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Kinetic Resolution *via* Oxidation of *endo*-Bicyclic Octen- and Heptenols with *Bacillus stearothermophilus*

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Abstract - Kinetic resolution of the racemic *endo*-bicyclic octenol and heptenols *via* oxidation with *Bacillus stearothermophilus* is described The enantiomerically pure ketones and alcohols can be obtained varying the oxidation time

Bicyclic heptenols and octenols anf their corresponding ketones are versatile intermediates and/or starting materials for the stereocontrolled synthesis of more complex structures In particular bicyclo[3 3 0]oct-7-en-2-ol is an inexpensive starting material for the stereoselective synthesis of cyclopentenoid natural products[1] and prostacyclin analogues,[2] bicyclo[3 2 0]heptenones are used for the synthesis of prostaglandins,[3] hirsutic acid-C[4] and pentalenolactone E and F[5] while the compounds possessing norbornane-type framework are employed as important intermediates to obtain prostaglandins,[6] terpenes,[7] steroids[8] and alkaloids [9]

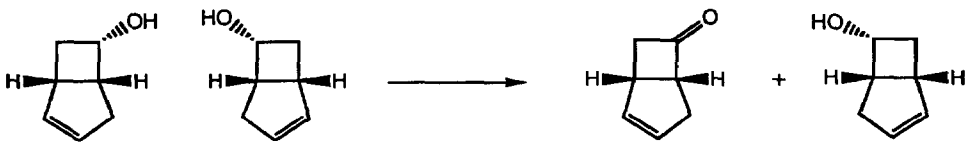
On the basis of these considerations the EPC-syntheses[10] of these compounds hold a great interest as demonstrated by recent work[11] where the resolution of the racemic alcohol has been realised by chiral reagents In order to achieve this same goal we have utilize the oxidation with *Bacillus stearothermophilus* that has given excellent results in the kinetic resolution of 1-aryl ethanols [12] The results are summarized in the Table

Table Kinetic resolution of the racemic bicyclic alcohols **1**, **3** and **5** *via* oxidation with *Bacillus stearothermophilus*

alcohol	time(h)	ketone (%)	ee (abs conf)	alcohol (%)	ee (abs conf)
1	1 5	**2** (41)	99 (1*S*,5*R*)	**1** (53)	80 (1*R*,5*S*,6*R*)
1	3	**2** (52)	70 (1*S*,5*R*)	**1** (40)	100 (1*R*,5*S*,6*R*)
3	4	**4** (43)	100 (1*S*,5*S*)	**3** (54)	82 (1*R*,2*R*,5*R*)
3	8	**4** (54)	64 (1*S*,5*S*)	**3** (42)	100 (1*R*,2*R*,5*R*)
5	2	**6** (30)	82 (1*S*,4*S*)	**5** (60)	32 (1*R*,2*R*,4*R*)
5	8	**6** (55)	40(1*S*,4*S*)	**5** (28)	97 (1*R*,2*R*,4*R*)

Racemic 6-*endo*-bicyclo[3.2.0]hept-2-en-6-ol (**1**), dissolved in DMF, was added on preparative scale to a culture of *Bacillus stearothermophilus* and oxidized to give after 1.5 h (1*S*,5*R*)-bicyclo-heptenone **2** in 41% yield (ee 99%) (Scheme 1, Table).

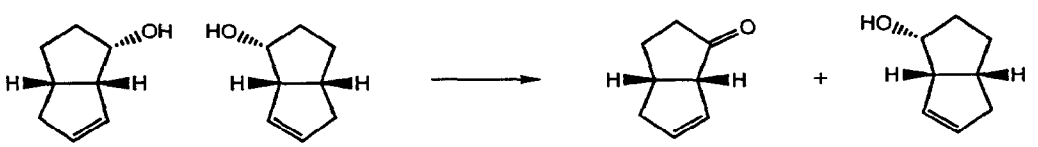
Scheme 1



(rac)-**1** (1S,5R)-**2** (1R,5S,6R)-**1**

The recovered alcohol showed an ee of 80% of the (1*R*,5*S*,6*R*)-enantiomer. The reaction was repeated and stopped at 3 h to give (1*S*,5*R*)-bicyclic ketone **2** (52%, ee 70%) and the enantiomerically pure bicyclic alcohol (1*R*,5*S*,6*R*)-**1** (40%, ee 100%). Practically the same results were obtained in the microbial oxidation of the racemic 2-*endo*-bicyclo[3.3.0]oct-7-en-2-ol (**3**) but the reaction rate was slower (Scheme 2).

Scheme 2

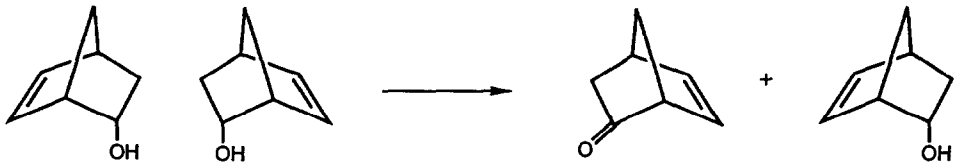


(rac)-**3** (1S,5S)-**4** (1R,2R,5R)-**3**

In fact only after 4 h the conversion to (1*S*,5*S*)-bicyclo-octenone **4** reached to 43% with 100% of ee. The residual alcohol (54%) showed the (1*R*, 2*R*, 5*R*)-configuration (ee 82%). In an other experiment the reaction was stopped at 8 h obtaining 54% of bicyclic ketone **4** [(1*S*,5*S*)-configuration, ee 64%] and the enantiomerically pure (1*R*,2*R*,5*R*)-bicyclo-octenol **3** (42%, ee 100%) was separated from the reaction mixture. The same approach was applied to the racemic 2-*endo*-norbon-5-en-2-ol (**5**) but with worse results (Scheme 3). The compound was added to the culture of *Bac. stearotherm.* in DMSO[13] and after 2 h from the reaction mixture was separated the (1*S*,4*S*)-norborn-5-en-2-one (**6**) (30%, ee 82%). The recovered alcohol **5** (60%) showed the (1*R*,2*R*,4*R*)-configuration (ee 32%). In the same conditions after 8 h the enantiomerically pure (1*R*,2*R*, 4*R*)-norbornenol **5** was obtained (28%, ee 97%) with the corresponding (1*S*,4*S*)-ketone **6** (55%, ee 40%).

These results are obtained after the reactions were carried out on analytical scale to get ready the conditions of incubation and the time. With regard to this some considerations can be made: i) non pathogenic aerobic bacterium *Bacillus stearothermophilus* ATCC 2027 is grown at 39° C in a non selective nutrient medium as a Bacto m Plate count broth (pH 7) at 50% of concentrations of the nutrients; ii) the reaction is carried out by the

Scheme 3



(rac)-**5** (1S,4S)-**6** (1R,2R,4R)-**5**

cells;[14] iii) variation of pH (from 6 to 8)[15] did not affect the oxidation rate and enantioselectivity for compound **1** and **3** while for compound **5** increased only the rate; iv) the exo diastereomers have not been oxidized in any case; v) increasing the concentration of the substrate until threefold, the reaction rate increased without variation of the yields in comparison with the enantiomeric excess.

In conclusion the oxidation with *Bac. stearother.* of these bicyclic systems with high synthetic value permit a kinetic resolution to obtain compounds enantiomerically pure with different oxidation states in comparison with other processes where lipases or chiral reagents are used as resolving reagents only for the alcohols. Moreover this methodology is very promising to prepare these enantiomerically pure compounds on a multigram scale. On the other hand, the mild conditions of the microbial oxidation give an effective alternative to the chemical oxidation. In fact at longer time (8-24 h) all the reaction afforded in good yields the ketone.

Experimental

Oxidations on Analytical Scale. General Procedure. A nutrient broth was prepared dissolving bactotryptone (5 g), yeast extract (2.5 g) and glucose (1 g) in 1 L of distilled water. A sterilized nutrient broth (8 mL) was inoculated with a loopful of *Bacillus stearothermophilus* ATCC (American type culture collection) 2027. The mixture was incubated for 2 days at 38-39° C on a reciprocatory shaker. To the resulting suspension of grown cells the racemic alcohols **1** and **3** (10 mg) in DMF (0.1 mL) and the racemic alcohol **5** (10 mg) in DMSO (0.1 mL) were added. Aliquots were withdrawing periodically and monitored by GLC on a chiral column: Megadex 5 column (25 m X 0.25 mm) containing *n*-pentyl dimetyl b-cyclodextrine in OV 1701 from Mega snc. The enantiomeric separations of the alcohols was achieved after acylation (acetic anhydride and pyridine).

Oxidations on Preparative Scale. Genaral Procedure. The reactions were repeated on preparative scale inoculating 200 mL of the nutrient broth and adding, after 48 h of incubation, the racemic alcohols (0.5 g) in the appropriate solvent (2.5 mL) . After the suitable time (see Scheme), the reaction mixtures were extracted with diethyl ether (100 mL) with a continous liquid-liquid extractor, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The products were purified by column chromatography (silica gel, petroleum ether/diethyl ether 7/3) to give the ketones and the unreacted alcohols. The yields, the absolute configurations and the enantiomeric excesses are listed in the Table. The absolute configurations of compounds **1** and **2**,[16] **3**,[17] **5**,[18] **6**[19] were assigned on the basis of the specific rotations of the literature. The absolute configuration of the ketone **4** is determined by comparison with the alcohol **3** obtained from the same reaction. Optical rotations were measured on a Perkin Elmer Model 241 polarimeter: for (1*R*,5*S*,6*R*)-**1**

$[\alpha]_D$ = -68 (*c* 1.1, $CHCl_3$); for (1*S*,5*R*)-**2** $[\alpha]_D$ = -63 (*c* 1.2, $CHCl_3$); for (1*R*,2*R*,5*R*)-**3** $[\alpha]_D$ = 151 (*c* 1.5, $CHCl_3$); for (1*S*,5*S*)-**4** $[\alpha]_D$ = -502 (*c* 1.3, $CHCl_3$); for (1*R*,2*R*,4*R*)-**5** $[\alpha]_D$ = 160 (*c* 0.5, $CHCl_3$); for (1*S*,4*S*)-**6** $[\alpha]_D$ = -930 (*c* 1.1, $CHCl_3$). The enantiomeric excesses, however, are determined by GLC on chiral column (see above).

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13. The reaction carried out in DMF gave lower enantioselectivity.
14. After the growth (48h), the culture was centrifugated and the recovered cells were suspended in a phosphate buffer solution (8 mL) at pH 7. The alcohol added to the cells in phosphate buffer was oxidized while the alcohol added to the sumatant was recovered unaltered.
15. The cells were centrifugated and added to phosphate buffer (8 mL) at the appropriate pH.
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