Tetrahedron: Asymmetry Vol. 2, No. 6, pp. 471-479, 1991
Printed in Great Britain

0957-4166/91 $3.00+.00
Pergamon Press plc

Stereoselective formation of metabolites from 2-methyl-2-hepten-6-one by Botrytis cinerea

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(*Received* 20 *May* 1991)

Abstract. Controlled conversion of 2-methyl-2-hepten-6-one (**1**) by *Botrytis cinerea* resulted in the formation of (*S*)-(+)-2-methyl-2-hepten-6-ol (sulcatol) (**2**) of 90% ee. In addition, (2*R*,5*R*)- (**3a**), (2*S*,5*S*)- (**3a'**), (2*R*,5*S*)- (**3b**) and (2*S*,5*R*)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran (**3b'**), (3*S*,6*R*)- (**4a**), (3*R*,6*S*)- (**4a'**), (3*R*,6*R*)- (**4b**) and (3*S*,6*S*)-tetrahydro-2,2,6-trimethyl-2*H*-pyran-3-ol (**4b'**) as well as the diastereomeric dihydro-3-hydroxy-5-methyl-5-(2'-methyl-pent-2-en-5'-yl)-2(3*H*)-furanones (**5a/b**) were found as biotransformations products of **1**. Chiral analysis carried out by on-line multidimensional gas chromatography-mass spectrometry (MDGC-MS) revealed the following ee values: 46 % (**3a**); 80 % (**3b**); 56 % (**4a**); and 60 % (**4b'**). As confirmed by nOe experiments, **5a/b** were formed in a ratio of 6:1.

Introduction

In bioorganic chemistry, microbial conversions have been acknowledged as a powerful alternative for the selective production of chemicals[1]. In flavour chemistry, the usefulness of microbial transformations for the preparation of various aroma compounds has been demonstrated, in particular, at the example of monoterpenes[2] and norisoprenoids[3]. As an

extension of our studies carried out on the microbial conversion of isoprenoids by *Botrytis cinerea*, a fungus well-known in winemaking, the conversion of 2-methyl-2-hepten-6-one (1) by this microorganism was investigated. Ketone 1 plays an important industrial role as starting material for the widely used monoterpene alcohol linalool[7] and has previously been employed as an educt for biological conversions[8]. This paper mainly concerns the identification and the stereochemistry of bioconversion products of 1 formed by *B. cinerea*.

Experimental

Chemicals. All commercial chemicals used were of analytical grade quality. Solvents were redistilled before use.

Incubation conditions. Sterilized grape must (sugar 193 g/l; acid, 8.0 g/l) (700 ml) was inoculated with a pure *B.cinerea* strain (5882/1; Bayer. Landesanstalt für Weinbau und Gartenbau, Würzburg) and incubated with 1 (Aldrich) (35 mg) at 25°C for 14 days. The mycelium was removed by filtration and the solutions analyzed by HRGC, HRGC-MS, and HRGC-FTIR after extractive sample preparation[6]. In the same manner, blank tests both without *B.c.* and 1 were carried out. In additional experiments, the time course of bioconversion of 1 was studied by variation of incubation time from 1 to 14 days.

Separation of bioconversion products. After addition of an internal standard (0.4 mg/l 2-methyl-1-pentanol) to the above mentioned filtrate, solvent extraction was carried out continuously over 24 h at 40°C using n-pentane-CH_2Cl_2 (2+1). The organic phase was dried over anhydrous Na_2SO_4 and carefully concentrated to 1 ml using a Vigreux column (45°C) for subsequent HRGC, HRGC-MS, and HRGC-FTIR analysis.

Isolation of dihydro-3-hydroxy-5-methyl-5-(2'-methyl-pent-2-en-5'-yl)-2(3H)-furanone (5a/b). Pure 5a/b (30 mg) was isolated from extracts obtained in the above described manner from 20 *B.c.* incubations by preparative TLC (Kieselgel 60 PF_{254}; df = 1.25 mm; n-pentane-diethyl ether [2+1]; Rf = 0.11). R_i 2602. MS (*m/z* %) 43 (100) 41 (66) 68 (63) 69 (62) 55 (24) 67 (22) 109 (18) 110 (16) 198 (4). Vapor phase FTIR (ν, cm^{-1})

3592, 1799. ^1H NMR (200 MHz, $CDCl_3$, J in Hz, ppm) δ (TMS) 1.49 (s, 3H); 1.61 (s, 3H); 1.68 (s, 3H); 2.00 (dd, J_1= 13.1, J_2= 9.2, 1H); 2.06 (m, 2H); 2.57 (dd, J_1= 13.1, J_2= 8.8, 1H); 4.60 (t; J =9.0, 1H); 5.06 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 17.7 (C-1) 22.8 (C-5) 25.6 (C-2) 26.9 (C-8) 40.7/41.2 (C-6/C-9) 68.7 (C-10) 84.9 (C-7) 122.7 (C-4) 132.8 (C-3) 177.2 (C-11).

HRGC and on-line coupled HRGC techniques. HRGC, HRGC-MS, and HRGC-FTIR analyses were carried out on a J & W DB-Wax capillary column (30 m x 0.259 mm i.d.; df = 0.25 µm) as recently described in detail[6]. Results of analyses were verified by comparison of HRGC retention (R_t), mass spectral, and FTIR vapor phase spectra with those of authentic reference substances.

On-line multidimensional gas chromatography-mass spectrometry. MDGC-MS using the combination of an achiral and chiral column (DB-5/CP-Cyclodextrine-2,3,6-M-19) was performed as recently described[9]. Identities and order of elution were determined by means of authentic racemic and enantiomerically pure reference substances.

Chiral evaluation of 2-methyl-2-hepten-6-ol (sulcatol) (2). Enantiodifferentiation of microbially produced 2 was performed by HRGC on a J & W DB-5 capillary column (30 m x 0.25 mm i.d.; df = 0.25 µm) after derivatization with (R)-(+)-1-phenylethylisocyanate (PEIC) as previously described[10].

NMR. NMR spectra were recorded on Bruker AC 200 and WM 400 spectrometers using $CDCl_3$ and benzene-D_6 as solvents and Me_4Si as internal standard. NOe experiments were carried out at ambient temperature by irradiation of the different proton chemical shift frequencies for 8 s. Before changing the irradiation frequency, there was a relaxation delay of 15 s.

Reference compounds. A mixture of diastereomers 3 and 4 was obtained by *p*-chloroperbenzoic acid oxidation of 2 according to the method of Mihailovic and Marinkovic[11]. MS- and IR data were in agreement with previously published data[12-14]. Optically enriched (*S*)-(+)-2 was prepared by porcine pancreas lipase catalyzed kinetic resolution of

(*R*,*S*)-2 as described[15].

Results and discussion

Quantitative HRGC revealed the formation of 24 % of volatile products formed from 1 by *B. cinerea* In blank tests carried out without addition of 1 these substances were not detectable. As confirmed by comparison of R_i, mass spectral and vapor phase FTIR data with those of an authentic reference compound, 60 % of total volatiles consisted of 2-methyl-2-hepten-6-ol (sulcatol) (2), the well-known pheromone of *Gnathotrichus sulcatus* and *G. retusus*[16,17]. Chiral evaluation of microbially formed 2 carried out by HRGC after derivatization with (R)-(+)-PEIC revealed the occurrence of (*S*)-(+)-sulcatol of 90 % ee. In accordance with bioconversion studies of 1 previously performed with *Geotrichum candidum*[8], a strong dependance of ee values on the duration of cultivation was observed. Whereas in an early stage of cultivation a racemic mixture of 2 was found, incubation of 14 days gave the above mentioned ee value. Changing ee values of product 2 could result from differing stabilities of (*R*) and (*S*) selective hydrogenases[18] and/or differing metabolic stabilities of the alcohols in *B. cinerea*.

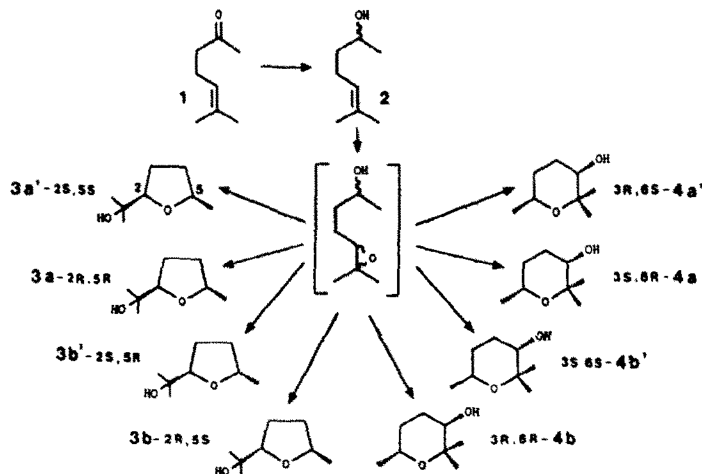

Figure 1. Structures of biotransformation products formed from 1 by *Botrytis cinerea*. **2** sulcatol; **3a** (2*R*,5*R*)-; **3a'** (2*S*,5*S*)-; **3b** (2*R*,5*S*)- **3b'** (2*S*,5*R*)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran; **4a** (3*S*,6*R*)-; **4a'** (3*R*,6*S*)-; **4b** (3*R*,6*R*)-; **4b'** (3*S*,6*S*)-tetrahydro-2,2,6-trimethyl-2*H*-pyran-3-ol. Formation of 3/4 from 2 *via* an epoxy derivative postulated according to lit.[18].

Together with 2 five additional biotransformation products were detected in higher amounts. Four of these products (14 % of total volatiles) exhibited mass spectra that were identical with those previously published for *cis*- and *trans*-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran (pityol) (3) as well as *cis*- and *trans*-tetrahydro-2,2,6-trimethyl-2*H*-pyran-3-ol (4)[12-14] (Fig. 1). Structural conformation was ob-

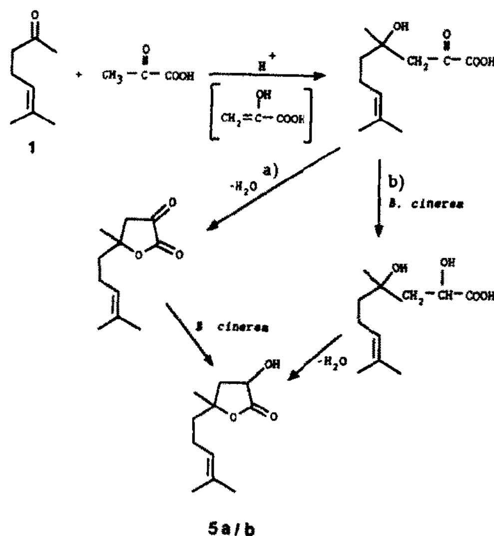

Figure 2. Potential pathway of the formation of diastereomeric dihydro-3-hydroxy-5-methyl-5-(2'-methyl-pent-2-en-5'-yl)-2(3*H*)-furanones (**5a/b**) by *Botrytis cinerea*

tained by synthesis of an authentic mixture of 3/4 and subsequent chromatographic and spectroscopic comparison of the data with those of the bioconversion products. Compounds 3/4 may be regarded as secondary products formed from 2 *via* a postulated epoxide (Fig. 1). A similar pathway has previously been reported for the formation of the structurally related linalool oxides[19].

Chiral analysis using MDGC-MS allowed the separation of all the stereoisomers of 3/4. Quantitative evaluation revealed the following data (total amount of 3/4 = 100 %): **3a** (13 %); **3a'** (5 %); **3b** (13 %); **3b'** (2 %); **4a** (8 %); **4a'** (2 %); **4b** (12 %); **4b'** (45 %). The following ee values

were determined: 46 % (**3a**); 80 % (**3b**); 56 % (**4a**); 60 % (**4b'**). From these enantiomers 3b has been found previously as a component of a male secreted aggregation pheromone of the spruce bark beetle, *Pityophtorus pityographus*[20]. Both 3a and **4a'** have been isolated as volatile compounds from the elm bark beetle, *Pteleobius vittatus*[21].

As to the on-line recorded spectroscopic information (MS, M^+ 198; vapour phase FTIR, O-H and C=O) of the remaining bioconversion product 5 (16 % of total volatiles; an additional number of minor products has been described elsewhere[22]), literature data were not available. After isolation of a sufficient amount of pure 5 by preparative TLC, structural elucidation was achieved by ^1H and ^{13}C NMR spectroscopy. From NMR data previously published for 1[23], dihydro-3-hydroxy-3,5,5-trimethyl-2(3*H*)-furanone[24], and (3S,5R)-5-[3'-(furanyl-3''-yl)-3'-oxopropyl]-3-hydroxy-5-methyldihydrofuran-2(3*H*)-one[25] 5 was assigned to be dihydro-3-hydroxy-5-methyl-5-(2'-methyl-pent-2-en-5'yl)-2(3*H*)-furanone (Fig. 2).

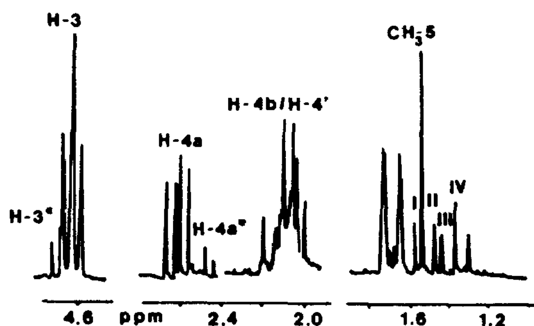

Figure 3. Part of ^1H NMR spectrum (200 MHz; $CDCl_3$) of the mixture of diastereomers 5a/b.

From a detailed study of ^1H NMR data of 5 (Fig. 3) the presence of a diastereomeric pair 5a/b (6:1) was evaluated. The assignment of the diastereomers was achieved by nOe experiments. As to the diastereomer present in higher amounts (**5a**) (Table 1), irradiation of CH_3-5 did not result in a nOe for the H-3 proton, indicating the *trans*-configuration. In addition, only a nOe for H-4b, but not for the H-4a proton was observed. Thus, axial position of CH_3-5 and the H-4a proton is required. The assigned conformation A shown in Fig. 4 was confirmed by irradia-

tion of H-4a and H-4b leading in both cases in a nOe of proton H-3 (equatorial position).

As to the diastereomer present in lower amounts (**5b**) irradiation of H-4b* was not possible due to overlapping with H-4b. In addition, CH_3-5* could not be assigned exactly. Thus, H-4a*, H-3*, and the sig-

Table 1 Results of nOe experiments carried out with the major diastereomeric product 5a (values in %).

irradiated: H-3	H-4a	H-4b	CH_3-5	observed
	6	1		H-3
1		15		H-4a
	18		4	H-4b
		1		CH_3-5

nals marked as I-IV in Fig. 3 were irradiated (Table 2). The assigned *cis*-configuration for 5b was confirmed by irradiation of signal III (potentially CH_3-5*) resulting in a nOe for the H-3* proton. Thus, *cis*-configuration with 1,3-diaxial positions of H-3* and CH_3-5* (conformation C in Fig. 4) is required.

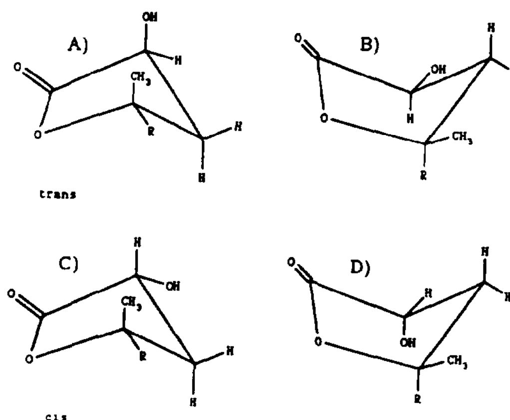

Figure 4. Conformations of *trans*- (A and B) as well as *cis*-dihydro-3-hydroxy-5-methyl-5-(2'-methyl-pent-2-en-5'-yl)-2(3*H*)-furanone (C and D).

As to the potential biogenetic formation of the newly described **5a/b**, aldol addition of **1** with pyruvate originating from the nutrient medium, followed by (i) lactonisation and reduction (a in Fig. 2), or (ii)

Table 2 Results of nOe experiments carried out with the minor diastereomeric product **5b** (values in %; [+] = quantitative evaluation not possible)

irradiated: H-3*	H-4a*	I	II	III	IV	observed
	3			4		H-3*
+				1		H-4a*
						I
						II
+	1					III
						IV

reduction and lactonisation (b in Fig. 2) is postulated.

Acknowledgments. Dr.I. Benda, Bayerische Landesanstalt für Weinbau und Gartenbau, Würzburg, is thanked for providing *B c* and various support during the cultivation experiments. Dr. D. Scheutzow's and E. Ruckdeschel's (Institut für Organische Chemie) NMR analyses (400 MHz) are gratefully acknowledged. The authors are indebted to E. Schindler and T. Schmitt for their skillful technical assistance.

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