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BIOTRANSFORMATION OF (+)- AND (−)-CAMPHORQUINONES BY FUNGI

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Key Word Index—*Aspergillus niger*; *Fusarium solani*; *Glomerella cingulata*; *Mucor mucedo*; *Rhizoctonia solani*; biotransformation; (+)-camphorquinone; (−)-camphorquinone; 2-*exo*-hydroxyepicamphor; 2-*endo*-hydroxyepicamphor; 3-*exo*-hydroxycamphor; 3-*endo*-hydroxycamphor; regioselectivity; stereoselectivity.

Abstract—(+)- and (−)-Camphorquinones were readily reduced by various fungi. The reduction of (−)-camphorquinone by *Aspergillus niger* produced mainly (+)-2*R*-*endo*-hydroxyepicamphor with high stereoselectivity whereas reduction of (+)-camphorquinone by *Glomerella cingulata* and *Mucor mucedo* afforded (−)-3*S*-*exo*-hydroxycamphor with stereoselectivity.

INTRODUCTION

We have shown that the monoterpene ketones fenchone [1], pulegone [2, 3] and piperitone [4, 5] are oxidized to new ketoalcohols by various fungi. The biotransformation of camphorquinone by microorganisms has been the subject of a few studies. Thus the biotransformation of (+)-camphorquinone (**1a**) by *Absidia orchidis* yielded (+)-3*S*-*exo*-hydroxycamphor (**1a-3**) as the sole product [6]. The biotransformation of (±)-camphorquinone (**1**) by *Acinetobacter* sp. produced (+)-2*R*-*exo*-hydroxyepicamphor (**1a-1**) from (**1a**) and (+)-3*R*-*exo*-hydroxycamphor (**1b-3**) from the (−)-form (**1b**) [7].

(+)-Camphorquinone (**1a**) was reduced by baker's yeast to give (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) as the major product [8]. In an attempt to further characterize the stereoselectivity and possible regioselectivity of such a potentially useful reduction process, the biotransformation of the bicyclic 2,3-dionetype monoterpene (+)- and (−)-camphorquinones (**1a** and **b**) by *Aspergillus niger*, *Fusarium solani*, *Glomerella cingulata*, *Mucor mucedo* and *Rhizoctonia solani* has been investigated.

RESULTS AND DISCUSSION

(+)-Camphorquinone (**1a**) and (−)-camphorquinone (**1b**) were readily reduced by *A. niger*, *F. solani*, *G. cingulata*, *M. mucedo* and *R. solani* (Figs 1-5). (−)-Camphorquinone (**1b**) afforded a mixture of diastereomeric isomers of four α-ketoalcohols: (−)-(2*S*)-2-*exo*-hydroxy-1,7,7-trimethylbicyclo[2.2.1]heptan-3-one [(−)-2*S*-*exo*-hydroxyepicamphor, **1b-1**], (+)-(2*R*)-2-*endo*-hydroxy-1,7,7-trimethylbicyclo[2.2.1]heptan-3-one [(+)-2*R*-*endo*-hydroxyepicamphor, **1b-2**], (+)-(3*R*)-3-*exo*-hydroxy-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one [(+)-3*R*-*exo*-hydroxycamphor, **1b-3**] and (−)-(3*S*)-3-*endo*-hydroxy-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one [(−)-3*S*-*endo*-hydroxycamphor, **1b-4**], whereas (+)-camphorquinone (**1a**) afforded a mixture of the corresponding enantiomeric isomers of three α-ketoalcohols: (+)-2*R*-*exo*-hydroxyepicamphor (**1a-1**), (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) and (+)-3*R*-*endo*-hydroxycamphor (**1a-4**). (−)-2*S*-*endo*-Hydroxyepicamphor (**1a-2**) was not formed from the (+)-form (**1a**) by any of the five fungi used in this study (Schemes 1 and 2).

When **1a** was added to cultures of *A. niger*, it was easily transformed to give (+)-2*R*-*exo*-hydroxyepicamphor (**1a-1**, 50%) as the major product after a 24 hr incubation period (Fig. 1). Compound **1b** was transformed to (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**, 80%) as the major product. In case of *F. solani*, **1a** and **b** had completely disappeared after a 72 hr incubation period (Fig. 2). Compound **1a** was transformed to (+)-2*R*-*exo*-hydroxycamphor (**1a-1**), (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) and (+)-3*R*-*endo*-hydroxycamphor (**1a-4**) in equal amounts. Compound **1b** was converted to (+)-3*R*-*exo*-hydroxycamphor (**1b-3**, 60%) as the major product after 72 hr. Compound **1a** rapidly disappeared from cultures of *G. cingulata* and was undetectable after a 9 hr incubation period (Fig. 3). It was transformed to (−)-3*S*-*exo*-hydroxycamphor (**1a-3**, 70%). Compound **1b** was transformed to (+)-3*R*-*exo*-hydroxycamphor (**1b-3**, 50%) as

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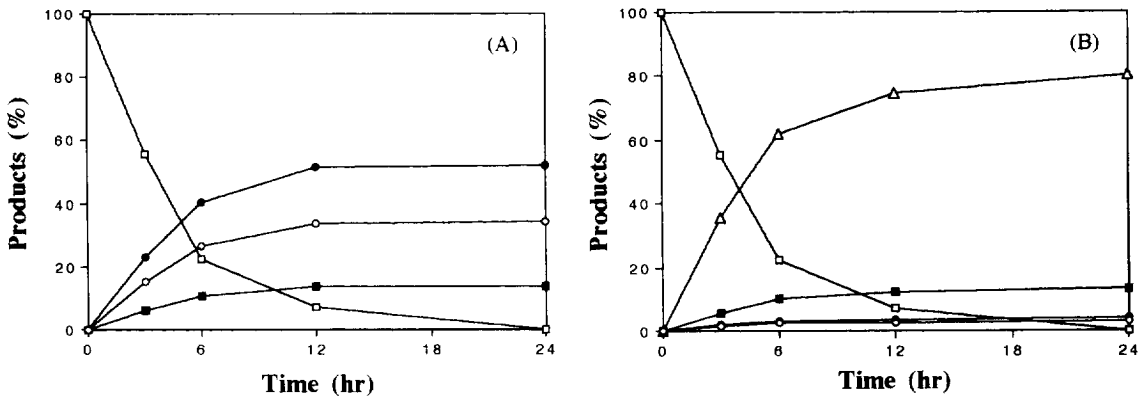


Fig. 1. Biotransformation of (+)-camphorquinone (**1a**) (A) and of (−)-camphorquinone (**1b**) (B) by *A. niger*. □, (+)-Camphorquinone (**1a**); □, (−)-camphorquinone (**1b**); ●, 2-*exo*-hydroxyepicamphor (**1-1**); △, 2-*endo*-hydroxyepicamphor (**1-2**); ■, 3-*exo*-hydroxycamphor (**1-3**); ○, 3-*endo*-hydroxycamphor (**1-4**).

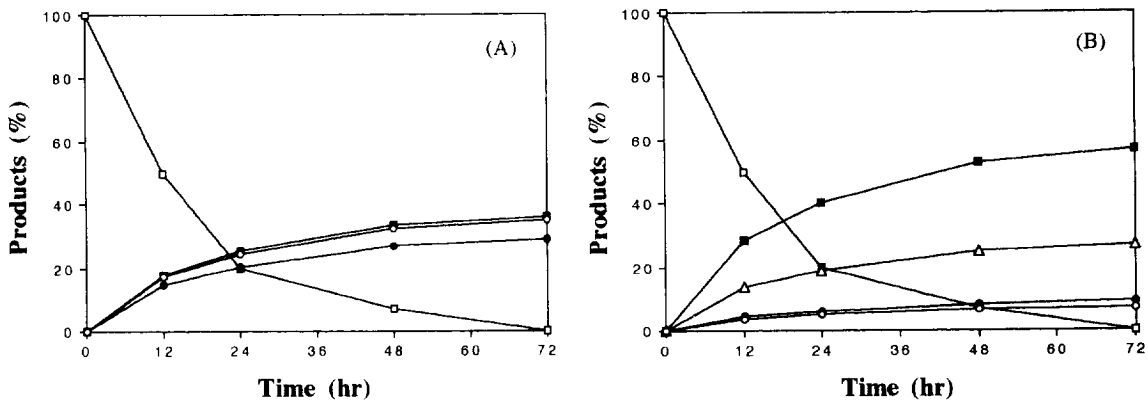


Fig. 2. Biotransformation of (+)-camphorquinone (**1a**) (A) and of (−)-camphorquinone (**1b**) (B) by *F. solani*. Symbols as in Fig. 1.

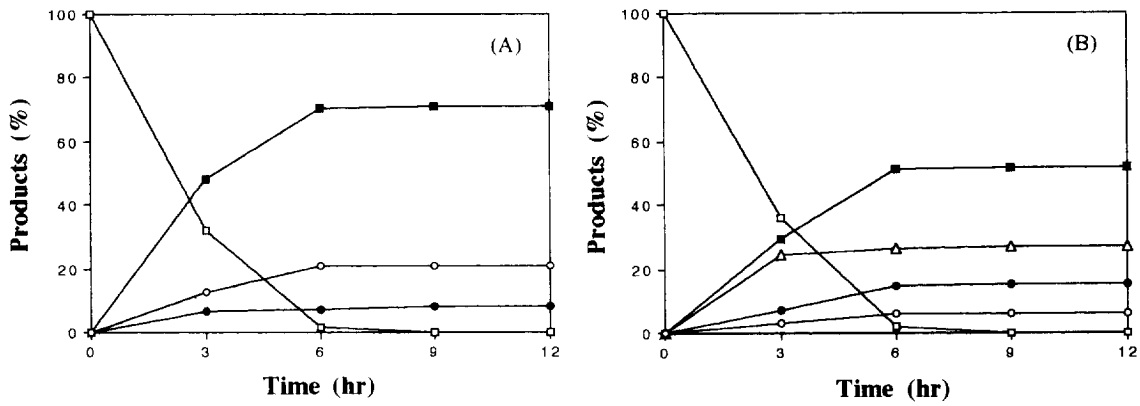


Fig. 3. Biotransformation of (+)-camphorquinone (**1a**) (A) and of (−)-camphorquinone (**1b**) (B) by *G. cingulata*. Symbols as in Fig. 1.

the major product after 9 hr (Fig. 3). In the case of *M. mucedo*, **1a** and **b** were undetectable after a 24 hr incubation period (Fig. 4). Compound **1a** was transformed to (−)-3*S*-*exo*-hydroxycamphor (**1a-3**, 70%) as the major product, and **1b** was transformed to (−)-3*S*-*endo*-hydroxycamphor (**1b-4**, 45% after 24 hr). In the case of *R. solani*, **1a** and **b** had disappeared after 24 hr (Fig. 5). Compound **1a** was transformed to (+)-3*R*-*endo*-hydro-

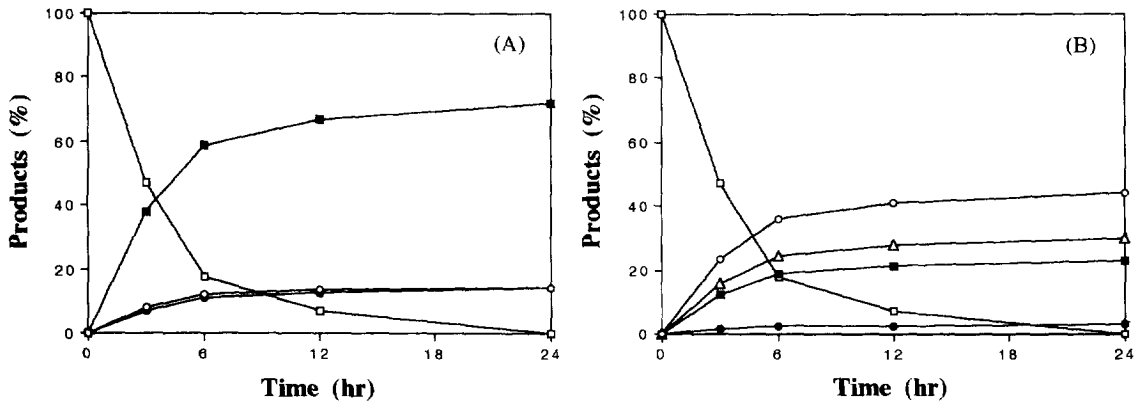


Fig. 4. Biotransformation of (+)-camphorquinone (**1a**) (A) and of (−)-camphorquinone (**1b**) (B) by *M. mucedo*. Symbols as in Fig. 1.

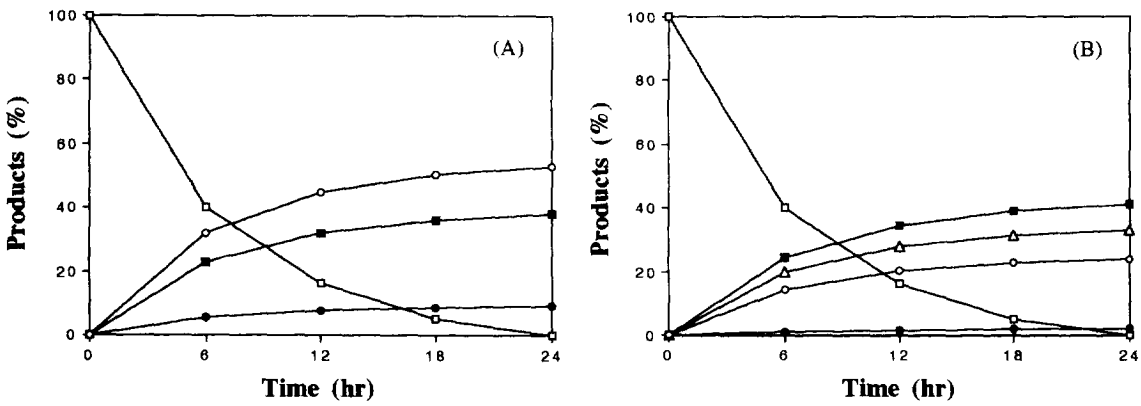
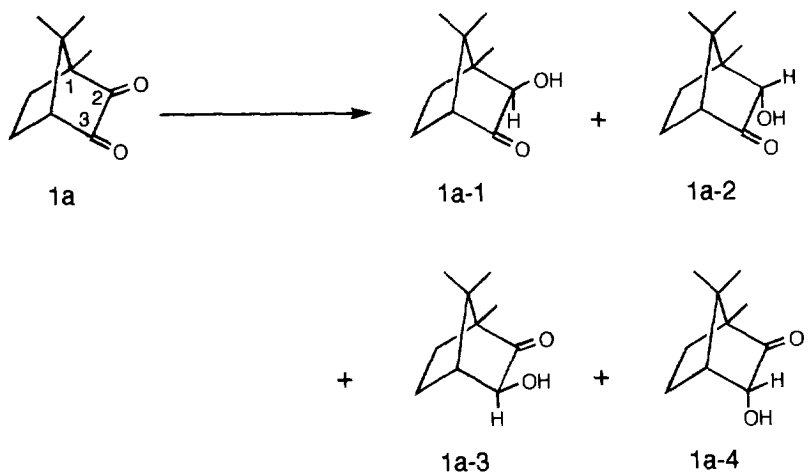


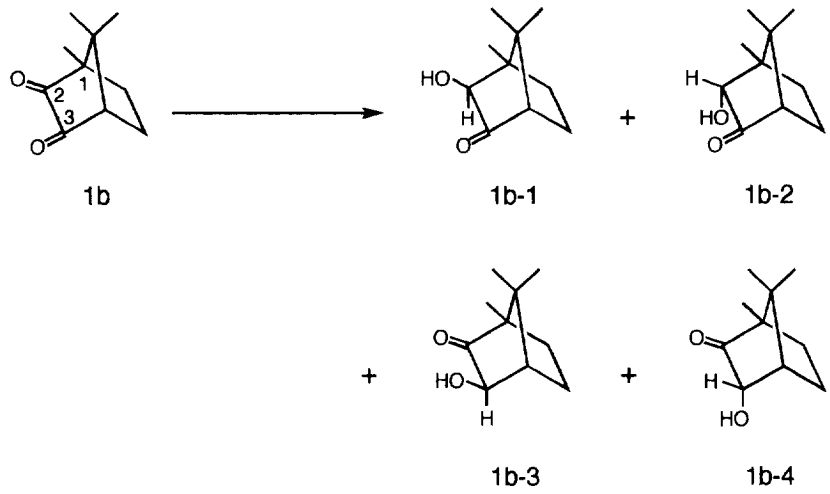
Fig. 5. Biotransformation of (+)-camphorquinone (**1a**) (A) and of (−)-camphorquinone (**1b**) (B) by *R. solani*. Symbols as in Fig. 1.



Scheme 1. Reduction of (+)-camphorquinone.

xycamphor (**1a-4**, 50%) and (−)-3*S*-*exo*-hydroxycamphor (**1a-3**, 40%) as major products at the end of 24 hr. Compound **1b** was converted to (+)-3*R*-*exo*-hydroxycamphor (**1b-3**, 40%).

The structures and relative yields of the products were determined on the basis of the ^{1}H NMR spectra [6, 9]. The mixture of three or four isomeric products was separated by silica gel column chromatography and the



Scheme 2. Reduction of (−)-camphorquinone.

Table 1. Reduction of (+)- and (−)-camphorquinones by various microorganisms

Substrates	Microorganisms	Incubation time (hr)	Yield of product (wt%)	Product ratio (%)			
				2-*exo*-Hydroxy-epicamphor	2-*endo*-Hydroxy-epicamphor	3-*exo*-Hydroxy-camphor	3-*endo*-Hydroxy-camphor
(+)-Camphorquinone (**1a**)				**1a-1**	**1a-2**	**1a-3**	**1a-4**
	A. niger	24	97	51	0	13	33
	F. solani	72	97	28	0	35	34
	G. cingulata	9	99	7	0	70	22
	M. mucedo	24	99	14	0	71	14
	R. solani	24	98	8	0	37	53
(−)-Camphorquinone (**1b**)				**1b-1**	**1b-2**	**1b-3**	**1b-4**
	A. niger	24	98	3	80	12	3
	F. solani	72	98	8	26	57	7
	G. cingulata	9	98	17	24	51	6
	M. mucedo	24	97	2	30	22	43
	R. solani	24	99	2	33	40	24

amounts present in the mixture calculated. The yield of products (**1a-1–4**, **1b-1–4**) formed by each of the five fungi is summarized in Table 1.

The results (Table 1) indicate that the reduction of (−)-camphorquinone (**1a**) by *A. niger* produced (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**) with high stereoselectivity, and compares favourably with previously reported biotransformation of **1a** and **b** by various microorganisms [6–8]. In the previous studies, it was shown that in mammals **1a** is transformed to 3-hydroxycamphor by rats [10] and that racemic camphorquinone is converted to 3-*endo*-hydroxycamphor (major product) by rabbits [11]. Thus the biotransformation of **1a** to afford (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**) has not been reported for mammals. (+)-Camphorquinone (**1a**) was reduced by *G. cingulata* and *M. mucedo* to afford (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) with stereoselectivity. The opposite stereoselectivity is observed for the reduction of **1a** by *Absidia orchidis* [6] and *Acinetobacter* sp. [7].

Though the biotransformation of (−)-camphorquinone (**1b**) by each of the five fungi yielded (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**, 24–80%), (+)-camphorquinone (**1a**) was not converted to (−)-2*S*-*endo*-hydroxyepicamphor (**1a-2**) by any of the five fungi. The reduction of **1a** by *Absidia orchidis* [6] and baker's yeast [8] did not produce (−)-2*S*-*endo*-hydroxyepicamphor (**1a-2**), either. The stereoselective reduction of camphorquinone has been reported by a chiral NAD(P)H model [12]. The chiral NAD(P)H model was able to produce (−)-2*S*-*endo*-hydroxyepicamphor (**1a-2**).

As the carbonyl reductase of *A. niger* exhibits substrate specificity and gives α-ketoalcohols (**1b-2**) with high stereospecificity as products, *A. niger* may be useful for the general synthesis of chiral α-hydroxy ketones.

EXPERIMENTAL

General. (+)- and (−)-Camphorquinones were purchased from Kanto Chemical Co., Inc. ^1H and ^{13}C NMR: 270.05 and 67.80 MHz, respectively; GC-MS: 20 eV (ion voltage) and 250° (ion source) using OV-1 (0.25 mm ×30 m) capillary column GC; GC: column temp. 4° min^{-1} from 140 to 240°, injection temp. 240°; TLC: silica gel 60 F_{254} pre-coated (layer thickness 0.25 mm, Merck) with *n*-hexane–EtOAc (1:1; CC : silica gel with *n*-hexane–EtOAc gradients.

Microorganisms and culture condition. A. niger IFO 4414 and *M. mucedo* IFO 5776 were purchased from the Institute of Fermentation Osaka. *F. solani, G. cingulata* and *R. solani* AG4 H-98 were used from store in Gifu university. These five fungi were maintained on nutrient agar slants at 10° and were used to inoculate autoclaved culture medium.

Components of the culture media. A. niger, F. solani and *G. cingulata*: sucrose 15 g, glucose 15 g, polypeptone 5 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, K_2HPO_4 1 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, H_2O 1 l. *M. mucedo*: glucose 50 g, $NaNO_3$ 3 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, yeast extract 1 g, H_2O 1 l. *R. solani*: glucose 50 g, KNO_3 10 g, K_2HPO_4 5 g, $MgSO_4 \cdot 7H_2O$ 2.5 g, $FeCl_3 \cdot 6H_2O$ 0.02 g, H_2O 1 l.

Cultivation of A. niger. The spores were shaken with culture medium at 28° for 2 days in the incubator. Mycelia were then transferred to the culture medium (15 ml in a 50 ml Petri dish) and incubated for 36–42 hr (until mycelia occupied 60–80% of the surface area of a culture medium) at 28° under static conditions. After growth of the fungus, the substrates (**1a** and **b**) were added directly to each medium, (30 mg/15 ml) and the incubation continued under the same conditions for 24 hr.

Cultivation of F. solani. The spores were shaken with culture medium at 26° for 3 days in the incubator. Mycelia were then transferred to the culture medium (15 ml in a 50 ml Petri dish) and incubated for 5 days (until mycelia occupied 80–90% of the surface area of a culture medium) at 26° under static conditions. After growth of the fungus, the substrates (**1a** and **b**) were added directly to each medium (30 mg/15 ml) and the incubation continued under the same conditions for 72 hr.

Cultivation of G. cingulata. The spores were shaken with culture medium at 27° for 3 days in the incubator. Mycelia were then transferred to the culture medium (150 ml in a 300 ml conical flask) and stirred for 3 days at 27°. After growth of the fungus, the substrates (**1a** and **b**) were added directly to each medium (300 mg/150 ml) and the incubation continued under the same conditions for 24 hr.

Cultivation of M. mucedo. The spores were shaken with the culture medium and then incubated at 26° for 6 days under static conditions. Mycelia were then transferred to the culture medium (15 ml in a 50 ml Petri dish) and incubated for 3 days (until mycelia occupied 60–80% of the surface area of a culture medium) under the same conditions. After growth of the fungus, the substrates (**1a** and **b**) were added directly to each medium (30 mg/15 ml) and incubated under the same conditions for 24 hr.

Cultivation of R. solani. The mycelial fragments were shaken with culture medium at 26° for 5 days in the incubator. Mycelia were then transferred to the culture medium (15 ml in a 50 ml Petri dish) and incubated for 4 days (until mycelia occupied 60–70% of the surface area of a culture medium) at 26° under static conditions. After growth of the fungus, the substrates (**1a and b**) were added directly to each medium (15 mg/15 ml) and the incubation continued under the same conditions for 24 hr.

Purification of the metabolic products. At the end of the incubation period, the culture medium was collected, saturated with NaCl, and extracted with EtOAc. The mycelia were also collected and extracted with EtOAc. The EtOAc extracts were combined and the solvent removed under red. pres. The extract was purified by CC on silica gel with a *n*-hexane–EtOAc gradient, to afford a mixture of reduction products. The mixture was weighed to give the yield of products.

Analysis of the ^1H NMR spectra of the products in $CDCl_3$ led to the assignment of the mixture of three or four isomeric α-ketoalcohols **1-1**, **1-2**, **1-3** and **1-4**: δ 3.55 (*s*, 1H for **1-1**), 3.75 (*s*, 1H for **1-2**), 3.85 (*s*, 1H for **1-3**), and 4.22 (*d*, 1H for **1-4**) [6, 9]. The yields of the three or four isomeric products were calculated from the peak areas for each products.

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