C_{17} Polyacetylenic Alcohols as the Major Constituents in Roots of *Petroselinum crispum* Mill. ssp. *tuberosum*

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Falcarinol [heptadeca-1,9(*Z*)-diene-4,6-diyn-3-ol] and falcarindiol [heptadeca-1,9(*Z*)-diene-4,6-diyne-3,8-diol] were extracted from edible parsley root and purified by means of multilayer coil countercurrent chromatography (MLCCC). The structures of the compounds were determined by spectroscopic and chemical derivatization methods. The quantities of falcarinol and falcarindiol were 30% and 9% of the extractable oil, respectively. Their influence on seed germination was examined.

INTRODUCTION

During the investigation of volatile constituents of parsley root (Nitz et al., 1990), two C_{17} polyacetylenic compounds were observed. Acetylenic substances occur in many plant species (Bohlmann et al., 1973) and have often been shown to be toxic to bacteria, nematodes, mammals, and fungi (Kemp, 1978; Kern et al., 1979). Their possible role in the resistance of plants to disease has received little attention so far. They also can effect sensibilization and allergic skin reactions in minute concentrations (Bruhn et al., 1987; Hausen et al., 1987; Hansen et al., 1986). With respect to their possible biological activity and due to the fact that parsley root serves as a flavor enhancer and is used as an ingredient in foods, it was of importance to determine the structure of the aforementioned compounds. IR, MS, and NMR spectroscopies of the purified substances supplemented by chemical conversions and investigation of the reaction products showed both to be identical with heptadeca-1,9-(*Z*)-diene-4,6-diyn-3-ol (1) [i.e., falcarinol, Bohlmann et al.

Falcarinol [1]

Falcarindiol [2]

(1966); panaxynol, Takahashi and Yoshikura, (1966); carotatoxin, Bentley et al. (1969)] and heptadeca-1,9(*Z*)-diene-4,6-diyne-3,8-diol (2) [falcarindiol, Bohlmann et al. (1966)]. Their isolation, identification, stability, and inhibitory effect on seed germination will be reported in this paper.

MATERIALS AND METHODS

Plant Materials. *Petroselinum crispum* (Mill.) Nyman syn. P. *sativum* Hoffm., var. Mooskrause, Hilmar was cultivated in a greenhouse at Freising-Weihenstephan, FRG. *P. crispum* Mill. ssp. *tuberosum* (parsley root) were bought from Fa. Neumüller, grown at field locations nearby Hallbergmoos-Munich.

Sample Preparation. Fresh parsley roots (150 g) were grounded in liquid nitrogen with pestle and mortar. Ethyl ether (350 mL) was added, and the slurry was filtered. The organic extract (−10 °C) was concentrated under reduced pressure at ambient temperature in the dark.

Multilayer Coil Countercurrent Chromatography (MLCCC). MLCCC was performed on a epicyclic coil planet centrifuge, the Ito multi-layer coil separator-extractor, manufactured by PC Inc. (Potomac, MD). The solvent system used was hexane–acetonitrile–*tert*-butyl methyl ether (10:10:1). The mobile phase was the acetonitrile solution, and the stationary phase was the hexane solution, retained in the column by centrifugal means. The injected sample was 350 mg of pure extract, dissolved in 0.3 mL of acetonitrile solution. Falcarindiol (4 mg) was isolated in fractions 8–11 and falcarinol (17 mg) in fractions 55–62, both with a purity over 98%. The flow direction was from head to tail. The physical parameters were as follows: column, 1.6 mm i.d., 130 m long, 285 mL capacity; speed, 800 rpm; flow rate, 0.8 mL/min.

Falcarinol. R_f, UV, and MS data are in agreemeent with the data reported by Hansen and Boll (1986), but not with MS data from Bentley et al. (1969). MS (70 eV) data are shown in Table I, GC–FTIR data in Table II, and $^1H/^{13}C$ NMR data in Table III. The Kovats index is 2035 (SE 54). Acetylation (acetic anhydride–pyridine) and silylation (TMCS/HMDS) gave the corresponding derivatives. Oxidation with $KMnO_4/NaIO_4$ leads to the main products hexadecanoic acid and octanoic acid. The main product of ozonolysis was octanal. Hydrogenation with H_2/PtO_2 for 4 h at 20 °C in ethanol gave the following product distribution: 44% *n*-heptadecane, 13% heptadecan-3-one, and 40% heptadecan-3-ol.

Falcarindiol. UV data are in agreement with the data reported by Bentley et al. (1969) and Lemmich (1979) and ^{13}C NMR data with those from Kern and Cardellina (1982) and Cichy et al. (1984). MS (70 eV) data are shown in Table I, GC–FTIR data in Table II, and $^1H/^{13}C$ NMR data in Table III. The Kovats index is 2190 (SE 54). Acetylation (acetic anhydride–pyridine) and silylation (TMCS/HMDS) gave the corresponding derivatives. Oxidation with $KMnO_4/NaIO_4$ leads to the same main products as for falcarinol. Hydrogenation with H_2/PtO_2 for 4 h at 20 °C in ethanol gave the following product distribution: 25% *n*-heptadecane, 8% heptadecan-8-one, 13% heptadecan-3-one, 26% heptadecan-3-ol, and 13% heptadecane-3,8-dione. The hydrogenation results were comparable with those from Lemmich (1981).

Gas Chromatography. Siemens Sichromat I was equipped with a 26 m × 0.25 mm i.d. fused silica capillary column coated with 0.3-μm cross-linked SE 54. The carrier gas was 1.7 mL/min H_2. The temperature program was 60 °C (5 min), raised at 2 °C/min to 250 °C; the injector and detector temperatures were

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Table I. Mass Spectral Data of Falcarinol (1) and Falcarindiol (2)

	intensity, %			intensity, %	
m/z	1	2	m/z	1	2
39	35	35	91	54	41
41	70	79	103	15	6
43	68	64	105	14	16
51	19	19	115	30	25
53	17	19	117	25	12
54	21		128	13	29
55	100	100	129	18	36
57	17	20	131	18	7
63	14	12	141	12	5
65	20	19	157	3	11
67	21	17	159	14	1
69	6	13	171	0.2	4
77	27	35	173	3	0.3
78	19	13	231		0.1
79	19	24	242		0.2
81	20	13			

Table II. GC–FTIR Spectral Peak Assignments of 1 and 2

ν_{max} (cm^{-1}), intensity		
falcarinol (1)	falcarindiol (2)	vibration
3641, w	3641, m	O—H streching (free OH)
3030, m	3029, m	C—H stretching (C=C—H)
2934, vs	2934, vs	C—H stretching (asym)
2867, s	2868, s	C—H stretching (sym)
2249, m	2145, w	C≡C stretching (disubstituted)
1646, m	1651, m	C=C stretching
1462, m	1459, m	C—H in plane deformation
1349, m	1318, m	O—H in plane deformation
1286, m		C—O stretching
1225, m	1228, m	
1116, m	1114, m	
1016, s	1020, s	
932, s	934, s	C—H out-of-plane deformation (1.1-disubst CH_2-wagging or 1.2-trans-CH-wagging)

Table III. ^{13}C and ^{1}H NMR Spectral Peak Assignments of 1 and 2

	falcarinol (1)			falcarindiol (2)		
posn	δ_C	δ_H	couplings	δ_C	δ_H	couplings
1	117.1	5.23	1 H, ddd	117.4	5.24	1 H, ddd
		5.47	1 H, ddd		5.49	1 H, ddd
2	136.2	5.92	1 H, ddd	135.9	5.93	1 H, ddd
3	63.6	4.89	1 H, dd	63.5	4.92	1 H, dd
4	80.1			79.9		
5	71.4			70.4		
6	64.1			68.8		
7	74.3			78.9		
8	17.8	3.02	2 H, d	58.7	5.19	1 H, d
9	121.9	5.37	1 H, dtt	127.8	5.50	1 H, ddt
10	133.2	5.50	1 H, dtt	134.8	5.59	1 H, dtd
11	27.3	2.01	2 H, td	27.8	2.09	2 H, tdd
12	29.3	1.35	2 H, m	29.2	1.37	2 H, m
13	29.3	1.27	2 H, m	29.3	1.27	2 H, m
14	29.2	1.27	2 H, m	29.2	1.27	2 H, m
15	31.9	1.27	2 H, m	31.9	1.27	2 H, m
16	22.7	1.27	2 H, m	22.7	1.27	2 H, m
17	14.2	0.87	3 H, t	14.2	0.87	3 H, t

150 and 250 °C, respectively. The Siemens PTV (temperature programmed vaporizer) program was 40 °C (3 min) raised to 250 °C (splitless injection).

GC–FTIR. Hewlett-Packard Model 5890/5965 A(IRD) was equipped with a 30 m × 0.25 mm fused silica capillary column coated with 0.5-μm OV-101. Capillary conditions as above were used.

NMR. NMR spectra were recorded with a Bruker AM X500 NMR spectrometer at 500-MHz ^{1}H and 125-MHz ^{13}C operating frequency, both in chloroform-*d*. The ^{13}C and ^{1}H NMR spectral peak assignments of [1] and [2] were made on the basis of homonuclear and heteronuclear COSY (Friebolin, 1988).

GC–MS. Finnigan 1020 (quadrupole) was linked to an Incos data processing system, directly coupled to a Sigma III (Perkin-Elmer) GC. J&W 30 m × 0.25 mm i.d. fused silica capillary column was coated with 0.25-μm bonded DB 5. The carrier gas was 1.2 mL/min He. The temperature program was 60 °C (5 min) raised at 2 °C/min to 250 °C; the injector and transferline temperatures were 200 °C. The ionization energy was 70 eV.

Bioassay. Inhibition of seed germination was determined by germination tests with *Amaranthus caudatus* and *Lepidium sativum*, described by Oster (1988).

RESULTS AND DISCUSSION

In the beginning the first natural acetylenes were isolated mainly by steam distillation using the methods of essential oil production. In the actual case, this method proved to be inadequate because of the instability and polarity of the investigated polyacetylenes. Only careful solvent extraction leads to higher recoveries. The combined methods of solvent extraction, column chromatography, and HPLC (Yates and England, 1982; Wulf et al., 1978) for the isolation and purification of falcarinol and falcarindiol in higher amounts proved to be nonsuitable, because multiple chromatographic steps are necessary to achieve separation from other parsley root constituents like coumarins, phenylpropenes, and phthalides. Additionally, due to their sensitivity to oxygen, light, and heat (Bohlmann et al., 1973), a careful one-step separation and purification method was designed. The separation obtained by means of multilayer coil countercurrent chromatography yielded almost pure (>98%) falcarinol and falcarindiol. Countercurrent chromatography is a new form of partition chromatography that eliminates the use of solid supports. The MLCCC system has retained the merits of the countercurrent distribution method (CCD) and liquid–liquid partiton chromatography (LC) while discarding their disadvantages. It yields a high partition efficiency by continuous elution as does LC and yet retains the good sample recovery, excellent reproducibility, and high purity of fractions inherent to CCD (Ito and Conway, 1984). The two-phase system hexane–acetonitrile–*tert*-butyl methyl ether (10:10:1) leads to the best resolution of the two polyacetylenes; slight changes in the proportion of *tert*-butyl methyl ether or the replacement of hexane against octane deteriorates the separation. A flow of 0.8 mL/min with 350 mg of solvent extract stand the test (Fischer et al., 1990). The content of the different fractions was determined by GC. The quantified amounts were strongly dependent on the injection temperature. The best results were obained by using a PTV injector, a technique that is proved to be suitable for chromatography of thermally instable compounds. Distinct elution from GC columns with polar coatings like Carbowax 20M was not possible.

Although much work about polyacetylenes is found in the literature, the spectroscopic data reported are fragmentary, mainly in the case of falcarindiol. Therefore, the assignment of 500-MHz ^{1}H NMR and 125-MHz ^{13}C NMR signals as well as the derivatization results are given in this paper for comprehensive characterization. Two-dimensional ^{1}H and ^{13}C NMR (H,H-COSY and H-C-COSY) spectra of **2** are shown in Figures 1 and 2. The ^{1}H cross peaks for each of the carbon resonances observed in the heteronuclear COSY as well as the proton coupling constants observed in the homonuclear COSY are given in Table III.

Most often, falcarindiol has been isolated in minute quantities only. However, root material of *P. crispum* Mill. ssp. *tuberosum* proves to be a rich source of falcarinol

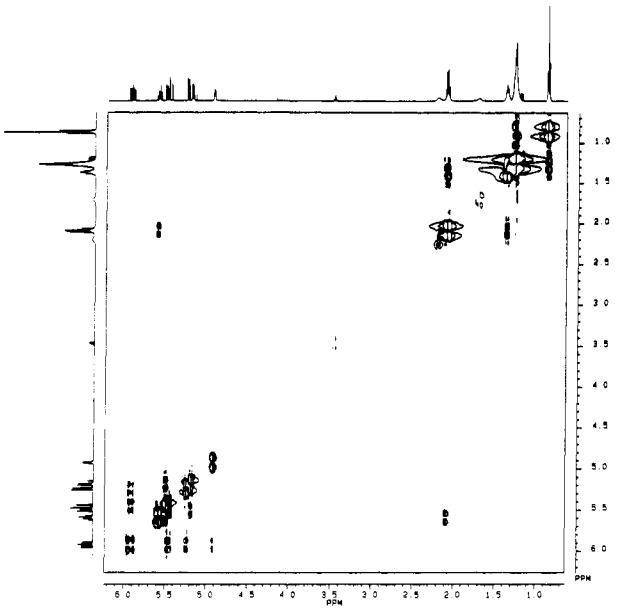

Figure 1. Homonuclear COSY spectrum of falcarindiol (2), recorded in $CDCl_3$ at ambient temperature.

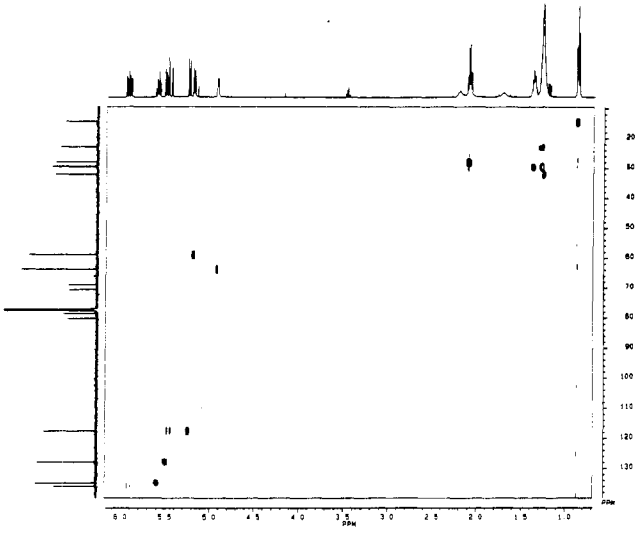

Figure 2. Heteronuclear COSY spectrum of falcarindiol (2), recorded in $CDCl_3$ at ambient temperature.

(approximately 30 mg/kg) and falcarindiol (approximately 10 mg/kg); minor amounts are found in the roots of *P. crispum* Mill. ssp. *crispum*. As long as the classical essential oils are used in food manufacturing, there is no concern about their biological activity, because of their low content of polyacetylenes. The GC–MS examination of two commercially available essential oils from parsley root showed them to be free from polyacetylenes. It can be expected, however, that a large increase in concentration will result if solvent or supercritical fluid extraction is used for oil manufacturing. The toxicological properties of parsley root seem to be comparable to those of other Umbelliferae already reported in literature (Bohlmann et al., 1973; Cichy et al., 1984; Teuscher and Lindequist, 1987). Allergic contact dermatitis and skin irritation as a result of the contact with those oils cannot be excluded.

The antifungal properties of both compounds were determined by Kemp (1987); his results demonstrated falcarindiol to be a strong inhibitor. Similar results were found with regard to the effects on seed germination, which showed falcarindiol to be a potent inhibitor to *A. caudatus* and *L. sativum*. Experiments referred to structure–activity relationship are in progress.

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LITERATURE CITED

Bentley, R. K.; Bhattacharjee, D.; Jones, R. H.; Thaller, V. Natural Acetylenes. Part XXVII. *J. Chem. Soc. C* **1969**, 685–688.

Bohlmann, F.; Niedballa, U.; Rode, K.-M. Über neue Polyine mit C_{17}-Kette. *Chem. Ber.* **1966**, *99*, 3552–3558.

Bohlmann, F.; Burkhardt, T.; Zdero, C. Introduction. In *Naturally Occurring Acetylenes*; Academic Press: London, 1973.

Bruhn, G.; Faasch, H.; Hahn, H.; Hausen, B. M.; Bröhan, J.; König, W. A. Natürliche Allergene, I. Das Auftreten von Falcarinol und Didehydrofalcarinol in Efeu. *Z. Naturforsch.* **1987**, *42B*, 1328–1332.

Cichy, M.; Wray, V.; Höfle, G. Neue Inhaltsstoffe von Levisticum officinale Koch (Liebstöckel). *Justus Liebigs Ann. Chem.* **1984**, 397–400.

Fischer, N.; Weinreich, B.; Nitz, S.; Drawert, F. 1990, unpublished results.

Friebolin, H. Zweidimensionale NMR-Spektroskopie. In *Ein- und zweidimensionale NMR-Spektroskopie*; VCH: Weinheim, 1988.

Garrod, B.; Lea, E. A.; Lewis, B. G. Studies on The Mechanism Of Action Of The Antifungal Compound Falcarindiol. *New Phytol.* **1979**, *83*, 463–471.

Hansen, L.; Boll, P. M. The Polyacetylenic Falcarinol As The Major Allergen in Schefflera Arboricola. *Phytochemistry* **1986**, *25*, 529–530.

Hansen, L.; Hammershoy, O.; Boll, P. M. Allergic contact dermatitis from falcarinol isolated from Schefflera arboricola. *Contact Dermatitis* **1986**, *14*, 91–93.

Hausen, B. M.; Bröhan, J.; König, W. A.; Faasch, H.; Hahn, H.; Bruhn, G. Allergic and irritant contact dermatitis from falcarinol and didehydrofalcarinol in comon ivy. *Contact Dermatitis* **1987**, *17*, 1–9.

Ito, Y.; Conway, W. Development of Countercurrent Chromatography. *Anal. Chem.* **1984**, *56*, 524A.

Kemp, M. S. Falcarindiol: An Antifungal Polyacetylene from Aegopodium Podagraria. *Phytochemistry* **1978**, *17*, 1002.

Kern, J. R.; Cardellina, J. H. Native American Medicinal Plants. Falcarindiol And 3-O-Methylfalcarindiol from Osmorhiza Occidentalis. *J. Nat. Prod.* **1982**, *45*, 774–776.

Lemmich, E. Peucelinendiol, A New Acyclic Diterpenoid From Peucedanum Oreoselinum. *Phytochemistry* **1979**, *18*, 1195–1197.

Lemmich, E. The Absolute Configuration Of The Acetylenic Compound Falcarindiol. *Phytochemistry* **1981**, *20*, 1419–1420.

Nitz, S.; Spraul, M. H.; Kollmansberger, H.; Drawert, F. 1990, unpublished results.

Oster, U. Dissertation LMU-Munich, 1988, pp 33–34.

Takahashi, M.; Yoshikura, M. J. Studies on the components of *Panax Ginseng* C. A. Meyer. V. On the Structure of a New Acetylene Derivative "Panaxynol". *J. Pharm. Soc. Jpn.* **1966**, *86*, 1053–1056.

Teuscher, E.; Lindequist, U. Polyacetylene. In *Biogene Gifte*; Fischer Verlag: Stuttgart, 1987.

Wulf, L. W.; Nagel, C. W.; Branen, A. L. High-Pressure Liquid Chromatographic Separation of The Naturally Occurring Toxicants Myristicin, Related Aromatic Ethers And Falcarinol. *J. Chromatogr.* **1978**, *161*, 271–278.

Yates, S. G.; England, R. E. Isolation and Analysis of Carrot Consistuents: Myristicin, Falcarinol, and Falcarindiol. *J. Agric. Food Chem.* **1982**, *30*, 317–320.

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