2-(2-Butenylidene)-3,3-dimethyl-5-(2-oxopropyl)tetrahydrofuran: A New Degradation Product of 3-Hydroxy-5,6-epoxy-β-ionol

Wolfgang Neugebauer, Peter Winterhalter, and Peter Schreier*

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

Identification of 2-(2-butenylidene)-3,3-dimethyl-5-(2-oxopropyl)tetrahydrofuran diastereomers (**4**, **5**) in various leaves [stinging nettle; sloe tree; strawberry; vine (Riesling); sweet silique (*Bunias orientalis*)] was achieved by comparison of HRGC retention and spectral (MS; vapor phase FTIR) data with those of synthetic references. Model experiments revealed their acid-catalyzed formation from 3-hydroxy-5,6-epoxy-β-ionol that was present in glycosidically bound form in the above-mentioned leaves. Assignment of the isomers **4a/4b** and **5a/5b** was established by NOE experiments. Enantiodifferentiation carried out by on-line coupled multidimensional gas chromatography–mass spectrometry revealed the occurrence of enantiomerically pure 5*S* enantiomers **4b/5b** in the natural sources. A pathway for the selective formation of **4b/5b** in nature is proposed.

Keywords: *2-(2-Butenylidene)-3,3-dimethyl-5-(2-oxopropyl)tetrahydrofuran; 3-hydroxy-5,6-epoxy-β-ionol; norisoprenoid degradation; multidimensional gas chromatography–mass spectrometry*

INTRODUCTION

In a recent study, glycosidically bound 3-hydroxy-5,6-epoxy-β-ionol has been elucidated as a natural precursor of isomeric actinidols (**2**) and a C_{13} substance whose tentative structure has been assigned on the basis of spectral data as 3,4-didehydro-5,6-epoxy-β-ionol (**3**) (Humpf and Schreier, 1992) (Figure 1). The detection of **3** in an increasing number of glycosidic hydrolysates, obtained from various species, has made the determination of the exact chemical structure of degradation product **3** a necessity. In this paper, the previously suggested structure of **3** is revised and reported to be that of 2-(2-butenylidene)-3,3-dimethyl-5-(2-oxopropyl)-tetrahydrofuran diastereomers (**4**, **5**).

EXPERIMENTAL PROCEDURES

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

Plant Material. Leaves from stinging nettle, sloe tree, strawberry, vine (Riesling), and sweet silique (*Bunias orientalis* L.) were plucked in the summer of 1993 from plants grown in the Würzburg area.

Isolation of a Glycosidic Extract. After the mixing of 500 g of 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 washing 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.

Acid Hydrolysis. A solution of 500 μg of glycosidic extract in 100 mL of distilled water (pH 2.5) 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.

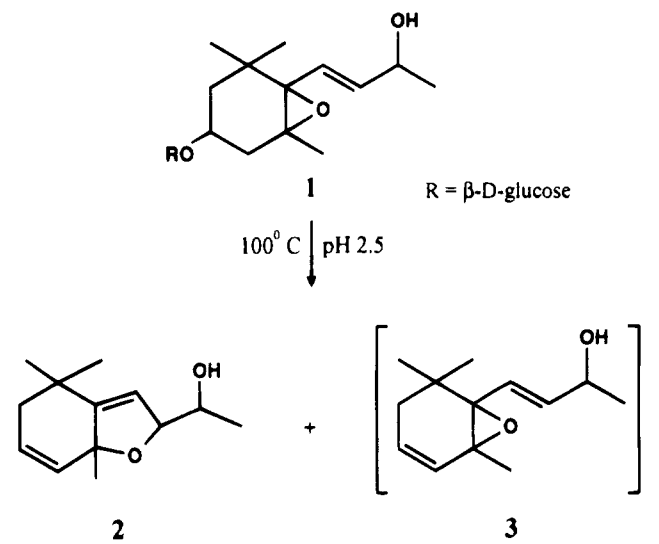

Figure 1. Acid-catalyzed degradation of 3-hydroxy-5,6-epoxy-β-ionol β-D-glucopyranoside (**1**) (SDE, pH 2.5) (Humpf and Schreier, 1992).

Synthesis of 2-(2-Butenylidene)-3,3-dimethyl-5-(2-oxopropyl)tetrahydrofuran. *(a) Reduction of 3,4-Didehydro-β-ionone.* A solution of 6 g of 3,4-didehydro-β-ionone, synthesized from β-ionone according to the method of Henbest et al. (1951), in 30 mL of dry diethyl ether was added to a suspension of 600 mg of $LiAlH_4$ in 50 mL of diethyl ether. After 2 h of stirring at room temperature 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): yield 4.2 g. Chromatographic and spectral data of 3,4-didehydro-β-ionol were identical with published data (Winterhalter and Schreier, 1988).

(b) Epoxidation of 3-Hydroxy-β-ionol. Preparation of 3-hydroxy-β-ionol from 3,4-didehydro-β-ionol was performed according to the method of Kienzle and Minder (1975): yield 1.3 g. For the epoxidation the method described by Kametani et al. (1978) was modified as follows: To a mixture of 1 g of 3-hydroxy-β-ionol, 30 mL of dichloromethane, and 20 mL of saturated sodium hydrogen carbonate solution was added 1 g of *m*-chloroperbenzoic acid in small portions under ice cooling.

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

δ	signal	J	atom
1.20[a]	3 H, s		H_3C-C3
1.25[a]	3 H, s		H_3C-C3
1.73	3 H, dd	6.7/1.6	H_3C1'
2.24	3 H, s		H_3C1''
2.61	1 H, dd	16.2/5.8	H C3″a
2.93	1 H, dd	16.2/6.9	H C3″b
4.65	1 H, m		H C5
4.85[b] (5.08)[c]	1 H, d	10.3	H C4′
5.46	1 H, dq	15.7/6.64	H C2′
6.25	1 H, ddq	15.7/10.3/1.73	H C3′

[a] Interchangeable values. [b] Signal isomer **4**. [c] Signal isomer **5**.

Table 2. ^{13}C NMR Spectral Data (50 MHz, $CDCl_3$) of Compounds 4 and 5 (δ Relative to TMS)

δ	DEPT	atom
18.21	CH_3	C1′
27.18	CH_3	C-C3[a]
28.4	CH_3	C-C3[a]
30.85	CH_3	C1″
41.17	C	C3
46.1	CH_2	C4
49.34	CH_2	C3″
75.09	CH	C5
95.01	CH	C4′
122.76	CH	C2′
125.55	CH	C3′
206.15	C	C2″

[a] Interchangeable values.

The reaction was controlled by TLC (detection by vanillin/sulfuric acid). The organic layer was separated and washed with water (2 × 20 mL), 2% sodium hydroxide solution (2 × 20 mL), and saturated sodium chloride solution (2 × 10 mL). After purification by flash chromatography on silica gel, 75 mg of pure 3-hydroxy-5,6-epoxy-β-ionol was obtained. The structure was confirmed by comparison with published data (Takagi et al., 1978).

(c) Acid Hydrolysis of 3-Hydroxy-5,6-epoxy-β-ionol. To a solution of 65 mg of 3-hydroxy-5,6-epoxy-β-ionol in 1 mL of methanol was added 150 mL of distilled water. SDE was carried out at pH 2.5 over 2 h. The organic phase was dried over anhydrous sodium sulfate and carefully concentrated by a Vigreux column (45 °C). Purification by HPLC yielded two diastereomers (yields 2.1 and 0.7 mg) exhibiting the following data.

Diastereomer **4**: R_i (DB-Wax) 2094; EI-MS (m/z, %) 209 (M + 1, 3), 208 (M^+, 17), 207 (4), 166 (4), 135 (5), 126 (4), 125 (38), 124 (7), 109 (10), 107 (9), 95 (3), 91 (6), 83 (10), 82 (22), 81 (13), 79 (7), 77 (4), 69 (6), 67 (4), 65 (3), 55 (23), 54 (10), 53 (10), 44 (6), 43 (100), 41 (23); ESI-MS (m/z, %) 209 (M + 1); FTIR (vapor phase, ν, cm^{-1}) 3035, 2968, 2935, 1732, 1678, 1458, 1358, 1323, 1254, 1065, 964, 914, 825. NMR data cf. Tables 1 and 2.

Diastereomer **5**: R_i (DB-Wax) 2099; EI-MS (m/z, %) 209 (M + 1, 2), 208 (M^+, 13), 207 (3), 166 (4), 135 (4), 126 (4), 125 (33), 124 (6), 109 (9), 107 (9), 95 (3), 91 (5), 83 (10), 82 (21), 81 (13), 79 (7), 77 (4), 69 (6), 67 (4), 65 (4), 55 (23), 54 (10), 53 (10), 44 (14), 43 (100), 41 (23); ESI-MS (m/z, %) 209 (M + 1); FTIR (vapor phase, ν, cm^{-1}) 3039, 2967, 2931, 1732, 1670, 1620, 1458, 1369, 1319, 1203, 1076, 968, 918, 810, 721. NMR data cf. Tables 1 and 2.

Capillary Gas Chromatography (HRGC). *(a)* 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 carrier gas was 2.0 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 220 and 260 °C respectively.

(b) A Hewlett-Packard 5890A gas chromatograph with FID equipped with a J&W fused silica DB-5 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 from 60 to 300 °C at 5 °C/min. The flow rate for carrier gas was 1.6 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 250 °C.

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 44 mass spectrometer with PCDS data system. The same type of columns and the same temperature programs as mentioned above for HRGC were used: 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 with 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 from 70 to 240 °C at 7 °C/min. The flow rate for 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). Split injection (1:20) was employed. The temperature was programmed from 110 to 240 °C at 2 °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: temperature program, 40 min isothermal at 100 °C, increased from 100 to 200 °C at 2 °C/min. The following cuts were used: diastereomer **4**, 41.2–41.5 min; diastereomer **5**, 41.6–41.9 min. Helium was used as 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 each 30 mL/min hydrogen and 300 mL/min air. The coupling of the MDGC system with a Finnigan MAT 44 mass spectrometer was achieved by a variable effluent splitter (Siemens) working as a second "live" switching device. The temperature of the ion source and of the transfer line was 200 °C. The electron energy was 70 eV and the cathodic current 0.7 mA.

Electrospray Interface Mass Spectrometry (ESI-MS). ESI-MS was performed with a Finnigan TSQ 7000 triple-stage quadrupole mass spectrometer equipped with a Finnigan electrospray interface (ESI) at atmospheric pressure and room temperature employing mass range from 150 to 500. The potential of the capillary was 5000 kV.

Nuclear Magnetic Resonance (NMR). ^{1}H and ^{13}C NMR spectra were recorded on a Fourier transform Bruker AC 200 spectrometer 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.

RESULTS AND DISCUSSION

In the course of our ongoing studies on C_{13} norisoprenoid flavor precursors, glycosidic extracts were examined from leaves of stinging nettle, sloe tree, straw-

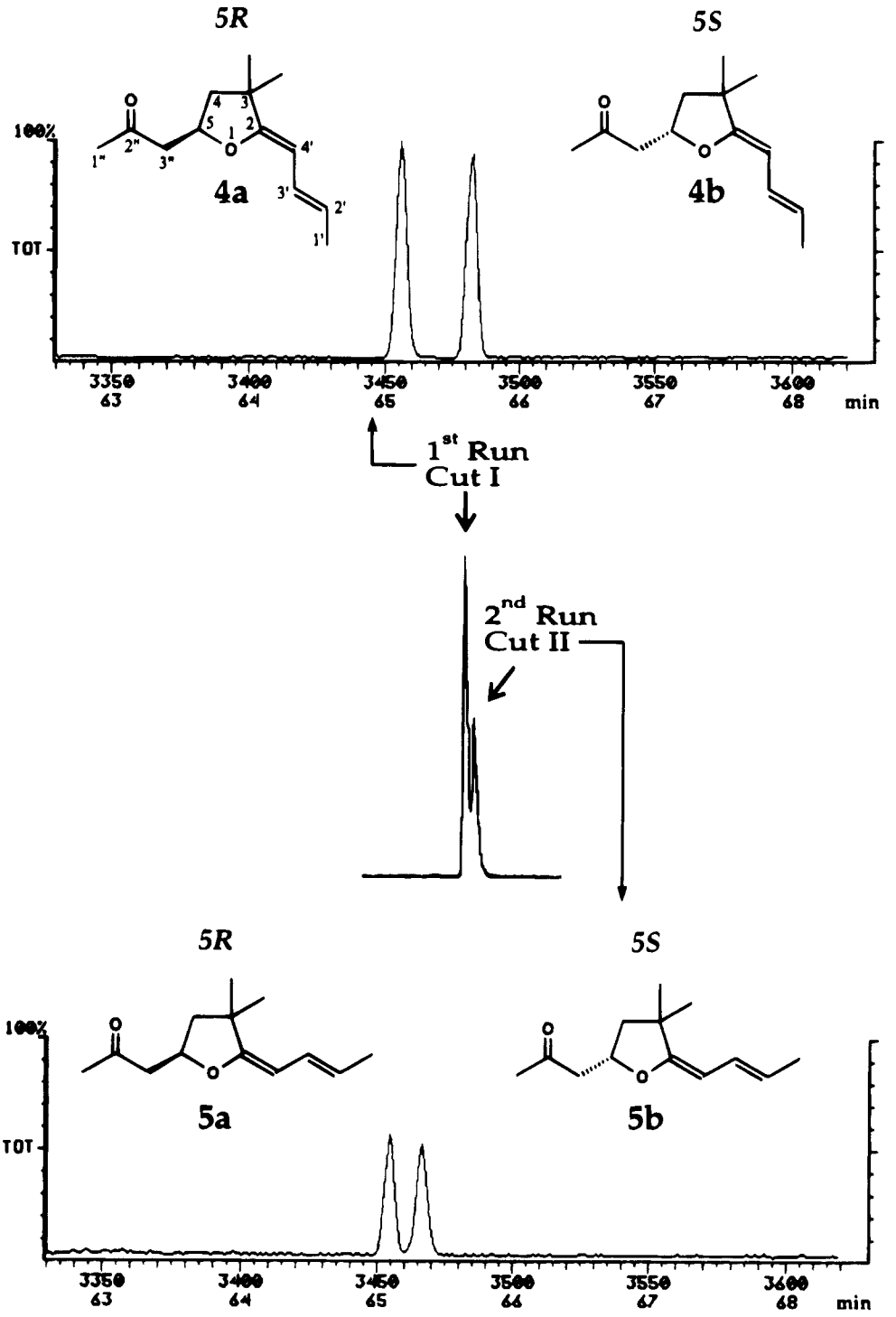

Figure 2. MDGC–MS separation of the four stereoisomeres of synthetic 2-(2-butenylidene)-3,3-dimethyl-5-(2-oxopropyl)-tetrahydrofuran **4a/4b** and **5a/5b**; preseparation of diastereomeric pairs **4a/4b** and **5a/5b** and two-step chiral separation (for MDGC conditions see Experimental Procedures).

berry, vine (Riesling), and sweet silique (*Bunias orientalis* L.). After acid hydrolyses using SDE at pH 2.5, two major products were observed that showed identical chromatographic and mass spectral data with those reported for the tentatively identified degradation product **3** of 3-hydroxy-5,6-epoxy-β-ionol (Humpf and Schreier, 1992). Since the absolute concentrations were too small for NMR experiments, additional structural information was obtained by on-line coupled HRGC–FTIR spectroscopy. The vapor-phase FTIR data recorded for the degradation products showed *inter alia* a strong carbonyl band at 1732 cm^{-1} and no signal for hydroxy group. This contradicted the previously suggested structure **3** and prompted us to finally verify the correct chemical structure. Thus, by degradation of synthetic 3-hydroxy-5,6-epoxy-β-ionol the unknown degradation products were produced in milligram scale and purified by HPLC. From the ^1H and ^{13}C NMR spectral data (cf. Tables 1 and 2) the presence of a tetrahydrofuran ring substituted with a 2-oxopropyl group (MeCO at δ 2.24 and ABX system at δ 2.61, 2.93 and 4.65) was apparent. The substituents included two geminal methyl groups (δ 1.20 and 1.25) as well as a butenylidene chain with an *E*-configured double bond ($J = 15.7$ Hz). From these data the degradation product was identified as 2-(2-butenylidene)-3,3-dimethyl-5-(2-oxopropyl)tetrahydrofuran (**4/5**). The data obtained by ESI-MS supported the

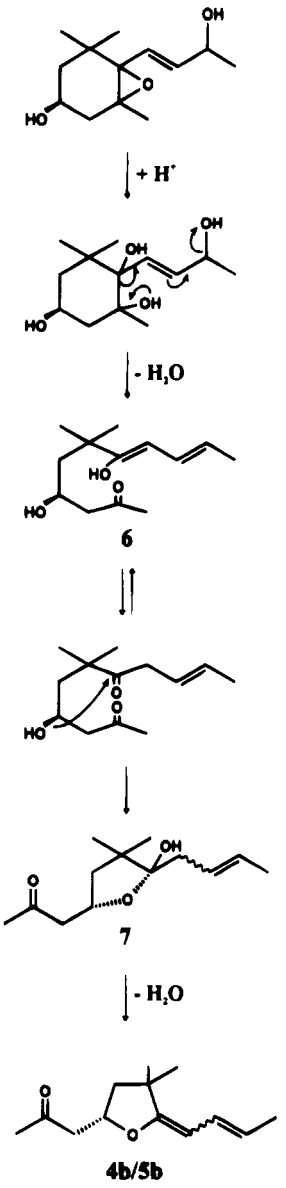

Figure 3. Proposed pathway for selective formation of **4b/5b** by acid-catalyzed degradation of **1**.

structure of **4/5**; only one peak was registered at m/z 209 (M + 1).

As elaborated by NOE experiments the diastereomers **4** and **5** differ in the configuration of the butenylidene chain. To the major isomer **4** irradiation of the methyl groups of C3 resulted in a NOE at the methine proton at C4′, whereas in the case of the minor isomer **5** the C4′ proton was not affected. The ratio of the isomers was 3:1 (**4**:**5**). Purified isomers reisomerized within 24 h, yielding the same ratio.

Using on-line coupled multidimensional gas chromatography–mass spectrometry (MDGC–MS) (Bernreuther and Schreier, 1991), enantiodifferentiation of **4a/4b** and **5a/5b** (Figure 2) in a number of natural sources was carried out. Assuming the configuration at C5 of 2-(2-butenylidene)-3,3-dimethyl-5-(2-oxopropyl)tetrahydrofuran remains *S* as it is at C3 in the precursor 3-hydroxy-5,6-epoxy-β-ionol according to natural 5,6-epoxycarotenoids (Eugster and Märki-Fischer, 1991), only the 5*S* enantiomers (**4b/5b**) were found in the plant material under study. Thus, for the degradation of natural 3-hydroxy-5,6-epoxy-β-ionol a pathway is postulated as shown in Figure 3. The first step is considered to be an acid-catalyzed ring opening leading to keto–diol **6**. Keto–enol tautomery and formation of hemiketal **7** can be regarded as the following steps. Finally, **4b/5b** are suggested to be formed by dehydration of **7**.

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