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MICROBIAL HYDROXYLATION AND FUNCTIONALIZATION OF SYNTHETIC BICYCLIC ENONES

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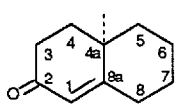
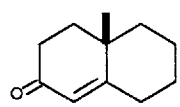
Abstract: The regio- and stereoselective hydroxylation of substituted octalones by various fungal strains has been evaluated. Several hydroxylated derivatives have been obtained and the potential of this method for the preparation of useful chiral synthons is discussed.

The functionalization of selected non-activated methylene groups of hydrocarbon compounds is considered as a crucial problem in organic synthesis. However, hydroxylation reactions, while constituting the simplest functionalization operation, remains a difficult chemical reaction. On the other hand, regio- and stereoselective microbial hydroxylation of natural cyclic substances, such as steroids[1,2], alkaloids or terpenes[1], has been extensively used for the production of numerous derivatives unapproachable by chemical methods. However, only few applications of such techniques to the regioselective functionalization of relatively simpler synthetic cyclic molecules, producing asymmetric specific synthons, have been described[3].

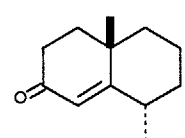
A very efficient method to prepare enantiomerically pure hexahydronaphthalenone **1**, bearing a methyl group on the quaternary asymmetric carbon has been described[4] and extended to the stereospecific synthesis of more substituted enones like **2** [5]. Such products may constitute pivotal intermediates in the total synthesis of terpenoids or steroids[4-7] with definite stereochemistry, provided that a regio- (and eventually stereo-) selective functionalization of the B ring may be easily achieved.



R-1



S-1



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The first data about hydroxy derivatives of hydronaphthalenones **1** arose from microbial reduction of the corresponding racemic 2,5-diketocompound (known as the Wieland-Miescher ketone) to diastereoisomeric mixtures of nearly optically pure (5S)-hydroxylated enones[8,9], thus achieving one of the possible enantio- and regioselective functionalizations of the B ring. The same reduction has been more recently applied to the elaboration of the corresponding 1-carboxymethyl substituted compound[10]. Unfortunately this microbial reduction only allows the preparation of (5S)-hydroxy derivatives. As regards to direct hydroxylation reactions, the only published data concern hydroxylation of **1** by *Rhizopus arrhizus*, as a model for steroid hydroxylations[11,13]. Mainly *cis*-8-hydroxy derivatives were obtained, a rather disappointing result as such compounds are easily accessible by (electro)chemical oxidation of the starting enone[12] or its enol ether[13].

We have thoroughly investigated the biotransformation of these substrates with several

commonly used fungal strains in order to detect the formation of different regioselectively hydroxylated products. Moreover, as enantiomers of **1** were available[4], it was possible to test whether a different behaviour of each enantiomer could be demonstrated.

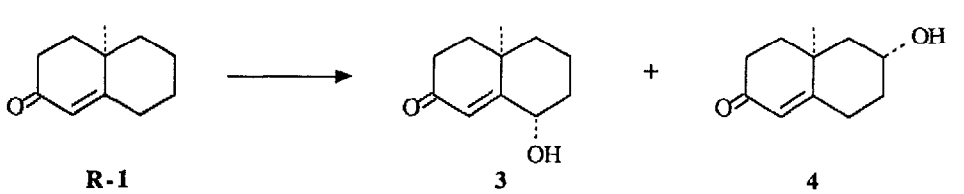
A number of fungal strains (see Table) were found to be able to convert the R-enantiomer of hydronaphthalenone **1**, added to the aerated culture medium, into several hydroxylated products, as shown by GC-analysis of the incubation supernatant. Most frequently, the main product was **3**, identified as the known *cis*-(4aR,8S)-8-hydroxy enone by comparison with published data[12,13], with no significant formation of the *trans*-isomer. This assignment[14] was confirmed by the ^{1}H-nmr data[15] and the analysis of correlation patterns observed in the 2D COSY spectra[16] which show a C*H*OH signal (δ= 4.29 ppm, t, J= 2.8 Hz) typical of an equatorial proton. 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 methyl signal of **3** with respect to **1** (Δδ= + 0.2 ppm) are in agreement with a 8-axial position of the newly introduced hydroxy group.

Table: Formation of hydroxylated products from **1** by incubation with various fungal strains[a]

Strains	incubation time (days)	%products obtained[b] from (R)-1 (R)-1	3	4	incubation time (days)	%products obtained[b] from (S)-1 (S)-1	ent-3	ent-4
Cunninghamella echinulata NRRL 3655	9	33	17	11	-	-	-	-
Mucor plumbeus MMP 430	9	42	47	-	-	-	-	-
Mucor plumbeus CBS 110-16	3	1	66	22	2	-	77[c]	-
Mucor aromaticus NRRL 1701	10	14	44	26	-	-	-	-
Mucor janssenii NRRL 3826	9	37	21	6	-	-	-	-
Mucor racemosus	9	74	20	1	-	-	-	-
Mucor rouxii NRRL 1430	9	58	21	-	-	-	-	-
Curvularia lunata NRRL 2380	8	16[c]	5[c]	42[c]	4	-	45[c]	-
Absidia glauca	8	14[c]	51[c]	8[c]	-	-	-	-
Beauveria bassiana ATCC 7159	5	4[c]	6[c]	36[c]	6	63	15	15

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**, 320 sec.; **3**, 606 sec.; **4**, 808 sec.). c) several other peaks present.

Interestingly, some strains produced another compound **4**, sometimes in equivalent amounts. Both products were easily obtained after incubation of R-**1** (0.45 g) with *Mucor plumbeus* CBS 110-16 [17], followed by extraction and separation by silica gel chromatography: **3** (125 mg), m.p.= 68°C, $[\alpha]_D^{21}$= –95°(c 1.04,$CHCl_3$); **4** (75 mg) m.p.=122-124°C, $[\alpha]_D^{21}$= –175° (c 1.01, $CHCl_3$).



R-1 **3** **4**

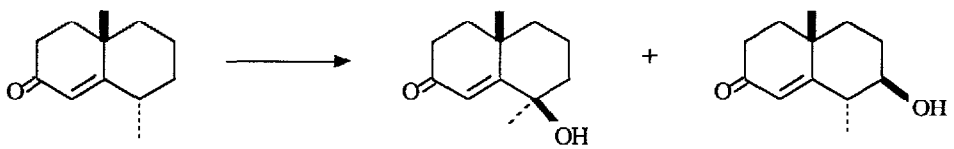
The structure of compound **4** was easily deduced from mono- and bidimensional ^{1}H-NMR experiments[15,16]. As for compound **3**, no modification of H-3 and H-4 coupling pattern was

observed, and the methyl group signal was shifted downfield by about + 0 2 ppm as a result of a 1,3-diaxial interaction with a *cis*-OH group; the equatorial 6β-hydrogen at 4.3 ppm resonated as a quintuplet (J= 2.8 Hz) characteristic of ax-eq and eq-eq couplings The 6α-ax position of the OH group was confirmed by the correlations observed in the 2D COSY 45 spectra, with a C*H*OH signal coupled to CH_2-5 and -7 (CH_2-8 was assigned thanks to the ^4J long range coupling between H-1 and H-8ax and the H-8ax, H-8eq correlation).

It is important to note that with several strains (see Table), nearly all the substrate was converted in the conditions used and that compounds **3** and **4** generally represented more than 70% of the crude conversion product, as estimated by GPC.

In the same incubation conditions, using S-**1** (0.2 g) as substrate, only **ent-3** (135 mg), m.p. 70-72°C, $[\alpha]_D^{21}$= + 96° (c 1.07,$CHCl_3$) could be obtained. As a rule, S-**1** was more rapidly hydroxylated than R-**1** (see Table), giving essentially (excepted with *B bassiana*) **ent-3**[19].

Using a similar incubation procedure, (4aS,8S)-**2** (0.5 g) was converted by incubation (4 days) with *M.plumbeus* CBS 110-16 into a mixture of (8R) and (7R)-hydroxy derivatives: **5** (40%) m.p.= 93-94°C, $[\alpha]_D^{21}$= + 71° (c 0.21,$CHCl_3$); **6** (27%) m.p.= 102-103°C, $[\alpha]_D^{21}$= + 62 °(c 0.47, $CHCl_3$). Both compounds were easily identified by ^{1}H-NMR[20]: the methyl groups signals of **5** resonated as singlets and consequently the hydroxy group had been introduced on C-8 and in a β-*cis* situation to the angular 4a-methyl group, in agreement with an observed Δδ of *ca.* +0.2 ppm with respect to the corresponding singlet of **2**. This was confirmed by the signals of the β-side protons (H-7eq, H-6ax and H-3ax), identified in the 2D COSY spectrum, which were also shifted downfield (*ca* +0.15, +0.2 and +0.15 ppm respectively). On the other hand, hydroxylation in **6** was assigned to a 7β(eq)-position owing to the correlation patterns observed in the 2D COSY spectrum[16] 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)- **2** **5** **6**

These results show that the microbial hydroxylation of substituted octalones may easily afford new and valuable regio- and stereoselectively functionalized synthons. Experiments are in progress to extend this concept to a whole range of other variously substituted hydronaphthalenones and hydrindenones.

References and notes:

1- See for example the corresponding chapters in "Biotechnology, a Comprehensive Treatise", H.-J.Rehm and G.Reed ed., vol.6a "*Biotransformations*", Verlag Chemie, Weinheim, 1984; H.Iizuka and A.Naito, "*Microbial Conversion of Steroids and Alkaloids*", University of Tokyo Press, Springer Verlag, Berlin, 1981.
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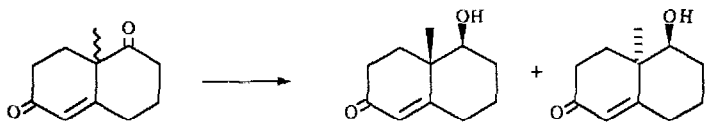
D.Dütting and V.Prelog, *ibid* , **41**, 1424 (1958); W.Aklin, V Prelog and A.P.Prieto, *ibid* , **41**, 1416 (1958); W.Aklin, V.Prelog and D.Zäch, *ibid* , **41**, 1428 (1958).

9- In order to obtain comparative analytical data, the (5S)-reduction products of (±) Wieland-Miescher ketone (tetrahydro-4a-methyl-2,5-naphthalenedione) were easily prepared by a procedure similar to that described by Prelog and coworkers (see ref 8), using *C lunata* NRRL 2380 as a reducing microorganism:



7 8

7 (4aS,5S), 46%, $[\alpha]_D^{21}$= +159° (c 0.6,MeOH); +183° (c 0.4,benzene) (lit. +203°)[9], ^{1}H-nmr ($CDCl_3$), δ ppm, J Hz: 5.71 (C*H*=,d,1.7), 3.35 (C*H*OH,dd,4,11), 2.73 (O*H*,br.s), 2.10-2.45 (2xCH_2,2m), 1.55-1.85 (2xCH_2,m), 1.30-1.42 (CH_2,m), 1.13(CH_3,s). **8** (4aR,5S), 36%, m.p. 88-90°C (lit. 94-95°C)[9]; $[\alpha]_D^{21}$= -125° (c 0.53,MeOH) (lit. -129°)[9]; ^{1}H-nmr ($CDCl_3$), δ ppm, J Hz: 5.85 (C*H*=,d,1.2), 3.63 (C*H*OH,br.s), 2.22-2.64 (2xCH_2 +O*H*,m+s), 1.46-2.08 (3xCH_2,m), 1 22(CH_3,s).

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14- Molecular modeling of the flexible bicyclic enone system, using the Alchemy II program (Tripos Associates Inc.), indicated that the most stable conformation of all compounds corresponded to a B cycle in a chair conformation, and the H-4 *trans* to the 4a methyl group in a quasi-axial position.

15-^{1}H-nmr ($CDCl_3$, 250 MHz), δ ppm, J Hz: **3**, 5.80(-C*H*=,s), 4.29(C*H*OH,t,2.8), 2.57(*H*-3ax,ddd,17.5, 14.5,5.5), 2.37 (*H*-3eq,dddd,17.5,4,3,1.5), 1.85-2.10 and 1.50-1.85 (4xCH_2,2m), 1.43 (CH_3,s); **4**, 5.74(-C*H*=,d,2), 4.24(C*H*OH,quint,2.8), 2.84(*H*-8ax,tdd,14.5,6,2), 2.50(*H*-3ax,ddd,17,14.5,5.2), 2.34 (*H*-3eq,dtd, J=17,2.8,1.2), 2.12(*H*-8eq,ddd,14.5,4.5,2.8), 1.53-1.95(3xCH_2,m), 1.46(CH_3,s). ^{13}C-nmr ($CDCl_3$, 62.9 MHz), δ ppm: **3**,.200.6(CO), 167.8(C-8a), 126.3(CH-1), 72.4(CHOH-8), 35 3(C-4a), 41.1, 39.3, 34.2, 33.2, 16.1(CH_2-3 to -7), 24.0(CH_3); **4**, 199.4(CO), 170.6(C-8a), 124.1(CH-1), 66.5 (CHOH-6), 35.5(C-4a), 47.0, 38.4, 33.6, 27.2(CH_2-3 to -5,-7 and -8), 24.9(CH_3).

16- 2D ^{1}H,^{1}H COSY nmr spectra were acquired at 250 MHz. A delay period of 0.08 s was used to emphasize long-range or small couplings (see ref.18). ^{13}C multiplicity was obtained from DEPT 135 experiments [D.M.Dodderell, D.T.Pegg and M.R.Bendall, *J Magn.Reson.*, **48**, 323 (1982)]

17- 250 ml erlenmeyer flasks containing each 100 ml of liquid medium (Corn steep liquor, 10g; K_2HPO_4, 2g; KH_2PO_4, 1g; $NaNO_3$, 2g; KCl, 0.5g; $MgSO_4,7H_2O$, 0.5g; $FeSO_4,7H_2O$, 0.02g; Glucose, 30g in water, 1L.) were inoculated with freshly obtained spores of *M.plumbeus* CBS 110-16. After 48 hours growth in an orbital shaker (27°C, 290 rpm), crystalline substrate (50mg) was added to each flask, and incubation was continued in the same conditions. After several days, when the substrate had nearly disappeared, the incubation medium was filtered with celite and the filtrate was repetitively extracted with EtOAc. The extract was evaporated to give the crude transformation product which was then submitted to silica gel flash chromatography.

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19-The original description (see.ref.13) of biohydroxylation of (±)**1** by *R.arrhizus* did not mention any data about the optical activity of the 8-*cis*-hydroxylated products. However, enrichment of the remaining substrate into the R(-)-compound, as observed by optical rotation measurements, indicated some enantioselectivity in the hydroxylation reaction (H.L.Holland, personal communication).

20-^{1}H-nmr (CDCl3, 250 MHz), δ ppm, J Hz: **5**, 6.01(C*H*=,s), 2.58(*H*-3ax,ddd,17.5,14.5,5.5), 2.37(*H*-3eq, ddd,17.5,4,2.5), 2.08 (*H*-7eq,dt,14.5,3.5), 1.50-1.90 (7xC*H*,4m), 1.42, 1.39 (CH_3-4a and -8, 2s); **6**,.5.88 (C*H*=,d,2,$w_{1/2}$=4), 3.22 (C*H*OH,dt,4.5,10.5), 2.50 (*H*-3ax,m), 2.35 (*H*-3eq,m), 1.69-2.35 (6xC*H*,4m), 1.41(*H*-5ax,dt,4.5,14), 1.23(CH_3-4a,s), 1.15(CH_3-8,d,6.5). ^{13}C-nmr (*CDCl*$_3$, 62.9 MHz), δ ppm: **5**, 201.1(CO), 169.8(C-8a), 123.2(CH-1), 71.4(COH-8), 35.8(C-4a), 41.2, 40.2, 40.1, 34.1, 17.3 (CH_2-3 to -7), 29.0, 24.5(CH_3-4a and-8); **6**, 199.8(CO), 170.3(C-8a), 123.5(CH-1), 75.9(CHOH-7), 42.1(CH-8), 38.5, 37.9, 33.5, 30.9(CH_2-3 to -6), 35.7(C-4a), 22.9(CH_3-4a), 13.2(CH_3-8).

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