49. The Allylic Oxidation of Geraniol Catalyzed by Cytochrome P-450$_{Cath.}$, Proceeding with Retention of Configuration[1])

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Dedicated to Prof. *D. Arigoni* on the occasion of his 60th birthday

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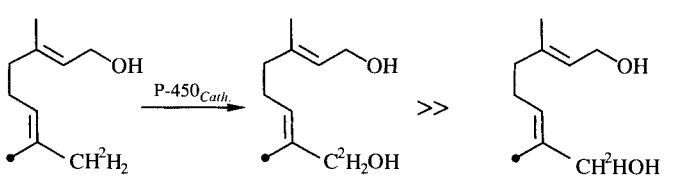
Incubation of the geraniols (*R*)-(8-$^2H_1$)[8-^3H_t]-**1** and (*S*)-(8-$^2H_1$)[8-^3H_t]-**1** with microsomal cytochrome P-450$_{Cath.}$ from the subtropical plant *Catharanthus roseus* (L.) G. DON resulted in the formation of the chiral 8-hydroxygeraniols (*S*)-(8-$^2H_1$)[8-^3H_t]-**2** and (*R*)-(8-$^2H_1$)[8-^3H_t]-**2**. Their absolute configuration was assigned on the basis of the ^{1}H-decoupled ^{3}H-NMR spectra of the corresponding dicamphanates (*S*)-(8-$^2H_1$)[8-^3H_t]-**9** and (*R*)-(8-$^2H_1$)[8-^3H_t]-**9**, of which the configurations are established in relation to the synthetic reference samples. The results clearly indicate retention of configuration during the allylic oxidation of **1**.

Introduction. – Information on the mechanism of the cytochrome-P-450-catalyzed allylic oxidation is limited to two fundamental studies by *Groves* and *Subramanian* [1] and *Nelson* and coworkers [2]. In the cases investigated, cyclic substrates were oxidized by microsomal P-450 to yield considerable amounts of stereochemically scrambled products, indicating that the allyl radical, presumably formed as an intermediate, is unexpectedly prone to allylic rearrangement faster than being trapped by the hydroxyl radical delivered from the porphyrin-Fe. Since it is not evident, whether this is a general behaviour of cytochrome P-450, related to the P-450 source, or due to the cyclic nature of the substrates, it is of general interest to investigate other systems.

Recently, we have shown that the allylic hydroxylation of geraniol (**1**) catalyzed by microsomal cytochrome P-450$_{Cath.}$ from the subtropical plant *Catharanthus roseus* (L.) G. DON proceeds regiospecifically in favor of the Me group (*E*)-configurated with respect to the chain at C(6) of **1** (*Scheme 1*) to yield 8-hydroxygeraniol as the only product (35–45%).

Scheme 1



(7-*methyl*-^{13}C, 8,8-$^2H_2$)-**1** (7-*methyl*-^{13}C, 8,8-$^2H_2$)-**2** (7-*methyl*-^{13}C, 8-$^2H_1$)-**2**

[1]) Presented in part at the '22nd Reaction Mechanisms Conference', Pittsburgh, 12.6.-16.6.88, at the '6th International Conference on Biochemistry and Biophysics of Cytochrome P-450', Vienna, 3.7.-8.7.88, and at the meeting on the 'Biocatalytic Synthesis of Organic Compounds', Saratoga Springs, NY, 8.-12.8.88.

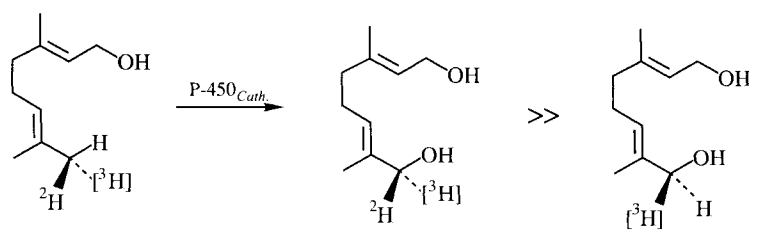
The 'product-determining' step displays a substantial intramolecular isotope effect of $k_H/k_D = 8.0$ [3]. These results are a prerequisite in order to investigate the stereospecificity of this reaction using geraniol samples which, due to isotopic labelling, are chiral at CH_3–C(7).

Since the pioneering work of *Cornforth et al.* [4] and *Arigoni* and coworkers [5], determination of the sense of chirality of isotopically modified Me and CH_2 groups by enzymatic analysis of the corresponding chirally labelled acetic acids has been established as the main source of evidence concerning the stereospecificity of enzymatic reactions involving substitution at or transfer of Me groups. In many laboratories, this type of analysis has become almost a routine, as excellently reviewed by *Floss* [6][7].

We reasoned that the mixture of the chirally labelled 8-hydroxygeraniols, *e.g.* (*R*)-(8-$^2H_1$)[8-$^3H_1$]-**2** and (*S*)-[8-$^3H_1$]-**2** originating from (*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1** under retention of configuration (*Scheme 2*), could be analysed in a way similar to the enzymatic analysis by only considering few molecules being chiral due to the ^{3}H-label. This can be accomplished, in principle, by analysis of the ^{1}H-decoupled ^{3}H-NMR spectra of appropriate derivatives of 8-hydroxygeraniol (**2**), which display magnetic non-equivalence of the ^{3}H-labels for the (*R*)- and (*S*)-configuration of **2**. Assignment of the absolute configuration is possible by correlation to reference samples of known absolute configuration.

Only few examples are known, which demonstrate the use of ^{3}H-NMR in order to follow the enzymatic transformations of chiral Me groups. *Altman et al.* [8] investigated the formation of the cyclopropane ring during the biosynthesis of cycloartenol, and *Crout* and coworkers [9] studied the conversion of valine, chirally labelled in one Me group, into cephalosporin. *Aberhart* and *Tann* [10] determined the stereochemical course of the dehydrogenation of isobutyryl CoA .

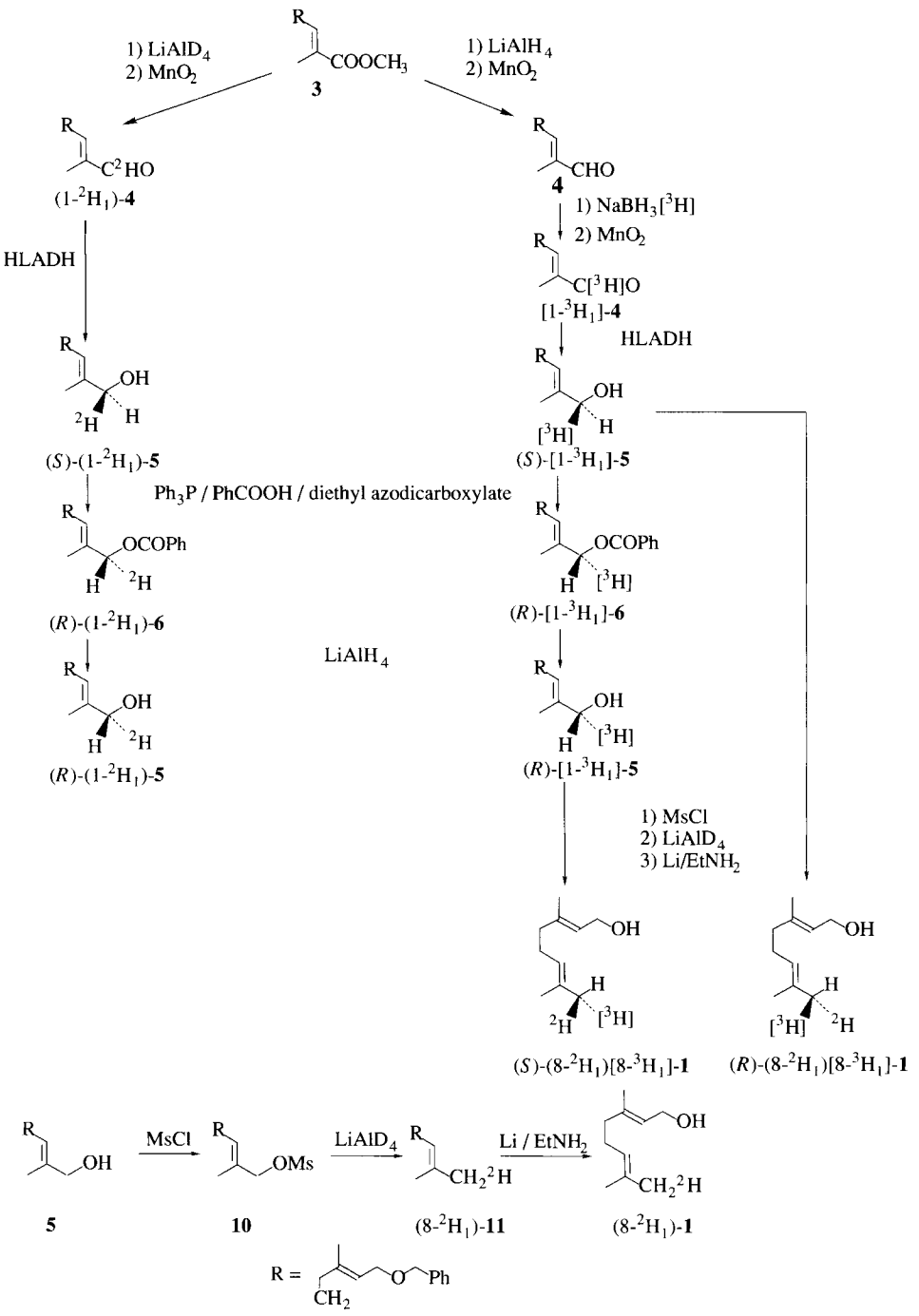
Scheme 2



(*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1** (*R*)-(8-$^2H_1$)[8-$^3H_1$]-**2** (*S*)-[8-$^3H_1$]-**2**

Results and Discussion. – The geraniols chiral at CH_3–C(7) due to isotopic labelling were synthesized as depicted in *Scheme 3*. The readily available ester **3** [3] as the starting material was converted into the three aldehydes **4**, [1-$^3H_1$]-**4**, and (1-$^2H_1$)-**4** according to established methods [3]. Subsequently, the labelled aldehydes were incubated with horse liver alcohol dehydrogenase (HLADH) to yield the chiral allyl alcohols (*S*)-[1-$^3H_1$]-**5** and (*S*)-(1-$^2H_1$)-**5**. Inversion of configuration was achieved according to the method of *Mitsunobu* and *Eguchi* [11] yielding the esters (*R*)-(1-$^2H_1$)-**6** and (*R*)-[1-$^3H_1$]-**6** which, after reduction, furnished the alcohols (*R*)-(1-$^2H_1$)-**5** and (*R*)-[1-$^3H_1$]-**5**, respectively. The alcohols (*R*)- and (*S*)-[1-$^3H_1$]-**5** were then mesylated, subsequent reduction with $LiAlD_4$ led to the formation of the chiral Me groups, and removal of the benzyl group with Li/$EtNH_2$ finally afforded geraniols (*R*)-(8-$^2H_1$)[8-$^3H_1$]-**1** and (*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1** displaying specific activities of 140 mCi/mmol and 162 mCi/mmol, respectively.

Scheme 3

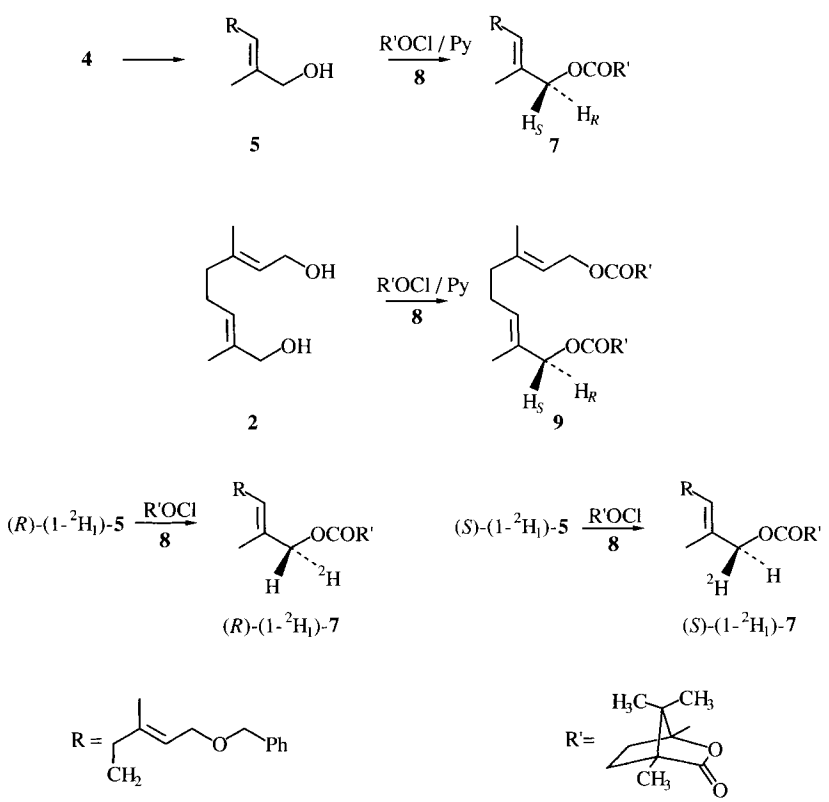

1) LiAlD4
2) MnO2
3
1) LiAlH4
2) MnO2
(1-2H1)-4
4
1) NaBH3[3H]
2) MnO2
[1-3H1]-4
HLADH
HLADH
(S)-(1-2H1)-5
(S)-[1-3H1]-5
Ph3P / PhCOOH / diethyl azodicarboxylate
(R)-(1-2H1)-6
(R)-[1-3H1]-6
LiAlH4
(R)-(1-2H1)-5
(R)-[1-3H1]-5
1) MsCl
2) LiAlD4
3) Li/EtNH2
(S)-(8-2H1)[8-3H1]-1
(R)-(8-2H1)[8-3H1]-1
MsCl
LiAlD4
Li / EtNH2
5
10
(8-2H1)-11
(8-2H1)-1
R =
Ph

The assignment of the absolute configuration of these geraniol samples is based on the (*re*)-selectivity of HLADH [12] and on consecutive reactions proceeding with either clean inversion or retention of configuration at the relevant C-atom.

The tritiated and deuterated chiral alcohols (*S*)-[1-$^3H_1$]-**5** and (*R*)-[1-$^3H_1$]-**5**, and (*S*)-(1-$^2H_1$)-**5** and (*R*)-(1-$^2H_1$)-**5**, respectively, were envisaged as reference material in order to correlate the ^{3}H-NMR analysis to the sense of chirality of the 8-hydroxygeraniol samples, enzymatically formed from geraniols of opposite configuration. *Gerlach* and *Zagalak* [13] had already shown that the (–)-camphanates (= (1*S*)-4,7,7-trimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carboxylates) of several primary alcohols display magnetic non-equivalence of the protons of the CH_2–O group in the presence of $Eu(dpm)_3$. Accordingly, the resonances of the *pro-R* H-atoms appear at higher field than those of the *pro-S* H-atoms.

Following the procedure in [13], camphanates **7** were prepared quantitatively from the corresponding chiral and achiral alcohols **5** by addition of the (–)-camphanoyl chloride **8** in abs. pyridine (*Scheme 4*). Surprisingly, the ^{1}H-NMR spectrum of unlabelled **7** displayed signals for 2 H–C(1) as two clearly separated *d* at 4.61 and 4.58 ppm in $CDCl_3$ without the addition of shift reagent. This is also valid for the resonances of the corresponding protons of the dicamphanate **9**, prepared from enzymatically formed 8-hydroxygeraniol (**2**). According

Scheme 4



to the ^{1}H-NMR spectra of the chiral (*R*)-(1-$^2H_1$)-**7** and (*S*)-(1-$^2H_1$)-**7**, the high-field signal corresponds to the *pro-R* H-atom and the low-field signal to the *pro-S* H-atom; the deviation of 0.01 ppm is attributed to an upfield isotopic shift due to the presence of the ge-minal ^{2}H [14]. In the ^{1}H-decoupled ^{3}H-NMR spectrum of the tritiated ester (*RS*)-[1-$^3H_1$]-**7** (specific activity 67.0 mCi/mmol), two *s* at 4.66 and 4.63 ppm have been observed, which were assigned to the *pro-S* and the *pro-R* H-atoms, respectively, according to the appearance of the corresponding signals of the pure diastereoisomeric esters (*S*)-[1-$^3H_1$]-**7** and (*R*)-[1-$^3H_1$]-**7** (*Fig. 1*).

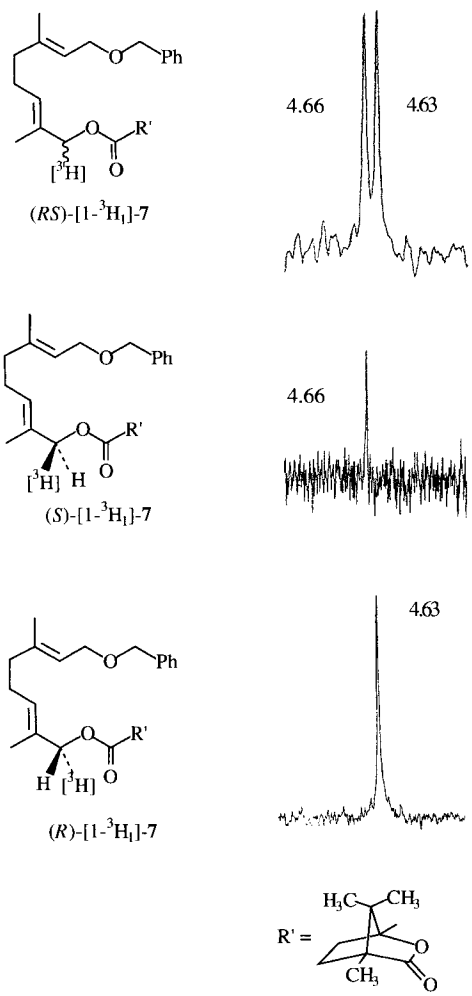


Fig. 1. *384-MHz ^{1}H-Decoupled ^{3}H-NMR spectra* (in $CDCl_3$) *of synthetic reference compounds.* Chemical shifts are given in ppm (δ (TMS) = 0).

Incubation of the chiral geraniols (*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1** and (*R*)-(8-$^2H_1$)[8-$^3H_1$]-**1** under standard conditions [3] with microsomal cytochrome P-450$_{Cath.}$ (isolated from 5-days-old seedlings of *Catharanthus roseus* (L.) G. DON) yielded radioactive 8-hydoxygeraniol samples in 40–45% yield; reaction with an excess of (–)-camphanoyl chloride **8** gave quantitatively two samples of dicamphanates **9** (specific activities: 132 mCi/mmol and 126 mCi/mmol, respectively), which were subjected to ^{1}H-decoupled ^{3}H-NMR spectroscopy. As shown in *Fig. 2*, the mixture of camphanates **9** originating from (*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1** contains (*R*)-(1-$^2H_1$)[1-$^3H_1$]-**9** and (*S*)-[1-$^3H_1$]-**9** in the ratio of 8:1, confirming the already determined intramolecular isotope effect of this reaction [3]. The ^{3}H-NMR signal of (*S*)-[1-$^3H_1$]-**9** appears at the same position as for the reference (*S*)-[1-$^3H_1$]-**7** (4.66 ppm), whereas the ^{3}H-NMR resonance of (*R*)-(1-$^2H_1$)[1-$^3H_1$]-**9** (4.61 ppm) is shifted upfield by 0.02 ppm, relative to the reference material (*R*)-[1-$^3H_1$]-**7** due to the presence of the geminal ^{2}H.

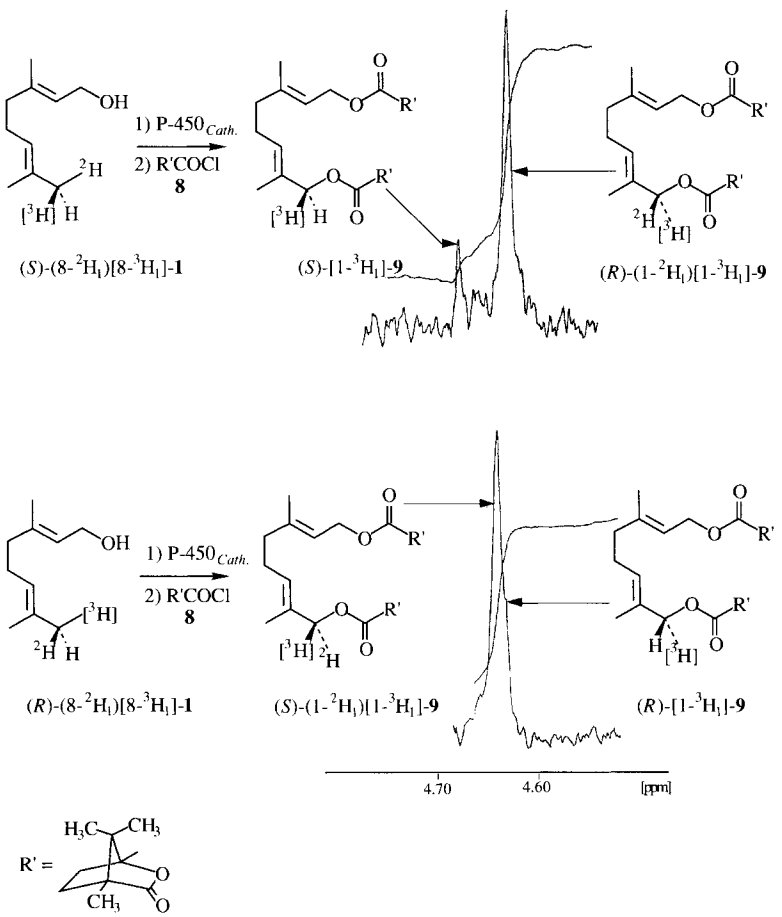
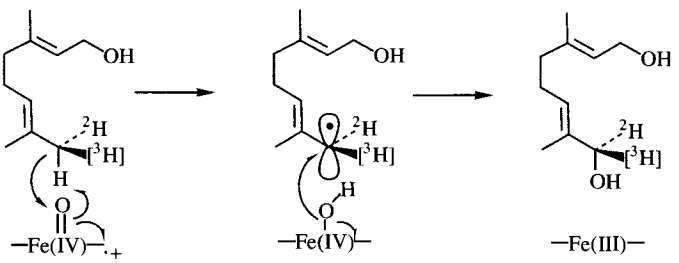


Fig. 2. *384-MHz ^{1}H-Decoupled ^{3}H-NMR spectra* (in $CDCl_3$) *of the derivatives of 8-hydroxygeraniol, obtained from enzymatic hydroxylation of geraniols with microsomal cytochrome P-450$_{Cath.}$.* Chemical shifts are given in ppm (δ(TMS) = 0).

In contrast, the enzymatic hydroxylation of (*R*)-(8-$^2H_1$)[8-$^3H_1$]-**1** yields hydroxygeraniols leading to a mixture of esters, (*S*)-(1-$^2H_1$)[1-$^3H_1$]-**9** being the major component, which gives rise to a ^{3}H-NMR signal at 4.64 ppm, shifted upfield by 0.02 ppm. The presence of (*R*)-[1-$^3H_1$]-**9** is deduced from a shoulder at 4.63 ppm. From these ^{3}H-NMR spectra, it can be estimated that the ^{3}H-labelled 8-hydroxygeraniols have been formed with >90% ee. Both experiments provide interlocking evidence that the P-450$_{Cath.}$-catalyzed allylic oxidation of geraniol proceeds with retention of configuration, indicating that trapping of the allylic radical by HO• transfer from the hydroxylated porphyrin-Fe(IV) is much faster than racemization (see **B**, *Scheme 5*). This result supports the general behaviour of P-450 based on the reaction-mechanism studies on P-450 hydroxylations in non-activated positions [15], however, this is the first example for a regio- and stereospecific allylic oxidation proceeding without rearrangement.

Scheme 5



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We are very much indebted to *U. Piantini* for recording the ^{1}H-NMR spectra.

Experimental Part

General. See [3]. Horse liver alcohol dehydrogenase (HLADH, *EC 1.1.1.1*; spec. act. 2.7 U/mg, 25°, EtOH as substrate) was purchased from *Boehringer*, Mannheim; NAD$^+$ and NADPH from *Fluka AG*, Buchs. *Plant Material and Preparation of Microsomes.* See [3]. *Standard Incubation and Assay Conditions.* See [3]. Chemical purity of all compounds was checked by TLC and GLC (> 95%) [3], the labelled compounds were identified by R_f and t_R of the corrresponding non-labelled parents. Radiochemical purity was checked by TLC radioactivity scanning [3]. The radioactivity of the ^{3}H-labelled compounds was determined by measuring aliquots in a *Packard Tri-Carb* liquid scintillation analyzer, model *2000 CA*. $[\alpha]_D$: *Perkin-Elmer 242* Polarimeter. ^{3}H-NMR spectra: *Bruker WH 360* (*Sandoz AG*, Basel; *H.-R. Loosli* and *L. Oberer*).

(2E,6E)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dienal (**4**) was obtained from the reduction ($LiAlH_4$) of *methyl (2E,6E)-8-(benzyloxy)-2,6-dimethylocta-2,6-dienoate* (**3**) [3], followed by MnO_2 oxidation in AcOEt. ^{1}H-NMR (200 MHz): 9.39 (*s*, 1 H); 7.37–7.27 (*m*, 5 H); 6.47 (*tq*, *J* = 7.1, 1.3, 1 H); 5.45 (*tq*, *J* = 6.7, 1.3, 1 H); 4.51 (*s*, 2 H); 4.04 (br. *d*, *J* = 6.7, 2 H); 2.55–2.19 (*m*, 4 H); 1.75 (br. *s*, 3 H); 1.68 (*s*, 3 H).

(2E,6E)-8-(Benzyloxy)-2,6-dimethyl(1-$^2H_1$)octa-2,6-dienal ((1-$^2H_1$)-**4**). Compound (1-$^2H_1$)-**4** was prepared by reduction ($LiAlD_4$) of **3** followed by MnO_2 oxidation. ^{1}H-NMR (200 MHz): identical chemical shifts as for **4**, but lacking the *s* at 9.39 ppm.

(2E,6E)-8-(Benzyloxy)-2,6-dimethyl[1-$^3H_1$]octa-2,6-dienal ([1-$^3H_1$]-**4**). A soln. of **4** (55.84 mg, 0.216 mmol) in i-PrOH (1.0 ml) was treated, at r.t. under Ar, with $NaBH_3[^3H]$ (0.396 mg, 0.0104 mmol; 100 mCi, 9.6 Ci/mmol,

according to *Amersham*, UK) for 2 h, then further $NaBH_4$ (3.80 mg, 0.1 mmol) was added to complete the reduction. After 15 min, the solvent was evaporated, the residue hydrolysed with H_2O (2 ml), and the aq. soln. extracted with CH_2Cl_2 (4 × 4 ml). The combined org. layers were dried (Na_2SO_4) and then evaporated to afford (*RS*)-[1-$^3H_1$]-**5** (55.40 mg, 98.5%; 68.7 mCi, 323 mCi/ mmol). Without further purification, a soln. of (*RS*)-[1-$^3H_1$]-**5** (52.17 mg, 0.20 mmol; 64.7 mCi) in AcOEt (2.8 ml) was subjected to oxidation with MnO_2 (0.5 g, 5.75 mmol) by stirring for 2 d at r.t. After filtration through *Celite*, evaporation of the solvent and TLC on SiO_2 ((i-Pr)$_2$O) afforded [1-$^3H_1$]-**4** (36.76 mg, 71%; 52.0 mCi, 80%, 365 mCi/ mmol).

(1S,2E,6E)-8-(Benzyloxy)-2,6-dimethyl(1-$^2H_1$)octa-2,6-dien-1-ol ((*S*)-(1-$^2H_1$)-**5**). A soln. of (1-$^2H_1$)-**4** (109.00 mg,0.42 mmol) in EtOH (15 ml) was introduced dropwise to a gently stirred potassium-phosphate buffer (600 ml, 0.05M, pH 7.0) containing edta (1 mM), albumine (60 mg), NAD^+ (100 mg), EtOH (2.5 ml), and HLADH (20 mg) during 1 h at 30°. After stirring for 40 h, the mixture was extracted with Et_2O (3 × 200 ml). The combined org. layers were dried (Na_2SO_4) and evaporated to yield a colourless oily residue from which, after separation by CC on SiO_2 (5 g, Et_2O/pentane 1:2), unreacted (1-$^2H_1$)-**4** (29.00 mg, 26.6%) and (*S*)-(1-$^2H_1$)-**5** (76.83 mg, 70.0%) were obtained: $[\alpha]_{598}^{22}$ = +0.25 (*c* = 2.02, EtOH). IR ($CHCl_3$): 2150*w*. ^{1}H-NMR (400 MHz): 7.35–7.27 (*m*, 5 H); 5.42–5.36 (*m*, 2 H); 4.51 (*s*, 2 H); 4.02 (*d*, *J* = 6.8, 2 H); 3.97 (br. *s*, H–C(1)); 2.19–2.06 (*m*, 4 H); 1.66 (br. *s*, 3 H); 1.65 (br. *s*, 3 H). Anal. calc. for $C_{17}H_{23}DO_2$ (261.38): C 78.12; found: C 77.90.

(1S,2E,6E)-8-(Benzyloxy)-2,6-dimethyl[1-$^3H_1$]octa-2,6-dien-1-ol ((*S*)-[1-$^3H_1$]-**5**). As decribed for (*S*)-(1-$^2H_1$)-**5**, a soln. of [1-$^3H_1$]-**4** (35.26 mg, 0.136 mmol; 49 mCi,360 mCi/mmol) in EtOH (5 ml) was reduced in a buffered soln. (100 ml; 20 mg albumine, 35 mg NAD^+, 0.8 ml EtOH) with HLADH (20 mg). Usual workup by extraction with Et_2O (5 × 20 ml) yielded, after separation by TLC on SiO_2 (3 plates, (i-Pr)$_2$O), unreacted [1-$^3H_4$]-**4** (9.35 mg, 27%; 13.8 mCi, 28%) and (*S*)-[1-$^3H_1$]-**5** (21.99 mg, 62%; 33.2 mCi, 68%, 393 mCi/mmol).

(1R,2E,6E)-8-(Benzyloxy)-2,6-dimethyl(1-$^2H_1$)octa-2,6-dien-1-yl Benzoate ((*R*)-(1-$^2H_1$)-**6**). According to the procedure of *Mitsunobu* and *Eguchi* [11], diethyl azodicarboxylate (DEAD; 175.0 mg, 1.0 mmol) in abs.THF (1.0 ml) was added dropwise to a soln. of (*S*)-(1-$^2H_1$)-**5** (130.50 mg, 80.50 mmol), Ph_3P (650.0 mg, 2.48 mmol), and benzoic acid (122.90 mg, 1.0 mmol) in THF (6.0 ml) at r.t. To complete the reaction, the mixture was stirred for further 20 min, the solvent was removed, and the product isolated from the residue by CC on SiO_2 (10 g, Et_2O/ pentane 1:9) to yield (*R*)-(1-$^2H_1$)-**6** (174.50 mg, 95.3%) as a colourless oil. ^{1}H-NMR (200 MHz): 8.05 (*m*, 2 H); 7.54 (*m*, 1 H); 7.45 (*m*, 2 H); 7.45 (*m*, 2 H); 7.40–7.26 (*m*, 5 H); 5.55 (*m*, 1 H); 5.42 (*m*, 1 H); 4.70 (br. *s*, H–C(1)); 4.50 (*s*, 2 H); 4.03 (*d*, 2 H); 2.25–2.06 (*m*, 4 H); 1.74, 1.66 (2*s*, 6 H).

Subsequent reduction of (*R*)-(1-$^2H_1$)-**6** by $LiAlH_4$ (20.15 mg, 0.53 mmol) in abs. Et_2O (3.5 ml) at 0–4° afforded, after usual workup and CC on SiO_2 (5 g, Et_2O/pentane 1:2), (*R*)-(1-$^2H_1$)-**5** (101.60 mg, 77.8% overall yield), spectroscopically (IR,^{1}H-NMR) identical with (*S*)-(1-$^2H_1$)-**5**. $[\alpha]_{589}^{22}$ = –0.25 (*c* = 3.54, EtOH). Anal. calc. for $C_{17}H_{23}DO_2$ (261.38): C 78.12; found: C 77.90 .

(1R,2E,6E)-8-(Benzyloxy)-2,6-dimethyl[1-$^3H_1$]octa-2,6-dien-1-yl Benzoate ((*R*)-[1-$^3H_1$]-**6**). As described above, (*S*)-[1-$^3H_1$]-**5** (32.46 mg, 0.125 mmol; 22 mCi, 176 mCi/mmol) was converted to (*R*)-[1-$^3H_1$]-**6** (43.68 mg, 96%; 19.4 mCi, 88%), which was then reduced with $LiAlH_4$ (4.60 mg, 0.12 mmol) in abs. Et_2O (0.9 ml) at 0–4° to give, after usual workup, (*R*)-[1-$^3H_1$]-**5** (30.67 mg, 94.5%; 19 mCi, 86%, 161 mCi/mmol).

(8-$^2H_1$)Geraniol (= *(E)-3,7-Dimethyl(8-$^2H_1$)octa-2,6-dien-1-ol*; (8-$^2H_1$)-**1**). As decribed in [3], **5** (104.10 mg, 0.40 mmol) was treated with MsCl (50.90 mg, 0.44 mmol) in the presence of Et_3N (160.0 mg, 1.58 mmol) and the resulting crude mesylate (1-$^2H_1$)-**10** immediately reduced with $LiAlD_4$ (42.0 mg, 1.0 mmol) in abs. THF (2.0 ml) at –10° to yield, after CC on SiO_2 (5 g, Et_2O/pentane 1:9), (8-$^2H_1$)-**11** (= *1-(benzyloxy)-2,6-dimethyl (8-$^2H_1$)octa-2,6-diene*; 62.70 mg, 64%). ^{1}H-NMR (200 MHz): 7.38–7.27 (*m*, 5 H); 5.40 (*tq*, *J* = 6.9, 1.2, 1 H); 5.10 (br. *m*, 1 H); 4.50 (*s*, 2 H); 4.03 (*d*, *J* = 6.9, 2 H); 2.13–2.02 (*m*, 4 H); 1.65 (br. *s*, 3 H); 1.60 (br. *s*, 2 H–C(8)); 1.57 (*s*, 3 H). Reductive ether cleavage of (8-$^2H_1$)-**11** (52.00 mg, 0.21mmol) by Li/1% Na afforded, after CC on SiO_2 (1.2 g, Et_2O/pentane 1:1) and bulb-to-bulb destillation (12 Torr), (8-$^2H_1$)-**1** (27.55 mg, 84%). IR ($CHCl_3$): 3620*m*, 3600–3300*w*, 3000*s*, 2970*m*, 2920*s*, 2170*w* (br.), 1670*m*, 1450*m*, 1385*s*, 990*s*. ^{1}H-NMR (400 MHz): 5.41 (*tq*, *J* = 7.0, 1.1, H–C(2)); 5.08 (*m*, H–C(6)); 4.15 (br. *d*, *J* = 7.0, 2 H–C(1)); 2.11–2.00 (*m*, 2 H–C(4), 2 H–C(5)); 1.67 (*s*, CH_3–C(3)); 1.66 (*t*, *J*(^{1}H,^{2}H) = 0.9, 2 H–C(8)); 1.59 (*s*, CH_3–C(7)); 1.13 (br. *s*, OH). EI-MS: 155 (2, $M^{+\cdot}$), 137 (5), 124 (7), 111 (7), 93 (27), 80 (10), 71 (11) 70 (100), 69 (19), 68 (22), 67 (10). Anal. calc. for $C_{10}H_{17}DO$: C 77.36; found: C 77.14 .

(S)-(8-$^2H_1$)[8-$^3H_1$]Geraniol ((*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1**). In the presence of Et_3N (42.80 mg, 0.424 mmol) (*R*)-[1-$^3H_1$]-**5** (27.50 mg, 0.106 mmol; 17.7 mCi, 167 mCi/ mmol) was treated with MsCl (13.60 mg, 0.118 mmol) as described above, the resulting crude mesylate (*R*)-[1-$^3H_1$]-**10** immediately reduced with $LiAlD_4$ (8.40 mg, 0.20 mmol) in abs.THF (2.5 ml) at –10°, to yield, after usual workup, (*S*)-(8-$^2H_1$)[8-$^3H_1$]-**11** (24.10 mg, 93%; 16.5 mCi, 168 mCi/ mmol). Without further purification, reductive ether cleavage by Li/1% Na was carried out to afford,

after usual workup and TLC on SiO_2 ((i-Pr)$_2$O), pure (*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1** (12.78 mg, 77.7%; 13.4 mCi, 75.7%, 162 mCi/mmol).

(R)-(8-$^2H_1$)[8-$^3H_1$]Geraniol ((*R*)-(8-$^2H_1$)[8-$^3H_1$]-**1**). Conversion of (*S*)-[1-$^3H_1$]-**5** (22.52 mg, 0.086 mmol; 14.7 mCi, 171 mCi/mmol) to (*R*)-(8-$^2H_1$)[8-$^3H_1$]-**11** (18.94 mg, 89%; 11.6 mCi, 79%) was accomplished as described above. Reductive ether cleavage by Li/1% Na afforded, after usual workup and TLC on SiO_2 ((i-Pr)$_2$O), pure (*R*)-[8-$^3H_1$](8-$^2H_1$)-**1** (9.74 mg, 73%; 8.8 mCi, 60%, 140 mCi/mmol).

Preparation of (–)-Camphanates According to Gerlach *and* Zagalak [13]. a) *(2E,6E)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dien-1-yl (1S)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate* (**7**). A soln. of **5** (19.37 mg, 0.074 mmol) in pyridine (0.15 ml) was treated with (–)-camphanoyl chloride (**8**), 17.80 mg, 0.082 mmol) at r.t. for 5 h. The pyridine was evaporated and the residue purified by CC on SiO_2 (1 g, Et$_2$O/pentane 1:1) to yield **7** (31.49 mg, 97.5%) pure by TLC (SiO_2, (i-Pr)$_2$O): R_f 0.36. IR ($CHCl_3$): 3040*w*, 3000*m*, 2970*m*, 2930*m*, 2860*m*, 1785*s*, 1730*s*, 1670*w*, 1500*w*, 1450*m*, 1400*w*, 1380*w*, 1340*w*, 1310*m*, 1270*s*, 1170*m*, 1105*s*, 1065*s*, 1020*w*, 990*w*, 930*w*. ^{1}H-NMR (400 MHz): 7.35–7.25 (*m*, 5 arom. H); 5.48 (*tq*, *J* = 6.8, 1.0, H–C(3)); 5.38 (*tq*, *J* = 6.8 ,1.2, H–C(7)); 4.61 (*d*, J_{gem} = 11.9, H_S–C(1)); 4.58 (*d*, J_{gem} = 11.9, H_R–C(1)); 4.51 (*s*, 2 PhCH_2); 4.02 (*d*, *J* = 6.8, 2 H–C(8)); 2.18–2.04 (*m*, 2 H–C(4), 2 H–C(5)); 2.45–2.36, 2.02–1.97, 1.92–1.85, 1.73–1.60 (4*m*, 4 H); 1.65, 1.63 (br. *s*, 6 H, CH_3–C(2), CH_3–C(6)); 1.09, 1.03, 0.94 (*s*, 9 H). CI-MS: 441(7, M^+ + 1), 349 (5), 333 (56), 135 (100). Anal. calc. for $C_{27}H_{36}O_5$ (440.58): C 73.61, H 8.24; found: C 73.34, H 8.54.

b) *(S)-(1-$^2H_1$)-***7**. As described above, (*S*)-(1-$^2H_1$)-**5** (13.07 mg, 0.05 mmol) was converted with **8** (11.92 mg, 0.055 mmol) in pyridine (0.1 ml) to yield, after TLC on SiO_2 (2 plates, (i-Pr)$_2$O, R_f 0.36), (*S*)-(1-$^2H_1$)-**7** (18.60 mg, 84.2%). IR ($CHCl_3$): 2150*w* (br.). ^{1}H-NMR (400 MHz): 4.57 (br. *s*, H–C(1)). CI-MS: 442 (2, M^+ + 1), 350 (5), 334 (100), 136 (81). Anal. calc. for $C_{27}H_{35}DO_5$ (441.59): C 73.44; found: C 73.23.

c) *(R)-(1-$^2H_1$)-***7**. In a similar reaction as described in *b*, (*R*)-(1-$^2H_1$)-**5** afforded (*R*)-(1-$^2H_1$)-**7** (20.20 mg, 91.5%), showing identical R_f, IR, and MS as (*S*)-(1-$^2H_1$)-**7**. ^{1}H-NMR (400 MHz): 4.60 (br. *s*, H–C(1)). Anal. calc. for $C_{27}H_{35}DO_5$ (441.59): C 73.44; found: C 73.17.

d) *(RS)-[1-$^3H_1$]-***7**. Esterification of (*RS*)-[1-$^3H_1$]-**5** (15.34 mg, 0.059 mmol; 4.01 mCi, 68 mCi/mmol) with **8** (14.08 mg , 0.065 mmol) in pyridine (0.15 ml) furnished (*RS*)-[1-$^3H_1$]-**7** (22.62 mg, 87%; 3.43 mCi, 85.5%, 67 mCi/mmol). ^{1}H-NMR (360 MHz): identical resonances as for **7**. ^{3}H-NMR (384 MHz); *i*) ^{1}H-coupled spectrum: 4.66 (*d*) and 4.63 (*d*) with $J(^3H,^1H)$ = 12. *ii*) ^{1}H-decoupled spectrum: 4.66 (*s*) and 4.63 (*s*) with the same intensity.

e) *(S)-[1-$^3H_1$]-***7**. Esterification of (*S*)-[1-$^3H_1$]-**5** (7.02 mg, 0.027 mmol; 2.1 mCi, 78mCi/mmol) with **8** (7.00 mg, 0.032 mmol) in pyridine (0.1 ml) furnished (*S*)-[1-$^3H_1$]-**7** (10.90 mg, 91.5%; 2.0 mCi, 95%, 81mCi/mmol). ^{1}H-NMR (360 MHz): identical resonances as for **7**. ^{3}H-NMR (384 MHz, ^{1}H-decoupled): 4.66 (*s*, [^{3}H]–C(1)); lacking 4.63 (*s*).

f) *(R)-[1-$^3H_1$]-***7**. Esterification of (*R*)-[1-$^3H_1$]-**5** (7.08 mg, 0.027 mmol; 1.4 mCi, 52 mCi/mmol) with **8** (7.00 mg) in pyridine (0.1 ml) furnished (*R*)-[1-$^3H_1$]-**7** (10.69 mg, 90%; 1.2 mCi, 86%, 50 mCi/ mmol). ^{1}H-NMR (360 MHz): identical resonances as for **7**. ^{3}H-NMR (384 MHz, ^{1}H-decoupled): 4.63 (*s*, [^{3}H]–C(1)); lacking 4.66 (*s*).

g) *Dicamphanate of 8-Hydroxygeraniol (= 2,6-Dimethylocta-2,6-diene-1,8-diyl Di(4,7,7-trimethyl-3-oxo-2-oxa[2.2.1]heptane-1-carboxylate)*; **9**). In a similar reaction, **2** (10.20 mg, 0.06 mmol) was treated with **8** (31.20 mg, 0.14 mmol) in pyridine (0.25 ml) to yield, after evaporation and CC on SiO_2 (4 g, Et$_2$O), **9** (27.56 mg, 86.6%), pure by TLC (SiO_2, Et$_2$O, R_f 0.54). IR ($CHCl_3$): 3020*m*, 2970*m*, 2930*m*, 1785*s*, 1730*s*, 1670*w*, 1450*m*, 1400*m*, 1315*m*, 1275*s*, 1170*s*, 1100*s*, 1060*s*, 1020*m*, 990*w*, 960*w*, 910*m*. ^{1}H-NMR (400 MHz): 5.48 (*tq*, *J* = 7.0,1.0, H–C(3)); 5.37 (*tq*, *J* = 7.1, 1.2, H–C(7)); 4.76 (*dd*, *J* = 12, 7, H_S–C(8)); 4.71 (*dd*, *J* = 12, 7, H_R–C(8)); 4.61 (*d*, *J* = 11.9, H_S–C(1)); 4.58 (*d*, *J* = 11.9, H_R–C(1)); 2.46–2.39 (*m*, 2 H); 2.20–2.07 (*m*, 2 H–C(4),2 H–C(5)); 2.07–1.99 (*m*, 2 H); 1.96–1.88 (*m*, 2 H); 1.73 (br. *s*, CH_3–C(6)); 1.67 (*s*, CH_3–C(2)); 1.11, 1.10, 1.06, 1.05, 0.96, 0.95 (*s*, 18 H). CI-MS: 531 (2, M^+ + 1), 333 (33), 135 (100).

*Incubation of (S)-(8-$^2H_1$)[8-$^3H_1$]-***1** *with Cytochrome P-450$_{Cath.}$*. A soln. of 2.97 mg of (*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1** (0.019 mmol, 3.13 mCi) in 1.5 ml of acetone was incubated under standard conditions in two identical assays, as described in [3], for 45 min. After usual workup, the substrate ((*S*)-(8-$^2H_1$)[8-$^3H_1$]-**1**/(8-$^2H_1$)-**1**; 1.26 mCi, 40.3%) and the product ((*R*)-(8-$^2H_1$)[8-$^3H_1$]-**2**/(*S*)-[8-$^3H_1$]-**2**/(*R*)-(8-$^2H_1$)-**2**/(*S*)-(8-$^2H_1$)-**2/2**; 1.22 mCi, 40.0%) were separated by chromatography on 2 anal. plates (Al_2O_3, $CHCl_3$/MeOH 19:1). The product fraction was purified by TLC (SiO_2, Et$_2$O) and the isolated pure mixture ((*R*)-(8-$^2H_1$)[8-$^3H_1$]-**2**/(*S*)-[8-$^3H_1$]-**2**/(*R*)-(8-$^2H_1$)-**2**/(*S*)-(8-$^2H_1$)-**2/2**; 1.09 mCi, 34.8%) was analysed by ^{1}H-NMR (400 MHz): all resonances identical with those of **2** [3], except the signal at 4.00 (H–C(8)), splitted in a *s* at 4.00 and a br. *s* at 3.98, due to 2 H–C(8) of **2** and 2 H–C(8) of (*R*)-(8-$^2H_1$)-**2**/(*S*)-(8-$^2H_1$)-**2**, resp. The mixture (1.09 mCi, 0.0067 mmol) was treated with **8** (4.0 mg, 0.018 mmmol) in pyridine (0.1 ml) at r.t. for 2h. Evaporation of the pyridine followed by TLC purification (SiO_2, (i-Pr)$_2$O) afforded a pure fraction ((*R*)-(1-$^3H_1$)[1-$^3H_1$]-**9**/(*S*)-[1-$^3H_1$]-**9**/(*R*)-(1-$^2H_1$)-**9**/(*S*)-(1-$^2H_1$)-**9/9**; 3.27 mg; 1.06 mCi, 97.2%), identical with a synthetic reference sample of **9** by TLC (Et$_2$O). ^{1}H-NMR (400 MHz): 4.61, 4.58 (2*d*) due to H_S–C(1) and H_R–C(1)

of **9**, resp, overlapped by br. *s* at 4.60 and 4.57 (H–C(1)) originating from *ca.* equal amounts (estimated by integral) of (*R*)-(1-$^2H_1$)-**9** and (*S*)-(1-$^2H_1$)-**9**, resp. ^{3}H-NMR (384 MHz): peak area at 4.61(*s*, [^{3}H]–C(1) of (*R*)-(1-$^2H_1$)[1-$^3H_1$]-**9**)/peak area at 4.66([^{3}H]–C(1) of (*S*)-[1-$^3H_1$]-**9**) = 8.

*Incubation of (R)-(8-$^2H_1$)[8-$^3H_1$]-**1** with Cytochrome P-450$_{Cath.}$.* A soln. of 2.05 mg (*R*)-(8-$^2H_1$)[8-$^3H_1$]-**1** (0.013 mmol; 1.85 mCi) in 1.0 ml of acetone was incubated under standard conditions in two identical assays described in [3] for 45 min. As described above, separation of the substrate ((*R*)-(8-$^2H_1$)[8-$^3H_1$]-**1**/(8-$^2H_1$)-**1**; 0.85 mCi, 45.9%) and the product ((*S*)-(8-$^2H_1$)[8-$^3H_1$]-**2**/(*R*)-[8-$^3H_1$]-**2**/(*R*)-(8-$^2H_1$)-**2**/(*S*)-(8-$^2H_1$)-**2/2**; 0.75 mCi, 40.5%) and purification of the latter fraction gave a pure probe ((*R*)-(8-$^2H_1$)[8-$^3H_1$]-**2**/(*R*)-[8-$^3H_1$]-**2**/((*R*)-(8-$^2H_1$)-**2**/(*S*)-(8-$^2H_1$)-**2/2**; 0.68 mCi, 36.8%) analysed by ^{1}H-NMR (400 MHz): all resonances identical with those of a synthetic reference sample of **2** [3], except 4.00 (H–C(8)). This signal is splitted in a *s* at 4.00 and a br. *s* at 3.98 ppm, due to 2 H–C(8) of **2** and H–C(8) of (*R*)-(8-$^2H_1$)-**2**/(*S*)-(8-$^2H_1$)-**2**, resp. The mixture (0.68 mCi, 0.0049 mmol) was treated with **8** (4.0 mg, 0.018 mmol) in pyridine (0.1 ml) at r.t. for 2 h. Evaporation of the pyridine followed by TLC purification (SiO_2, (i-Pr)$_2$O) afforded the pure mixture (*R*)-(1-$^2H_1$)[1-$^3H_1$]-**9**/(*R*)-[1-$^3H_1$]-**9**/(*R*)-(1-$^2H_1$)-**9**/(*S*)-(1-$^2H_1$)-**9/9** (2.49 mg; 0.63 mCi, 92,6%), identical with a synthetic sample of **9** by TLC (Et_2O). ^{1}H-NMR (400 MHz): as for the mixture (*R*)-(1-$^2H_1$)[1-$^3H_1$]-**9**/(*R*)-[1-$^3H_1$]-**9**/(*R*)-(1-$^2H_1$)-**9**/(*S*)-(1-$^2H_1$)-**9/9**. ^{3}H-NMR (384 MHz): 4.64(*s*, [^{3}H]–C(1) of (*S*)-(1-$^2H_1$)[1-$^3H_1$]-**9**); 4.63(br. *s*, [^{3}H]–C(1) of (*R*)-[1-$^3H_1$]-**9**).

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