3-Butyl-5,6-dihydro-4*H*-isobenzofuran-1-one, a Sensorial Active Phthalide in Parsley Roots

Siegfried Nitz,* Martin H. Spraul, and Friedrich Drawert†

Institut für Lebensmitteltechnologie und Analytische Chemie der T.U.M. Weihenstephan, W-8050 Freising-Weihenstephan, Germany

Manfred Spraul

Bruker Analytische Messtechnik GmbH, W-7512 Rheinstetten/Karlsruhe, Germany

The analysis of parsley root revealed the presence of a novel phthalide derivative with low odor threshold. The structure 3-butyl-5,6-dihydro-4*H*-isobenzofuran-1-one followed from interpretation of MS, GC-FTIR, and NMR spectroscopic data. It can be derived from sedanenolide. Due to its restricted stability, a high-speed countercurrent chromatography isolation and purification method, which allows separation from other phthalides, had to be elaborated.

INTRODUCTION

Petroselinum crispum (Mill.) Nym. ex A. W. Hill, var. Tuberosum (Bernh.) crov., is cultivated for its root which mainly is used as a cooked vegetable in soups but which is also eaten raw, especially in salads. During our previous studies on parsley roots (Spraul, 1991; Nitz et al., 1990; Spraul et al., 1991), phthalides were found to be responsible for the typical sensory properties of the root. They are important flavor components of umbelliferous vegetables and are frequently used as flavoring agents in the food industry (Gijbels et al., 1979; MacLeod et al., 1988; Tang et al., 1990; Crosby and Anderson, 1963). The identification and nomenclature of phthalides are confusing (Uhlig et al., 1987; Gijbels et al., 1985); in some cases several trivial names and also systematic names were indistinctly used for the description of the same constituent. This is probably due to the fact that some of them were difficult to resolve on packed gas chromatographic columns, which resulted in ambiguous structure assignment.

In this paper, we report the separation of phthalides from *P. crispum* (Mill.) Nym. ex A. W. Hill, var. Tuberosum (Bernh.) crov., by means of high-speed countercurrent chromatography (HSCCC) and the structure elucidation of a new phthalide with an extremely low odor threshold.

MATERIALS AND METHODS

Plant Materials. A curled-type cultivar, *P. crispum* (Mill.) Nym. ex A. W. Hill Mooskrause, was cultivated in a greenhouse at Freising-Weihenstephan, FRG. *P. crispum* (Mill.) Nym. ex A. W. Hill, var. Tuberosum (Bernh.) crov., grown at field locations nearby Hallbergmoos-Munich, was purchased from Fa. Neumüller.

Sample Preparation. Fresh parsley roots (150 g) were ground in liquid nitrogen with pestle and mortar. Ethyl ether (350 mL) was added and the slurry filtered through a glass wool layer. The organic extract was allowed to warm up to −10 °C and was then concentrated under reduced pressure at ambient temperature in the dark.

High-Speed Countercurrent Chromatography (HSCCC). HSCCC was performed on an epicyclic coil planet centrifuge, the Ito multi-layer coil separator-extractor, manufactured by PC Inc. (Potomac, MD). The solvent systems used were hexane–ethyl acetate–methanol–water (70:30:14:10, system 1) and hexane–acetonitrile–*tert*-butyl methyl ether (10:10:1, system 2). In the case of system 1, the mobile phase was the hexane solution; in the case of system 2, the mobile phase was the acetonitrile solution. The flow directions were from tail to head for system 1 and from head to tail for system 2. The physical parameters were as follows: column, 1.6 mm i.d./130 m long, 285-mL capacity; speed, 800 rpm; flow rate, 1.0 mL/min.

Gas Chromatography (GC). Siemens Sichromat I equipped with a 26 m × 0.25 mm i.d. fused silica capillary column coated with 0.3 μm of cross-linked SE54 was used. Other parameters were as follows: carrier gas, 1.7 mL/min H_2; temperature program, 60 °C (5 min)–2 °C/min–250 °C; injector and detector temperatures, 250 °C; Siemens PTV (temperature-programmed vaporizer) program, 40 °C (3 min)–250 °C (splitless injection).

GC-FTIR. A Nicolet 710 SX equipped with an Siemens Si-Chromat I was used. GC parameters were as above.

NMR. NMR spectra were recorded with a Bruker AMX 500 NMR spectrometer at 500 MHz for ^1H and at 125 MHz for ^{13}C operating frequencies, both in CD_2Cl_2. The ^{13}C and ^1H NMR spectral peak assignments of 1 were made on the basis of homonuclear and heteronuclear COSY.

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

HRMS. A Kratos MS 80 RFA was used. Other parameters were as follows: ion source temperature, 200 °C; ionization energy, 70 eV.

Synthesis and Isolation of 3-Butyl-5,6-dihydro-4*H*-isobenzofuran-1-one (1). Forty individual on-column injections of 10 μL each of sedanenolide in ether (10 mg/mL) on a fused silica column (0.53 mm × 50 m, 1.5-μm film thickness, SE54) operated at 160 °C oven temperature with reduced column flow (1 mL/min) were performed. The eluate from the column was collected in a trap containing ether precooled at −20 °C. After evaporation of the solvent, the reaction mixture was separated by means of HSCCC with solvent system 1 right before NMR measurements.

1: MS *m/e* (high resolution/relative intensity) 192 ($C_{12}H_{16}O_2$/37), 163 ($C_{10}H_{11}O_2$/5), 150 ($C_9H_{10}O_2$/30), 135 ($C_8H_7O_2$/10), 122 ($C_8H_{10}O$/13), 108 (C_7H_8O/100), 79 (C_6H_7/52), 77 (C_6H_5/47), 57 (C_4H_9/25); IR ν_{max} 2942 (m), 2879 (w), 1805 (s), 1671 (w), 1435 (w), 1396 (w), 1255 (w), 1183 (w), 1109 (w), 1050 (w), 1018 (w), 940 (m), 742 (w) cm^{-1}.

Sedanonic Acid Methyl Ester (2): MS *m/e* (relative intensity) 167 (18), 140 (20), 85 (100), 79 (32), 57 (73), 41 (22); IR ν_{max} 3026 (w), 2949 (m), 2883 (w), 1729 (s), 1651 (w), 1440 (w),

* Author to whom correspondence should be addressed.
† In memorial.

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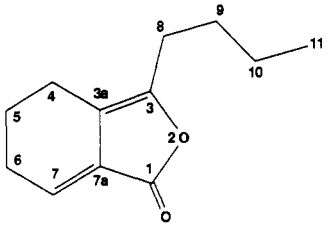
Table I. Concentration, Sensory Evaluation, and Kovats Indices of Phthalides from Parsley Roots Extracts (Punkert, 1990)

compound	parsley root, mg/kg: turnip-rooted type	parsley root, mg/kg: curled type	odor quality	intensity[a]	threshold, ng/stimulus	Kovats retention indices: SE54	Kovats retention indices: CW20M
butyl phthalide	7.3	15.0	like celery	++++	>700	1639	2466
(*E*)-butylidene phthalide	3.6	12.2	like celery	+++		1663	2464
compound 1	1.1	4.2	like celery, spicy	+++++	>0.5	1665	2350
(*Z*)-butylidene phthalide	4.0	tr	like celery	+++		1702	2557
sedanenolide	50.2	44.4	like celery, spicy	++++	>500	1707	2543
(*E*)-ligustilide	22.0	54.3	spicy	+++		1720	2511
trans-sedanolide	0.7	1.0				1711	2470
cis-sedanolide	(isolated from celery)		spicy, like celery	++++	>200	1723	2514
(*Z*)-ligustilide	0.80	0.18	spicy	++		1770	2600

[a] Arbitrary five-point intensity scale: +, very low; +++++, very high. [b] tr, trace quantities.

Table II. ^{13}C and ^{1}H NMR Spectral Peak Assignment of Compound 1



posn	δ_H	δ_C	no. of prot, multiplicity	HH-COSY (^1H–^1H couplings)
3		149, 120, 111[a]		
3a		149, 129, 111[a]		
4	2.20	20–26[a]	2 H, m	4–5
5	1.56	22	2 H, m	5–4, 6
6	2.20	20–26[a]	2 H, m	6–5
7	6.65	137	1 H, t	7–5, 6
7a		149, 129, 111[a]		
8	2.20	20–26[a]	2 H, m	8–11, 10, 9
9	1.38	29	2 H, m	9–11, 10, 8
10	1.13	23	2 H, m	10–11, 9, 8
11	0.71	14	3 H, t	11–10, 9, 8

[a] No definite assignment.

1368 (w), 1252 (s), 1208 (w), 1149 (w), 1083 (m), 975 (w), 746 (w) cm^{-1}; ^1H NMR (CD_2Cl_2) δ 6.98 (t, 1 H), 3.50 (s, 3 H), 3.39 (m, 1 H), 2.37 (t, 2 H), 2.05 (m, 2 H), 1.66 (m, 1 H), 1.55 (m, 1 H), 1.38 (m, 2 H), 1.37 (m, 2 H), 1.14 (m, 2 H), 0.75 (t, 3 H).

RESULTS AND DISCUSSION

The phthalides isolated from fresh parsley roots are listed in Table I. The assignment of chemical structure of known phthalides was accomplished by comparing MS, GC-FTIR, and NMR data with those available from literature (Fischer and Gjibels, 1987; Yamagishi and Kaneshima, 1977; Gijbels et al., 1982; Bjeldanes and Kim, 1977). On the basis of these results Kovats retention indices for apolar (SE54) and polar (CW20M) chromatographic phases could be determined (see Table I).

With regard to the organoleptic properties compound 1, first detected by sniffing GC, possesses a very low odor threshold in comparison with the other isolated phthalides (Table I). In recent studies it could also be identified in *Apium graveolens* L. and *Levisticum officinale* Koch at trace levels (unpublished data). Due to its instability on certain GC columns coated with SE54 or CW20M, the preparative isolation of this substance by means of gas chromatography was unsuccessful. Consequently, GC enrichment over several days was accompanied by isomerization to sedanenolide and dehydration to butyl phthalide. The reasons for the instability on certain capillary columns are not known. We believe that it could be attributed to residues of peroxides in the mobile phase if cross-linking was performed with such type of reagents. Injection temperature (PTV injector, 100–250 °C) had no influence on the distribution of isomerization and dehydration products. MS and NMR measurements showed that compound 1 slowly undergoes ring opening in the presence of water or methanol to yield sedanonic acid or the corresponding methyl ester (compound 2). The conversion of sedanenolide to butyl phthalide and compound 1 can also be achieved limitedly by interaction with GC column material (see Figure 1). Thermal treatment of sedanenolide or compound 1 leads to butyl phthalide. As such, the quantitative data presented for compound 1, sedanenolide, and butyl phthalide have to be considered with caution. Synthesis and isolation of compound 1 at preparative scale were carried out by means of the aforementioned GC interaction followed by HSCCC. The separation of the phthalides, achieved with solvent system 1, was monitored by off-line GC analyses of collected fractions due to the elution of strong UV–vis-absorbing pigments at trace level (Figure 2). This graphical representation clearly shows that the peaks belonging to

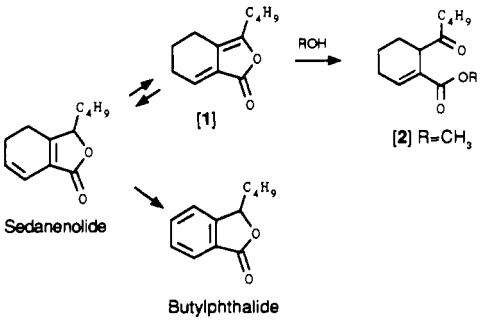


Figure 1. Isomerization, dehydration, and ring opening of compound 1.

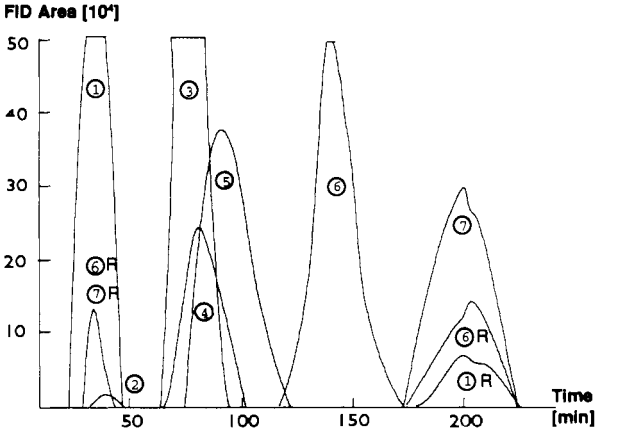


Figure 2. HSCCC separation of phthalides.

compound 1 (peak 1) and sedanenolide (peak 7) are superimposed with the corresponding reaction products. That these findings were a consequence of the GC analysis was confirmed by NMR measurements of the same fractions, which possessed high NMR purity and were absent of signals corresponding to other phthalides. (*E*)-Ligustilide, (*Z*)-butylidene phthalide, and *cis*-sedanolide (Figure 2, peaks 3–5) could be well separated by means of HSCCC using solvent system 2 (not shown). Structure elucidation of compound 1 is based on the interpretation of spectroscopic data. The molecular formula, determined by high-resolution mass spectrometry, is $C_{12}H_{16}O_2$ (192 amu), which results in five double bond equivalents. Comparison of GC-FTIR data with all discussed phthalides, especially their characteristic carbonyl absorption near 1800 cm^{-1}, revealed the presence of a typical dehydrophthalide skeleton. The assignments of 500-MHz ^1H NMR and 125-MHz ^{13}C NMR signals are given in Table II for comprehensive characterization. In combination with two-dimensional ^1H and ^{13}C NMR (HH-COSY and CH-COSY) experiments, the definite structure was determined to be 3-butyl-5,6-dihydro-4*H*-isobenzofuran-1-one.

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Registry No. 1, 141120-37-8; 2 (R = CH_3), 62006-38-6; butyl phthalide, 6066-49-5; (*E*)-butylidene phthalide, 76681-73-7; (*Z*)-butylidene phthalide, 72917-31-8; sedanenolide, 62006-39-7; (*E*)-ligustilide, 81944-08-3; *trans*-sedanolide, 3553-29-5; *cis*-sedanolide, 2550-44-9; (*Z*)-ligustilide, 81944-09-4.