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Microbial Hydroxylation and Functionalization of Synthetic Polycyclic Enones

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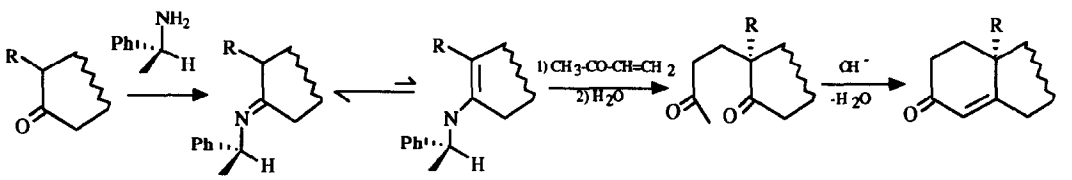
Abstract: The regio- and stereoselective hydroxylation of variously substituted asymmetric 4a-methyl octalenones by selected fungal strains has been shown to afford, sometimes in high yields, several optically pure derivatives, generally hydroxylated in the B-ring. The potential of this method for the preparation of functionalized (hydroxylated) chiral synthons is evaluated and discussed.

INTRODUCTION

Efficient deracemizing alkylations of 2-substituted cyclic ketones leading to optically active functionalized 2,2-disubstituted cyclanones have been described and thoroughly investigated[1-6]. Such carbonyl compounds bearing an α-quaternary center, obtained in high chemical yield and enantiomeric excess, are easily converted to bicyclic enones which may constitute useful building blocks for total synthesis in the field of steroids, terpenes, alkaloids and related molecules[7].



However, the functionalization of selected carbons of these synthons, particularly in the B-ring, which is necessary for further elaboration and which is not always attainable by chemical methods, remains a difficult challenge. In fact, most of the published methods use as starting material the corresponding racemic or optically active 2,5-diketones (known as the Miescher-Wieland ketones[8]), which present a second carbonyl group in the B-ring, and which can be further elaborated to useful synthons[9-11]. The microbial reduction of the 5-keto group has been, for example, currently used to produce stereoselectively hydroxylated derivatives[12-16]. A direct microbial hydroxylation of one of the methylenic groups of the rings, though frequently found in the practice of steroid[17-21] or terpene[17,22] biotransformations, has been poorly explored in that respect, excepted for allylic hydroxylation in position -8 of the racemic 4a-methyl-octalenone **1** by *Rhizopus arrhizus*[23], extended in recent

papers to other octalenone derivatives[24,25]. We have recently described preliminary results about the biotransformations of both enantiomers of octalenone **1** and of a methyl-substituted derivative **6** by several fungal strains, and their potential in the production of regio- and stereoselectively hydroxylated derivatives[26]. We report in the present paper detailed accounts of these results and their extension to variously substituted bi- and polycyclic enones, available as pure or enriched enantiomers through the previously described alkylation method[1-6].

RESULTS AND DISCUSSION

Hexahydronaphthalenone derivatives.

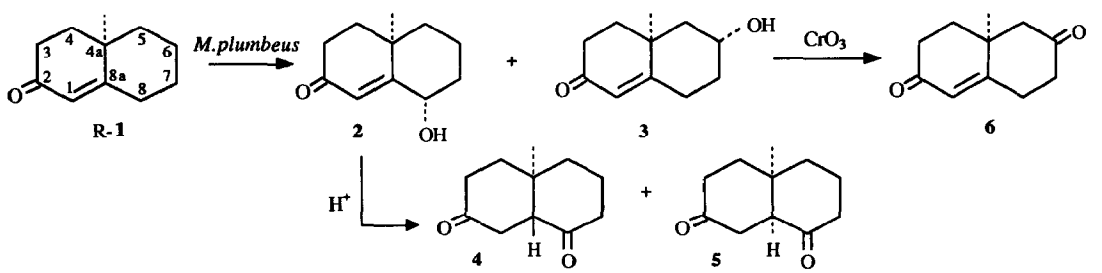
R- and S-enantiomers of hydronaphthalenone **1**, which were available as nearly optically pure material, were selected as model compounds for this study. A number of microorganisms, and specially fungal strains, were found to be able to metabolize these compounds, either added to the grown cultures or to a buffered suspension of the washed mycelium in water, in strongly aerated conditions. A limited number of products were detected by GC and/or TLC of organic extracts of the incubation medium, depending on the strain, the stereochemistry of the substrate and the incubation time (Table 1).

Table 1. Formation of Hydroxylated Products from **1** (0.5 g/L of Culture Medium) by Incubation with Grown Cultures of Various Fungal Strains

Strains[a]	incubation time (days)	% products obtained[b] from R-1			incubation time (days)	% products obtained[b] from S-1		
		R-1	2	3		S-1	ent-2	ent-3
Absidia glauca	8	14[c]	51[c]	8[c]	7	43	50	-
Beauveria bassiana ATCC 7159	5	4[c]	6[c]	36[c]	7	8	35	39
Cunninghamella echinulata NRRL 3655	9	33	17	11	7	28[c]	33[c]	6[c]
Curvularia lunata NRRL 2380	8	16[c]	5[c]	42[c]	4	-	45[c]	-
Cylindrocarpon radicicola MMP 1197	8	88	10	-	-	-	-	-
Mucor aromaticus NRRL 1701	10	14	44	26	-	-	-	-
Mucor janssenii NRRL 3826	9	37	21	6	7	35[c]	45[c]	-
Mucor plumbeus MMP 430	9	42	47	-	-	-	-	-
Mucor plumbeus CBS 110-16	3	1	66	22	2	-	77[c]	-
Mucor racemosus	9	74	20	1	7	56[c]	30[c]	-
Mucor rouxii NRRL 1430	9	58	21	-	-	-	-	-

a) Origin of strains: ATCC, American Type Culture Collection (Rockville, Maryland, USA); CBS, Centraalbureau voor Schimmelcultures (Baarn, Netherlands); MMP, Muséum d'Histoire Naturelle (Paris, France); NRRL, Northern Utilization Research (Peoria, Illinois, USA). Other strains are from local origin. b) % area of the corresponding chromatographic peaks (25 m BP-10 capillary column run at 200°C with helium; retention times: **1**, 5.3 min; **2**, 10.1 min; **3**, 13.5 min). c) several other peaks present.

For example, R-1 was converted rapidly and quantitatively by *M.plumbeus* CBS 110-16, either by direct incubation with the mycelium in the culture medium, or using the washed mycelium incubated in a buffered solution, into a mixture of two main polar products, easily separated by flash chromatography. The major product was identified as the (4aR,8aS)-enantiomer of the known *cis*-4a-methyl-8-hydroxy-octalenone **2**, previously



Scheme 1

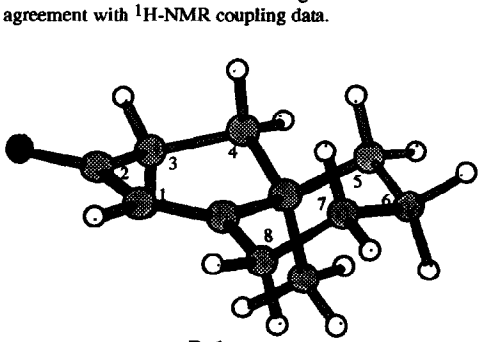
obtained by allylic (electro)chemical oxidation of **1** or its enol ether[27-30] or, in low yields, from the incubation of **1** with *Rhizopus arrhizus* cultures[23]. Compound **2** was characterized by its ^{1}H-NMR spectrum (Table 2), which shows a C*H*OH signal (triplet at 4.29 ppm, J = 2.8 Hz) typical of an equatorial proton[23]. The analysis of the correlation patterns observed in the 2D-COSY spectrum of **2**, the disappearing of the ^{4}J long range coupling between H-1 and H-8ax normally observed for **1***, and the downfield shift of the 4a-methyl signal of **2** with respect to **1** ($\Delta\delta$= +0.2 ppm), are in agreement with an 8-axial position of the newly introduced hydroxy group.

An acidic treatment (HCl/MeOH) converted quantitatively compound **2** into a mixture of *trans* and *cis*-fused diketones **4** and **5** (mainly **4** upon prolonged reaction)[27,29,30] identified by ^{1}H-NMR[31], as expected from allylic proton elimination and double bond rearrangement, and contrarily to the previously assumed *cis* to *trans* isomerisation of **2**[23].

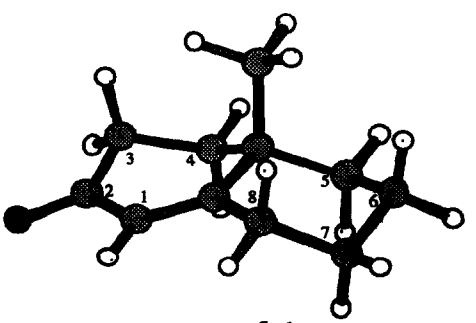
Table 2. ^{1}H-NMR Spectra of Hexahydronaphthalenone **1** and Derivatives **2-6** in $CDCl_3$: δ ppm (J Hz)

	1	**2**	**3**	**4**	**5**	**6**
H-1	5.75 (br.s,$w_{1/2}$= 5)	5.80 (s,$w_{1/2}$= 2)	5.74 (d, 2)		2.71(dd, 10, 3.7) 2.3-2.4 (m)	5.90 (br.s,$w_{1/2}$= 4)
H-3ax	2.5 (ddd, 17, 14.5, 5)	2.57 (ddd, 17.5, 14.5, 5.5)	2.53 (ddd, 17, 14.5, 5.2)	2.2-2.6 (m)	2.25-2.45 (m)	2.43 (m)
H-3eq	2.30 (ddd, 17, 4, 3)	2.37 ((dddd, 17.5, 4, 3, 1.5)	2.34 (dddd,17.5,5.5,2.8,1.2)			2.5 (dt, 18, 5.5)
H-4ax	1.7-1.9 (m)	1.85 (dt, 5, 13.5)	1.8 (m)		1.75-2.0 (m)	1.99 (dt, 5,13.5)
H-4eq	1.7-1.9 (m)	1.7 (m)	1.8 (m)			1.88 (ddd, 13.5, 5, 4.5)
H-5ax	1.3-1.4 (m)	1.3 (dt, 3.5, 13.5)	1.53 (m)	1.55-2.05 (m)	1.38 (m)	2.45 (m)
H-5eq	1.6-1.7 (m)	1.68 (m)	1.90 (m)			2.45 (m)
H-6ax	1.6-1.7 (m)	2.05 (m)	-		1.75-2.0 (m)	-
H-6eq	1.6-1.7 (m)	1.5 (m)	4.24 (quint, 2.8)			-
H-7ax	1.35 (m)	1.6 (m)	1.76 (m)	2.2-2.6 (m)	2.25-2.45 (m)	2.40 (m)
H-7eq	1.85 (m)	2.0 (m)	1.95 (m)			2.40 (m)
H-8ax	2.3-2.4 (m)	-	2.84 (tdd,14.5, 6, 2)	-	H-8a: 2.2 (m)	2.81 (dddd,15.5,11.5,7.5,2)
H-8eq	2.2-2.3 (m)	4.29 (t, 2.8)	2.12 (ddd,14.5,4.5,2.8)	-		2.69 (ddd, 15.5, 6.5, 3.5)
CH_3-4a(ax)	1.23 (s)	1.43 (s)	1.46 (s)	0.96 (s)	1.29 (s)	1.23 (s)

* Molecular modeling of the flexible bicyclic enone system, using Alchemy II® (Tripos Ass.Inc.), indicated for all compounds an energy-minimized conformation with the B-ring in a chair conformation, and H-4 *trans* to the 4a-methyl group in a quasi-axial position, in agreement with ^{1}H-NMR coupling data.



R-1



S-1

The second main product from the incubation of R-**1** with *M.plumbeus* CBS 110-16, which was also present in the incubation mixture of other strains, was identified as a different hydroxylated product, the (4aS,6R)-*cis*-4a-methyl-6-hydroxy octalenone **3**, by 1D- (Table 2) and 2D-^{1}H-NMR analysis (Fig.1). As reported for compound **2**, no alteration of the coupling pattern of H-3 and H-4 was observed; a ^{4}J long range coupling pattern between H-1 and H-8ax, and the correlation between H-8ax and H-8eq were used to assign the 8-CH_2 hydrogens. A C*H*OH signal resonating as a quintuplet at 4.24 ppm indicated hydroxylation in positions -6 or -7, while the low value of the coupling constant (J = 2.8 Hz) was characteristic of an equatorial hydrogen; the downfield shift of the methyl group signal ($\Delta\delta$= + 0.2 ppm) suggested a 1,3-diaxial interaction with a *cis*-6α-hydroxy group, in agreement with the coupling pattern observed for the C*H*OH proton. The 6α-position of the newly introduced hydroxy group was confirmed by the correlations observed in the 2D-COSY spectra, with the coupling of the C*H*OH signal to CH_2-5 and CH_2-7. Mild chromic acid oxidation of **3** afforded the new diketone **6** characterized by ^{1}H- (Table 2) and ^{13}C-NMR (Table 7).

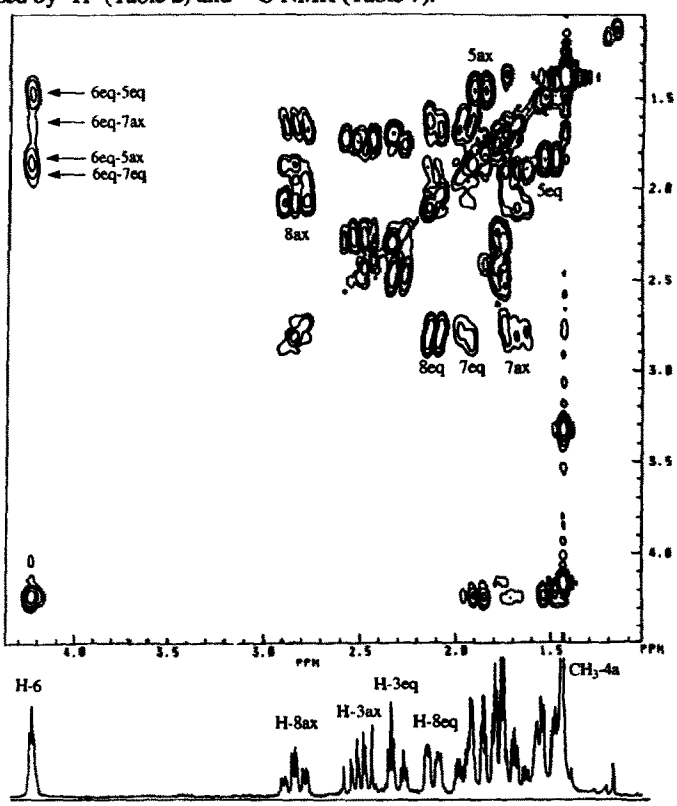
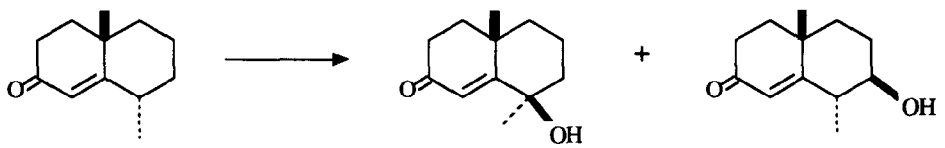


Figure 1: COSY-45 Proton-Proton Shift Correlation of ^{1}H-NMR Spectra of *cis*-(4aS,6R)-4a-methyl-6-hydroxy-octalenone **3** (250 MHz; 20 mg in 0.4 ml of $CDCl_3$, Room Temperature).

Using the same incubation conditions, S-**1** was more rapidly metabolized than R-**1** (Table 1). With *M.plumbeus*, essentially **ent-2** could be obtained. However, other strains (e.g.*B.bassiana*) were able to produce **ent-3**, but at a lower rate. No significant formation of a *trans* isomer could be detected. The difference observed in the hydroxylation rates of each enantiomer indicates some enantioselectivity of the hydroxylating systems; in the original description[23] of the 8-hydroxylation of (±)-**1** by *Rhizopus arrhizus*, no mention was made of the absolute configuration of the products; as a matter of fact, small but significant optical activities were measured in the *cis*-8-hydroxylated product ($[\alpha]_D$= +2) and in the residual substrate ($[\alpha]_D$= – 5)[32], corresponding to a faster hydroxylation of the S-enantiomer.

Without optimization, the amount of main hydroxylated compounds from both enantiomers in the crude conversion product was generally higher than 70%, as estimated by GC. On a 500mg scale, using *M.plumbeus* CBS 110-16, 40-65% of isolated pure hydroxylated compounds were recovered after simple extraction of the incubation mixture and silicagel chromatography.

The microbiological transformation was extended to more substituted optically active octalenones, which had been prepared by the same previously described method. Using a similar incubation procedure, (4aS,8S)-**7**[4] was converted by *M.plumbeus* CBS 110-16 into a mixture of (8R)- and (7R)-hydroxy derivatives **8** (40%) and **9** (27%). Both compounds were easily identified by ^{1}H-NMR (Table 3): the methyl groups signals of **8** resonated as singlets and consequently the hydroxy group had been introduced on C-8 and in a β(ax)-*cis* position with respect to the angular 4a-methyl group, in agreement with an observed Δδ of *ca.* +0.2 ppm compared to the corresponding singlet of **7**. This was confirmed by the signals of the β-side protons (H-7eq, H-6ax and H-3ax), identified in the 2D ^{1}H-COSY spectrum, which were also shifted downfield (*ca* +0.15, +0.2 and +0.15 ppm respectively). On the other hand, hydroxylation in **9** was assigned to a 7β(eq)-position owing to the correlation patterns observed in the 2D COSY spectrum and to the large coupling constants of the C*H*OH signal with axial H-8 and H-6 and the smaller one observed with H-6eq (δ= 3.2 ppm, dt, J = 4.5 and 10.5 Hz)



(4aS,8S)-**7** **8** **9**

Table 3. ^{1}H-NMR Spectra of 4a,8-Dimethyl-hexahydronaphthalenones **7** and Hydroxylated Derivatives **8** and **9** in $CDCl_3$: δ ppm (J Hz)

	7	**8**	**9**
H-1	5.75 (br.s, $w_{1/2}$= 5)	6.01 (s, $w_{1/2}$= 2)	5.85 (br.d, 2, $w_{1/2}$= 4)
H-3ax	2.44 (ddd,16.5, 11.5, 6.5)	2.58 (ddd,17.5, 14.5, 5.5)	2.5 (m, 17)
H-3eq	2.32 (ddd, 12, 3.5, 3)	2.37 (ddd, 17.5, 4, 2.5)	2.35 (m)
H-4ax	1.8 (m)	1.9 (m)	1.8 (m)
H-4eq	1.8 (m)	1.7 (m)	1.8 (m)
H-5ax	1.35 (dt)	1.3 (m)	1.4 (dt, 4.5, 14)
H-5eq	1.65 (m)	1.5 (m)	1.65 (m)
H-6ax	1.6-1.9 (m)	1.9 (m)	1.75 (m)
H-6eq	1.6-1.9 (m)	1.65 (m)	1.9 (m)
H-7ax	1.15 (dt, 12.5, 3.8)	1.3 (m)	3.26 (dt, 4.8, 10.6)
H-7eq	1.85 (m)	2.1 (dt, 14, 3.5)	-
H-8ax	2.35 (m)	-	2.3 (m)
CH_3-4a (ax)	1.21 (s)	1.42 (s)	1.26 (s)
CH_3-8a (eq)	1.04 (d, 6.5)	1.39 (s)	1.18 (d, 6.5)

Similarly, the (S)-1,4a-dimethyl octalenone **10** was converted by *M.plumbeus* to a complex mixture of hydroxylated derivatives from which major products **11** (23%), **12** (11.5%), **13** (about 15%) and **14** (21%) could be isolated and purified by repeated chromatography. However, other hydroxylated compounds certainly occur as minor products.

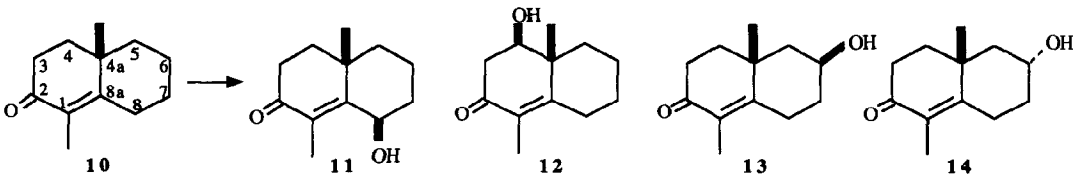


Table 4. ^{1}H-NMR Spectra of 1,4a-Dimethyl-4,4a,5,6,7,8-Hexahydro-2(3*H*)naphthalenone **10** and Hydroxylated Derivatives **11-14** in $CDCl_3$: δ ppm (J Hz)

	10	**11**	**12**	**13**[a]	**14**
H-3ax	2.51 (ddd, 16.8, 13.5, 5.8)	2.62 (ddd, 17.5, 15, 5.5)	2.57 and 2.65 (AB part of an ABX system, 17.5, 14, 5)	2,49 (ddd, 17, 12.6, 5.7)	2.51 (ddd, 17, 13.8, 5.5)
H-3eq	2.35 (ddd, 17, 5, 3.8)	2.42 (ddd, 17.5, 4.5, 2.5)		2,35 (ddd, 17, 4.5, 3.2)	2.38 (ddd, 17, 4.8, 3.5)
H-4ax	1.7(m)	1.75 (m)	3.80 (dd, 14, 5)	1,7(m)	1.8 (m)
H-4eq	"	"	-	"	"
H-5ax				1.36 (dt, 3.6, 14)	1.9 (dm, 12)
H-5eq				1.83 (dt, 14, 3)	"
H-6ax	1.6(m)	1.5 (m)	1.9 (m)	-	4.05 (tt, 11, 4)
H-6eq	"	"	1.7 (m)	4.18 (quint, 3.4)	-
H-7ax	1.35(m)	1.6 (m)	1,4 (m)	1.7 (m)	1.32 (dm, 13)
H-7eq	1,87 (m)	2.03 (dm, 14)	2.1 (m)	1.90 (dm, 13.3)	2.18 (m)
H-8ax	2.04 (ddd, 15, 15, 5.5)	-	2.1 (m)	1.70 (m)	2.1 (m)
H-8eq	2,70 (dm, 15)	4.92 (t, 2.9)	2.77 (dm, 15)	2,56(dd, 7.5, 3.8))	2.80(ddd, 15, 7, 3)
CH_3-1	1.73 (s)	1.83 (s)	1.74 (d, 1.4)	1.77 (s)	1.76 (d, 1)
CH_3-4a(ax)	1.19 (s)	1.38 (s)	1.16 (s)	1.41 (s)	1.22 (s)

a) major component of an unseparated mixture of two hydroxylated products; the minor component was characterized by signals at 3.66 (1H, tt, 11.2, 4.5), 2.95 (1H, ddd, 12, 4.7, 2.3), 2.09 (1H, br.t, 13.5), 1.77 (3H, s) and 1.22 (3H, s) ppm.

Compound **11** was easily identified as a 8-*cis*-hydroxylated product by ^{1}H-NMR (Table 4): the C*H*OH signal (triplet at 4.92 ppm, J = 2.9 Hz) is typical of an equatorial proton[23] and the downfield shift of the 4a-methyl signal (Δ = 0.1 ppm) is is agreement with a *cis*-axial position of the newly introduced hydroxyl group. This product was partially converted during isolation and under acidic conditions to an isomeric saturated diketone in a rearrangement similar to that observed for compound **2**. The pattern of the C*H*OH signal in compound **12** was unusual (doublet of doublets at 3.80 ppm, J = 5 and 14 Hz). 2D-^{1}H-NMR and spectra simulations confirmed an ABX coupling with both H-3 (around 2.6 ppm), indicating a 4(β)eq-*cis*-hydroxylated derivative. Compound **13** was obtained in mixture with a minor product: the major component was characterized by a C*H*OH signal as a quintuplet (4.18 ppm, J= 3.5 Hz), without any coupling with H-8eq, thus corresponding to a 6(β)-axially hydroxylated compound, as confirmed by the downfield shift of the 4a-methyl signal (Δ = 0.22 ppm). The minor component was probably a 7eq-hydroxy derivative as demonstrated by a C*H*OH signal resonating as a relatively highfield signal (triplet of triplets at 3.66 ppm, J= 11.5 and 4.7 Hz), coupled with H-8eq (ddd at 2.95 ppm, J = 12, 4.7 and 2.3 Hz). The C*H*OH signal of **14** (triplet of triplets at 4.05 ppm, J = 11 and 4 Hz) could be again attributed to a hydroxylated product in position C-6 or C-7, the signals of H-3, H-4 and 4a-methyl protons beeing unchanged. The 6α(eq)-position of the hydroxyl group was finally deduced from 2D-^{1}H-NMR spectra and from the absence of coupling of the C*H*OH signal with H-8eq.

It is remarkable that the simple addition of a methyl group on the enone system induces such a variety of hydroxylation sites, including the new 4-position, comparatively to other octalenones.

Tetrahydrophenanthrenone derivatives.

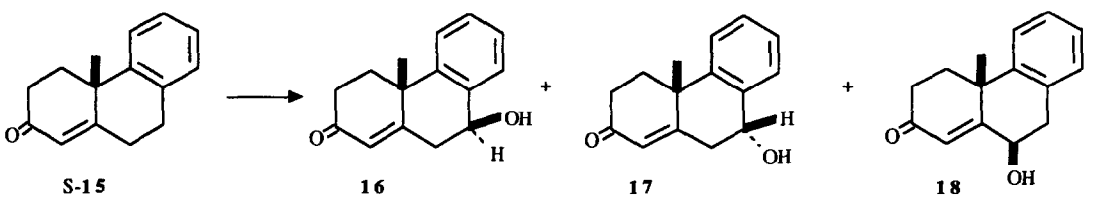
The biotransformation of hydrophenanthrenone **15** enantiomers was interesting to investigate: a preliminary screening (Table 5) showed that *M.plumbeus* was again the best strain for a complete biotransformation of S-**15**, affording one major product **16** and several other minor products. The R-enantiomer was better oxidized by *Curvularia lunata* NRRL 2380, affording mainly **ent-16**. In a preparative incubation with *M.plumbeus*, 350 mg of the S-enantiomer were completely converted in 3 days into more polar products, from which **16** (33%), **17** (13.5%) and a very small amount of **18** were isolated.

Table 5. Formation of Hydroxylated Products from **15** (0.5 g/L of Culture Medium).

Strains[a]	incubation time (days)	% products observed[b] from S-15			% products observed[b] from R-15		
		S-15	16	others	R-15	ent-16	others
Beauveria bassiana ATCC 7159	1	-	-	-	14.5	64.3	16.8[d]
	2	-	-	-	12.0	66.2	16.5[d]
Curvularia lunata NRRL 2380	1	88.1	11.9	-	64.7	35.3	-
	2	64.7	18.8	-	34.6	65.4	-
	7	18.4	32.5	28.1[d]	11.7	88.3	-
Mucor plumbeus CBS 110-16	2	23.0	43.8	22.8[c]	65.3	34.7	-
	4	3.9	59.5	28.0[c]	42.0	38.0	-

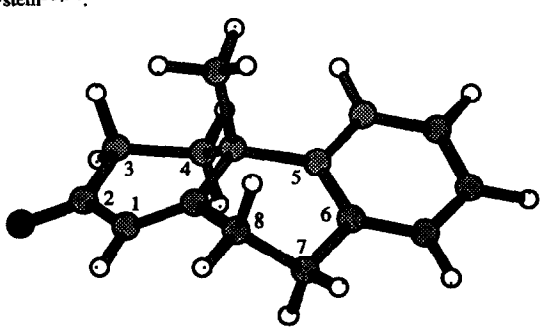
a) Origin of strains: see Table 1. b) % area of the corresponding chromatographic peaks (25 m.BP-10 capillary column run at 250°C with helium; retention times: **15**, 6.6 min; **16**, 7.4 min. c) mainly **17**. d) several other peaks present.

Compounds **16** and **17** were identified as epimeric benzylic hydroxylation products, with an OH group in the 7eq or 7ax position respectively *, as demonstrated.by 1D- (Table 6) and 2D-COSY ^{1}H-NMR: H-3 and H-4 signals are not modified with respect to the substrate compound and the ^{4}J long range coupling constant between H-1 and H-8ax is always observed, while this H-8ax signal is also coupled to the geminal H-8eq and to a C*H*OH signal; in compound **16**, the hydroxy group is located in an equatorial position as shown by the



S-15 16 17 18

* Similarly to the previously examined simple octalenones, hexahydrophenanthrenone **15** exhibited by computer modelisation a minimal energy conformation involving H-4 *trans* to the 4a-methyl group and H-8 *cis* to the same methyl group in quasi axial positions, in agreement with ^{1}H-NMR coupling data and with the literature dedicated to the conformational analysis of the 4a-methyl perhydrophenanthrene system[33,34].



S-15

coupling pattern of the C*H*OH signal (dd, J = 11.5 and 5.5 Hz), in agreement with *ax-ax* and *ax-eq* coupling with H-8ax and H-8eq respectively. This assignment is confirmed by the downfield shift of a single hydrogen of the phenyl ring (appearing as a doublet) and corresponding to the hydrogen born by the aromatic carbon atom next to C-6. The hydroxyl group of **17** is located in an axial position as shown again by the coupling pattern of the C*H*OH signal (t, J = 3.2 Hz), in agreement with *eq-ax* and *eq-eq* coupling with H-8ax and H-8eq respectively.

The minor compound **18** was similarly identified by ^{1}H-NMR spectroscopy (Table 6) as a 8ax-hydroxylation product: the ^{4}J long range.coupling constant between H-1 and H-8ax disappeared and the H-1 signal shows a smaller half height-width ($w_{1/2}$ = 2 Hz); the axial position of the hydroxyl group is demonstrated by the coupling pattern of the C*H*OH signal (t, J = 3.5 Hz), in agreement with eq-ax and eq-eq coupling with H-7ax and H-7eq respectively. This assignment is confirmed by the downfield shift of the methyl group (Δ = +0.14 ppm), compared with the starting compound, and corresponding to an axial-axial interaction with the newly introduced OH group.

Table 6: ^{1}H-NMR of Hydrophenanthrenone **15** and Hydroxylated Derivatives **16-18** in $CDCl_3$: δ ppm (J Hz).

	15	**16**	**17**	**18**
H-1	5.88 (br.s,$w_{1/2}$= 3.5)	5.93 (br.s,$w_{1/2}$= 3.5)	6.0 (br.s,$w_{1/2}$= 3.5)	5.96 (s,$w_{1/2}$=2)
H-3ax	2.7	2.7 (ddd,16.5, 13.5, 5)	2.7 (ddd,16.5, 13.5, 5)	2.7 (m)
H-3eq	2.50 (dddd,16.5, 5, 2.5, 1.5)	2.5 (dddd,16.5, 5, 2.5, 1.5)	2.55 (dddd,16.5, 5, 2.5, 1.5)	2.5 (m)
H-4ax	2.04 (dt, 5, 13.5)	2.04 (dt, 5, 13.5)	2.08 (dt, 5, 13.5)	2.05
H-4eq	2.37 (ddd, 13.5, 5, 2.5)	2.37 (ddd,13.5, 5, 2.5)	2.42 (ddd,13.5, 5, 2.5)	2.35
H-7ax	2.85 (ddd, 16, 12, 5)	4.82 (dd, 11.5, 5.5)	-	3.12 (d, 3.5)
H-7eq	3.02 (ddd, 16, 6.5, 3)	-	4.91 (t, 3.2)	3.12 (d, 3.5)
H-8ax	2.7 (m) and 2.55 (m)	2.69 (dt, 1.5, 11.5)	2.92 (ddd,13.5, 3.2, 1.5)	-
H-8eq		2.84 (dd,11.5, 5. 5)	2.7 (dd,13.5, 3.2)	4.63 (t, 3.5)
CH_3-4a	1.56 (s)	1.59 (s)	1.53 (s)	1.70 (s)
ArH	7.05-7.30 (m,4H)	7.58(d, 7, 1H) 7.30 (m, 3H)	7.2-7.4 (m,4H)	7.2-7.4 (m,4H)

Such results show that the benzylic oxidation process of **15** is somewhat favored compared to the allylic position. However, the only product of allylic hydroxylation, when detected, is the *cis* product, as previously found for the simpler 4a-methyl octalenones. No phenolic compound resulting from aromatic ring hydroxylation could be apparently detected.

CONCLUSION

From these results, it is obvious that, at this moment, no clear-cut model can be drawn for the microbial hydroxylation of the various 4a-methyl octalenone derivatives investigated, even by considering only the results obtained by using a single fungal strain (*M.plumbeus*). As a general rule in this series, it appears that, unexpectedly, the introduction of the hydroxyl in a more hindered *cis*(ax) position, relatively to the 4a-methyl group, is favored. However, the regioselectivity of the hydroxylation reaction is unpredictable, as shown by the difference in the hydroxylation pattern of enantiomeric substrates, or the diversity of hydroxylated positions found when an additional 1-methyl group was present. Nevertheless, in some examples, useful new hydroxylated synthons, functionalized in the B-ring, are formed in adequate amounts, in very simple reactions.

EXPERIMENTAL PART

General. Melting points are uncorrected. ^{1}H-NMR spectra were generally obtained in $CDCl_3$ at 250 MHz on a WM 250 FT Brucker spectrometer and occasionnally on an AMX 500 Brucker spectrometer at 500 MHz. Chemical shifts are expressed in ppm from tetramethylsilane (using as a reference residual $CHCl_3$). Multiplicities are reported as br. (broad), s (singlet), d (doublet), t (triplet) q (quadruplet) and m (multiplet). ^{13}C-NMR spectra were recorded at 62.9 MHz and multiplicities were assigned by polarization transfer using a DEPT 135 sequence. 2D-^{1}H-^{1}H COSY NMR spectra were acquired at 250 MHz with sweep width of 2000 Hz into 1024 data points in f2. The relaxation delay was 1s and each FID was acquired with 64 scans after 4 dummy scans. The data were zero-filled to 2048 points in f_2 and f_1 prior to double fourier transform, with unshifted sinebell window functions in both dimensions. A delay period of 0.08 s was used to emphasize long-range or small couplings[35,36]. Thin layer chromatography on Merck $60F_{254}$ precoated plates and GC were routinely used to monitor the bioconversion experiments. BP-10, DBWax or SE-30 capillary columns (0.2 mm x 25-30 m) were used on Varian 3700 or Shimadzu G6 instruments equipped with flame ionization detectors and Shimadzu CR-6A integrating-recorders. Optical purity of the enone substrates was determined or verified by GC on a Chiraldex G-TA capillary column (0.25 mm x 30 m, Astec). Flash chromatography on silica gel (Merck 60, 230-400 mesh) was used for products separation with cyclohexane or methylene chloride containing 25-70% ethyl acetate as solvent. Optical rotations were measured in a 1 dm-cell on a Perkin Elmer 241 spectropolarimeter. Elemental analyses of crystalline samples were performed at the CNRS Central Microanalysis Laboratory, Gif-sur-Yvette, France. Mass spectra (EI mode) and high resolution mass measurements (HRMS) were supplied by the University P. and M. Curie (Paris), Spectrochemistry Centre. Commercial reagents and solvents were used without further purification.

Starting materials. (S)-(+) and (R)-(–)-4a-methyl-4,4a,5,6,7,8-hexahydro-2(3*H*)-naphthalenone (**1**) enantiomers, $[\alpha]_D^{21}$= +195 (c 1.95, EtOH)[37] and $[\alpha]_D^{21}$= –205 (c 1.73, EtOH)[37] respectively (ee ≥ 95%), (4aS,8S)-4a,8-dimethyl-4,4a,5,6,7,8-hexahydro-2(3*H*)-naphthalenone (**7**), m.p. 56°C, $[\alpha]_D^{21}$= +94 (c 1.58, $CHCl_3$)[4,38], (S)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-2(3*H*)-naphthalenone (**10**), $[\alpha]_D^{21}$= +148 (c 1.55, EtOH) (ee 90%)[4], and (S)-(+) and (R)-(–)-4a-methyl-4,4a,9,10-tetrahydro-2(3*H*)-phenanthrenone (**15**) enantiomers, m.p. 64-65°C, $[\alpha]_D^{21}$= +324 (c 1, EtOH) and $[\alpha]_D^{21}$= – 320 (c 1.05, EtOH) respectively (ee ≥ 98%)[2,39] were synthesized as previously described[1,2,4].

Microorganisms, culture and incubation conditions. Screening of microorganisms from international collections was effected using a standard liquid medium[26] (100 ml) in orbitally shaken (200-250 r.p.m.) conical flasks, inoculated with spores (freshly obtained from a solid medium) and incubated for 48-72 hours at 27°C in order to obtain maximal growth; substrates (50 mg) were directly added to the cultures, sometimes after dilution into a small volume of ethanol, and incubation was continued in the same conditions. Progress of the biotransformation was followed by GC and TLC.

Preparative incubations were effected either through a simple scaling up of the screening procedure, or using a pelleted mycelium previously grown in a Biolafitte fermentor (7.5 L), filtered and washed with tap water then resuspended in a 0.1M potassium phosphate buffer, pH 7.0 (about 20 g.dry weight mycelium/L.) to which the substrate (0.5-2 g/L.) was added.

Isolation and purification of biotransformation products. The incubation medium was filtered with celite and the filtrate, saturated with sodium chloride (and eventually filtered again) was repeatedly extracted with ethyl acetate. In most cases, owing to the large amount of material extracted, compared with the minute amount of useful product detected, the mycelium extract was discarded; in some few cases, it was extracted apart and the extract pooled with the filtrate extract for further purification.

Biotransformation of (R)-4a-methyl-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone (**1**)

a) after incubation of R-1 (0.5 g in 1L) during 2-3 days with *M.plumbeus* CBS 110-16 in the culture

medium, only a small amount of residual substrate was detectable. Extraction and purification of the products by flash chromatography (cyclohexane-EtOAc, 1:1) afforded, following the elution order, **2** (125mg, 23%), M.p.= 68-69°C, $[\alpha]_D^{21}$= -95 (c 1.04, $CHCl_3$) and **3** (75 mg, 14%), M.p.= 122-124°C then 129-130°C (after crystallisation in ether-hexane), $[\alpha]_D^{21}$= -210, $[\alpha]_{578}^{21}$= - 220, $[\alpha]_{546}^{21}$= - 258, $[\alpha]_{436}^{21}$= - 528. (c 1.025, $CHCl_3$). Anal. $C_{11}H_{16}O_2$ (180.25), calc.%:C, 73.29, H, 8.94; found %: C, 72.67, H, 8.56. MS, 180(55) M^+, 162(47) M-H_2O, 152(60) M-CO, 134(47), 123(72), 108(100). ^1H- and ^{13}C-NMR see Tables 2 and 7

b) R-**1** (370 mg) was distributed into two flasks containing 0.75 L.of phosphate buffer and 20 g/L (dry weight) of washed mycelium from a 48 hours culture of *M.plumbeus* CBS 110-16. Incubation was performed at 27°C during 3 days, when no more substrate disappeared. Extraction and purification as above from the filtrate afforded, besides residual **1** (57 mg), hydroxyoctalenones **2** (83 mg, 20%) and **3** (91 mg, 22%).

Oxidation of (**3**) *to 4a-methyl-4,4a,7,8-tetrahydronaphthalene-2(3H),6(5H)-dione* (**6**). 20 mg of **3** in acetone (0.4 ml) were treated at 0°C with 40 µl of a solution of chromic acid (1g) in water-H_2SO_4 (5 ml, 4:1). After 5 min, water was added and the mixture was extracted with ethyl ether. Preparative TLC afforded 12 mg of the diketone **6**, M.p.= 90-91°C, $[\alpha]_D^{21}$= -115, $[\alpha]_{579}^{21}$= -119, $[\alpha]_{546}^{21}$= -139, $[\alpha]_{436}^{21}$= -301 (c 0.3, $CHCl_3$). ^1H- and ^{13}C-NMR see Tables 2 and 7

Biotransformation of (S)-4a-methyl-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone (**ent-1**)

a) from **S-1** (200 mg) incubated as above in the culture medium during 2 days, 135 mg (62%) of **ent-2** were recovered; M.p.= 72°C (after crystallisation in ether-hexane); $[\alpha]_D^{21}$= +96.5, $[\alpha]_{578}^{21}$= +102, $[\alpha]_{546}^{21}$= +120, $[\alpha]_{436}^{21}$= +266 (c 1.025, $CHCl_3$). Anal. $C_{11}H_{16}O_2$ (180,25), calc.%: C, 73.29, H, 8.94; found %: C, 72.77, H, 8.66. MS, 180(46) M^+, 151(100), 137(25), 123(68). ^1H- and ^{13}C-NMR see Tables 2 and 7

b) from **S-1** (370 mg) incubated as above in phosphate buffer with washed mycelium, 199 mg (49 %) of **ent-2** were recovered. A small amount of undetermined more polar products (about 50 mg) was observed.

Biotransformation of (4aS,8S)-4a,8-dimethyl-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone. **7** (100mg) was incubated in 200 ml of phosphate buffer with washed mycelium of *M.plumbeus* CBS 110-16 (20 g dry weight/L). After 4 days and complete disappearance of the substrate, two main polar products (45 and 36%) were detected by GPC. After extraction (160 mg) and flash chromatography, 40 mg (37%) of **8**, M.p.= 93-94°C, $[\alpha]_D^{21}$= +71 (c 0.21, $CHCl_3$), and 27.3 mg of **9** (25%), M.p. 102-103°C, $[\alpha]_D^{21}$= +62 (c 0.47, $CHCl_3$), were recovered. ^{13}C- and ^1H-NMR see Tables 3 and 7.

Biotransformation of (S)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone (**10**). **10** (300 mg) was incubated in the culture medium of *M.plumbeus* CBS 110-16 (ten 100 ml-containing flasks). After 5 days, most of the substrate had disappeared. Extraction (420 mg) and preparative thin layer chromatography afforded residual substrate (9 mg) and (following elution order) 4 main products: **11** (75 mg), colorless oil; $[\alpha]_D^{21}$= +40, $[\alpha]_{578}^{21}$= +42, $[\alpha]_{546}^{21}$= +50, $[\alpha]_{436}^{21}$= +131 (c 3.7, $CHCl_3$); HRMS, calc.for $C_{12}H_{18}O_2$, 194.130678, found 194.130735; MS, 194(100) M^+, 179(16), 161(20), 151(20), 137(41), 123(50). **12** (37.5 mg), pale yellow oil, crystallized in ether-hexane. **13** (70 mg), colorless oil containing two unseparated products. **14** (68 mg), M.p. 81-82°C (from ether-hexane); $[\alpha]_D^{21}$= +164, $[\alpha]_{578}^{21}$= +171, $[\alpha]_{546}^{21}$= +199, $[\alpha]_{436}^{21}$= + 391 (c 1.34, $CHCl_3$);HRMS, calc.for $C_{12}H_{18}O_2$, 194.130678, found 194.130735; MS, 194(15) M^+, 176(100) M-H_2O, 161(45), 148(18), 137(36), 119(28). ^{13}C- and ^1H-NMR see Tables 4 and 7.

Biotransformation of (S)-4a-methyl-4,4a,9,10-tetrahydro-2(3H)-phenanthrenone (**15**). S-**15** (350 mg) was incubated in the culture medium with *M.plumbeus* CBS 110-16 (seven 100 ml-containing flasks). After 2 days, all the substrate had disappeared and several polar products are detected by TLC. Extraction of the filtrate and of the mycelium (1.6 g) followed by flash chromatography afforded as main products: **16** (124 mg, 33%), pale yellow oil; $[\alpha]_D^{21}$= +208 (c 1.85, $CHCl_3$); **17** (61 mg, 16%), M.p. 145-148°C (amorphous solid from ether); $[\alpha]_D^{21}$= +105 (c 0.6, $CHCl_3$); a small amount of **18** (pale yellow oil, about 10 mg) could be isolated and purified by preparative thin layer chromatography. ^1H- and ^{13}C-NMR see Tables 5 and 7

Table 7: ^{13}C-NMR spectra of ketones **1**, **6**, **7**, **10** and **15** and Hydroxylated Derivatives (δ in ppm, $CDCl_3$). Multiplicity from DEPT 135 experiments.

	1	**2**	**3**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13#**	**14**	**15**	**16**	**17**
C-1	124.13	126.28	124.13	125.53	121.48	123.17	123.46	128.32	131.26	128.13	128.55	128.99	128.6*	128.2*	129.6*
C-2	199.67	200.56	199.44	208.19	199.92	201.06	199.75	199.15	200.22	197.42	198.87	198.92	198.7	198.9	198.1
C-3	34.00	34.20	33.64	33.58	33.74	34.60	33.45	33.82	34.09	37.60	33.25	33.28	34.7	34.6	34.6
C-4	38.90	39.33	38.44	39.74	38.29	40.23*	38.51*	37.68	39.09	74.37	37.78	37.46*	36.9	36.7	36.6
C-4a	35.96	35.27	35.53	38.84	36.24	35.82	35.70	36.21	35.24	41.64	35.77	36.92*	39.2	39.4	39.1
C-5	41.50	41.09	47.04	53.85	41.77	41.21*	37.88*	42.11	41.45	42.38	47.61	50.30	143.7**	142.5**	143.4**
C-6	21.78	16.12	66.51	198.46	21.62	17.30	30.91	21.46	15.63	21.03	66.27	66.13	134.7**	138.1**	135.5**
C-7	27.18	33.21	33.64	31.27	36.24	40.13*	75.85*	26.85*	33.25	26.04*	33.40	35.24	31.1	69.5	68.9
C-8	32.78	72.35	27.19	37.27	34.11	71.42	42.08	27.73*	66.62	27.59*	22.25	26.25	31.1	41.2	38.9
C-8a	170.55	167.84	170.57	170.39	173.66	169.83	171.31	163.02	159.62	162.22	162.62	160.00	169.8	166.4	164.8
C-9	-	-	-	-	-	-	-	-	-	-	-	-	126.9*	126.6*	129.4*
C-10	-	-	-	-	-	-	-	-	-	-	-	-	126.1*	125.4*	127.6*
C-11	-	-	-	-	-	-	-	-	-	-	-	-	126.1*	125.4*	129.9*
C-12	-	-	-	-	-	-	-	-	-	-	-	-	124.2*	125.6*	126.2*
CH_3-1	-	-	-	-	-	-	-	10.83	10.37	10.89	10.84	11.02	-	-	-
CH_3-4a	22.10	23.89	24.89	24.06	22.94	24.48	22.91	22.48	24.56	21.03	25.12	23.24	27.7	27.4	26.7
CH_3-8a	-	-	-	-	18.04	29.01	13.18	-	-	-	-	-	-	-	-

major component.
* or **, assigments could be reversed between the labelled carbons.

Biotransformation of (R)-4a-methyl-4,4a,9,10-tetrahydro-2(3H)-phenanthrenone (**ent-15**). R-**15** (150 mg) was incubated in the culture medium with *C.lunata* NRRL 2380 (three 100 ml-containing flasks). After 4 days, only 10 % of the substrate remained and one main product identical to **16** by TLC and GC was detected and purified from the filtrate by extraction and preparative thin layer chromatography (75 mg, 46%).

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