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Chemobiological Transformations of Octalone and Hydrindenone Derivatives

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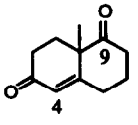
Abstract : Examples are given for allylic hydroxylations with *Rhizopus arrhizus* ATCC 11145. Chemical allylic hydroxylations are compared with microbiological ones. Stereoselective lipase-catalyzed hydrolyses are used efficiently.

The Wieland-Miescher ketone **1** and its lower homolog the Hajos-Parrish ketone **2** are easily available in both enantiomerically homogenous forms. They have been used in a large variety of



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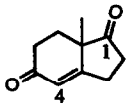

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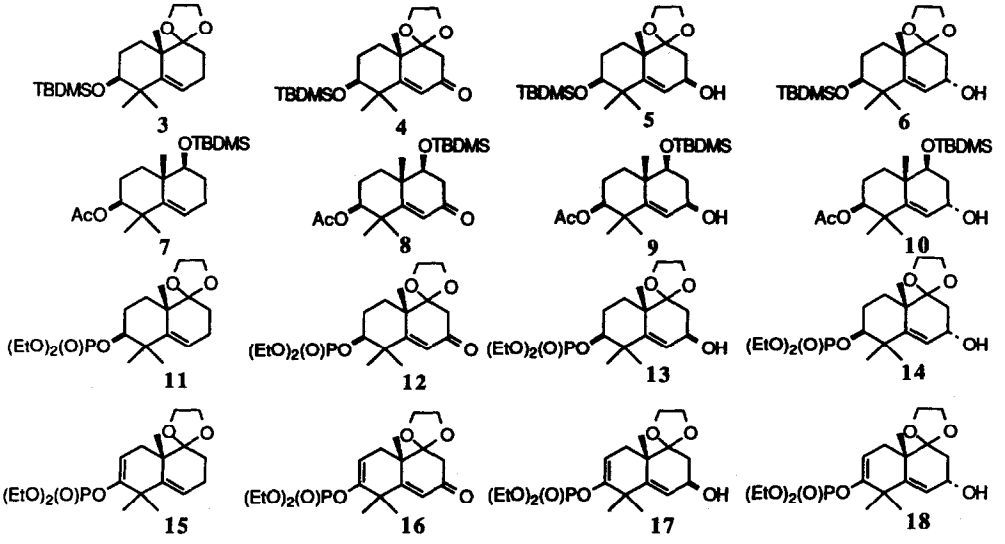
classical syntheses of steroid derivatives, and we have ourselves used S(+)-**1** and R(-)-**2** to build rings A/B and D/E respectively of pentacyclic precursors of isoarborinol and analogues.[1] If they could be transformed chemo- and stereoselectively into more heavily oxygenated derivatives, they would provide very useful precursors for the syntheses of many biologically active polyterpenoids. We have thus undertaken to functionalize further some of their derivatives. Various chemical oxidations have been first studied, in the 4,4-dimethyl decalin series. Their relative poor performance has then led us to study on the same substrates and on hydrindane and other decalin derivatives the use of microbiological oxidations, with much more success. This article describes both approaches, as well as some results obtained in the combined use of these microbiological oxidations and of enzymatic hydrolyses.

Our substrates have been either directly derived from ketones **1** and **2**, or substituted at C-4 (e.g. 4-methoxycarbonylated, 4-methylated, or mostly 4,4-dimethylated).

Chemical transformations

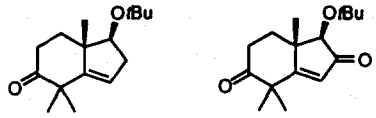
The allylic oxidation of substrates **3, 7, 11 and 15** was studied using classical methods. While selenium dioxide and its variants were unsuccesful, the best results in chromic oxidation were obtained with *t*-

butyl hydroperoxide in the presence of chromium hexacarbonyl;[2] the yields of 7-ketones remained low to moderate (38 - 64 %). The reduction of the 7-ketone **8** follows an easily predictable course : as in the steroid series, the α-side is unhindered, and the 7β–alcohol **9** is obtained very selectively, whatever the hydride used (sodium borohydride or S-Selectride®). For the 7-ketones bearing at C-9 the dioxolane ring, *i.e.* carrying at C-9 an α- C-O substituent, quasi-axial, α- attack of a large reagent must be disfavored; L-Selectride® gave indeed exclusively the 7α-alcohols **6, 14 and 18**, while the less sterically demanding sodium borohydride gave a mixture of the α- and the β- alcohols.



The maximum overall yield achieved was 33 % for the β-, 31 % for the α-hydroxylation, not counting the impediment and yield decrease inherent in the protection-deprotection steps. The chemical route to allylic hydroxylation was therefore quite unattractive in the decalin series.

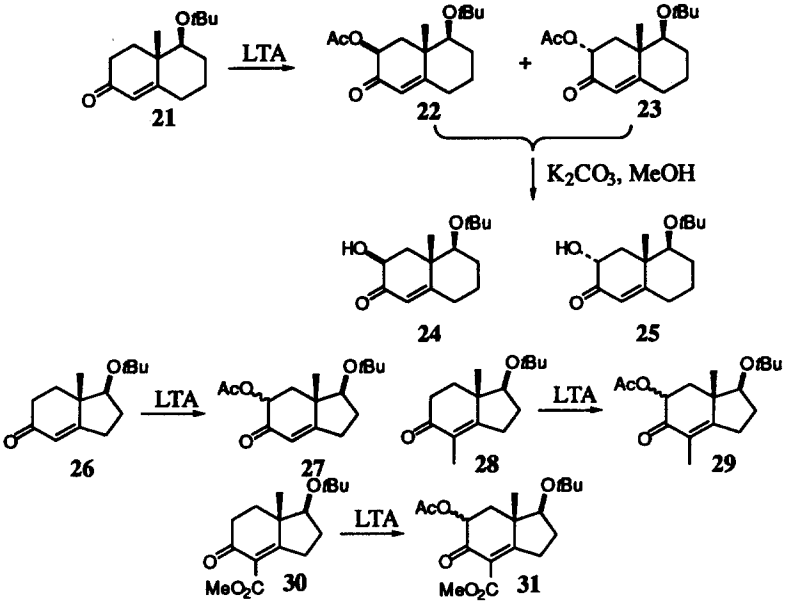
It was not more efficient in the hydrindane series. Oxidation of ketone **19** with *t*-butyl hydroperoxide in the presence of chromium hexacarbonyl gave only a 25 % yield of the conjugated ketone **20.** In both series, we shall see that allylic biohydroxylation is much more efficient.



We have also used another classical chemical method to introduce hydroxyls in the bicyclic systems : oxidation α to a ketone with lead tetracetate in refluxing benzene. This followed published procedures[1] on several substrates. In the decalone series, the *t*-butyl ether **21** gave quantitatively a 60 :40 mixture of the two acetates **22** and **23** which gave, upon alkaline hydrolysis at room temperature, the mixture of alcohols **24** and **25** (we shall see below how an enzymatic hydrolysis gave access to optically pure **24** and **25**). The same has now been found to hold (in 70 % yield at least) for the analogous substrates **28** and **30**. In the hydrindane series, the t-butyl ether **26** had given us, with lead tetracetate, a quantitative yield of the unseparable mixture of acetates **27**.[1] Chemical hydrolysis (K_2CO_3-MeOH) led to some epimerization : at room temperature, only the unwanted 6α-alcohol was obtained and, at 0°C, a 80-20 mixture of the 6α and 6β

alcohols. A further drawback of the chemical hydrolysis is that it may give poor yields[3] and often, upon scaling-up, a considerable amount of unidentified rearrangement products was obtained.

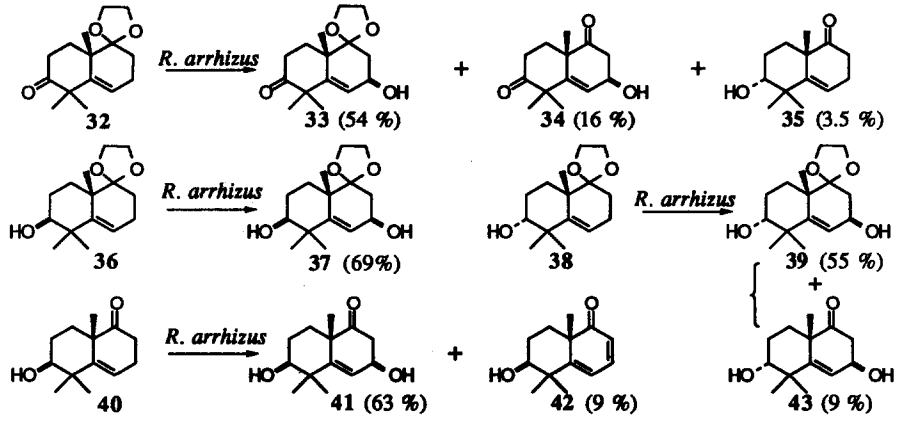
The chemical route to hydroxylation α to ketones was therefore also unattractive, and this was the motivation of our study of enzymatic hydrolysis, described below, which gave access to the pure



diastereomeric alcohols corresponding to **27**, **29** and **31** (OH instead of OAc), and hence, by acetylation, to the pure acetates **27** α and β, **29** α and β, **31** α and β.

Allylic Biohydroxylations. The Wieland-Miescher Series.

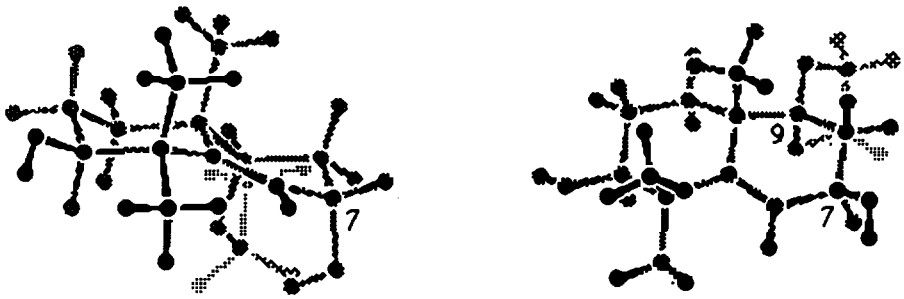
The 11α-hydroxylation of the non-activated 11-position of progesterone by the fungus *Rhizopus arrhizus* was the first reported bioconversion of synthetic significance.[4] Since then, a very large variety of



similar reactions have been studied,[5] using the same microorganism or others. We have found it to be very efficient for our goals.

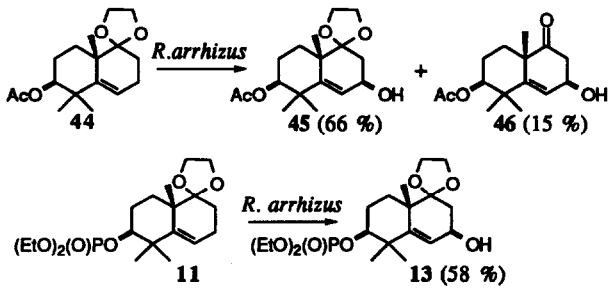
In our case, *R. arrhizus* (strain ATCC 11145) was found to hydroxylate the allylic positions of a wide range of unsaturated decalin and hydrindane derivatives regio-, stereo- and chemoselectively, in 1/2 - 2 days, often with excellent yields.[6]

For instance, the allylic hydroxylation of **32** was efficient (11 h) without protection of the 3-keto group; it gave some hydrolysis of the 9-ketal, and only very little reduction of the free ketone (to the 3α-alcohol **35**); the 7-alcohols obtained (**33** and **34**) were exclusively the β-diastereomers (70 % 7β hydroxylation), and the separation of the three products **33-35** was easy by flash chromatography. The corresponding 3β and 3α alcohols **36** and **38** were transformed, again without protection at C-3, to the allylic alcohols **37** (69%) and **39** + **43** (64%) respectively. The structure of the products was deduced from their ^{1}H-NMR spectra, which could be compared with those published for 7α- and 7β-hydroxycholesterols[7]. Such a comparison implies of course that the conformations of the bicyclic and the steroidal substances be identical, which is not obvious as the bicyclic system could well be sufficiently flexible to adopt a different conformation. We thus ran a computer-assisted analysis of **6** (3-OH instead of TBS) and **37** via Still's MacroModel program and, from the dihedral angles, we calculated by the Karplus equation the vicinal coupling constants between H-7 and its first neighbours. These vicinal coupling constants are in excellent agreement with those measured from the 400 MHz ^{1}H-NMR spectra. The 7β-hydroxyl group of **37** is indeed quasi-equatorial, as the dihedral angles of H-7α with H-6, H-8α and H-8β lead to predicted Js of 9.1, 7.0 and 3.2 Hz, very close to the observed values of 9.6, 7.2 and 3.0 Hz. For the 7α–diastereomer **6** (3-OH instead of 3-OTBS), obtained by the chemical route, the calculated conformation (Figure 1) leads to predicted



Conformations by MacroModel of products **6** and **37** (3-OH)
Figure 1

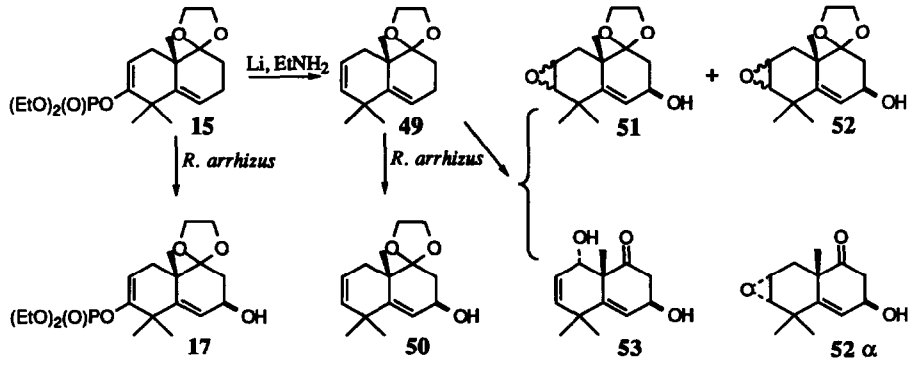
values of the Js, for H-7β (equatorial) with H-6, H-8α and H-8β, of 5.9, 4.0 and 1.5 Hz (found : 4.7, 4.6 and 1.0 Hz).



The 3-position may carry other functions than a free hydroxyl or a ketone. The 3-acetate **44** and the 3-diethyl phosphate **11** gave the corresponding 7β-hydroxy derivatives **45** and **13** , after a relatively short

incubation period (48 h) with *R. arrhizus*. These 7β-alcohols are those unavailable by the chemical route. However, the biohydroxylation of acetate **44** also gave some deprotection at C-9 to **46**.

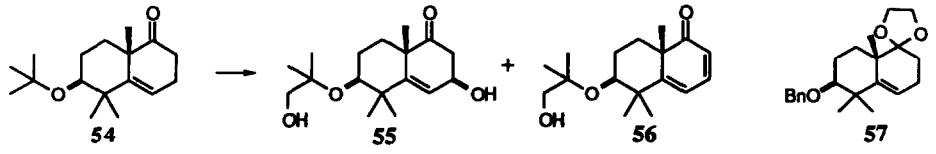
The above results gave us hope to obtain, by allylic hydroxylation of Δ^2 olefins, 1-hydroxy derivatives of interest for some syntheses such as that of forskolin[8]. Dehydration of the 3β-alcohol **36**, using literature conditions (TfCl, DMAP, DCM),[9] failed to give **49** in a reasonable yield. We therefore prepared the enol phosphate **15**, by treatment of ketone **32** first with LDA and then with diethyl chlorophosphate.



This enol phosphate **15** (95 % after SiO_2 flash chromatography), was deoxygenated with Li in $EtNH_2$,[10] and gave the desired product **49** in 83% yield. Incubation of **15** with *R. arrhizus* afforded in analogy to previous cases the 7β-alcohol **17** (76%). Incubation of **49** gave initially, at 40 % conversion, after some 6 hours, the non-epoxidized 7β-alcohol **50**, accompanied by a little of the 9-deprotected ketone. However, incubation of **49** for 20 h gave products resulting from more extensive reactions : 7β-hydroxylation (products **51-53**), additional 1-hydroxylation (**53**, 10 % only), and epoxydation of the Δ^2-double bond (**51**, **52**, 60 %). Only one of the diastereomeric epoxides **52**, α from its NMR spectrum, was obtained pure. This route is therefore of no use to provide reasonable amounts of the 1-hydroxy derivatives.

Limitations

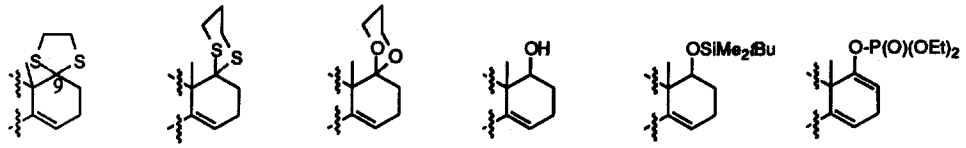
Other cases demonstrate some limitations. For instance, the *t*-butyl ether **54** was transformed in 48 h to a mixture of two products having both suffered a hydroxylation on one of the non-activated methyl



groups of the *t*-butyl moiety : **55**, with the expected allylic oxidation at 7 (54 %), and its β–elimination product **56** (31 %). The reaction on the *t*-butyl group is favored over the allylic hydroxylation, as no product with an intact *t*-butyl group could be isolated.

With other substrates, the incubation simply failed to give hydroxylated derivatives. This was the case for the benzyl ether **57** which appeared to be used as a carbon source : we recovered only minute amounts of unidentified low-molecular weight products. In the Wieland-Miescher series, protection of the 9-keto group by a dithioketal or by a six-membered m-dioxane, or reduction of the carbonyl to the

corresponding alcohol, free or substituted by several of the usual protective groups, are other cases where no biohydroxylation at all was observed.



Octalone derivatives **not** hydroxylated by *R. arrhizus*

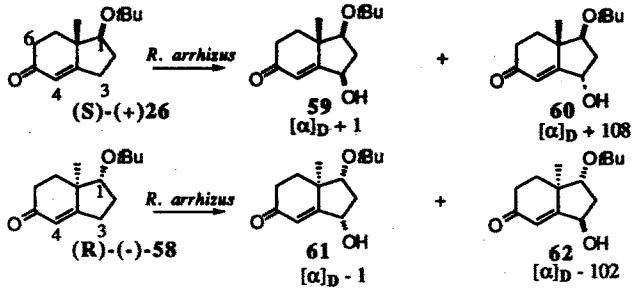
Another obvious limitation, not surmounted so far, lies with water-insoluble substrates : cholesterol, for instance, could not be hydroxylated so far.

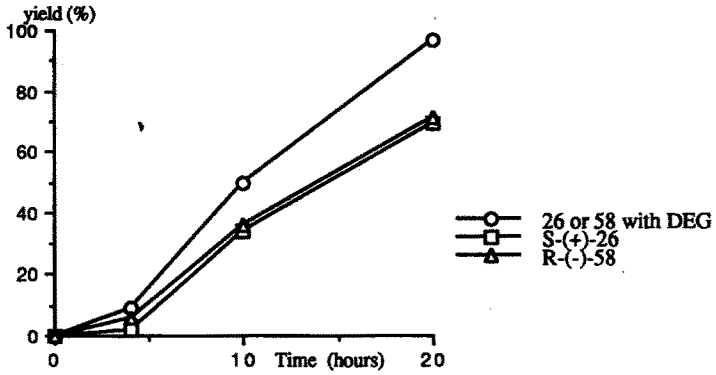
Allylic Biohydroxylations. The Hajos-Parrish series.

In the **hydrindane series**, we have studied two sets of substrates, chosen for the potential use of their hydroxylated derivatives in syntheses of several heavily oxygenated polyterpenes : the simplest ones are the immediate derivatives of the Hajos-Parrish hydrindenone, both in the S(+) and in the R(-) series; in the others, we have taken advantage of the 5-keto group to firstly introduce an oxygenated function at C-6 with lead tetracetate, as shown above. *In both enantiomeric series*, with the 1-*t*-butyl ethers **26** and **58**, the fungus acts as a nearly quantitative oxidizing reagent (> 95 % overall yield), but with poor stereoselectivity in the steroid-like series, the 3β/3α **59:60** ratio being 88:12; in the enantiomeric series, the selectivity is even poorer, the ratio **61:62** being 56:44.

This complete lack of sensitivity to the absolute configuration of the substrate is further reflected in the rates of reaction, nearly identical with both enantiomers **58** and **26**. Time-course studies in the hydrindenone series (Figure 2) showed that there is no "kinetic resolution", both enantiomers being hydroxylated with practically identical rates, either in the standard conditions or with addition of diethyleneglycol to the incubation medium (this addition accelerated the process in both steroid and enantio-steroid series, the biohydroxylation reaching an almost quantitative yield after only 20 h at room temperature).

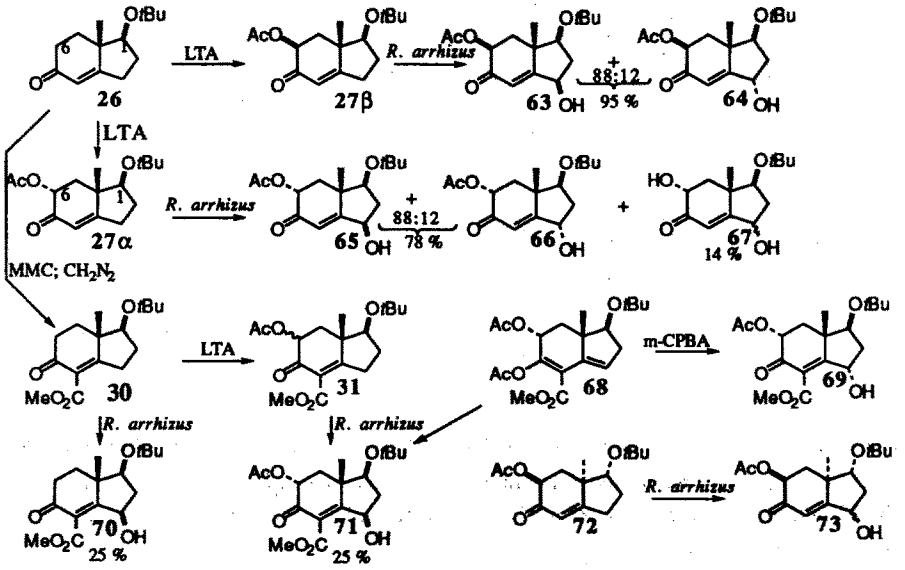
The 4-methoxycarbonylated derivative **30** was prepared by the action of magnesium methyl carbonate (MMC),[11] and the 6–acetoxylated derivatives **27α, 27β, 29** and **31**, by lead tetracetate (LTA) oxidation as mentioned above. Incubation with *R. arrhizus* proceeded as well and as rapidly whichever the absolute configuration, to give nearly quantitatively mixtures of the epimeric 3-hydroxy derivatives **63/64, 65/66** and 3β+3α **67**. At worst, with 1.11 g of substrate in 500 ml water and 200 g of fresh biomass, a 70% yield was isolated after 22 h; a little unreacted material was easily recovered. To obtain the acetoxy carbomethoxy-enones **31**, the two steps required (acetoxylation with lead tetraacetate and carboxymethylation with magnesium methyl carbonate) can be followed in either order. When the carboxymethylation is run last, the basic conditions prevailing lead to the corresponding alcohols **31** (6-OH instead of OAc); reacetylation with Ac_2O/Py at 0°C for 15h gave a mixture of products : the starting alcohols (5%), the acetate **31** α-OAc (56%) and the dienol acetate **68** (38%), which thus became available for a study of its biotransformation. Incubation of this dienol acetate with *R. arrhizus* gave the 3β-alcohol **71** (also obtained by incubation of the enone **31** α-OAc). The epimeric 3α-alcohol **69** was obtained by treatment with mCPBA under buffered conditions[12]. Attempts to increase the yields of these potentially useful transformations (e.g. by increasing the time up to 5 days, increasing the amount of biomass up to 4 times the usual amount of 50 g per 200 mg of substrate and addition of fresh biomass every 24 h for 4 days) were not successful.





Conditions : 27°C : substrate : 0.4 g, H_2O : 400 mL (4.4 mM), biomass : 125 g wet; conversion 100%.
Same conditions, but 10 mM substrate concentration : 70 % conversion.
The 3α/3β- OH ratio does not vary, in both series, between 4, 10 and 20 hrs.

Figure 2

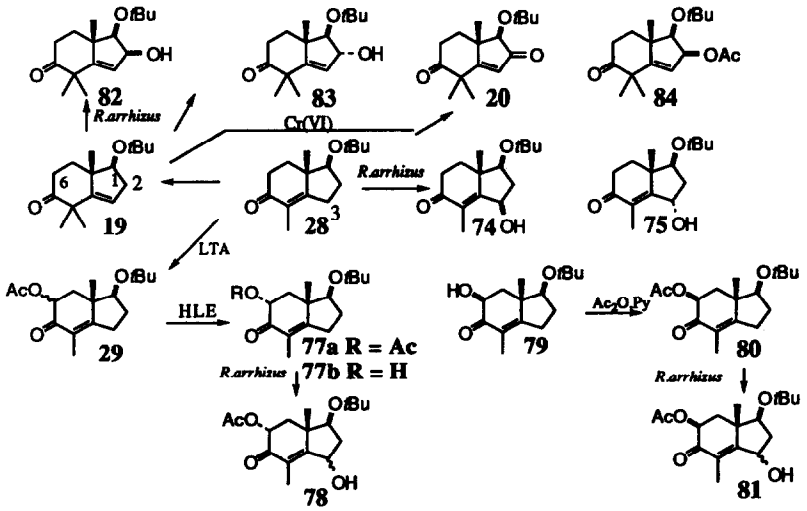


The 4-carbomethoxy enone **30** gave only a mediocre yield of the 3β-hydroxy derivative **70** (25%). Other strains, such as *Mucor mucedo, M. plumbeus, Cylindrocarpon radicicola, Curvularia lunata,* either gave lower yields, with comparable 3α/3β ratios (*Mucor* species) or unchanged starting material. The only side-reaction observed with *Rhizopus arrhizus* was again some hydroxylation of one of the methyls of the *t*-butyl protecting group. A product directly related to **70** (free carboxylic acid, 3-OH) has been obtained earlier chemically, by a more complex procedure, from the acid corresponding to **30**, by NBS photobromination followed by silver acetate substitution, with 38% overall yield.[13]

The configuration at C-3 of the products was established unambiguously by 400 MHz ^{1}H-NMR with 1D and 2D-experiments both for resonance assignments (homo- and hetero-correlation spectra, different shifts of the δ of the angular methyls with *cis* or *trans* hydroxyls) and for spatial relationship (difference n.O.e. technique).[14] A downfield shift of the angular methyl group signals of the 3β-OH derivatives was observed with respect to the methyl resonance in the 3α–hydroxy series (angular methyl anti to 3-OH) (Δδ ≈+ 0.2 ppm), as a result of a 1,3-interaction with the *cis* hydroxy group. An analogous downfield shift on the H-1 proton (Δδ ≈ + 0.3 ppm) is observed when the 3-OH group is on the α–face and again a downfield shift of the H-3 resonance (Δδ ≈ + 0.2 ppm) when H-3 lies on the β–face of the molecule. Another set of very useful data arose from the observation, upon irradiation of the angular methyl group, of n.O.e.'s with every single one of the β protons. The NMR raw data are given in the Experimental Part but, for convenience, our detailed assignments are given in an Annex.

Biohydroxylations - Methylated Hydrindane Derivatives.

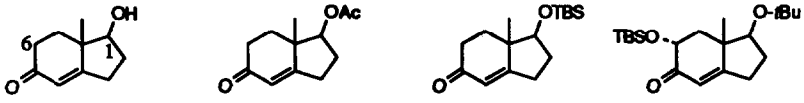
In preparation for some polyterpene syntheses, we have also investigated the biohydroxylations of 4-methylated derivatives of the hydrindenones mentioned above. The 4-methyl and 4,4-dimethyl derivatives **28** and **19** were prepared as described earlier[15] and incubated with *R. arrhizus*. Despite the difference of electronic nature of the double-bonds in **28** and **19**, in both cases the allylic positions - 3 and 2 respectively -



were attacked with high regiospecificity, but poor stereoselectivity. Incubation of **19** with *R. arrhizus* led to a mixture of three compounds, **82, 20** and **83**, in rather low yields (16%, 9% and 17% respectively).

The 2β-hydroxylated compound **82** has been characterized as the corresponding acetate **84**, due to the difficulties encountered during chromatographic separations.

Like in the Wieland-Miescher series, there are structural limitations in the hydrindenone series to the biohydroxylation by *R. arrhizus*. In particular, modifying the *t*-butyl protective group at C-1 also led to a



Hydrindenone derivatives not hydroxylated by *R. arrhizus* (both antipodal series)

failure : the free hydroxy, as well as the acetoxy and silyloxy protections, render the bioconversion totally inefficient.

Lipase-mediated hydrolyses in the hydrindane series.

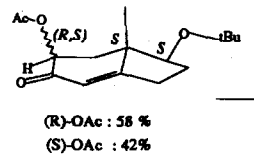
We have mentioned that, in several cases, a limitation to the facile obtention of pure substances lies in the difficulty of separation of diastereomeric acetates in the case of lead tetracetate oxidations. One possible solution appeared to be available with the remarkable efficiency of some enzymatic hydrolyses of esters, from which one could hope to obtain, from a mixture of R and S acetates, the selective hydrolysis of only one of the components, leading to the obviously very easy separation of an acetate and an alcohol.

For example, we have described above the straightforward preparation of some 6–acetoxy enones, and mentioned the difficult separation of their epimeric mixtures (Formulas **21-31**). In one case already mentioned, chemical hydrolysis of the acetates **27** had led to some epimerisation and to the formation of unidentified side-products : at room temperature, only the unwanted 6α–alcohol (equatorial), and even at 0°C, a 4:1 mixture of the 6α– and 6β–alcohols were obtained. We were thus led to start a screening with relatively cheap lipases, searching for a diastereoselective hydrolysis.

In both the steroid-like and enantio-steroid-like series, the crude lipase preparation of *Candida cylindracea* and porcine pancreatic lipase were ineffective, the starting material being recovered intact. However, in the steroid-like series, incubation with horse liver esterase (HLE, acetone powder) diastereoselectively cleaved the (S)-acetate **27β** (the desired one) with a high selectivity even after prolonged treatment and the use of a large excess of the lipase; a small amount of toluene (1 ml for 30 ml of buffer) can be used to solubilize the sample, but this is not even necessary. Usually, equal masses of the enzyme and the substrate were used, but in some cases, it was enough to use a lipase/substrate ratio of 0.5 for the hydrolysis to run at a comparable rate. The procedures described below in the Experimental Part are deceptively simple, and these enzymatic hydrolyses can be performed on a multigram scale. In a similar experiment, pig liver esterase (PLE) also preferentially hydrolyzed the (S)-acetoxy group and afforded the corresponding (S)-alcohol with an acceptable diastereomeric excess (Table 1). We have also tested the use of HLE to effect diastereoselective hydrolyses on optically homogeneous epimeric 6-acetates in both steroid-like and enantio-steroid-like series, and finally, on the racemic material, and we obtained cleanly the optically pure (S)-alcohols and (R)-acetates, easily separable by flash silica gel chromatography. Enantiodifferentiation of chiral secondary alcohols was carried out by diastereomeric derivatization with (S)-O-acetyllactyl chloride as a chiral auxiliary[16] and e.e.'s were measured by gas-liquid chromatography and high-field ^{1}H-NMR.

In the 4-methylated series, the 6-acetoxy derivatives **29**, obtained as an epimeric mixture as for all cases investigated, were subjected to HLE-catalyzed hydrolysis to provide the 6α acetate **77a** and the 6β alcohol **79**. The first was hydroxylated by *R. arrhizus* in 65 % yield to the mixture of 3α and 3β alcohols **78** (β/α ratio 93:3), while the epimeric alcohol **79** was first acetylated to **80**, itself hydroxylated by *R.*

arrhizus in 74 % to give the epimeric mixture of 3-alcohols **81** with a poorer β/α selectivity (62:38). Table 1 summarizes the results obtained with the mixture of acetates **27α+ 27β**.



	K_2CO_3 - MeOH		HLE	PLE	LCC	LPP
	1h-RT	1h-0°C	20°C, 24h, pH=7 (phosph.buffer, 20mM)			
(S)-OAc	0	0	0	0	42	42
(R)-OAc	0	0	56	55	56	56
(R)-OH	100	81	0	5	0	0
(S)-OH	0	19	44	40	0	0

Hydrolyses of the mixture of acetates 27α + 27β

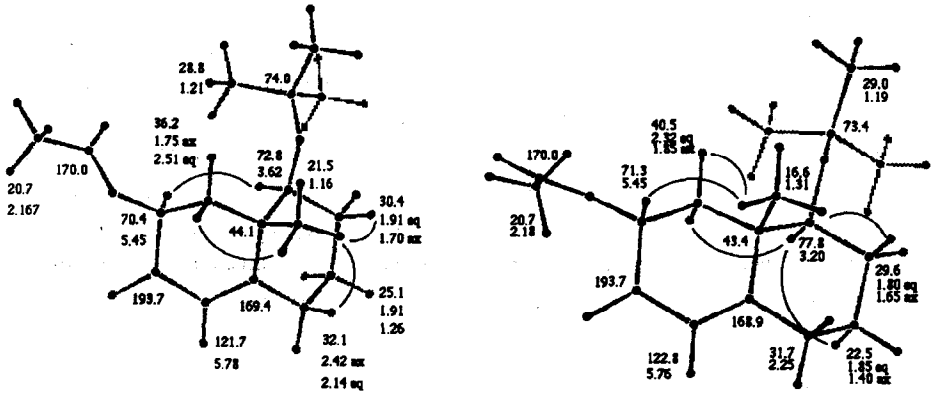
Table 1

Lipase-mediated hydrolyses in the Wieland-Miescher series.

The success encountered with the use of lipases in the hydrindane series led us to study it also in the decalin series. The results thus obtained show that the efficiency is, as expected, as good in this series.

This made available a series of acetoxy-enones, which present interesting conformational problems. For preparative purposes, the most efficient route to optically pure acetates and alcohols proved to be recrystallization from heptane, which separated the α– from the β–acetates, the former crystallizing out, and subsequent enzymatic hydrolysis.

We have studied the conformation of these acetates by a combined NMR/molecular mechanics approach, and showed that both 2α and 2β-acetoxy derivatives have an equatorial acetoxy group. Figure 3 shows the structures of the lowest energy conformations using the MM2 force field, together with NMR



Lowest energy conformations of 22 and 23. The numerical values indicate the ^1H and ^{13}C NMR assignments; the arcs indicate observed diagnostic n.O.e.'s.

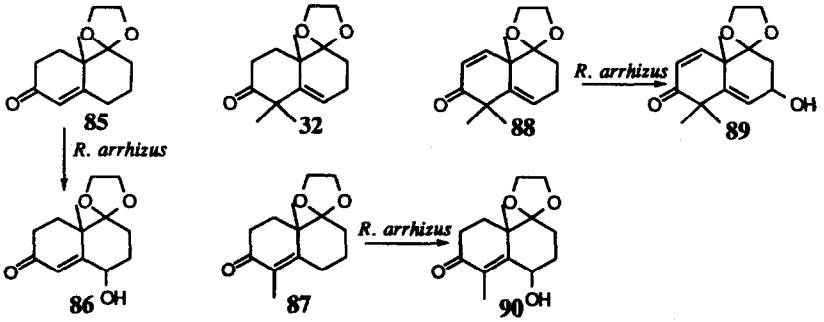
Figure 3

assignments. The coupling patterns for H-2 were similar (5.2, 14.2 and 5.5, 14.5 Hz), and the configuration of the C-2 acetoxy group was found to be equatorial in both **22** and **23**; the signal of the C-2 proton appeared at δ 5.45 ppm as a doublet of doublets with coupling constants of 5.2, 14.2 Hz (**23**) and 5.5, 14.5 Hz (**22**) which is consistent with the dihedral angles calculated for a half-chair conformation of ring A in **23** and an inverted half-chair conformation for **22**. Diagnostic n.O.e.'s were observed upon examination of the 1D-Difference n.O.e. spectra. Thus, selective saturating irradiation of the angular methyl signal at 1.31 ppm resulted in an n.O.e. enhancement on H-2β axial (5.45 ppm) and H-1β (2.32 ppm) equatorial for **23**,

whereas similar selective irradiation of the angular methyl signal at 1.16 ppm on **22**, resulted in an enhancement on H-1β axial proton (1.75 ppm). Still more informative was the irradiation of H-1, H-2 and H-9 protons on both compounds. Thus selective irradiation of H-1 α axial (1.85 ppm) on **23** produced an enhancement on H-9 (3.20 ppm) whereas irradiation on H-2α axial (5.45 ppm) on **22** resulted in an enhanced peak due to H-9 (3.62 ppm), in complete agreement with the lowest energy conformations defined by molecular mechanics.

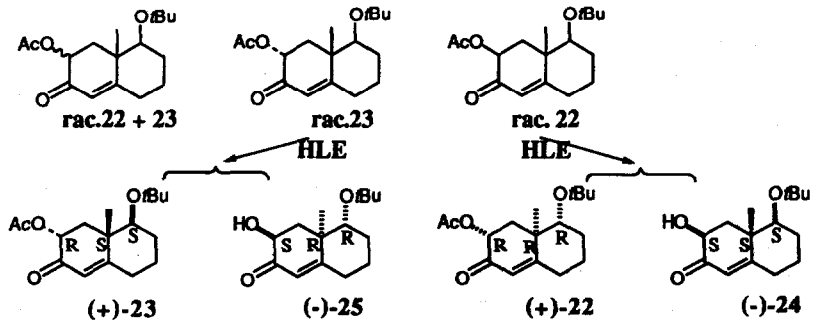
Observations in the racemic series

We have indicated above that the biohydroxylation with *R. arrhizus* displayed practically no sensitivity to the absolute configuration in the hydrindane series. The same is true in the Wieland-Miescher series. Only a slight selectivity in favor of the steroid-like series when incubated with the C-9 ketal-protected racemic Wieland-Miescher ketone derivatives **85** and **88**; they afforded [±]-**86** (25%),[±]-**89** (66%) and [±]-**90**



(40%) with low e.e.'s (≈10%, by comparing the optical rotations of the same compounds obtained by bioconversion of the corresponding optically pure compounds and by derivatization with Mosher chloride or (S)-O-acetyllactyl chloride). [±]-**88** was prepared from [±]-**32** in a three-step sequence (TMSOTf, collidine, -10°C, 15 min, then NBS, THF, -78°C, 10 min and finally $CaCO_3$, dimethylacetamide, 1h reflux; 51% overall yield).

In contrast, the HLE was much more selective. Racemic **23** was subjected to enzymatic hydrolysis with horse liver esterase (40 mg of HLE for 100 mg of (R,S)-**23**, 30 ml of phosphate buffer pH = 7, 1 ml



toluene, 18.5 h at room temperature) to yield the (R)-acetate **23** (steroid-like series) and the (S) alcohol **25** (enantiosteroid-like series), thus exhibiting an (S)-specificity. Hydrolysis of racemic **22** with HLE (equal

masses of the enzyme and substrate) required longer incubation times (approximately 3 days) and led to the (R)-acetate **22** (enantiosteroid series) and (S)- alcohol **24** (steroid series).

Extensions

A number of other strains were tested, such as : *Aspergillus niger* ATCC 9142, *Mucor mucedo* ATCC 7941, *Mucor plumbeus* CBS 11016, *Beauveria sulfurescens* ATCC 7157, *Curvularia lunata* NRRL 2388, *Cylindrocarpon radicicola* ATCC 11011, *Geotrichum candidum* CBS 23376, *Fusarium oxysporum* AP 68M LPPON. None of them presented the broad spectrum of *Rhizopus arrhizus*. In one case, the (±)-4-methyl octalone **87**, *Aspergillus niger*, gave a 25% hydroxylation at the 6β-position (*R. arrhizus* gave 40 % of the same product, with a very low e.e.) and *Mucor mucedo* with both steroid-like **58** and enantiosteroid-like **26** hydrindenones gave the same products as *R. arrhizus* in approximately the same β/α ratios **59/60** and **62/61**. The yield was only about 40 % (>95 % with *R. arrhizus*.).

Of course, it is probable that other microorganisms could be identified by a proper screening to effect these or other hydroxylations even better, but *R. arrhizus* remains for the time being a microorganism of choice.

Conclusion

Thus, *Rhizopus arrhizus* ATCC 11145, a safe and easily handled fungus, smoothly hydroxylates many bicyclic derivatives in good yield at 27°C in water. Isolation is extremely simple, and cost is very low. No over-hydroxylation has been observed, except on the O-*t*-butyl group. One can easily work on a 2 mmolar scale of the organic substrate in water, and reactions are complete within at most 48 h (usually less than one day). Routine use of this fungus and of horse liver esterase does not present any problem; all that is required is to throw the proper amount into an erlenmeyer flask together with the substrate, in water, and to stir. The yields of allylic biohydroxylations are often higher than those obtained by chemical means and no significant amounts of by-products are obtained. The remaining mass usually consists of unreacted starting material. The process is compatible with the presence of a number of functional groups, which remain intact, and direct one-step hydroxylations on substrates containing free hydroxy or keto groups can be achieved in high yield in the absence of toxic chemicals and organic solvents and without the use of the expensive protection-deprotection steps. As it has already often been emphasized, the use of microbial hydroxylations of this type, and even more of enzymes like lipases should find place in the general methodology of organic chemistry.

Experimental section :

Flash chromatographies are run on silica gel (Merck 60, 230-400 mesh) with the solvent mixture indicated. Thin layer chromatography is performed on commercial silica gel glass plates developped by immersion in 5% phosphomolybdic acid in 95% ethanol. Experiments requiring an inert atmosphere are carried out under dry argon or nitrogen in a flame dried glass system. THF and benzene are freshly distilled from $LiAlH_4$ and sodium wire respectively, and are transferred by a syringe. Methylene chloride is distilled from P_2O_5. Triethyl and diisopropyl amines are distilled from KOH pellets. Commercial reagents are purchased from Aldrich Chemicals and used as received. "Usual workup" means washing of the organic layer with brine, drying on anhydrous $MgSO_4$, and evaporating *in vacuo* with a rotary evaporator at aspirator pressure. **Optical rotations** are recorded in $CHCl_3$ solution in a 1 dm cell using a Perkin-Elmer 243 polarimeter. **IR** spectra are recorded on a Nicolet 205 FTIR instrument, neat or in chloroform. Melting points are uncorrected. **^{1}H-NMR** spectra are obtained on Bruker AM 400, AM300, AC250 (400, 300 and 250 MHz respectively) instruments in $CDCl_3$. Chemical shifts are expressed in ppm downfield from TMS (the 1H-NMR

data are presented in the order : δ value of the signal, integrated number of protons, peak multiplicity (abbreviations used are as follows : s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet) and coupling constants in Hertz. Nuclear Overhauser enhancements by the NOEDIFF (ref. 14) method are obtained with the aid of the Aspect 3000 microprograms, which allow direct accumulations of difference FID's. N.O.e's are successfully obtained with extremely low irradiating power levels (40 dB); 320 transients are acquired in n.O.e. experiments, and an exponential line broadening of 0.3 Hz is used. ^{13}C spectra are measured at 62.5 and 75 MHz and the chemical shifts are reported relative to $CDCl_3$ triplet centered at 77.00 ppm. For all compounds investigated, multiplicities of ^{13}C resonances are assigned by the SEFT technique.[17] Two-dimensional homo and heteronuclear correlation experiments are performed with standard Bruker software. Mass spectra (MS), recorded on an AEI MS-50 (electron impact spectra, EI), an AEI MS-9 (chemical ionization spectra, CI), or a Kratos MS-50 (high resolution mass spectra, HR) instruments are reported in the form : "m/z (intensity relative to base peak=100%)". Molecular mechanics calculations are run using Still's Macromodel program version 3.1 operated on a Silicon Graphics work-station. Structures are constructed by means of the interactive graphics input and then subjected to the MM2 minimization using the Monte Carlo option of the program for the search of all conformers and the evaluation of their energy (indicated solvent : chloroform).

Lipases were purchased from Sigma Chemical Co. *R. arrhizus* was purchased from the American Type Culture Collection, Rockville, and maintained on agar slants (Diagnostics Pasteur, Paris). Spores of *R.arrhizus* have been used to inoculate the liquid culture medium containing 1g KH_2PO_4, 2g K_2HPO_4, 10g corn steep liquor, 0.5g $MgSO_4$, $7H_2O$, 2g $NaNO_3$, 0.02g $FeSO_4$, $7H_2O$, 0.5g KCl, 30g of glucose per litre of distilled water. Cultures are grown in erlenmeyer flasks at 27°C on a rotatory shaker (150 rpm). The 3 days old mycelium is recovered by filtration, washed and incubated in the culture medium containing the substrate at the desired concentration. The mixture is then placed on a rotary shaker at 27°C and 220 rpm. TLC monitoring, removal of the cellular mass by filtration, extraction with ethyl acetate and usual work-up are used regularly.

The enantiomeric composition of the microbial products is determined by GC and ^1H-NMR analyses of the corresponding (S)-O-acetyllactates. The corresponding (S)-O-acetyllactates, derived from racemic starting materials, are used as control standards. For all the examples studied, the enantiomeric purity of the microbial products is in excess of 97% (as established by a control experiment where 1% of diastereomer was detected and quantified by GC analysis). The conditions of fungal hydroxylations and lipase mediated hydrolyses are kept standard for all substrates to observe any effect of functional group modification on conversion efficiency and selectivity.

General procedure for chemical allylic oxidations. A 0.2 M solution of the olefin in acetonitrile is prepared. To this is added one equivalent of chromium hexacarbonyl and three equivalents of t-butyl hydroperoxide. The mixture is heated under reflux for 24 h, then cooled and diluted with DCM. After washing with brine and water the washings are back-extracted with DCM. The combined organic layer is dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The residue is then chromatographed on silica gel.

General procedure for the reduction of α,β-unsaturated ketones

Method 1 : The enone is dissolved in EtOH-DCM (1:1) to a concentration of 0.2M then cooled to -78°C. One equivalent of ceric chloride heptahydrate is added and the mixture stirred at this temperature for 15 min. 2.5 equiv. of $NaBH_4$ are then added and the stirring is continued for a further 0.5-1h. The reaction is quenched by the addition of 2N HCl to pH7. The mixture is diluted with DCM, washed with brine, then water and worked up as usual.

Method 2 : A solution of the α,β–unsaturated ketone in methanol (0.2M) is prepared and cooled to 0°C. 2.5 equiv. of $NaBH_4$ are added and the mixture is stirred for 1-2h (TLC monitoring). The remainder of the procedure is identical to that of method 1.

Method 3 : A 0.2M solution of the enone in THF is cooled to -78°C. A 1M solution of L-Selectride in THF is then added and the resulting solution is stirred for 15-30 min at this temperature. The reaction is quenched with water and the organoborane is oxidized with 6M sodium hydroxide and 30% H_2O_2. Most of the THF is removed under reduced pressure and the crude product worked up as usual.

General procedure for the acetylation of alcohols

To a stirred solution of the alcohol (1 mmol) in 2 ml of pyridine and 0.05 mmol of DMAP is added 2.5 mmol of acetic anhydride. The reaction mixture is stirred at 0°C and TLC monitored. After completion, water is added and the reaction mixture is extracted with dichloromethane, washed with 1 M hydrochloric acid , then with saturated sodium bicarbonate and finally with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue is chromatographed on silica gel, the yields are often quantitative.

General procedure for the preparation of unsaturated α-ketols

The unsaturated α-ketols are synthesized from the corresponding α,β--unsaturated ketones using a slightly modified (see ref 1) lead tetraacetate oxidation using a large excess of the reagent to ensure very high isolated yields leading to an almost single spot reaction crude. To 68.5 mmol of enone in a two necked flask is added 225 mmol of lead tetraacetate and the reaction vessel is vacuum dried while flashed with argon. 240 ml of freshly distilled benzene (calcium hydride, argon) is then added and the reaction mixture is refluxed for four days under inert atmosphere. After cooling to room temperature, a large volume of ether is added (five-six times the volume of the solvent) and the reaction mixture stirred for an additional hour, filtered, and the filtrate washed with brine and water. The organic layer is then dried over anhydrous magnesium sulfate, concentrated under reduced pressure and chromatographed on silica gel.

General procedure for biohydroxylation

The biomass is weighed and added directly to the culture medium containing the substrate at the desired concentration. The mixture is placed in an orbital incubator and shaken at 27°C at a speed of 220 rpm. The reaction time is different for each substrate, but is usually between 24 and 72 hours. Once the reaction is complete the culture medium is diluted with the same volume of ethyl acetate, filtered and further extracted twice with the same solvent. The combined organic phase is dried over anhydrous $MgSO_4$, and the solvent removed under reduced pressure. The residue is chromatographed on silica and the isolated products are analyzed by NMR and G.C.

General procedure for HLE-catalyzed hydrolysis of the acetates : To a mixture of acetate (100mg) in 20 mM phosphate buffer (pH=7, 30ml) and 1ml of toluene as cosolvent, is added commercially available horse liver esterase (100 mg, acetone powder) at room temperature. The mixture is stirred (magnetic stirrer) for 12 to 24 h while TLC and G.C.(capillary column) monitored. Typically the reaction is complete after 24 h. After the indicated incubation time the mixture is diluted with ethyl acetate and filtered through Celite. The extracts are washed with brine, dried with magnesium sulfate and concentrated in vacuo. The residue is chromatographed on silica gel. This procedure can be scaled up without problem; the ratio of enzyme to substrate can in many cases be lowered to 0.5 without effect on the rate.

General procedure for determination of the enantiomeric purity : In all cases investigated the enantiomeric excess is measured after diastereomeric derivatization with (S)-O-acetyllactyl chloride as described in reference 16. The acetates are first converted to the corresponding alcohols. The acetate (1 mmol) is treated with solid potassium carbonate (2 mmol) in methanol (3 ml) at 0°C. The mixture is vigorously stirred for 2 h (TLC monitoring). Methanol is removed *in vacuo* and the resulting mixture diluted with water then extracted with dichloromethane, dried and concentrated to give the corresponding alcohol. Both (-)- and (+)-alcohols were converted to the corresponding lactates by treatment with (S)-O-acetyllactyl chloride, pyridine (or Et_3N), DCM, DMAP at room temperature for several minutes. Their GC analyses were carried out on a ST1 capillary column (0.32mm x 25m) operated at 170°C with helium as carrier gas (60Kpa), and compared to the racemic mixture. The enantiomeric purities were ≈ 98%.

Alcohol 3 : Protection of the alcohol **36** with *t*-butyl-dimethyl-silyl chloride, DMF, imidazole, r.t., gave alcohol 3 (95%); silica gel (EtOAc/heptane, 1:2), $[\alpha]_D$: - 17 (c= 0.97). IR : 2958, 2931, 2879, 2858, 1474, 1462, 1380, 1363, 1257, 1136, 1109, 1089, 1042, 855, 835, 775. ^{1}H-NMR : -0.01 (3H, s); 0.24 (3H, s); 0.89 (9H, s); 1.04 (3H, s); 1.10 (3H, s); 1.31 (3H, s); 1.39 (1H, m); 1.61 (2H, m); 1.73 (2H, dt, J=2.7, 13.1), 1.89 (1H, m); 2.17 (1H, d, J= 4.8); 2.25 (1H, ddd, J= 2.8, 6.9, 10.8); 3.28 (1H, dd, J= 2.02, 8.7); 3.90-4.05 (4H, m); 5.59 (1H, t, J=

3.1). **^{13}C-NMR** :-4.9, -3.8, 18.1, 24.4, 25.7, 25.9, 26.1, 27.1, 27.2, 29.8, 42.7, 64.6, 65.4, 77.6, 112.9, 119.0, 148.7.

Oxidation of 3 is achieved according to the general procedure for allylic oxidations. The residue is chromatographed on silica gel (EtOAc/heptane, 1:2) to give 4 in 38% yield. 20 % of starting material is also recovered. 4 : $[\alpha]_D$: + 3 (c= 0.83). IR : 2955, 2931, 2885, 2861, 1675, 1466, 1255, 1130, 1097, 1034, 888, 851, 835, 775. ^{1}H-NMR : 0.05 (3H, s); 0.08 (3H, s); 0.91 (9H, s); 1.13 (3H, s); 1.19 (3H, s); 1.46 (3H, s); 1.53 (1H, m); 1.60-1.90 (3H, m); 2.55 (1H, d, J= 17); 2.81 (1H, d, J= 17); 3.42 (1H, dd, J= 3.2, 12.2); 3.80-4.05 (4H, m); 6.10 (1H, s). ^{13}C-NMR : -4.9, -3.9, 18.0, 24.1, 24.6, 25.8 (3), 26.2, 26.3, 26.6, 43.7, 44.7, 65.2, 65.4, 76.2, 112.9, 124.4, 175.6, 197.6. EIMS : 380 ($M^{+\cdot}$, 16), 323 (58), 294 (68), 237 (77), 171 (55), 162 (57), 147 (42), 113 (86), 73, 75 (100). CIMS : 381 $[M+H]^+$, 339 $[M-42+H]^+$.

Reduction of compound 4 According to method 1; a mixture of allylic alcohols 5 (7β) and 6 (7α) are obtained in 92% yield. The ratio between the two as determined by ^{1}H-NMR was 78:22 respectively. According to method 3, only the 7α alcohol 6 is obtained, in 81% yield.

Deprotection of 6 : A solution of the alcohol 6, (60 mg, 0.16 mmol) in THF (1 ml) is stirred at room temperature overnight with a 1.0 M solution of tetra-n-butyl ammonium fluoride in THF (0.47 ml, 0.47 mmol). The solution is then poured onto ice and extracted three times with DCM. The organic layers are washed once with water. Usual work up leads to a crude product which is purified by chromatography on silica gel (EtOAc/heptane, 1:1) to give diol 6-3β OH (35 mg, 83%) together with 8% of recovered starting material. 6-3β OH : $[\alpha]_D$: - 9 (c= 0.86). IR : 3409, 2944, 2881, 1468, 1406, 1361, 1289, 1139, 1116, 1039, 1011. ^{1}H-NMR : 1.07 (3H, s); 1.22 (3H, s);1.27 (3H, s); 1.50 (1H, dd, J= 3.3, 12.5 Hz); 1.65-1.80 (3H, m); 1.90 (1H, d, J= 14.2); 2.18 (1H, dd, J= 5.6, 14.2); 3.34 (1H, dd, J= 5.3, 10); 3.85-4.05 (4H, m); 4.17 (1H, ddd, J= 4.6, 4.7, 1.0); 5.81 (1H, d, J= 4.6). ^{13}C-NMR : 23.4, 25.6, 26.8, 26.9, 36.4, 40.7, 43.0, 64.7, 65.4, 67.2, 76.8, 112.9, 123.4, 150.5. EIMS : 268 ($M^{+\cdot}$, 5), 164 (16), 182 (19), 149 (38), 123 (35), 87 (100), 43 (49). HREIMS : calcd for $C_{15}H_{24}O_4$: m/z 268.1674, found : 268.1675.

Oxidation of compound 11 : The oxidation of compound 11 is done according to the general procedure for allylic oxidations. The residue is chromatographed on silica gel (EtOAc) to give compound 12 (55 %) : $[\alpha]_D$: + 19 (c= 1.01). IR : 2985, 2896, 1675, 1602, 1473, 1315, 1265, 1134, 1032, 986. ^{1}H-NMR : 1.21 (3H, s); 1.29 (3H, s); 1.35 (6H, t, J= 6.9 Hz); 1.48 (3H, s); 1.60 (1H, m); 1.80-2.30 (4H, m); 2.56 (1H, d, J= 17.2); 2.81 (1H,d, J= 17.2); 3.80-4.20 (8H, m); 6.09 (1H, s). ^{13}C-NMR : 16.0 ($^3J_{C-P}$= 4.9) (2), 24.1, 24.3, 24.6, 25.7, 26.0, 42.3 ($^3J_{C-P}$= 5.0), 43.6, 44.5, 63.6 ($^2J_{C-P}$= 5.1) (2), 65.2, 65.4, 82.2 ($^2J_{C-P}$= 5.8), 112.5, 124.8, 173.3, 197.2. EIMS : 402 ($M^{+\cdot}$, 5), 248 (7), 163 (27), 162 (100), 147 (42), 119 (99), 99 (18). HREIMS : calcd for $C_{19}H_{31}O_7P$: m/z 402.1964, found : 402.1800.

Reduction of compound 12 : A) Performed according to method 1 : A mixture of allylic alcohols 13 and 14 are obtained in 89% yield. The ratio between them was 68:32 respectively as determined by ^{1}H-NMR. B) Performed according to method 3 : Only the alcohol 14 is obtained in 66% yield. $[\alpha]_D$: - 59 (c= 0.65). IR : 3461, 2984, 2944, 2914, 2884, 1262, 1144, 1038, 1002, 984. ^{1}H-NMR : 1.12 (3H, s); 1.24 (3H, s); 1..28 (3H, s); 1.35 (6H, t, J= 7.1); 1.50 (1H, dd, J= 4.3, 17.8); 1.74 (1H, dd, J= 4.0, 12.5); 1.85-2.00 (3H, m); 2.09 (1H, m), 2.18 (1H, dd, J= 5.7, 14.4); 3.80-4.20 (9H, m); 5.82 (1H, d, J= 4.5). ^{13}C-NMR : 16.2 ($^3J_{C-P}$= 6.4) (2), 24.1, 24.3, 24.6, 26.7, 26.8, 34.5, 36.5, 42.7, 63.5 (2), 64.8, 65.0, 65.6, 83.7 ($^2J_{C-P}$= 6.9), 112.8, 123.2, 149.8. EIMS : 404 ($M^{+\cdot}$, 0.5), 386 (6), 318 (7), 232 (9), 164 (68), 155 (26), 149 (34), 99 (46), 87 (100).

Acetylation of alcohol 14 : According to the general procedure described above, alcohol 14 afforded in 95% isolated yield the corresponding acetate 14, OAc instead of OH : $[\alpha]_D$: - 77 (c= 0.65). IR : 2984, 2884, 1732, 1472, 1386, 1247, 1108, 1005, 984, 755. ^{1}H-NMR : 1.13 (3H, s); 1.25 (3H, s); 1.29 (3H, s); 1.33 (6H, t, J= 7); 1.51 (1H, dd, J= 3.4, 9.8); 1.88 (2H, m); 2.09 (3H, s), 2.27 (1H, dd, J= 7.1, 15.4); 3.80-4.20 (9H, m); 5.45 (1H, dd, J= 4.2, 6.9); 5.69 (1H, d, J= 3.9). ^{13}C-NMR :16.2 ($^3J_{C-P}$= 6.3) (2), 21.4, 24.5, 24.6, 26.1, 26.8, 32.5, 42.3 ($^3J_{C-P}$= 6.9), 43.0, 63.5 (2), 64.9, 65.2, 67.9, 83.3 ($^2J_{C-P}$= 6.9 Hz), 110.0, 118.8, 153.8, 172.3. EIMS : 403 (1), 386 (12), 232 (100), 217 (38), 164 (68), 160 (53), 99 (56), 73 (29).

Enol phosphate 15 : Starting from ketone 32, the procedure of Ireland (ref 10) was repeated on a 1 mmol scale, to afford the desired enol phosphate in 95% yield. 15 : $[\alpha]_D$: - 21 (c= 2.0). IR : 2980, 1686, 1273, 1120, 1039. ^{1}H-NMR : 1.27 (3H, s); 1.27 (3H, s); 1.29 (3H, s); 1.59 (6H, m); 1.65 (1H, m); 1.79-1.96 (2H, m); 2.10-2.35 (2H, m); 2.35 (1H, d, J= 16); 3.97 (8H, m); 5.63 (2H, m). ^{13}C-NMR : 15.9 ($^3J_{C-P}$= 6.0) (2), 23.9, 25.0,

26.1, 27.0, 27.8, 29.4, 39.1 ($^3J_{C-P}$= 6.3), 42.3, 63.9 ($^2J_{C-P}$= 5.6) (2), 64.4, 65.0, 105.3, 111.5, 119.3, 147.6, 150.7 ($^2J_{C-P}$= 9.2). **EIMS** : 386 ($M^{+\cdot}$, 18), 371 (9), 324 (7), 309 (25), 285 (52), 230 (43), 156 (34), 147 (59), 132 (46), 87 (100). **HREIMS** : calcd for $C_{19}H_{31}O_6P$: m/z 386.1859; found : 386.1862.

Oxidation of compound 15 : The oxidation of compound **15** is carried out according to the general procedure for allylic oxidations. The residue thus obtained is chromatographed on silica gel (EtOAc) to give **16** (26%) together with 55% of unreacted starting material. **16** : $[\alpha]_D$ - 0.1 (c= 1.2). **IR** : 2983, 2938, 2911, 1675, 1609, 1282, 1130, 1034, 960. **^{1}H-NMR** : 1.35 (6H, m); 1.38 (3H, s); 1.41 (3H, s); 1.45 (3H, s); 2.02 (1H, dd, J= 7.4, 16.3); 2.59 (2H, dd, J= 4.2, 16.7); 2.85 (1H, d, J= 16.6); 3.80-4.40 (8H, m); 5.72 (1H, d, J= 7.4); 6.11 (1H, s). **^{13}C-NMR** : 16.1 ($^3J_{C-P}$= 6.1) (2), 23.6, 26.1, 27.2, 28.8, 40.2, 43.7, 44.6, 64.3 ($^2J_{C-P}$= 3.1) (2), 65.2, 65.5, 105.1, 111.8, 124.5, 149.4, 173.9,196.9. **EIMS** : 352 (22), 308 (6), 221 (15), 99 (100), 85 (39).

Reduction of compound 16. Method 1 : Starting material recovered unchanged. Method 2 : A mixture of two allylic alcohols **17** (7β) and **18** (7α) is obtained in 89% combined yield and in a ratio of 1:1. Method 3 : Only the alcohol **18** (7α) alcohol is obtained in 95% yield. Acetylation of **18** as above afforded a 95% isolated yield of **18, OAc instead of OH** : $[\alpha]_D$: - 4 (c= 1.03). **IR** : 2984, 2937, 1735, 1688, 1668, 1647, 1251, 1038. **^{1}H-NMR** : 1.27 (3H, s); 1.29 (3H, s); 1.33 (3H, s);1.36 (6H, q, J= 6.9, 12.2); 1.92 (2H, dd, J= 6.2, 18.8); 2.09 (3H, s); 2.24 (1H, m); 2.54 (1H, d, J= 17.6); 3.90-4.20 (8H, m); 5.47 (1H, dd, J= 2.0, 6.3); 5.64 (1H, d, J= 7.2); 5.71 (1H, d, J= 4.3). **^{13}C-NMR** : 16.1 ($^3J_{C-P}$= 6.8) (2), 21.5, 23.5, 27.3, 27.7, 29.4, 32.2, 39.6, 42.5, 64.2 ($^2J_{C-P}$= 3.9) (2), 64.8, 65.1, 68.1, 105.4 ($^2J_{C-P}$= 2.7), 110.2, 118.3, 150.2, 153.9, 171.0. **EIMS** : 444 ($M^{+\cdot}$, 2), 384 (35), 369 (100), 230 (30), 215 (26), 162 (45), 87 (96).

Preparation of 11. The protection of the alcohol **36** (100 mg, 0.39 mmol) is achieved according to the procedure of Ireland et al. and gave 136 mg (90%) of diethylphosphate **11**. $[\alpha]_D$: - 17 (c= 1.0). **IR** : 2990, 2975, 2950, 2940, 1475, 1265, 1035, 1000, 980. **^{1}H-NMR** : 1.12 (3H, s); 1.19 (3H, s); 1.32 (3H, s); 1.33 (6H, t, J= 7.3); 1.5-2.5 (8H, m); 3.2-4.2 (9H, m); 5.62 (1H, t, J= 2.75). **^{13}C-NMR** : 16.1 ($^3J_{C-P}$= 6.2) (2), 24.1, 24.4, 24.6, 25.5, 25.8, 26.5, 26.7, 41.4, 42.4, 63.3 ($^2J_{C-P}$= 6.2) (2), 64.6, 65.2, 84.1, 112.4, 120.1, 147.1. **EIMS** : 388 ($M^{+\cdot}$, 12), 234 (21), 219 (16), 155 (49), 148 (100), 133 (91), 97 (92), 96 (37).

Bioconversion of 11. 120 mg (0.31 mmol), 30 g of fresh biomass, 300 ml of water, 48 h. Silica gel chromatography (EtOAc/MeOH, 97:3) gave **13** (73 mg, 58%) **m.p.** : 215-217°C (heptane). $[\alpha]_D$: - 7 (c= 1.72). **IR** : 3414, 3970, 2929, 2871, 1646, 1436, 1357, 1257, 1125, 1031, 1000, 968. **^{1}H-NMR** : 1.16 (3H, s); 1.22 (3H, s); 1.33 (6H, m); 1.37 (3H, s); 1.40-2.30 (6H, m); 3.8-4.2 (9H, m); 4.39 (1H, ddd, J= 2.7, 7.2, 9.6); 5.68 (1H, d, J= 2.3). **^{13}C-NMR** : 16.1 ($^3J_{C-P}$= 4.0) (2), 24.1, 24.6, 25.5, 26.5, 26.7, 36.1, 41.3 ($^2J_{C-P}$= 7.6), 42.7, 63.5, (2), 64.6, 65.3, 66.7, 83.8, ($^3J_{C-P}$= 6.8), 112.8, 124.4, 149.0. **EIMS** : 388 (9), 164 (55), 155 (24), 149 (27), 99 (26), 87 (100). **CIMS** : 405 $[M + H]^+$, 387 $[M - H_2O + H]^+$.

Preparation of compound 7. Starting from the (S)-(+)-Wieland-Miescher ketone the C-9 carbonyl was selectively reduced and dimethylated in C-4 according to known literature procedures (see ref 1). The corresponding 9β-alcohol (760 mg, 4.22 mmol) was dissolved in a mixture of DMF and DCM (2:8). To this solution were added *t*-butyl-dimethyl-silyl chloride (1.27 g, 8.44 mmol), then imidazole (574 mg, 8.44 mmol). The mixture was stirred for 60 h at room temperature. After removal of the solvent under reduced pressure, the residue was diluted with DCM. Extraction with water followed by standard treatment leads to a crude product which is purified by chromatography on silica gel (EtOAc/heptane, 1:4). This affords the 9-protected alcohol (970 mg, 78%) and 12% of recovered starting material. **7, 3=O instead of -OAc** : $[\alpha]_D$: - 1 (c = 1.7). **IR** : 2956, 2931, 2890, 2859, 1713, 1465, 1381, 1255, 1106, 1025, 837,774. **^{1}H-NMR** : 0.03 (3H, s); 0.05 (3H, s); 0.88 (9H, s); 0.93 (3H, s); 1.19 (3H, s); 1.23 (3H, s); 1.67 (2H, m); 2.00-2.20 (4H, m); 2.49 (2H, m); 3.48 (1H, dd, J= 4.6, 11.2); 5.48 (1H, t, J= 3.6). **^{13}C-NMR** : -4.9, -4.0, 17.9, 18.2, 24.6 , 25.8, 26.6, 27.5, 28.8, 31.6, 33.8, 39.6, 48.6, 76.5, 119.7, 148.3, 215.4. **EIMS** : 322 ($M^{+\cdot}$, 4), 307 (7), 265 (100), 190 (9), 173 (13), 164 (18), 75 (47), 73 (22).

Reduction of the 9-protected ketone 7, 3=O instead of -OAc at its C-3 carbonyl was achieved as follows : A solution of the silyl ether (100 mg, 0.31 mmol) in a mixture of EtOH-DCM (1:1, 4 ml) was cooled to -78°C. Solid sodium borohydride (38 mg, 1 mmol) was added and the mixture stirred for 12 h before quenching with 2N HCl. The ethanol was removed under reduced pressure and the remaining solution diluted with water. Extraction with water followed by standard treatment gave a crude product. This was chromatographed on silica gel (EtOAc/heptane, 1:4) to afford solely the 3β-alcohol **7, 3-OH instead of 3-OAc** : $[\alpha]_D$: - 4 (c = 1.5). **IR** :

3391, 2951, 2857, 1472, 1366, 1254, 1111, 1095, 1017, 1001, 836, 774. **^{1}H-NMR** : 0.01 (3H, s); 0.04 (3H, s); 0.89 (9H, s); 1.04 (3H, s); 1.09 (3H, s); 1.12 (3H, s); 1.50-1.90 (6H, m); 2.10 (2H, m); 3.22 (1H, t, J= 7.8); 3.33 (1H, dd, J= 3.9, 11.6); 5.49 (1H, t, J= 3.2). **^{13}C-NMR** : -4.8, -4.0, 18.0, 19.9, 23.1, 25.0, 25.9, 26.7, 26.8, 27.2, 35.9, 39.4, 41.4, 77.5, 78.7, 119.2, 148.7. **EIMS** : 324 ($M^{+\cdot}$, 1), 268 (22), 250 (26), 176 (48), 149 (100), 134 (70), 120 (28), 76 (78), 74 (38).

Acetylation of the alcohol thus obtained afforded a 95% isolated yield of the desired 3 β-acetate **7** : $[\alpha]_D$: - 2 (c = 1.49). **IR** : 3022, 2954, 2930, 2859, 1728, 1473, 1373, 1260, 1114, 1096, 1001. **^{1}H-NMR** : 0.04 (3H, s); 0.06 (3H, s); 0.90 (9H, s); 1.02 (3H, s); 1.13 (6H, s); 1.56-1.80 (6H, m); 1.87 (1H, ddd, J= 3.5, 6.8, 13.4); 2.07 (3H, s); 2.13 (1H, dd, J= 3.7, 6.6); 3.37 (1H, dd, J= 3.8, 12.0); 4.48 (1H, dd, J= 5.1, 10.9); 5.51 (1H, t, J= 3.8). **^{13}C-NMR** : -4.9, -4.2, 17.8, 19.8, 20.9, 23.5, 24.2, 24.8, 25.7, 26.5, 27.5, 35.4, 39.2, 40.0, 78.4, 79.2, 119.6, 147.7, 170.0. **EIMS** : 366 ($M^{+\cdot}$, 4), 351 (8), 308 (32), 268 (11), 250 (30), 175 (68), 148 (100), 133 (87), 75 (32).

Oxidation of 7 according to the general procedure for allylic oxidation led to a residue which was chromatographed on silica gel (EtOAc/heptane, 1:2) to give the enone **8** in 40% yield. We obtained a better yield with the following procedure : A solution of CrO_3 (10.3 mg, 0.103 mmol) in DCM (4 ml) was prepared. A 70% solution of t-butyl hydroperoxide (0.25 ml, 1.80 mmol) was added, followed by a solution of **7** (100mg, 0.27 mmol) in 1 ml of DCM, and the mixture was stirred for 6 hours before diluting with DCM. Usual work up afforded **8** in 64% yield together with unreacted starting material (33%). **8** $[\alpha]_D$: + 1 (c = 1.49). **IR** : 3021, 2957, 2931, 2861, 1731, 1663, 1256, 1113, 1033, 1000. **^{1}H-NMR** : 0.05 (3H, s); 0.06 (3H, s); 0.89 (9H, s); 1.12 (3H, s); 1.22 (3H, s); 1.26 (3H, s); 1.70-2.00 (4H, m); 2.09 (3H, s); 2.57 (2H, dd, J= 4.1, 16.4); 3.73 (1H, dd, J= 7.1, 10.3); 4.54 (1H, dd, J= 5.8, 8.1); 6.04 (1H, s). **^{13}C-NMR** : -5.1, -4.3, 17.7, 18.7, 20.8, 22.9, 24.3, 25.6, 25.7, 33.8, 40.8, 41.1, 42.7, 75.6, 77.1, 125.0, 169.6, 174.2, 197.4. **EIMS** : 380 ($M^{+\cdot}$, 2), 365 (9), 323 (64), 281 (23), 263 (29), 222 (45), 189 (34), 147 (50), 117 (77), 75 (86), 43 (100).

Reduction of 8 : A) Performed according to method 1, affords the 7β-alcohol **9** in quantitative yield. B) Performed according to method 3, affords the 7β-alcohol **9** in 60% yield. **9 m.p.** : 97-99°C (heptane). $[\alpha]_D$: + 1 (c = 1.18). **IR** : 3408, 2955, 2883, 2858, 1740, 1717, 1472, 1373, 1250, 1111, 1030, 866, 838, 776. **^{1}H-NMR** : 0.01 (3H, s); 0.04 (3H, s); 0.86 (9H, s); 1.02 (3H, s); 1.14 (3H, s); 1.15 (3H, s); 1.50-2.00 (6H, m); 2.05 (3H, s); 3.28 (1H, dd, J= 2.9, 12.4); 4.29 (1H, t, J= 7.6); 4.40 (1H, dd, J= 6.2, 9.2); 5.51 (1H, d, J= 1.3). **^{13}C-NMR** : -4.9, -4.1, 17.9, 20.1, 21.2, 23.6, 24.1, 25.8, 26.7, 35.2, 37.1, 39.8, 40.3, 66.9, 76.1, 79.1, 124.1, 150.6, 170.7. **EIMS** : 365 (4), 337(6), 324 (8), 264 (13), 223 (16), 190 (100), 172 (63), 75 (29).

Bioconversion of 15 : 280 mg (0.73 mmol), 70 g of fresh biomass, 500 ml of water, 48 h. Silica gel chromatography (EtOAc) gave **17** (223 mg, 76% yield). $[\alpha]_D$: - 4 (c= 2.0). **IR** : 3416, 2975, 2925, 2875, 1685, 1654, 1269, 1125, 1038. **^{1}H-NMR** : 1.29 (3H, s); 1.292 (3H, s); 1.32 (3H, s); 1.35 (6H, m); 1.82-1.88 (2H, m); 2.11 (1H, dd, J= 12.5, 6.6 Hz); 2.37 (1H, d, J= 16.3 Hz); 3.95-4.02 (8H, m); 4.39 (1H, ddd, J= 9.3, 6.3, 3.0 Hz); 5.59 (1H, ddd, J= 7.3, 3.6, 1.8 Hz); 5.67 (1H, d, J= 3.0 Hz). **^{13}C-NMR** : 16.0 ($^3J_{C\text{-}P}$= 6.4 Hz) (2), 24.8, 27.0, 28.0, 29.1, 36.3, 39.3 ($^3J_{C\text{-}P}$= 6.8 Hz), 42.5, 64.2 ($^2J_{C\text{-}P}$= 5.5 Hz) (2), 64.6, 65.1, 66.7, 105.4, 111.9, 124.0, 149.2, 150.5 ($^2J_{C\text{-}P}$= 8.5 Hz). **CIMS** : 403 (0.8, M+H), 385 (100, M+H-H_2O).

Bioconversion of (S)-(+)-26 : 1.11 g (5 mmol), 200 g of fresh biomass, 500 ml of water, 22 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to give a mixture of alcohols **59** and **60** in 95% isolated yield and 88 : 12 ratio. The two epimers are separated by HPLC using heptane/isopropanol 97 : 3 as eluent.

59 : $[\alpha]_D$ +1 (c = 1.0). **IR** : 3409, 2975, 2939, 1668, 1391, 1364, 1263, 1198, 1100, 1093, 1060, 1027, 737. **^{1}H-NMR** : 1.18 (9H, s); 1.27 (3H, s); 1.71 (1H, ddd, J= 5.0, 13.7, 12.7); 1.88 (1H, ddd, J= 7.3, 10.4, 14.0); 2.05 (1H, dd, J= 5.1, 2.4); 2.40 (1H, ddd, J= 18.0, 5.0, 5.0); 2.44 (1H, ddd, J= 14.0, 7.0, 8.0); 2.56 (1H, ddd, J= 18.0, 14.0, 5.0); 3.49 (1H, dd, J= 7.4, 10.4); 4.57 (1H, t, J= 7.5); 5.98 (1H, s). **^{13}C-NMR** :16.9, 28.6, 33.4, 36.6, 35.0, 40.4, 70.0, 73.2, 76.9, 124.8, 173.8, 200.4. **EIMS** : 238 ($M^{+\cdot}$, 11), 183 (12), 182 (100), 180 (17), 57 (49). **HREIMS** : calcd for $C_{14}H_{22}O_3$: m/z 238.1568, found 238.1588.

60: $[\alpha]_D$: + 108 (c= 0.54). **IR** : 3422, 2981, 2937, 2872, 1669, 1364, 1197, 1098, 886. **^{1}H-NMR** : 1.12 (3H, s); 1.17 (9H, s); 1.95-2.09 (2H, m); 2.20 (1H, m); 2.47 (1H, ddd, J= 18.0, 14.0, 5.0); 3.80 (1H, t, J= 8.7); 4.85 (1H, ddd, J= 9.8, 3.3, 1.6); 6.02 (1H, d, J= 1.6). **^{13}C-NMR** : 16.8, 28.6 (3), 33.5, 33.8, 35.3, 41.6, 69.4, 73.3, 77.9, 122.0, 175.8, 199.8. **EIMS** : 238 ($M^{+\cdot}$, 5), 182 (100), 154 (18), 140 (43), 135 (41), 57 (79), 41 (27).

Bioconversion of (R)-(-)-58: 1.11 g (5 mmol), 200 g of fresh biomass, 500 ml of water, 22 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to give a mixture of alcohols **61** and **62** in 95% isolated yield and 56 : 44 ratio. The two epimers are separated by HPLC as above.

61 : $[\alpha]_D$: -1 (c=0.8). **62** : $[\alpha]_D$ -102 (c=0.89).

Bioconversion of 32. 300 mg (1.2 mmol), 75 g of fresh biomass, 500 ml of water, 11 h. The residue is chromatographed (EtOAc/heptane, 1:2) to give a mixture of two hydroxylated compounds **33** and **34** in 70% combined yield plus the reduction product **35** (3.5%).

33 : 166 mg (54%). **m.p.** : 95-97°C (heptane). $[\alpha]_D$: + 39 (c = 1.44). **IR** : 3412, 3105, 2990, 1706, 1651, 1140, 1055. ^{1}**H-NMR** : 1.15 (3H, s); 1.28 (3H, s); 1.31 (3H, s); 1.69 (1H, m); 1.87 (2H, m); 2.19 (2H, m); 2.52 (2H, m); 3.80-4.10 (4H, m); 4.41 (1H, ddd, J = 2.8, 6.8, 9.2); 5.65 (1H, d, J= 2.5). ^{13}**C-NMR** : 23.0, 24.3, 26.6, 29.0, 33.7, 36.1, 42.4, 48.7, 64.7, 65.0, 66.8, 112.1, 123.7, 149.3, 209.3. **CIMS** : 267 $[M + H]^+$, 249 $[M - H_2O + H]^+$.

34 : 62 mg (16%). **IR** : 3430, 3105, 2995, 1719, 1706, 1647, 1140, 855. ^{1}**H-NMR** : 1.30 (3H, s); 1.35 (3H, s); 1.39 (3H, s); 2.00-3.00 (7H, m); 4.10 (1H, m); 5.95 (1H, d, J= 4.0). ^{13}**C-NMR** : 25.5, 26.8, 27.6, 29.0, 33.7, 36.1, 44.6, 66.5, 112.1, 124.4, 151.5, 210.3, 211.5. **EIMS** : 266 ($M^{+\cdot}$, 3), 249 (12), 222 (22), 149 (27), 123 (25), 87 (100).

Bioconversion of 36. 300 mg (1.42 mmol), 50 g of fresh biomass, 500 ml of water, 18 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to give a mixture of two compounds **37** and **41** in 69% combined yield.

37 : 162 mg, (42%) **m.p.** : 170-172°C (heptane). $[\alpha]_D$: - 15 (c= 0.35, MeOH). **IR** : 3420, 2982, 2942, 2878, 1654, 1622, 1562, 1466, 1364, 1062, 1036, 1007, 821. ^{1}**H-NMR** : 1.11 (3H, s); 1.21 (3H, s);1.36 (3H, s); 1.4-1.8 (4H, m); 1.85 (1H, dd, J= 12.5, 9.6); 2.13 (1H, dd, J= 12.5, 7.2); 3.32 (1H, dd, J= 8.8, 6.7); 3.94 (4H, m); 4.39 (1H, ddd, J= 9.6, 7.2, 3.0); 5.65 (1H, d, J= 3). ^{13}**C-NMR** : 23.4, 25.6, 26.8, 26.9, 26.95, 36.3, 41.6, 42.9, 64.7, 65.4, 67.2, 76.8, 112.9, 123.4, 150.4. **EIMS** : 268 ($M^{+\cdot}$, 1), 182 (9), 164 (14), 149 (30), 87 (100). **HREIMS** : calcd for $C_{15}H_{24}O_4$: m/z 268.1675, found : 268.1678.

41 : 87 mg, (27%). **m.p.** : 99-101°C (heptane). $[\alpha]_D$: - 17 (c= 1.0, MeOH). **IR** : 3376, 2974, 2943, 2876, 1707, 1463, 1043. ^{1}**H-NMR** : 1.18 (3H, s); 1.29 (3H, s);1.43 (3H, s); 1.50 (1H, m); 1.81 (3H, m); 2.73 (1H, dd, J= 12.8, 6.2); 2.84 (1H, dd, J= 12.8, 8.4); 3.23 (1H, dd, J= 10.7, 4.7); 4.51 (1H, ddd, J= 8.4, 6.2, 3.2); 5.87 (1H, d, J= 3.2). ^{13}**C-NMR** : 22.6, 26.8, 26.9, 27.0, 30.7, 42.3, 44.8, 47.0, 67.2, 76.7, 124.4, 150.2, 211.6. **EIMS** : 224 ($M^{+\cdot}$, 6), 206 (47), 149 (100), 135 (23), 121 (34), 91 (19). **HREIMS** : calcd for $C_{13}H_{20}O_3$: m/z 224.1413, found : 224.1414.

Bioconversion of 38. 120 mg (0.47 mmol), 30 g of fresh biomass, 375 ml of water, 18 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to give a mixture of two compounds **39** and **43** in 64% combined yield. 20 % of starting material is also recovered.

39 : 70 mg, (55%) : **m.p.** : 173-175°C (MeOH). $[\alpha]_D$: - 28 (c= 1.0). **IR** :3491, 3365, 2949, 2884, 1467, 1306, 1159, 1135, 1058, 1040, 755. ^{1}**H-NMR** : 1.19 (3H, s); 1.21 (3H, s);1.39 (3H, s); 1.69 (1H, m); 1.87 (1H, dd, J= 12.2, 9.6); 2.08 (4H, m); 2.14 (1H, dd, J= 12.2, 7.0); 3.49 (1H, d, J= 3.6); 3.97 (4H, m); 4.40 (1H, ddd, J= 9.6, 7.0, 2.3); 5.58 (1H, d, J= 2.3). ^{13}**C-NMR** : 22.1, 24.4, 26.5, 27.7, 29.7, 36.2, 40.7, 42.5, 64.7, 65.3, 66.7, 76.2, 113.1, 124.9, 148.0. **EIMS** : 268 ($M^{+\cdot}$, 15), 251 (31), 182 (95), 164 (95), 149 (98), 135 (63), 123 (90), 87 (100). **HREIMS** : calcd for $C_{15}H_{24}O_4$: m/z 268.1675, found : 268.1690.

43 : 10 mg, (9%) : $[\alpha]_D$: -25 (c= 1.2). **IR** : 3475, 3360, 2972, 2870, 1710, 1325, 1159, 1035, 755. ^{1}**H-NMR** : 1.17 (3H, s); 1.20 (3H, s);1.35 (3H, s); 1.73 (1H, m); 1.87-2.08 (5H, m); 2.14 (1H, dd, J= 12.1, 7); 3.35 (1H, d, J= 3.3); 4.39 (1H, ddd, J= 9.5, 7, 1.9); 5.54 (1H, d, J= 2.3). ^{13}**C-NMR** : 22.5, 26.8, 27.2, 27.3, 29.9, 42.4, 44.5, 47.0, 62.0, 75.5, 122.6, 149.9, 211.2. **EIMS** : 224 ($M^{+\cdot}$, 12), 164 (65), 149 (98), 123 (90), 57 (100).

Bioconversion of 40 : 170 mg (0.81 mmol), 42.5 g of fresh biomass, 425 ml of water, 24 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to give **41** (115 mg, 63%) and **42** (15 mg, 9%). **42** : $[\alpha]_D$: - 117 (c= 1.0, MeOH). **IR** : 3430, 2976, 2855, 1675, 1120, 1042, 986. ^{1}**H-NMR** : 1.16 (3H, s); 1.20 (3H, s); 1.38 (3H, s); 1.50-2.20 (4H, m); 3.30 (1H, dd, J= 9.7, 6.0); 5.93 (3H, m). ^{13}**C-NMR** : 22.8, 23.4, 26.3, 27.5, 30.0, 42.9, 50.9, 76.7, 116.4, 122.6, 141.5, 165.0, 207.8. **EIMS** : 188 (M-18, 31), 148 (M-58, 100)

Preparation of 44 : According to the general procedure described above, alcohol **36** was converted to the corresponding acetate. Standard treatment leads to a crude product which is purified on silica gel

(EtOAc/heptane, 1:2) to give 280 mg (100%) of the acetate **44** : $[\alpha]_D$: - 48 (c= 0.81). **IR** : 2960, 2880, 1734, 1472, 1361, 1243, 1138, 1111, 1041, 988. **^{1}H-NMR** : 1.06 (3H, s); 1.15 (3H, s); 1.33 (3H, s); 1.4-2.0 (6H, m); 2.05 (3H, s); 2.1-2.4 (2H, m); 3.95 (4H, m); 4.55 (1H, dd, J= 7.7, 10.8); 5.59 (1H, t, J= 7.2). **^{13}C-NMR** : 21.0, 23.1, 24.1, 24.8, 25.6, 25.8, 26.5, 26.7, 40.1, 42.5, 64.5, 65.2, 78.8, 112.3, 119.6, 147.2, 170.2. **EIMS** : 294 ($M^{+\cdot}$, 23), 173 (88), 148 (94), 87 (100), 86 (65).

Bioconversion of 44. 200 mg (0.68 mmol), 50 g of fresh biomass, 500 ml of water, 24 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to give **45** and **46** in 81% combined yield. Only **45** could be obtained pure.

45 : 139 mg, (66%) : $[\alpha]_D$: - 13 (c= 0.83). **IR** : 3420, 2977, 2877, 1732, 1645, 1469, 1377, 1247, 1138, 1046, 1000. **^{1}H-NMR** : 1.08 (3H, s); 1.18 (3H, s); 1.37 (3H, s); 1.6-2.2 (6H, m); 2.06 (3H, s); 3.8-4.2 (4H, m); 4.39 (1H, ddd, J= 2.7, 7.2, 9.6); 4.54 (1H, dd, J= 7.8, 9.5); 5.64 (1H, d, J= 2.4). **^{13}C-NMR** : 21.1, 23.1, 24.6, 25.6, 26.5, 26.7, 30.2, 40.2, 42.8, 64.6, 65.3, 66.8, 78.6, 112.8, 123.9, 149.2, 170.6. **EIMS** : 310 ($M^{+\cdot}$, 4), 211 (42), 164 (32), 149 (48), 142 (56), 129 (82), 115 (53), 87 (100). **HREIMS** : calcd for $C_{17}H_{26}O_5$: m/z 310.1780; found : 310.1770.

Bioconversion of 54 : 160 mg (0.61 mmol), 40 g of fresh biomass, 400 ml of water, 48 h. Elution with EtOAc/heptane, 1:2 afforded **55** and **56**.

55 : 98 mg, (54%) : $[\alpha]_D$: - 12 (c= 3.0). **IR** : 3406, 2972, 2866, 1724, 1635, 1455, 1051, 775. **^{1}H-NMR** : 1.15 (6H, s); 1.16 (3H, s); 1.19 (3H, s); 1.43 (3H, s); 1.60-2.00 (3H, m); 2.60-3.00 (3H, m); 3.07 (1H, dd, J= 6.0, 8.6); 3.40 (2H, s); 4.50 (1H, ddd, J= 2.7, 7.1, 9.8); 5.68 (1H, d, J= 2.7). **^{13}C-NMR** : 22.7 (2), 23.3, 23.6, 26.3, 27.0, 27.6, 30.5, 42.2, 44.7, 46.6, 67.0, 71.1, 76.3, 124.2, 149.9, 211.7. **EIMS** : 207 ($[(M-18)-71]^+$, 100), 192 (74), 180 (98), 149 (75), 121 (50), 55 (25). **CIMS** : 297 $[M + H]^+$, 279 $[M - H_2O + H]^+$.

56 :54 mg, (31%) : **^{1}H-NMR** : 1.15 (3H, s); 1.20 (6H, s); 1.25 (3H, s); 1.37 (3H, s); 1.60-2.00 (2H, m); 2.60-3.0 (2H, m); 3.17 (1H, dd, J= 5.7, 9.6); 3.41 (2H, s); 5.94 (1H, d, J= 9.6 Hz,); 6.24 (1H, d, J= 6.4 Hz); 7.02 (1H, dd, J= 6.2, 9.5 Hz). **^{13}C-NMR** : 22.7, 23.4, 26.2, 27.4, 29.1 (2), 42.9, 44.6, 67.4, 71.3, 76.4, 116.4, 122.8, 123.8, 141.7, 151.3, 211.5. **CIMS** : 279 $[M + H]^+$, 261 $[M - H_2O + H]^+$.

Preparation of compound 49 : A 250 ml two-necked flask equipped with a dry-ice condenser is charged with a solution of enol phosphate **15** (2 g, 5.2 mmol) in dry THF (30 ml). Next ethylamine is condensed into the flask at -78°C. t-Butanol (4 ml) is added followed by lithium (500 mg, 70 mmol). During the addition of the lithium a blue colour appears. The mixture is stirred at -78°C for 30 min. then ether (30 ml) is added. After warming the reaction to room temperature the solvents are removed under reduced pressure. The residue is dissolved in ether and washed twice with water. Usual workup leads to a crude product which is purified by chromatography on silica gel (EtOAc: heptane, 1:3) to give 927 mg (83%) of **49**. $[\alpha]_D$: - 44 (c= 1.95). **IR** : 3117, 3110, 3050, 2950, 1665, 1475, 1210, 1140, 1090, 1043. **^{1}H-NMR** : 1.13 (3H, s); 1.17 (3H, s); 1.26 (3H, s); 1.5-2.5 (6H, m); 3.95 (4H, m); 5.37 (1H, dd, J= 2.9, 9.9); 5.60 (2H, m). **^{13}C-NMR** : 24.0, 25.3, 26.5, 28.4, 31.3, 33.2, 36.2, 42.8, 64.6, 65.2, 112.0, 118.4, 121.8, 136.3, 148.2. **EIMS** : 234 ($M^{+\cdot}$, 21), 210 (6), 149 (30), 133 (100), 91 (37), 87 (73), 86 (42). **HREIMS** : calcd for $C_{15}H_{22}O_2$: m/z 234.1619 ; found : 234.1627.

Bioconversion of hexalin 49 stopped at low conversion : 100 mg (0.42 mmol), 25 g of fresh biomass, 250 ml of water, 6 h. Silica gel chromatography (EtOAc/heptane, 1:2) gave 40% of the 7β-hydroxylated compound **50**, and mainly unreacted starting material. **50** : $[\alpha]_D$: - 5 (c= 1.5). **IR** : 3360, 3020, 2960, 2925, 2860, 1664, 1648, 1137, 1054. **^{1}H-NMR** : 1.16 (3H, s); 1.22 (3H, s); 1.29 (3H, s); 1.79 (1H, dd, J= 11.8, 6.6); 1.88 (1H, dd, J= 12.0, 9.3); 2.13 (1H, dd, J= 12.0, 6.6); 2.29 (1H, d, J= 11.8); 3.98 (4H, m); 4.41 (1H, ddd, J= 9.3, 6.6, 2.6); 5.38 (1H, dd, J= 9.9, 3.0); 5.83 (2H, m). **^{13}C-NMR** : 24.9, 28.4, 31.3, 32.6, 36.3, 36.5, 42.8, 64.6, 65.2, 66.9, 112.2, 121.7, 122.7, 135.9, 150.2. **EIMS** : 250 ($M^{+\cdot}$, 4), 232 (3), 217 (2), 87 (100). **CIMS** : 233 (100, M+H-H_2O).

Bioconversion of hexalin 49 at high conversion : 600 mg (2.56 mmol), 150 g of fresh biomass, 1.5 l of water, 20 h. Silica gel chromatography afforded a mixture of epoxides **51** and **52** (only the α isomer of which could be isolated pure) accounting for 60% yield and 10% of the diol **53**.

52α : $[\alpha]_D$ -5 (c= 1.5). **IR** : 3424, 2975, 2937, 2887, 1715, 1650, 1054, 1008. **^{1}H-NMR** : 1.35 (3H, s); 1.38 (3H, s); 1.42 (3H, s); 2.10 (2H, m); 2.72 (2H, d, J= 6.4); 2.89 (1H, d, J= 3.8); 3.34 (1H, m); 4.50 (1H, dt, J= 2.8, 6.4); 5.89 (1H, d, J= 3.7). **^{13}C-NMR** : 27.6, 28.1, 29.6, 32.7, 36.2, 44.8, 45.5, 51.3, 60.6, 66.1, 125.3, 122.7, 210.4.

53 : $[\alpha]_D$: - 2 (c= 0.41). **IR** : 3404, 2950, 2900, 1713, 1652, 1053, 1006. **^{1}H-NMR** : 1.29 (6H, s); 1.32 (3H, s); 2.78 (2H, t, J= 6.9); 4.30 (1H, d, J= 6.1); 4.64 (1H, dd, J= 4.7, 8.9); 5.65 (1H, d, J= 9.8); 5.85 (1H, dd, J= 6.1, 10.0); 6.16 (1H, d, J= 3.8). **^{13}C-NMR** : 25.3, 27.4, 29.6, 31.5, 46.9, 66.2, 69.8, 122.4, 126.1, 140.9. **EIMS** : 222 ($M^{+\cdot}$, 24), 204 (29), 149 (49), 133 (62), 91 (76), 87 (100), 55 (70), 41 (95).

Bioconversion of (R)-(-)-58 : 1.11 g (5 mmol), 200 g of fresh biomass, 500 ml of water, 22 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to give a mixture of alcohols **61** and **62** in a 95% combined yield and 88:12 ratio. The two epimers are separated by HPLC using heptane/isopropanol, 97:3 as eluent.

61 : $[\alpha]_D$: - 1 (c= 1.0). **IR** : 3409, 2975, 2939, 1668, 1391, 1364, 1263, 1198, 1100, 1093, 1060, 1027, 737. **^{1}H-NMR** : 1.18 (9H, s); 1.27 (3H, s); 1.71 (1H, ddd, J= 5.0, 13.7, 12.7); 1.88 (1H, ddd, J= 7.3, 10.4, 14.0); 2.05 (1H, dd, J= 5.1, 2.4); 2.40 (1H, ddd, J= 18.0, 5.0, 5.0); 2.44 (1H, ddd, J= 14.0, 7.0, 8.0); 2.56 (1H, ddd, J= 18.0, 14.0, 5.0); 3.49 (1H, dd, J= 7.4, 10.4); 4.57 (1H, t, J= 7.5); 5.98 (1H, s). **^{13}C-NMR** :16.9, 28.6, 33.4, 36.6, 35.0, 40.4, 70.0, 73.2, 76.9, 124.8, 173.8, 200.4. **EIMS** : 238 ($M^{+\cdot}$, 11), 183 (12), 182 (100), 180 (17), 57 (49). **HREIMS** : calcd for $C_{14}H_{22}O_3$: m/z 238.1568, found : 238.1588.

62 : $[\alpha]_D$: - 102 (c= 0.54). **IR** : 3422, 2981, 2937, 2872, 1669, 1364, 1197, 1098, 886. **^{1}H-NMR** : 1.12 (3H, s); 1.17 (9H, s); 1.95-2.09 (2H, m); 2.20 (1H, m); 2.47 (1H, ddd, J= 18.0, 14.0, 5.0); 3.80 (1H, t, J= 8.7); 4.85 (1H, ddd, J= 9.8, 3.3, 1.6); 6.02 (1H, d, J= 1.6). **^{13}C-NMR** : 16.8, 28.6 (3), 33.5, 33.8, 35.3, 41.6, 69.4, 73.3, 77.9, 122.0, 175.8, 199.8. **EIMS** : 238 ($M^{+\cdot}$, 5), 182 (100), 154 (18), 140 (43), 135 (41), 57 (79), 41 (27).

Preparation, enzymatic hydrolysis and bioconversion of 6-acetylated hydrindenone derivatives in both steroid and antisteroid series. The procedure described in ref. 1 is used to convert **(S)-(+)-26**, **(R)-(-)-58** and their racemic mixture to the 6-acetoxy derivatives, obtained as an epimeric mixture in a 58:42 ratio and 95% isolated yield. Subjection of the epimeric mixture of the 6-acetates from steroid and anti-steroid series separately confirmed the (S)-specificity of horse liver esterase as only the (S)-acetates are hydrolyzed, the (R)-acetates remaining intact even after much longer incubation time (up to 72 h). We kept the 1:1 enzyme to substrate ratio throughout all our experiments, even though on several substrates we observed that a much lower ratio (as low as 1:2) is sufficient. All experiments with this lipase are easily performed on the bench with magnetic stirring and no special care is needed.

27α : $[\alpha]_D$: +52 (c=1.0). **IR** : 2977, 1748, 1691, 1638, 1377, 1237, 1207, 1095. **^{1}H-NMR** : 1.18 (9H, s), 1.24 (3H, s), 1.75-2.10 (3H, m), 2.17 (3H, s), 2.22-2.47 (2H, m), 2.73 (1H, m), 3.62 (1H, t, J= 8.7), 5.56 (1H, dd, J= 5.3, 13.5), 5.61 (1H, br.s). **^{13}C-NMR** : 16.4, 20.7, 26.8, 28.5, 29.4, 40.4, 46.3, 70.6, 73.1, 79.3, 121.4, 170.0, 174.5, 193.1. **EIMS** : 224 (46), 164 (94), 136 (57), 122 (51), 57 (100). **CIMS** : 281 (M+H, 100), 225 (39), 147 (15). **HREIMS** : calcd for $C_{16}H_{25}O_4$ M+H : 281.1753, found : 281.1743.

Alkaline hydrolysis of **27α** (K_2CO_3-MeOH-H_2O, 0°C) gave, after chromatography (EtOAc/heptane, 1:2), the corresponding alcohol **27α, OH instead of OAc**, which is in all respects identical with the same alcohol obtained via the (S)-proline catalyzed asymmetric Robinson annelation.

27α, OH instead of OAc : $[\alpha]_D$: +67 (c=1.0). **IR** ($CHCl_3$) : 3490, 2980, 1673, 1150, 1095, 1073. **^{1}H-NMR** : 1.17 (9H, s), 1.20 (3H, s), 1.64 (1H, t, J= 12.7), 1.80 (1H, m), 1.98 (1H, m), 2.37 (1H, m), 2.42 (1H, m), 2.73 (1H, m), 3.60 (1H, t, J= 9.0), 4.33 (1H, dd, J= 5.5, 13.2), 5.85 (1H, s). **^{13}C-NMR** : 16.2, 27.1, 28.5, 29.1, 43.0, 46.2, 69.1, 73.0, 79.2, 119.8, 176.6, 199.2. **EIMS** : 238 ($M^{+\cdot}$, 0.5), 223 (0.6), 182 (72), 57 (100).

27β, OH instead of OAc : $[\alpha]_D$: -22 (c=1.0). **IR** : 3409, 2977, 1663, 1640, 1470, 1397, 1364, 1224, 1204, 1098, 1052, 886. **^{1}H-NMR** : 1.19 (9H, s), 1.21 (3H, s), 1.85 (1H, m), 2.04 (3H, m), 2.34 (1H, m), 2.75 (1H, m), 3.63 (1H, t, J= 8.4), 4.08 (1H, t, J= 5.3), 5.87 (1H, s). **^{13}C-NMR** : 21.0, 27.4, 28.6, 29.8, 38.2, 44.9, 69.1, 73.1, 77.9, 120.1, 176.7, 199.0. **EIMS** : 238 ($M^{+\cdot}$, 1), 205 (47), 183 (11), 182 (73), 57 (100).

Acetylation of **27β, OH instead of OAc**, using the procedure described above (Ac_2O, Py, DMAP, 0°C), gave the 6β-acetate **27β** : $[\alpha]_D$: -6 (c=2.3). **IR** : 2977, 1742, 1669, 1364, 1224, 1091, 1032, 892. **^{1}H-NMR** : 1.17 (9H, s), 1.20 (3H, s), 1.75-2.03 (3H, m), 2.10 (3H, s), 2.20-2.50 (2H, m), 2.77 (1H, m), 3.57 (1H, dd, J= 7.9, 9.3), 5.23 (1H, dd, J= 1.9, 5.3), 5.90 (1H, s). **^{13}C-NMR** : 19.4, 20.8, 27.1, 28.3, 29.1, 38.3, 44.0, 69.4, 72.8, 78.9, 121.4, 168.2, 176.8, 190.4. **EIMS** : 280 ($M^{+\cdot}$, 4), 224 (61), 164 (100), 146 (81), 122 (54), 57 (39).

Bioconversion of 27α : 300 mg (1.07 mmol), 50 g of fresh biomass, 400 ml of water, 24 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:2) to afford **65** and **66** in 78.5% combined yield and 88:12 ratio, together with 14% of deacetylated alcohol **67**. The epimeric alcohols **65** and **66** are separated by HPLC using the above mentioned conditions.

65 : $[\alpha]_D$: + 13 (c= 1.2). **IR** : 3447, 2975, 2934, 2873, 1749, 1693, 1462, 1371, 1237, 1211, 1072, 1044, 889, 757. ^{1}**H-NMR** : 1.18 (9H, s); 1.42 (3H, s);1.80-1.93 (2H, m); 2.19 (3H, s); 2.29 (1H, dd, J= 12.0, 5.2); 2.50 (1H, m); 3.52 (1H, dd, J= 9.3, 7.9); 4.57 (1H, t, J= 7.2); 5.61 (1H, dd, J= 13.5, 5.2); 6.01 (1H, s). ^{13}**C-NMR** : 17.4, 20.8, 28.6, 40.3, 41.3, 45.6, 69.6, 71.1, 73.3, 76.8, 123.7, 170.4, 173.5, 194.7. **EIMS** : 296 ($M^{+\cdot}$, 5), 240 (50), 180 (100), 162 (42), 57 (66.7). **CIMS** : 297 $[M+H]^+$, 241 $[M-56+H]^+$.

66 : $[\alpha]_D$: + 27 (c= 0.16). **IR** : 3414, 3020, 2976, 2934, 1746, 1685, 1376, 1364, 1239, 1098, 1070. ^{1}**H-NMR** : 1.17 (9H, s); 1.23 (3H, s); 1.94 (1H, t, J= 13.6); 2.02 (1H, m); 2.18 (3H, s); 2.21-2.29 (2H, m); 3.83 (1H, t, J= 8.6); 4.87 (1H, m); 5.53 (1H, dd, J= 13.6, 5.1); 6.08 (1H, d, J= 1.7). ^{13}**C-NMR** : 17.5, 20.8, 28.5 (3), 41.1, 41.2, 46.4, 69.1, 70.7, 73.5, 77.7, 120.3, 170.3, 175.2, 193.8.

Bioconversion of 27β : The procedure used for **27α** is repeated. Column chromatography (eluent EtOAc/heptane, 1:1) gave a 95% combined yield of **63** and **64** in 88:12 ratio respectively.

63 : **IR** : 3442, 2971, 1745, 1679, 1463, 1369, 1232, 1194, 1093, 1027, 976, 887. ^{1}**H-NMR** : 1.17 (9H, s), 1.32 (3H, s), 1.90 (1H, m), 2.04 (1H, m), 2.11 (3H, s), 2.21 (1H, m), 2.44 (1H, m), 3.47 (1H, dd, J= 7.1, 14.1), 4.58 (1H, t, 7.7), 5.26 (1H, dd, 1.0, 5.3), 6.12 (1H, s). ^{13}**C-NMR** : 21.1, 21.2, 39.3, 40.1, 43.8, 28.6, 69.6, 69.9, 73.4, 76.4, 123.6, 170.0, 175.8, 193.9. **EIMS** : 296 ($M^{+\cdot}$, 4), 240 (24), 180 (28), 137 (36), 136 (28), 109 (24), 57 (100).

64 : $[\alpha]_D$: + 20 (c = 1.23). **IR** : 3449, 2977, 2918, 2851, 1748, 1677, 1461, 1367, 1227, 1093, 1060, 1024, 961, 889, 752. ^{1}**H-NMR** : 1.18 (9H, s), 1.26 (3H, s), 2.11 (3H, s), 1.92-2.42 (4H, m), 3.85 (1H, t, J= 8.6), 4.94 (1H, ddd, J= 1.5, 3.8, 10.0), 5.24 (1H, t, J= 5.0), 6.15 (1H, d, J= 1.5). ^{13}**C-NMR** : 21.1, 21.3, 28.7, 38.1, 42.0, 44.5, 69.5, 70.3, 73.6, 76.6, 120.2, 170.0, 177.0, 193.6. **EIMS** : 297 (M+1, 3), 240 (67), 198 (18), 180 (97), 163 (41), 162 (97), 151 (31), 145 (25), 138 (90), 137 (97), 136 (95), 135 (31), 134 (61), 133 (100), 109 (79), 108 (66), 91 (33), 81 (31), 57 (74).

Bioconversion of 30 : 200 mg (0.71 mmol), 50 g of fresh biomass, 500 ml of water, 24 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:1) to afford 32.5% of unchanged starting material and 25% of a single hydroxylated compound **70** : $[\alpha]_D$: + 95 (c = 0.91). **IR** : 3500, 2975, 1723, 1674, 1366, 1264, 1234, 1199, 1098. ^{1}**H-NMR** : 1.17 (9H, s); 1.29 (3H, s); 1.69 (2H, m); 1.85-2.13 (2H, m); 2.40-2.64 (3H, m); 3.49 (1H, dd, J= 10.7, 7.2); 3.66 (3H, s); 4.65 (1H, t, J= 7.9). ^{13}**C-NMR** : 16.1, 28.6 (3), 33.1, 33.7, 38.5, 45.4, 52.6, 68.7, 73.4, 76.3, 128.7, 167.2, 178.5, 195.1. **EIMS** : 224 (26), 208 (100), 192 (48), 166 (42), 164 (52), 131 (35), 57 (45).

Reaction of compound 27α with magnesium methyl carbonate (MMC) : 20 ml of 1M solution of MMC in DMF are added to 4.0 g (14.9 mmol) of **27α** and the reaction vessel is placed in a preheated oil bath (125°C) and stirred for 2h under inert atmosphere. The reaction mixture is chilled, poured into water and acidified with concentrated HCl. Extraction of the aqueous phase with ether gave, after careful removal of the solvent under reduced pressure and immediate treatment with diazomethane in ether, 1.5 g (33%) of the desired ester **31, OH instead of OAc** : $[\alpha]_D$: + 40 (c = 0.77). **IR** : 3428, 2990, 1733, 1683, 1638, 1196, 1102. ^{1}**H-NMR** : 1.17 (9H, s); 1.26 (3H, s); 1.60-2.20 (4H, m); 2.43 (1H, dd, J= 5.4, 12); 2.80 (1H, m); 3.65 (1H, dd, J= 7.8, 9.7); 3.82 (3H, s); 4.39 (1H, dd, J= 5.6, 13.6). ^{13}**C-NMR** : 16.8, 27.2, 28.6 (3), 29.0, 42.1, 46.7, 52.1, 69.1, 73.5, 78.8, 126.5, 165.0, 170.3, 189.5. **EIMS** : 296 ($M^{+\cdot}$, 5), 254 (9), 240 (48), 208 (100), 91 (13), 57 (27). **HREIMS** : calcd for $C_{16}H_{24}O_5$: m/z 296.3667, found 296.1631.

31, OH instead of OAc (1.5g, 5.28 mmol) was acetylated according to the general procedure to give 964 mg (56%) of **31**, 762 mg (38%) of **68** and 5% of recovered starting material (silica gel chromatography, eluent EtOAc/heptane, 1:3).

31 : $[\alpha]_D$: + 59 (c= 1.47). **IR** : 2980, 1750, 1694, 1642, 1374, 1234, 1101. ^{1}**H-NMR** : 1.18 (9H, s), 1.29 (3H, s), 1.80-2.04 (3H, m), 2.17 (3H, s), 2.28 (1H, dd, J= 5.4, 12.0), 2.58-2.88 (2H, m), 3.67 (1H, dd, J= 7.7, 9.9), 3.79 (3H, s), 5.59 (1H, dd, 5.4, 13.6). ^{13}**C-NMR** : 16.7, 20.6, 26.7, 28.5, 29.2, 39.4, 46.6, 51.8, 70.2, 73.4, 78.8, 126.6, 165.0, 170.0, 176.5, 189.5. **EIMS** : 338 ($M^{+\cdot}$, 22), 282 (19), 222 (58), 190 (92), 162 (100), 161 (95), 57 (39).

68 : $[\alpha]_D$: + 28 (c= 1.39). **IR** : 2970, 1777, 1737, 1637, 1367, 1236, 1225. ^{1}**H-NMR** : 1.09 (3H, s); 1.17 (9H, s); 1.4-1.7 (1H, m); 2.08 (3H, s); 2.13 (3H, s); 2.25, (1H, m); 2.45 (2H, m); 3.78 (3H, s); 3.90 (1H, m); 5.85 (2H, m). ^{13}**C-NMR** : 16.4, 20.8, 28.6 (3), 33.5, 38.9, 39.1, 46.7, 51.9, 67.7, 72.8, 79.4, 119.3, 124.7, 138.2,

147.9, 164.5, 168.0, 172.1. **EIMS** : 380 ($M^{+\cdot}$, 9), 338 (89), 222 (35), 208 (27), 193 (41), 190 (45), 162 (57), 161 (100).

Bioconversion of 31 : 150 g (0.44 mmol), 50 g of fresh biomass, 400 ml of water, 22 h. The residue is chromatographed on silica gel (EtOAc/heptane, 1:3) to give 40 mg (25%) of a single allylic alcohol **71** together with 40 % of unreacted starting material. **71** : $[\alpha]_D$: + 37 (c= 0.94). **IR** : 3500, 2950, 2870, 1753, 1702, 1654, 1232. ^{1}**H-NMR** : 1.17 (9H, s); 1.45 (3H, s); 1.88 (1H, t, J= 13 Hz); 1.98 (1H, m); 2.17 (3H, s); 2.28 (1H, m); 2.46 (1H, m); 3.55 (1H, dd, J= 7.4, 10.5 Hz); 3.87 (3H, s); 4.67 (1H, t, J= 7.7 Hz); 5.56 (1H, dd, J= 5.5, 13.7 Hz). ^{13}**C-NMR** : 17.1, 20.7, 28.5 (3), 38.2, 39.4, 46.1, 52.7, 68.9, 70.6, 73.5, 76.0, 127.2, 166.1, 170.0, 178.6, 189.8. **EIMS** : 354 ($M^{+\cdot}$, 3), 298 (67), 206 (100), 179 (56), 177 (65), 163 (66), 162 (65), 57 (44). **HREIMS** : calcd for $C_{18}H_{26}O_7$: m/z 354.1678, found : 354.1676.

Bioconversion of 68 : 200 mg (0.53 mmol), 50 g of fresh biomass, 400 ml of water, 20 h. The residue is chromatographed to give 53 mg (25%) of a single allylic alcohol **71**.

Oxidation of dienolacetate 68 with mCPBA : A solution of compound **68** (50 mg, 0.132 mmol) in a mixture of buffer (0.5 ml, pH8) and dioxane (0.5 ml) is cooled to 0°C. To the above solution is added dropwise a solution of 85% mCPBA (27.5 mg, 0.158 mmol) in 0.5 ml of dioxane and 0.5 ml of the buffer. The reaction mixture is stirred at 0°C for 24 h then quenched by adding solid sodium bicarbonate (53 mg) and 27 mg of sodium thiosulphate. After stirring a further 30 min. the mixture is diluted with ether and the ether washed twice with water. The aqueous phase is then extracted twice with ether and the combined organic layer is worked up in standard fashion to give a crude product which upon silica gel chromatography (EtOAc/heptane, 1:2) afforded allylic alcohol **69** (52%) as the sole product.

69 : $[\alpha]_D$: + 17 (c= 1.02). **IR** : 3488, 2976, 2875, 1745, 1693, 1649, 1436, 1369, 1236. ^{1}**H-NMR** : 1.19 (9H, s); 1.29 (3H, s); 2.07 (1H, t, J= 12.1 Hz); 2.10-2.18 (2H, m); 2.18 (3H, s); 2.33 (1H, dd, J= 5.6, 12.0); 3.85 (3H, s); 4.04 (1H, t, J= 8.0 Hz); 4.88 (1H, dd, J= 1.7, 7.4 Hz); 5.65 (1H, dd, J= 5.6, 13.5 Hz). ^{13}**C-NMR** : 18.6, 20.7, 28.5 (3), 39.4, 39.9, 47.3, 52.7, 68.5, 70.4, 73.6, 76.9, 126.8, 166.9, 170.1, 179.2, 189.9. **EIMS** : 354 ($M^{+\cdot}$, 3), 206 (95), 194 (61), 188 (56), 179 (67), 177 (75), 163 (100), 162 (80).

Preparation of 19 and 28. Starting from the C-1-O-tBu hydrindenone derivative (S)-(+)-**26**, and proceeding as described in ref. 1, the 4-methyl **28** and the 4,4-dimethyl hydrindenones **19** were obtained in 26.5 and 46.5 % yield respectively (EtOAc/heptane, 1:4).

28 : m.p. : 73-75°C (pentane). $[\alpha]_D$: +14 (c= 1.00). **IR** : 2969, 2928, 2874, 1661, 1460, 1391, 1197, 1103, 1031. ^{1}**H-NMR** : 1.07 (3H, s), 1.18 (9H, s), 1.65 (3H, s), 1.78 (2H, m), 2.00 (2H, m), 2.25-2.65 (4H,m), 3.55 (1H, dd, J=7.6, 10.5 Hz). ^{13}**C-NMR** : 10.5, 15.7, 25.9, 28.6,29.8, 33.3, 34.2, 44.5, 72.8, 79.9, 128.2, 167.9, 198.7. **EIMS** : 236 ($M^{+\cdot}$, 1), 194 (52), 180 (39), 57 (100).

19 : m.p. : 40-42°C (pentane). $[\alpha]_D$: +49 (c= 1.00). **IR** : 2975, 2933, 2871, 1716, 1462, 1362, 1198, 1104, 1040. ^{1}**H-NMR** : 1.17 (3H, s), 1.18 (9H, s), 1.21 (3H, s), 1.27 (3H, s), 1.61-1.93 (2H, m), 2.19-2.39 (3H, m), 2.62 (1H, m), 3.76 (1H, t, J=8.1), 5.41 (1H, t, J=2.0). ^{13}**C-NMR** : 17.8, 23.8, 27.9, 28.5, 33.8, 34.7, 38.1, 45.6, 48.1, 72.4, 80.7, 119.9, 154.0, 214.5. **EIMS** : 250 ($M^{+\cdot}$, 8), 194 (100), 179 (12), 166 (12), 165 (18), 110 (68), 57 (71).

Bioconversion of 19 : 250 mg (1 mmol), 70 g of fresh biomass, 400 ml of water, 22 h. Work up then silica gel flash chromatography (EtOAc/heptane, 1:5) afforded 17% of the 2β-hydroxylated **82** (characterized as its acetate **84**), 16% of the 2α-hydroxylated **83**, 9% of the 2-keto derivative **20**, along with unreacted starting material.

20 : m.p. : 92-94° C (pentane). $[\alpha]_D$: +177 (c= 1.1). **IR** : 2955, 2938, 2911, 2855, 1723, 1708, 1610, 1462, 1378, 1261, 1198, 1115, 1068, 896, 664. ^{1}**H-NMR** : 1.27 (12H, s), 1.37 (3H, s), 1.41 (3H, s), 1.91 (1H, dt, J= 5.7, 13.0), 2.16 (1H, ddd, J= 3.6, 6.5, 13.6), 2.53 (1H, ddd, J= 3.4, 5.7, 17.1), 2.79 (1H, ddd, J= 6.3, 12.4, 17.1), 3.84 (1H, s), 6.01 (1H, s). ^{13}**C-NMR** : 22.1, 25.0, 27.6, 28.6, 32.6, 34.3, 46.1, 49.8, 74.7, 83.1, 124.9, 186.0, 205.8, 211.1. **EIMS** : 264 ($M^{+\cdot}$, 33), 249 (17), 208 (100), 193 (40), 179 (19), 164 (60), 151 (14), 149 (13), 137 (60), 127 (43), 121 (17), 119 (16), 107 (16), 91 (22), 85 (33), 67 (13), 57 (73).

83 : m.p. : 59-61° C (pentane). $[\alpha]_D$: -3 (c= 1.0). **IR** : 3425, 3059, 2937, 2937, 2873, 1711, 1639, 1462, 1392, 1380, 1366, 1322, 1259, 1235, 1194, 1108, 1064, 1003, 984, 895, 734, 662. ^{1}**H-NMR** : 1.21 (3H, s), 1.24 (9H, s), 1.25 (3H, s), 1.28 (3H, s), 1.73 (1H, td, J= 5.2, 12.6), 1.90 (1H, dt, J= 5.3, 13.3), 2.36 (1H, dt, J= 5.2, 16.0), 2.68 (1H, ddd, J= 5.7, 11.8, 16.0), 3.59 (1H, d, J= 6.3), 4.64 (1H, dd, J= 6.3, 1.1), 5.45 (1H, bs). ^{13}**C-**

NMR : 20.0, 23.9, 28.2, 29.3, 34.7, 34.8, 46.7, 48.2, 73.1, 80.5, 88.6, 124.2, 155.5, 214.2. **EIMS** : 266 ($M^{+\cdot}$, 2), 249 (3), 210 (96), 195 (46), 192 (98), 181 (53), 177 (93), 163 (52), 149 (38), 137 (65), 123 (59), 107 (24), 91 (21), 81 (15), 69 (16), 57 (100).

84 : m.p. : 77-79° C (pentane). $[\alpha]_D$: +175 (c= 1.1). **IR** : 2964, 2891, 2871, 1741, 1720, 1627, 1462, 1378, 1233, 1194, 1167, 1125, 1105, 1015, 886, 723, 668. **^{1}H-NMR** : 1.20 (9H, s), 1.25 (3H, s), 1.32 (3H, s), 1.35 (3H, s), 1.65 (1H, td, J= 5.2, 13.0), 1.97 (1H, dt, J= 5.1, 13.2), 2.08 (3H, s), 2.32 (1H, dt, J= 4.8, 16.1), 2.71 (1H, ddd, J= 5.7, 12.2, 16.1), 3.59 (1H, d, J= 5.6), 5.43 (1H, dd, J= 3.3, 5.6), 5.66 (1H, d, J= 3.3). **^{13}C-NMR** : 21.3, 21.5, 24.0, 28.2, 28.3, 33.9, 34.2, 46.5, 48.7, 73.6, 76.5, 78.7, 119.7, 163.3, 170.8, 213.6. **EIMS** : 308 ($M^{+\cdot}$, 2), 237 (5), 210 (12), 209 (11), 192 (100), 177 (45), 163 (21), 149 (14), 135 (14), 121 (12), 107 (9), 91 (10), 85 (22), 57 (62), 43 (43), 41 (26).

Bioconversion of 28 : 198 mg (0.83 mmol), 60g of fresh biomass, 300 ml of water, 24 h. Silica gel flash chromatography (elution with dichloromethane/ether, 5:1) afforded a 60% combined yield of alcohols **74** and **75** in a 60:40 ratio.

74 : 117 mg, (36%) : m.p. : 96-8° C (pentane). $[\alpha]_D$: + 21 (c= 1.00). **IR** : 3415, 3236, 2977, 1650, 1474, 1451, 1420, 1390, 1359, 1318, 1289, 1268, 1193, 1120, 1101, 1052, 1036, 956, 907, 874, 839, 736. **^{1}H-NMR** : 1.16 (9H, s); 1.23 (3H, s); 1.55-2.70 (6H, m); 1.81 (3H, s); 3.43 (1H, dd, J= 7.2, 10.3); 4.72 (1H, t, J= 7.6). **^{13}C-NMR** : 10.2, 16.6, 28.6, 33.5, 34.8, 41.0, 41.1, 68.9, 73.0, 76.9, 131.5, 163.9, 200.1. **EIMS** : 253 ($M+1^+$, 1), 252 ($M^{+\cdot}$, 2), 210 (2), 197 (36), 196 (100), 178 (13), 161 (22), 154 (99), 152 (78), 149 (99), 135 (18), 123 (12), 57 (10). **HREIMS** : calcd. for $C_{15}H_{24}O_3$: m/z 252.1725, found : 252.1726.

75 (75 mg, 24%) : $[\alpha]_D$: + 17 (c= 1.00). **IR** : 3415, 2977, 2937, 2871, 1669, 1650, 1460, 1418, 1392, 1378, 1366, 1261, 1195, 1167, 1106, 1057, 1017, 905, 865. **^{1}H-NMR** : 1.04 (3H, s); 1.19 (9H, s); 1.70-2.65 (6H, m); 1.85 (3H, s); 3.93 (1H, dd, J= 7.3, 10.4), 4.84 (1H, d, J= 6.8). **^{13}C-NMR** : 11.6, 17.3, 28.5, 33.3, 34.3, 41.4, 44.8, 68.6, 73.1, 77.6, 131.4, 167.9, 200.1. **EIMS** : 252 ($M^{+\cdot}$, 3), 214 (3), 197 (11), 196 (100), 178 (6), 161 (8), 154 (22), 149 (30), 140 (28), 123 (11), 107 (5), 93 (10), 91 (8), 79 (5), 67 (4), 57 (50), 41 (20). **HREIMS** : calcd. for $C_{15}H_{24}O_3$: m/z 252.1725, found : 252.1733.

Preparation, enzymatic hydrolysis and bioconversion of 6α and 6β acetates 29.

The procedure of ref. 1 was repeated using 4 molar equiv. of lead tetraacetate to afford, after refluxing for 60 h and chromatography on silica gel using EtOAc/ heptane 1:15, 90% of **29** as a 55:45 mixture. Upon subjection to enzymatic hydrolysis, using the general procedure, the latter mixture afforded the (6R) acetate **77a** and (6S) alcohol **79**. The latter was acetylated using the conventional method described above and both (6R)-**77a** and (6S)-**80** acetates were biotransformed with *Rhizopus arrhizus*.

77a : $[\alpha]_D$: + 66 (c= 1.0). **IR** : 2971, 2935, 1745, 1681, 1648, 1468, 1372, 1234, 1105, 904. **^{1}H-NMR** : 1.17 (9H, s); 1.20 (3H, s); 1.68 (3H, s); 1.70-2.70 (6H, m); 2.18 (3H, s); 3.59 (1H, dd, J= 7.6, 9.8); 5.59 (1H, dd, J=5.3, 13.6). **^{13}C-NMR** : 10.6, 16.6, 20.7, 25.8, 28.5, 29.2, 40.5, 45.3, 70.7, 72.9, 79.4, 127.2, 167.4, 170.1, 193.2. **EIMS** : 295 (M+1, 2), 252 (11), 238 (88), 197 (6), 192 (12), 178 (94), 160 (54), 150 (46), 136 (62), 122 (39), 106 (34), 91 (34), 79 (23), 58 (100), 43 (100), 41 (88). **HREIMS** : calcd. for $C_{17}H_{26}O_4$: m/z 294.1831, found : 294.1860.

79 : m.p. : 56-58°C (pentane). $[\alpha]_D$: - 54 (c= 1.0). **IR** : 3455, 2973, 2938, 2912, 1652, 1470, 1419, 1389, 1361, 1263, 1195, 1162, 1104, 1048, 996, 903. **^{1}H-NMR** : 1.14 (3H, s); 1.19 (9H, s); 1.60-2.70 (6H, m); 1.73 (3H, t, J= 1.0); 3.02 (1H, bs); 3.61 (1H, dd, J= 7.4, 9.8); 4.06 (1H, dd, J= 4.5, 6.5). **^{13}C-NMR** : 11.0, 21.3, 26.1, 28.6, 29.8, 38.1, 44.3, 69.0, 72.9, 78.4, 126.3, 169.2, 198.9. **EIMS** : 252 ($M^{+\cdot}$, 1), 210 (2), 196 (100), 178 (10), 160 (16), 150 (14), 136 (37), 122 (15), 106 (24), 91 (18), 79 (12), 57 (99), 41 (32).

80 : m.p. : 62-64 °C (pentane). $[\alpha]_D$: - 26 (c= 0.97). **IR** : 2975, 1750, 1669, 1644, 1462, 1431, 1369, 1325, 1300, 1231, 1162, 1100, 1031, 956, 906. **^{1}H-NMR** : 1.16 (3H, s); 1.17 (9H, s); 1.72 (3H, s); 1.70-2.70 (6H, m); 2.11 (3H, s); 3.56 (1H, dd, J= 7.4, 9.9); 5.24 (1H, dd, J=1.9, 5.7). **^{13}C-NMR** : 10.6 19.8, 20.9, 26.1, 28.4, 29.4, 38.3, 43.7, 69.4, 72.8, 79.3, 127.5, 167.6, 169.7, 192.2. **EIMS** : 295 (M+1, 1), 238 (67), 178 (100), 160 (67), 150 (44), 135 (70), 122 (33), 106 (24), 91 (18), 79 (7), 57 (37), 43 (15), 41 (11).

Chemical hydrolysis of **77** (K_2CO_3, MeOH-H_2O, r.t., 1h), proceeded with complete epimerization and in 58% yield to give **77b** : $[\alpha]_D$: + 73 (c= 0.97). **IR** : 3487, 2975, 2937, 2868, 1662, 1462, 1381, 1362, 1319, 1300, 1194, 1106, 1069, 981, 900. **^{1}H-NMR** : 1.17 (12H, s); 1.60-2.65 (6H, m); 1.71 (3H, s); 3.56 (1H, dd, J= 7.7, 9.8); 3.68 (1H, d, J=1.0); 4.34 (1H, dd, J= 5.6, 12.9). **^{13}C-NMR** : 10.6, 16.6, 26.1, 28.6, 29.1, 43.2, 45.3, 69.1,

73.1, 79.6, 125.9, 169.5, 199.8. **EIMS** : 252 ($M^{+\cdot}$, 1), 210 (23), 196 (100), 179 (6), 160 (15), 150 (15), 136 (30), 122 (10), 106 (12), 91 (7), 79 (4), 57 (26), 41 (6).

Bioconversion of 80 : 191 mg (0.65 mmol), 60 g of fresh biomass, 300 ml of water, 24 h. The combined yield of this biohydroxylation is 79%, affording the 3α and 3β–alcohols accompanied to some extent (variable, depending incubation time and work-up) with their corresponding diols. It is not clear whether partial hydrolysis of the acetates occurs during incubation or work up or both. Elution with dichloromethane/ether, 10:1 gave 60 mg (30%) of **81βOH**, 58 mg (36%) of **81αOH**, plus the diols and unreacted starting material.

81βOH : Oil. $[\alpha]_D$: - 29 (c= 1.10). **IR** : 3469, 2977, 1745, 1671, 1462, 1432, 1373, 1301, 1233, 1165, 1113, 1094, 1080, 1036, 968, 907, 738. **^{1}H-NMR** : 1.17 (9H, s); 1.31 (3H, s); 1.80-2.60 (4H, m); 1.88 (3H, s); 2.11 (3H, s); 3.43 (1H, dd, J= 7.1, 10.3), 4.75 (1H, t, J= 7.6); 5.25 (1H, dd, J= 2.0, 5.8). **^{13}C-NMR** : 10.5, 20.9, 21.0, 28.5, 38.9, 40.7, 43.4, 68.8, 69.5, 73.2, 76.4, 131.0, 166.3, 169.9, 194.0. **EIMS** : 311 (M+1, 1), 254 (100), 212 (4), 194 (14), 176 (21), 164 (14), 151 (30), 150 (45), 122 (23), 105 (10), 94 (13), 79 (12), 67 (8), 57 (99), 43 (48). **HREIMS** : calcd. for $C_{17}H_{27}O_5$ (M+1) : m/z 311.1858, found : 311.1863.

81αOH : Oil. $[\alpha]_D$: - 42 (c= 1.00). **IR** : 3455, 2977, 2944, 1746, 1673, 1460, 1432, 1368, 1326, 1301, 1235, 1174, 1104, 1052, 1017, 959, 902, 867, 738. **^{1}H-NMR** : 1.09 (3H, s), 1.18 (9H, s), 1.85-2.25 (4H, m), 1.91 (3H, d, J= 1.0), 2.11 (3H, s), 3.92 (1H, dd, J= 7.4, 10.2), 4.89 (1H, d, J= 6.5), 5.30 (1H, dd, J= 1.9, 6.5). **^{13}C-NMR** : 11.9, 21.1, 21.6, 28.6, 38.8, 41.4, 44.2, 68.9, 69.2, 73.3, 77.3, 131.0, 168.5, 170.0, 194.4. **EIMS** : 311 (M+1, 1), 252 (100), 212 (9), 194 (36), 176 (60), 165 (42), 150 (84), 149 (100), 147 (50), 133 (13), 123 (36), 122 (43), 105 (14), 94 (15), 79 (13), 57 (86), 43 (52). **HREIMS** : calcd. for $C_{17}H_{27}O_5$ (M+1) : m/z 311.1858, found : 311.1853.

Bioconversion of 77a : 179 mg (0.61 mmol), 60 g of fresh biomass, 300 ml of water, 24 h. Usual work up and chromatography (dichloromethane/ether, 10:1) afforded the 3β and 3α alcohols **78βOH** and **78αOH** in 65% combined yield and 97:3 ratio respectively.

78βOH : $[\alpha]_D$: - 48 (c= 1.0). **IR** : 3462, 2977, 2937, 2871, 1751, 1681, 1462, 1376, 1237, 1197, 1165, 1104, 1036, 977, 910, 860, 755, 738. **^{1}H-NMR** : 1.16 (9H, s); 1.36 (3H, s); 1.70-2.60 (4H, m); 1.84 (3H, s); 2.17 (3H, s); 3.45 (1H, dd, J= 7.3, 10.3); 4.70 (1H, t, J= 7.5); 5.60 (1H, dd, J= 5.3, 13.6). **^{13}C-NMR** : 10.2, 17.3, 20.8, 28.5, 40.7, 41.2, 44.8, 68.6, 71.0, 73.2, 76.7, 130.7, 164.5, 170.3, 194.9. **EIMS** : 311 (M+1, 1), 279 (3), 268 (3), 254 (100), 212 (6), 194 (23), 176 (32), 165 (21), 150 (58), 122 (12), 94 (9) 57 (40), 43 (23).

Preparation and enzymatic hydrolysis of racemic 22 and 23 : Starting from racemic Wieland-Miescher ketone, selective reduction then protection of the unconjugated C-9 carbonyl using literature procedures gave the C-9 O-tBu protected derivative which was subsequently treated with lead tetraacetate in refluxing benzene as described in ref. 1 to afford an epimeric mixture of the C-2 acetates. Crystallization in heptane precipitates the 2α-acetates [±]-**23** while leaving the 2β-acetates [±]-**22** in solution. The racemic mixture of the 2α acetates was then subjected to enzymatic hydrolysis with horse liver esterase according to the general procedure (18 h 30 min) to afford the optically pure (S)-(-)-alcohol **25** alongside (R)-(+)-acetate **23**.

(2R, 9S, 10S)-(+)-23. $[\alpha]_D$: +119 (c= 0.75). **IR** : 2964, 2878, 1753, 1695, 1617, 1446, 1369, 1231, 1191, 1109, 1078, 1064, 1038, 994, 874. **^{1}H-NMR** : 1.19 (9H, s); 1.31 (3H, s); 1.40 (1H, m); 1.65 (1H, m); 1.80 (1H, m); 1.82 (1H, m); 1.85 (1H, dt, J=12.6, 14.2); 2.18 (3H, s); 2.32 (1H, dd, J= 5.2, 12.6); 2.35 (2H, m); 3.20 (1H, dd, J= 3.7, 10.7); 5.46 (1H, dd, J= 5.2, 14.2); 5.76 (1H, s). **^{13}C-NMR** : 16.6, 20.7, 22.6, 29.0, 29.6, 31.7, 40.6, 43.4, 71.3, 73.4, 77.8, 122.8, 168.9, 170.0, 193.7. **EIMS** : 238 (63), 179 (75), 161 (14), 150 (33), 135 (20), 134 (100), 122 (26), 121 (26), 57 (47).

(2S, 9R, 10R)-(-)-25 :. **m.p.** : 50-52°C (pentane). $[\alpha]_D$ -134 (c=1.0). **IR** : ($CHCl_3$) : 3469, 2977, 2864, 1684, 1661, 1616, 1463, 1362, 1225, 1105, 1080, 1062. **^{1}H-NMR** : 1.19 (9H, s); 1.28 (3H, s); 1.58 (1H, t, J=13.3); 1.67 (2H, m); 1.85 (2H, m); 2.28 (2H, m); 2.50 (1H, dd, J=5.6, 12.9); 3.19 (1H, dd, J=4.0, 11.2); 4.26 (1H, dd, J= 5.6, 13.8); 5.82 (1H, s). **^{13}C-NMR** : 16.8, 22.9, 29.1, 29.7, 30.3, 43.2, 43.3, 69.7, 73.9, 77.9, 121.5, 171.5, 200.0. **EIMS** : 252 ($M^{+\cdot}$, 1), 197 (14), 196 (100), 165 (43), 134 (42), 57 (46). **HREIMS** : calcd. for $C_{15}H_{25}O_3$ m/z 253.1804 (M+H); found : 253.1813; calcd. for $C_{15}H_{24}O_3$.m/z 252.1725 ($M^{+\cdot}$); found 252.1695.

Enzymatic hydrolysis of racemic 22 : The racemic mixture of the 2β-acetates [±]-**22** required longer incubation time than the 2α-acetates [±]-**23**. Thus upon subjection to enzymatic hydrolysis with horse liver

esterase according to the general procedure, the optically pure (S)-(-)-alcohol **24** and the (R)-(+)- acetate **22** were obtained after 72 h.

(2R, 9R, 10R)-(+)-22 : $[\alpha]_D$: +33 (c=1.15). **IR** : 2977, 2864, 1751, 1697, 1458, 1371, 1228, 1071, 982, 923, 865, 757. **^{1}H-NMR** : 1.16 (3H, s); 1.21 (9H, s); 1.26 (1H, m); 1.70 (1H, m); 1.75 (1H, dd, J= 14.0, 11.2 Hz); 1.91 (2H, m); 2.14 (1H, m); 2.17 (3H, s); 2.42 (1H, dddd, J= 12.7, 11.6, 4.0, 1.0); 2.51 (1H, dd, J= 11.2, 5.5); 3.62 (1H, dd, J= 4.7, 10.7); 5.45 (1H, dd, J= 5.5, 14.0); 5.78 (1H, d, J= 1.0). **^{13}C-NMR** : 20.7, 21.5, 25.5, 28.8, 30.4, 32.1, 36.2, 44.1, 70.4, 72.8, 74.0, 121.7, 169.4, 170.0, 193.7. **EIMS** : 238 (23), 178 (55), 150 (28), 135 (19), 134 (100), 122 (25), 121 (28), 57 (47). **HREIMS** : calcd for $C_{17}H_{27}O_4$: m/z 295.1909 (M+H); found : 295.1918.

(2S, 9S, 10S)-(-)-24 : **m.p.** : 44-50°C (pentane). $[\alpha]_D$: -99 (c= 1.27). **IR** : 3449, 2971, 2878, 1688, 1620, 1460, 1390, 1364, 1364, 1231, 1198, 1111, 1083, 1062, 1020, 980, 961, 900, 870, 668. **^{1}H-NMR** : 1.14 (3H, s); 1.25 (9H, s); 1.45 (1H, t, J=13.9 Hz), 1.72 (2H, m), 1.94 (2H, m), 2.15 (1H, m), 2.40 (1H, m), 2.71 (1H, dd, J= 6.0, 13.7); 3.60 (1H, dd, J= 4.8, 10.5); 4.23 (1H, dd, J= 6.1, 14.0); 5.81 (1H, d, J= 1.0). **^{13}C-NMR** : 21.7, 25.3, 28.8, 30.4, 32.3, 38.5, 44.2, 68.8, 72.5, 74.0, 120.1, 171.2, 199.9. **EIMS** : 252 (1), 197 (12), 196 (100), 140 (14), 134 (28), 57 (16).

Bioconversion of (S)-(+)-85 : 24h, gave 25% of **(+)-86** : $[\alpha]_D$: +21 (c=1.0). **IR** : 3422, 2944, 2884, 1665, 1620, 1083, 1062. **^{1}H-NMR** : 1.55 (3H, s), 1.62 (2H, m), 1.95 (2H, m), 2.30 (2H, m), 2.43 (2H, m), 3.94 (4H, m), 4.33 (1H, t, J= 2.9), 5.88 (1H, s). **^{13}C-NMR** : 22.1, 25.3, 27.8, 28.9, 33.9, 44.3, 65.0, 65.3, 71.6, 112.2, 127.8, 166.4, 200.3. **EIMS** : 238 ($M^{+\cdot}$, 16), 180 (14), 124 (19), 109 (20), 99 (100).

Bioconversion of racemic 85 : Stopped after 19h, afforded a mixture of **86** (28%) which upon derivatization showed a 2:1 ratio of lactates, the steroid series being hydroxylated faster than the enantiosteroid one.

Bioconversion of racemic 87 : The hydroxylated compound **90** thus obtained shows no optical activity and upon derivatization leads to two lactates. **[±]-90** (73 mg, 40%) **m.p.** : 160-162° C (pentane). **IR** : 3356, 2964, 2937, 2871, 1645, 1611, 1462, 1378, 1269, 1209, 1188, 1168, 1152, 1122, 1096, 1071, 982, 919, 892. **^{1}H-NMR** : 1.53 (3H, s); 1.87 (3H, s); 1.20-2.60 (8H, m); 3.97 (4H, A_2B_2, J= 5.2); 4.93 (1H, t, J= 2.7); 5.45 (1H, bs). **^{13}C-NMR** : 10.8, 22.3, 24.5, 27.2, 28.9, 33.5, 44.2, 64.9, 65.2, 65.6, 112.3, 132.6, 158.4, 199.9. **EIMS** : 252 ($M^{+\cdot}$, 100), 194 (20), 133 (49), 100 (98), 99 (95), 91 (65), 86 (98). **HREIMS** : calcd for $C_{14}H_{20}O_4$ m/z 252.1361, found 252.1356.

Preparation of racemic 88 : Racemic ketone **32** (100 mg, 0.4 mmol) is converted to its trimethylsilyl enol ether (0.8 ml, 2 equiv. of TMSOTf, 0.16 ml, 1.2 mmol of collidine in 4 ml of DCM, 15 min stirring at 0°C) followed by bromination (85 mg, 0.48 mmol of NBS, in 2 ml of THF, -78°C, 15 min). Dehydrobromination (280 mg of $CaCO_3$, 5 ml of dimethylacetamide, 1 h reflux) gave the conjugated ketone **88** after silica gel chromatography (eluent EtOAc/heptane, 1:4) in 51% overall yield. **[±]-88** : **IR** : 2984, 2878, 1681, 1463, 1385, 1362, 1266, 1158, 1139, 1099, 1083, 1043, 1034, 949, 909, 851, 827, 731. **^{1}H-NMR** : 1.34 (3H, s), 1.35 (3H, s), 1.51 (3H, s), 1.73 (1H, m), 1.99 (1H, m), 2.29 (2H, m), 4.00 (4H, m), 5.65 (1H, t, J= 3.7), 6.00 (1H, d, J= 10.3), 6.88 (1H, d, J= 10.3). **^{13}C-NMR** : 23.7, 24.2, 25.4, 26.8, 31.0, 45.0, 47.7, 65.0, 111.5, 120.6, 126.2, 143.3, 150.6, 202.4. **EIMS** : 248 ($M^{+\cdot}$, 100), 233 (12), 147 (19), 134 (19), 99 (13), 87 (17), 86 (58).

Bioconversion of [±]-88 : 160 mg (0.65 mmol), 60 g of fresh biomass, in 400 ml of water, 24 h. Elution with EtOAc/heptane, 1:1, gave a 66% combined yield of **89** along with some C-9 deprotected 7β-hydroxylated enone. Compound **89** has $[\alpha]_D$: +15 (c=1.17). Derivatization as its (S)-O-acetyl lactyl derivative and NMR analysis of the diastereomeric lactates confirmed the fact that the fungus shows very little preference towards the steroid series, the enantiomeric excess being around 10%.

[±]-89 : **IR** : 3482, 3349, 2971, 2898, 1681, 1460, 1388, 1270, 1223, 1160, 1088, 1052, 982, 949, 865, 734. **^{1}H-NMR** : 1.36 (3H, s), 1.39 (3H, s), 1.56 (3H, s), 1.96 (1H, d, J= 9.5,13.2), 2.24 (1H, dd, J= 7.4, 13.2), 4.03 (4H, m), 4.42 (1H, ddd, J= 5.7, 7.6, 9.5), 5.72 (1H, d, J= 2.7), 6.01 (1H, d, J= 10.3), 6.85 (1H, d, J= 10.3). **^{13}C-NMR** : 24.3, 26.9, 30.5, 35.4, 45.1, 47.6, 65.0, 66.1, 111.9, 124.6, 126.0, 144.9, 150.4, 202.5. **EIMS** : 264 ($M^{+\cdot}$, 6), 247 (23), 207 (34), 179 (20), 178 (49), 163 (39), 150 (100), 121 (46), 115 (45), 105 (23), 91 (35), 88 (28), 87 (80), 86 (43). **HREIMS** : calcd. for $C_{15}H_{20}O_4$ m/z 264.1361; found : 264.1359.

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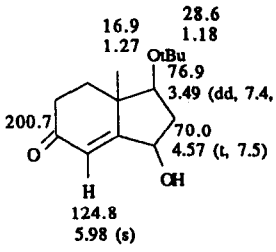
ANNEX

NMR assignments, hydrindenone series



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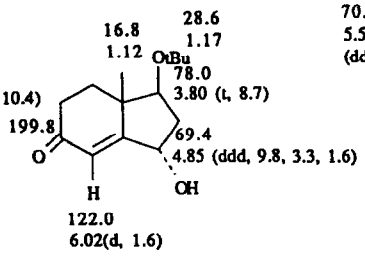

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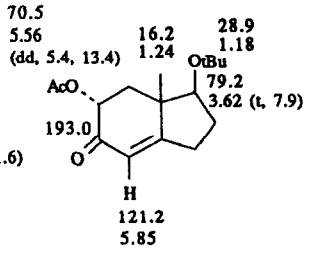

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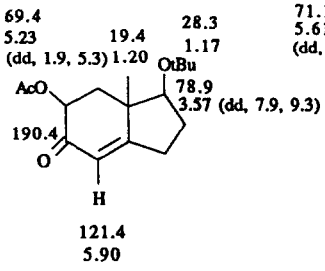

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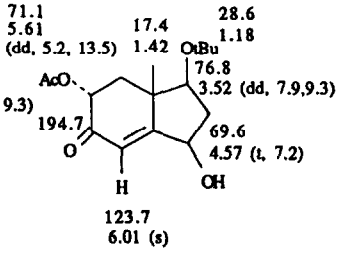

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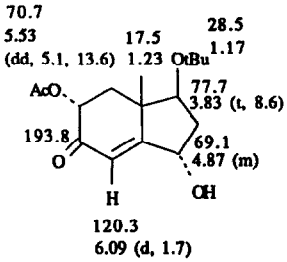

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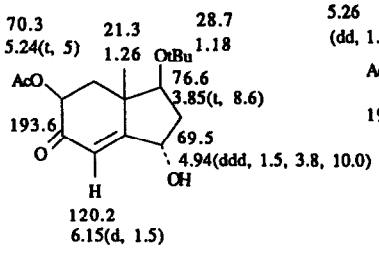

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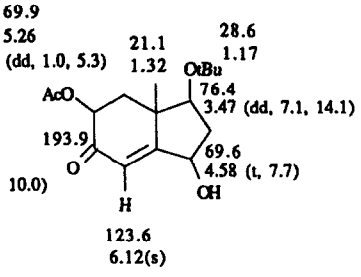

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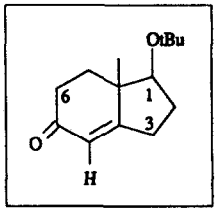

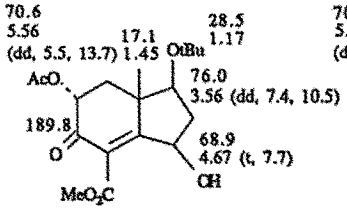

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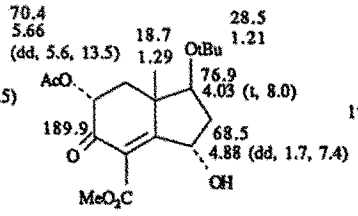

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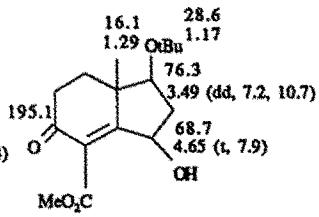

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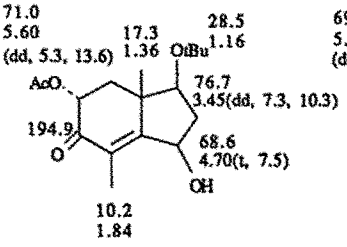

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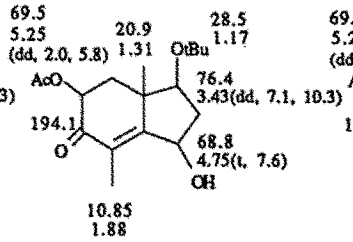

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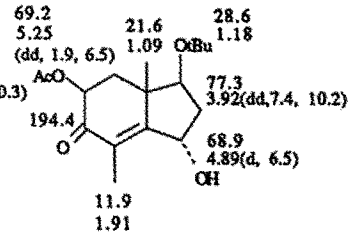

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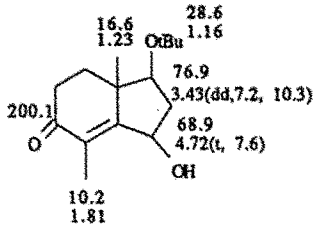

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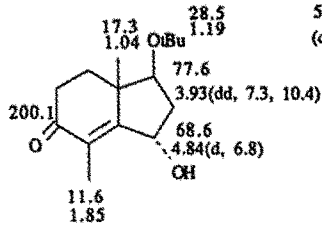

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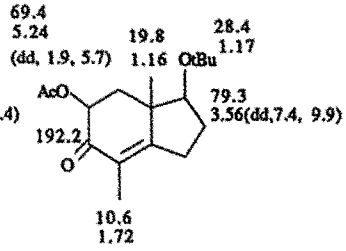

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