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# Asymmetric reduction of enones with *Synechococcus* sp. PCC 7942

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**Abstract**—*Synechococcus* sp. PCC 7942, a cyanobacterium, reduced both the endocyclic C–C double bond of s-*trans* enones and the exocyclic C–C double bond of s-*cis* enones with high enantioselectivity to afford the corresponding (*S*)-ketones under illumination.


Optically active α-substituted ketones are versatile chiral building blocks for asymmetric synthesis.[1] Asymmetric reduction of enones by living whole cells is very attractive and useful for the practical preparation of α-chiral ketones because of high enantioselectivity, no cofactor requirement and ease of scaling up. Microorganisms and plants capable of reducing s-*trans* enones to (*R*)-ketones have been reported so far.[2–4] However, a cell-mediated process in which s-*trans* enones are reduced to (*S*)-ketones is still unavailable. On the other hand, yeast-catalyzed reduction of s-*cis* enones afforded (*S*)-ketones.[4] Over the course of developing a new cell-mediated reduction for asymmetric induction, we investigated the asymmetric reduction of enones by *Synechococcus* sp. PCC 7942.

First, s-*trans* enones **1**–**9** (10 mg each) with an endocyclic C–C double bond were administered to 50 mL of a suspension of *Synechococcus* sp. PCC 7942 cells (2 g)[5,6] in 50 mM Na-phosphate buffer (pH 7.0) and incubated at 25 °C for 1 or 3 days under illumination.[7] The yields of the products were determined by GLC analyses. Extraction from the cell broth with ether followed by purification using column chromatography on silica gel with pentane/ethyl acetate (95:5, v/v) gave the products. It was found that the C–C double bonds of **1**–**3** were reduced to give the corresponding (*S*)-ketones, as shown in Table 1.[8–11] The enantiomeric purities of the resulting ketones were determined based on the peak area of the corresponding enantiomers in the GLC analyses on CP cyclodextrin β 236M-19.[12] Enone **1** was the best substrate, allowing us to achieve the highest enantiomeric excess (98% ee) and yield (>99%). In the case of **2**, the reduction of the C–C double bond of **2** was accompanied by the formation of minor saturated (*S*)-alcohols **17** (>99% ee in 7% yield) and **18** (>99% ee in 2% yield).[13–15] No reduction occurred in the case of **4**, which had an

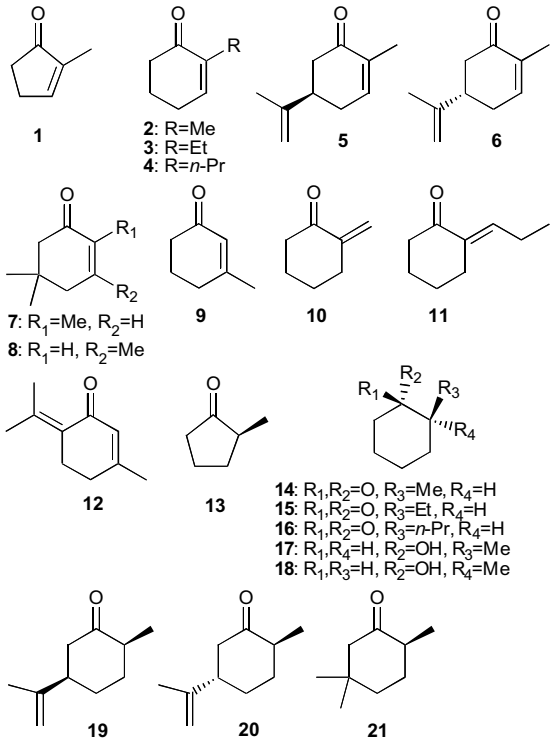


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**Table 1.** Reduction of enones by *Synechococcus* sp. PCC 7942

| Substrates | Products | Reaction time (day) | Conversion (%)[a] | Ee (%) | Configuration[b] |
|---|---|---|---|---|---|
| **1** | **13** | 1 | >99 | 98 | *S* |
| **2** | **14** | 1 | 86 | 85 | *S* |
| **3** | **15** | 3 | 17 | 83 | *S* |
| **4** | — | 3 | 0 | — | — |
| **5** | **19** | 3 | >99 | 80[c] | *S* |
| **6** | **20** | 3 | >99 | 81[c] | *S* |
| **7** | **21** | 3 | 15 | 86 | *S* |
| **8** | — | 3 | 0 | — | — |
| **9** | — | 3 | 0 | — | — |
| **10** | **14** | 1 | 82 | 71 | *S* |
| **11** | **16** | 1 | 7 | 72 | *S* |
| **12** | — | 3 | 0 | — | — |

[a] Percentage of the products in the reaction mixture on the basis of GLC analyses.
[b] Preferred configuration at the α-position to the carbonyl group of the products.
[c] Diastereomeric excess.

*n*-propyl group as the α-substituent. After three days incubation, **5**–**7** were reduced to the corresponding (*S*)-ketones **19**–**21**. *Synechococcus* sp. PCC 7942 cells were not able to reduce β-substituted substrates **8** and **9**. The results obtained here reveal that *Synechococcus* sp. PCC 7942 cells have (i) the ability of catalyzing enantioface differentiating reduction of s-*trans* enones to afford (*S*)-ketones and (ii) similar substrate specificity to microorganisms, which reduce s-*trans* enones if the substituent at the β-position to the carbonyl group is hydrogen and if the α-substituent is not too bulky.[2,4]

Next, s-*cis* enones **10** and **11**[16,17] with an exocyclic C–C double bond were subjected to the same reduction system. **10** was smoothly reduced to give (*S*)-ketone **14** in 82% yield, and the hydrogenation at the α-position showed relatively low enantioselectivity (71% ee). Saturated alcohols **17** (>99% ee) and **18** (>99% ee) were formed as minor products in 7% yield (4:1). The reduction of **11** gave (*S*)-ketone **16** with 72% ee in 7% yield. On the other hand, substrate **12**, which had both endocyclic and exocyclic C–C double bonds was not reduced by the cells probably due to the existence of the β-methyl group. These results demonstrate that *Synechococcus* sp. PCC 7942 cells have the same enantioselectivity in the reduction of s-*cis* enones as yeast.[4]

Thus, the asymmetric reductions of s-*trans* and s-*cis* enones have been accomplished and optically active α-substituted (*S*)-ketones have been prepared by using *Synechococcus* sp. PCC 7942 as biocatalyst. It is worth noting that this new biocatalyst has opposite enantioselectivity in the reduction of s-*trans* enones to other microorganisms[2,4] and plants[3] and that each enantiomer of the α-substituted ketones can be synthesized by selective use of the whole cells. Recently, two enone reductases have been isolated from *Nicotiana tabacum*; s-*trans* enone reductase, which was responsible for the reduction of the endocyclic C–C double bond and s-*cis* enone reductase, which was capable of reducing the exocyclic C–C double bond.[18] In *Synechococcus* sp. PCC 7942 s-*trans* enone reductases with an opposite enantioselectivity to those from yeast and *N. tabacum* might exist. Further investigations using the enzyme preparation from *Synechococcus* sp. PCC 7942 are currently in progress.

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reduced to **13** with 70% ee in 42% yield after one day's incubation in the dark.

8. Product **13**: $[\alpha]_D^{25} = +114.9$ ($c$ 0.52, $CHCl_3$) {lit.[9] $[\alpha]_D^{25} = -110.5$ for (*R*)-enantiomer}; **14** converted from **2**: CD $[\theta]_{288} = +887$ ($c$ 0.75, MeOH) {lit.[10] $[\theta]_{288} = -987$ for (*R*)-enantiomer}; **14** from **10**: CD $[\theta]_{288} = +701$ ($c$ 0.68, MeOH); **15**: CD $[\theta]_{288} = +1914$ ($c$ 0.32, MeOH) {lit.[11] $[\theta]_{288} = +2200$}; **16**: CD $[\theta]_{288} = +1860$ ($c$ 0.15, MeOH) {lit.[11] $[\theta]_{288} = +2480$}; **21**: CD $[\theta]_{288} = +995$ ($c$ 0.14, MeOH).
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12. Conditions for capillary GLC analysis: column, CP cyclodextrin β 236M-19 (0.25 mm × 25 m); injection, 180 °C; detector, 180 °C; oven, 100 °C; carrier gas, $N_2$ (50 mL min$^{-1}$). Retention times for the products in the GLC were as follows: (*S*)- and (*R*)-**13**, 10.5 and 11.4 min; (*S*)- and (*R*)-**14**, 11.8 and 12.8 min; (*S*)- and (*R*)-**15**, 12.7 and 12.9 min; (*S*)- and (*R*)-**16**, 27.7 and 27.9 min; (*S*)- and (*R*)-**21**, 50.1 and 51.9 min.
13. Product **17**: $[\alpha]_D^{25} = +51.2$ ($c$ 0.4, MeOH) {lit.[14] $[\alpha]_D^{20} = +42.9$}; **18**: $[\alpha]_D^{25} = +25.7$ ($c$ 0.2, MeOH) {lit.[14] $[\alpha]_D^{20} = +24.3$}. The enantiomeric purities of **17** and **18** were determined by $^1$H NMR analyses of the corresponding MTPA esters as described previously.[15] In the cases of the other substrates, saturated alcohols were not obtained during the incubation time examined.
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