alkaline Hydrolysis of (VIII). A solution of 50 mg of (VIII) in 6 ml of 5% aqueous methanolic (1:1) NaOH was left at room temperature for 5 h. Then the hydrolysate was neutralized with KU-2 cation-exchange resin and was filtered and evaporated to dryness. According to its R_f value, the residue (71 mg) was identical with a sample of harpagide, $[\alpha]_D^{21}$ −150.8° (*c*, 1.7; water.

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