# The Essential Root Oil of *Peucedanum lancifolium* Lange and Chemotaxonomic Implications

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*Peucedanum lancifolium, Apiaceae,* Essential Oil, Irregular Sesquiterpenoids, *trans*-Sesquilavandulol

The composition of the essential root oil of *Peucedanum lancifolium* Lange (Apiaceae), growing in the Atlantic coast regions of France, North-West Spain und Portugal was investigated by chromatographic and spectroscopic methods. Forty five terpenoids, many of them of already known structure, and several aliphatic esters have been identified. Besides the main constituent, the irregular sesquiterpene alcohol *trans*-sesquilavandulol, representing 53.37% of the total oil, some hitherto unknown sesquiterpenoids with an irregular carbon skeleton were found. The possible chemotaxonomic value of the essential oil composition with regard to the close botanical relationship of *Peucedanum lancifolium* Lange and *Peucedanum palustre* (L.) Moench is briefly discussed. With the exception of these two species, *trans*-sesquilavandulol was not found in any other member of the genus *Peucedanum*.

## Introduction

In the course of our systematic investigations of the essential oils from Central- and West European *Peucedanum* species, the essential root oil of *Peucedanum lancifolium* Lange *(Apiaceae)* was analyzed. This rarely occurring perennial plant, which reaches a height of 80 to 120 cm, grows at wet places such as swampy meadows, peat bogs, marshes and banks of lakes. Its distribution is restricted to the atlantic coast regions of France, North-West Spain und Portugal [1, 2].

In the present paper, we report on the qualitative and quantitative composition of the hydrodistilled root oil of *Peucedanum lancifolium,* which has not been subject of investigations before. Identification of most of the constituents was performed by comparison of capillary GC retention times and mass spectral data with those of authentic reference substances. In addition, some of the components were isolated by means of preparative chromatographic techniques and their structures elucidated by IR- and NMR-spectroscopy.

## Experimental

### Plant material and essential oil recovery

The plant material was collected in the "Grande Brière", a large marsh area in Brittany (France) in September 1986. A voucher specimen of the examined plants is deposited in the herbarium of the university of Würzburg. The essential oil was obtained by hydrodistillation of chopped fresh roots for 6 h using the distillation apparatus described in [3].

### Column chromatography

Dry column chromatography: For the separation into hydrocarbons and oxygenated components, the essential oil, dissolved in *n*-pentane, was subjected to dry column chromatography with silica gel (ICN Silica TSC, 60 A, ICN Biomedicals). After isolation of the hydrocarbons using *n*-pentane as mobile phase, the oxygenated compounds were eluted with diethylether. Both fractions were concentrated by evaporation of the solvent under nitrogen at 30 °C and reduced pressure.

Low pressure liquid chromatography: For the isolation of oxygenated constituents, the polar fraction was subsequently separated by low pressure liquid chromatography (Merck type B, LoBar® column packed with Merck LiChrosorb Si 60, 40–63 μm). A mixture of *n*-pentane and diethylether was used as mobile phase, and 9 fractions of different volumes were collected according to UV-absorption at

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220 nm. After repeated chromatography (10 runs, sample amount: 1 g) the individual fractions were analyzed by capillary GLC.

### *Gaschromatography and GC/MS*

Capillary gas chromatograph: Fractovap 2900 (Carlo Erba Strumentazione) with FID (200 °C), column: 50 m × 0.25 mm WG 11 glass capillary column (Werner Günther Analysentechnik, F.R.G.), flow: 1.5 ml/min $N_2$, temperature program: 7 min at 70 °C, 70–200 °C (3 °C/min), injection port: Grob-type splitter (1:20), 200 °C.

Preparative gas chromatograph: Varian Aerograph 1400 with FID (200 °C, split 1:50), column: 2 m × 3/8″ aluminium column packed with 20% Carbowax 20000 on Volaspher A2® Merck, flow: 150 ml/min $N_2$.

GC/MS-system: Hewlett-Packard (GC: 5890 A; MSD 5970; Data system 59970 A), column: 30 m × 0.25 mm DB-WAX (film: 0.25 μm) fused silica capillary (J & W Scientific), directly led into the ion source (220 °C), carrier gas: He, flow: 1 ml/min, temperature program: 60–200 °C (2 °C/min), ionization energy: 70 eV.

### *IR spectroscopy*

The IR-spectra were obtained with a Perkin Elmer grating spectrometer model 597 as films between KBr pellets (cell path: 0.015 μm). The absorption range was from 4000 – 400 $cm^{-1}$.

### *NMR spectroscopy*

The $^{13}C$ NMR spectra were measured in $C_6D_6$ solution at 50.3 MHz in the Fourier Transform mode with a Bruker AC 200 spectrometer. The solvent was used as internal lock. The $^1H$ NMR spectra were recorded at 200 MHz.

### *Identification*

The monoterpenoids were identified by computer search of a user-generated reference library, comprising GC retention times and mass spectral data. The same holds true for most of the sesquiterpenoids found; for this purpose a library was created by isolating authentic compounds from various essential oils [4]. In several cases, sesquiterpene hydrocarbons were prepared as reference substances by gas phase dehydration from the corresponding alcohols [4].

*Cis*-sesquilavandulol could be identified by comparison of its retention time and mass spectrum with those of a synthetic sample, which was prepared from neryl acetone using the same preparation steps as described by us for the synthesis of the *trans*-isomer. *Trans*-sesquilavandulyl acetate was prepared from authentic *trans*-sesquilavandulol (from *Peucedanum palustre* [5] by reaction with acetic anhydride in pyridine. The $^{13}C$ NMR assignments of these two compounds were performed by comparative studies of their $^{13}C$ NMR chemical shifts with those of *trans*-sesquilavandulol and lavandulol [5, 6]. Structure elucidations of *trans*-cyclobutyl-sesquilavandulol and lancifolol were carried out mainly by homo- and heteronuclear correlated NMR-spectroscopy. The six aliphatic esters found in the essential root oil of *Peucedanum lancifolium* were identified only by GC/MS data. The mass spectra of three of them (*cis*-3-hexenyl acetate, isobutyl 2-methylbutanoate and isobutyl 3-methylbutanoate) were virtually identical with those from our own library. The structures of the remaining three esters, however, could be deduced only tentatively from their mass spectra.

## **Results and Discussion**

The capillary GC (Fig. 1) of the hydrodistilled root oil of *Peucedanum lancifolium* showed a very complex composition. Therefore, first a preseparation was carried out by dry column chromatography yielding two fractions, one containing only hydrocarbons and another comprising oxygenated compounds. The non polar fraction was directly submitted to GC/MS analysis, whereas the more complex polar fraction required further preseparation steps such as low pressure liquid column chromatography and subsequent preparative gas chromatography. In this way, we succeeded in isolating several of the polar chief constituents of this root oil, which could be identified by IR-, MS- and above all by NMR-spectroscopy.

The chromatographic and spectroscopic analysis revealed that the essential root oil of *Peucedanum lancifolium* consists of about 98% terpenoids and about 2% aliphatic esters. Among the terpenoids, oxygenated sesquiterpenes are dominating with about 75%. Monoterpene- and sesquiterpene hydrocarbons represent only about 10% and 8% of the total root oil, respectively. The group of monoter-

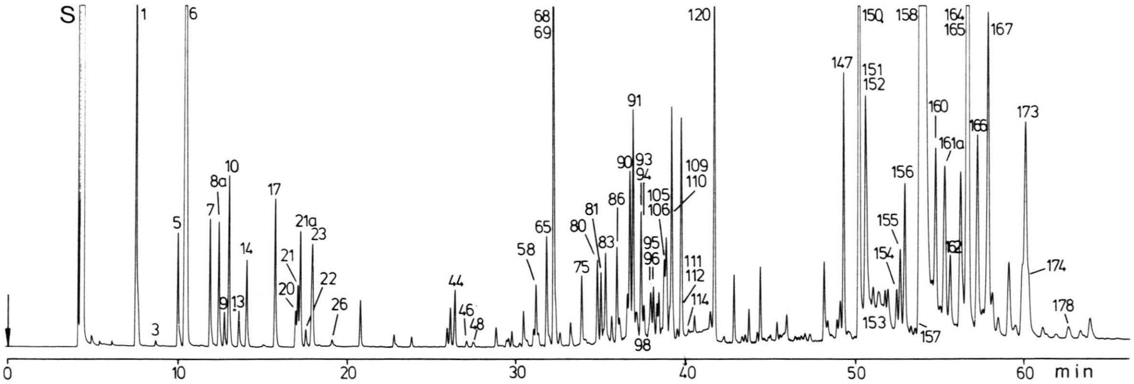

Fig. 1. Capillary gas chromatogram of the essential root oil of *Peucedanum lancifolium* Lange (S = solvent).

pene hydrocarbons present in the unpolar fraction is characterized by a high content of sabinene and α-pinene, amounting to about 7% and 2% of the total oil, respectively. In addition, traces of several other common monoterpene hydrocarbons occur (Table I). The sesquiterpene hydrocarbons proved to be a complex mixture of minor constituents among which germacrene B is dominating (1.2% of the total oil). Further sesquiterpenes with germacrane skeleton are germacrene A and germacrene D. The predominant portions of β- and γ-elemene must be interpreted as artifacts, which were formed by cope-rearrangement from the corresponding germacrenes during hydrodistillation and capillary GC analysis. In addition, thirteen other sesquiterpenes, occurring only in traces, could be identified (Table I).

The polar fraction, obtained by dry column chromatography, proved to be a complex mixture predominantly comprising oxygenated sesquiterpenoids. *Trans*-sesquilavandulol, an irregular sesquiterpene alcohol was found to be the chief constituent of the root oil (53%). This unusual terpenoid had already been isolated by us from the essential root oil of *Peucedanum palustre* and its structure elucidated by spectroscopic methods and confirmed by synthesis [5]. Apart from *trans*-sesquilavandulol, two further oxygenated sesquiterpenoids with the same carbon skeleton were established. One of them, which could be identified by comparison of GC retention time and mass spectral data with those of a synthesized sample, is the *cis*-isomer. The second irregular sesquiterpenoid was isolated and its structure elucidated to be *trans*-sesquilavandulyl acetate. One should note that these two sesquiterpenoids are described here for the first time as natural compounds. Their formulas, including the $^{13}$C NMR assignments are given in Fig. 2. Besides these sesquilavandulan derivatives, two further sesquiterpene alcohols could be isolated. Their structures were established mainly by 2D-NMR experiments and proved to represent two new types of carbon skeletons (Fig. 2). We suggest the common names *trans*-cyclobutyl-sesquilavandulol and lancifolol, respectively, for these compounds. In addition to these new sesquiterpenoids, some ubiquitous occurring members of this compound class could be detected, *e.g.* hedycaryol, its cope-rearrangement product elemol, germacradiene-1(10)*E*,5*E*-ol-4 and others (Table I).

As already mentioned before, the essential root oil of *Peucedanum lancifolium* also contains some aliphatic esters, together representing about 2% of the oil (Table I). Totally, in the essential root oil of *Peucedanum lancifolium* 51 compounds could be identified by detailed chromatographic and spectroscopic analysis, representing about 89% of this oil.

Among these, particularly the occurrence of several irregular sesquiterpenoids with *trans*-sesquilavandulol as main constituent is worth mentioning from the chemotaxonomic point of view. Due to the difficulty to distinctly characterize and delimitate the individual species of the genus *Peucedanum* by anatomical and morphological features, we have investigated the essential root oils of ten further Central and West European *Peucedanum* species (*Peucedanum palustre, P. austriacum, P. verticillare, P. oreoselinum, P. cervaria, P. officinale, P. longifolium, P. alsaticum, P. venetum* and *P. his-*

Table I. Composition of the essential root oil of *Peucedanum lancifolium* Lange (peak numbering refers to Fig. 1).

| Peak No. | Compound | [%] | Identification |
|---|---|---|---|
| 1 | α-Pinene | 2.03 | GC, MS |
| 3 | Camphene | trace | GC, MS |
| 5 | β-Pinene | 0.40 | GC, MS |
| 6 | Sabinene | 7.34 | GC, MS |
| 7 | Myrcene | 0.36 | GC, MS |
| 8a | Isobutyl 2-methylbutanoate | 0.37 | MS |
| 9 | α-Terpinene | 0.14 | GC, MS |
| 10 | Isobutyl 3-methylbutanoate | 0.52 | MS |
| 13 | Limonene | 0.11 | GC, MS |
| 14 | β-Phellandrene | 0.20 | GC, MS |
| 17 | γ-Terpinene | 0.47 | GC, MS |
| 20 | p-Cymene | 0.11 | GC, MS |
| 21 | 3-Methylbutyl 2-methylbutanoate | 0.20 | MS |
| 21a | 2-Methylbutyl 2-methylbutanoate | 0.38 | MS |
| 22 | Terpinolene | 0.06 | GC, MS |
| 23 | 2-Methylbutyl 3-methylbutanoate | 0.42 | MS |
| 26 | *cis*-3-Hexenyl acetate | trace | GC, MS |
| 44 | δ-Elemene | 0.18 | GC, MS |
| 46 | α-Ylangene | 0.06 | GC, MS |
| 48 | α-Copaene | 0.05 | GC, MS |
| 58 | $C_{15}H_{24}$ | 0.13 | MS |
| 65 | β-Elemene | 0.32 | GC, MS |
| 68 | β-Caryophyllene | 0.33 | GC, MS |
| 69 | Terpinen-4-ol | 1.86 | GC, MS |
| 75 | γ-Elemene | 0.16 | GC, MS |
| 80 | *E*-β-Farnesene | 0.24 | GC, MS |
| 83 | α-Humulene | 0.27 | GC, MS |
| 86 | γ-Muurolene | 0.36 | GC, MS |
| 90 | $C_{15}H_{24}$ | 0.59 | MS |
| 91 | Germacrene D | 0.87 | GC, MS |
| 93 | β-Selinene | 0.42 | GC, MS |
| 94 | α-Selinene | | GC, MS |
| 95 | Bicyclogermacrene | 0.10 | GC, MS |
| 96 | $C_{15}H_{24}$ | 0.18 | MS |
| 98 | Germacrene A | trace | GC, MS |
| 105 | δ-Cadinene | 0.17 | GC, MS |
| 106 | γ-Cadinene | 0.39 | GC, MS |
| 109 | ar-Curcumene | trace | GC, MS |
| 110 | $C_{15}H_{24}$ | 0.85 | MS |
| 112 | $C_{15}H_{24}$ | 0.80 | MS |
| 120 | Germacrene B | 1.20 | GC, MS |
| 147 | Germacradiene-1(10)*E*,5*E*-ol-4 | 0.80 | GC, MS, IR, $^1$H-, $^{13}$C NMR |
| 150 | Elemol | 3.65 | GC, MS, IR, $^1$H-, $^{13}$C NMR |
| 151 | Guajol | 1.48 | GC, MS |
| 152 | *trans*-Sesquilavandulyl acetate | | GC, MS, IR, $^1$H-, $^{13}$C NMR |
| 153 | Hedycaryol | –* | GC, MS |
| 154 | *cis*-Sesquilavandulol | 0.16 | GC, MS, IR, $^1$H-, $^{13}$C NMR |
| 155 | $C_{15}H_{26}O$ | 0.33 | MS |
| 156 | $C_{15}H_{26}O$ | 0.65 | MS |
| 157 | T-Cadinol | trace | GC, MS |
| 158 | *trans*-Sesquilavandulol | 53.37 | GC, MS, IR, $^1$H-, $^{13}$C NMR |
| 160 | $C_{15}H_{26}O$ | 0.60 | MS |
| 161a | $C_{15}H_{26}O$ | 0.77 | MS |
| 162 | Bulnesol | 0.26 | GC, MS |
| 164 | β-Eudesmol | 5.90 | GC, MS |
| 165 | α-Cadinol | | GC, MS, IR, $^1$H-, $^{13}$C NMR |
| 166 | *trans*-Cyclobutyl sesquilavandulol | 1.05 | MS, IR, $^1$H-, $^{13}$C NMR |
| 167 | $C_{15}H_{26}O$ | 2.02 | MS |
| 173 | Lancifolol | 1.96 | MS, IR, $^1$H-, $^{13}$C NMR |
| 174 | 2*Z*,6*E*-Farnesol | | GC, MS, IR, $^1$H-, $^{13}$C NMR |
| 178 | 2*E*,6*E*-Farnesol | 0.12 | GC, MS |

* No integration value; estimated peak area approximately 3%.

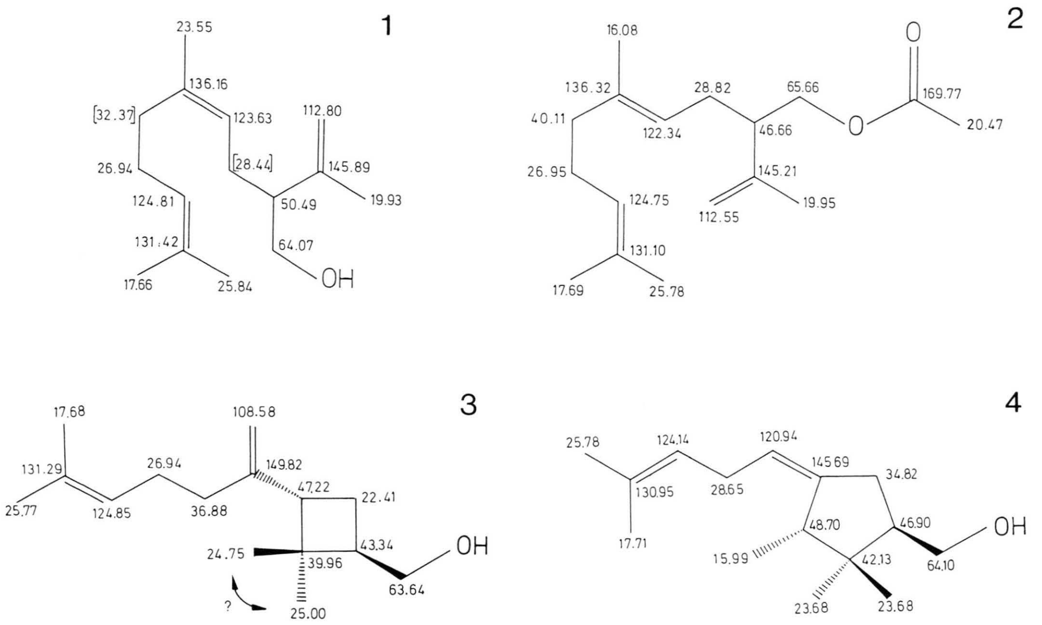

Fig. 2. Structural formulas and $^{13}$C NMR assignments of several new sesquiterpenoids identified in the essential root oil of *Peucedanum lancifolium*. (1) *cis*-sesquilavandulol, (2) *trans*-sesquilavandulyl acetate, (3) *trans*-cyclobutyl sesquilavandulol, (4) lancifolol.

*panicum)*, in order to examine the chemotaxonomic value of the uncommon sesquiterpenoid *trans*-sesquilavandulol. Apart from *Peucedanum lancifolium*, this constituent was only present in the roots of one other species, namely *Peucedanum palustre*, in which it has already been described by us before [5]. This result, along with the fact, that the essential root oils of these two *Peucedanum* species show a very similar composition [7] may be taken as a further indication of the close botanical relationship of these two species. In this connection, it is of interest that, based mainly on the anatomical features, a separate genus called *Thysselinum* comprising only the two species *Peucedanum lancifolium* and *Peucedanum palustre* has been proposed [8].

Although the root oils of these two plants are very similar in their composition, a reliable differentiation is yet possible. The volatile root oil of *Peucedanum lancifolium* is characterized by several aliphatic esters not occurring in *Peucedanum palustre;* in contrast, the root oil of the latter contains a number of benzyl esters which are absent in *Peucedanum lancifolium*.

The essential root oils of the other *Peucedanum* species mentioned before, which were also investigated by us, showed significant differences in their chemical composition. It is remarkable that besides mono- and sesquiterpene hydrocarbons, members of some other compound classes like C13-polyacetylenes, hemiterpenoids and phenylpropanoids could be observed.

The significant differences in the composition of the essential root oils from various *Peucedanum* species are in good accordance with the polymorphism of this genus. Regarding the great anatomical and morphological variability within the genus *Peucedanum*, Chamberlain wrote in the Flora of Turkey [9] that "it seems unlikely that *Peucedanum* represents a natural assemblage of species; it is more probably a collection of taxa brought together by exclusion from other genera".

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