

**Phenylpropanoids and Polyacetylenes from
Ligusticum mutellina (Apiaceae) of Tyrolean Origin**

Renate Spitaler¹, Ernst-Peter Ellmerer-Müller², Christian Zidorn^{1*}, and Hermann Stuppner¹

¹Institut für Pharmazie, Abteilung Pharmakognosie, Leopold-Franzens-Universität Innsbruck,
Innrain 52, A-6020 Innsbruck, Austria

²Institut für Organische Chemie, Leopold-Franzens-Universität Innsbruck, Innrain 52a, A-6020
Innsbruck, Austria

Key words - Apiaceae, *Ligusticum*, Phenylpropanoids, Polyacetylenes, Falcarindiol-3-O-acetate.

Abstract

Roots of *Ligusticum mutellina* (L.) CRANTZ afforded five major compounds, the phenylpropanoids trans-isoolemicin (**1**), sarisan (**2**), and trans-isomyristicin (**3**), and the polyacetylenes falcarindiol (**4**) and falcarindiol-3-O-acetate (**5**). Structures were assigned by 1D- and 2D-NMR spectroscopy. Close inspection of the NMR spectra of falcarindiol-3-O-acetate (**5**) and comparison with the literature revealed that published NMR data for this compound are most probably attributable to *cis* or *trans* Δ^2 -isofalcarindiol-1-O-acetate (**6a/6b**). ¹H and ¹³C NMR data for falcarindiol-3-O-acetate are given and trans-isoolemicin (**1**), sarisan (**2**), and trans-isomyristicin (**3**), falcarindiol (**4**), and falcarindiol-3-O-acetate (**5**) are reported for the first time from *Ligusticum mutellina*. Chemosystematic and ethnopharmacological implications of the findings are discussed briefly.

Introduction

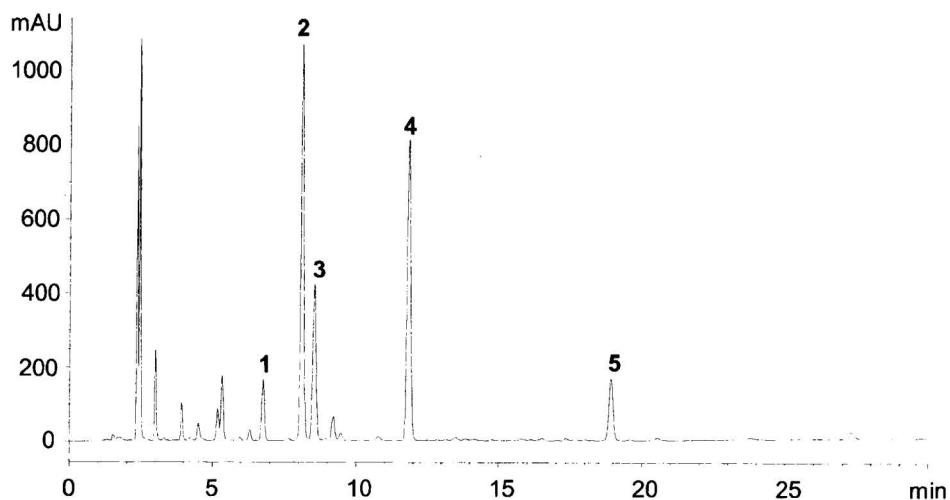
Ligusticum mutellina L. (German name: Alpen-Mutterwurz, Tyrolean folk names: Madaun, Mataun, Medaun, Muttern, Padaun, Roter Bärenfenchel)¹⁻³ is an aromatic umbelliferous herb of 10-50 cm height, distributed over Central and Southern Europe from Southern Central France to the Carpathians and Southern Bulgaria.⁴ In the Alps it is widespread and grows mainly in alpine and subalpine meadows.⁵ Leaves, flowers and roots were formerly used in folk medicine against colds, obstipation, liver disorders, and kidney and bladder diseases. Furthermore "Mutterwurz" was used as a spice like parsley (*Petroselinum crispum*, German: Petersilie),⁶ to flavor goat cheese,⁷ and to brew liquor similar to that made from *Meum athamanticum* (German name: Bärwurz).⁷ Analyses of the essential oils of *L. mutellina* roots from the Bavarian Forest revealed ligustilide as the main compound (> 20 %), other main compounds with a portion of over 5 % each were the monoterpenes α -phellandrene, β -phellandrene, and camphene as well as the C₁₁-substance viridene.⁸ The phenylpropanoids myristicin and dillapiol were also detected in reasonably high amounts (3.7 and 13.1 %, respectively).⁸ Analyses of plants collected in the Black Forest showed pronounced differences between root, herb and fruit oils.⁹ Phenylpropanoids were amongst the main compounds in all three oils, while phtalides were detectable in the underground parts only.⁹ Non-volatile compounds of *Ligusticum mutellina* have not been investigated yet.

Results and Discussion

The HPL-chromatogram of the dichloromethane extract of roots of *Ligusticum mutellina* showed five major peaks in the apolar region (Figure 1). After isolation by repeated silica gel column chromatography (CC) and subsequent Lobar and Sephadex LH-20 CC, the appertaining compounds were identified as trans-isoelemicin (1), sarisan (2), and trans-isomyristicin (3), falcarindiol (4) and falcarindiol-3-O-acetate (5). ¹H NMR and ¹³C NMR data of trans-isoelemicin

(1),¹⁰ sarisan (2),¹¹ trans-isomyristicin (3)¹², and falcarindiol (4)¹³ were in agreement with those reported in the literature.

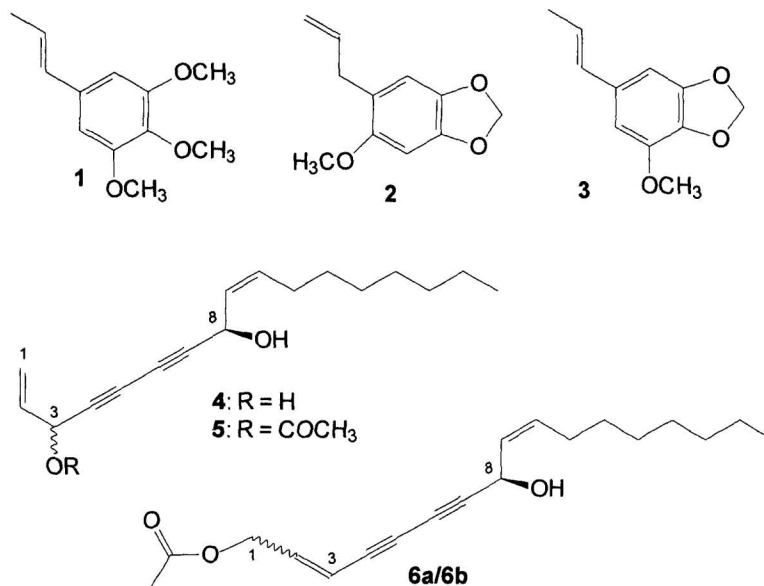
Figure 1. HPL chromatogram of a dichloromethane extract from roots of *Ligusticum mutellina*.



HPLC parameters: Mobile phase A: H₂O, mobile phase B: MeCN; linear gradient: 0 min: 50% B, 20 min: 75% B, 30 min: 95% B; stop time: 35 min; flow rate: 1.00 ml/min; column: Zorbax SB-C18 4.6 x 150 mm; column material: 3.5 μm; injection volume: 10 μl; detection wavelength: 205 nm.

Spectra for compound **5** were very similar to those of compound **4**. The spectra of **5** differed only in the occurrence of additional signals assignable to an acetyl moiety (¹H NMR: δ_H 2.10 3H, s; ¹³C NMR: δ_C 169.6 C=O, 21.0 CH₃), in the downfield-shift of the ¹H NMR signal for the proton in position 3 and in minor ¹³C NMR shift differences for carbons C-1, C-2, C-3, C-4, C-5, and C-6. ¹H and ¹³C NMR data of compounds **4** and **5** are given in Table 1. All signal assignments were verified by 2D-NMR experiments (HSQC, HMBC). Important HMBC crosspeaks observed for compound **5** are shown in Figure 2. Though there was no direct correlation from H-3 to the carbonyl of the

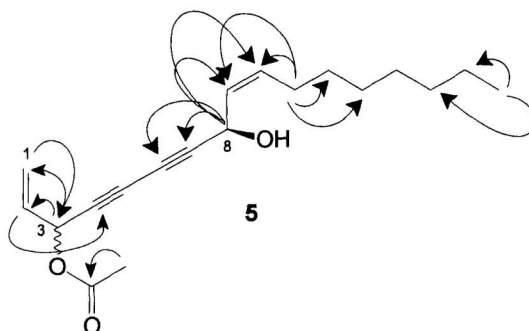
acetyl moiety observable, available ^1H NMR, ^{13}C NMR, HSQC and HMBC data are congruent with the assumption that compound **5** is falcarindiol-3-O-acetate.



Falcarindiol-3-O-acetate (**5**) has so far only been reported from *Daucus carota* L. (carrot, German name: Möhre, Austrian name: Karotte)¹⁴⁻¹⁹ and *Pituranthus tortuosus* (Desf.) Benth. & Hook.²⁰ Lund *et al.*¹⁷ were the first to report ^{13}C NMR data of falcarindiol-3-O-acetate (**5**). However, when compared with our data some significant differences were observed (Table 1). Lund *et al.*¹⁷ reported shift values for carbons C-11 to C-16 in increasing order from C-11 to C-16 without actually assigning the respective positions of each signal. This fact might be explained by the unavailability of 2D-hetero experiments at the time of the publication. Further shift differences occur in the polyene part (C-4 to C-7) of the molecule. However, the main difference was observed

in the ^{13}C NMR shift value given for carbon C-1 ($\delta_{\text{C}} = 110.6$ versus $\delta_{\text{C}} = 119.8$). There are no other signals in this region, so this difference can not be explained by a mix-up of signal assignments.

Figure 2. HMBC correlations observed for falcarindiol-3-O-acetate (**5**).



The shift differences between falcarindiol (Table 1) and the compound isolated by Lund *et al.* are not explainable by a simple substitution of the oxygen in position 3 with an acetyl moiety. As Lund¹⁹ pointed out in a later publication, falcarindiol-3-O-esters tend to isomerize to their Δ^2 -1-O-acyl isomers. Therefore, data published earlier for falcarindiol-3-O-acetate¹⁷ are most probably attributable to Δ^2 -isofalcarindiol-1-O-acetate (**6a/6b**). The fact that the compound isolated from *Ligusticum* was indeed falcarindiol-3-O-acetate was also supported by the HSQC data, which showed signals for a terminal methylene group ($\delta_{\text{H}} = 5.53$ and 5.34 ; $\delta_{\text{C}} = 119.8$). Conclusively this is the first reliable - verified by HSQC and HMBC spectroscopy - report about the NMR properties of falcarindiol-3-O-acetate **5**.

The HPLC data (Figure 1) from our collection of subaerial parts of *Ligusticum mutellina* from the Hahntennjoch (Tyrol/Austria) showed that compounds **1-5** are the prevalent compounds in that extract. Brandt and Schultze⁹ used GC analysis for their investigation of essential oils from

subaerial parts of *L. mutellina* from Southern German low mountain ranges. Therefore, it is not surprising that these authors did not observe any polyacetylenes.

Table 1. ^1H and ^{13}C NMR data of falcarindiol (4) and falcarindiol-3-O-acetate (5).

	Falcarindiol (4) ^a		Falcarindiol-3-O-acetate (5) ^a		"Falcarindiol-3-O-acetate" ¹⁶
	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR	^{13}C NMR
1	5.44, 1H, ddd (17.0, 1.5, 1.0) 5.26, 1H, ddd (10.0, 1.5, 1.0)	117.4	5.53 1H, m* 5.34 1H, br dd (10.0, 1.0)	119.8	110.6
2	5.94, 1H dd (17.0, 10.0, 5.0)	136.0	5.91 1H, m*, ^a	132.1	132.0
3	4.94, 1H, br d (5.0)	63.7	5.83 1H, dd (16.5, 6.5) ^a	64.6	64.4
4		78.4		75.0	72.8
5 ^a		70.5		71.0	74.8
6 ^a		69.4		68.8	75.7
7		80.1		80.2	n.o.
8	5.20, 1H br d (8.0)	58.8	5.19 1H, br d (8.0)	58.8	58.5
9	5.50, 1H ddt (10.5, 8.0, 1.0)	127.9	5.50 1H, m*	127.8	127.7
10	5.63, 1H ddd (10.5, 7.5, 1.0)	134.9	5.62 1H, m	134.9	134.6
11	2.11, 1H qd (7.5, 1.0)	27.9	2.10, 1H qd (7.5, 1.0)	27.9	22.6
12 ^b	1.25-1.35, 2H m*	29.4	1.25-1.35, 2H m*	29.4 ^b	27.7
13 ^b	1.25-1.35, 2H m*	29.3	1.25-1.35, 2H m*	29.3 ^b	29.1
14 ^b	1.25-1.35, 2H m*	29.2	1.25-1.35, 2H m*	29.2 ^b	29.1
15 ^c	1.25-1.35, 2H m*	22.8	1.25-1.35, 2H m*	22.8	29.3
16 ^c	1.25-1.35, 2H m*	31.9	1.25-1.35, 2H m*	31.9	31.8
17	0.88, 3H, t (7.0)	14.2	0.88 3H, t (7.0)	14.2	14.0
α				169.6	n.g.
β			2.10 3H, s	21.0	n.g.

^a Measured in CDCl_3 at 300 and 75 MHz, respectively. ^b Exchangeable signals. * Signals not resolved. n.g. not given. n.o. not observed.

However, the phenylpropanoid spectrum observed in the German samples also differed considerably from our data. While Brandt and Schultze⁹ observed myristicin and phtalides as the main phenylpropanoids, extracts of Tyrolean origin mainly contained the myristicin isomeres

sarisan (**1**) and trans-isomyristicin (**2**) and no major amounts of myristicin or phtalides were detectable (Figure 1).

Apparently different chemotypes of *Ligusticum mutellina* are inhabiting the German low mountain ranges (Black Forest, Bavarian Forest) and the Tyrolean alps. Phenylpropanoids possess pronounced biological activity, e.g. as anticarcinogenic;²¹ antiplatelet and hypolipidemic;¹² and psychotropic²² compounds. The activity profiles of the particular phenylpropanoids differ considerably and therefore the qualitative and quantitative differences observed between extra-alpine and Tyrolean samples of *Ligusticum mutellina* imply a different spectrum and degree of biological activity of the extracts of plant samples collected in different areas. Further comparative chemosystematic studies of plants collected in different areas and analyzed by the same technique (GC and/or HPLC) might therefore solve ethnopharmacological and botanical questions alike.

Experimental

General. - NMR spectra were recorded in CDCl₃ at 300 and 75 MHz, respectively. ¹H NMR spectra were referenced to solvent residual signals of CHCl₃ at 7.25 ppm and ¹³C NMR spectra were referenced to solvent signals at 77.16 ppm.²³

Plant material. - *Ligusticum mutellina* was collected in July 1999 at the Faselfeijöchel N of the Anhalter Hütte near the Hahntennjoch/Tyrol/Austria (altitude: 1980 m; coordinates (WGS84): N 47°18'43"; E 10°40'14"). A voucher specimen (CZ-99-00334) was deposited in the herbarium of the Institut für Pharmazie, Abteilung Pharmakognosie.

Extraction and isolation. - 172 g of air dried were ground and extracted exhaustively with dichloromethane. The crude extract (11.9 g) obtained after evaporating the solvent *in vacuo* was fractionated by silica gel column chromatography (CC) using a gradient from petrol ether to dichloromethane and from dichloromethane to methanol. Fractions containing **1** and **2** (885 mg)

were further fractionated by silica gel CC employing a gradient from petrol ether and dichloromethane. Enriched fractions of **1** (383 mg) were finally purified by RP18 Lobar CC using a gradient from H₂O and methanol to yield 17.9 mg of pure compound **1**. Enriched fractions of **2** (226 mg) were purified by Sephadex LH-20 CC - using the eluant methanol - yielding 23.4 mg of compound **2**. More polar silica gel CC fractions containing prevalently compound **3** (207 mg) were further fractionated by repeated Sephadex LH-20 CC (eluant methanol) to give 21.7 mg of compound **3**. Silica gel fractions containing **4** and **5** (1.45 g) were fractionated by silica gel CC again using a gradient of petrol ether, dichloromethane, and methanol. Enriched fractions of **4** (482 mg) were further purified by silica gel CC employing a gradient of dichloromethane and acetone. Further enrichment of impure compound **4** (63.5 mg) was performed by Lobar RP18 CC using a gradient of H₂O and MeCN to yield a fraction of 23.9 mg containing prevalently compound **4**. This was finally purified by Sephadex LH-20 CC (eluant methanol) to yield 17.0 mg of pure **4**. Enriched silica gel fractions of compound **5** were purified by Sephadex LH-20 CC (eluant methanol) yielding 11.2 mg of compound **5**.

Acknowledgement

The authors wish to thank Dr. M. J. Dobner for helping collect the plant material.

References

- [1] Pritzel, G., Jessen, C. (1882), Die deutschen Volksnamen der Pflanzen, Philipp Cohen, Hannover, p. 238.
- [2] Dalla Torre, K. W. (1895), Die volkstümlichen Namen in Tirol und Vorarlberg, A. Edlinger, Innsbruck, p. 43.
- [3] Malfer, V. (1953), Der Schlern 27: 369.

- [4] Tutin, T. G. (1968), *Ligusticum*, Tutin, T. G., Hywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., Webb, D. A. (Eds.), *Flora Europaea* Vol. 2 Rosaceae to Umbelliferae, Cambridge University Press, Cambridge, p. 356.
- [5] Adler, W., Oswald, K., Fischer, R. (1994), *Exkursionsflora für Österreich*, E. Ulmer, Stuttgart - Wien, 1180 p.
- [6] French, D. H. (1971), *Ethnobotany of the Umbelliferae*, Heywood, V. H. (Ed.), *The Biology and Chemistry of the Umbelliferae*, Bot. J. Linn. Soc., Vol. 64, Suppl. 1, London, pp. 385-412.
- [7] Hegi, G. (1925), *Illustrierte Flora von Mitteleuropa*, Band V, 2. Teil, J. Lehmann, München, pp. 1318-1325.
- [8] Kubeczka, K.-H., Bartsch, A., Ullmann, I. (1982), *Neuere Untersuchungen an Apiaceen Ölen*, Kubeczka, K.-H. (Ed.), *Ätherische Öle*, Thieme, Stuttgart, pp. 158-187.
- [9] Brandt, J. J., Schultze, W. (1995), *J. Essent. Oil Res.* 7: 231.
- [10] Poplawski, J., Lozowicka, B., Dubis, A. T., Lachowska, B., Witkowski, S., Siluk, D., Petruszewicz, J., Kaliszan, R., Cybulski, J., Strzalkowska, M., Chilmonczyk, Z. (2000), *J. Med. Chem.* 43: 3671.
- [11] Villegas, M., Vargas, D., Msonthii, J. D., Marston, A., Hostettmann, K. (1988): *Planta Medica* 54: 36.
- [12] Antunes, A. M., Palma, F. M. S. B., Santos, S. M. B. P. (1995), *Fitoterapia* 66: 554.
- [13] Zheng, G., Lu, W., Cai, J. (1999), *J. Nat. Prod.* 62: 626.
- [14] Bentley, R. K., Bhattacharjee, D., Jones, E. R. H., Thaller, V. (1969), *J. Chem. Soc. C*: 685.
- [15] Lund, E. D., White, J. M. (1987), *Proc. Fla. State Hort. Soc.* 99: 100.
- [16] Maki, A., Kitajima, J., Abe, F., Stewart, G., Ryan, M. F. (1989), *J. Chem. Ecol.* 15: 1883.
- [17] Lund, E. D., White, J. M. (1990), *J. Sci. Food Agric.* 51: 507.
- [18] Lund, E. D., Bruemmer, J. H. (1991), *J. Sci. Food Agric.* 54: 287.
- [19] Lund, E. D. (1992), *Phytochemistry* 31: 3621.
- [20] Schulte, K. E., Potter, B. (1977), *Arch. Pharm.* 310: 945.
- [21] Tseng, T.-H., Tsheng, Y.-M., Lee, Y.-J., Hsu, H.-L. (2000), *J. Chin. Chem. Soc.* 47: 1165.
- [22] Shulgin, A. T. (1966), *Nature* 210: 380.
- [23] Gottlieb, H. E., Kotlyar, V., Nudelman, A. (1997), *J. Org. Chem.* 62: 7512.