Insecticidal Activity of the Essential Oil of *Ligusticum mutellina* Roots

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The essential oil obtained from roots of different collections of *Ligusticum mutellina* was tested against 3rd instar armyworms, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae), for insecticidal activity. The main compounds were isolated and their structures were elucidated using 2D-NMR techniques. Our collections contained dillapiole, ligustilide and myristicin as major compounds. The previously reported sarisan was not present, moreover its occurrence in *L. mutellina* should be revised based on our findings.

Key words: Ligusticum mutellina, Essential Oil, Insecticidal Activity

Introduction

Ligusticum mutellina (L.) Crantz is a member of the Apiaceae family which occurs in the alpine and subalpine regions of Central and Southern Europe from France to the Carpathians and Southern Bulgaria (Tutin, 1968).

Its utilisation ranges from its use as a spice (French, 1971), similar to parsley, to the distillation of a liquor, similar to that made from *Meum athamanticum,* called "Bärwurz" in Bavaria (Hegi, 1925), to medicinal use as a stomachic and as a herbal remedy against female disorders (French, 1971; Hegi, 1925).

The essential oil and the CH_2Cl_2 extract, respectively, from roots of *L. mutellina* collected from the Black Forest (Brandt and Schultze, 1995) and from Tyrol (Spitaler *et al.,* 2002) were previously studied by different investigators. While Brandt and Schultze (1995) showed by GLC/MS studies in comparison to authentic samples, that the essential oil of their plants mainly contained ligustilide (**1**) (19%), dillapiole (**2**) (3%) and myristicin (**3**) (26%), Spitaler *et al.* (2002) isolated sarisan (**4**), a structural isomer of **3**, along with other phenylpropanoids and polyacetylenes from Tyrolean plants. No major amounts of **1** or **3** were found in their alpine collection. Based on these findings, different chemotypes were proposed (Spitaler *et al*., 2002).

In light of these discussions and in continuation of our work on the insecticidal activity of essential oils (Isman *et al*., 2001; Passreiter *et al*., 2004), we now have studied the root oil from wild and cultivated *L. mutellina* plants from Bavaria.

Materials and Methods

Plant material

Ligusticum mutellina roots collected in the wild (Bohemian forest) and cultivated in fields near Plattling (Bavaria) were obtained from Eckert Comp., Deggendorf, Germany. Plants were identified by CMP. A voucher specimen (Lig 0104) is on deposit at the Institute of Pharmaceutical Biology, Düsseldorf, Germany.

Extraction of the essential oil

Following the method for quantification of essential oils in roots of *Levisticum officinale* (Apiaceae) (Pharmacopoea Europea, 2002a), 40 g dried roots were ground and distilled with water for 30 min. The quantity (ml) of the obtained essential oil was then determined using the required distillation apparatus (Pharmacopoea Europea, 2002b).

Purification

Column chromatography of 0.9 g of the essential oil obtained from the cultivated *L. mutellina* roots on silica gel 60 with a mixture of toluene/ethyl acetate 95:5 (v/v) as mobile phase afforded 15 fractions. Compounds **2** (fr. 4, 15 mg) and **3** (fr.

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6, 106 mg) were directly obtained in pure form, while **1** (5.9 mg) was only isolated after further purification by preparative TLC (silica gel 60 F_{254}; CH_2Cl_2 100%) from fraction 8; R_f-values; **1** 0.53; **2** 0.61; **3** 0.72.

Isolation of myristicin from nutmeg oil

1 ml of nutmeg oil (Oleum macidis, Caesar & Loretz Comp., Hilden, Germany) was purified by preparative TLC using CH_2Cl_2 (100%) as mobile phase.

NMR (Bruker ARX 500, $CDCl_3$, calibrated on solvent signal): δ = 6.38 (d, J = 1.6 Hz, H-6), 6.35 [s (br.), H-2], 5.93 (s, $-OCH_2O-$), 5.92 (m, H-8), 5.08 (m, H-9), 3.89 (s, OCH_3), 3.29 (d, J = 6.9 Hz, H-7). – GC/MS: EI (70 eV) HP MSD 5972 with GC 5890 plus (HP); Optima-1 (MN), 25 m × 0.25 mm; 46 °C (3 min) to 220 °C at 2 °C min^{-1}; R_t (min): **1** 36.4; **2** 32.7; **3** 29.1. – MS (*m/z*, rel. int.): **1**: 190 $[M]^+$ (24), 161 (58), 148 (52), 105 (55), 91 (20), 77 (41), 55 (100), 41 (10); **2**: 222 $[M]^+$ (100), 177 (40), 149 (28), 121 (20), 106 (30), 91 (24), 77 (36), 53 (28) 41 (7); **3**: 192 $[M]^+$ (94), 177 (10), 165 (29), 161 (25), 147 (21), 131 (25), 119 (40), 105 (24), 91 (88), 77 (59), 65 (57), 53 (53), 41 (100).

Toxicity experiments

Samples of *Ligusticum* oils were applied topically using a repeating syringe to the dorsum of laboratory-reared 3rd instar armyworms (*Pseudaletia unipuncta*, Noctuidae) in 1 μl acetone as previously described (Isman *et al.*, 2001). LD_{50} values for each oil were determined by probit analysis, based on a 24 h mortality with 3 replicates of 4-doses, with ten larvae per dose. Concentrations ranged from 6.25–50 $\mu g/\mu l$.

Results and Discussion

The essential oil of *L. mutellina* grown in fields near Plattling (Bavaria) mainly consisted of three compounds. From the GC/MS spectra it was obvious that **2** (M^+ 222) was the major compound, followed by **1** (M^+ 190) and a third compound displaying its molecular ion at *m/z* 192, consistent with the molecular weight of the isomeric compounds **3** and **4** (Fig. 1).

After purification by column chromatography and preparative TLC all three compounds were isolated in pure form and their structures established by NMR. The assignment of all data was additionally proven by homo- and hetereonuclear

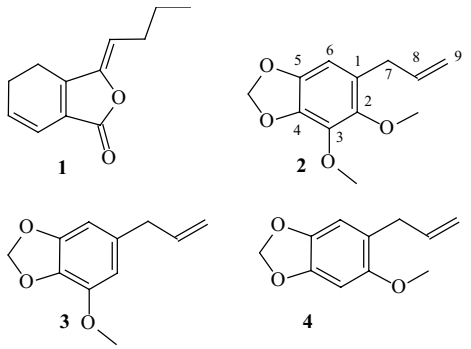

Fig. 1. Structures of isolated compounds ligustilide (**1**), dillapiole (**2**) and myristicin (**3**) as well as sarisan (**4**).

correlation experiments (COSY, HMQC and HMBC). The structure of **1** was unambiguously proven by its ^1H and ^{13}C NMR spectra, which displayed all signals at shift values previously reported for ligustilide (**1**) (Beck and Stermitz, 1995). The signals in the NMR spectra of **2** were found at shift values previously reported for dillapiole (**2**) (Benevides *et al.*, 1999), but the assignment of the carbon signals given there has to be corrected. The correct assignment is given in Table I.

The structure elucidation of the third compound was more complex. Spitaler *et al.* (2002) only gave reference to a paper published by Villegas *et al.* (1988), who isolated **4** from *Heteromorpha trifoliata*. Our data herein are identical to those published in the latter paper (see Table I). Strikingly, different shift values are given for **4** in a paper published earlier by Yakushijin *et al.* (1983), while

Table I. ^{13}C NMR data of compounds **2–4** (125 MHz, $CDCl_3$).

Carbon	**2**	**3**	**4**[a]
1	126.0	133.4	133.4
2	137.6	107.6	134.4
3	144.3	143.5	107.8
4	144.6	134.6	148.7
5	135.9	148.8	143.3
6	102.7	102.7	102.5
7	33.9	40.2	40.1
8	137.4	137.3	137.2
9	115.5	115.8	115.6
OCH_3 at C-2	61.3		56.4
OCH_3 at C-3	59.6	56.5	
OCH_2O	101.1	101.2	101.0

[a] Data taken from Villegas *et al.* (1988).

Table II. Toxicity [LD_{50} (95% confidence limits) in μg per larva] of root oils and pure compounds to 3rd instar armyworms.

Essential oil/compound	LD_{50} [μg/larva]	Ref.
L. mutellina cultivated	25.1 (12.5–38.4)	present paper
L. mutellina wild	12.6 (9.4–14.2)	present paper
Origanum creticum	66.0 (58.0–75.1)	Isman *et al.* (2001)
Satureja hortensis	48.4 (44.2–53.1)	Isman *et al.* (2001)
Thymus vulgaris	46.7 (41–47)	Hummelbrunner and Isman (2001)
Dillapiole (**2**)	5.8 (3.2–9.6)	present paper

identical values were given for myristicin (**3**) by the same authors.

Although the data given by Yakushijin *et al.* (1983) are more consistent with the structures of **3** and **4**, we decided to record a 2D-NOESY spectrum, to confirm the position of the methoxyl group. By the contacts between H-6 and H-7 of the side chain and those between H-2 and the OCH_3 as well as H-7 it was obvious that our isolate **3** was myristicin and not sarisan. Additionally, we isolated myristicin from nutmeg oil for comparison. Both compounds were absolutely identical, indicating that the reports of Spitaler *et al.* (2002) and Villegas *et al.* (1988) require correction.

The essential oil from roots collected in the wild was proven by GLC/MS to be qualitatively identical to that distilled from cultivated plants. Differences were only found in the proportions of the major compounds. However, **4** was also not found in the wild sample.

Oil obtained from the cultivated plants (near Plattling, Bavaria) was toxic to armyworms, with an LD_{50} value of 25.1 μg per larva (see Table II). As such, this oil is approx. ten times more toxic to the armyworm than rosemary oil (from *Rosmarinus officinalis* L., Isman, unpubl. data). It also appears considerably more toxic than oils from *Satureja hortensis* L. (LD_{50} = 48.4 μg/larva), *Origanum creticum* L. (LD_{50} = 66.0 μg/larva) (Isman *et al.*, 2001), or *Thymus vulgaris* L. (LD_{50} = 46.7 μg/larva) (Hummelbrunner and Isman, 2001), although the latter oils were tested on the related tobacco cutworm, *Spodoptera litura* Fab.

Oil from wild *Ligusticum mutellina* plants collected at Boehmerwald was twice as toxic to armyworms (LD_{50} = 12.6 μg/larva) as that from the cultivated plants (see Table II). This result is not surprising because oil from the wild plants contained ~ 3 times more of **1** and ~ 1.6 times more of **2** than the oil from the cultivated plants. Pure dillapiole (**2**) has a LD_{50} of 5.8 μg/larva for the armyworm. Further investigations on the activities of individual compounds and combinations of these from *L. mutellina* root oil are planned.

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