THE STRUCTURE OF INULICIN – A NEW SESQUITERPENE LACTONE FROM Inula japonica

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Inula japonica Thunb. (Japanese inula) is widely used in popular medicine as a rapid-healing agent [1]. However, its active principle has not so far been studied.

From the epigeal part of Japanese inula we have isolated [2] a new sesquiterpene lactone – inulicin (I) $C_{17}H_{24}O_5$, with mp 125.5-126.5°C, $[\alpha]_D^{20}+90.11°$ (c 4.69; chloroform). IR spectrum of the lactone: ν_{max} 3502 cm^{-1} (OH), and 1745, 1660, and 1260 cm^{-1} (α-methylene-γ-lactone and $OCOCH_3$). The UV spectrum has an absorption maximum with λ_{max} 204 nm, ε 13,284 – which is typical for an α-methylene-γ-lactone [3].

Inulicin contains one hydroxy group as determined by the Chugaev–Tserevitinov [Zerewitinoff] method, and with acetic anhydride in pyridine it forms a monoacetyl derivative (II), $C_{19}H_{26}O_6$, mp 83.5-85.5°C.

The hydrogenation of inulicin over an Adams Pt catalyst in ethanol gave dihydroinulicin (III), $C_{17}H_{26}O_5$, from which was obtained acetyldihydroinulicin (IV), $C_{19}H_{28}O_6$, in the IR spectrum of which there was no absorption band of a hydroxy group.

The hydroxy group is secondary, as was shown by the oxidation of dihydroinulicin to an α,β-unsaturated ketone (V), $C_{17}H_{24}O_5$. Its UV spectrum exhibited a new absorption maximum, with λ_{max} 250 nm, ε 7200.

The saponification of inulicin consumed two equivalents of alkali; from the hydrolysis products after acidification desacetylinulicin (XIII), $C_{15}H_{22}O_4$ (a vitreous product), was obtained.

The hydrolysis of dihydroinulicin gave two substances: desacetyldihydroinulicin (VI), $C_{15}H_{24}O_4$ (vitreous product), and a substance (VII), $C_{15}H_{22}O_3$, with mp 162-164°C, in the IR spectra of which there was no hydroxyl absorption band. The dehydrogenation of a mixture of (VI) and (VII) gave chamazulene (XV) in extremely low yield.

The hydrogenation of inulicin over a Pt catalyst (from Pt_2O) in acetic acid gave two vitreous products: tetrahydroinulicin (VIII), $C_{17}H_{28}O_5$, and deoxytetrahydroinulicin (IX), $C_{17}H_{28}O_4$. The hydrolysis of (IX) formed a hydroxy lactone (X), $C_{15}H_{26}O_3$ (vitreous product). Its oxidation with chromic acid gave an acid (XI), $C_{15}H_{24}O_4$ in the form of a vitreous product.

The facts given above permit the conclusion that inulicin contains one secondary hydroxy group, two double bonds, a γ-lactone system, and an acetoxy group, the latter being attached to a primary carbon atom. The composition $C_{17}H_{25}O_5$ (the molecular weight of inulicin was determined mass spectrometrically) and the presence of the functional groups mentioned show that inulicin is a monocyclic sesquiterpene lactone.

At the present time, four types of carbon skeletons of natural monocyclic sesquiterpene lactones are known: elemane (XVI), germacrane (XVII), xanthane (XVIII), and psilostachane (XIX).

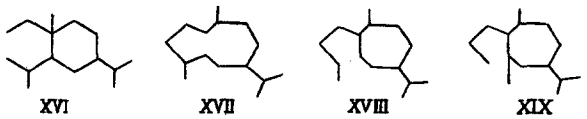

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TABLE 1. Chemical Shifts and Coupling Constants of the Protons of Inulicin and Its Derivatives, ppm

Substance	Solvent	C_2	C_3	C_4	C_5	C_6	C_8	C_{10}	C_{13}	C_{14}	C_{15}
I	Pyridine; HMDS	2,61 m	4,30 t J=7 Hz	3,59 m	4,95 m	2,26 q 2,94 m	—	3,95 m	5,58 6,22 2 d J=3 Hz	1,52 d J=2 Hz	1,08 d
II		2,51 m	5,25 d J=2,2 Hz	3,50 m	4,85 m	2,29 2,61 2 q	—	3,91 t	5,80 6,28 2 d J=3 Hz	1,52 d J=2 Hz	0,77 d
III		—	4,25 s	2,90	4,86 m	2,45 2,75 2 q	—	4,04 t	1,28 d	1,79 s	1,14 d
IV		—	5,40 s	—	4,86 m	—	—	4,02 t	1,37 d	1,83 s	0,91 d
VII	Chloroform; TMS	—	—	—	4,79 m	—	5,45 d	—	1,12 d	1,22 d	1,29 s
XII		—	—	3,15 t J=12Hz	4,78 m	—	—	3,93 t J=14 Hz	1,50 d	0,75 d	0,75 d
XIII		—	5,02 d J=8 Hz	—	4,02 q	—	—	—	5,90 6,26 2 d	1,72 s	1,05 d

Note. TMS – tetramethylsilane; HMDS – hexamethyldisiloxane; m – multiplet; t – triplet; d – doublet; s – singlet; q – quartet.

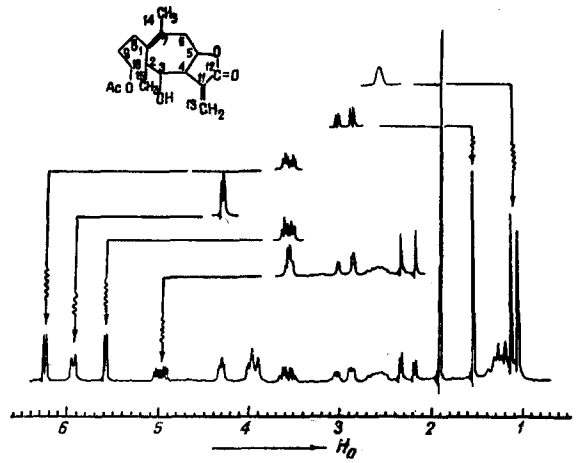


Fig. 1. NMR and double-resonance spectra of inulicin.

The first type is excluded on the basis of the NMR spectrum of inulicin, since there is no signal of the methylene singlet at 0.8-1.1 ppm, that is characteristic for the angular methyl of the elemanolides.

The NMR spectrum of inulicin has the signals of the protons of a secondary methyl (a doublet at 1.08 ppm), of a methyl adjacent to a double bond (a doublet at 1.52 ppm), of an acetylmethyl a singlet at 1.95 ppm), and of an exocyclic methylene conjugated with a γ-lactone carbonyl [two doublets at 5.58 and 6.22 ppm (J = 3 Hz)] and a 1-H triplet at 4.30 ppm which we have assigned to the signal of a hemihydroxylic proton, since in acetylinulicin and acetyldihydroinulicin its chemical shift is found in the weak-field region (Table 1). A 1-H multiplet with a center at 4.95 ppm is the signal of a lactone proton, a 2-H multiplet at 3.95 ppm the signal of methylene protons connected with an acetoxy group, and a 1-H multiplet at 3.59 ppm the signal of a proton at C_4. In dihydroinulicin, this signal is shifted upfield, and the signals of the exocyclic methylene have disappeared.

To assign the signals in inulicin, we used double resonance with a strong radiofrequency field (H_2 r.f. field). Figure 1 shows the spectrum of inulicin and the double-resonance spectra. The assignment of the signal at 3.59 ppm to the C_4 proton is due to the change in the multiplicity of this signal on the irradiation with the H_2 r.f. field of the signals of the exocyclic vinyl group. The H_4 proton, as well as the C_{13} protons, interacts with the protons the signals of which are located at 4.30 and 4.95 ppm, their coupling constants being 3 and 8.5 Hz, respectively.

The large coupling constant (J = 8.5 Hz) [4, 5] shows that the signal at 4.95 ppm is due to the lactone proton. None of the other coupling constants of the H_4 proton exceeds 3 Hz. The signal at 4.30 ppm is ascribed to the geminal (to the hydroxy group) proton. A double bond in the α position to the methylene protons at C_6 is shown by the comparatively large value of the geminal constant (J = 17 Hz) between the H_6 and H_6' protons [6, 7]. In the fragment $-\overset{|}{C}-CH_2-\overset{|}{C}-$ the geminal constant does not exceed 13 Hz, as a rule [8].

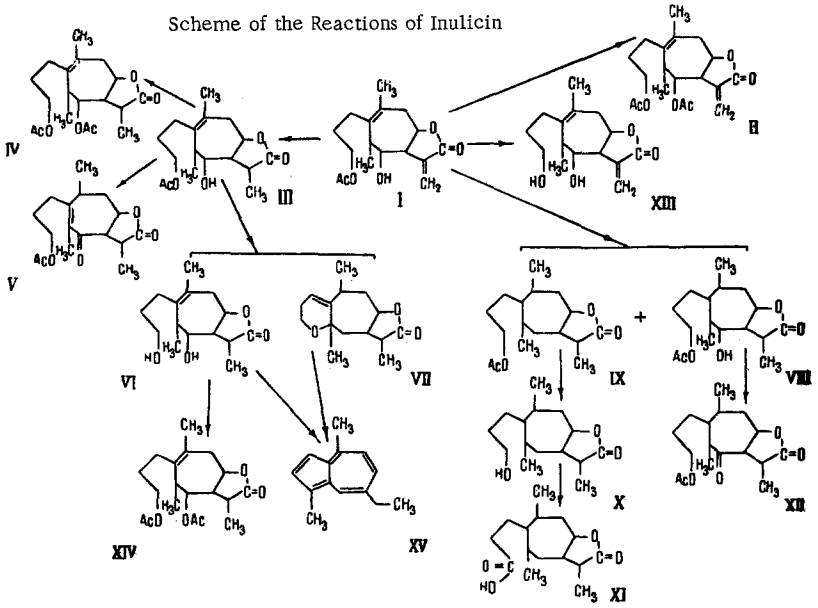
The existence of splitting of the signal of the hydroxy proton on coupling with the vicinal H_3 proton is extremely interesting, since this is generally absent from the spectra of organic compounds because of the rapid exchange of the mobile hydrogens in solution. The reasons for this phenomenon in inulicin remain obscure. The absence of vinyl protons in (I) while the signal of methyl protons adjacent to a double bond is present enables the germacrane carbon skeleton to be excluded for inulicin. The formation of the acid (XI) also includes the xanthane type.

The results of the chemical transformations and the NMR spectra and also the formation of chamazulene (XV) in the dehydrogenation of (VI) and (VII) permit the assumption for inulicin of the psilostachane carbon skeleton. To determine the position of the hydroxy group and the lactone ring in the lactone, tetrahydroinulicin (VIII) was oxidized to the keto derivative (XII), $C_{17}H_{26}O_5$, (mp 113-115.5°C), in the IR spectrum of which a strong absorption band appeared at 1720 cm^{-1} (cycloheptanone). Substance (XII) gave a negative Zimmerman test for $CO-CH_2$. Consequently, the hydroxy group can be present only at C_3, while for the lactone ring the C_4-C_5 position remains. This conclusion is in full harmony with the single- and double-resonance NMR spectra of inulicin, from the results of which it follows that there is a methylene group adjacent to the lactone proton. Thus, structure (I) is possible for inulicin.

In the NMR spectrum of desacetylinulicin (XIII) the lactone proton is represented by a doublet. This shows a reorientation of the lactone ring, which is found not infrequently in sesquiterpene lactones with a hydroxyl in a neighboring position [9]. The appearance of an α,β-unsaturated ketone in the oxidation of (III) is due to the migration of the double bond, the readiness of which to shift can probably explain the formation of (IX) in the hydrogenation of inulicin in an acid medium.

The NMR spectrum of (VII) lacks the signal of a methyl at a double bond, while it contains a singlet at 1.29 ppm – methyl attached to oxygen; two doublets at 1.11 and 1.22 ppm – $2CH_3CH$; a multiplet at 4.79 ppm – a lactone proton; and a doublet at 5.45 ppm (1H) – a vinyl proton. This shows that the substance $C_{15}H_{22}O_3$ must have the structure (VII). However, we do not exclude the possibility that the double bond is located at C_3-C_4.

Scheme of the Reactions of Inulicin



EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrophotometer, the UV spectra on an SF-4 instrument, and the NMR spectra on a Varian HA instrument, 100 MHz. Thin-layer chromatography (TLC) was performed on neutral alumina (activity grade IV) in the benzene–methanol (9 : 1) and diethyl ether systems; in both cases the spots were revealed with a 0.5% solution of $KMnO_4$ in 0.5% H_2SO_4.

The analytical results for all the compounds corresponded to the calculated figures.

Acetylinulicin (II). A mixture of 0.20 g of inulicin, 2 ml of acetic anhydride, and 4 ml of pyridine was heated at 50°C for 1 h, diluted with water, and extracted with chloroform. The chloroform extracts were washed with 3% HCl solution and with water. After the chloroform had been distilled off, crystals of (II), $C_{19}H_{26}O_6$, mp 83.5-85.5°C (from ethanol), formed. Yield 0.21 g. IR spectrum, ν_{max}, cm^{-1}: 1765 (γ-lactone), 1735 and 1230-1270 ($OCOCH_3$), 1660 (C=C).

Dihydroinulicin (III). In 45 ml of ethanol, 0.50 g of inulicin was hydrogenated in the presence of 0.075 g of PtO_2 (Adams). Hydrogenation stopped 15 min after the absorption of 1 mole of hydrogen. The catalyst and solvent were removed, and the residue consisted of dihydroinulicin, $C_{17}H_{26}O_5$, mp 60-61°C (from ether). Yield 0.4 g. IR spectrum, ν_{max}, cm^{-1}: 3510 (OH), 1745 (γ-lactone), 1250-1270 ($OCOCH_3$), 1670 (C=C).

Similar results were obtained on hydrogenation over a Raney Ni catalyst (in ethanol).

Acetyldihydroinulicin (IV). A mixture of 0.22 g of (III), 4.5 ml of pyridine, and 2.5 ml of acetic anhydride was heated at 50°C for 1 h. The reaction mixture was treated as in the preparation of (II). The residue consisted of a viscous crystallizing liquid from which, on the addition of petroleum ether, crystals of (IV) deposited with the composition $C_{19}H_{28}O_6$, mp 69-71°C. Yield 0.23 g. IR spectrum, ν_{max}, cm^{-1}: 1740-1770 and 1245-1265 (γ-lactone and $OCOCH_3$).

Dehydrodihydroinulicin (V). A mixture of 0.65 g of (III), 12 ml of pyridine, and 0.65 g of CrO_3 was left at 0°C for 24 h, diluted with water, and extracted with a mixture of benzene and diethyl ether (1 : 1). The extract was washed with 3% HCl solution and with water. The residue after the elimination of the solvents was chromatographed on 3.5 g of neutral alumina (activity grade IV). The column was washed with benzene. The benzene–ether fraction yielded a colorless vitreous substance, $C_{17}H_{24}O_5$. Yield 0.28 g. IR spectrum, ν_{max}, cm^{-1}: 1780 (γ-lactone), 1745 and 1250 ($OCOCH_3$), 1660 (C=C), 1630 (α,β-unsaturated ketone).

Hydrolysis of (I): Preparation of Desacetylinulicin (XIII). A. A mixture of 0.1166 g of (I) in 15 ml of ethanol and 15 ml of 0.1 N NaOH solution was heated at 70°C: for 5 min in the first experiment and for 20 min in the second. The unconsumed alkali was back titrated with 0.1 N H_2SO_4; two equivs. of alkali had been consumed.

B. A mixture of 0.5 g of (I) and 25 ml of 4% KOH solution was left at room temperature for 39 h, after which it was acidified with 10% H_2SO_4 to pH 1 and was extracted with ethyl acetate; the extract was washed with 5% $NaHCO_3$ and with water and was distilled under vacuum. This gave a vitreous product, $C_{15}H_{22}O_4$ (on TLC in the diethyl ether system, R_f 0.18). Yield 0.17 g. IR spectrum, ν_{max}, cm^{-1}: 3440 (OH), 1750 (γ-lactone), 1660 (C=C).

Hydrolysis of (III). Preparation of (VI) and (VII). A mixture of 4.6 g of (III), 80 ml of ethanol, and 100 ml of 4% NaOH solution was heated at 55-60°C for 1 h, and then the ethanol was distilled off under vacuum and the residue was acidified with 10% HCl to pH 2. The reaction product was extracted with chloroform, the extract being washed with 5% K_2CO_3 solution and with water. Elimination of the chloroform gave 3 g of a colorless viscous liquid [on TLC in the benzene–methanol (9:1) system, R_f 0.38], which partially crystallized on drying. On the addition of diethyl ether, crystals of (VII), $C_{15}H_{22}O_3$, precipitated with mp 162-164°C. IR spectrum, ν_{max}, cm^{-1}: 1780 (γ-lactone).

The residue, after the separation of the crystals, was chromatographed on 10 g of neutral Al_2O_3 (activity grade IV). The column was washed with benzene, and then a benzene–chloroform eluate gave desacetyldihydroinulicin (VI), $C_{15}H_{24}O_4$, in the form of a colorless vitreous product. IR spectrum, ν_{max}, cm^{-1}: 3435 (OH), 1762 (γ-lactone), 1660 (C=C).

The Diacetyl Derivative of (VI) – (XIV). A mixture of 0.5 g of (VI), 10 ml of pyridine, and 5 ml of acetic anhydride was heated at 50-60°C for 1 h and was then treated as described above. This gave 0.6 g of a liquid which was purified on 23 g of neutral alumina (activity grade IV). The column was eluted with benzene. The benzene fraction yielded a yellowish liquid which on rechromatography with diethyl ether gave a colorless vitreous substance with the composition $C_{19}H_{28}O_6$ (on TLC in the diethyl ether system, R_f 0.63). Yield 0.4 g. IR spectrum, ν_{max}, cm^{-1}: 1780 (γ-lactone), 1745 and 1245 ($OCOCH_3$), 1660 (C=C).

Deoxytetrahydroinulicin (IX) and Tetrahydroinulicin (VIII). In the presence of 0.1 g of PtO_2, 5 g of (I) in 75 ml of glacial acetic acid was hydrogenated until the absorption of hydrogen ceased. The consumption of hydrogen was 2.5 moles. After the catalyst had been eliminated, the solution was diluted with water and the reaction product was extracted with chloroform. The chloroform extract was washed with 5% $NaHCO_3$ solution and with water. After the solvent had been distilled off, the viscous liquid (on TLC in the ether system, R_f 0.10 and 0.50) was chromatographed on 92 g of neutral Al_2O_3 (activity grade IV). The column was eluted with ether. The colorless vitreous product obtained in the third fraction (on TLC in diethyl ether, R_f 0.73) was deoxytetrahydroinulicin (IX), $C_{17}H_{28}O_4$. Yield 1.07 g. IR spectrum, ν_{max}, cm^{-1}: 1777 (γ-lactone), 1742 and 1246 ($OCOCH_3$).

On subsequent elution with diethyl ether, the fifth fraction gave a yellowish vitreous substance $C_{17}H_{28}O_5$ (on TLC in the diethyl ether system, R_f 0.48) – tetrahydroinulicin (VIII). Yield 0.51 g. IR spectrum, ν_{max}, cm^{-1}: 3500 (OH), 1772 (γ-lactone), 1748 and 1242 ($OCOCH_3$).

Hydrolysis of (IX) to (X). A mixture of 0.9 g of (IX), 15 ml of ethanol, and 20 ml of 4% NaOH solution was heated at 55-60°C for 1 h. The ethanol was distilled off under vacuum, and the solution was cooled and acidified with 10% HCl to pH 2 and extracted with ethyl acetate; the extract was washed with 5% K_2CO_3 solution and with water, and the solvent was distilled off under vacuum. This gave a vitreous substance, $C_{15}H_{26}O_3$. Yield 0.48 g. IR spectrum, ν_{max}, cm^{-1}: 3420-3500 (OH), 1775 (γ-lactone).

Oxidation of (X): Preparation of the Acid (XI). A mixture of 70 mg of (X), 10 ml of acetone, and 0.4 ml of 8 N chromic acid (prepared from 2.67 g of CrO_3, 2.30 ml of H_2SO_4, and 4.00 ml of water, diluted to 10 ml with water) was left at +4°C for 30 min. Then 0.5 ml of methanol was added, and the reaction mixture was diluted with water and extracted with chloroform; the extract was washed with water, and the solvent was distilled off under vacuum. This gave a yellowish vitreous product, $C_{15}H_{24}O_4$. Yield 50 mg.

The NMR spectrum had a 1-H signal at 10.27 ppm (carboxy proton). IR spectrum, ν_{max}, cm^{-1}: 1775 (γ-lactone), 1711 (carboxy carbonyl).

Dehydrotetrahydroinulicin (XII). A mixture of 0.26 g of (VIII), 7 ml of 90% CH_3COOH, and 0.26 g of CrO_3 was left at 0°C for 2 h. Then it was diluted with water and extracted with chloroform, and the extract

was washed with 5% $NaHCO_3$ solution and with water. The solvent was eliminated under vacuum. This gave a liquid the addition of which to diethyl ether precipitated crystals with the composition $C_{17}H_{26}O_5$, mp 113-115.5°C. Yield 0.19 g. IR spectrum, ν_{max}, cm^{-1}: 1780 (γ-lactone), 1720 (cycloheptanone), 1735 cm^{-1}.

Dehydrogenation of (VI) and (VII): Preparation of Chamazulene (XV). A mixture of 1.5 g of saponified dihydroinulicin [a mixture of (VI) and (VII)] and 1.5 g of Se was heated at 330°C for 40 min. The reaction product was extracted with petroleum ether and was chromatographed on 3 g of Al_2O_3 (activity grade II). Petroleum ether eluted 5 mg of a blue liquid identified by its R_f value (TLC in petroleum ether) in the presence of a marker as chamazulene.

SUMMARY

From the epigeal part of Inula japonica Thunb. a new sesquiterpene lactone has been isolated which we have called inulicin. Structure (I) is proposed for it.

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