

PLANT SUBSTANCES. XXXVI.*

COMPONENTS OF *Laserpitium siler* L. FRUITS

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From the light petroleum extract of the *Laserpitium siler* L. fruits of Slovenian origin paraffins C_{16} to C_{31} have been isolated in addition to four sesquiterpenic lactones (acetylismontanolide, isomontanolide and two new ones). Calamenene and calacorene, an aliphatic C_{29} ketone, a mixture of phytosterols with β -sitosterol as the main component, and a mixture of aliphatic alcohols, C_{26} , C_{28} and C_{30} have also been identified. The predominant part of the extract is composed of fats. In the fruits an essential oil is present (4.2%) composed of (+)-limonene and (+)-perillaldehyde.

In one of the preceding papers we described¹ the isolation and the determination of the structure of a new sesquiterpenic lactone, isomontanolide, which is present in *Laserpitium siler* L. fruits of Slovenian origin. Simultaneously with its isolation we also carried out an analysis of the light petroleum extract and prepared an essential oil, that we describe in this paper. The fruits, which are characterised by an intense odour, afford on steam distillation 4.2% of essential oil. According to gas-liquid chromatographic analysis it is an essential oil with a very simple composition, as it contains 10.5% of (+)-limonene and 89.5% of (+)-perillaldehyde.

The light petroleum extract was chromatographed on deactivated silica gel. The forefractions, eluted with light petroleum, represented a liquid mixture of hydrocarbons, which on distillation gave (+)-limonene as the main component. The distillation residue was chromatographed on silver nitrate impregnated silica gel to afford, in the first fractions a mixture of paraffinic hydrocarbons from C_{16} to C_{31} , as followed from a gas-liquid chromatographic analysis, in which C_{27} and C_{29} hydrocarbons prevailed. This mixture was not further investigated. In subsequent fractions of the distillation residue sesquiterpenic hydrocarbons were present, as followed from gas liquid-mass spectroscopy analysis, in which only calamenene and calacorene could be identified.

In the fractions which followed the hydrocarbons liquid components were present — esters according to IR spectra — evidently fats, from which a crystalline substance

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separated on standing. This substance was composed of two components even after repeated crystallisations, as shown by thin layer chromatography. Repeated chromatography gave the pure more polar component of m.p. 74.5°C, which according to IR spectrum contained a carbonyl group (1706 cm^{-1}). On the basis of its high resolution mass spectrum its composition is $\text{C}_{29}\text{H}_{58}\text{O}$, and according to its fragmentation pattern it is an aliphatic ketone of the composition $\text{C}_{16}\text{H}_{33}\text{CH}_2\cdot\text{COCH}_2\text{C}_{10}\text{H}_{21}$.

Further fractions contained fats which are the main part of the extract. After transformation to methyl esters the present fatty acids were analysed by gas-liquid chromatography. The presence of palmitic and linolic acid was shown in addition to an unsaturated C_{18} acid, most probably oleic acid which represents the main component of this mixture. Fractions 105–140 contained, in addition to fats, a mixture of sterols. According to mass spectroscopy their composition was similar to that commonly met with in vegetal material². After acetylation the product obtained was shown to be identical with β -sitosterol acetate on the basis of its mixed melting point with an authentic specimen.

After rechromatography of subsequent fractions 141–164 which according to IR spectra contained substances of lactonic character (1780 cm^{-1}) four sesquiterpene lactones were isolated. The least polar lactone, eluted first, was acetylismontanolid of m.p. 134–135°C which was isolated earlier from the roots of this plant¹. It was identified in the conventional manner (IR spectrum, physical constants, mixture melting point). From the fractions following after acetylismontanolid a substance was isolated, m.p. 166°C, $[\alpha]_{\text{D}}^{20} + 50.6^\circ$, $\text{C}_{20}\text{H}_{28}\text{O}_5$ (high resolution mass spectrometry). Its IR spectrum contains a lactone band (1766 cm^{-1}), a band at 1700 cm^{-1} due most probably to the α,β -unsaturated ester group, a band of a double bond (1646 cm^{-1}) and of a free hydroxyl (3520 cm^{-1}). Other eluted fractions also contained a sesquiterpene lactone (band at 1780 cm^{-1}) of m.p. 154°C, $[\alpha]_{\text{D}}^{20} - 50.9^\circ$, and the composition $\text{C}_{25}\text{H}_{34}\text{O}_7$. The IR spectrum of this substance shows further bands at 1695 and 1706 cm^{-1} characteristic of α,β -unsaturated esters, a double bond band at 1643 cm^{-1} and a band at 3520 cm^{-1} typical of a free hydroxy group. From the last fractions a fourth lactone was eventually obtained (IR band at 1792 cm^{-1}), m.p. 179°C, which according to its IR spectrum is identical with the previously described isomontanolid¹ and which melted undepressed on admixture with its authentic specimen.

From the last chromatographic fractions a component of m.p. 80–86°C was obtained the IR spectrum of which displayed the frequency (3610 cm^{-1}) of a hydroxy group. According to gas-liquid chromatography it was a mixture of three substances, according to a comparison with standard substances a mixture of aliphatic alcohols C_{26} , C_{28} and C_{30} . In accordance with this the mass spectrum of this mixture contained a peak with maximum mass m/e 420, corresponding to a dehydrated alcohol $\text{C}_{30}\text{H}_{62}\text{O}$.

EXPERIMENTAL

The infrared spectra were measured on Unicam S. P. 200 and Zeiss UR-10 (Jena) spectrographs in chloroform solution, the PMR spectra in deuteriochloroform were recorded with a Varian HA-100 apparatus using tetramethylsilane as internal standard, and the mass spectra were taken with a AEI MS 902 spectrograph. For column chromatography a silica gel identical with that from the preceding paper¹ was used or a silica gel impregnated with 12% of silver nitrate. Light petroleum was a fraction boiling at 40–60°C. Thin layer chromatography was carried out using silica gel G Merck. The melting points were determined on a Kofler block and they were uncorrected. Optical activity was measured with an objective polarimeter.

Essential Oil

Ground seeds (100 g) were mixed with 300 ml of water and the mixture heated in a one litre flask at 110°C for five hours under gradual addition of the water which was distilled off. The separated essential oil (4.2 g) was dried over sodium sulfate and it had d_4^{20} 0.8963, n_D^{20} 1.4882 and $[\alpha]_D^{20}$ +125.8° (liquid). On prolonged heating (12–36 hours) another small amount of essential oil was obtained which has a weak blue colour. According to gas-liquid chromatography (Perkin-Elmer F 11 chromatograph with FID; column length 2 m, i.d. 2.2 mm, support Chromosorb W (80–100 mesh) wetted with 10% of Carbowax 400, temperature 120°C, carrier gas nitrogen, calibration with standards) it contained 10.5% of limonene (IR), and 89.5% of perillaldehyde (IR, PMR).

Chromatography of the Light Petroleum Extract

The extract was obtained from the seeds as described earlier¹. The part of the extract (350 g) with a typical perillaldehyde smell was chromatographed on a column of deactivated silica gel (6 kg). The average volume of single fractions was about 1.75 l.

Hydrocarbons. Fraction 1–7 (light petroleum, 12 l, residue 55.3 g) was distilled at 10 Torr. The distillate (b.p. 80°C) consisted of (+)-limonene (IR (ref.³), GLC—conditions as in the analysis of the essential oil); the distillation residue (2.8 g) was chromatographed on silica gel impregnated with silver nitrate. The forefractions (280 mg) obtained on elution with light petroleum contained according to GLC (Pye 104 provided with FID; column length 1.5 m, o.d. 1/4 inch; support Chromosorb G impregnated with 3% of OV-17; temperature 150–260°C, carrier gas nitrogen) a mixture of hydrocarbons. Comparison with standards of paraffinic hydrocarbons indicated the presence of hydrocarbons from C₁₆ to C₃₁ with C₂₇ and C₂₉ hydrocarbons prevailing. The subsequent fraction (1400 mg) contained according to GLC-MS (Pye 104; glass column 1.5 m long, i.d. 4 mm, support Diatomite CQ (100–200 mesh) impregnated with 3% SE 30; temperature 120°C, carrier gas helium) 16 substances of *m/e* 204. The mass spectra of two of them were identical with the spectrum of calamenene⁴ and calacorene (comparison with a standard).

Aliphatic ketone C₂₉. Fraction 8–25 (light petroleum, 35 l, residue 17.3 g) gave on standing a precipitate of m.p. 67–74°C (1500 mg), IR: 1720, 1734 cm⁻¹ (C=O), according to thin layer chromatography in light petroleum–chloroform giving two spots. Chromatography on a hundred-fold amount of deactivated silica gel with light petroleum gave a fraction (200 mg) substantially enriched with the more polar component. Rechromatography of this fraction on a two-hundred-fold amount of silica gel with tetrachloromethane afforded fractions containing according to TLC (in tetrachloromethane *R_F* 0.3) and GLC (Pye 104; column length 1.5 m, o.d. 1/4 inch; support Gas-Chrom Z impregnated with 3% of SE-30, temperature 240°C, carrier gas nitrogen) a single substance, m.p. 74.5°C (tetrachloromethane); IR: 1706 cm⁻¹ (—CO—); UV: λ_{\max} 281 nm (log ϵ = 1.54); high resolution mass spectrum: *m/e* 422.4504; for C₂₉H₅₈O₂ calculated:

422-4487. According to GLC the less polar spot was composed of several components; IR: 1716, 1734 cm^{-1} (—CO—).

Fats. Fats were present in fractions 26—68 (light petroleum—ether 1—4%, 76 l, residue 215.3 g) and 69—104 (light petroleum—ether 4—5%, 76 l, residue 25.3 g). Fraction 26—68 (100 mg) was heated in a sealed ampoule with 3.5 ml of methanol, containing 3% of HCl, and 3.5 ml of tetrachloromethane at 100°C for 6 hours and then poured into water. The tetrachloromethane layer was separated and the aqueous layer was extracted with ether. Combined organic phases were washed with aqueous sodium hydrogen carbonate solution and dried. After distilling off of the solvents a liquid was obtained which was analysed by GLC (Pye 12000; column length 120 cm, i.d. 1/4 inch; support Chromosorb W impregnated with 10% of CAR 1 500 A; temperature 175°C, carrier gas argon). Comparison with standards indicated the presence of methyl palmitate, methyl ester of a C_{18} acid with one double bond (most probably methyl oleate, main component), and methyl linolate.

Phytosterols. Fractions 105—140 (light petroleum—ether 5—13%, 60 l, 14.6 g) were rechromatographed on deactivated silica gel (800 g) using light petroleum—ether mixture (3 : 1) as eluent. In medium fractions crystals were present of m.p. 134—136°C (ethanol), $[\alpha]_{\text{D}}^{20} - 35.0^\circ$ (c 0.5, chloroform), IR: 1603 cm^{-1} (C=C) and 3450, 3605 cm^{-1} (OH). The mass spectrum indicated a mixture of substances of the following molecular weights: 400, 412 and 414. Heating with acetic anhydride in a sealed ampoule gave acetate of m.p. 128—129°C (ethanol), IR spectrum identical with that of β -sitosterol acetate. On admixture of an authentic specimen the melting point remained undepressed.

Sesquiterpenic lactones. Fractions 141—164 (light petroleum—ether, 13—20%, 40 l, residue 13.7 g) were rechromatographed on 1500 g of deactivated silica gel. Elution with light petroleum—ether mixture (88 : 12), fraction volume 3 l. From fraction 21 a substance of m.p. 134—135°C (ethanol) and $[\alpha]_{\text{D}}^{20} - 69.2^\circ$ (c 0.5, chloroform) was obtained after evaporation of the solvent which melted undepressed on admixture of authentic acetylismontanolide¹; the IR spectra of both substances were also identical. Fraction 23 after evaporation of the solvent gave a substance of m.p. 166°C (ethanol), $[\alpha]_{\text{D}}^{20} + 50.6^\circ$ (c 0.5, chloroform). For $\text{C}_{20}\text{H}_{28}\text{O}_5$ (348.4) calculated: 68.94% C, 8.10% H, 0.29% H act.; found: 68.84% C, 8.22% H, 0.45% act.; molecular weight 348 (mass spectrometry). From fractions 26 and 27 a substance of m.p. 154°C (ethanol), $[\alpha]_{\text{D}}^{20} - 50.9^\circ$ (c 0.7, chloroform) was obtained in a similar manner. For $\text{C}_{25}\text{H}_{34}\text{O}_7$ (446.5) calculated: 67.24% C, 7.68% H; found: 67.35% C, 7.75% H, Molecular weight: 446 (mass spectrometry). Fraction 29 afforded in a similar manner a substance of m.p. 179—180°C (ethanol), $[\alpha]_{\text{D}}^{20} - 18.9^\circ$ (c 0.5, chloroform). Mixture melting point with authentic isomontanolide¹ was undepressed and the IR spectra of both substances were identical.

Aliphatic alcohols. After distilling off the solvent from fractions 165—180 (ether, 20 l, residue 6.8 g) a strongly viscous residue was obtained which partly crystallised on standing; on separation of the crystals on a porous clay plate and recrystallisation from light petroleum—acetone mixture a substance melting at 80—86°C was obtained. According to GLC (Pye 104; glass column of 140 cm length, i.d. 0.4 cm, packed with Gas-Chrom Z 100—120 mesh impregnated with 3% SE-30; temperature 250°C, carrier gas nitrogen) the product contained 5.9% of a C_{26} alcohol, 52.6% of a C_{28} alcohol and 36.4% of a C_{30} alcohol. The IR spectrum displayed the frequency (3610 cm^{-1}) of an OH group; mass spectrometry: base peak m/e 420 (M-18); molecular weight for $\text{C}_{30}\text{H}_{62}\text{O}$ is 438.

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