STRUCTURE OF NEW SESQUITERPENE LACTONES FROM Inula germanica

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From the leaves and flowerheads of Inula germanica L. we have isolated a colorless crystalline substance, mp 155-159°C (decomp.), with the composition $C_{20}H_{24-26}O_6$, identical according to its IR spectrum with a substance previously isolated from this plant species [1].

The substance dissolved completely in a cold 5% solution of $NaHCO_3$. On acidification of this solution, the initial substance was obtained, which shows the presence of a free carboxy group in its molecule. On thin-layer chromatograms in various adsorbents and solvent systems, one spot appeared; on column chromatography under various conditions the melting point of the substance scarcely changed. However, according to NMR and mass spectroscopy it is a mixture of two substances with similar structures having M^+ 362 and 360. After numerous experiments on the purification of the mixture, we succeeded in obtaining one substance, contaminated with about 5% of the other, with the composition $C_{20}H_{26}O_6$, mp 150-153°C (decomp.), $[\alpha]_D^{20}$ + 17.8° (c 3.96; ethanol); IR spectrum, cm^{-1}: ν_{max}w 2710, 2650, 2580, 1670 ($\mathrm{C{=}C{-}C}\genfrac{}{}{0pt}{}{\nearrow \mathrm{O}}{\searrow \mathrm{OH}}$), 1770 ($\gamma$-lactone), 1732 (OCO), and 1635 (C = C); UV spectrum: λ_{max} 201 nm (ε 27,219). We have called this lactone germanin A (for the IR and NMR spectra of germanin A, see Figs. 1 and 2).

Germanin A dissolves readily in KOH solution in the cold, undergoing hydrolysis with the formation of α-methylbutyric acid (II) and a hydroxy lactone (III) with the composition $C_{15}H_{18}O_5 \cdot \frac{1}{2}H_2O$.

The hydrogenation of germanin A (I) over a Ni catalyst led to a dihydro derivative (V); hydrogenation over a Pt catalyst in ethanol gave a tetrahydro derivative (VI) the IR spectrum of the methyl ester of which (VII) lacked the absorption band of a carboxylic hydroxyl. The hydrogenation of germanin A over a Pt catalyst in acetic acid formed a hexahydro derivative (VIII) which, on treatment with diazomethane, was also converted into a methyl ester (IX). It follows from the facts given that germanin A contains a γ-lactone ring, a carboxy group, an ester group, and also three double bonds. With these functional groups and the given composition, the hydrocarbon skeleton of germanin A is monocyclic.

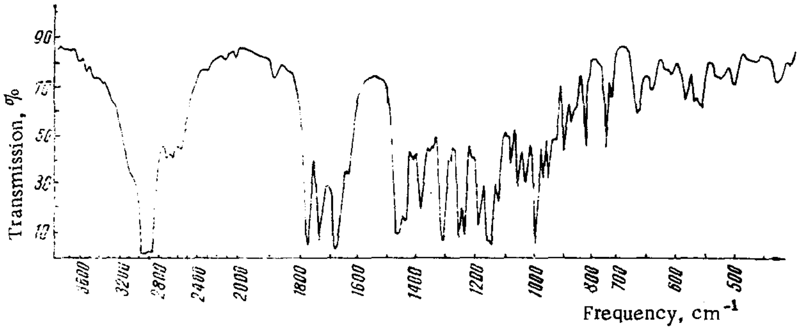

Fig. 1. IR spectrum of germanin A (paraffin oil).

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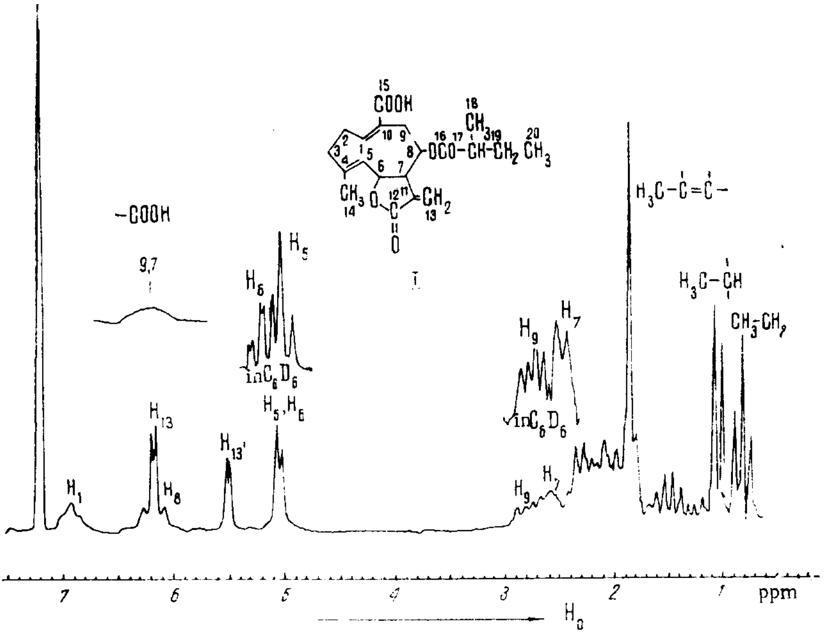


Fig. 2. NMR spectrum of germanin A in $CDCl_3$ with fragments in C_6D_6 (100 MHz).

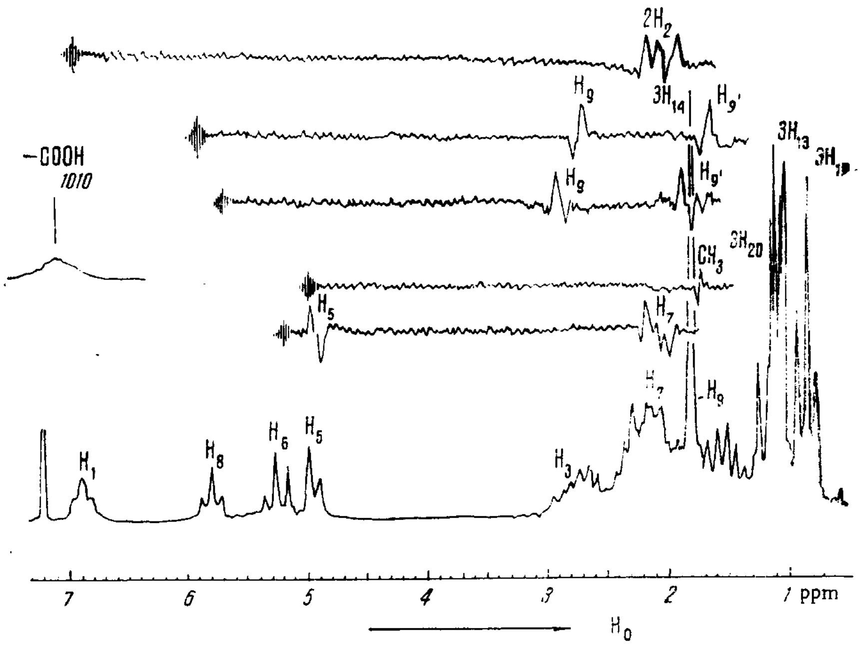


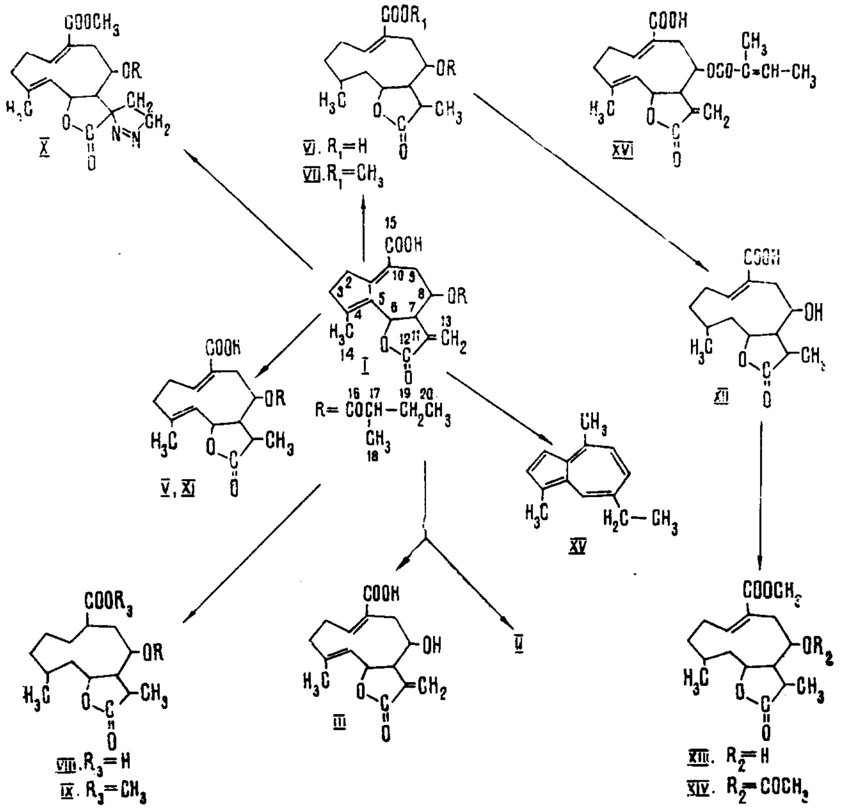
Fig. 3. NMR spectrum of dihydrogermanin A (V) in $CDCl_3$ (100 MHz).

When germanin A was treated with diazomethane, in addition to the methylation of the carboxylic hydroxyl, the addition of diazomethane took place with the formation of a pyrazoline derivative (X) having the composition $C_{22}H_{30}O_6N_2$, which confirms the indications of the NMR spectra (Table 1) on the presence in germanin A of an exocyclic methylene attached to a γ-lactone ring. The hydrogenation of germanin A over a Ni catalyst led to the reduction of the exocyclic methylene to a CH_3 group (V), and the treatment of germanin A with sodium borohydride led to the production of its epimer (XI).

To determine the position of the acyl residue we hydrolyzed tetrahydrogermanin A. The product obtained (XII) had the absorption band of a hydroxyl in the IR spectrum (ν_{max} 3440 cm^{-1}). Substance (XII) was converted into its methyl ester (XIII). Acetylation of the latter gave an acetate (XIV), in the IR spectrum of which the absorption band of the hydroxyl was absent. In the NMR spectra of (XII) and (XIII) an upfield chemical shift of one of the signals (of one proton unit) and in the NMR spectrum of (XIV) a downfield shift of the same signal was observed (see Table 1). Consequently, there is one geminal proton, i.e., the acyl residue in germanin A is located on a secondary carbon atom.

To investigate the carbon skeleton of germanin A, it was dehydrogenated over Se at 280-350°C. This gave chamazulene (XV) in low yield. The production of this substance from the monocyclic skeleton established above enables germanin A to be assigned to the germacranolides.

The chemical shifts of the signals and the coupling constants of the protons in germanin A and its derivatives can be judged from the figures in Table 1 which were obtained by a direct consideration of the spectra, and also by means of the INDOR method.



In the weak-field region of the NMR spectrum of germanin A there are the signals of seven protons, of which four are superposed on one another. The following assignments can be made directly from the spectrum: carboxyl proton (broadened signal at 9.7 ppm); proton on a double bond conjugated with the carboxyl (broadened triplet at 6.95 ppm); protons of an exocyclic methylene group conjugated with the lactone carbonyl (d, 6.16, and d, 5.51 ppm); a broadened singlet at 1.84 ppm corresponding to the protons of a vinyl methyl; a doublet at 1.05 ppm and a triplet at 0.82 ppm assigned to the methyl protons of the α-methylbutyryl group: they disappear in the products of the hydrolysis of germanin A and tetrahydrogermanin A. In the NMR spectrum of germanin A taken in deuterobenzene, the signals superposed in the former spectrum separate (see Fig. 2 and Table 1). The high value of the coupling constant between the protons giving the signals in the form of a sextet at 4.88 ppm and of the doublet with broadened components at 4.65 ppm ($J_{5,6} = 11.2$; $J_{6,3} = 2.0$; $J_{6,7} = 10.0$) show the vicinal position of these protons.

In tetrahydrogermanin A (VI), the signal in the form of a doublet has disappeared. Consequently, this signal belongs to the vinyl proton. The position of the proton vicinal to it changes little in the spectra of the derivatives of germanin A, which permits it to be assigned to the lactone proton.

On analysis of the spectrum of dihydrogermanin A by the INDOR method (Fig. 3) it was established that the vinyl proton of the strong field (d, 4.95 ppm) and the protons of the methyl group of the double bond (broadened singlet at 1.85 ppm) interact with one another, i.e., the lactone proton is in the α position with respect to the vinyl proton, and the methyl group is in the β position with respect to the latter. In its turn, it follows from this that the carboxy group is located at C_{10}.

TABLE 1. Characteristics of the NMR Spectra of Germanin A and Its Derivatives

Compound	Protons at										Solvent
	C_1	C_5	C_6	C_8	C_9	C_{13}	C_{11}	C_{19}	C_{20}	other C's	
I	br.t. 6,95 ($\Sigma J=16{,}0$)	d. (2H)	5,05	t 6,16 ($\Sigma J=18{,}0$)	—	d 6,16 ($J=3{,}8$) d 5,51 ($J=3{,}5$)	br.s. 1,84	t 0,82	d 1,05	br.sig. 9,7—carboxy proton	$CDCl_3$
	—	d.w.br. com. 4,65 ($J_{5,6}=11{,}2$)	sex 4,88 ($J_{5,6}=11{,}2$, $J_{6,7}=10.0$; $J_{6,8}\sim2{,}0$)		H_9— qu 2,75 ($J_{9,9'}=15{,}0$; $J_{9,8}=7{,}0$) H_9, overlapped by the methyl signals	—	—	—	—	H_7—d.w.br. com. 2,48 ($J_{7,6}=9{,}2$; $J_{7,8}<1$)	C_6D_6
V	t 6,89 ($J=16{,}0$)	d.w.br. com. 4,95 ($J=9{,}0$)	t 5,26 ($\Sigma J=20{,}2$)	t 5,79 ($\Sigma J=17{,}0$)	qu 2,84 ($J_{9,8}=8{,}2$; $J_{9,9'}=15{,}2$) qu. 1,85 ($J_{9',8}=9{,}3$) superposition of the signals of methyl protons	d 1,10	br.s. 1,85	t 0,85	d 1,08	br.sig. 10,1—carboxy proton H_7 qu 2,12 ($J_{7,8}<1$; $J_{7,6}=10{,}2$; $J_{7,11}=8{,}0$)	$CDCl_3$
VI	qu 6,96 ($J=4{,}2$; 12,4)	—	m. 4,98	m. 5,31	qu 3,05 ($J_{9,8}=6{,}3$; $J_{9,9'}=15{,}0$)	d 1,03	br.s. 0,98	t 0,83	d 1,03	br.sig. 10,82—carboxy proton	$CDCl_3$
VII	qu. 6,81 ($J=5{,}2$; 11,9)	—	m. 4,98	m. 5,30	qu 2,98 ($J_{9,8}=6{,}5$; $J_{9,9'}=14{,}0$)	d 1,05	d 0,89	t 0,83	d 1,03	s, 3,71—OCH_3	$CDCl_3$
XII	qu 7,0	—	m. 5,17	m. 4,73	qu 3,16 ($J_{9,8}=6{,}4$; $J_{9,9'}=13{,}0$)	d 1,31	br.s. 0,71	—	—	—	C_5D_5N
XIII	qu 6,76 ($J=4{,}8$; 12,2)	—	m. 4,95	m. 4,27	—	d 1,21	d 0,91	—	—	s. 3,78—OCH_3	$CDCl_3$
XIV	qu 6,82 ($J=4{,}8$; 12,2)	—	m. 4,95	m. 5,26	qu 2,96 ($J_{9,8}=6{,}8$; $J_{9,9'}=13{,}9$) qu 2,49 ($J_{9',8}=10{,}0$; $J_{9,9'}=13{,}9$)	d 1,09	d 0,91	—	—	s, 1,93—$OCOCH_3$ s, 3,71—OCH_3	$CDCl_3$

Note. Figures given in ppm relative to HMDS; the coupling constants are given in parentheses and are expressed in Hz; s — singlet; br. sig. — broadened signal; d — doublet; d. w. br. com. — doublet with broadened components; t — triplet; qu — quartet; sex — sextet; m — multiplet. The spectra were taken on an HA-100D spectrometer.

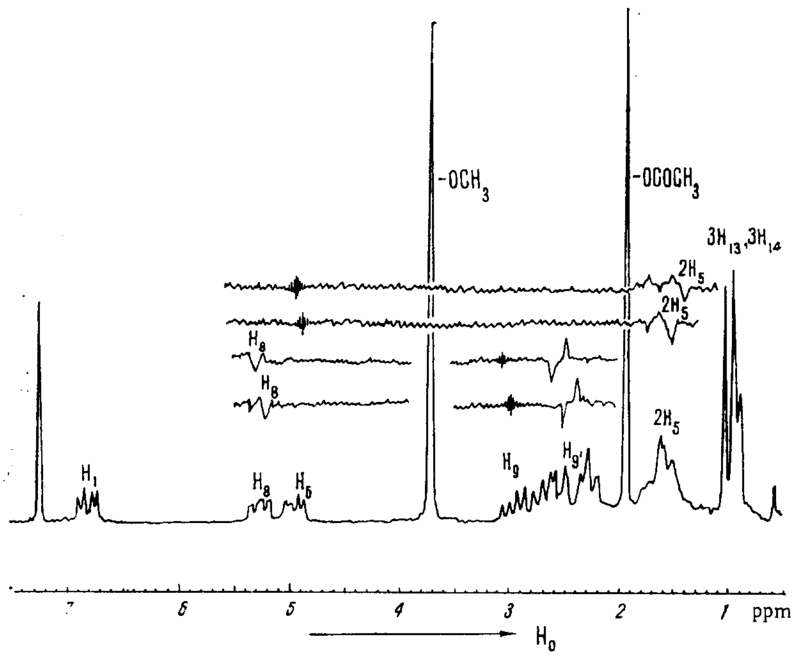


Fig. 4. NMR spectrum of (XIV) in $CDCl_3$ (100 MHz).

The results of an analysis of the signals of the proton vicinal to the acyl proton, the position and structure of which were established by the INDOR method (see Figs. 3 and 4), showed that a methylene group is adjacent to the acyl group. The value of the geminal constant (J = 15.2 Hz) and also the position of the signal of one of the protons [quartet at 2.84 ppm in (V)] show the α location of the methylene group with respect to the double bond.

The structure of the H_7 signal in the spectra of (I) and (V) indicates that there is only one $J_{7,8}$ constant, which is close to zero, and this is possible only when the substituent is present at C_8, i.e., the ester group is attached to this carbon atom. In the spectra of (VI) and (VII), the signal of the geminal proton is split additionally, which is due to the increase in the $J_{7,8}$ constant because of the change in the dihedral angle between the H_7 and H_8 protons.

The position of the acyl group at C_8 is also shown by the shift of the signal of the methyl protons of the lactone ring on passing from (VII) to (XIII) and from (XIII) to (XIV).

Thus, the facts given above permit structure (I) to be proposed for germanin A.

We attempted to obtain the other lactone, which we have called germanin B in the individual form, but so far unsuccessfully; however, we have succeeded in determining its structure in the following way. The combined lactones were treated with diazomethane in ether, and the reaction product was chromatographed. This gave two substances: the pyrazoline derivative of germanin A (X) and the pyrazoline derivative of germanin B (Fig. 5) in the form of white crystals with the composition $C_{22}H_{28}O_6N_2$, mp 132-134°C. As can be seen from Fig. 5, the NMR spectrum of the pyrazoline derivative of germanin B has no signals of methyls at CH_2 and CH, and it has a singlet (6H) at 1.90 ppm and a doublet (3H) at 2.0 ppm, corresponding to three vinyl methyls. In the 5.5-6.2-ppm region there are the signals of three protons, while in the spectrum of (X) there are signals of only two protons in this region, i.e., in germanin B there is one more vinyl proton than in germanin A. In the NMR spectrum of the combined lactones taken in deuterobenzene, the signal of this proton is a quartet at 5.60 ppm; two doublets are also found, at 1.40 and 1.60 ppm, which enables these signals to be assigned to the protons of an angelic acid residue. When the combined lactones were hydrolyzed, in addition to α-methylbutyric acid, we obtained angelic acid, which was identified by GLC.

As already mentioned, the mass spectrum of the combined lactones also have M^+ 360 and a peak with m/e 100 (angelic acid). All these facts show that germanin B differs from germanin A only by the acyl residue, i.e., germanin B has the structure (XVI) (see Scheme, p. 593).

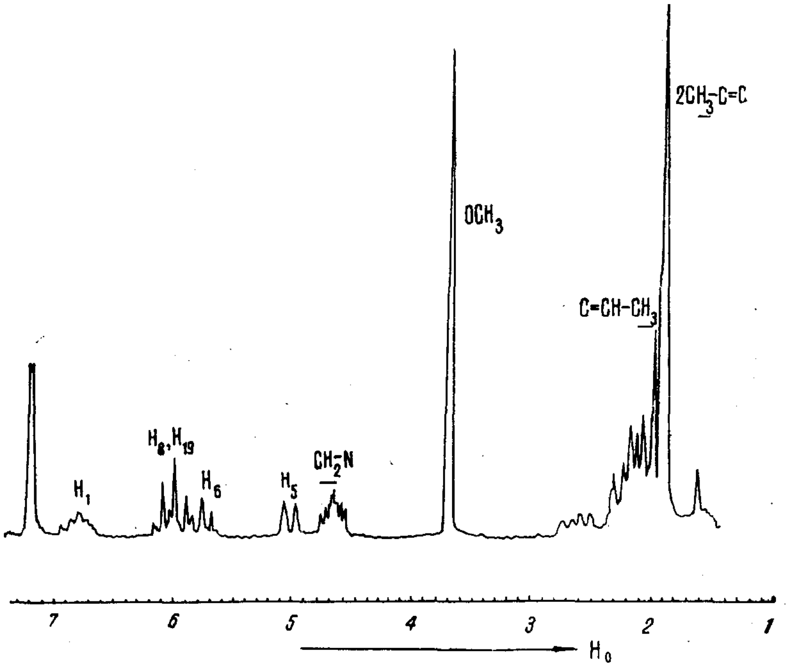


Fig. 5. NMR spectrum of the pyrazoline derivative of germanin B in $CDCl_3$ (100 MHz).

EXPERIMENTAL

Isolation of the Lactones. The leaves and flowerheads of Inula germanica collected in the flowering phase in Azerbaidzhan (10 kg) were steeped in hot water three times (1 h each). The lactones were extracted from the aqueous solution with chloroform. After the chloroform had been driven off, a dark viscous mass was obtained, and this was treated with ether. The ethereal extract was partially evaporated, and an excess of petroleum ether was added. On standing, white crystals deposited which were soluble in organic solvents other than petroleum ether; they were recrystallized from a mixture of petroleum ether and diethyl ether or of petroleum ether and ethyl acetate. In all cases, colorless crystals were obtained which, after drying for 1 h in a vacuum pistol over P_2O_5 had mp 155-159°C (decomp.); on TLC in the ether, the benzene–methanol (9: 1), and other systems they gave a single spot. Yield 0.15%, composition $C_{20}H_{24-26}O_6$. IR spectrum, cm^{-1}: ν_{max} w 2710, 2660, 2570, and br. 1670 ($C{=}C{-}C\genfrac{}{}{0pt}{}{\diagup O}{\diagdown OH}$), br. 1770 (γ-lactone), 1730 and 1720 (2 OCO), 1630 (C=C). Mass spectrum: M^+ 362 and 360 in almost equal proportions.

The combined lactones obtained were chromatographed on silica gel. Elution was performed with petroleum ether, mixtures of petroleum ether and diethyl ether in various proportions, and diethyl ether. In all cases, mixtures of the two lactones were obtained in proportions of 1 : 1, 2 : 1, and 3 : 1. On chromatography in benzene, the lactones were retained strongly by silica gel and were not eluted by either benzene or ether. When the crystals obtained were stored over $Mg(ClO_4)_2$ or over $CaCl_2$ a gradual fall in the melting point and in the carbon content was observed, i.e., the substance reacted with atmospheric oxygen, as has been reported previously for other lactones [2-4]. Thus, on the fifteenth day of storage the mp was 119-123°C. The changes taking place were irreversible; in the mass spectrum the molecular weight had increased more than twofold. The combined lactones obtained were stored in the moist state.

Isolation of Germanin A (I). A solution of 5 g of the combined lactones in 50 ml of ethanol was treated with water until a slight opalescence appeared, and the solution was extracted with a mixture of petroleum ether and diethyl ether (8 : 2). Evaporation of the solvent gave 2.5 g of crystals in the form of colorless needles which were treated again in the same way. Colorless needles were again isolated, and after drying in a vacuum pistol over P_2O_5 for 1 h or over $CaCl_2$ for a day they had mp 150-153°C (decomp.) and the composition $C_{20}H_{26}O_6$. Mass spectrum: M^+ 362, intense peaks with m/e 278 (362 − 84) and 260 (362 − 102). IR spectrum, cm^{-1}: ν_{max} w 2710, 2650, 2580 (carboxy hydroxyl), 1770 (γ-lactone), 1732 (OCO), 1670 (C=C−COOH), and 1635 (C=C). UV spectrum: λ_{max} 201 nm (ε 27,219).

Treatment of Germanin A with $NaHCO_3$. A solution of 0.5 g of germanin A in 20 ml of 5% $NaHCO_3$ solution was acidified with 10% H_2SO_4 to pH 1. A white precipitate deposited, which was filtered off, washed with water, and dried; mp 151-154°C (decomp.). The IR and NMR spectra were identical with those of the starting material.

Hydrolysis of Germanin A. Formation of (II) and (III). A 1-g solution of germanin A in 100 ml of 4% KOH solution was left at room temperature for 10 days and was then acidified with 10% H_2SO_4 solution to pH 1 and was extracted six times with ether. The ethereal extract was washed five times with a 5% solution of $NaHCO_3$ and then with water to neutrality. After the elimination of the ether, a substance was obtained in traces.

The sodium bicarbonate extract was acidified with a 10% solution of H_2SO_4 and extracted five times with ether. The ethereal extract was washed with water until the reaction to universal indicator was neutral. The residue after the elimination of the ether gave on TLC (in the ether system) three spots with R_f 0.79, 0.59, and 0.26. Part of the products of the hydrolysis of germanin A was dissolved in ether and analyzed by the GLC method; α-methylbutyric acid (II) was found.

The remainder was dissolved in a mixture of petroleum ether and diethyl ether (1: 1). This gave crystals with mp 152-156°C (the initial compound according to its IR spectrum) and an oily residue. The residue was treated with ether several times. The ethereal extract deposited colorless rhombs. After recrystallization from ether and drying, the substance had mp 198-210°C (Koffler), and on TLC (in the ether system) it gave one spot with R_f 0.27 (III); composition $C_{15}H_{18}O_5 \cdot {}^1\!/_2H_2O$. Mass spectrum: M^+ 278 (here and below the results of analysis corresponded to the calculated figures).

IR spectrum: ν_{max} of an absorption band in the 3540-3430-cm^{-1} region (OH), broad band with its center at 2620 cm^{-1} (carboxylic hydroxyl), 1770 cm^{-1} (γ-lactone), 1705 cm^{-1} (CO), 1640 cm^{-1} (C = C). UV spectrum: λ_{max} 202 nm (ε 27,503).

Hydrogenation of Germanin A over Raney Ni Catalyst. Production of (V). The hydrogenation of 1 g of germanin A in 100 ml of ethanol was performed in the presence of Raney nickel catalyst W-4 at room temperature. The amount of hydrogen absorbed was 1 mole, and no further hydrogenation took place. After the catalyst and solvent had been removed, a syrupy product was obtained which gave on TLC (in the ether system) a single spot, and this was chromatographed on silica gel with elution by ether. Colorless crystals were isolated with mp 87-90°C (Koffler) from a mixture of petroleum ether and diethyl ether. After drying over $CaCl_2$, the composition was $C_{20}H_{28}O_6 \cdot {}^1\!/_2C_4H_{10}O$ (diethyl ether).

IR spectrum, cm^{-1}: ν_{max} 1779 (γ-lactone), 1734 (OCO), 1690 (CO), 1645 (C = C). UV spectrum: λ_{max} 201 nm (ε 39,150). The NMR spectrum showed the signals of the protons of diethyl ether, which disappeared when the substance was recrystallized from CCl_4.

Hydrogenation over a Pt-catalyst.

A. Production of the Tetrahydro Derivative (VI). The hydrogenation of 4 g of germanin A in 100 ml of ethanol in the presence of 0.2 g of PtO_2 was performed until the absorption of hydrogen ceased, the amount absorbed being 2 moles. After the catalyst and the ethanol had been eliminated, a colorless liquid was obtained which was dissolved in ether. On standing, colorless rhombs deposited. After drying in a vacuum pistol over P_2O_5 with heating by means of ethanol for 1 h, they had the composition $C_{20}H_{30}O_6 \cdot {}^1\!/_2C_4H_{10}O$, mp 168-170°C. Mass spectrum: M^+ 366.

IR spectrum, cm^{-1}: ν_{max} 2710, 2590 (carboxylic hydroxyl), 1780 (γ-lactone), 1750 (OCO), 1700 (CO), 1650 (C = C). UV spectrum: λ_{max} 213 nm (ε 23,994). The NMR spectrum showed the signals of the protons of diethyl ether.

The crystals with mp 168-170°C (0.2 g) were dissolved in CCl_4, and a white powder was obtained. After drying in a vacuum pistol over P_2O_5 with heating by ethanol for 1 h, their composition was $C_{20}H_{30}O_6 \cdot {}^1\!/_3CCl_4$, mp 135-152°C. The NMR spectrum showed no signals of the protons of diethyl ether.

B. Production of the Hexahydro Derivative (VIII). The hydrogenation of 0.5 g of germanin A in 30 ml of glacial acetic acid in the presence of 0.06 g of PtO_2 was performed until the absorption of hydrogen ceased, the amount absorbed being 3 moles. The catalyst was filtered off, the filtrate was diluted with a fivefold amount of water, the reaction product was extracted four times with chloroform, and the extract was washed with water to neutrality. After the solvent had been driven off, a vitreous product was isolated which gave a single spot with R_f 0.55 on TLC (in the ether system); it crystallized from ether in the form of colorless crystals which, after drying in a vacuum pistol over P_2O_5, had the composition $C_{20}H_{32}O_6 \cdot \frac{1}{2}C_4H_{10}O$, mp 134-150°C (Koffler).

IR spectrum, cm^{-1}: ν_{max} 2700, 2600 (carboxylic hydroxyl), 1785 (γ-lactone), 1740 (OCO), 1700 (CO). The NMR spectrum lacked the signals of vinyl protons but contained the signals of the protons of diethyl ether.

Treatment of (VI) with CH_2N_2. Production of (VII). An ethereal solution of diazomethane was added to a solution of 0.5 g of tetrahydrogermanin A in ether until a permanent yellow coloration was obtained. Then the ether was driven off and colorless crystals were obtained which gave a single spot with R_f 0.58 on TLC in the ether system; after recrystallization from a mixture of petroleum ether and diethyl ether and drying, they had the composition $C_{21}H_{32}O_6$, mp 123.5-125°C.

IR spectrum, cm^{-1}: ν_{max} 1780 (γ-lactone), 1750 and 1740 (OCO), 1655 (C = C). UV spectrum: λ_{max} 215 nm (ε 32,815).

Treatment of (VIII) with CH_2N_2. Production of (IX). An ethereal solution of diazomethane was added to a solution of 0.1 g of (VIII) in ether until a permanent yellow coloration was produced. After the solvent had been driven off, a vitreous product was isolated which gave a single spot with R_f 0.65 on TLC (in the ether system). The NMR signal lacked the signals of vinyl protons and contained a singlet at 3.75 ppm $-OCH_3$.

Hydrolysis of Tetrahydrogermanin A. Production of (XII). A solution of 0.5 g of tetrahydrogermanin A in 50 ml of 4% KOH solution was left at room temperature for 10 days and was then acidified with 10% H_2SO_4 to pH 1 and extracted ten times with ethyl acetate, the extract then being washed with water to neutrality. After the solvent had been driven off, a vitreous residue was obtained, two recrystallizations of which from ether yielded colorless crystals with the composition $C_{15}H_{22}O_5$, mp 249-251°C (Koffler).

IR spectrum, cm^{-1}: ν_{max} 3440 (OH), 2650 and 2590 (carboxylic hydroxyl), 1740 (γ-lactone), 1700 (CO), 1660 (C = C). UV spectrum: λ_{max} 206 nm (ε 21,370).

Treatment of (XII) with CH_2N_2. Production of XIII. An ethereal solution of diazomethane was added to a solution of 0.5 g of (XII) in ethanol until a permanent yellow coloration was obtained. After the solvent had been driven off, a vitreous product was obtained which gave a single spot with R_f 0.36 on TLC (in the ether system); it was impossible to crystallize it, and after drying in the vacuum pistol over P_2O_5 without heating for 40 h it had the composition $C_{16}H_{24}O_5 \cdot \frac{1}{2}H_2O$.

Acetylation of (XIII). Production of (XIV). A solution of 0.12 g of (XIII) in 1 ml of pyridine was mixed with 1 ml of acetic anhydride in 1 ml of pyridine, and the mixture was left at room temperature for a day. Then it was diluted with water and extracted with ether, and the extract was washed three times with 5% HCl and then with water to neutrality. After the elimination of the solvent, crystals deposited which were recrystallized from a mixture of petroleum ether and diethyl ether; composition $C_{18}H_{26}O_6$, mp 157-158°C (Koffler).

IR spectrum, cm^{-1}: ν_{max} 1780 (γ-lactone), 1750 (OCO), 1655 (C = C).

Reduction of Germanin A with $NaBH_4$. Production of (XI). A solution of 0.37 g of germanin A in 5 ml of methanol was mixed with 0.5 g of $NaBH_4$ in 20 ml of methanol and the mixture was left for 20 min, after which it was diluted with water and acidified with 10% H_2SO_4, and the reaction product was extracted three times with ethyl acetate. The extract was washed with water to neutrality. After the solvent had been distilled off, a faintly colored syrupy product was obtained which gave two spots with R_f 0.71 and 0.42 on TLC [in the benzene–ethanol (8 : 2) system]. It was chromatographed on silica gel with ether as the eluent. This gave colorless crystals with the composition on $C_{20}H_{28}O_6$, mp 198-202°C.

IR spectrum, cm^{-1}: ν_{max} 2700, 2650, 2580 (carboxylic hydroxyl), 1790 (γ-lactone), 1740 (OCO), 1690 (CO), 1640 (C = C). UV spectrum: λ_{max} 201 nm [ε 44,863 (C = C–COOH)].

Treatment of the Combined Lactones with CH_2N_2. A solution of 1 g of the combined lactones in 20 ml of ether was treated with an ethereal solution of diazomethane until a permanent yellow coloration appeared. The reaction product was chromatographed on silica gel. Elution was performed with petroleum ether, a mixture of petroleum ether and diethyl ether, and then with ether. Petroleum ether–diethyl ether (6 : 4) eluted 0.5 g of a vitreous substance. After drying in the vacuum pistol over P_2O_5 it formed a white amorphous powder with the composition $C_{22}H_{30}O_6N_2$ (X), mp 50-53°C.

IR spectrum, cm^{-1}: ν_{max} 1780 (γ-lactone), 1730 (OCO), 1705 (OCO), 1635 (C = C).

The same mixture eluted colorless crystals with the composition $C_{22}H_{28}O_6N_2$, mp 132-134°C (Koffler). IR spectrum, cm^{-1}: 1775 (γ-lactone), 1715 (OCO), 1650, 1630 (C = C).

Substance (X) was obtained by treating germanin A with diazomethane under similar conditions.

Dehydrogenation of Germanin A. A mixture of 0.5 g of germanin A and 1 g of Se was heated at 280-350°C for 2 h, the reaction products were extracted with petroleum ether, and the residue after the elimination of the solvent was chromatographed on neutral alumina (activity grade II). A blue liquid was isolated which on TLC gave a single spot at the level of a spot of chamazulene (marker).

SUMMARY

It has been established that the substance isolated previously from *Inula germanica* L. is a mixture of two new sesquiterpene lactones: germanin A and germanin B.

On the basis of chemical reactions and a study of IR, UV, NMR, and mass spectra, structures (I) and (XVI), respectively, are proposed for germanin A and germanin B.

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