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PREPARATION OF BOTH ENANTIOMERS OF A CHIRAL LACTONE THROUGH COMBINED MICROBIOLOGICAL REDUCTION AND OXIDATION.

An application of an enantioselective biological Baeyer-Villiger reaction

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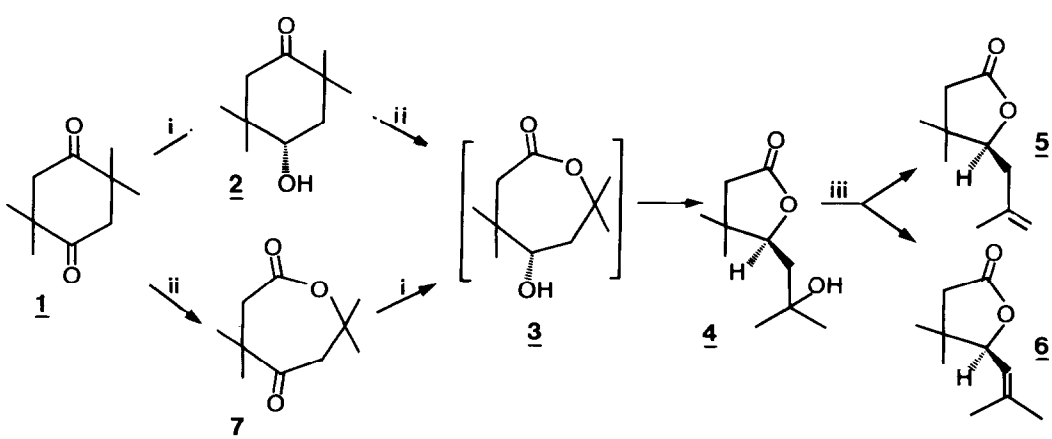
Abstract: The Baeyer-Villiger-like oxidation of (**R,S**)-2,2,5,5-tetramethyl-4-hydroxy -cyclohexanone by several fungal strains was highly enantioselective, affording a rearranged (**S**)-hydroxy-γ-lactone. The recovery of the nearly optically pure (**R**)-hydroxyketone allowed its conversion to the enantiomeric (**R**)-hydroxylactone through a classical Baeyer-Villiger oxidation.

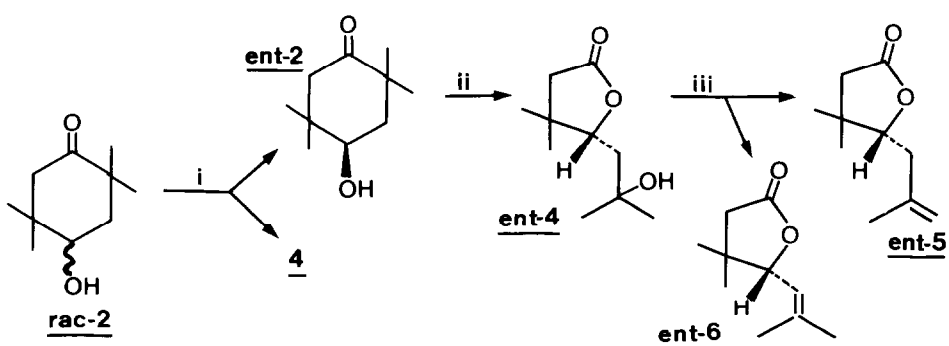
The microbiological reduction of 2,2,5,5-tetramethyl-1,4-cyclohexanedione **1** to the optically pure (**S**)-ketol **2**, a valuable synthon for the synthesis of optically active cis-chrysanthemic acid, has been precedently described[1,2]. When submitted to m-chloroperbenzoic acid oxidation, in the conditions of a usual Baeyer-Villiger reaction, the (**S**)-ketol **2** is converted quantitatively to a presumed seven-membered lactone **3** (Scheme 1) which rearranges spontaneously to the hydroxy-γ-lactone **4**, $[\alpha]_D^{21}$ -110° (c 1.6, MeOH), retaining the initial chirality[2]; **4** can be easily dehydrated by $POCl_3$ treatment in HMPA-pyridine[3] to a 2:1 mixture of unsaturated (**S**)-lactones **5**, $[\alpha]_D^{21}$ -107.5° (c 1, MeOH) and **6**, mp 48-49°C (from EtOAc-cyclohexane), $[\alpha]_D^{21}$ -86.6° (c 0.7, MeOH)[4-6].



Scheme 1: **i**, reduction by C.lunata; **ii**, MCPBA/CH_2Cl_2; **iii**, $POCl_3$,C_6H_5N/HMPA,100°C.

As the hydroxylactone 4 and the unsaturated lactones 5 and 6 may constitute other valuable chiral synthons, or at least interesting models for the preparation of asymmetric lactones, it was worthwhile to have a method for the preparation of both enantiomers. As none of the fungal or yeast strains tested were able to reduce the dione 1 to the (R)-ketol[2], the dione was submitted first to m-chloroperbenzoic acid (1 equiv.) oxidation to give 75% of the keto-lactone 7 [5], mp 129-130°C (from cyclohexane), which, in turn, was submitted to fungal reduction (Scheme 1): the optically pure (S)-hydroxylactone 4 was again obtained.

On the other hand, it was observed that, during the fungal reduction of the dione to the ketol, if glucose (the reducing equivalent source) was omitted, the already formed (S)-ketol 2 was slowly converted to the same hydroxylactone 4, indicating the occurrence of an oxygenase-mediated reaction analogous to the Baeyer-Villiger oxidation. Such a reaction has been occasionally described for bacterial strains adapted to growth on cyclic alkanes, ketones or alcohols[7,8], or fungi acting on steroidal ketones[9]; the camphor ketolactonase complex[10], and more recently cyclohexanone oxygenase[11], both flavoprotein enzymes, have been extensively purified and the mechanism of oxygen insertion closely examined[12]. Only scattered informations about the stereospecificity of these enzymes, and their use for the synthesis of chiral lactones, have been published: for example, the specificity of camphor ketolactonases is complete and exclusive towards the D(+) or the L(-) camphor enantiomers, but much less exacting with respect to the substituants in the 5-position[13]. As a rule, these monooxygenases seem to participate in induced degradative pathways which are used by microorganisms to metabolize cyclic alkane, alkanone or alkanol molecules.



Scheme 2: **i**, *C.lunata*; **ii**, MCPBA/CH_2Cl_2; **iii**, $POCl_3$,C_6H_5N/HMPA,100°C.

It was found, after optimizing the incubation conditions, specially by increasing the aeration of the culture medium, that the oxidation of the (S)-ketol 2 was fairly rapid, allowing a quantitative accumulation of the lactone 4, without apparent further degradation. Moreover, when a 2 g/liter solution of the racemic ketol rac-2 (obtained by careful reduction of the dione 1 with limited amounts of $NaBH_4$ [14]) was submitted to the oxidation by a *Curvularia lunata* suspension [15], the (S)-ketol disappeared rapidly (fig.1), leaving in a few

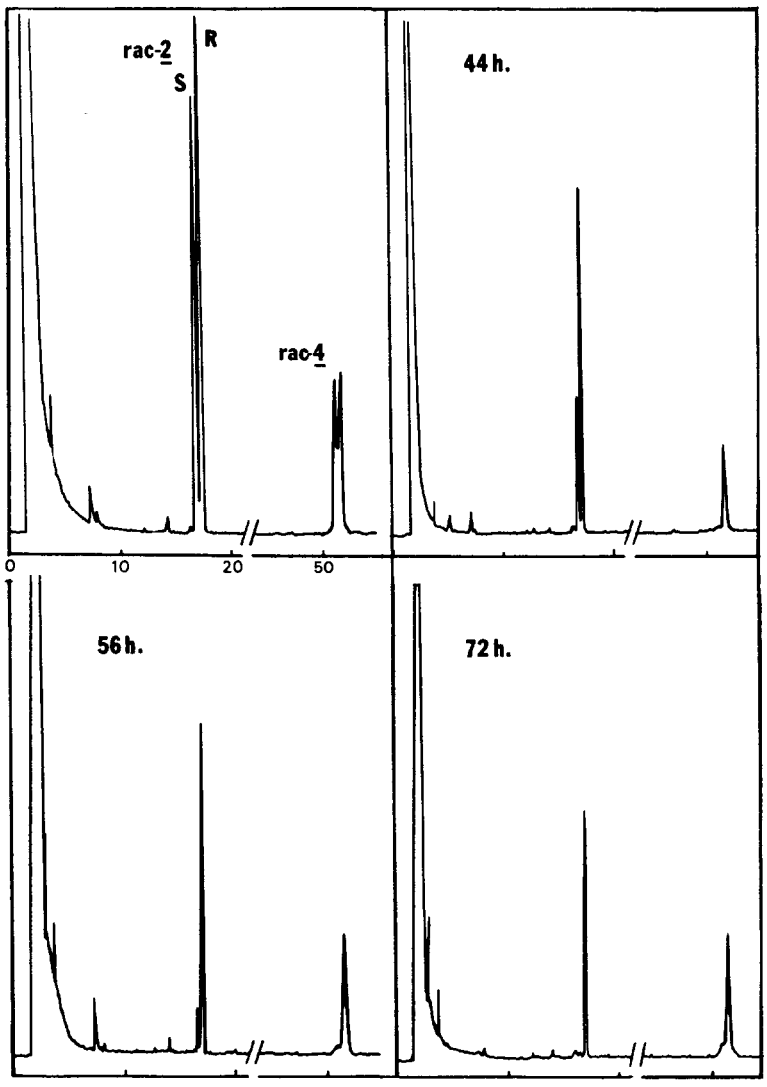


Figure 1: Time course of the conversion of racemic 2,2,5,5-tetramethyl-4-hydroxy-cyclohexanone to the (S)-hydroxylactone 4. Aliquots from the incubation with C.lunata[15] were extracted, derivatized with isopropyl isocyanate and chromatographed at 170°C on a chiral column[4].

days the unchanged nearly optically pure (R)-ketol. Thus it appears that the Baeyer-Villiger-like oxidation of rac-2 by C.lunata is able to discriminate between two stereoisomers differing only in a stereochemical feature located in a relatively remote position from the reaction center. After usual processing of the extracted mixture, a simple chromatographic separation on silicagel H_{60} (cyclohexane-EtOAc, 6:4) afforded the (S)-lactone 4 ($[\alpha]_D^{21}$ -89°, c 1.5, MeOH, 63% yield) and the unchanged (R)-ketol ($[\alpha]_D^{21}$ +86.9°, c 1.25, MeOH , 97% ee, 83% yield), which was then treated by m-chloroperbenzoic acid in the usual conditions to give the (R)-hydroxylactone ent-4, $[\alpha]_D^{21}$ +105.9° (c 0.96, MeOH); this lactone was in turn converted to the (R)-lactones ent-5, $[\alpha]_D^{21}$ +107.1° (c 0.57, MeOH) and ent-6, $[\alpha]_D^{21}$ +84° (c 0.6, MeOH), by the same dehydrating treatment[3] (Scheme 2).

The reactions described constitute a useful synthesis of some alkyl substituted chiral lactones and are currently extended to other less substituted prochiral 1,4- and 1,3-cyclic diketones, the reduction/oxidation of which should allow the generation of optically pure lactones containing more than one asymmetric carbon atom.

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References and Notes

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3- S.Torii, T.Inokuchi and R.Oi, J.Org.Chem., **48**, 1983, 1944. The method described in the same reference and using a rhodium salt catalyst for a quantitative conversion of methylene-compounds like **5** to dimethylallyl-compounds like **6** was inefficient; however, slightly modified dehydrating conditions (pyridine added before $POCl_3$) afforded lactones **5** and **6** in a 91:9 ratio. On the contrary, a 14:86 ratio was obtained by refluxing the lactone **4** with p-toluenesulfonic acid in benzene.
4- Optical purity of products were best assessed by GL-chromatographic separation of isopropyl urethane derivatives (see ref.16) of enantiomers on a Chrompack (25 m x 0.25 mm) XE-60-polysiloxane S-valine-S-α-phenylethylamide column, operated at 170 °C with helium (1.5 bar) as carrier gas (compound, absolute configuration, retention time): lactone **4**, **S**, 52.5 min; **R**, 51.8 min.; ketol **2**(see ref.2), **S**, 17.0 min.; **R**, 17.4 min.
5- ^{1}H-NMR data (250 MHz, $CDCl_3$): **4**, δ 4.32 (dd, 1H,-CH-O, J_1= 10, J_2= 2 Hz), 2.40, 2.28 (2d, 2H,-CH_2-CO-, J= 17 Hz), 1.74 (dd, 1H,-CH-, J_1= 15 Hz, J_2= 10 Hz), 1.68 (s, 1H,-OH), 1.62 (dd, 1H,-CH=, J_1= 15 Hz, J_2= 2 Hz), 1.30, 1.28, 1.12, 0.99 (4s, 12H,-CH_3). **5**, δ 4.86, 4.83 (2m, 2H,=CH_2), 4.22 (dd, 1H,-CH-O, J_1= 16.5, J_2= 10 Hz), 2.41, 2.30 (2d, 2H,-CH_2-CO-, J= 16.5 Hz), 2.23 (m, 2H,-CH_2), 1.79 (s, 3H,CH_3-C=), 1.16, 1.03 (2s, 6H,-CH_3). **6**, δ 5.18 (dq, 1H,-CH=, J_1= 9.5, J_2= 1.3 Hz), 4.80 (d, 1H,-CH-O, J= 9.5 Hz), 2.41, 2.32 (2d, 2H,-CH_2-CO-, J= 16.7 Hz), 1.79, 1.71 (2d, 6H, CH_3-C=, J= 1.3 Hz), 1.11, 1.0 (2s, 6H,-CH_3). **7**, δ 2.98, 2.75 (2s, 4H, -CH_2-O), 1.49, 1.20 (2s, 12H,-CH_3).
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