

The isolation from the leaves of *Inula grandis* of a sesquiterpene hydroxylactone - grandulin - and a sesquiterpene oxolactone - grandicin - has been reported previously [1]. Further investigations have shown that the composition of the lactones and their ratio depend fundamentally on the growth site of the plants. Thus, in the leaves of *inula* growing in the Almalyk region (Tashkent oblast) we found grandulin and a considerable amount of grandicin, while in the same plant growing in the region of the town of Bol'shoi Chimgan other lactones were found, with only a small amount of grandicin (0.07%), and granulin was completely absent.

From the raw material collected in the spring during the period of stem formation on the slopes of the Chimgan we have isolated by chromatography a new lactone, $C_{15}H_{20}O_4$, mp 197-198°C (from methanol), $[\alpha]_D^{25} + 147.9$ (acetone; c 1.02), R_f 0.44, M^+ 264, which we have called granilin. It is readily soluble in acetone, sparingly in chloroform, benzene, and ethanol, and insoluble in carbon tetrachloride and water.

The UV spectrum of granilin has a maximum in the 208 nm region ($\log \epsilon$ 4.20) which shows the presence in it of an exocyclic methylene group conjugated with a lactone carbonyl [2]. The IR spectrum (Fig. 1a) has maxima at 1757 cm^{-1} (carbonyl of an unsaturated γ -lactone), 1640 and 1653 cm^{-1} (stretching vibrations of a C=C bond), 3450-3150 cm^{-1} (hydroxy groups), and 2860, 2900, and 2930 cm^{-1} (C-methyl groups). In addition there are bands due to the deformation vibrations of the C-H bonds of exocyclic methylene groups, one of which is conjugated with a carbonyl (910 cm^{-1}) while the second (797 cm^{-1}) is adjacent to an oxygen atom [3].

The presence of two hydroxy groups was confirmed by the preparation of a diacetate with mp 118-123°C, M^+ 348, the NMR spectrum of which showed two three-proton singlets at 2.05 and 1.95 ppm. The lactone contains two double bonds and on catalytic hydrogenation over PtO_2 in acetic acid it absorbs 2 moles of hydrogen, forming a tetrahydro derivative with mp 82-83°C (decomp.), M^+ 268, the IR spectrum of which (Fig. 1b) does not contain the absorption bands of 1640, 1656, 910, and 797 cm^{-1} corresponding to double bonds. Its dehydrogenation with selenium did not lead to the formation of azulenes. With the given composition and the presence of a methyl group on a tertiary carbon atom and of two double bonds, it cannot be either a guaianolide, a germacranolide, or an elemanolide and must be assigned to the group of eudesmane derivatives.

The insolubility of the substance in $CHCl_3$, CCl_4 , and pyridine made it difficult to obtain its NMR spectrum. This could be obtained only in CF_3COOH , which, however, complicated its interpretation since in this case the positions of the signals proved to be shifted downfield by 0.2-0.25 ppm in comparison with the spectra of its derivatives taken in other solvents. In the NMR spectrum of granilin (Fig. 2a) there are four broadened one-proton singlets at 6.35, 5.97, 4.92, and 5.26 ppm, and in the spectrum of the diacetate there are signals at 6.07, 5.56, 4.77, and 5.17 ppm due to exocyclic methylene groups. By analogy with ivasperin [4], ivalin [5], and telekin and its derivatives [6, 8], the first pair of singlets corresponds to the protons of a conjugated methylene group located in a lactone ring and the second to the protons of a non-conjugated methylene at C-4. On considering literature information, it can be seen that the positions of the signals of the protons of this group at C-4 depend to a certain extent on the substituents present in the vicinal position (C-3). Thus, in ivasperin [4], telekin [8], and asperilin [7] with no electron-accepting groups in position C-3, these signals are in the 4.90 and 4.59 ppm regions, while in erivanin [9] and dihydro-

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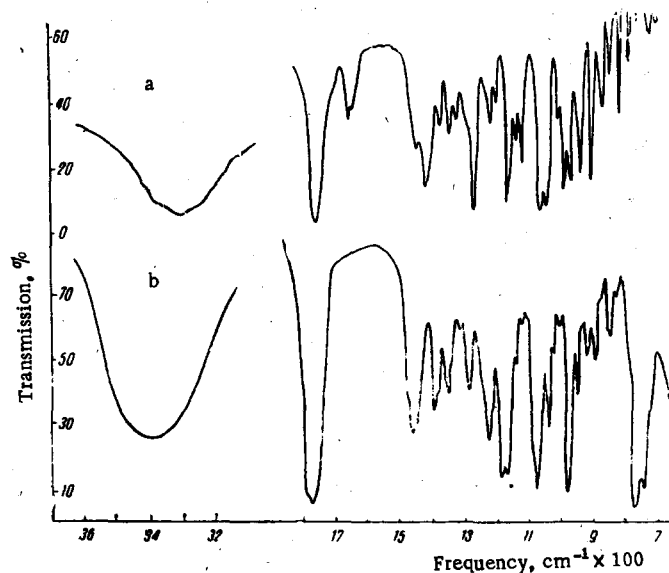


Fig. 1. IR spectra (KBr) of granilin (a) and of tetrahydrogranilin (b).

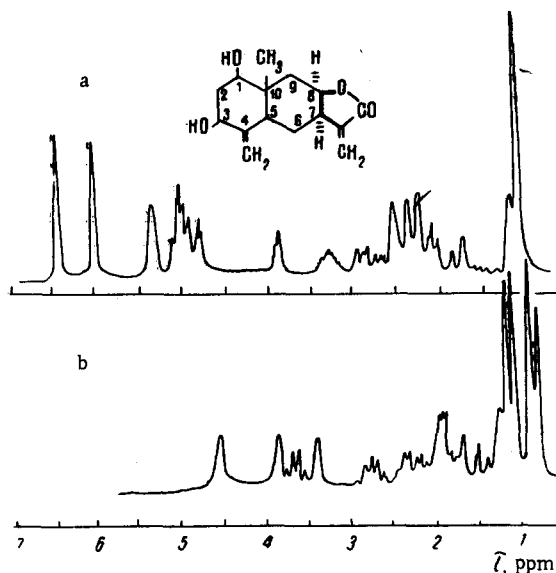


Fig. 2. NMR spectra of granilin (a) and of tetrahydrogranilin (b).

3-epiisotelekin [8] (with C_3 -OH) they are, respectively, at 5.13 and 4.95 and at 5.10 and 4.62 ppm, and in 3-oxoiso- and 3-oxodihydroisoalantolactones [8] (with C_3 =O) they are at 5.9 and 5.1 ppm. Taking this and the chemical shift of the signals of the same protons of granilin into account, it may be considered that in the molecule of the latter one of the hydroxy groups is present in position 3. In the NMR spectrum of tetrahydrogranilin the signals of the protons of the exocyclic methylene groups had disappeared, and two three-proton doublets had appeared at 1.14 and 0.83 ppm, $J=7$ Hz, corresponding to the protons of two methyl groups on secondary carbon atoms.

The signal of the lactone proton is present in the spectrum in the form of a multiplet at 4.92 ppm (4.56 ppm in the spectrum of the diacetate; 4.52 ppm in the tetrahydro derivative; and 4.45 ppm in the diacetate of the tetrahydro derivative). Its multiplicity, particularly well observed in the spectrum of the diacetate of tetrahydrogranilin, and its small half-width (8 Hz) permit the conclusion [9] that the granilin molecule is linear and the lactone ring is linked in the cis position, as in biogenetically close compounds – alantolactone [10], telekin [8], and others isolated from *Inula helenium* L. and *Telekia speciosum* Schreib.

A quintet at 2.72 ppm, $J=7.5$ Hz (1H), and a quartet at 3.62 ppm, $J_1=14$ Hz, $J_2=7.0$ Hz (1H each), observed in the spectrum of tetrahydrogranilin, in agreement with those of 3-epiisotelekin and 3-oxoisalantolactone [8], may be assigned respectively to the protons at C-5 and C-7. Both hydroxy groups of granilin are secondary, as is shown by the presence of the signals of the two methine protons present in the geminal position at 4.71 and 4.92 ppm. In the NMR spectrum of granilin diacetate, these signals undergo a paramagnetic shift and appear in the form of poorly resolved triplets at 5.30 (1H) and 4.56 ppm (2H); the half-width of the first is 8 Hz, but the half-width of the second could not be determined since this signal overlaps the signal of the lactone proton. In the spectrum of the tetrahydro derivative (Fig. 2b), these protons give two multiplets at 3.35 and 3.84 ppm (half-width of each 8 Hz) which, in the diacetyl derivative, are

likewise shifted downfield [multiplets at 4.68 (1H) and 4.45 ppm (2H)]. The latter overlaps the multiplet of the lactone proton. The small width of the signals mentioned and their poor resolution do not permit an unambiguous answer to the question of whether they are in the axial or the equatorial position. In the IR spectrum (KBr) of granilin acetate the stretching vibrations of the C-OAc group give bands at 1020 and 1030 cm^{-1} corresponding to the equatorial position of the acyl residues, but in the analogous spectra of granilin bands are present at 1000 and 1015 cm^{-1} and in the tetrahydro derivative at 1012 and 1031 cm^{-1} which may be assigned to the stretching vibrations of an axial C-OH group.

Since both hydroxy groups are secondary, this excludes their presence in the C-5, C-7, and C-8 positions. One of them, as has been shown above, is most probably at C-3. Granilin is not oxidized by periodic acid, which excludes the location of the hydroxyls on adjacent carbon atoms (C-3, C-2), and therefore the second hydroxyl may be in position 1, 6, or 9. To determine the position of this hydroxyl, we acetylated tetrahydrogranilin, which caused a paramagnetic shift of the signals of the protons on the neighboring carbon atoms. In the NMR spectrum of tetrahydrogranilin acetate there were singlets at 1.94 and 1.98 ppm (3H each) due to the two acetyl groups, and a paramagnetic shift of the signals of the methyl groups at C-4 and C-10. The shift of the first signal was 0.07 ppm and that of the second 0.14 ppm. The shift of the signal of the angular methyl group showed the presence of an acetyl group at C-1 or C-9. The stable position of the signal of the lactone proton and the nature of its resolution excludes the possibility of the second variant, and therefore the hydroxy groups in granilin are located at C-1 and C-3. Consequently, granilin has the structure of 1,3-dihydroxyeudesma-4(14),11(13)-dien-7,8-olide.

EXPERIMENTAL

Isolation of Granilin. The dried comminuted raw material was treated with methanol, the extract was concentrated in vacuum to small volume, and it was extracted successively with gasoline, carbon tetrachloride, and chloroform. The chloroform extract was evaporated and passed through a column filled with Kapron and alumina (activity grade IV). When the latter sorbent was washed with benzene and the eluate was concentrated, large colorless crystals deposited with mp 197-198°C (from methanol), R_f 0.44. Yield 0.04%.

Granilin diacetate was obtained by heating the substance with acetic anhydride in pyridine for 8 h in the form of colorless crystals with mp 118-123°C, R_f 0.77.

Tetrahydrogranilin. The hydrogenation of 0.1978 g of the substance in solution in 15 ml of acetic acid was performed in the presence of 0.0253 g of PtO_2 at room temperature until the absorption of hydrogen ceased. The catalyst was filtered off, and the filtrate was diluted with water and treated with ether. The ethereal extract was washed with sodium carbonate solution and with water and was dried and distilled. The residue was dissolved in 2 ml of ethyl acetate, 10 ml of petroleum ether was added, and the mixture was left in the refrigerator. The oily residue containing scattered crystals was separated by decantation and triturated with 5 ml of ether until the solvent had evaporated off. This gave a colorless crystalline substance with mp 82-83°C (decomp.).

Tetrahydrogranilin diacetate was obtained by heating the substance with acetic anhydride and sodium acetate for 3 h. A colorless amorphous substance was isolated.

The IR spectra were taken on a UR-20 spectrometer (KBr), the NMR spectra on a JNM-100/100-4H instrument at 100 MHz (solution of granilin in CF_3COOH and of granilin acetate and tetrahydrogranilin in CDCl_3) and on a JEOL instrument at 60 MHz (solutions of tetrahydrogranilin and its acetate in CDCl_3); the signals are given in the δ scale from the signal of HMDS taken as 0. The mass spectra were taken on an MKh-1303 instrument. The purity of the substances was checked by thin-layer chromatography in silica gel (Silufol) in the benzene-methanol-ethyl acetate (12:3:1) system, the spots being revealed with conc. H_2SO_4 containing 1% of vanillin.

SUMMARY

From the epigeal part of *Inula grandis* Schrenk., collected in the rosette-incipient stem formation period, a new sesquiterpene lactone has been isolated with the formula $\text{C}_{15}\text{H}_{20}\text{O}_4$, mp 197-198°C, $[\alpha]_D^{25} + 147.9^\circ$ (acetone), and we have called it granilin. On the basis of the NMR spectra of the initial substance and the products of its hydrogenation and acetylation, it has been established that granilin has the most probable structure of 1,3-dihydroxyeudesma-4(14),11(13)-dien-7,8-olide.

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