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AN OXIDATIVE METABOLITE OF PERILLALDEHYDE FROM *PERILLA FRUTESCENS*

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Key Word Index—*Perilla frutescens*; Labiatea; hydroxyperillaldehyde.

Abstract—(3*S*,4*R*)3-Hydroxy-4-(1-methylethenyl)-1-cyclohexene-1-carboxaldehyde, an oxidative metabolite of perillaldehyde, was isolated from *Perilla frutescens*. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The leaf of *Perilla frutescens* Britton (Japanese name: shiso) is one of the most popular garnishes in Japan [1] and is also used in folk medicine in China and Japan as a detoxicant, an antitussive, an antibiotic, an antipyretic, and for the treatment of intestinal disorders and allergies [2]. Perillaldehyde, the major constituent, is found in amounts of *ca* 50% in the essential oil of *P. frutescens*. In addition, numerous other constituents from *P. frutescens* have been reported [3–6]. A practical problem encountered when *P. frutescens* oil is used in food products is that the constituents have a tendency to be easily oxidized in air. Previously we reported about the photooxidation of perillaldehyde [7]. We report here the isolation and structural elucidation of a novel metabolite of perillaldehyde from *P. frutescens* Britton var. *crispa* (Thunb.) (Japanese name: aochirimen-shiso).

RESULTS AND DISCUSSION

A methanol extract of the aerial parts of *P. frutescens* was concentrated and the residue was washed with hexane. The undissolved material was partitioned between ethyl acetate and water. The ethyl acetate was evaporated and the residue was chromatographed on silica gel with hexane–ethyl acetate. The fractions were purified by repeated HPLC on ODS and gel permeation columns to give a novel metabolite (**1**) of perillaldehyde (**2**).

(3S,4R)3-*Hydroxy*-4-(1-*methylethenyl*)-1-*cyclohexene*-1-*carboxaldehyde* (**1**)

The ^{1}H and ^{13}C NMR spectra of **1** resemble those of (**2**). The difference between the spectra of **1** [HR mass spectrum *m/z* 166.1025 $[M]^+$ (calc. for $C_{10}H_{14}O_2$: 166.0994); $\delta_H(CDCl_3)$: 4.39 (1H, *dddd*, $J = 9.6$, 3.3, 1.6 and 1.6 Hz); $\delta_C(CD_3OD)$: 68.7 and Ir (3400 m^{-1})] and **2** suggested that **1** has a hydroxyl group at C-3 of **4**. The coupling constants ($J_{4,5} = 9.6$ Hz; $J_{3,4} = 3.3$ Hz) confirm that **1** is (3*S*,4*R*)3-hydroxy-4-(1-methylethenyl)-1-cyclohexene-1-carboxaldehyde. As the content of **1** in dry *P. frutescens* was *ca* 0.097%, which is *ca* 1/6–1/7 of **2** (0.64%), **1** could be one of the important flavour compounds in this herb. The next problem was whether **1** is produced by biogenesis or autooxidation from perillaldehyde.

Autooxidation of perillaldehyde (**2**)

In our previous research on the photooxidation of **2** [7], **1** was not obtained. Autooxidation of **2** was reexamined in chloroform for nine days at ambient temperature. This gave diastereomeric mixtures of epoxy-aldehyde **3** and dihydroxy-aldehydes **4** and **5** with recovery of **2**; however, no 3-hydroxyperillaldehyde was detected. These data suggest that **1** may be biogenetically synthesized from **2** in the harvested plant. The formation of this compound could change the flavour of the essential oil.

EXPERIMENTAL

Mps: uncorr.; ^{1}H and ^{13}C NMR: 270 and 68 MHz, respectively, $CDCl_3$, TMS as int. standard; CC: silica gel 60 (Merck, 70–230 mesh) with hexane and EtOAc; HPLC: ODS column (LiChrospher RP-18 and Inertsil PREP-ODS, eluted with MeOH–H_2O) and gel permeation column (Asahipak GS310, eluted with EtOAc) with UV detector.

Plant materials. Plant material (leaves and stems) of *P. frutescens* Britton var. *crispa* (Thunb.) was harvested at Kitami farm, Ogawa & Co., Hokkaido, in August 1993.

Extraction and isolation. Dry leaves and stems (2.12 kg) were extracted with MeOH at room temp. for

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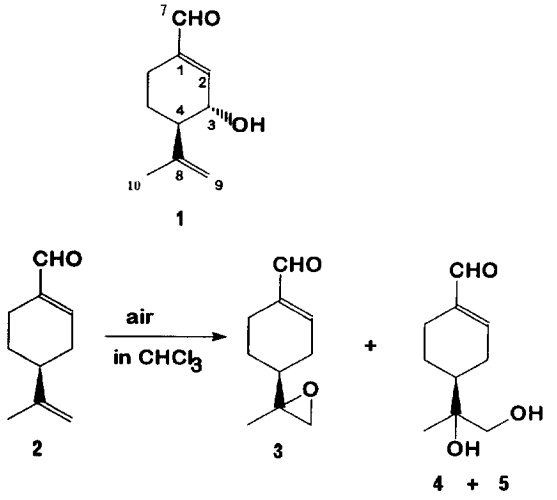

14 days. The extract was concd *in vacuo* and the residue partitioned between hexane and H_2O. The MeOH-soluble portion was evapd and the residue was partitioned between EtOAc and H_2O. The EtOAc-soluble fr. was evapd. The residue (36.2 g) was sepd into several frs by silica gel CC, eluting with hexane–EtOAc. The less polar fr. was further sepd by HPLC on an ODS column eluted with MeOH–H_2O, followed by a Asahipac (GS310) column eluted with EtOAc to give **1** (258.8 mg).

(3S,4R)3-*Hydroxy*-4-(1-*methylethenyl*)-1-*cyclohexene*-1-*carboxaldehyde* (**1**). Crystals, mp 51–53°, $[\alpha]_D$ +120° (EtOH, *c* 0.064); HRMS *m/z*: 166.1025 $[M]^+$ (cal. for $C_{10}H_{14}O_2$: 166.0994); $\delta_H(CDCl_3)$: 9.49 (1H, *s*), 6.74 (1H, *dd*, $J = 3.3$, 2.3 Hz), 4.95 (1H, *m*), 4.90 (1H, *s*), 4.39 (1H, *dddd*, $J = 9.6$, 3.3, 1.6, 1.6 *Hz*), 2.41 (1H, *m*), 2.21 (1H, *m*) 2.16–2.04 (1H, *m*), 1.87 (1H, *dddd*, $J = 13.9$, 9.5, 6.1, 1.9 Hz), 1.77 (3H, *d*, $J =$ 1.3 Hz), 1.7–1.52 (1H, *m*); $\delta_c(CDCl_3)$: 194.0, 150.6, 146.1, 141.2, 113.1, 68.7, 50.2, 25.3, 21.5, 19.1; IR ν_{max}^{NaCl} cm^{-1}: 3400 (br), 2940, 1680, 1060; UV λ_{max}^{EtOH} nm (ε): 231 (12 000).

Autooxidation of perillaldehyde (**2**). $CHCl_3$ (10 ml) soln of **2** (320 mg) was stirred at ambient temp. for 9 days under air. The soln was evapd and the residue dissolved in EtOAc. The soln was washed with brine and evapd. The residue was subjected to CC on silica gel eluted with hexane–EtOAc to give **3** (9.1 mg), **4** (5.8 mg) and **5** (3.7 mg) with the starting material. Compound **3**, oil; MS *m/z*: 166 $[M]^+$; $\delta_H(CDCl_3)$: 9.43, 9.44 (1H, *s*), 6.80, 6.82 (1H, *m*), 2.68, 2.69 (2H, m), 2.61 (1H, *d*, $J = 4.3$ Hz), 2.57–2.35 (2H, *m*), 2.30–1.90 (3H, *m*), 1.70–1.53 (1H, *m*), 1.40–1.20 (1H, *m*), 1.32, 1.30 (3H, *s*); $\delta_C(CDCl_3)$: 193.8, 149.9 and 149.7, 141.4 and 141.3, 58.8 and 58.6, 53.0 and 52.8, 39.8 and 39.6, 28.4 and 28.3, 23.7 and 23.6, 21.6 and 21.2, 18.5 and 18.2; IR ν_{max}^{NaCl} cm^{-1}: 2930, 1680, 1640 and 1175; λ_{max}^{EtOH} nm (ε): 229 (10 000). Compound **4**, oil; MS *m/z*: 184 $[M]^+$; $\delta_H(CDCl_3)$: 9.44 (1H, *s*), 6.80 (1H, *m*), 2.64–2.37 (2H, *m*), 2.02–2.00 (3H, *m*), 1.98–1.84 (1H, *m*), 1.35–1.18 (1H, *m*), 1.24 (3H, *s*); $\delta_C(CDCl_3)$: 193.7, 149.6, 141.7, 73.3, 53.4, 41.0, 28.0, 22.0, 21.7, 21.0; IR ν_{max}^{NaCl} cm^{-1} 3450 (br), 2930, 2620, 1680, 1645, 1175; UV λ_{max}^{EtOH} nm (ε): 230 (10 000). Compound **5**, Oil; $\delta_H(CDCl_3)$: 9.44 (1H, *s*), 6.86 (1H, *m*), 2.64–2.37 (2H, *m*), 2.36–2.18 (1H, *m*), 2.18–1.82 (3H, *m*), 1.35–1.18 (1H, *m*), 1.26 (3H, *s*).

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