Biotransformation of β-Damascone by *Botrytis cinerea*

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Biotransformation of β-damascone (**1**) was studied with four strains of *Botrytis cinerea* using grape must (A) and a mixture of A with synthetic medium (B). Whereas in A 4-hydroxy-β-damascone (**2**), 3-hydroxy-β-damascone (**3**), and 2-hydroxy-β-damascone (**4**) were found as volatile bioconversion products, in B additionally 4-oxo-β-damascone (**5**) was detected. Furthermore, 9-hydroxy-8,9-dihydro-β-damascone (**6**) was identified as chemically formed by-product. Quantitatively, the results were strongly dependent on the strain and medium used. Incubation of **1** with two *B. c.* strains in medium B led to an almost selective formation (93 and 95%) of **2** and the highest yield of products (30 and 66%). The bioconversion products were identified by capillary gas chromatography (HRGC) and coupled HRGC techniques, *i.e.* – mass spectrometry (HRGC-MS) and – Fourier transform infrared spectroscopy (HRGC-FTIR) after extractive sample preparation.

Introduction

Due to their attractive sensory properties, there is a considerable industrial interest in C_{13} norisoprenoids and their derivatives [1]. The usefulness of microbial conversion for the preparation of oxygenated C_{13}-cyclic terpenoid ketones, such as ionone and damascone derivatives, has previously been demonstrated [2–4]. This paper concerns the microbial conversion of β-damascone (**1**) by *Botrytis cinerea*. In our previous work, *B. cinerea* has been already found to be an useful microorganism for the biotransformation of monoterpene alcohols [5].

Materials and Methods

Botrytis cinerea strains

The *B. c.* strains 5882/1, 5899/4, 5901/2, and 5909/1 under study were obtained from the collection of the Bayerische Landesanstalt für Weinbau und Gartenbau, Würzburg. From the original cultures, a part was transferred to malt agar slants and incubated at 25 °C for 7 days.

Media and incubation conditions

A. Grape must. The grape must (*cv.* Müller-Thurgau) used showed sugar and acid contents of 193 g/l and 8.0 g/l (pH 3.2), respectively.

B. Grape must/synthetic medium. To 700 ml of a synthetic medium containing (per l) $NaNO_3$ 3 g; K_2HPO_4 1 g; $MgSO_4 \times 7H_2O$ 0.5 g; KCl 0.5 g, and $FeSO_4$ 0.01 g (pH adjusted to 3.5 using 1 N HCl) 1 ml of grape must was added [5].

The medium (A, B) (700 ml) was filled into 1 l Erlenmeyer flasks and sterilized (30 min at 110 °C). After addition of 35 mg β-damascone (**1**) (in 1 ml ethanol) each flask was inoculated with a pure *B. c.* strain and incubated at 25 °C for 14 days. The mycelium was removed by filtration and the solutions analyzed by capillary gas chromatography (HRGC), capillary gas chromatography-mass spectrometry (HRGC-MS) and, in part, capillary gas chromatography-Fourier transform infrared spectroscopy (HRGC-FTIR) after extractive sample preparation. In the same manner, blank tests without *B. c.* incubation and without **1** were carried out.

Isolation of bioconversion products

After addition of 0.4 mg/l 2-methyl-1-pentanol (internal standard) to the above mentioned filtrates, continuous solvent extraction was carried out over 24 h using pentane–dichloromethane

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(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, in part, HRGC-FTIR analysis.

Capillary gas chromatography (HRGC)

A Carlo Erba Fractovap 4100 gas chromatograph with FID equipped with a J & W fused silica DB-Wax capillary column (30 m × 0.25 mm i.d.; d_f = 0.25 µm) was used. Split injection (1:20) was employed. The temperature program was isothermal for 3 min at 50 °C, then to 240 °C at 4 °C/min. The flow rates for the carrier gas were 1.6 ml/min He, for the make-up gas 30 ml/min N_2, and for the detector gases 30 ml/min H_2 and 300 ml/min air, respectively. The temperatures of injector and detector were both kept at 250 °C.

Results of qualitative analyses were verified by comparison of HRGC retention (R_i), mass spectral and vapour phase FTIR spectra with those of authentic reference compounds. Quantitative HRGC determinations were carried out by standard controlled calculations using a Shimadzu CR-6-A computing integrator without consideration of calibration factors (F = 1.0).

Capillary gas chromatography-mass spectrometry (HRGC-MS)

A Varian Aerograph 1440 apparatus directly coupled to a Finnigan MAT 44 mass spectrometer with PCDS data system was used. The same column as described for HRGC analysis and split injection (1:50) were used. The conditions were as follows: temperature program 50–240 °C at 4 °C/min; carrier gas 1.8 ml/min He; temperature of the ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA.

Capillary gas chromatography – FTIR spectroscopy (HRGC-FTIR)

A Nicolet 20 SXB system interfaced by a DANI 6500 gas chromatograph equipped with FID was used. A J & W DB-Wax fused silica capillary column (30 m × 0.32 mm i.d.; d_f = 0.25 µm) was employed. Total sample injection mode using PTV (40–240 °C, 0.1 min) was performed. The temperature program was 50 °C to 240 °C at 4 °C/min. Light pipe and transfer line were held at 240 °C. He (2.0 ml/min) was employed as carrier gas. Vapour phase FTIR spectra were recorded from 400–4000 cm^{-1} with a resolution of 8 cm^{-1}.

Reference compounds

4-Hydroxy-β-damascone (**2**) and 4-oxo-β-damascone (**5**). Syntheses were accomplished according to the method of Helmlinger and Krasnobajew [6]. **2:** R_i: 2451. MS (*m/z*, %): 69 (100), 43 (90), 41 (84), 139 (73), 55 (26), 193 (25), 208 (16), 109 (15). FTIR (vapour phase, ν, cm^{-1}): not available due to decomposition in the light-pipe. **5:** R_i: 2247. MS (*m/z*, %): 69 (100), 41 (43), 138 (12), 206 (12), 55 (10), 53 (10), 191 (8), 43 (9), 79 (7). FTIR (vapour phase, ν, cm^{-1}): 3030, 2970, 2934, 2871, 1696, 1666, 1629, 1232, 1163, 972.

9-Hydroxy-8,9-dihydro-β-damascone (**6**). A solution of 5 g **1** in 200 ml of dist. water (adjusted to pH 1 with 2 N HCl) was stirred at 25 °C for 14 days. After extraction with pentane–dichloromethane (2:1) LC purification on silica gel (Kieselgel 60 Merck, 0.2–0.5 mm; *n*-pentane–diethyl ether [5:1] to remove **1;** *n*-pentane–diethyl ether [3:1]) yielded 350 mg of pure **6.** R_i: 2100. MS (*m/z*, %): 151 (100), 123 (76), 43 (72), 81 (72), 41 (49), 45 (30), 55 (21), 135 (15), 109 (16), 69 (16), 210 (10). FTIR (vapour phase, ν, cm^{-1}): 3579, 2971, 2944, 2880, 1691.

2- (**4**) and 3-hydroxy-β-damascone (**3**) were available from our own laboratory collection of flavour substances. **3:** R_i: 2510. MS (*m/z*, %): 69 (100), 41 (81), 121 (39), 175 (33), 193 (30), 208 (30), 43 (23), 55 (20), 147 (18), 149 (16). **4:** R_i: 2460. MS (*m/z*, %): 69 (100), 121 (63), 41 (60), 149 (46), 165 (37), 175 (24), 93 (23), 208 (22), 79 (22), 55 (22).

Results and Discussion

In grape must (A) and in a 1:700 mixture of grape must/synthetic medium (B) [5], β-damascone (**1**) was metabolized by the four *Botrytis cinerea* strains used. In both fermentation media the ring hydroxylated products **2–4** shown in Fig. 1 were identified after extractive sample preparation using capillary gas chromatography (HRGC), capillary gas chromatography-mass spectrometry (HRGC-MS), and, in part, capillary gas chromatography-FTIR spectroscopy (HRGC-FTIR). Using medium B, 4-oxo-β-damascone (**5**) was detected as additional bioconversion product of **1.** Moreover, in A and B 9-hydroxy-8,9-dihydro-β-damascone (**6**) was detected. However, as shown

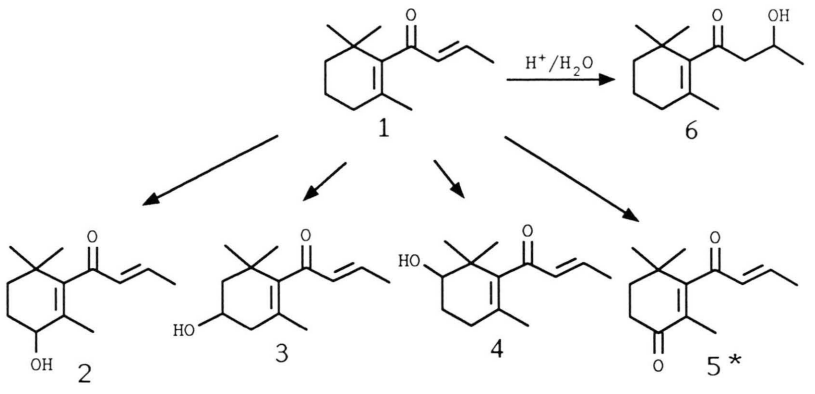

Fig. 1. Structures of bioconversion products formed from β-damascone (**1**) by *Botrytis cinerea* strains. (**2**) 4-hydroxy-β-damascone; (**3**) 3-hydroxy-β-damascone; (**4**) 2-hydroxy-β-damascone; (**5**) 4-oxo-β-damascone. Chemically formed: 9-hydroxy-8,9-dihydro-β-damascone (**6**). * Only detected in medium B.

Table I. Remaining educt (mg/l), yields of products (mg/l) and distribution (%) of bioconversion products formed from β-damascone by *Botrytis cinerea* strains in grape must (A) and a 1:700 mixture of grape must/synthetic medium (B) [5].

Strain	Medium	**1** [mg/l]	Yield [mg/l]	Percentage of yield for products **2**	**3**	**4**	**5**
5882/1	A	4.6	2.6	59	16	25	–
	B	3.1	10.2	78	10	9	3
5899/4	A	1.9	6.6	80	9	11	–
	B	0.6	15.0	93	2	1	4
5901/2	A	2.8	2.2	70	18	12	–
	B	3.9	33.3	95	1	2	2
5909/1	A	1.7	1.6	62	18	20	–
	B	1.9	12.2	82	8	8	2

from blank tests as well as by model experiments carried out with **1** in water at pH 1, this compound was formed chemically by hydratation of the 8,9-double bond of **1.**

As shown from Table I, **1** was not completely metabolized by *B. c.* In both media (A, B) predominant conversion of **1** to 4-hydroxy-β-damascone (**2**) occurred. The highest and most selective transformations were observed in medium B. Quantitatively, the results were strongly dependent on the strains used. With strains 5901/2 and 5899/4 selective formation (93 and 95%, respectively) of **2** was observed. The highest yields of bioconversion products were also obtained with these strains (30 and 66%, respectively).

Whereas the prevailing hydroxylation in the allylic position of the ring double bond of **1** (Fig. 1) has been previously observed using various fungi [6], compounds **3** and **5** have not been described as bioconversion products of **1** as yet.

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