

Plants of the genus *Ligularia* (family Compositae) are widely distributed in Central Asia [1, 2]. We have investigated the roots of *L. macrophylla* D. C. Prodr. and *L. thomsonii* (Clark) Pojark [1, 2], collected in the Aksu-Dzhabaglinskii reserve, in the upper reaches of the Rivers Ugam and Ak-su, in the mountains of Karatau, and in the Kzylchi-sai gorge.

Substances were found which gave the lactone test, and the IR spectra of chloroform extracts showed an absorption band at 1780 cm^{-1} which is characteristic for sesquiterpene lactones (these substances were absent from the epigeal part of the plant). TLC revealed seven substances, with R_f 0.98 (violet coloration), 0.95 (crimson), 0.85 (violet-blue), 0.76 (red), 0.65 (red), 0.48 (lilac), and 0.3 (blue). The qualitative compositions of these substances in different species were similar and differences were shown only in the ratio of the individual components.

When a concentrated ethanolic extract of the roots of *L. macrophylla* was treated with petroleum ether, a lactone was obtained with the compositions $C_{15}H_{18}O_3$, mp $136-137^\circ\text{C}$, $[\alpha]_D^{20} -50^\circ$ (c 0.5; dioxane). IR spectrum, ν_{\max} , cm^{-1} : 1770 (carbonyl of a γ -lactone), 1640, 1570 (double bond).

NMR spectrum (in CDCl_3): singlets at 7.01 and 5.03 ppm, 1 H each (proton of a furan ring and lactone proton, respectively), 1.96 and 1.21 ppm (3 H each, methyl on a double bond and angular methyl, respectively), and a doublet at 2.56 ppm, $J = 7.5\text{ Hz}$, 2 H (methylene protons at C-9). Reduction of the lactone with NaBH_4 gave a known ether of a diol $C_{15}H_{20}O_2$ with mp $114-115^\circ\text{C}$ [3]. On the basis of its physicochemical constants and IR and NMR spectra, this lactone was identified as furoeremophilan-14 β ,6 α -olide isolated previously from *L. hodgsonii* Hook. [3], *L. gaurieri* and *L. angusta* [4]. By chromatography, we isolated the same lactone from the roots of *L. thomsonii* collected in the Kzylchi-sai gorge. This is the first time that furoeremophilan-14 β ,6 α -olide has been isolated from plants of the genus *Ligularia* growing in the Soviet Union.

By chromatography of an ethanolic extract of *L. macrophylla* we isolated two hydrocarbons with the compositions $C_{11}H_{18}$, $M^+ 150$, and $C_{17}H_{26}$, $M^+ 240$, and a crystalline substance with mp 136°C identified by a mixed melting point as β -sitosterol.

Elution with acidified methanol gave a second sesquiterpene lactone with the composition $C_{16}H_{22}O_4$, mp $191-192^\circ\text{C}$, which proved to be new and which we have called ligolide.

In its UV spectrum, maxima at 225 nm ($\log \epsilon 4.38$) and 265 nm (inflection) show the presence of three multiple bonds in the molecule. The IR spectrum (Fig. 1) shows adsorption bands at 3200-3500 (hydroxy group), 2860, 2930, and 2960 (C-methyl groups), a broadened band at 1730-1750 (carbonyl of an unsaturated five-membered lactone), and bands at 1700 and 1660 (double bond in a lactone ring). The substance was not saponified by alkali and did not react with 2,4-dinitrophenylhydrazine.

The mass spectra contained the peaks of the molecular ion, $M^+ 278$, and fragments with m/e 260 [$M^+ - \text{H}_2\text{O}$] $^+$ and 246 [$M - \text{OCH}_2$] $^+$, showing the presence of one hydroxy and one methoxy group, and also at 218 [$M^+ - \text{OCH}_2 - \text{CO}$] $^+$, 203 [$M^+ - \text{OCH}_2 - \text{CO} - \text{CH}_3$] $^+$, and 188 [$M^+ - \text{OCH}_3 - \text{CO} - 2\text{CH}_3$] $^+$, which are characteristic for eremophilanolides [8].

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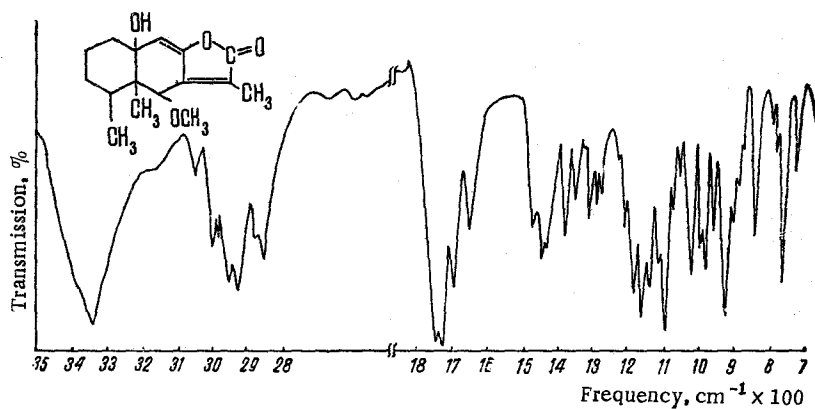


Fig. 1. IR spectrum of ligolide (in KBr).

The NMR spectrum (in deuteropyridine) of the lactone showed three-proton signals in the form of a singlet at 1.85 ppm (methyl on a double bond), a singlet at 0.8 ppm (angular methyl), and a doublet at 0.86 ppm (secondary methyl). The presence of these signals is characteristic for ligularenolide [5]. However, as compared with the latter, the absorption maximum in the UV spectrum of ligolide is further towards the shortwave region, which shows the presence of not four but three multiple bonds, i.e., the absence of a double bond at C₁-C₁₀. This is confirmed by the NMR spectrum, in which there is the signal of only one olefinic proton — a sharp singlet at 5.70 ppm. The fact that it is not split shows that there is no proton on the neighboring carbon atom (C₁₀), which is possible only if a hydroxy group is present in this position.

The NMR spectrum also contains a three-proton singlet at 3.02 ppm and a broadened one-proton singlet at 4.15 ppm caused by a methoxy group and a methine proton in a CH₃OCH fragment, respectively. Since the latter signal is not split, it may be concluded that the adjacent carbon atoms are quaternary and, consequently, the methoxy group is located in position 6. This conclusion is in harmony with biogenetic considerations [6, 7]. The nature of the signal of the hemimethoxy proton and literature information [3] permit the assumption that the methoxyl at C₆ and the hydroxyl at C₁₀ each have the β orientation. Thus, ligolide most probably has the structure of 10 β -hydroxy-6 β -methoxyeremophilan-7-olide or 6-methoxy-10-hydroxyligularenolide, as shown in Fig. 1.

By a chromatographic method, the roots of *L. thomsonii* yielded a lactone with mp 190°C which we have designated L2. Its IR spectrum contains bands at 1730-1760 cm⁻¹ (carbonyl of an unsaturated lactone), 1440-1460 cm⁻¹ (double bonds), 3400-3500 cm⁻¹ (hydroxyl), and 2860, 2920, 2950, and 2980 cm⁻¹ (C-methyl groups). A comparison of its IR spectrum with that of ligolide and a mixed melting point showed that they were different. In addition to the lactones mentioned we have established the presence of noncrystallizing lactones in both species the study of which is a matter of undoubted interest.

EXPERIMENTAL

The NMR spectra were taken on a "Jeol" instrument at 60 MHz, the IR spectra on a Hitachi spectrophotometer, and the mass spectra on an MKh-1303 instrument. TLC was performed on "Silufol" plates in the benzene-methanol-ethyl acetate (12:3:1) system. The spots were revealed by spraying the chromatograms with a 1% solution of vanillin in concentrated H₂SO₄.

L. macrophylla. Furoereniophilan-14 β ,6 α -olide. The comminuted roots collected in the Karatau mountains in the flowering period (6.5 kg) were comminuted and extracted with ethanol (3 \times 6 liters). The extracts were combined, the ethanol was distilled off, and the concentrated extract was diluted with water (1:3). The curd-like precipitate that deposited (197 g) was filtered off with suction, and 57 g of it was extracted with petroleum ether in a Soxhlet apparatus. Concentration of the extract yielded 30 g (1.6%) of a crystalline substance with mp 136-137°C (from petroleum ether) with the composition C₁₅H₁₈O₃, M⁺ 246, R_f 0.76-0.8 (red coloration).

Diol Ether. To 0.5 g of the substance in 25 ml of ethanol was added 2.5 g of sodium tetrahydroborate, and the mixture was left for 12 days. Then the solvent was distilled off

and the residue was mixed with water and treated with ether. Evaporation of the ethereal extract yielded colorless crystals with mp 114-115°C (from ethanol).

Hydrocarbons. The aqueous solution (the mother solution after the separation of the furoeremophilanolide) was treated with ether. The extracts were combined, the ether was evaporated off, and 30 g of the resin obtained was chromatographed on a column of silica gel (22 × 8.0 cm). On elution with hexane the first fractions yielded by reprecipitation from methanol two neutral paraffin-wax-like hydrocarbons with R_f 0.98 (violet coloration) and 0.95 (crimson) with the compositions $C_{11}H_{18}$ and $C_{17}H_{36}$, respectively.

β -Sitosterol. When the column was eluted with benzene and the product was crystallized from ethanol, a substance was obtained with mp 136°C, R_f 0.65 (red coloration). A mixture of the substance with sitosterol showed no depression of the melting point.

Ligolide. By washing the column with hexane-ethyl acetate (9:1) and crystallization from petroleum ether, we obtained a substance with mp 137°C, which was identified by a mixed melting point with furoeremophilanolide. When the column was then washed with methanol acidified with 1% acetic acid, a colorless substance was isolated which was recrystallized from ethanol, giving crystals with mp 191-192°C, R_f 0.3 (blue coloration), M^+ 278, readily soluble in organic solvents and in aqueous ethanol.

L. thomsonii. The dried roots collected in the Kuraminskii range (Kzylchi-sai gorge) (50 g) were comminuted and extracted with chloroform. This gave 3.8 g (7.6%) of resin which was transferred to a column of silica gel (13 × 6 cm). When it was washed with gasoline (mp 120°C), the first fractions yielded two hydrocarbons with R_f 0.98 (violet coloration) and 0.88 (crimson).

Elution with benzene gave a substance with mp 136-137°C, R_f 0.76 (red coloration). A mixture of the substance with furoeremophilanolide isolated from *L. macrophylla* showed no depression of the melting point.

The latter fractions, obtained by washing the column with benzene-ethyl acetate (9:1) yielded, on recrystallization from ethanol, a colorless crystalline substance with mp 190-192°C (L2).

SUMMARY

From the roots of *Ligularia macrophylla* D. C. Prodr. we have isolated a new sesquiterpene lactone ligolide with the composition $C_{16}H_{22}O_4$, for which the structure of 10 β -hydroxy-6 β -methoxyeremophilan-7-olide is proposed. From the same plant and from *L. thomsonii* (Clark) Pojark. we have isolated and identified furoeremophilan-14 β ,6 α -olide, β -sitosterol, and hydrocarbons with the compositions $C_{11}H_{18}$ and $C_{17}H_{36}$.

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