2-Hydroxy-2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-en-8-one: Precursor of 8,9-Dehydrotheaspirone in White-Fleshed Nectarines†

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2-Hydroxy-2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-en-8-one (**2**) was identified as precursor of 2,6,-10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (8,9-dehydrotheaspirone, **1**) in an Et_2O extract from white-fleshed nectarine (*Prunus persica* Batsch var. *nucipersica* Schneid) juice. The identification was verified by comparison of chromatographic and spectral data (MS, ^1H and ^{13}C NMR) of the new natural product with those of an authentic reference compound synthesized from dehydrovomifoliol (**3**). NMR spectral data revealed that the precursor compound **2** is present in 5:1 mixture of diastereomeric hemiacetals **2a/b**. The absolute stereochemistry of the diastereoisomers (major isomer, 2*S*,5*S*; minor isomer, 2*R*,5*S*) was elucidated by NOE experiments and dehydration to optically pure (*S*-configured) spiroether **1**. In addition to hemiacetals **2a/b**, (6*S*)-dehydrovomifoliol (**3**) as well as the β-D-glucoside of (3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-β-ionone (**5**) was also identified in nectarine juice.

Keywords: *2-Hydroxy-2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-en-8-one; 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one; 8,9-dehydrotheaspirone; enantiodifferentiation; dehydrovomifoliol; 3-hydroxy-5,6-epoxy-β-ionone 3-O-β-D-glucoside; white-fleshed nectarines; Prunus persica Batsch var. nucipersica Schneid; Rosaceae*

INTRODUCTION

There have been only limited studies on the volatile constituents of nectarines (Engel et al., 1988a,b; Takeoka et al., 1988, 1992). Flavor differences that are observed between the white- and yellow-fleshed cultivars, i.e. an additional flowery note in the white-fleshed cultivar, have attracted particular attention (Takeoka et al., 1992). The results published by Takeoka et al. (1992) clearly demonstrated the importance of carotenoid-derived volatiles (so-called C_{13}-norisoprenoids) for the specific flavor of the white-fleshed cultivar, with β-ionone and β-damascenone being the major contributors to the overall flavor impression. In continuation of this work, we now report on the formation of 8,9-dehydrotheaspirone (**1**). This spirocyclic C_{13}-ether, which was detected as a major volatile constituent in an Et_2O extract of white-fleshed nectarine juice, has also been considered as a likely contributor to the unique flavor of the white-fleshed cultivar (Engel et al., 1988a).

MATERIALS AND METHODS

Chemicals. All commercial chemicals used were of analytical grade quality. Dehydrovomifoliol (1-hydroxy-4-keto-2-ionone) was obtained from Sigma Chemical Co. (St. Louis, MO). Solvents were redistilled before use.

Plant Material. Fresh white-fleshed nectarines, grown in Greece, were purchased from the local market in summer of 1995.

High-Resolution Gas Chromatography (HRGC). DANI educational (DANI Strumentazione Analitica spa., Monza, Italy) gas chromatographs equipped with either a J&W Scientific (Folsom, CA) fused silica DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) or a J&W fused silica DB-Wax capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) were used. Split injection (1:20) was employed. The temperature program was from 60 °C (2 min isothermal) to 300 °C at 5 °C/min for DB-5 and from 50 °C (3 min isothermal) to 215 °C at 4 °C/min for DB-Wax, respectively. The flow rate for the carrier gas was 1.5 mL of He/min at the initial temperature. A flame ionization detector (FID) was used with the flow rates of 30 mL of N_2/min for the makeup gas and for the detector gases 37 mL of H_2/min and 280 mL of air/min. The injector temperature was kept at 250 °C and the detector temperature at 280 °C for DB-5; these temperatures for DB-Wax were 220 and 250 °C, respectively. For chiral analyses the HRGC system was equipped with a permethylated β-cyclodextrin column (J&W C-DEX B, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was 150 °C isothermal. The flow rate of the carrier gas was 1.7 mL/min. The linear retention index (R_i) is based on a series of *n*-hydrocarbons.

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). HRGC-MS was performed with a Hewlett-Packard GCD system (Hewlett-Packard Co., Palo Alto, CA) equipped with a programmed-temperature-vaporizer (PTV) injector (KAS-system, Gerstel, Mülheim, Germany). The same types of columns and the same temperature programs as mentioned above for HRGC analysis were used. Other conditions were as follows: carrier gas flow rate, 1.2 mL of He/min, temperature of ion source, 180 °C; electron energy, 70 eV; injection volumes, 1 μL.

Nuclear Magnetic Resonance (NMR). ^1H and ^{13}C NMR spectra were recorded on Fourier transform Bruker AM 360 and AC 250 spectrometers (Bruker, Rheinstetten, Germany) with $CDCl_3$ as solvent and Me_4Si as reference standard. Using an automatic technique, nuclear Overhauser enhancement (NOE) measurements of the carefully degassed samples were performed at ambient temperature by irridation of the different proton chemical shift frequencies for 4.8 s.

Circular Dichroism (CD). CD spectra were recorded in MeOH (20 °C) using a JASCO J-710 (Jasco, Gross-Umstadt, Germany) polarimeter.

Isolation of 2-Hydroxy-2,6,10,10-tetramethyl-1-oxaspiro-[4.5]dec-6-en-8-one (2a/b) and (6*S*)-Dehydrovomifoliol (3). *(a) Isolation of Free Volatiles by Continuous Et_2O Extraction.* Freshly prepared juice was obtained from 30 kg of fruit

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after homogenization with 0.1 M phosphate buffer (pH 7) in a Waring blender, neutralization by addition of an aqueous solution of NaOH (2.5 N), and centrifugation at 2000*g* for 30 min. The clear juice (27 L) was continuously liquid−liquid extracted (24 h) using diethyl ether (14 L). After drying over Na_2SO_4, the Et_2O extract was carefully concentrated to 1 mL at a temperature not exceeding 35 °C using a Vigreux column. The concentrated extract was then fractionated by flash chromatography (Still et al., 1978) on silica gel using a pentane/diethyl ether gradient. Five fractions were separated: fraction I (200 mL of pentane), fraction II (200 mL of pentane/Et_2O, 3:1), fraction III (200 mL of pentane/Et_2O, 1:1), fraction IV (200 mL of pentane/Et_2O, 1:3), and fraction 5 (1000 mL of Et_2O). All eluates were dried over anhydrous sodium sulfate and concentrated to 0.5 mL prior to HRGC and HRGC-MS analyses. Fractions III and IV were further fractionated by HPLC.

*(b) Purification of **2a/b** Using HPLC.* Concentrated flash-fraction III was separated by HPLC (Knauer, Berlin, Germany) on an Eurospher Si 100 column (5 μm, 250 × 4 mm, Knauer, flow rate 1.5 mL/min, UV detection at 230 nm) using pentane/methyl *tert*-butyl ether (1:1) as eluent. Hemiacetals **2a/b** (12 mg, ratio 5:1), which were inseparable by HPLC as well as HRGC, showed the following chromatographic and spectral data: HPLC retention time, 10.7 min; R_i (DB-5) 1774; UV λ_{max} 233 nm (pentane/Et_2O; 1:1); EI-MS, *m/z* (%) 206 (20 [M − H_2O]$^+$), 191 (6), 168 (32), 150 (38), 136 (16), 135 (15), 125 (18), 121 (16), 111 (100), 108 (52), 93 (28), 91 (16), 79 (19), 77 (18), 69 (13), 55 (16), 53 (13), 43 (95); ^{1}H NMR (360 MHz, $CDCl_3$) major diastereoisomer **2a** δ 0.98 and 1.02 (2 × 3H, 2s, $2CH_3$-C10), 1.63 (3H, s, CH_3-C2), 2.08 (3H, d, J = 1.2 Hz, CH_3-C6), 1.8−2.3 (4H, m, H_2C3 and H_2C4), 2.25 and 2.42 (2H, 2d, J = 17 Hz, H_2C9), 5.89 (1H, br s, HC7); minor diastereoisomer **2b** δ 1.07 ($2CH_3$-C10), 1.60 (CH_3-C2), 1.97 (CH_3-C6), 5.75 (HC7); ^{13}C NMR (63 MHz, $CDCl_3$) **2a** δ 20.7 (Me-C6), 23.0 and 24.9 (2Me-C10), 27.6 (Me-C2), 30.8 (C3), 38.2 (C4), 41.6 (C10), 49.9 (C9), 91.0 (C5), 106.7 (C2), 125.8 (C7), 166.8 (C6), 198.0 (C8). Signals for the isomer **2b** showed slightly different chemical shifts ($\Delta\delta \leq 0.4$).

*(c) Purification of (6S)-Dehydrovomifoliol (**3**) Using HPLC.* Concentrated flash-fraction IV was separated by HPLC on the same type of column as mentioned above, using a flow rate of 2.0 mL and pentane/methyl *tert*-butyl ether (1:4) as eluent. **3** showed the following chromatographic and spectral data: HPLC retention time, 5.04 min; R_i (DB-5) 1814; UV λ_{max} 233 nm; CD (*c* 0.01% in MeOH) $[\Theta]_{318}$ −10 800, $[\Theta]_{244}$ +149 300, $[\Theta]_{210}$ −128 700; CD data are in good agreement with those published for (6*S*)-**3** by Mori (1974); EI-MS, *m/z* (%) 222 (0.5 [M]$^+$), 180 (2), 166 (13), 125 (9), 124 (100), 123 (8), 43 (53); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.03 and 1.11 (2 × 3H, 2s, $2CH_3$-C1), 1.89 (3H, d, J = 1.5 Hz, CH_3-C5), 2.31 (3H, s, CH_3-C9), 2.34 and 2.51 (2H, 2d, J = 17 Hz, H_2C2), 5.96 (1H, br s, HC4), 6.47 and 6.83 (2H, 2d, J = 16 Hz, HC7 and HC8); ^{13}C NMR (63 MHz, $CDCl_3$) δ 18.6 (Me-C5), 22.9 and 24.3 (2Me-C1), 28.4 (Me-C9), 41.4 (C1), 49.6 (C2), 79.3 (C6), 127.8 (C4), 130.4 (C8), 144.9 (C7), 160.2 (C5), 196.8 (C9), 197.3 (C3).

Isolation of 3-Hydroxy-5,6-epoxy-β-ionone 3-*O*-β-D-Glucoside (5). The Et_2O-stripped nectarine juice was passed through a column (72 cm × 5 cm) of Amberlite XAD-2 (Günata et al., 1985). After a rinse with water (2 L), a glycosidic extract was obtained by elution with MeOH (2 L). The methanolic eluate was concentrated under reduced pressure to dryness and fractionated by multilayer coil countercurrent chromatography (MLCCC) using $CHCl_3$/MeOH/H_2O (7:13:8) as solvent system (Ito, 1986; Roscher and Winterhalter, 1993). Acetylation of MLCCC fraction III followed by flash chromatography as well as preseparation on an Eurospher Si 100 column (5 μm, 250 × 16 mm, Knauer, flow rate 10 mL/min; eluent, pentane/methyl *tert*-butyl ether (2:8); UV detection 210 nm) and final purification by reversed-phase HPLC (Eurospher Si 100-C_{18} column, 5 μm, 250 × 16 mm, Knauer, flow rate 7.5 mL/min; MeOH/H_2O gradient) yielded peracetylated glucoside **5** (8 mg). Spectral data were identical with those previously published (Skouroumounis and Winterhalter, 1994).

Preparation of Reference Compounds. *(a) Preparation of Hemiacetals **2a/b**.* A solution of 100 mg (0.45 mmol) of racemic dehydrovomifoliol (**3**) in MeOH (10 mL) was hydrogenated (20 min) at atmospheric pressure in the presence of 80 mg of Pd (5% on $BaSO_4$), then filtrated, and concentrated *in vacuo*. The reaction mixture was separated by preparative HPLC (Eurospher Si 100 column, 5 μm, 250 × 16 mm, Knauer, flow rate 15 mL/min, UV detection 230 nm) using a pentane/methyl *tert*-butyl ether gradient. Besides unreacted starting material **3**, the mixture was found to contain an inseparable mixture of hemiacetals **2a/b** (16.4 mg) as well as the methyl derivatives **4a** and **4b** (18.2 mg). ^{1}H and ^{13}C NMR data of **2a/b** were identical with those of natural **2a/b**. Spectral data of 2-methoxy-2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-en-8-one (**4a/b**): ^{1}H NMR (360 MHz, $CDCl_3$) major diastereoisomer **4a** δ 0.97 and 1.02 (2 × 3H, 2s, $2CH_3$-C10), 1.52 (3H, s, CH_3-C2), 2.02 (3H, d, J = 1 Hz, CH_3-C6), 1.8−2.3 (4H, m, H_2C3 and H_2C4), 2.24 and 2.40 (2H, 2d, J = 18 Hz, H_2C9), 3.29 (3H, s, OCH_3), 5.78 (1H, br s, HC7); minor diastereoisomer **4b** δ 1.07 (6H, s, $2CH_3$-C10), 1.54 (3H, s, CH_3-C2), 1.97 (3H, d, J = 1 Hz, CH_3-C6), 1.8−2.7 (6H, m, H_2C3, H_2C4, H_2C9), 3.38 (3H, s, OCH_3), 5.75 (1H, br s, HC7). Variation of the conditions used for the hydrogenation of **3** (i.e. 5% Pd on Al_2O_3; solvent, THF) gave the hemiacetals **2a/b** as sole product.

*(b) Dehydration of **2a/b** to 2,6,10,10-Tetramethyl-1-oxaspiro-[4.5]deca-2,6-dien-8-one (8,9-Dehydrotheaspirone, **1**).* To 14 mg (0.06 mmol) of **2a/b** in 1 mL of triethylamine were added under ice cooling and stirring 5 drops of $POCl_3$. Stirring was continued for 10 min at 0 °C. After addition of ice−water (3 mL) and neutralization with $NaHCO_3$ solution, the crude product was purified by flash chromatography. Spiroether **1** (12 mg) showed the following chromatographic and spectral data: R_i (DB-5) 1511, R_i (DB-Wax) 2031; EI-MS, *m/z* (%) 206 (32 [M]$^+$), 191 (8), 150 (35), 149 (20), 136 (24), 135 (20), 121 (21), 109 (15), 108 (92), 107 (14), 105 (10), 93 (45), 91 (19), 79 (16), 77 (22), 55 (12), 53 (15), 43 (100); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.00 and 1.09 (2 × 3H, 2s, $2CH_3$-C10), 1.81 (3H, dxdxd, J = 2.0, 2.0, 2.0 Hz, CH_3-C2), 1.97 (3H, d, J = 1.5 Hz, CH_3-C6), 2.23 and 2.41 (2H, 2d, J = 17.0 Hz, H_2C9), 2.46 and 3.03 (2H, 2 dxdxq, J = 16.0, 2.0, 2.0 Hz, H_2C4), 4.50 (1H, m, HC3), 5.70 (1H, br s, HC7); ^{13}C NMR (63 MHz, $CDCl_3$) δ 13.2 (Me-C2), 18.3 (Me-C6), 22.8 and 22.9 (2Me-C10), 37.1 (C4), 40.7 (C10), 48.8 (C9), 90.7 (C5), 93.8 (C3), 123.9 (C7), 155.2 (C2), 164.5 (C6), 198.1 (C8). A partial conversion of **2a/b** into the target compound **1** was also observed under conditions of simultaneous distillation/extraction (SDE; 2 h, pH 2.5) using the SDE apparatus described by Schultz et al. (1977).

*(c) Preparation of Optically Pure 8,9-Dehydrotheaspirones **1** (cf. Figure 1).* Fifteen milligrams of racemic **3** was separated by HPLC into the enantiomers using a LiChroCART (R,R)-Whelk-O1 column [5 μm, 250 mm × 4 mm, chiral selector: (3*R*,4*R*)-4-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene; Merck, Darmstadt, Germany] and *n*-hexane/2-propanol (85:15) as eluent (flow rate = 1 mL/min): first eluting isomer *S*-(+)-**3**, HPLC retention time 13.5 min; second eluting isomer *R*-(−)-**3**, HPLC retention time 14.8 min. CD data were in good agreement with those published by Mori (1974). Six milligrams of each of the enantiomers in THF (1 mL) was hydrogenated in the presence of Pd (2 mg, 5% on Al_2O_3) for 30 min, then filtrated, and concentrated *in vacuo*. After dehydration with $POCl_3$ in triethylamine (1 mL) and workup as described above, optically pure specimens of 8,9-dehydrotheaspirone (**1**), i.e. dextrorotatory (5*S*)-**1** and laevorotatory (5*R*)-**1**, were obtained. CD data of the pure enantiomers are shown in Figure 2. The sense of optical rotation was measured with a Jasco OR-990 polarimetric detector. Other spectral data are identical with those of racemic **1**. The optical purity was checked by HRGC and HRGC-MS using a permethylated β-cyclodextrin column. HRGC retention times were 12.9 min for (5*R*)-**1** and 13.3 min for (5*S*)-**1**.

RESULTS AND DISCUSSION

In 1988, Engel and coworkers investigated the volatile constituents of nectarines. In the white-fleshed cultivar P 89-56 an unknown ionone-type compound (apparent molecular weight 206) was detected at an approximate

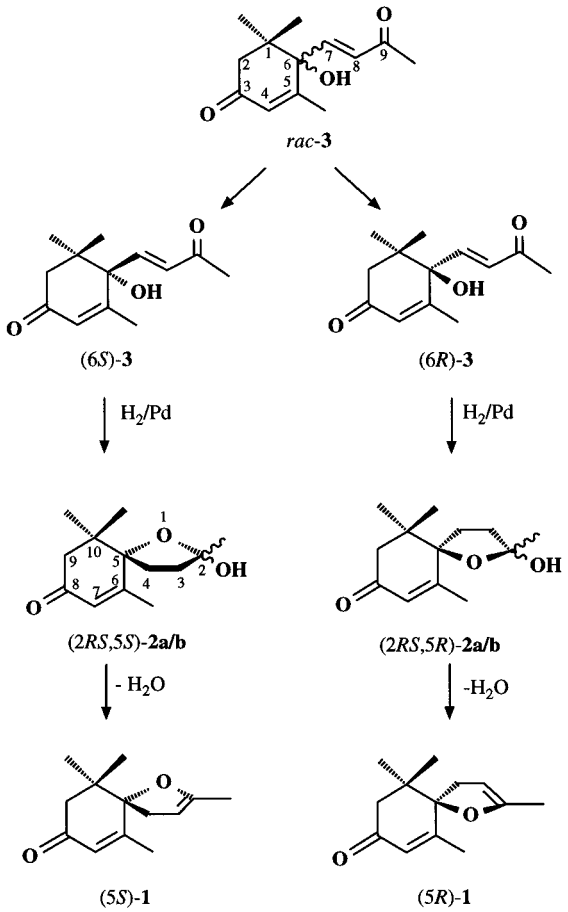


Figure 1. Synthesis of optically pure isomers of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (8,9-dehydrotheaspirone, **1**).

concentration of 4000 μg/kg. On the basis of sensory studies, the compound was considered as a likely contributor to the unique flavor of the white-fleshed cultivar. The same compound was now observed in the course of HRGC-MS analyses of aroma extracts isolated from white-fleshed nectarines grown in Greece. By comparison of the GC retention index as well as mass spectral data of the unknown constituent with those of an authentic reference (Winterhalter et al., 1990), the compound was identified as 8,9-dehydrotheaspirone (**1**). Importantly, the concentration of spiroether **1** was found to vary strongly depending on the conditions used for HRGC analyses. With an injector temperature of 350 °C, maximum concentrations of **1** (parts per million range) were obtained. Injection at 150 °C reduced the amount of **1** and revealed the co-occurence of a structurally related compound **2** eluting as a broad peak at a later retention time. To clarify the structure of this presumed precursor of **1**, the aroma extract of white-fleshed nectarines was fractionated by means of flash chromatography (FC) using a pentane/diethyl ether gradient. HRGC analyses of separated FC fractions confirmed the presence of a labile progenitor of **1**. Only a small portion of genuine **1** (5−10% of total) was detectable in the least polar FC fraction I. The predominant portion of **1** was thermally formed from a more polar progenitor **2**, which eluted in FC fraction III (eluent, pentane/Et_2O 1:1).

Identification and Synthesis of 2-Hydroxy-2,6,-10,10-tetramethyl-1-oxaspiro[4.5]dec-6-en-8-one (2). FC fraction III was further separated by HPLC, thus enabling the isolation of 12 mg of a pure product. The UV absorption maximum at 233 nm suggested the

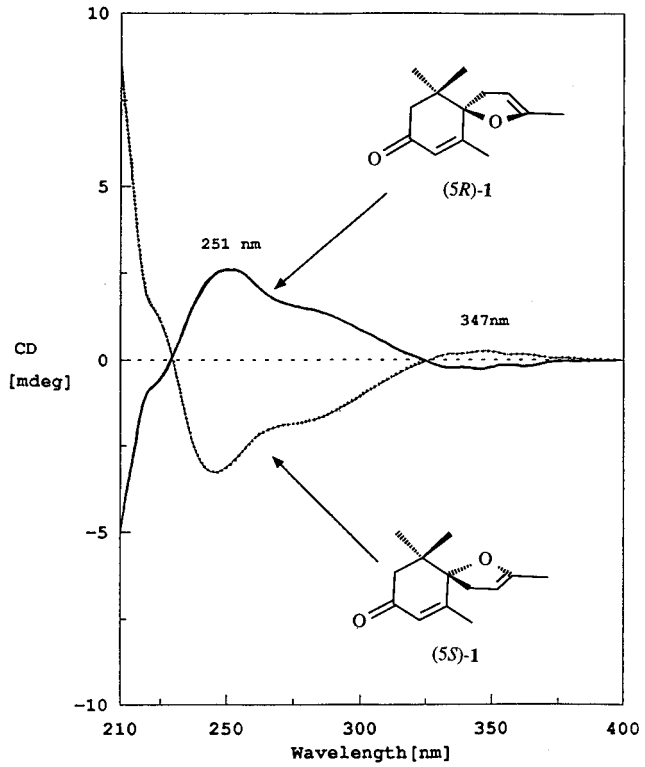
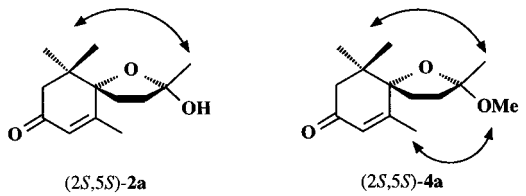


Figure 2. CD spectra (MeOH) of optically pure isomers of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (8,9-dehydrotheaspirone, **1**).

presence of a conjugated carbonyl group. High-field NMR spectra revealed that the isolated compound was a mixture of two diastereoisomers (ratio 5:1). The proton spectrum of the major diastereoisomer **2a** included three three-proton singlets at δ 0.98, 1.02, and 1.63 as well as an AB system centered at δ 2.34. A broad singlet for an olefinic proton at δ 5.89 showed a long-range coupling with a three-proton doublet at δ 2.08. All other protons resonated between δ 1.8 and 2.3. From the ^{13}C NMR and DEPT spectra, the presence of an unsaturated ketone (δ 125.8, 166.8, 198.0) with four methyl (δ 20.7, 23.0, 24.9, 27.6) and three methylene groups (δ 30.8, 38.2, 49.9) as well as a hemiacetal function (δ 106.7) was apparent. The structures **2a/b** assigned to the isolated diastereoisomers were confirmed by synthesis.

Hemiacetals **2a/b** were obtained by hydrogenation (Pd on Al_2O_3; solvent, THF) of racemic dehydrovomifoliol (**3**). The synthesized hemiacetals, which were formed in a ratio of 5:1, showed identical NMR spectral data as obtained for the natural nectarine constituents **2a/b**. Variation of the hydrogenation conditions (Pd on $BaSO_4$; solvent, MeOH) yielded, in addition to the hemiacetals **2a/b**, the diastereoisomeric methyl derivatives **4a/b**.



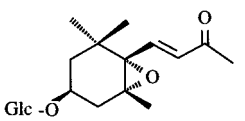
These acetals could be separated into the pure diastereoisomers by means of normal phase HPLC. For the assignment of the relative stereochemistry at C-2, NOE measurements were carried out. For the major diastereoisomer **4a**, irradiation at δ 1.52 (CH_3-C2) gave a NOE

at δ 0.97 and 1.02 ($2CH_3$-C10), whereas irradiation at δ 3.29 (OCH_3) showed a NOE at δ 2.02 (CH_3-C6). Opposite effects were observed for the minor isomer **4b**. In the case of the inseparable mixture of natural hemiacetals **2a/b**, NOE measurements were only performed with the major isomer **2a**. Importantly, irradiation at δ 1.63 (CH_3-C2) gave a NOE at δ 0.98 and 1.02 ($2CH_3$-C10).

Absolute Configuration of 2-Hydroxy-2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-en-8-ones (2a/b) and Enantiodifferentiation of 8,9-Dehydrotheaspirone (1). To establish the configuration at the chiral center C-5, the hemiacetals **2a/b** were prepared from optically pure dehydrovomifoliol (6*S*)-**3** and (6*R*)-**3** as outlined in Figure 1. Subsequent dehydration reaction yielded optically pure spiroethers (5*S*)-**1** and (5*R*)-**1**. The optical purity was checked by HRGC as well as HRGC-MS using a permethylated β-cyclodextrin column (first eluting peak, *R*-enantiomer). Since the dehydration of the natural product yielded only the *S*-configured enantiomer, the absolute configuration of the natural hemiacetals could be assigned as 2*S*,5*S* (major isomer) and 2*R*,5*S* (minor isomer), respectively.

To our knowledge, the hemiacetals **2a/b** have not been previously reported as natural compounds. The dehydration product **1**, however, was identified as a volatile constituent in tobacco (Fujimori et al., 1981), Riesling wine (Winterhalter et al., 1990), nectarines (Takeoka et al., 1992), and *Reseda odorata* flowers (Surburg et al., 1993). The previous odor description of **1** ("strong flowery-woody") by Fujimori et al. (1981) could not be confirmed in the present study. Both of the pure enanantiomers of **1** were found to be too weak for odor evaluation using GC sniffing. The most plausible explanation for this discrepancy is that the isolated natural material contained at least one concomitant having a very strong smell. This is a fundamental problem of all sensory evaluation of compounds isolated from natural sources, since even rigorous purification methods will not necessarily lead to a completely pure substance. Therefore, an olfactory identity is only given if the isolated and synthesized compounds smell the same or very similar (Weyerstahl and Meisel, 1994). In summary, it can be concluded that C_{13}-spiroether **1** does not play any decisive role in the flavor of white-fleshed nectarines.

Biogenetic Considerations. In addition to 8,9-dehydrotheaspirone (**1**) and its immediate precursors **2a/b**, two structurally related compounds were also isolated from the juice of white-fleshed nectarines, i.e. (6*S*)-dehydrovomifoliol (**3**) and—in glucoconjugated form—(3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-β-ionone (**5**). The



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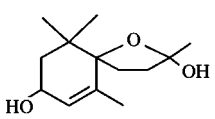

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detection of C_{13}-hydroxyketones **3** and **5** supports an earlier suggestion of Enzell et al. (1977) for the formation of theaspirone derivatives from ubiquitous epoxycarotenoids, such as violaxanthin or neoxanthin.

Importantly, the reduced forms of hemiacetals **2a/b**, i.e. 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diols **6**, are known constituents of Riesling wine (Winterhalter, 1991). Acid-catalyzed degradation of **6** is known to generate the hydrocarbon 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), which at higher concentrations causes a "kerosene—petrol-like" off-flavor in wine. The question as to what extent the oxo group in **2a/b** might be reduced during juice fermentation, thus giving rise to a formation of TDN precursor **6**, must be addressed in future studies.

ABBREVIATIONS USED

HRGC, high-resolution gas chromatography; R_i, linear retention index; MS, mass spectrometry; PTV, programmed-temperature vaporizer; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; CD, circular dichroism; HPLC, high-performance liquid chromatography; EI, electron impact; MLCCC, multilayer coil countercurrent chromatography; SDE, simultaneous distillation/extraction; FC, flash chromatography; DEPT, distortionless enhancement by polarization transfer; FID, flame ionization detector; IUPAC names for compounds **1**—**6**: 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-2,6-dien-8-one (**1**); 2-hydroxy-2,6,10,-10-tetramethyl-1-oxaspiro[4.5]dec-6-en-8-one (**2**); 4-hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-enyl)cyclohex-2-enone (**3**); 2-methoxy-2,6,10,10-tetramethyl-1-oxaspiro-[4.5]dec-6-en-8-one (**4**); 4-(4-β-D-glucopyranosyloxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-buten-2-one (**5**); 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol (**6**).

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