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The Highly Selective Equatorial Hydride Delivery by Biocatalysis: Chemoenzymatic Synthesis of *trans*-2-(4-Propylcyclohexyl)-1,3-propanediol via *cis*-4-Propylcyclohexanol

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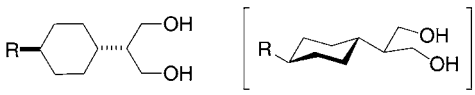
Abstract:

4-Propylcyclohexanone 10a (69 g/L) is reduced by the catalysis of *Galactomyces geotrichum* JCM 6359 using *i*-PrOH (4.0 equiv) as an auxiliary substrate for recycling externally supplemented NAD^+ (0.001 equiv) in 40 mM potassium phosphate buffer (pH 7.5) for 20 h to provide a mixture of *cis*-4-propylcyclohexanol 3a [*cis*/*trans* (99:0.5); 74%] and unconsumed 10a (22%). Practically pure 3a can be isolated in 69% yield after removing the entailed ketone 10a via bisulfite adduct formation. In the meantime, the crude reduction product [3a/10a (74:22)], without further purification, can be elaborated into *trans*-2-(4-propylcyclohexyl)-1,3-propanediol 1a, a compound deemed versatile in liquid-crystals development, in 30% overall yield from 10a in four steps.

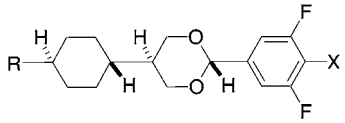
Introduction

Recent material research has witnessed *trans*-2-(4-alkylcyclohexyl)-1,3-propanediol **1** exhibiting liquid crystalline properties in themselves[1] and serving as immediate precursors to *trans*-1,3-dioxane-based liquid crystals **2**[2] (Figure 1). However, starting with *trans*-4-alkylcyclohexanol **5**, the hitherto reported synthesis of *trans*-1,4-disubstituted cyclohexane **1** (Scheme 1) would end up suffering practical issues, which are tactically interrelated with each other as outlined below.

(1) Preparation of *trans*-4-alkylcyclohexanol **5**: While no specific method to prepare **5** has been recorded in the literature dealing with the synthesis of **1** (Scheme 1),[1,2] Eliel's protocol should be applicable to setting up the hydroxy group

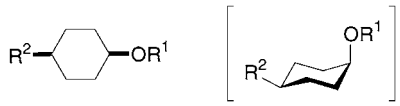


1a R = *n*-C_3H_7; **b** R = C_2H_5; **c** R = *n*-C_5H_{11}



2

R = Alkyl (from C_1 to C_{15})
X = F, Cl, OCF_3, $OCHF_2$, OCH_2CF_3, $OCHFCF_3$
$OCH{=}CF_2$, $OCF{=}CF_2$, OCF_2CF_3



3 R^1 = H
a R^2 = *n*-C_3H_7; **b** R^2 = C_2H_5; **c** R^2 = *n*-C_5H_{11} **d** R^2= *tert*-C_4H_9
4 R^1 = SO_2Me (Ms)
a R^2 = *n*-C_3H_7; **b** R^2 = C_2H_5; **c** R^2 = *n*-C_5H_{11} **d** R^2= *tert*-C_4H_9

Figure 1. **Structures of *trans*-2-(4-alkylcyclohexyl)-1,3-propanediol 1, its derived liquid-crystal 2, *cis*-4-alkylcyclohexanol 3, and *cis*-4-alkylcyclohexyl mesylate 4.**

with an equatorial orientation in reducing 4-alkylcyclohexanone **10** to **5**, wherein mixed hydride [$AlCl_3$/$LiAlH_4$ (3.6:1)] was used to advantage, coupled with equilibration between *trans*-4-alkylcyclohexanol **5** and its *cis*-isomer **3** in favor of the former.[3] Reportedly, this classical method was so effective that 4-*tert*-butylcyclohexanone **10d** was reduced to *trans*-4-*tert*-butylcyclohexanol **5d** in 99.3% yield, with **3d** (Figure 1) and **10d**[4] contaminating it only 0.3% and 0.4%, respectively. However, such high stereoselectivity notwithstanding, use of pyrophoric reducing agents such as $LiAlH_4$ might detract from the industrial viability of this ingenious preparative method.

(2) Homologation with double stereochemical inversion: To install the *trans*-oriented 1,3-propanediol appendage onto

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⊥ Fukuchiyama Factory 2, Nagase ChemteX Corporation.

(1) (a) Diele, S.; Geissler, E.; Vorbrodt, H.-M.; Zaschke, H. *Mol. Cryst. Liq. Cryst. Lett.* **1984**, *102*, 181. (b) Tschierske, C.; Altmann, H.; Zaschke, H.; Brezesinski, G.; Kuschel, F. *Mol. Cryst. Liq. Cryst.* **1990**, *191*, 295.
(2) (a) Kirsch, P.; Poetsch, E. *Adv. Mater.* **1998**, *10*, 602. (b) Binder, W.; Krause, J.; Meyer, V.; Pötsch, E.; Schön, S.; Tarumi, K. (Merck Patent GMBH). German Patent DE 19522529, 1997. (c) Obikawa, T.; Ikukawa, S.; Nakayama, J. (Seiko Epson Corporation). Eur. Pat. Appl.EP 0 400 861, 1990.
(3) Eliel, E. L.; Martin, R. J. L.; Nasipuri, D. *Organic Syntheses*; Wiley & Sons: New York, 1973; Collect. Vol. V, pp 175−178.

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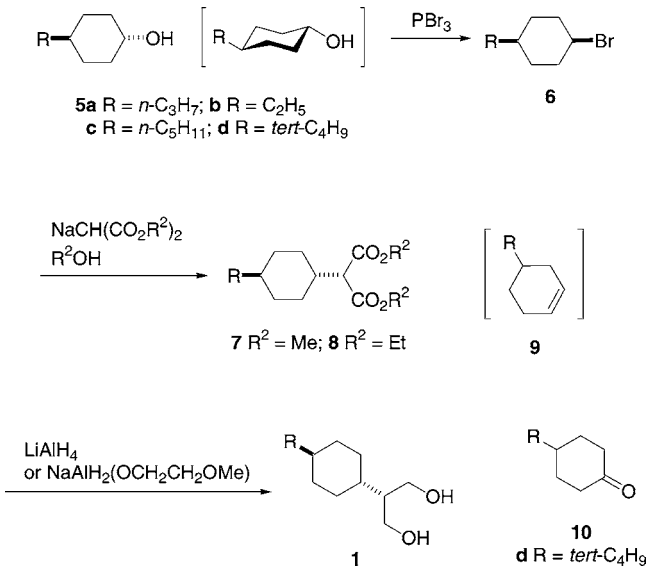
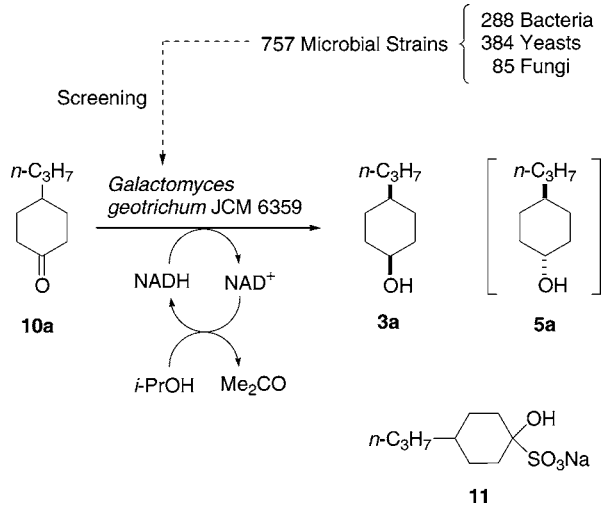
Scheme 1. **Existing route to *trans*-2-(4-alkylcyclohexyl)-1,3-propanediol 1 from *trans*-4-alkylcyclohexanol 5**



the cyclohexane framework **5**, the hydroxy group in *trans*-4-alkylcyclohexanol **5** was supposed to undergo bromination (**5** → **6**) and nucleophilic substitution with malonate ester (**6** → **7**/**8**) each with stereochemical inversion (Scheme 1). When **5** was treated neat with PBr_3, bromination was reported to proceed uneventfully, giving *cis*-1-alkyl-4-bromocyclohexane **6**, and the yields reported in the literature were as follows: **6a**,[2c] 88% yield; **6b**,[5a] 92% yield; **6c**,[5b] 55% yield.[6] However, bromide **6** proved to be so liable to β-elimination that nucleophilic substitution with sodiomalonate [$NaCH(CO_2R^2)_2$] produced **8a** in a mediocre yield of 43% with the elimination product, 4-propylcyclohexene **9a**, being generated in 46% yield, as shown by literature precedent.[2c]

(3) Metal-hydride reduction of diester **7**/**8** to 1,3-diol **1**: For the reduction of diester **7**/**8** to 1,3-diol **1**, use of $LiAlH_4$[1b] and $NaAlH_2(OCH_2CH_2OMe)_2$[2c,5] was reported in the literature; diethyl *trans*-(4-propylcyclohexyl)malonate **8a** was reduced to *trans*-2-(4-propylcyclohexyl)-1,3-propanediol **1a** with $NaAlH_2(OCH_2CH_2OMe)_2$ in 45% yield,[2c] while no specific yield was described for the $LiAlH_4$-mediated reduction of **7a** (Scheme 1).[1b] In addition to such mediocre yield, both the pyrophoric nature and the relatively high cost of the reagents used would also afflict the practical aspects of the production of **1**.

Scheme 2. **Reduction of 4-propylcyclohexanone 10a with Galactomyces geotrichum JCM 6359**



Thus, as part of our process research and development program, we explored more industrially viable approaches to *trans*-2-(4-propylcyclohexyl)-1,3-propanediol **1a**, which have culminated in scalable construction of *cis*-4-propylcyclohexanol **3a** through the microbial equatorial hydride delivery to its ketone progenitor and have paved a practical route for *trans*-1,4-disubstituted **1a** via *cis*-4-propylcyclohexyl mesylate **4a** with stereochemical inversion (Figure 1).[4]

Results and Discussion

Tactics for Process Development. To resolve the problems mentioned above, we chose to adopt the following tactics in developing new and improved processes to access *trans*-2-(4-propylcyclohexyl)-1,3-propanediol **1a**: (1) stereoselective reduction of 4-propylcyclohexanone **10a** to *cis*-4-propylcyclohexanol **3a**, which was eventually achieved by the use of biocatalysis (Scheme 2); (2) use of *cis*-4-propylcyclohexyl mesylate **4a** as an electrophile, instead of bromide **6a**, in the alkylation of dimethyl sodiomalonate [$NaCH(CO_2Me)_2$] (Scheme 3);[7] (3) $NaBH_4$-mediated reduction of diester **7a** to 1,3-diol **1a** (Scheme 3).

Equatorial Hydride Delivery via Microbial Reduction. For the malonate alkylation to be viable with *cis*-4-propylcyclohexyl mesylate **4a** on scale (Scheme 3), its immediate precursor, *cis*-4-propylcyclohexanol **3a** (Figure 1), should be secured in quantity. However, the modern arsenal of synthetic chemistry is still short of industrially viable methods to build an axial alcohol in the reduction of 4-alkylcyclohexanone **10** (Scheme 2).

Indeed, *tert*-butylcyclohexanone **10d** was reported to undergo equatorial hydride delivery to give *cis*-4-*tert*-butylcyclohexanol **3d** (Figure 1) with high selectivity when bulky hydride agents were used, typical examples of which are as follows: $Li[tert\text{-}Bu(Et)_2CO]_3AlH$, THF, 20 °C, **3d**/**5d** (95:5);[8a] $Li[Me_2CH(Me)CH]_2BH$, THF, −78 °C,

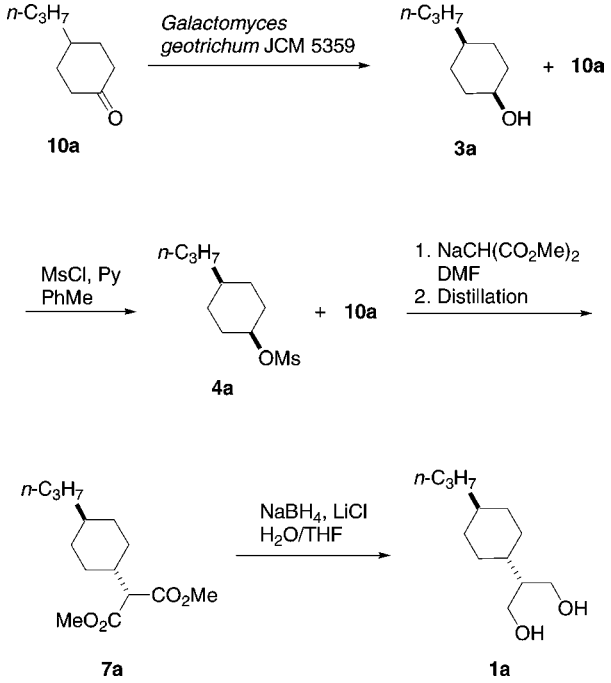
(4) Part of this work was discussed briefly in the keynote lecture entitled "Biocatalysis from the Perspective of an Industrial Practitioner: Let a Biocatalysis Do a Job That No Chemocatalyst Can" given by M.I. at Symposium C (Material Design in Catalysis) in the 2nd International Conference on Materials for Advanced Technologies (ICMAT), Singapore, December 7−12, 2003.

(5) (a) Obikawa, T.; Ikukawa, S.; Nakayama, J. (Seiko Epson Corporation). Jpn. Kokai Tokkyo Koho 94−25216, 1994. (b) Obikawa, T.; Ikukawa, S. (Seiko Epson Corporation). Jpn. Kokai Tokkyo Koho 91-141274, 1991.

(6) More elaborate procedures to prepare *cis*-4-*tert*-butylcyclohexyl bromide **6d** than treatment of *trans*-4-*tert*-butylcyclohexanol **3d** with PBr_3 were also reported: (a) Treatment of **3d** with PBr_5 prepared from PBr_3 and Br_2 in CH_2Cl_2: Eliel, E. L.; Haber, R. G. *J. Org. Chem.* **1959**, *24*, 143. (b) Preparation of *trans*-4-*tert*-butylcyclohexyl *p*-nitrobenzenesulfonate from **3d** and treatment of the former with LiBr and $CaCO_3$ in acetone: Eliel, E. L.; Martin, R. J. L. *J. Am. Chem. Soc.* **1968**, *90*, 689. (c) Preparation of *trans*-4-*tert*-butylcyclohexyl(pyridine)cobaloxime from *cis*-*tert*-butylcyclohexyl tosylate and treatment of the former with Br_2 in CH_2Cl_2: Shinozaki, H.; Ogawa, H.; Tada, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 775. (d) Treatment of **3d** with Ph_3PBr_2 in MeCN: Kitching, W.; Olszowy, H.; Waugh, J. *J. Org. Chem.* **1978**, *43*, 898.

(7) Osako, A.; Moriya, N.; Suenaga, H. (Teikoku Chemical Industry Co., Ltd.). Jpn. Kokai Tokkyo Koho 2002-226431, 2002.

Scheme 3. **Conversion of crude *cis*-4-propylcyclohexanol 3a into *trans*-2-(4-propylcyclohexyl)-1,3-propanediol 1a**



3d/**5d** (>99.5:<0.5);[8b] Li[2,4-(*tert*-Bu)$_2$C$_6$H$_3$O](*tert*-BuCH$_2$O)AlH, THF/Et$_2$O, 25 °C, **3d**/**5d** (64:5).[8c] However, their use should rather be confined to the laboratory because of their limited availability and safety concerns arising from their pyrophoric nature.[9]

Except for such modified hydride agents, hydrogenation over Rh catalyst in *i*-PrOH or THF in the presence of a small amount of 37% aqueous HCl at 25 °C under H_2 at atmospheric pressure was also reported to be effective for reducing *tert*-butylcyclohexanone **10d** to *cis*-4-*tert*-butylcyclohexanol **3d**: **3d**/**5d** (99.2:0.5) in *i*-PrOH; **3d**/**5d** (99.3:0.7) in THF.[10] However, this catalytic hydrogenation method should be less scalable due to its acidic conditions, the reason being that plant hydrogenation facilities are not usually glass-lined so that they would rust on exposure to acid.

In view of such technical limitations to both hydride reduction and catalytic hydrogenation, we embarked on exploring microbial reduction for the possibility of reducing 4-propylcyclohexanone **10a** to *cis*-4-propylcyclohexanol **3a** while knowing that there was no such track record for the microbial reduction (Scheme 2).[11,12] When cultures of 757 microbial strains (288 bacteria, 384 yeasts, and 85 fungi) were each treated with **10a** at a concentration of 4 g/L in 0.1 M potassium phosphate buffer (pH 7.0) in the presence of both NADH (0.5 equiv) and NADPH (0.5 equiv) at 25 °C for 2 h, GLC analysis (TC-WAX, GL Science) showed that 32 strains (10 bacteria and 22 yeasts) met the screening criteria that were set somewhat arbitrarily as follows: 2-h conversion > 10% with *cis*-selectivity > 80%.

Table 1. **Microbial strains able to reduce 4-propylcyclohexanone 10a to *cis*-4-propylcyclohexanol 3a[a,b]**

	NADH (1.0 equiv)		NADPH (1.0 equiv)		none[c]	
microbial strains	conv (%)[d]	*cis* (%)[d]	conv (%)[d]	*cis* (%)[d]	conv (%)[d]	*cis* %)[d]
Candida intermedia NBRC 0761[e]	46	99	10	91	1	93
Schizosaccharomyces octosporus JCM 1801[f]	42	99	7	80	3	86
Galactomyces geotrichum JCM 1945[f]	37	99	7	80	4	85
G. geotrichum JCM 6539[f]	26	99	6	89	1	92
Stenotrophomonas maltophilia JCM 1987[f]	14	98	5	88	0	*g*
Candida albicans JCM 1542[f]	14	98	5	89	1	92
Flavobacterium gleum CDC 11 b[h]	14	99	4	98	1	95

[a] For the experimental conditions in detail, see Optimization of the Microbial Reduction in Experimental Section. [b] [**10a**] = 13 g/L. [c] The microbial reduction was conducted in the absence of either nicotinamide cofactor. [d] The 2-h conversion and *cis*-selectivity were both determined by GLC; for the conditions in detail, see Microbial Screening in Experimental Section. [e] Available from NITE (National Institute of Technology and Evaluation) Biological Resource Center. [f] Available from Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research). [g] Not detected. [h] Identified in the microbial library collected at Research and Development Center, Nagase & Co., Ltd.

The selected 32 strains were next evaluated for the ability to reduce **10a** at elevated concentrations and for the dependence on nicotinamide cofactors, NAD^+ and $NADP^+$. When a 13 g/L suspension of **10a** in 0.1 M potassium phosphate buffer (pH 7.0) was treated with an aqueous suspension of each the 32 microbial cells in the presence of either NADH (1.0 equiv) or NADPH (1.0 equiv) for 2 h, only seven strains (one bacterium and six yeasts) were able to achieve the 2-h conversion > 10% with *cis*-selectivity > 80%, both being assessed by GLC under the same conditions as mentioned above. As regards the dependence on nicotinamide cofactors, the seven strains all showed significant preference for NAD^+ since each of them exhibited the much higher 2-h conversion in the presence of NADH than in the presence of NADPH as summarized in Table 1.

To reduce the NAD^+ usage to a catalytic level, different commercially available organic substrates, such as sugars,

(8) (a) Boireau, G.; Deberly, A.; Toneva, R. *Synlett* **1993**, 585. (b) Krishnamurthy, S.; Brown, H. C. *J. Am. Chem. Soc.* **1976**, *98*, 3383. (c) Haubenstock, H. *J. Org. Chem.* **1975**, *40*, 926.

(9) For the *cis*-selective reduction of 4-*tert*-butylcyclohexanone **10d** to **3d** via hydride transfer catalyzed by much less available $IrCl_4$, see: Eliel, E. L.; Doyle, T. W.; Hutchins, R. O.; Gilbert, C. E. *Organic Syntheses*; Wiley & Sons: New York, 1988; Collect. Vol. VI, pp 215−218.

(10) Nishimura, S.; Ishige, M.; Shiota, M. *Chem. Lett.* **1977**, 963.

(11) For the microbial reduction with little, if any, selectivity, see: (a) Okamura, S.; Miyazawa, M.; Yamaguchi, M.; Kameoka, H. *J. Jpn. Oil Chem. Soc.* **2000**, *49*, 343. (b) García-Pajón, C. M.; Hernández-Galán, R.; Collado, I. G. *Tetrahedron: Asymmetry* **2003**, *14*, 1229. (c) van Osselaer, T.; Lemière, G. L.; Lepoivre, J. A.; Alderweireldt, F. C. *Bull. Soc. Chim. Belg.* **1980**, *89*, 133.

(12) For treatises on biocatalytic reduction in general, see: (a) Drauz, K., Waldmann, H., Eds. Reduction Reactions. In *Enzyme Catalysis in Organic Synthesis: A Comprehensive Handbook*, 2nd ed.; Wiley-VCH: Weinheim, 2002; Vol. III, Chapter 15, pp 991−1063. (b) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659. (c) Hummel, W. New Alcohol Dehydrogenases for the Synthesis of Chiral Compounds. In *New Enzymes for Organic Synthesis: Screening, Supply and Engineering*; Scheper, T., Ed.; Springer: Berlin, 1999; pp 145−184. (d) Carnell, A. J. Stereoinversions Using Microbial Redox-Reactions. In *Biotransformations*; Faber, K., Ed.; Springer: Berlin, 2000; pp 58−72.

Table 2. **Auxiliary substrates to recycle NAD^+ in the reduction of 4-propylcyclohexanone 10a to *cis*-4-propylcyclohexanol 3a[a,b]**

microbial strains	2-h conversion (%) in the presence of NAD^+ (0.01 equiv)[c,d]										
	glc[e]	suc[f]	fru[g]	gly[h]	glu[i]	fra[j]	pgl[k]	lac[l]	glr[m]	etl[n]	ipa[o]
C. intermedia NBRC 0761[p]	8.4	5.6	3.7	4.4	4.8	5.2	6.8	4.9	4.7	9.8	40.8
S. octosporus JCM 1801[q]	5.3	5.3	2.8	4.9	5.3	4.8	7.1	4.4	6.0	7.6	45.5
G. geotrichum JCM 1945[q]	5.3	5.2	3	5	5.7	5.5	6.8	5.0	5.8	7.0	35.5
G. geotrichum JCM 6539[q]	2.0	2.0	1.1	1.9	2.1	2.3	5.6	1.5	3.0	4.5	52.4
S. maltophilia JCM 1987[q]	0.2	0.8	0.2	0.2	0.4	0.4	0.5	0.2	0.7	1.7	4.3
C. albicans JCM 1542[q]	1.9	1.3	1.0	1.1	1.8	4.1	4.0	1.1	1.3	1.9	9.6
F. gleum CDC 11 b[r]	1.8	0.8	0.7	0.7	1.3	0.8	1.0	1.0	0.9	1.8	6.6

[a] For the experimental conditions in detail, see Optimization of the Microbial Reduction in Experimental Section. [b] [**10a**] = 15 g/L. [c] Each auxiliary substrate, 2.0 equiv. [d] The 2-h conversion was determined by GLC; for the conditions in detail, see Microbial Screening in Experimental Section. [e] Glucose. [f] Sucrose. [g] Fructose. [h] Glycine. [i] Glutamic acid. [j] Formic acid. [k] 1,3-Propanediol. [l] Lactic acid. [m] Glycerol. [n] Ethanol. [o] 2-Propanol. [p] Available from NITE (National Institute of Technology and Evaluation) Biological Resource Center. [q] Available from Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research). [r] Identified in the microbial library collected at Research and Development Center, Nagase & Co., Ltd.

amino acids, alcohols, and formic acid, were evaluated for the ability to recycle NAD^+ in the microbial reduction in question, whether in direct conjugation with the enzymatic reduction of **10a** or via independent catabolic pathways (Table 2). When the microbial reduction of **10a** (15 g/L) was conducted in 0.1 M potassium phosphate buffer (pH 7.0) in the presence of each auxiliary substrate (2.0 equiv) along with NAD^+ (0.01 equiv), *i*-PrOH was identified as the best auxiliary substrate (hydride source) helping all seven microbes attain the highest conversion at 2 h. In particular, four yeast strains, *Galactomyces geotrichum* JCM 6359, *G. geotrichum* JCM 1945, *Candida intermedia* NBRC 0761, and *Schizosaccharomyces octosporus* JCM 1801, showed the 2-h conversion to be worth further investigation; *G. geotrichum* JCM 6359, 52%; *G. geotrichum* JCM 1945, 36%; *C. intermedia* NBRC 0761, 41%; *S. octosporus* JCM 1801, 46%.

The four yeasts were then tested for the reduction of **10a** (60 g/L) in 0.1 M potassium phosphate buffer (pH 7.0) in the presence of NAD^+ (0.01 equiv) and *i*-PrOH (1.0−4.0 equiv). With increasing amounts of *i*-PrOH, the reduction of **10a** proceeded further in the presence of *G. geotrichum* JCM 6539 (Table 3); specifically, when 4.0 equiv of *i*-PrOH was used, the reduction was driven to 75.4% conversion after 20 h. In contrast, increase in the amount of *i*-PrOH caused no such incremental effect on the conversion of the reduction with the other three yeast strains, *G. geotrichum* JCM 1945, *C. intermedia* NBRC 0761, and *S. octosporus* JCM 1801 (Table 3); in fact, each yeast showed the 20-h conversion in the presence of 4 equiv of *i*-PrOH less than that in the presence of 2 equiv of *i*-PrOH (Table 3). Hence, *G. geotrichum* JCM 6359 was eventually identified as the biocatalyst of choice for reducing 4-propylcyclohexanone **10a** to *cis*-4-propylcyclohexanol **3a**.

The *G. geotrichum* JCM 6359-catalyzed reduction was further explored to gain practical insight into parameters affecting its industrial viability. Use of *i*-PrOH in amounts >4 equiv failed to allow the reduction to proceed beyond 75% conversion, and this unfavorable result seemed to arise from acetone accumulating in conjugation with the reduction of **10a** to **3a** to such a degree that the responsible oxidoreductase suffered significant inhibition. To alleviate this suspected product inhibition and to drive the reduction close to completion, removal of acetone was then attempted. When the reaction mixture was exposed to reduced pressure (50 mmHg) at 25 °C for 20 min at 7 and 18 h after the initiation of the reaction {[**10a**] = 60 g/L; *i*-PrOH, 4.0 equiv; NAD^+, 0.01 equiv; 0.1 M potassium phosphate buffer, pH 7.0, 25 °C}, the 27-h conversion could be raised by 10% compared to that of 75% which was attained without such evacuation operations.

Table 3. **Effect of the increasing amounts of *i*-PrOH on the 20-h conversion in the reduction of 4-propylcyclohexanone 10a to *cis*-4-propylcyclohexanol 3a by the four yeast strains[a,b]**

yeast strains	20-h conversion (%)[c] amounts of *i*-PrOH (equiv)		
	1.0	2.0	4.0
G. geotrichum JCM 6539[d]	55.6	70.5	75.4
G. geotrichum JCM 1945[d]	38.6	44.1	32.9
C. intermedia NBRC 0761[e]	46.1	55.4	48.9
S. octosporus JCM 1801[d]	39.7	45.0	34.8

[a] For the experimental conditions in detail, see Optimization of the Microbial Reduction in Experimental Section. [b] [**10a**] = 60 g/L. [c] The 20-h conversion in the reduction of **10a** to **3a** was determined by GLC; for the conditions in detail, see Microbial Screening in Experimental Section. [d] Available from Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research). [e] Available from NITE (National Institute of Technology and Evaluation) Biological Resource Center.

To assess the effect of organic solvents on the reduction in question, the *G. geotrichum* JCM 6359-catalyzed reduction was conducted in the presence of an organic solvent in the same volume of **10a** (*d* 0.907) otherwise under the same conditions as specified above {[**10a**] = 60 g/L; *i*-PrOH, 4.0 equiv; NAD^+, 0.01 equiv; 0.1 M potassium phosphate buffer, pH 7.0; 25 °C, 20 h} and was analyzed quantitatively for the 20-h conversion. As a result, addition of organic solvents was found not to have any favorable effect on the progress of the reaction as demonstrated by decrease in the 20-h conversion: EtOAc, −32%; *n*-BuOAc, −15%; PhMe, −20%; *n*-hexane, −7%; *tert*-BuOMe (MTBE), −11%.

As regards the NAD^+ usage, further reduction from 0.01 to 0.001 equiv was achieved without any deleterious effect on the 20-h conversion. However, when the reduction was

run in the presence of 1.0×10^{-4} equiv of NAD^+, the 24-h conversion suffered 20% decrease compared to that in the presence of 1.0×10^{-3} equiv of NAD^+. Hence, the proper amount of NAD^+ to be used for the *G. geotrichum* JCM 6359-catalyzed reduction of **10a** should be 0.001 equiv.

The *G. geotrichum* JCM 6359-catalyzed reduction was also explored for the optimum pH and temperature, and they were determined as follows: the optimum pH, 7.3−8.1, and the optimum temperature, 22−33 °C.

Preparation of Pure *cis*-4-Propylcyclohexanol 3a. The select reaction parameters examined above were adapted for procedures to obtain *cis*-4-propylcyclohexanol **3a** in a pure state (Scheme 2): *G. geotrichum* JCM 6359 was grown in YM 5.5 medium for 2 days. The culture was centrifuged to harvest the cultivated cells, which were then added to a suspension of **10a** (69 g/L) in 40 mM potassium phosphate buffer (pH 7) containing *i*-PrOH (2.2 equiv) and NAD^+ (0.001 equiv). After stirring at 25 °C for 9 h, the reaction mixture was supplemented with *i*-PrOH (2.2 equiv). When the reduction reached 77% conversion in 27 h, the mixture was extracted with MTBE to provide a mixture of **3a** (*cis*-isomer; 74%), **5a** (*trans*-isomer; 0.4%), and unconsumed **10a** (22%) with the *cis*/*trans*ratio being 99.5:0.5.

To remove the unconsumed ketone **10a**, the crude reduction product was treated with an aqueous ethanol solution of $NaHSO_3$ to convert **10a** into its bisulfite adduct **11**[13] that, on formation, precipitated as insoluble solids. The solids were removed by filtration, and the filtrate was extracted with PhMe to furnish practically pure *cis*-4-propylcyclohexanol **3a** [*cis*/*trans* (99.6:0.4)] in 69% yield.

Completion of 2-(*trans*-4-Propylcyclohexyl)-1,3-propanediol 1a. The ultimate target being 2-(*trans*-4-propylcyclohexyl)-1,3-propanediol **1a**, the attempt was made to carry unpurified *cis*-4-propylcyclohexanol **3a**, contaminated with 4-propylcyclohexanone **10a**, into the ensuing steps leading to **1a** (Scheme 3). When the crude product [**3a**/**10a** (74:22)] arising from the *G. geotrichum* JCM 6359-catalyzed reduction was treated with MsCl in PhMe in the presence of pyridine at 50 °C for 5 h, mesylation proceeded quantitatively with participating **3a**. However, the resulting mesylate **4a** could not be separated from the ketone contaminant **10a** without recourse to silica gel chromatography. Hence, removal of the entailed ketone **10a** was postponed until the next homologation step.

The crude mesylation product [**4a**/**10a** (74:22)] was then treated with dimethyl sodiomalonate [$NaCH(CO_2Me)_2$] in DMF at 80 °C for 5 h, during which nucleophilic substitution on mesylate **4a** went to completion. In the meantime, ketone **10a** survived the malonate attack unaffected, and it was this situation that eventually allowed the homologated product **7a** to be separated from **10a** in a practical manner: pure malonate homologue **7a** was obtained as fractions boiling at 125−150 °C/7 mmHg in 50% overall yield after simple distillation where ketone **10a** was removed completely as lower-boiling fractions.

Last, reduction of diester **7a** to 1,3-diol **1a** was explored for safe practical procedures: when the purified diester **7a** was treated with $NaBH_4$ (1.8 equiv) and LiCl (1.8 equiv) in aqueous THF at room temperature for 16 h, *trans*-2-(4-propylcyclohexyl)-1,3-propanediol **1a** was obtained in 60% yield after single recrystallization from *n*-heptane, the four-step overall yield of **1a** being 30% from **10a**.

Conclusions

In summary, intensive microbial screening did identify *Galactomyces geotrichum* JCM 6359 as the best biocatalyst to deliver hydrides in equatorial disposition in reducing 4-propylcyclohexanone **10a** to *cis*-4-propylcyclohexanol **3a**. While use of *i*-PrOH (4.0 equiv) as the auxiliary substrate for recycling externally supplemented NAD^+ (0.001 equiv) made the *G. geotrichum* JCM 6359-catalyzed reduction catalytic with respect to the nicotinamide cofactor, the reduction failed to go to completion; however, ketone **10a** remaining unconsumed in 22% posed no serious problem since it could be removed in two practical ways: (1) formation of insoluble bisulfite adduct **11** or (2) simple distillation of the malonate-alkylation product arising from the 74:22 mixture of **3a** and **10a** via mesylation. In particular, the latter through processes paved a scalable, practical way for dimethyl *trans*-(4-propylcyclohexyl)malonate **7a**, which, on reduction with $NaBH_4$−LiCl, was converted to *trans*-2-(4-propylcyclohexyl)-1,3-propanediol **1a** in 30% overall yield from 4-propylcyclohexanone **10a**.

Indeed, the past two decades have seen remarkable progress in catalytic asymmetric reduction as demonstrated by Noyori's eventual triumph[14] over Baker's yeast-reduction.[15] However, there still remain process development issues to be addressed in the industrial setting, which include equatorial hydride delivery to 4-alkylcyclohexanone affording *cis*-4-alkylcyclohexanol, and it was biocatalysis that eventually succeeded in resolving this classical problem of stereochemistry in a practical manner as illustrated in this contribution when modern synthetic methodologies had long failed.[16]

Experimental Section

Melting points were measured on an Electrothermal 1A8104 melting point apparatus and are uncorrected. ^1H NMR spectra were recorded at 400 MHz on a Varian UNITY-400 spectrometer with tetramethylsilane as an internal standard in a solution of $CDCl_3$. FT-IR spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. Mass spectra were recorded with a Hewlett-Packard 5971 series mass selective detector connected to a Hewlett-Packard 5890 series gas chromatograph. Elemental analyses were performed on an Elementar vario EL analyzer. Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 plates (0.25 mm thick, art 1.05715). YM 5.5 medium was prepared by dissolving the following ingredients in tap water

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(15) Seebach, D.; Sutter, M. A.; Weber, R. H. *Org. Synth.* **1985**, *63*, 1.

(16) Zhu, Y.; Chuah, G.; Jaenicke, S. J. *J. Chem. Soc., Chem. Commun.* **2003**, 2734.

and adjusting the pH of the mixture to 5.5 with an aqueous 1 M HCl solution: glucose [1% (w/v)], peptone [0.5% (w/v), Polypepton, Nihon Pharmaceutical Co.], yeast extract [0.3% (w/v), dried, type S, Nihon Pharmaceutical Co.], and malt extract [0.3% (w/v), Bacto, Becton Dickinson Co.].

Microbial Screening. Bouillon or YM 5.5 medium (0.6 mL, autoclaved at 121 °C for 20 min) was dispensed to 96-deep-well plates (well volume, 2.0 mL), and each medium was inoculated with a glycerol stock culture (20 μL) of a microorganism. The cultures were incubated with shaking (1000 rpm) at 25 °C for 5 days. To each culture were added 0.1 M potassium phosphate buffer (pH 7.0, 250 μL), neat **10a** (Fluka, 2 μL, *d* 0.907, 7.2 nmol), NADH (5.1 mg, 7.2 nmol), and NADPH (6.0 mg, 7.2 nmol). The mixtures were shaken at 1000 rpm and 25 °C for 2 h. Each mixture was extracted with MTBE (1.0 mL), and an aliquot (1.0 μL) of the MTBE solution was analyzed for the progress of the reduction and the *cis*/*trans* selectivity by GLC: column, TC-WAX, 0.53 mm $\Phi \times$ 30 m, GL Science; injection temperature, 230 °C; column temperature, 150 °C; detection temperature, 230 °C; detection, FID; split ratio, 1:100; t_R 2.91 min for **10a**, 3.40 min for **3a** (*cis*-isomer), 3.62 min for **5a** (*trans*-isomer);[17] reference samples of **3a** and **5a** were prepared by reducing **10a** by the method of Nishimura et al.[10] and by the usual method using $LiAlH_4$,[18] respectively.

Optimization of the Microbial Reduction. Starter cultures were grown in bouillon or YM 5.5 medium (5.0 mL, autoclaved at 121 °C for 20 min) with shaking (160 rpm) at 30 °C overnight. The same medium (100 mL, autoclaved at 121 °C for 20 min) in a 500-mL baffled flask was inoculated with the starter culture (0.5 mL). The resulting cultures were grown at 30 °C with shaking (160 rpm) for 1−3 days, depending on the growth of each microbial strain. The cells were collected by centrifugation (11300*g* × 30 min) and suspended in H_2O (50 mL); the aqueous suspensions could be freeze-dried for storage if necessary. Microtubes of 2-mL capacity were each charged with the aqueous cell suspension (100 μL) and 0.1 M potassium phosphate buffer (pH 7.0, 100 μL). To the mixture were added a nicotinamide cofactor (NAD^+, NADH, $NADP^+$, or NADPH), an auxiliary substrate for recycling the nicotinamide cofactor, and **10a** such that each concentration was set to that specified in experimentation. The mixture was shaken at 25 °C, and the reaction was monitored at appropriate intervals by GLC for the conversion and the *cis*/*trans* selectivity according to the following procedures: From the reaction mixture was taken an aliquot (20 μL), and it was partitioned with MTBE (200 μL). A portion (1 μL) of the MTBE solution was injected to the gas chromatograph running under the above-mentioned conditions.

***cis*-4-Propylcyclohexanol 3a.** *Galactomyces geotrichum* JCM 6359 was grown in YM 5.5 medium (80 mL, autoclaved at 121 °C for 20 min) with shaking (160 rpm) at 30 °C for 2 days. The cells of *G. geotrichum* JCM 6359 were collected by centrifugation (11300*g* × 30 min) and suspended in tap water (80 mL). The cell suspension was added to a mixture of **10a** (4.0 g, 29 mmol), *i*-PrOH (3.8 g, 63 mmol), NAD^+ (17.3 mg, 26 μmol), and 40 mM potassium phosphate buffer (pH 7.5, 50 mL). The mixture was stirred at 25 °C for 9 h. *i*-PrOH (3.8 g, 63 mmol) was added, and the stirring was continued at 25 °C for 18 h. When the GLC analysis, the conditions of which are specified above, showed that the reduction reached 77% conversion, the mixture was extracted with MTBE (25 mL × 1). The MTBE extract was washed with H_2O (10 mL × 1). The aqueous washing was extracted with MTBE (20 mL × 1). The MTBE extracts were combined, dried ($MgSO_4$), and concentrated in vacuo to give an oily residue (3.87 g), which was analyzed by the GLC under the above-mentioned conditions for the composition: **10a**, 21.8%; **3a** (*cis*-isomer), 73.7%; **5a** (*trans*-isomer), 0.4%. To a portion (1.00 g) of this crude product was added a homogeneous mixture of $NaHSO_3$ (1.50 g), EtOH (1.60 mL), and H_2O (2.65 mL). The mixture was stirred at 25 °C for 2 h. To the heterogeneous mixture was added PhMe (10 mL) followed by Celite 545 (0.5 g). The mixture was filtered, and the filter cake was washed with PhMe (10 mL × 1). Layers of the filtrate were separated, and the PhMe layer was dried ($MgSO_4$). The PhMe solution was concentrated in vacuo to give **3a** (0.69 g, 68.5%). GLC analysis under the same conditions as mentioned above gave the following results: **10a** (0.4%), **3a** (*cis*-isomer, 99.2%), **5a** (*trans*-isomer, 0.2%); IR ν_{max} (KBr) 3356, 2926, 2858, 2667, 1713, 1456, 1443, 1371, 1335, 1254, 1144, 1034, 964, 729, 694 cm^{-1}; ^1H NMR δ 3.95 (1H, ddd, J = ca. 2.8 Hz), 1.69 (2H, m), 1.47−1.58 (5H, m), 1.27−1.38 (5H, m), 1.22 (2H, m), 0.89 (3H, t, J = 7.2 Hz); GC−MS (column, Ultra 1, 0.25 mm $\Phi \times$ 25 m, Hewlett-Packard; carrier gas, He, 35 KPa; injection temperature, 250 °C; column temperature, 150 °C; detection temperature, 280 °C; 70 eV) *m*/*z* 124 ($M^+ - H_2O$, 27), 109 (7), 98 (23), 95 (40), 82 (89), 81 (100), 67 (20), 57 (61), 43 (61).

***cis*-4-Propylcyclohexyl Mesylate 4a.** Under an atmosphere of N_2, neat MsCl (8.40 g, 73.8 mmol) was added dropwise to a stirred solution of crude **3a** (74%, contaminated with **10a** in 22%; 10.0 g, approximately 54 mmol for net **3a**) and pyridine (8.34 g, 105 mmol) in PhMe (15.0 mL) at 23 °C. The mixture was stirred and heated at 50 °C for 5 h. When complete consumption of **3a** was confirmed by TLC [$CHCl_3$, R_f 0.27 for **3a** and 0.57 for **4a**], PhMe (15 mL) and H_2O (30 mL) were added to the mixture, and the layers were separated. The PhMe layer was washed with 10% aqueous HCl solution (30 mL × 1), 10% aqueous $NaHCO_3$ solution (30 mL × 1), H_2O (30 mL × 1), and saturated aqueous NaCl solution (30 mL × 1). The PhMe solution was dried ($MgSO_4$) and concentrated in vacuo to give crude **4a** (14.6 g, contaminated with **10a** in 23% as estimated from integration of ^1H NMR signals): IR ν_{max} (KBr) 2930, 2862, 1446, 1352, 1175, 970, 854, 532 cm^{-1}; ^1H NMR δ 4.95 (1H, m), 3.00 (3H, s), 2.06−2.01 (2H, m), 1.67−1.50 (4H, m), 1.40−1.10 (7H, m), 0.89 (3H, t, J = 7.2 Hz). This was used in the next step without further purification.

Dimethyl *trans*-(4-Propylcyclohexyl)malonate 7a. Under an atmosphere of N_2, a methanolic solution of NaOMe

(17) For the application of GC−MS to determining the ratio of **3a** to **5a**, see ref 11a.

(18) Agami, C.; Kazakos, A.; Levisalles, J.; Sevin, A. *Tetrahedron* **1980**, *36*, 2977.

(28%; 41.6 g, 0.22 mol) was added dropwise to a stirred and heated solution of dimethyl malonate (30.0 g, 0.23 mol) in PhMe (90.0 mL) at 35−51 °C over a period of 45 min. The mixture was stirred and heated at 50 °C for 1.5 h. The mixture was cooled to 10 °C. Precipitated solids were collected by filtration, washed with PhMe (20 mL × 1), dried in vacuo at 15 mmHg at 25 °C for 5 h to give dimethyl sodiomalonate as a white powder (25.8 g, 73.6%). This was added in portions to a stirred solution of crude **4a** (77%, contaminated with **10a** in 23%; 14.5 g, approximately 54 mmol for net **4a**) in DMF (49.0 mL) at 23 °C under an atmosphere of N_2. The mixture was stirred with heating at 80 °C for 5 h. When complete consumption of **4a** was confirmed by TLC [AcOEt/*n*-hexane (1:4); R_f 0.38 for **4a** and 0.49 for **7a**], PhMe (50 mL) and H_2O (100 mL) were added to the mixture. The layers were separated, and the PhMe solution was washed with H_2O (50 mL × 1) and saturated aqueous NaCl solution (50 mL × 1). The PhMe solution was dried ($MgSO_4$) and concentrated in vacuo to give an oily residue (14.6 g), which was distilled in vacuo to give **7a** (7.21 g, 50.4% from **10a**) as a colorless oil: bp 125−150 °C/7 mmHg; IR ν_{max}(KBr) 2955, 2926, 2850, 1757, 1738, 1436, 1342, 1294, 1249, 1215, 1140, 1017 cm^{-1}; ^1H NMR δ 3.72 (6H, s), 3.16 (1H, d, J = 8.8 Hz), 2.45−1.90 (1H, m), 1.76−1.69 (3H, m), 1.56−1.42 (2H, m), 1.42−1.22 (2H, m), 1.17−1.14 (2H, m), 1.08−0.90 (4H, m), 0.87 (3H, t, J = 7.2 Hz).

***trans*-2-(4-Propylcyclohexyl)-1,3-propanediol 1a.** To a stirred solution of **7a** (7.00 g, 27.3 mmol) in THF (56.0 mL) was added $NaBH_4$ (1.86 g, 49.2 mmol) in portions at 25 °C. To the mixture was added an aqueous solution of LiCl (40%; 5.20 g, 49.2 mmol) with stirring over a period of 4 min. The mixture was stirred at 25 °C for 16 h. When complete consumption of **7a** was confirmed by TLC [AcOEt/*n*-hexane (1:1); R_f 0.91 for **7a** and 0.21 for **1a**], Me_2CO (2 mL) was added to destroy excess $NaBH_4$. To the mixture was added MTBE (22 mL) followed by H_2O (60 mL). The layers were separated, and the organic layer was washed with 10% aqueous HCl solution (50 mL × 1), 10% aqueous $NaHCO_3$ solution (50 mL × 1), H_2O (50 mL × 2), and saturated aqueous NaCl solution (50 mL × 1). The organic solution was dried ($MgSO_4$) and concentrated in vacuo to give a solid residue (5.05 g). This was dissolved in *n*-heptane (23 mL) with warming to 40 °C. The resulting solution was ice-cooled for 2 h. Precipitated solids were collected by filtration at 0 °C and air-dried at 25 °C overnight to give **1a** as a white powder (3.25 g, 59.4%): mp 99.0−100 °C; IR ν_{max}(KBr) 3340, 2955, 2908, 2845, 1435, 1379, 1026, 970, 669 cm^{-1}; ^1H NMR δ 3.87 (2H, dd, J = 4.0 Hz, 10.4 Hz), 3.81 (2H, dd, J = 7.6 Hz, 10.4 Hz), 2.05 (2H, br s), 1.79−1.70 (4H, m), 1.60−1.52 (1H, m), 1.42−1.24 (4H, m), 1.19−1.15 (3H, m), 1.08−0.96 (2H, m), 0.92−0.80 (4H, m); Anal. Calcd for $C_{12}H_{24}O_2 \cdot 0.1\ C_7H_{16}$ (*n*-heptane): C, 72.51; H, 12.29. Found: C, 72.5; H, 12.1.

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