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Microbial Synthesis of Optically Pure (R)-2,4,4-Trimethyl-3-(2'-hydroxyethyl)-cyclohex-2-en-1-ol, a New and Versatile Chiral Building Block for Terpene Synthesis.

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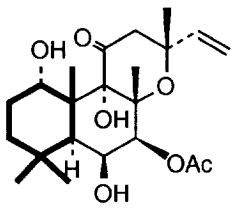
Abstract: The hydroxylation of 2,4,4-trimethyl-3-(2'-hydroxyethyl)-2-cyclohexene by *Mucor plumbeus*, after usual work up and a subsequent single crystallization, gave the corresponding optically pure (1R)-hydroxy synthon.

The preparation of homochiral building blocks is a constant target in modern organic chemistry focused towards the asymmetric synthesis of bioactive natural substances. If one considers biologically active molecules of the terpene family such as forskolin **1** [1], taxol **2** [2], strigol **3** [3], erigerol **4** [4], among many others, they are all characterized by a (1S)-1-hydroxy-2,4,4-trimethyl-2-cyclohexene (or cyclohexane) partial structure.



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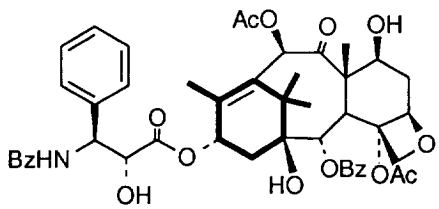

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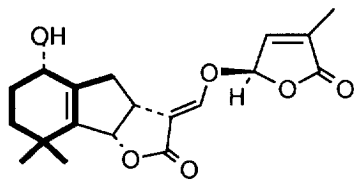

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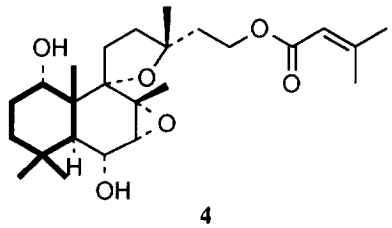

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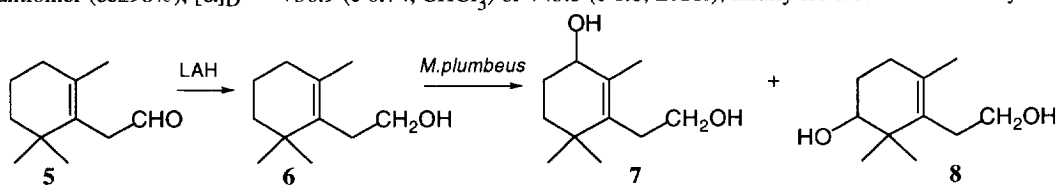
The use of racemic (or achiral) 1-oxygenated synthons derived from α- or β-cyclocitral and α- or β-ionones has been extensively explored for the synthesis of ring A(B) precursors of these terpenes [1,2,5-12]. Such an approach involves either a resolution step, or an asymmetric reduction of the 1-carbonyl function. On the other hand, a number of studies have been devoted to the elaboration of simplified homochiral 4,4-dimethyl-1-hydroxy-2-cyclohexene units; most of them call for a complementary introduction of other alkyl and/or functionalized substituents into the cyclohexene ring [13-17].

Our ongoing studies correspond in part to the elaboration of biologically active terpene structures by microbiological regio- and/or stereoselective hydroxylation reactions [18-24], and the purpose of our present investigation was the direct synthesis, as a model, of such a versatile highly substituted 1-hydroxylated cyclohexene-derived homochiral building block .

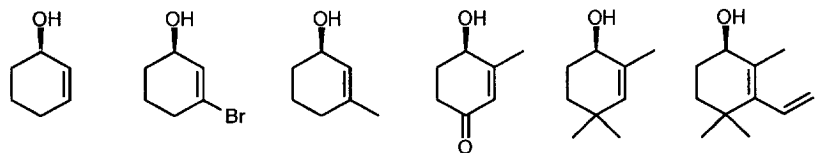
2,4,4-Trimethyl-3-(2'-hydroxymethyl)-2-cyclohexene **6**, easily obtained by reduction ($NaBH_4$/THF, or $LiAlH_4/Et_2O$) of the corresponding commercially available aldehyde **5**, itself obtained in high yield from β-cyclo-

citral [25,26], was incubated 2 days with *Mucor plumbeus* CBS 110-16, a powerful hydroxylating microorganism, which has been previously used for allylic hydroxylations [18-23]. After chromatography of the incubation products, a major 1-hydroxy derivative **7** (45-50 %) and a minor 3-hydroxy derivative **8** (3-5 %) were obtained (*Scheme 1*) and identified by usual spectroscopic methods [27]. Diol **7** was dextrorotatory, with $[\alpha]_D$ = +19.6 (c 1.4) in MeOH or +22.7 (c 2.1) in $CHCl_3$, and exhibited an enantiomeric excess of about 55 % [28], estimated by ^{1}H-NMR in the presence of an Eu(III)-chiral shift reagent. Dissolved in a CH_2Cl_2-pentane mixture, on standing at 4°C, diol **7** reproducibly deposited nice sheaves of crystals corresponding to the racemic diol (m.p. 81-81.5°C). The remaining colorless oil, obtained after evaporation of the mother liquors, turned out to be a pure enantiomer (ee≥98%), $[\alpha]_D^{22}$= +36.9 (c 0.74, $CHCl_3$) or +43.5 (c 1.8, EtOH), finally isolated in a 30-35% yield.



Scheme 1

An assignment of the absolute configuration of the 1-hydroxy metabolite **7**, based exclusively on previously established optical rotation measurements, was questionable: all known (R)-alcohols **I-VI** (*Scheme 2*) are dextrorotatory, either in chloroform or in methanol solution; their esters, when described, behave similarly, exhibiting again positive rotations in either solvent.



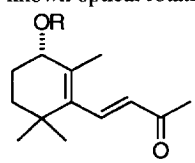
I [29] **II** [30] **III** [31] **IV** [14] **V** [15] **VI** [32]

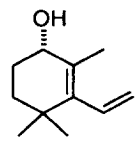
$[\alpha]_D$: +130.6 ($CHCl_3$) +40.9 ($CHCl_3$) +94.6 ($CHCl_3$) +48.8 ($CDCl_3$) +95.7 (MeOH) +53 ($CHCl_3$)

Scheme 2

However, deviating from this homogeneous family, the 3-substituted alcohol **9** and the corresponding esters **10** derived from β-ionone show anomalous optical rotations. (S)-Alcohol **9** is dextrorotatory in ethanol and levorotatory in chloroform solution [12,33]. Furthermore, the rotation of (S)-esters **10** (in EtOH) is opposite to that of the corresponding alcohol. So it was uncertain to deduce the absolute configuration of **7** from such conflicting data, and a conversion into a compound of known optical rotation was necessary.

9: R = H (S, $CHCl_3$) $[\alpha]_D$ -13
(S, EtOH) $[\alpha]_D$ +7
10: R= $COCH_3$ (S, EtOH) $[\alpha]_D$ -78
R= $COCH_2COCH_3$ (S, EtOH) $[\alpha]_D$ -51.4



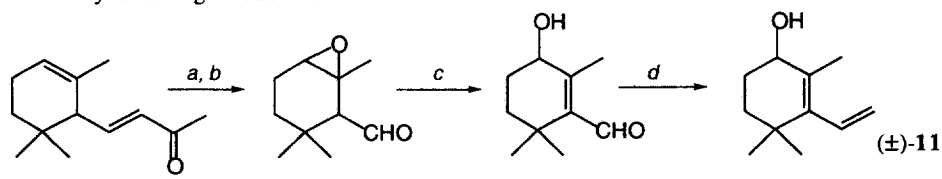


(S)-**11**

The levorotatory (S)-alcohol **11** has been previously obtained by borane reduction of the corresponding 1-ketone in the presence of an (R)-oxazoborolidine as a chiral catalyst [32]. In our hands, any attempt to perform the (enantioselective) reduction of the 1-keto derivative of **6**, following exactly the same protocole, or using a recently modified reagent [34], completely failed to give the desired diol **7**.

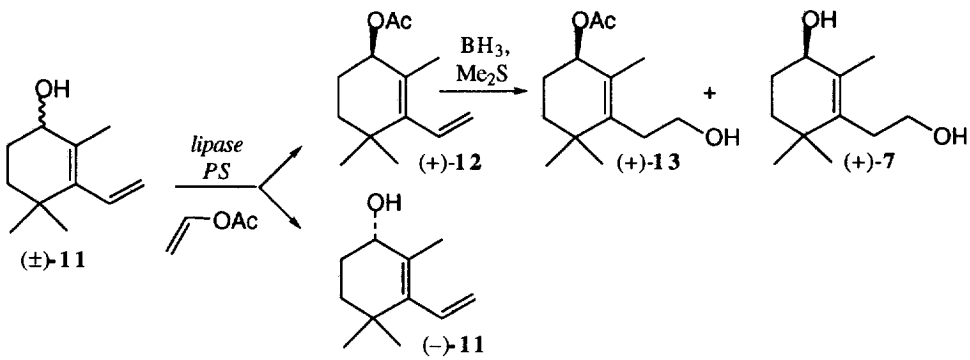
Another approach was a conversion of the microbiologically obtained diol **7** to the known 1-hydroxy diene **11** [32], by using mesylation or tosylation of the primary alcohol function, then elimination with NaI/DBU in acetonitrile, as previously and similarly described for the preparation of a conjugated diene from the corresponding hydroxyethyl compound **6**. The regioselective sulfonate ester formation at the primary alcohol function of diol **7** was easily realized, but the subsequent iodation and elimination by DBU were uneffective. Other methods for elimination of tosylate or mesylate with Li_2CO_3, Li_2CO_3/LiBr [35,36], LiBr/pyridine, LiBr/NaOAc [36]

in DMF solution were similarly uneffective. The unique positive result was, with the latter reagent, the isolation of a primary O-acetyl derivative as the major substitution product, and the presence of minute amounts of the desired conjugated 1-hydroxy diene (1-2 %), only detected by NMR in the crude reaction mixture. Such results can be hypothetically understood through the formation of delocalized cationic intermediates classically generated from an homoallylic leaving function [37].



Scheme 3: a) m-CPBA (1.5 eq.), CH_2Cl_2, 0°C, 16 h; b) O3, CH_2Cl_2-pyridine (4:1), -78°C, then Me_2S (5 eq.); c) 5% pyrrolidine, Et_2O, 4°C, 2 days; d) $(C_6H_5)_3P^+CH_3,Br^-$ (1.5 eq.), BuLi (1.5 eq.), THF, 0°C.

The reverse approach, starting from an enantiomerically enriched 1-hydroxydiene **11**, and using a hydroboration reaction to convert it to a diol **7** of known configuration, was more successful. The racemic hydroxydiene **11** was obtained from α-ionone (*Scheme 3*) following previously described methods [3,38,39]; epoxide opening and proton elimination were significantly improved (90-95% yield) by using 5% freshly distilled pyrrolidine in anhydrous ethyl ether at 4°C. Partial enzymic acetylation of (±)-**11** in freshly distilled vinyl acetate [40], catalyzed by lipase PS (Amano), allowed to obtain (*Scheme 4*, after chromatographic separation, the levorotatory (S)-alcohol **11** ($[\alpha]_D$ –5.5, c 1.55 in $CHCl_3$) and the dextrorotatory diene acetate **12** ($[\alpha]_D$ +18.7, c 1.8 in $CHCl_3$). Hydrolysis of this (+)-ester (NaOH/MeOH, 0°C) to the (R)-alcohol **11**, which was submitted again to the same enzymatic transesterification, resulted in an enriched (R)-acetate **12** ($[\alpha]_D$ +31, c 2 in $CHCl_3$; 56 % ee [41]).



Scheme 4

Hydroboration of the enantiomerically enriched (R)-acetate **12** with BH_3/Me_2S in THF at 0°C [42], followed by oxidation with alkaline hydrogen peroxide, allowed the recovery of a 2:1 mixture of the desired diol acetate **13** and diol **7** (resulting from a partial hydrolysis of the ester). The (R)-diol acetate **13** [43] was dextrorotatory in $CHCl_3$ and in EtOH ($[\alpha]_D$ +44.7, c 2.2), just as the (R)-diol **7** ($[\alpha]_D$ +19.5, c 1.25 in $CHCl_3$ and +24.4, c 1.25 in EtOH). The comparison of optical rotations of the authentic (R)-diol and of the microbial hydroxylation product **7** undoubtedly showed that the latter has the (R)-configuration.

Work is in progress to combine this microbial functionalization with a classical inversion of the (R)-allylic alcohol into its (S)-enantiomer by a Mitsunobu reaction, using previously described conditions [12]. Thus, the microbial hydroxylation may give access to a new 1-hydroxy-2,4,4-trimethyl-cyclohexene-derived chiral synthon in both enantiomeric forms. In addition, this reaction may be considered as an initial step for the elaboration of one of the appropriate 1,5-dioxygenated synthons commonly used for the building of the AB ring system of taxol [2].

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27. Diol **7**. IR (CCl_4) cm^{-1}: 3628, 3446, 2963, 2934, 1469, 1378, 1024. ^{1}H-NMR ($CDCl_3$) δppm, J Hz: 0.96, 1.04 (6H, 2s, 4–CH_3), 1.77 (3H, s, 2-CH_3), 2.36 (2H, m, 2'-CH_2), 3.91 (1H, br.t J= 4.3, 1-CHOH). ^{13}C-NMR ($CDCl_3$) δppm: 138.0, 131.1 (C-2 and C-3), 70.2 (C-1), 62.0 (C-2'), 35.3 (C-4), 34.5 (C-5), 32.4 (C-1'), 28.6 (C-6), 28.5, 27.2 (4-CH_3), 17.4 (2-CH_3). HRMS for $C_{11}H_{20}O_2$, calc 184.14633, found 184.14635. MS (EI, 70ev) m/z(%): 184(29) M^+, 169(11) $[M-CH_3]^+$, 166(22) $[M-H_2O]^+$, 151(10) $[M-(15+18)]^+$, 139(99) $[M-CH_2CH_2OH]^+$, 128(96), 109(38), 95(41), 72(41), 55(39), 43(100).
Diol **8**. Colorless oil. $[\alpha]_D^{22}$ -0.2 (c 1, $CHCl_3$). IR (CCl_4) cm^{-1}: 3630, 3574, 2929, 2853, 1464, 1379, 1263, 1034. ^{1}H-NMR ($CDCl_3$) δppm, J Hz: 1.03, 1.09 (6H, 2s, 4–CH_3), 1.67 (3H, s, 2-CH_3), 2.07 (2H, br.t J=6.1, 1-CH_2), 2.38 (2H, br.t J=8.1, 1'-CH_2), 3.51 (1H, dd J=8.6 and 3.3, 5-CHOH), 3.63 (2H, br.t J=8.1, 2'-CH_2). ^{13}C-NMR ($CDCl_3$) δppm: 131.4, 129.1 (C-2 and C-3), 76.0 (C-5), 62.6 (C-2'), 40.0 (C-4), 32.5 (C-1), 32.0 (C-1'), 26.7 (C-6), 26.6, 22.0 (4-CH_3), 20.1 (2-CH_3). MS (EI, 70ev) m/z(%): 184(5) M^+, 166(29) $[M-H_2O]^+$, 151(10) $[M-(15+18)]^+$, 136(32) $[M-(18+30)]^+$, 133(36) $[M-(36+15)]^+$, 121(96) $[M-(18+45)]^+$, 107(100), 93(60), 91(58), 81(81), 79(52), 67(74), 55(41), 43(67).
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43. Diol acetate **13**. IR (CCl_4) cm^{-1}: 3632, 2956, 2870, 1731, 1470, 1463, 1369, 1243, 1021. ^{1}H-NMR ($CDCl_3$) ppm, J Hz: 0.95, 1.05 (6H, 2s, 4–CH_3), 1.83 (3H, s, 2-CH_3), 2.05 (3H, s, $COCH_3$), 2.38 (2H, br.t J=8.3, 5-CH_2), 3.63 (2H, t J=8.3, 2'-CH_2), 5.11 (1H, br.t J=4.5,1-H). ^{13}C-NMR ($CDCl_3$) δppm: 171.2 (OCO), 140.4, 127.5 (C-2 and C-3), 72.9 (C-1), 62.0 (C-2'), 35.1 (C-4), 34.9 (C-5), 32.6 (C-1'), 28.4, 27.0 (4-CH_3), 25.4 (C-6), 21.4 ($OCOCH_3$), 16.9 (2-CH_3). HRMS for $C_{11}H_{20}O_2$ $[M-CH_2CO]$, calc 184.14633, found 184.14635. MS (EI, 70ev) m/z(%): 184(5) $[M-CH_2CO]^+$, 166(22) $[M-CH_3CO_2H]^+$, 151(25) $[166-CH_3]^+$, 133(61) $[151-H_2O]^+$, 121(100), 105(64), 91(62).
On standing at 4°C for several weeks, the (±)-diol acetate spontaneously crystallized; after recrystallization from CH_2Cl_2-pentane, M.p. 89-90°C. An enriched oily (R)-enantiomer was obtained from the mother liquors: $[\alpha]_D^{22}$ +65.8 (c 1.2, $CHCl_3$), +85.1 (c 0.9, EtOH); 94% ee determined by ^{1}H-NMR in the presence of an Eu(III)-chiral shift reagent.

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