Identification and Enantiodifferentiation of C_{13} Norisoprenoid Degradation Products of Glycosidically Bound 3-Hydroxy-α-ionol from Stinging Nettle (*Urtica dioica* L.)

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Acid-catalyzed degradation (simultaneous distillation–extraction; pH 2.5) of glycosidically bound 3-hydroxy-α-ionol from stinging nettle (*Urtica dioica* L.) yielded 2,2,6-trimethyl-7-methylenebicyclo-[4.3.0]nona-4,8-diene and 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,7,9-triene as well as 1-(2-butenylidene)-2,6,6-trimethylcyclohexa-2,4-diene (**1**), 1-(2-butenylidene)-6,6-dimethyl-2-methylenecyclohex-3-ene (**2**), and isomeric 4-(2,6,6-trimethylcyclohexa-2,4-dien-1-yl)but-3-en-2-ol (2,3-didehydro-α-ionol) (**3a–d**) and 4-(6,6-dimethyl-2-methylenecyclohex-3-en-1-yl)but-3-en-2-ol (3,4-didehydro-γ-ionol) (**4a–d**), which were identified by comparison of their chromatographic and spectral (MS; vapor phase FTIR) data with those of synthesized reference compounds. Configuration of the butenylidene chain of **1** and **2** was established by NOE experiments. The absolute configuration of the stereoisomers of **3** and **4** was elucidated using optically pure reference compounds. Enantiodifferentiation carried out by on-line coupled multidimensional gas chromatography–mass spectrometry revealed the occurrence of 1′*R*,2*R* and 1′*R*,2*S* diastereomers (**3a/3c** and **4a/4c**) in stinging nettle.

Keywords: *1-(2-Butenylidene)-2,6,6-trimethylcyclohexa-2,4-diene; 1-(2-butylidene)-6,6-dimethyl-2-methylenecyclohex-3-ene; 2,3-didehydro-α-ionol; 3,4-didehydro-γ-ionol; enantiodifferentiation; 3-hydroxy-α-ionol; stinging nettle*

INTRODUCTION

In the past, a considerable number of C_{13} norisoprenoid glycosides have been isolated and characterized from various plant tissues. Since they are recoverable aroma precursors, intensive studies have been carried out on their structural elucidation and reactivity (Williams et al., 1993). The actual state-of-the-art in this field has been summarized in a recent review, in which the importance of acid-catalyzed reactions leading to the liberation of aglycons and their subsequent conversions has been stressed (Winterhalter and Schreier, 1994). Thus, the formation of a number of attractive aroma compounds, such as β-damascenone (Näf et al., 1990), vitispirane (Humpf et al., 1991), and "Riesling acetal" (Humpf et al., 1992), is derived from glycosidically bound precursors.

Continuing our work on the acid-catalyzed formation of aroma compounds from their glycoconjugates, we report the identification and enantiodifferentiation of a number of C_{13} norisoprenoids formed from glycosidically bound 3-hydroxy-α-ionol in stinging nettle (*Urtica dioica* L.).

EXPERIMENTAL PROCEDURES

Chemicals. All commercial chemicals used were of analytical grade quality. α-Ionone was obtained from Fluka, Neu-Ulm, Germany. Solvents were redistilled before use.

Plant Material. Leaves from stinging nettle were plucked in the summer of 1993 from plants grown in the local area.

Isolation of a Glycosidic Extract and Its Acid Hydrolysis. After the mixing of 500 g of fresh leaves with 600 mL of methanol and macerization of the mixture (pH 7) at ambient temperature overnight, a clear extract was obtained by centrifugation (5000*g*, 30 min). Methanol was removed under reduced pressure (rotavapor). The aqueous residue was extracted three times with 100 mL of pentane and subsequently diethyl ether to remove chlorophyll and free volatiles, respectively, and then applied to an Amberlite XAD-2 column (25 × 900 mm, 10 mL/min) (Gunata et al., 1985). After a rinse with 1500 mL of distilled water, a glycosidic extract was obtained by elution with 500 mL of methanol. The methanol eluate was concentrated under reduced pressure to dryness (rotavapor) and redissolved in 15 mL of 0.1 M phosphate buffer (pH 7.0) (yields ranging from 2.3 to 4.1 g). Remaining volatiles were removed by diethyl ether extraction.

A solution of 500 μg of glycosidic extract in 100 mL of distilled water (pH 2.5; phosphoric acid) was subjected to simultaneous distillation–extraction (SDE) (Schultz et al., 1977) over 2 h. The organic phase was dried over anhydrous sodium sulfate and carefully concentrated to approximately 0.2 mL by a Vigreux column (45 °C) for subsequent HRGC and HRGC–MS analysis.

Reference Compounds. *(a) 3-Hydroxy-α-ionol.* A solution of 1.2 g of 3-oxo-α-ionol, synthesized from α-ionone according to the method of Sefton et al. (1989), in 20 mL of dry diethyl ether was added to a suspension of 110 mg of $LiAlH_4$ in 30 mL of diethyl ether. After 1 h of stirring under reflux and the addition of ice–water (50 mL), the organic layer was separated and the water phase extracted three times with 100 mL of diethyl ether. The combined organic layers were dried over anhydrous sodium sulfate and carefully concentrated (Vigreux column, 45 °C), yielding 820 mg. Chromatographic and spectral data of 3-hydroxy-α-ionol were identical with published data (Behr et al., 1978).

(b) Acid-Catalyzed Degradation of 3-Hydroxy-α-ionol. To a solution of 800 mg of 3-hydroxy-α-ionol in 2 mL of methanol was added 150 mL of distilled water. The solution was adjusted to pH 2.5 (phosphoric acid), and SDE was carried out over 2 h. The organic phase was dried over anhydrous sodium sulfate and carefully concentrated (Vigreux column, 45 °C) to approximately 2 mL. Separation by flash chromatography provided fraction I (pentane) and fraction II (pentane–diethyl ether 1:1). Argentation chromatography of fraction I (pentane–diethyl ether 97:3) using silver silica gel, prepared according to the method of De Vries (1962), yielded two hydrocarbons in pure form (2 and 6 mg, respectively), which were assigned to be 1-(2-butylidene)-2,6,6-trimethylcyclohexa-2,4-diene (**1**) and 1-(2-butylidene)-6,6-dimethyl-2-methylenecyclohex-3-ene (**2**) by means of their NMR spectra (cf. Tables 1–4). **1**: R_i 1668; EI-MS, *m/z* (%) 174 (M^+, 29), 160 (13), 159

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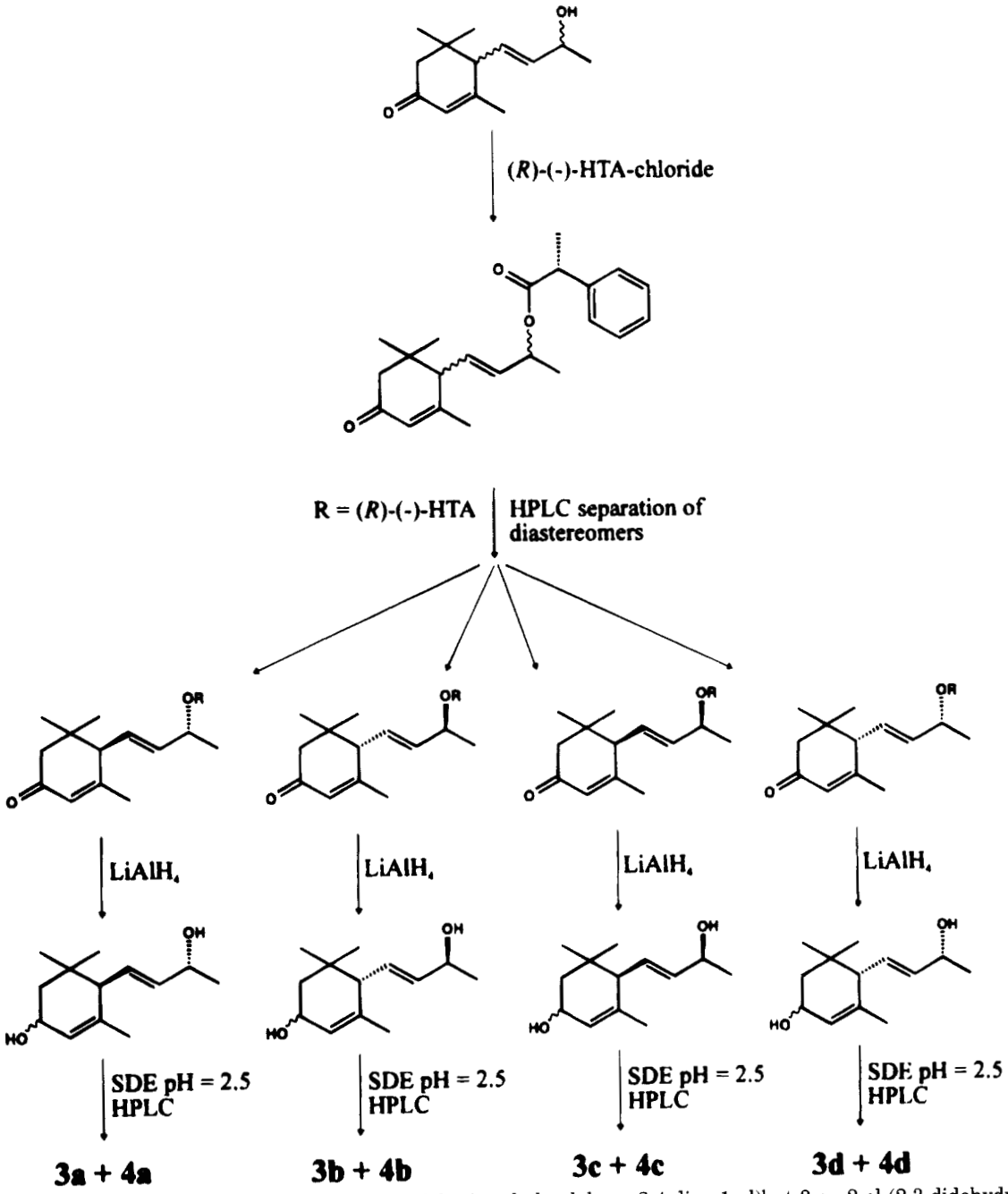


Figure 1. Synthesis of optically pure isomers of 4-(2,6,6-trimethylcyclohexa-2,4-dien-1-yl)but-3-en-2-ol (2,3-didehydro-α-ionol) (**3a–d**) and 4-(6,6-dimethyl-2-methylenecyclohex-3-en-1-yl)but-3-en-2-ol (3,4-didehydro-γ-ionol) (**4a–d**) from 3-oxo-α-ionol via its *R*-(−)-α-phenylpropionic acid esters, subsequent reductive hydrolysis, and acid-catalyzed (SDE, pH 2.5) dehydration (scheme).

(100), 144 (38), 131 (24), 130 (14), 129 (52), 128 (36), 119 (20), 117 (17), 115 (33), 105 (25), 91 (36), 79 (11), 78 (12), 77 (27), 65 (21), 63 (14), 53 (20), 51 (26), 41 (28); FTIR (vapor phase, ν, cm^{-1}) 3043, 2974, 2931, 2852, 1701, 1458, 1365, 1211, 1061, 964, 872, 798, 737. **2**: R_i 1582; EI-MS, m/z (%) 174 (M^+, 31), 160 (13), 159 (100), 144 (16), 141 (9), 131 (43), 130 (13), 129 (30), 128 (30), 127 (10), 118 (17), 117 (49), 116 (11), 115 (33), 105 (27), 91 (41), 79 (12), 78 (11), 77 (29), 71 (20), 65 (22), 63 (14), 53 (14), 51 (22), 41 (33); FTIR (vapor phase, ν, cm^{-1}) 3039, 2962, 2924, 2875, 2827, 1604, 1531, 1462, 1389, 1250, 1196, 1034, 849, 706. Fraction II was further purified by preparative HPLC using a Eurospher Si100 column (Knauer, Berlin; 5 μm, 250 × 16 mm; flow rate 5 mL/min; UV detection 230 nm) with pentane–methyl–*tert*-butyl ether (8:2) as eluent. Four compounds were obtained in pure form (6 and 4 mg as well as 27 and 25 mg, respectively), whose structures were elucidated to be 4-(2,6,6-trimethylcyclohexa-2,4-dien-1-yl)but-3-en-2-ol (2,3-didehydro-α-ionol) (**3a–d**) and 4-(6,6-dimethyl-2-methylenecyclohex-3-en-1-yl)but-3-en-2-ol (3,4-didehydro-γ-ionol) (**4a–d**) by means of their NMR spectra (cf. Tables 5 and 6). **3a/3b**: R_i 1865; EI-MS, m/z (%) 192 (M^+, 5), 174 ([M − H_2O]$^+$, 2), 159 (18), 148 (18), 147 (20), 134 (14), 133 (18), 122 (11), 121 (17), 120 (16), 119 (92), 117 (13), 115 (14), 107 (21), 106 (12), 105 (48), 93 (25), 92 (13), 91 (55), 79 (23), 77 (27), 69 (19), 67 (12), 65 (14), 55 (27), 53 (15), 51 (13), 45 (24), 43 (100), 41 (55); FTIR (vapor phase, ν, cm^{-1}) 3649, 3035, 2970, 2935, 2868, 1651, 1589, 1454, 1373, 1246, 1057, 972, 852, 725. **3c/3d**: R_i 1869; EI-MS and FTIR data are identical with those of **3a/3d**. **4a/4b**: R_i 1973; EI-MS, m/z (%) 192 (M^+, 1), 174 ([M − H_2O]$^+$, 3), 159 (40), 147 (9), 131 (13), 119 (27), 117 (10), 107 (21), 105 (32), 93 (18), 92 (13), 91 (38), 79 (20), 77 (18), 69 (10), 67 (10), 65 (10), 55 (18), 53 (10), 45 (16), 43 (100), 41 (39); FTIR (vapor phase, ν, cm^{-1}) 3649, 3035, 2970, 2935, 2868, 1651, 1589, 1454, 1373, 1246, 1057, 972, 852, 725. **4c/4d**: R_i 1977; EI-MS and FTIR data were identical with those of **4a/4b**.

*(c) Synthesis of Optically Pure Isomers of **3a–d** and **4a–d**.* The synthesis of 3-oxo-α-ionol and the separation into its optical isomers via the corresponding (*R*)-(−)-α-phenylpropionic acid esters was carried out as described earlier (Pabst et al., 1992) (Figure 1). Assignment of the absolute configuration

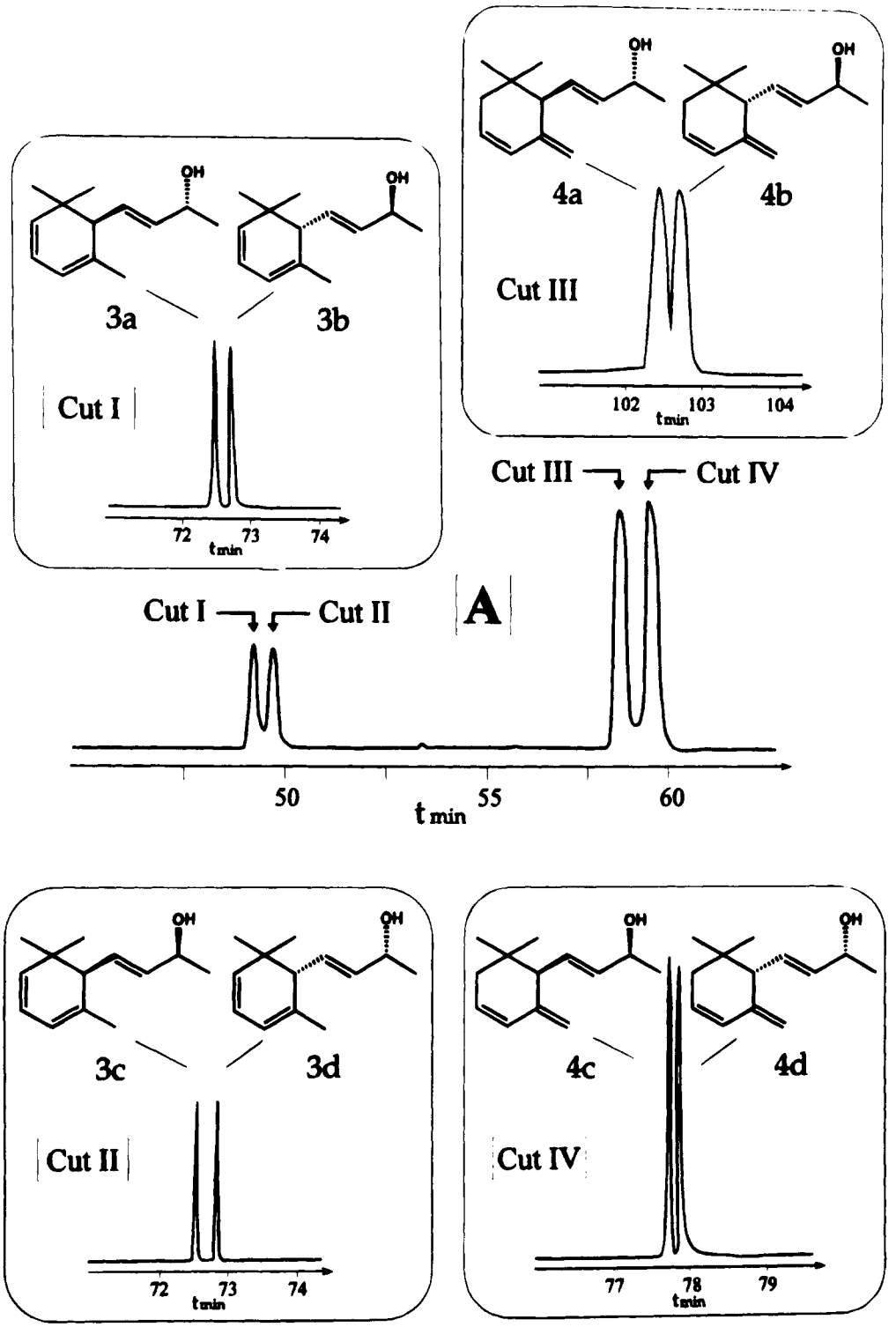


Figure 2. MDGC–MS separation of synthesized **3** and **4**: A, preseparation on DB-Wax; cut I–IV, separations on 2,6-dimethyl-3-pentyl(DMP)-β-cyclodextrin/OV 1701. (For MDGC conditions see Experimental Procedures.)

of the four separated isomers of 3-oxo-α-ionol was checked by ^{1}H NMR spectroscopy of the corresponding (*R*)-(−)-α-phenylpropionic acid esters and, after esterase hydrolysis of an aliquot of the esters and purification by HPLC (Pabst et al., 1992), by CD spectroscopy of the optically pure 3-oxo-α-ionol isomers. For reductive hydrolysis of the diastereomeric (*R*)-(−)-α-phenylpropionic acid esters, 5 mg (15 μmol) of each ester in dry diethyl ether (10 mL) was added to a stirred suspension of 5 mg (120 μmol) of $LiAlH_4$ in 5 mL of diethyl ether. After 2 h of stirring at room temperature and the addition of ice–water (10 mL), the organic layer was separated and the water phase extracted three times with 30 mL of diethyl ether. The combined organic layers were washed with brine (20 mL) and water (20 mL). After drying over anhydrous sodium sulfate and concentration (Vigreux column, 45 °C), the formed 3-hydroxy-α-ionol was subjected to SDE treatment at pH 2.5 for 2 h. Purification of the pure isomers **3a–d** and **4a–d** was achieved by HPLC as described above under (b). CD data (ethanol, nm, Δε): **3a**, 270 (+121); **3b**, 270 (−123); **3c**, 269 (+118); **3d**, 270 (−120) **4a**, 234 (−65), 272 (+5); **4b**, 232 (+62), 272 (−3); **4c**, 234 (−67), 271 (+6); **4d**, 233 (+62), 272 (−6).

Capillary Gas Chromatography (HRGC). A Carlo Erba Mega 5160 gas chromatograph with FID equipped with a J&W fused silica DB-Wax capillary column (30 m ×0.259 mm i.d., film thickness 0.25 μm) was used. Split injection (1:20) was employed. The temperature program was 3 min isothermal at 50 °C and then increased from 50 to 240 °C at 4 °C/min. The flow rate for the carrier gas was 2.0 mL/min He and for

Table 1. ^{1}H NMR Spectral Data (400 MHz, $CDCl_3$) of Compound 1 (Coupling Constants in Hertz, δ Relative to TMS)

δ	signal	J	atom
1.34	6 H, s		2 H_3C-C6
1.84	3 H, dd	6.9/1.6	H_3C1'
1.88	3 H, s		H_3C-C2
5.41[a]	1H, bd	10.7	H C5
5.6–5.8	2 H m		H C3/H C4
5.71	1 H, bdd	6.9/14.6	H C2′
6.23	1 H, d	11.7	H C4′
6.76	1 H, ddq	14.6/11.7/1.6	H C3′

[a] Assignment was based on a ^{1}H–^{1}H COSY experiment.

Table 2. ^{13}C NMR Spectral Data (100 MHz, $CDCl_3$) of Compound 1 (δ Relative to TMS)

δ	DEPT	atom
18.70	CH_3	C1′
20.95	CH_3	C-C2
29.97	CH_3	2 C-C6
37.96	C	C6
118.13[a]	CH	C3
121.88[a]	CH	C4
127.63[b]	CH	C2′
130.09[b]	CH	C3′
130.87[b]	CH	C4′
132.84	C	C2
139.89	CH	C5
142.56	C	C1

[a,b] Interchangeable values.

Table 3. ^{1}H NMR Spectral Data (400 MHz, $CDCl_3$) of Compound 2 (Coupling Constants in Hertz, δ Relative to TMS)

δ	signal	J	atom
1.02	3 H, s		H_3C-C6
1.10	3 H, s		H_3C-C6
1.76	3 H, dd	6.7/1.7	H_3C1'
2.06	2 H, brs		H_2C5
5.02	1 H, brs		H_aC-C2
5.18	1 H, brs		H_bC-C2
5.67	1 H, ddq	14.5/9.6/4.5	H C4
5.78	1 H, dq	14.9/6.7	H C2′
5.97	1 H, d	10.7	H C4′
6.10	1 H, d	9.6	H C3
6.54	1 H, ddq	14.9/10.7/1.7	H C3′

Table 4. ^{13}C NMR Spectral Data (100 MHz, $CDCl_3$) of Compound 2 (δ Relative to TMS)

δ	DEPT	atom
18.26	CH_3	C1′
29.80	CH_3	2 C-C6
36.29	C	C6
42.93	CH_2	C5
115.53	CH_2	C-C2
121.86	CH	C4
127.59[a]	CH	C3
128.81[a]	CH	C2′
129.1	C	C2
129.57[a]	CH	C3′
130.45[a]	CH	C4′
143.84	C	C1

[a] Interchangeable values.

the makeup gas 30 mL/min N_2; for the detector gases the flow rates were 30 mL/min H_2 and 300 mL/min air. Injector and detector temperatures were kept at 220 and 260 °C, respectively.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian 3300 gas chromatograph with split injector (1:20) was combined by direct coupling to a Finnigan MAT 44S mass spectrometer with PCDS data system. The same type of column and the same temperature program as mentioned above for HRGC were used. Other conditions: temperature of ion source and all connection parts, 220 °C; electron energy, 70 eV; cathodic current, 0.7 mA; mass range 41–250.

Capillary Gas Chromatography–Fourier Transform Infrared Spectroscopy (HRGC–FTIR). HRGC–FTIR analysis was carried out using a Bruker IFS 85 system interfaced with a Carlo Erba Fractovap 2101 AC gas chromatograph with FID equipped with a J&W fused silica DB-Wax capillary column (30 m ×0.32 mm i.d., film thickness 0.5 μm). Split injection (1:10) was employed. The temperature program was 3 min isothermal at 50 °C and then increased from 50 to 240 °C at 4 °C/min. The flow rate for the carrier gas was 1.3 mL/min He and for the makeup gas 30 mL/min N_2; for the detector gases the flow rates were 30 mL/min H_2 and 300 mL/min air. Injector and detector temperatures were kept at 200 °C. Light pipe and transfer line were held at 200 °C. Vapor-phase FTIR spectra were recorded from 600 to 4000 cm^{-1} with a resolution of 8 cm^{-1}.

Multidimensional Gas Chromatography–Mass Spectrometry (MDGC–MS). A Siemens Sichromat 2 double-oven gas chromatograph with split injection (250 °C, 1:20) and flame ionization detectors on ovens 1 and 2 (250 °C each) was used. Preseparation was achieved in oven 1 on a J&W fused silica DB-Wax capillary column (30 m × 0.259 mm i.d., film thickness 0.25 μm). The temperature was programmed from 100 to 240 °C at 1 °C/min. A "live" switching device (Schomburg et al., 1984) in oven 1 was used to perform effluent cuts onto a 2,6-dimethyl-3-pentyl(DMP)-β-cyclodextrin/OV 1701 column (30 m × 0.25 mm i.d.; film thickness 0.3 μm) in oven 2. The temperature program (cuts I, II, IV) was 60 min isothermal at 100 °C and then increased from 100 to 200 °C at 2 °C/min. The following cuts were used: cut I (**3a/3b**), 48.8–49.1 min; cut II (**3c/3d**), 49.6–49.9 min; cut IV (**4c/4d**), 59.1–59.4 min. Due to incomplete separation for **4a/4b** (cut III, 57.8–58.1 min) the temperature program of oven 2 was modified: 95 min isothermal at 100 °C and then increased from 100 to 200 °C at 1 °C/min. Cuts II and IV were carried out in one step. Helium was used as the carrier gas at 0.66 mL/min in oven 1 and at 1.96 mL/min in oven 2. The flow rates for the detector gases were 30 mL/min of H_2 and 300 mL/min air. The coupling of the MDGC system with a Finnigan MAT 44S mass spectrometer was achieved by a variable effluent splitter (Siemens) working as a second "live" switching device. The temperatures of the ion source and of the transfer line were 200 °C. The electron energy was 70 eV and the cathodic current 0.7 mA.

Capillary Gas Chromatography–Chemical Ionization Mass Spectrometry (HRGC–CI-MS). A Varian 3700 gas chromatograph was combined by direct coupling to a Finnigan MAT 8200 mass spectrometer with an SS300 data system using splitless mode. The same type of column and the same temperature program as mentioned above for HRGC were used. The flow rate for the carrier gas was 1.0 mL/min He. The temperature of the ion source was 160 °C and for connection parts 200 °C. Other conditions: electron energy, 70 eV; cathodic current, 0.05 mA; mass range 80–300. NH_3 was applied as reactand gas.

Nuclear Magnetic Resonance (NMR). ^{1}H and ^{13}C NMR spectra were recorded on Fourier transform Bruker AC 200 (200 MHz) and WM 400 (400 MHz) spectrometers 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 irradiation of the different proton chemical shift frequencies.

Circular Dichroism (CD). CD spectra were recorded on a Dichrograph CD 6 (Jobin Yvon) in ethanol solution using cells of different lengths.

RESULTS AND DISCUSSION

Acid-catalyzed degradation (SDE; pH 2.5) of a glycosidic fraction isolated from stinging nettle (*U. dioica* L.) leaves yielded six major volatiles, from which 2,2,6-trimethyl-7-methylenebicyclo[4.3.0]nona-4,8-diene and

Table 5. ^{1}H NMR Spectral Data of Compounds 3 (400 MHz, $CDCl_3$) and 4 (200 MHz, $CDCl_3$) (Coupling Constants in Hertz, δ Relative to TMS)

H	3a/3b	3c/3d	4a/4b	4c/4d
1	1.25 d (6.3)	1.26 (6.2)	1.26 d (6.3)	1.27 d (6.4)
2	4.29 quintet (6.3)	4.30 quintet (6.2)	4.35 quintet (6.3)	4.29 m
3	5.56 dd (15.4/6.2)	5.55 dd (15.2/6.2)	5.52–5.59 m	5.50–5.57 m
4	5.47 dd (15.4/9.2)	5.45 dd (15.2/8.9)	5.52–5.59 m	5.50–5.57 m
1′	2.12 d (9.2)	2.1 d (8.9)	2.55 br s	2.53 br s
3′	5.59 br d (5.2)	5.59 br d (5.2)	6.13 dt (9.9/2.0)	6.12 dt (9.8/2.0)
4′	5.73 dd (9.5/5.2)	5.76 dd (9.4/5.2)	5.69 m	5.69 m
5′	5.31 d (9.5)	5.31 d (9.4)	1.93 br s	1.93 br s
H_3C-C6′	0.99 s	1.01 s	0.89 s	0.87 s
H_3C-C6′	0.94 s	0.96 s	0.86 s	0.84 s
H_aC-C2′			4.77 br s	4.80 br s
H_bC-C2′			4.86 br s	4.87 br s
H_3C-C2′	1.7 s	1.68 s		

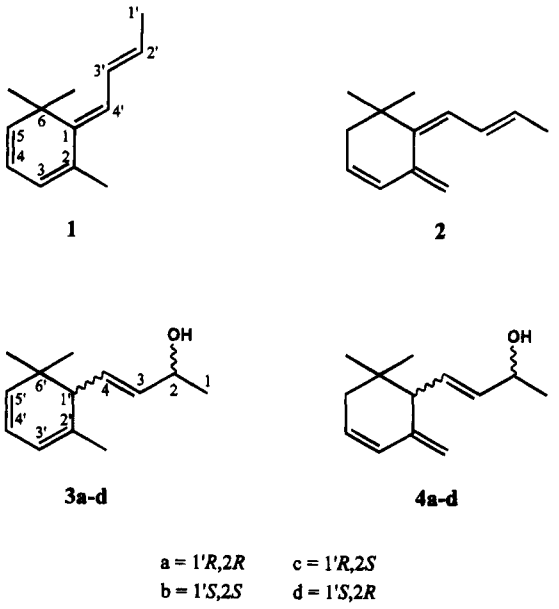
Table 6. ^{13}C NMR Spectral Data of Compounds 3 (200 MHz, $CDCl_3$) and 4 (100 MHz, $CDCl_3$) (Coupling Constants in Hertz, δ Relative to TMS)

C	3a/3b	3c/3d	4a/4b	4c/4d
1	23.54	23.65	23.50	23.57
2	68.81	68.71	68.79	68.89
3[a]	134.51	134.29	136.29	136.26
4[a]	128.34	128.50	127.73	127.73
1′	54.78	54.84	54.40	54.53
2′	136.81	136.68	145.43	145.41
3′[a]	117.88	117.95	129.70	129.94
4′[a]	122.08	121.93	128.24	128.20
5′	135.67	135.49	38.44	38.34
6′	34.39	34.22	32.89	32.91
C-C6′	25.57	25.44	25.15	25.30
C-C6′	26.64	26.80	28.34	28.27
C-C2′	21.93	22.02	112.81	112.87

[a] Assignments were based on comparison with data of similar compounds (Baranyai et al., 1984).

2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,7,9-triene were identified by HRGC–MS using authentic reference substances. From the HRGC–MS and HRGC–FTIR information the occurrence of two C_{13} norisoprenoid tetraenes and their corresponding diastereomeric alcohols was assumed for the unknown substances. The postulated structures suggested their formation from 3-hydroxy-α-ionol under acidic conditions. In fact, synthesis of 3-hydroxy-α-ionol and its subsequent SDE treatment at pH 2.5 led to a similar distribution of volatiles as observed with the glycosidic extract of stinging nettle.

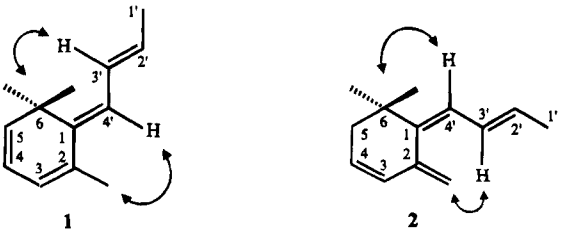
Identification of 1-(2-Butenylidene)-2,6,6-trimethylcyclohexa-2,4-diene (1) and 1-(2-Butenylidene)-6,6-dimethyl-2-methylenecyclohex-3-ene (2). The two tetraenes, whose structures have been postulated to be **1** and **2** due to their characteristic MS data, have been described tentatively as aroma compounds of quince fruit (Winterhalter et al., 1987) and starfruit (MacLeod and Ames, 1990), but to the best of our knowledge exact characterization has not been performed to date. The vapor phase FTIR data showed a *Z*-configured double bond in **1** (737 cm^{-1}) and **2** (706 cm^{-1}) and an exocyclic methylene group (849 cm^{-1}) in **2**. The ^{1}H NMR spectra of **1** and **2** (NMR data cf. Tables 1–4) exhibited signals [ddq at δ 6.76 (**1**) and 6.54 (**2**) and dd at δ 1.84 (**1**) and 1.76 (**2**)], which are typical for 2-butenylidene groups (Aasen et al., 1972; Winterhalter et al., 1990). While for **1** one six-proton singlet at δ 1.34 (2 × CH_3-C6) and one three-proton singlet at δ 1.88 was observed, **2** showed two corresponding singlets at δ 1.02 and 1.10 and two characteristic broad singlets for the protons of the methylene group at C-2′ (δ 5.02 and 5.18). For both molecules the presence of an *E*-configured double bond [J = 14.6 Hz (**1**), 14.9 Hz (**2**)] was apparent.



a = 1′*R*,2*R* c = 1′*R*,2*S*
b = 1′*S*,2*S* d = 1′*S*,2*R*

From the spectral data recorded the tetraenes were identified to be 1-(2-butenylidene)-2,6,6-trimethylcyclohexa-2,4-diene (**1**) and 1-(2-butenylidene)-6,6-dimethyl-2-methylenecyclohex-3-ene (**2**). The data obtained by HRGC–CI-MS supported the structural elucidations of **1** and **2**. For **1** and **2** one strong peak was registered at m/z 175 $[M + 1]^+$. In addition, for **2** two weak pseudomolecular ions, i.e. m/z 192 $[M + NH_4]^+$ and 209 $[M + NH_4 + NH_3]^+$, were recorded.

For the assignment of protons closely located in space, NOE experiments were carried out. Thus, for **1** irradiation of the methyl groups of C6 and of the proton at C4′ resulted in a NOE at the protons at C3′ and CH_3-C2, respectively, indicating *E*-configuration of the double



bond C1=C4′, whereas for **2** irradiation of the protons of the methyl groups of C6 and of the protons of C3′ showing a NOE at the protons at C4′ and CH_2-C2, respectively, made *Z*-configuration of the corresponding double bond evident.

Structure and Absolute Configuration of 4-(2,6,6-Trimethylcyclohexa-2,4-dien-1-yl)but-3-en-2-ol (3)

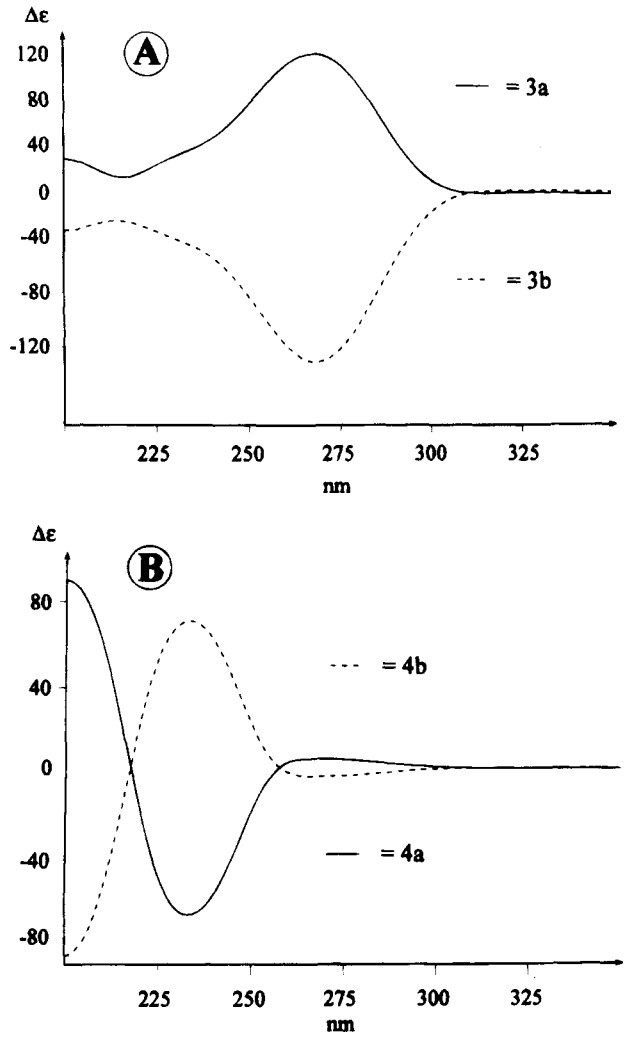


Figure 3. CD spectra of two optically pure stereoisomers of synthesized 2,3-didehydro-α-ionol **3a/3b** (A) and 3,4-didehydro-γ-ionol **4a/4b** (B) (the similar spectra of **3c/3d** and **4c/4d** are omitted).

and 4-(6,6-Dimethyl-2-methylenecyclohex-3-en-1-yl)but-3-en-2-ol (4). The two postulated alcohols have been described to be aroma constituents of rum (Ter Heide et al., 1981), but as far as we know no chromatographic and spectroscopic data have been published to date. Vapor phase FTIR analysis revealed the presence of a *Z*-configured double bond (725 cm^{-1}) in **3** and an exocyclic methylene group (887 cm^{-1}) in **4**. In addition, both compounds showed a characteristic OH absorption at 3649 cm^{-1}. The ^1H NMR spectra of the isomeric alcohols **3** exhibited four methyl groups, whereas for compounds **4** only three methyl groups were registered (NMR data cf. Tables 5 and 6). Correspondingly to **2**, two typical broad singlets for the protons of the methylene group at C2′ (**4a–d**) were present. From the ^1H and ^{13}C NMR (including DEPT) data the compounds were deduced to be 4-(2,6,6-trimethylcyclohexa-2,4-dien-1-yl)but-3-en-2-ol (**3**) and 4-(6,6-dimethyl-2-methylenecyclohex-3-en-1-yl)but-3-en-2-ol (**4**). The data recorded by HRGC–CI-MS were also in accordance with the structures of **3** and **4**; for **3** and **4** *m/z* 175 $[M - H_2O + 1]^+$ was registered. In addition, three other signals, i.e. *m/z* 192 $[M + NH_4 - H_2O]^+$, 210 $[M + NH_4]^+$, and 227 $[M + NH_4 + NH_3]^+$, were detected, indicating a molecular mass of 192.

Using on-line coupled multidimensional gas chromatography–mass spectrometry (MDGC–MS) (Bernreuther and Schreier, 1991), enantiodifferentiation of **3a–d** and **4a–d** (Figure 2) was carried out. Assignment of stereochemistry was achieved using optically pure reference compounds, which were synthesized via the corresponding (*R*)-(−)-α-phenylpropionic acid esters of racemic 3-oxo-α-ionol, the stereochemistry of which has been described earlier (Pabst et al., 1992). Reductive hydrolysis and subsequent SDE of separated isomers yielded after purification the four optically pure isomers **3a–d** and **4a–d** (Figure 1). CD data recorded for **3a** and **3c**, the absolute configurations of which were deduced from the corresponding 3-oxo-α-ionols to be 1′*R*,2*R* and 1′*R*,2*S*, respectively, showed positive maxima at 270 nm (Figure 3A). For **3b** (1′*S*,2*S*) and **3d** (1′*S*,2*R*) CD spectroscopy revealed negative maxima at 270 nm. The corresponding isomers **4a/4d** (1′*R*,2*R* / 1′*R*,2*S*) and **4b/4d** (1′*S*,2*S* / 1′*S*,2*R*) exhibited CD data (Figure 3B) that were almost inverse to those of **3a–d**. The influence of the chiral center at C-2 in **3** and **4** on the shape of the CD spectra was shown to be negligible. From these data the diastereomers of **3** and **4** originating from glycosidically bound 3-hydroxy-α-ionol in stinging nettle were assigned to be 1′*R*,2*R* and 1′*R*,2*S*, respectively. These configurations fit with the potential progenitors of 3-hydroxy-α-ionol, i.e. lutein and epilutein, which are known to exhibit 6*R* configuration (Buchecker et al., 1974, 1979).

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LITERATURE CITED

Aasen, A. J.; Kimland, B.; Almqvist, S.-O.; Enzell, C. R. Tobacco chemistry 15, new tobacco constituents—the structures of five isomeric megastigmatrienones. *Acta Chem. Scand.* **1972**, *26B*, 2573–2576.

Baranyai, M.; Radics, L.; Kajtar, H.; Kajtar, J.; Bujtas, Gy.; Szabolcs, J. The structure of deoxyluteins. *Acta Chim. Hung.* **1984**, *116*, 153–161.

Behr, D.; Wahlberg, I.; Toshiaki, N.; Enzell, C. R. Tobacco chemistry. 47. (3S,6R,7E,9R9- and (3S*,6R*,7E,9S*)-4,7-megastigmadiene-3,9-diol. Two new nor-carotenoids of greek tobacco. *Acta Chem. Scand.* **1978**, *B32*, 391–394.

Bernreuther, A.; Schreier, P. Multidimensional gas chromatography-mass spectrometry: A powerful tool for the direct chiral evaluation of aroma compounds in plant tissues. II. Linalool in essential oils and fruits. *Phytochem. Anal.* **1991**, *2*, 167–170.

Buchecker, R.; Eugster, C. H. Search for the presence of 3′-epilutein (= (3*R*,3′*S*,6′*R*)-β,ε-carotene-3,3′-diol) and 3′,*O*-dihydrolutein (= (3*R*,6′*R*)-3-hydroxy-β,ε-carotene-3′-on) in egg yolk, in *Caltha palustris* flowers, and in autumn leaves. *Helv. Chim. Acta* **1979**, *62*, 2817–2824.

Buchecker, R.; Hamm, P.; Eugster, C. H. Absolute configuration of xanthophyll (lutein). *Helv. Chim. Acta* **1974**, *57*, 631–656.

De Vries, B. Quantitative separations of lipid materials by column chromatography on silica impregnated with silver nitrate. *Chem. Ind.* **1962**, 1049–1050.

Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma constituents. *J. Chromatogr.* **1985**, *331*, 83–89.

Humpf, H. U.; Winterhalter, P.; Schreier, P. 3,4-Dihydroxy-7,8-dihydro-β-ionone β-D-glucopyranoside: Natural precursor of 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0^{1,6}]undec-4-ene (Riesling acetal) and 1,1,6-trimethyl-1,2-dihydronaphthalene in red currant (Ribes rubrum L.) leaves. *J. Agric. Food Chem.* **1991**, *39*, 1833–1835.

Humpf, H. U.; Wintoch, H.; Schreier, P. 3,4-Dihydroxy-7,8-dihydro-β-ionol β-D-glucopyranoside: Natural precursor of vitispiranes from gooseberry (Ribes uva crispa L.) and whitebeam (Sorbus aria) leaves. *J. Agric. Food Chem.* **1992**, *40*, 2060–2062.

MacLeod, G.; Ames, J. M. Volatile components of starfruit. *Phytochemistry* **1990**, *29*, 165–172.

Näf, R.; Velluz, A.; Thommen, W. Isolation of a glucosidic precursor of damascenone from *Lycium halimifolium* Mil. *Tetrahedron Lett.* **1990**, *31*, 6521–6522.

Neugebauer, W.; Winterhalter, P.; Schreier, P. 2-(2-Butenylidene)-3,3-dimethyl-5-(2-oxopropyl)tetrahydrofuran: A new degradation product of 3-hydroxy-5,6-epoxy-β-ionol. *J. Agric. Food Chem.* **1994**, *42*, 2885–2888.

Pabst, A.; Barron, D.; Semon, E.; Schreier, P. Two diastereomeric 3-oxo-α-ionol β-D-glucosides from raspberry fruit. *Phytochemistry* **1992**, *31*, 1649–1652.

Schomburg, G.; Husmann, H.; Podmaniczky, L.; Weeke, F. Coupled gas chromatographic methods for separation, identification, and quantitative analysis of complex mixtures: MDGC, GC-IR, LC-GC. In *Analysis of Volatiles*; Schreier, P., Ed.; de Gruyter: Berlin, 1984; pp 121–150.

Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. Isolation of volatile components from a model system. *J. Agric. Food Chem.* **1977**, *25*, 446–449.

Sefton, M. A.; Skouroumounis, G. K.; Massy-Westropp, R. A.; Williams, P. J. Norisoprenoids in Vitis vinifera white wine grapes and the identification of a precursor of damascenone in these fruits. *Aust. J. Chem.* **1989**, *42*, 2071–2084.

Ter Heide, R.; Schaap, H.; Wobben, H. J.; del Valois, P. J.; Timmer, R. Flavor constituents in rum. In *The quality of foods and beverages*; Charalambous, G., Inglett, G., Eds.; Academic Press: New York, 1981; Vol. 1, pp 183–200.

Williams, P. J.; Sefton, M. A.; Marinos, V. A. Hydrolytic flavor release from non-volatile precursors in fruits, wines and some other plant-derived foods. In *Recent developments in flavor and fragrance chemistry*; Hopp, R., Mori, K., Eds.; VCH Verlagsges.: Weinheim, 1993; pp 283–290.

Winterhalter, P.; Schreier, P. C_{13} norisoprenoid glycosides in plant tissues: An overview on their occurrence, composition, and role as flavour precursors. *Flavour Fragrance J.* **1994**, *9*, 281–287.

Winterhalter, P.; Lander, V.; Schreier, P. Influence of sample preparation on the composition of quince (Cydonia oblonga, Mill.) flavor. *J. Agric. Food Chem.* **1987**, *35*, 335–337.

Winterhalter, P.; Herderich, M.; Schreier, P. 4-Hydroxy-7,8-dihydro-β-ionone and isomeric megastigma-6,8-dien-4-ones: new C_{13}-norisoprenoids in quince (Cydonia oblonga, Mill.) fruit. *J. Agric. Food Chem.* **1990**, *38*, 796–799.

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