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OXYGENATED DERIVATIVES OF MENTHATRIENE IN PARSLEY LEAVES

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Key Word Index—*Petroselinum crispum*: Umbelliferae; parsley; aroma volatiles; *p*-mentha-1,3,8-triene; dioxetane; 1-methyl-4-(methylethenyl)-2,3-dioxabicyclo [2.2.2]oct-5-ene; diepoxide; 4-methyl-7-(methylethenyl)-3,8-dioxatricyclo [5.1.0^{2-4}]octane.

Abstract—The analysis of aroma volatiles of parsley revealed the presence of two novel oxygenated *p*-mentha-1,3,8-triene derivatives, the amounts of which increase during processing of plant material and deteriorate the typical fresh aroma. The two derivatives, which could be synthesized by means of a dye sensitized photooxygenation reaction, were identified as 1-methyl-4-(methylethenyl)-2,3-dioxabicyclo[2.2.2]oct-5-ene and 4-methyl-7-(methylethenyl)-3,8-dioxatricyclo [5.1.0^{2-4}] octane.

INTRODUCTION

To our knowledge *p*-mentha-1,3,8-triene (**1**) is unique to parsley [*Petroselinum crispum* (Mill.) Nyman syn. *P. sativum* Hoffm.] [1] and celery (*Apium graveolens* L.) [2]. This compound is believed to be essential for the characteristic parsley flavour [1]. The odour descriptions of menthatriene varies from "typical parsley" [3], "minty, parsley-like" [4] to "chewing gum, spearmint" [2]. It is known to be unstable, apparently to oxygen [5].

In our studies [6] of the volatiles from parsley leaves and their behaviour during technological processing, we observed a rapid deterioration in flavour upon freeze-storage and leaf-aging. This was accompanied by an increase of two volatile compounds, **2** and **3**, at the apparent expense of menthatriene (**1**). The structure elucidation of these compounds is reported in this paper.

RESULTS AND DISCUSSION

The fact that the increase in the amounts of the compounds **2** and **3** during processing of parsley is accompanied by a decrease in the amount of **1** suggested to us that **2** and **3** are derived from *p*-mentha-1,3,8-triene (**1**). The similarity of the mass spectra of **1** and **2** and the difference of 32 mass units between the molecular ions ($[M]^+$ 134 and 166 respectively) indicated that an addition of one molecule of oxygen to the menthatriene skeleton had taken place. By analogy to the conversion of α-terpinene (**4**) to ascaridole (**5**) [7], a natural endoperoxide occurring in some essential oils (e.g. *Chenopodium ambrosioides*, 'wormseed oil') [8], a 1,4-cycloaddition of oxygen on the cyclohexadiene skeleton was postulated.

If this is the case then **2** should be obtained by means of a (4+2)-cycloaddition of singlet oxygen [$O_2(^1\Delta g)$] to *p*-mentha-1,3,8-triene. To prove this assumption, we isolated **1** from parsley oil and subjected it to a dye-sensitized photooxygenation as well as to a chemically generated singlet-oxygen reaction, obtaining two main products with same retention index and mass spectra as **2** and **3** from parsley leaves.

Because the dioxetane from α-terpinene and that from **1** only differ in an exocyclic double bond, comparable spectral data were obtained for both substances. Hydrogenation of both endoperoxides (**2** and **5**) with H_2/Pd-C leads to the formation of *p*-menthane, *p*-menthan-1-ol and *p*-menthan-4-ol in approximately equal amounts, thus providing additional confirmation for the existence of a 1,4-substituted *p*-menthane skeleton.

Comparison of the mass spectral data of authentic ascaridole (**5**) and component **2** with the data reported for ascaridole in the literature [9–11] revealed that the published mass spectra were not consistent with the dioxetane structure, but were in fact similar to the fragmentation of the diepoxide **3** and identical with that of an authentic sample of isoascaridole (**6**). This can be attributed to the thermal and chemical instability of the studied dioxetanes which from our experience are easily converted mainly to the corresponding diepoxides. This is observed during GC analysis of the endoperoxides, especially at high injection temperatures (50% conversion at 260°) and with certain polar liquid phases (e.g. cross-linked CW20M, 100% decomposition). A possible mass spectrometric fragmentation scheme for the discussed oxygenated terpenes is shown in Fig. 2.

Generally, alkyl-substituted *cis*-1,3-diene systems such as α-terpinene (**4**) undergo 1,4-cycloaddition reactions with $^1O_2$ without formation of allylic hydroperoxides due to an ene-reaction [12]. The same behaviour is observed for **1**. Additionally, photooxygenation under different conditions leads to the formation of diepoxide **3** and higher amounts of *p*-cymenene (**7**). The photooxidation to an aromatic system (**9**) is also observed for α-terpinene and α-phellandrene. The time-dependent yield of photoproducts from **1** is shown in Fig. 3.

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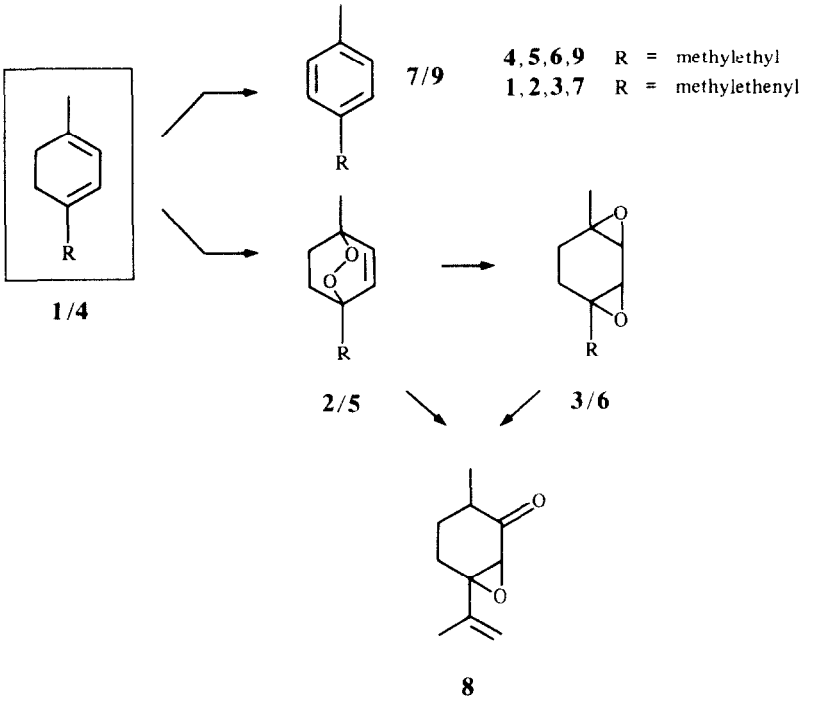

Fig. 1. Photooxidation of *p*-mentha-1,3,8-triene and α-terpinene.

The endoperoxide (**2**) formed, rearranges on irradiation to the bisepoxide (**3**), as in the thermal rearrangement, together with the ketoepoxide (**8**). Similar results have been reported [13–16] for the dye-sensitized photoconversion of α-terpinene (**4**) to ascaridole (**5**) and isoascaridole (**6**). With regard to the organoleptic properties it can be stated that both, the dioxetane and the corresponding bisepoxide, degrade the typical parsley flavour when present in higher amounts. The individual aroma character of the dioxetane (**2**) was described as fruity, fresh, sweet, not parsley-like whereas the diepoxide (**3**) was recorded as unpleasant and sweaty. Ascaridole (**5**), with a similar, but less fruity odour than **2** is known to possess anthelminthic and antibiotic properties [8, 17, 18]. If similar biological activities are exhibited by **2**, remains to be investigated.

The mechanism for the biotransformation of **1** to the oxygenated derivatives **2** and **3** in parsley is still uncertain. A photogeneration of $^1O_2$ by furanocoumarins [19] as well as a iodide peroxidase-catalysed synthesis [18] are viable routes for their biosynthesis.

The biological origin of singlet oxygen is, however, a subject of dispute, since numerous enzyme-catalysed transformations that yield products which can be rationalized in terms of singlet oxygen reactions have been shown to proceed by other mechanisms [18].

EXPERIMENTAL

Parsley [*Petroselinum crispum* (Mill.) Nyman syn. *P. sativum* Hoffm., var. 'Mooskrause', Hilmar/EWG Norm D 453 S-St] was cultivated in a greenhouse at 8050 Freising-Weihenstephan, F.R.G.

Sample preparation. Fresh parsley leaves (150 g) were ground in liquid N_2 with pestle and mortar. Et_2O (100 ml) was added and the slurry filtered. The organic extract (−15°) was concd under red. pres. at ambient temp. in the dark down to 10 ml. GC/MS-analysis revealed the presence of compounds **2** and **3**.

Isolation of compound **1**. Compound **1** was isolated from a commercial oil (Melchers & Co, Bremen, No. 4/2176/7670) by means of flash-chromatography [20]. The oil (300 μl) was placed on top of a water-cooled chromatographic column (24 mm i.d., length 37 cm), filled with 160 g of silica gel (Kieselgel 60, Merck, 63–200 μm). After the elution of 400 ml of pentane (flow rate 57 ml/min), 20 fractions (10 ml each) were collected. Fractions 14–18 contained **1** (*ca* 60 mg, purity 77–86%).

Photooxygenation. Dye-sensitized photooxygenation was carried out in an irradiation-vessel as described [21]. The sensitizer was prepared as follows: A soln of 10 mg Rose Bengal (Sigma) in 50 ml dist. H_2O and 10 g Dowex 1 × 8 (mesh size 100–200, anionic exchange resin, Serva) was stored for 12 hr, filtered and washed exhaustively with H_2O and Me_2CO. The fixed sensitizer (3 g) and a soln of 60 mg of **1** in Me_2CO (90 ml) was irradiated (Phillips HPK 125, high-pressure Hg-lamp) through Pyrex ($\lambda > 290$ nm) for 15 min under O_2 purge at a temp. of −5°. After filtration and concn the oxygenated products **2** (5 mg) and **3** (3 mg) could be identified by GC-MS. Experiments with solvents of different polarity (MeOH, Me_2CO, Et_2O and pentane) and/or treatment with DABCO (1,4-diazabicyclo[2.2.2]octane) and BHT (2,6-di-tert-butyl-*p*-cresol) were also performed. Commercially available α-terpinene (**4**) (Roth, Karlsruhe) was treated in the same way, resulting in **5** and **6**.

Chemosynthesis. 10 mg of **1** was treated with a filtered and cooled (2°) soln of 1 g Ca-hypochlorite in 3 ml of a 5% aq.–methanolic (2:1) soln of NaOH and 1 ml H_2O_2 (30%) for

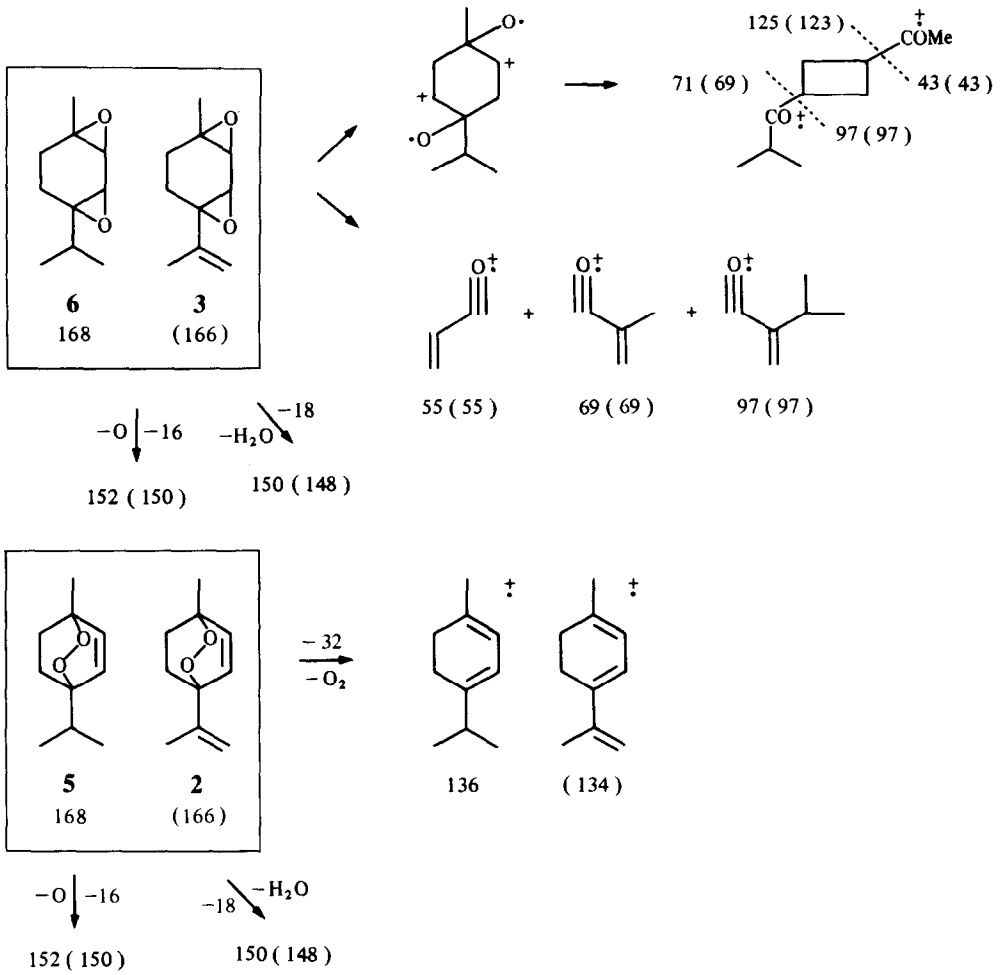

Fig. 2. Fragmentation scheme of the discussed oxygenated terpenes.

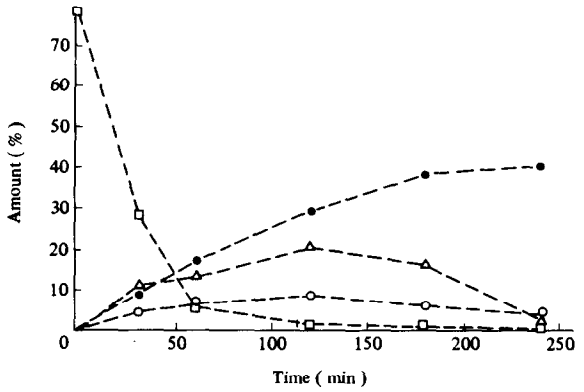

Fig. 3. Time dependent yield of photoproducts from **1**. □–□, *p*-Mentha-1,3,8-triene (**1**); △—△, *p*-cymenene (**7**); ○—○, dioxetane (**2**); ●—●, diepoxide (**3**).

24 hr. After neutralization, the reaction products were extracted with Et_2O and dried (Na_2SO_4). The concd Et_2O-extract contained 31% of **2** and 15% of **3**. α-terpinene (**4**) treated in the same way, gave 38 and 15% of **5** and **6** respectively.

Hydrogenation. The photooxygenation reaction products (**2**, **3**, **5**, **6**) were dissolved in pentane and hydrogenated with H_2 over Pd/C for 4 hr at 20°.

Isolation of **2** *and* **3**. The oxygenated photolysis products (**2**, **3**) were purified by means of flash-chromatography with a pentane–Et_2O gradient at −5°, leading to a fraction containing 65 and 15% of **2** and **3** respectively. Isolation of pure (>98%) components for structural analysis was achieved by means of prep. GC.

GC. Siemens Sichromat I equipped with a 26 m × 0.25 mm i.d. fused silica capillary column coated with 0.3 μm cross-linked SE 54. Carrier gas, 1.7 ml/min H_2; temp. programme, 60° (5 min)-2°/min-250°; injector and detector temperature, 150 and 250° respectively. Sniffing- and MDGC-analysis was performed as described elsewhere [22].

Preparative GC. Gas chromatograph Hupe & Busch Mod. 1075 A equipped with 4 m × 6 mm i.d. glass column packed with 10% cross-linked SE 54 on Chromosorb W. Carrier gas, 100 ml/min N_2; temp. programm. 60° (2 min)-2°/min-250°; injector and outlet manifold temp., 150 and 135° respectively. Injection vol., 30 μl per enrichment cycle.

GC/FTIR. Hewlett Packard Mod. 5890/5965 A(IRD) 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.

GC/MS. Finnigan 1020 (quadrupole) linked on-line to an Incos data processing system; directly coupled to a Sigma III (Perkin Elmer) GC. J&W 20 m × 0.25 mm i.d. fused silica capillary column coated with 0.25 μm bonded DB 5; carrier gas,

1.2 ml/min He; temp. programm., 60° (5 min)-2°/min-250°; injector and transfer-line temp., 150°; ionization energy, 70 eV.

Physical data

1-*Methyl*-4-(*methylethenyl*)-2,3-*dioxabicyclo* [2.2.2] *oct*-5-*ene* (**2**). ^{1}H NMR, (C_6D_6): 1.32 (*s*, 3H, H-12), 1.71 (*m*, 7H), 5.18 (*m*, 2H, H-10), 6.39 (*dd*, 2H, H-5, 6); ^{13}C NMR, (CD_2Cl_2): 21.6/23.7/29.9/31.6 (C-7, C-8, C-11, C-12), 115.6 (C-10), 136.1/138.6 (C-5, C-6). IR ν_{max} cm^{-1}: 2892 (s), 2940 (s), 1648 (m) 1452 (m), 1378 (m), 1141 (m), 905 (m). MS *m/z* (rel. int.): 166 (0.5), 150 (3), 148 (6), 135 (6), 134 (40), 133 (8), 132 (9), 122 (5), 119 (49), 117 (18), 115 (12), 108 (13), 107 (14), 105 (23), 95 (14), 93 (18), 92 (19), 91 (58), 79 (22), 77 (25), 69 (18), 67 (14), 65 (19), 55 (16), 53 (19), 52 (16), 51 (15), 43 (7), 41 (100), 39 (89). Kovats-Index: 1236 (SE54); decomposed on CW20M

4-*Methyl*-7-(*methylethenyl*)-3,8-*dioxatricyclo*[5.1.0$^{2-4}$]*octane* (**3**). ^{1}H NMR, (C_6D_6): 1.32 (*s*, 3H, H-12), 1.73 (*m*, 7H), 5.14 (*m*, 2H, H-10), 3.31 (*dd*, 2H, H-1, 2). IR ν_{max} cm^{-1}: 3091 (m), 2987 (s), 2937 (s), 1727 (w), 1643 (w), 1448 (m), 1383 (m), 1311 (w), 1203 (m), 1144 (m), 1090 (m), 1012 (m), 905 (s), 780 (m). MS *m/z* (rel. int.): 166 (0.6), 151 (1), 148 (2), 137 (4), 134 (4), 132 (6), 123 (4), 119 (6), 117 (8), 108 (7), 105 (8), 97 (19), 95 (11), 93 (9), 91 (20), 85 (14), 82 (20), 79 (18), 77 (12), 69 (38), 67 (26), 65 (15), 60 (27), 57 (9), 55 (14), 53 (16), 51 (11), 43 (7), 41 (100), 39 (57). Kovats-Index: 1295 (SE54); 1877 (CW20M).

1-*Methyl*-4-(*methylethyl*)-2,3-*dioxabicyclo*[2.2.2]*oct*-5-*ene* (**5**). ^{1}H NMR, (C_6D_6): 1.00 (*d*, 6H, H-10, 11), 1.34 (*s*, 3H, H-12), 1.77 (*m*, 5H), 6.40 (*dd*, 2H, H-5, 6). IR ν_{max} cm^{-1}: 3058 (m), 2974 (s), 2941 (s), 1715 (w), 1622 (w), 1464 (m), 1381 (m), 1308 (w), 1244 (w), 1207 (m), 1105 (m), 1019 (m), 933 (m), 889 (m). MS *m/z* (rel. int.): 168 (0.5), 152 (0.7), 137 (4), 136 (33), 134 (7), 122 (6), 121 (51), 119 (29), 109 (7), 107 (10), 105 (9), 97 (8), 94 (7), 93 (58), 92 (12), 91 (29), 81 (6), 80 (7), 79 (22), 77 (20), 69 (8), 67 (8), 65 (12), 55 (19), 53 (14), 51 (12), 43 (100), 42 (15), 41 (65), 39 (49). Kovats-Index: 1232 (SE54); decomposed on CW20M.

4-*Methyl*-7-(1-*methylethyl*)-3,8-*dioxatricyclo* [5.1.0$^{2-4}$] *octane* (**6**). IR ν_{max} cm^{-1}: 2972 (s), 2941 (s), 1450 (m), 1382 (m), 1309 (w), 1265 (w), 1210 (w), 1143 (w), 1090 (m), 1008 (m), 908 (m), 782 (m). MS *m/z* (rel. int.): 140 (2), 139 (6), 135 (4), 134 (3), 126 (3), 125 (10), 121 (2), 119 (5), 111 (3), 110 (5), 109 (5), 107 (10), 99 (4), 98 (6), 97 (39), 95 (11), 93 (9), 91 (6), 85 (11), 83 (9), 82 (16), 81 (9), 79 (13), 77 (6), 71 (20), 70 (5), 69 (30), 67 (11), 60 (12), 59 (4), 57 (7), 55 (29), 53 (9), 43 (100), 41 (60), 39 (17). Kovats-Index: 1297 (SE54); 1828 (CW20M).

3-*Methyl*-6-(1-*methylethenyl*)-7-*oxabicyclo*[4.1.0]*oct*-2-*one* (**8**). MS *m/z* (rel. int.): 166 (4), 149 (4), 148 (38), 133 (8), 123 (2), 119 (1), 109 (11), 108 (75), 107 (65), 105 (29), 97 (2), 95 (8), 93 (4), 91 (11), 90 (9), 83 (14), 82 (3), 80 (20), 79 (20), 77 (30), 69 (4), 67 (4), 65 (18), 63 (6), 55 (22), 53 (16), 51 (20), 43 (100), 41 (33), 39 (69). Kovats-Index: 1189 (SE54); 1827 (CW20M).

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