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DIASTEREO- AND ENANTIO-SELECTIVE REDUCTION OF ETHYL 2-METHYL-3-OXOBUTANOATE BY PLANT CELL CULTURES

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Key Word Index Biotransformation; reduction; *Marchantia polymorpha*; *Glycine max*; hydroxy ester; diastereoselective; reduction; enantioselective.

Abstract—Ethyl 2-methyl-3-oxobutanoate was reduced diastereo- and enantio-selectively to the corresponding anti- and syn-(*S*)-hydroxyester by callus of *Marchantia polymorpha* and *Glycine max*, respectively.

INTRODUCTION

Diastereo- and enantio-selective synthesis of 2-alkyl-3-hydroxyalkanoates is a challenging problem in the field of synthetic organic chemistry [1, 2]. Chemical methods have been developed which involve aldol condensation of chiral compounds [1] and reduction of α-alkyl-β-keto esters with BINAP–Ru complexes [2]. The latter method is effective for 2-alkylamino derivatives, but is not effective for 2-alkyl derivatives. Biotransformations such as microbial reductions in aqueous media [3, 4], and microbial reduction in organic media [5] are also effective. Herein, we report on a novel biotransformation for the reduction of ethyl 2-alkyl-3-hydroxyalkanoate by plant cell suspension cultures [(for review of plant cell biotransformations see refs [6–8]).

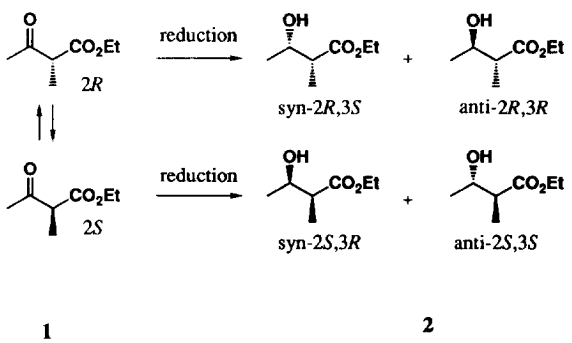
RESULTS AND DISCUSSION

Due to the β-keto ester structure, racemization of the enantiomers of ethyl 2-methyl-3-oxobutanoate (**1**), the starting material, is very rapid and the reduction product, 2-methyl-3-hydroxybutanoate (**2**), is hardly racemized [3]. The reduction would form one stereoisomer selectively out of the four possible stereoisomeric hydroxyl esters if the reduction proceeded diastereo- and enantioselectively (Scheme 1).

Several plant cell cultures were tested for the ability to reduce compound **1**, and the enantioselectivity and diastereoselectivity of the reduction were measured (Table 1).

The yield of the hydroxyl ester depends on the species of plant. Thus, *Glycine max*, *Catharanthus roseus* and *Marchantia polymorpha* afforded the hydroxyl ester in high yields while *Nicotiana tabacum*, *Pharaenopsis* sp. and *Phytolacca americana* gave only moderate yields. The diastereoselectivity of the reduction was species dependent. Callus from *C. roseus* afforded a low anti-selectivity (anti:syn = 61:39). Similar results were obtained with *P. americana* (anti:syn = 13:7) and *Pharaenopsis* sp. (anti:syn = 18:7). Although the diastereoselectivities of these calli were not satisfactory, we found that the anti-isomer could be prepared selectively by using callus of *M. polymorpha* as the reducing biocatalyst (anti:syn = 48.2:1.7). It is well known that BINAP–Ru catalyst exhibits high enantioselectivity when reducing **1**, whereas BINAP catalyst shows no diastereoselectivity towards 2-alkyl-3-oxobutanoate [2]. Thus, biocatalysts are superior to chemical catalysts in terms of diastereoselectivity. Several calli afforded syn-selectivity. Thus, the callus from *N. tabacum* (anti:syn = 19:31) showed low syn-selectivity while that of *G. max* (anti:syn = 2:23) gave excellent syn-selectivity.

The enantioselectivity of the reduction with *M. polymorpha* was excellent; the stereochemistry of the anti-isomer (2*S*,3*S*) was 3*S* and the enantiomeric excess (ee) was over 99%. The ee of the syn-isomer (2*R*,3*S*) obtained from *G. max* was also excellent (97%) (Scheme 2). Thus,



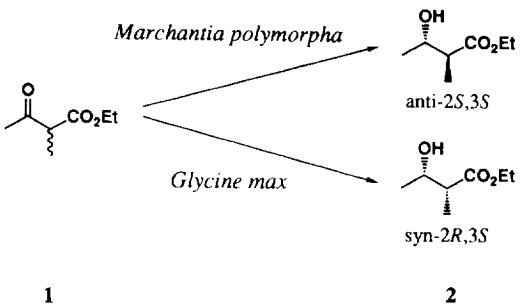
1 **2**

Scheme 1. Reduction products of **1**.

Table 1. Reduction of ethyl 2-methyl-3-oxobutanoate (**1**) by plant cell calli

Callus	Yield of **2** (%)	Anti(ee %):Syn(ee %)
Marchantia polymorpha	88	96(99*):4(13†)
Pharaenopsis sp	30	72(93*):28
Phytolacca americana	41	65:35
Catharanthus roseus	90	61(99*):39(84*)
Nicotiana tabacum (Bright yellow)	24	56:44
Nicotiana tabacum (Mild cure)	36	38:62
Glycine max	100	8(>95*):92(97*)

* The 3*S*-hydroxyl ester is obtained preferentially.
† The 3*R*-hydroxyl ester is obtained preferentially.



Scheme 2. Biotransformation of compound **1**.

only one stereoisomer out of four possible stereoisomers was obtained by using reduction with plant cell cultures.

EXPERIMENTAL

Analytical. Chemical yields and diastereomeric ratios were determined on a capillary GC column (HR-20M, 0.25 mm × 25 m, 120°, He, 2 ml min^{-1}) using tetradecane as an int. standard. The absolute configurations and enantiomeric excesses were determined on a chiral capillary GC column (Chiraldex G-TA, 0.25 mm × 30 m, 85°, He 2 ml min^{-1}). The standard sample of **2** was prepd according to ref. [3].

Cultivation of cells. Callus tissues were cultured on agarose with the following media: *C. roseus*, Schenk and Hildebrandt medium [9]; *G. max* and *N. tabacum* (Bright yellow and Mild cure), Murashige and Skoog's medium [10]; *M. polymorpha*, MSK-II medium [11]; Pharaenopsis, VWII medium [12].

Substrate. Compound **1** was purchased from Tokyo Kasei and used without further purification.

Biotransformation of **1**. Callus (10 g) was suspended in 30 ml K-Pi buffer (pH 7.0, 0.1 M) and 0.1 mmol **1** was added to the resulting mixt. After 24 hr, 2.5 mg tetradecane (GC int. standard) in 0.5 ml Et_2O was added to the reaction mixt., which was then extracted with EtOAc (10 ml × 3). Chemical yields and diastereomeric ratios were determined by GC.

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