methylene chloride and 4 mL of methanol was refluxed for 4 h. After vacuum removal of the volatile solvents the mixture was poured into water, neutralized with a saturated sodium bicarbonate solution, and extracted. Drying and evaporation of the latter, chromatography of the residue, and elution with 1:1 methylene chloride–ethyl acetate gave 65 mg (87%) of ketone **5b**: mp 285–290 °C dec (MeOH); IR (KBr) C=O 1730 (s), 1710 (s), C=C 1595 (m), SO_2 1355 (s), 1170 (s) cm^{-1}; ^1H NMR δ 2.30 (s, 3, Ts Me), 2.82 (d, 1, J = 19 Hz, H-16), 3.37 (s, 1, H-21), 3.62 (d, 1, J = 19 Hz, H-16), 3.78 (s, 3, OMe), 7.1–7.4 (m, 5, Ar Hs), 7.62 (d, 2, J = 8 Hz, Ts *o*-Hs), 7.90 (d, 1, J = 8 Hz, H-12); ^{13}C NMR δ 15.9 (C-14), 21.5 (Ts Me), 29.3 (C-19), 32.5 (C-16), 36.3 (C-15), 39.0 (C-6), 42.5 (C-18), 43.6 (C-20), 47.0 (C-3), 48.3 (C-5), 52.6 (OMe), 58.7 (C-7), 68.8 (C-21), 71.8 (C-2), 116.7 (C-12), 122.0 (C-10), 125.1 (C-9), 125.8 (Ts *o*-C), 128.3 (C-11), 129.7 (Ts *m*-C), 138.4 (C-13), 139.9 (C-8 or Ts *ipso*-C), 140.1 (Ts *ipso*-C or C-8), 143.7 (Ts *p*-C), 172.6 (CO_2), 210.1 (C-17); m/e 506 (M^+, 33), 420 (8), 351 (68), 293 (29), 265 (base), 91 (16); exact mass m/e 506.1854 (calcd for $C_{28}H_{30}O_5N_2S$ m/e 506.1875).

16,17-Didehydro-17-ethoxy-18(*R*)-(phenylsulfonyl)-1-(*p*-tolylsulfonyl)aspidofractinine (4b) and Its 18*S* Isomer (6). A solution of 280 mg (0.63 mmol) of diene **3** and 600 mg (3.6 mmol) of phenyl vinyl sulfone in 5 mL of dry toluene was stirred and refluxed under nitrogen for 48 h. It then was concentrated, and the residue was chromatographed twice on Merck alumina 90 (activity II–III). Elution with 4:1 ethyl acetate–cyclohexane yielded 56 mg (14%) of the colorless, crystalline adduct **6**: mp 258–260 °C (MeOH); IR C=C 1610 (s), SO_2 1350 (s), 1145 (s) cm^{-1}; ^1H NMR δ 1.29 (t, 3, J = 7.5 Hz, Me) 2.43 (s, 3, Ts Me), 3.4–3.6 (m, 1, OCH_2 H), 3.57 (s, 1, H-21), 3.6–3.9 (m, 1, OCH_2 H, H-18), 5.58 (s, 1, H-16), 7.1–7.6 (m, 8, Ar Hs), 7.71 (d, 2, J = 8 Hz, Ts *o*-Hs), 7.86 (d, 3, J = 8 Hz, Ar Hs); ^{13}C NMR δ 13.9 (Me), 16.0 (C-14), 21.4 (Ts Me), 29.9 (C-19), 37.1 (C-15), 37.4 (C-20), 42.2 (C-6), 46.7 (C-3), 47.9 (C-5), 62.2 (C-18), 63.6 (OCH_2), 65.7 (C-7), 66.3 (C-21), 76.8 (C-2), 95.6 (C-16), 116.3 (C-12), 161.5 (C-17); m/e 616 (M^+, 1), 475 (4), 461 (20), 448 (7), 320 (6), 293 (base), 125 (22), 91 (13). Anal. Calcd for $C_{34}H_{36}O_5N_2S_2$: C, 66.21; H, 5.88; N, 4.54. Found: C, 66.10; H, 5.96; N, 5.02.

Further elution led to 200 mg (52%) of colorless, crystalline adduct **4b**: mp 245–246 °C (MeOH); IR C=C 1612 (s), SO_2 1355 (s), 1145 (s) cm^{-1}; ^1H NMR δ 1.38 (t, 3, J = 7.5 Hz, Me), 1.64 (dd, 1, J = 13, 8 Hz, H-19), 1.87 (dd, 1, J = 13, 8 Hz, H-19), 2.31 (s, 3, Ts Me), 3.00 (s, 1, H-21), 3.58 (t, 1, J = 8 Hz, H-18), 3.7–3.9 (m, 1, OCH_2 H), 3.9–4.1 (m, 1, OCH_2 H), 5.92 (s, 1, H-16), 6.87 (d, 1, J = 8 Hz, Ar H), 7.0–7.2 (m, 5, Ar Hs), 7.4–7.7 (m, 5, Ar Hs), 7.82 (d, 2, J = 8 Hz, Ar Hs); ^{13}C NMR δ 14.0 (Me), 16.2 (C-14), 21.3 (Ts Me), 30.1 (C-19), 36.1 (C-15), 38.0 (C-20), 38.0 (C-6), 46.8 (C-3), 48.7 (C-5), 63.1 (C-18), 63.8 (OCH_2), 65.0 (C-7), 67.5 (C-21), 76.3 (C-2), 90.4 (C-16), 119.9 (C-12), 159.3 (C-17); m/e 616 (M^+, 3), 475 (5), 461 (base), 448 (26), 125 (13). Anal. Calcd for $C_{34}H_{36}O_5N_2S_2$: C, 66.21; H, 5.88; N, 4.54. Found: C, 65.98; H, 6.00; N, 5.08.

17-Oxo-18(*R*)-(phenylsulfonyl)-1-(*p*-tolylsulfonyl)aspidofractinine (5c). A solution of 220 mg (0.36 mmol) of enol ether **4b** and 10 mL of a 1 N hydrochloric acid solution in 30 mL of methanol was refluxed for 16 h. After vacuum removal of methanol, the mixture was poured into water, neutralized with a saturated sodium bicarbonate solution, and extracted. Drying of the extract, flash chromatography[10] of the residue, and elution with 49:1 methylene chloride–methanol gave 170 mg (81%) of the ketone **5c**: mp 257–259 °C (MeOH); IR C=O 1710 (s), C=C 1595 (m), SO_2 1360 (s), 1145 (s) cm^{-1}; ^1H NMR δ 2.31 (s, 3, Ts Me), 3.04 (dd, 1, J = 19, 2.5 Hz, H-16), 3.31 (s, 1, H-21), 4.20 (d, 1, J = 19 Hz, H-16), 6.74 (d, 1, J = 8 Hz, Ar H), 7.0–7.7 (m, 10, Ar Hs), 7.80 (d, 2, J = 8 Hz, Ts *o*-Hs); ^{13}C NMR δ 15.7 (C-14), 21.3 (Ts Me), 29.5 (C-19), 30.5 (C-16), 34.9 (C-15), 38.8 (C-6), 44.7 (C-20), 46.8 (C-3), 48.2 (C-5), 59.3 (C-18), 60.3 (C-7), 68.9 (C-21), 71.7 (C-2), 119.5 (C-12), 208.6 (C-17); m/e 588 (M^+, 12), 447 (7), 433 (46), 420 (12), 265 (base), 123 (35), 91 (38); exact mass m/e 588.1755 (calcd for $C_{32}H_{32}O_5N_2S_2$ m/e 588.1752).

17-Oxoaspidofractinine (5d). Hydrolysis of 50 mg of enol ether **6** under the above conditions and workup (5:1 ethyl acetate–cyclohexane elution) afforded 40 mg (84%) of colorless, amorphous ketone **7**: IR C=O 1715 (s), C=C 1600 (m), SO_2 1355 (s), 1150 (s) cm^{-1}; ^1H NMR δ 2.40 (s, 3, Ts Me), 2.05 (s, 1, H-21), 2.94 (d, 1, J = 19 Hz, H-16), 3.15 (d, 1, J = 19 Hz, H-16), 7.1–7.8 (m, 13, Ar Hs); m/e 588 (M^+, 17), 433 (32), 420 (31), 405 (11), 265 (base), 156 (15), 123 (12), 91 (18).

A mixture of 40 mg (0.067 mmol) of ketone **7**, 0.58 g of sodium monohydrogenphosphate, and 0.40 g of freshly prepared 6% sodium amalgam in 20 mL of methanol was stirred under nitrogen at room temperature for 12 h. It then was poured into water and filtered. Upon evaporation of the methanol of the filtrate the latter was extracted. The organic solution was washed with water, dried, and evaporated. Flash chromatography of the residue and elution with 19:1 methylene chloride–methanol gave 11 mg (55%) of colorless, amorphous ketone **5d**: IR NH 3330 (m), C=O 1705 (s), C=C 1605 (m) cm^{-1}; ^1H NMR δ 2.39 (d, 1, J = 19 Hz, H-16), 2.88 (dd, 1, J = 19, 3.5 Hz, H-16), 3.48 (s, 1, H-21), 6.72 (d, 1, J = 8 Hz, H-9), 7.08 (t, 1, J = 8 Hz, H-11), 6.85 (t, 1, J = 8 Hz, H-10), 7.30 (d, 1, J = 8 Hz, H-12); ^{13}C NMR δ 16.6 (C-14), 29.5 (C-15), 29.8 (C-19 or C-18), 30.0 (C-18 or C-19), 35.5 (C-16), 44.3 (C-20), 44.7 (C-6), 47.5 (C-3), 48.7 (C-5), 56.9 (C-7), 64.6 (C-21), 70.0 (C-2), 110.8 (C-12), 120.2 (C-10), 122.0 (C-9), 127.1 (C-11), 138.3 (C-8), 149.1 (C-13), 213.2 (C-17); m/e 294 (M^+, base), 266 (33), 251 (50), 238 (75), 144 (31), 143 (27), 130 (17), 123 (67), 109 (28), 95 (43); exact mass m/e 294.1734 (calcd for $C_{19}H_{22}ON_2$ m/e 294.1732).

Reduction of sulfone **5c** under the identical conditions led to a 70% yield of ketone **5d**.

1-Carbomethoxy-17-oxoaspidofractinine (5e). A solution of 0.25 mL of methyl chloroformate in 1 mL of anhydrous dioxane was added to a mixture of 19 mg (0.07 mmol) of ketone **5d** and 14 mg of sodium hydride (80% mineral oil dispersion) in 1 mL of dry dioxane. The mixture was stirred at 60 °C under nitrogen for 2 h. It then was poured into water and extracted. Evaporation of the extract, chromatography of the residue, and elution with 2:1 methylene chloride–ethyl acetate yielded 16 mg (70%) of colorless, amorphous ketone **5e**: UV λ_{max} 244 nm (log ϵ 4.19), 282 (3.56), 289 (3.54); IR C=O 1705 (s), C=C 1600 (w) cm^{-1}; ^1H NMR δ 3.0–3.3 (m, 3, H-3, H-16, H-5), 3.57 (s, 1, H-21), 3.87 (s, 3, OMe), 7.08 (t, 1, J = 7.5 Hz, H-10), 7.23 (t, 1, J = 7.5 Hz, H-11), 7.47 (d, 1, J = 7.5 Hz, H-9), 7.6–7.9 (m, 1, H-12); m/e 352 (M^+, base), 324 (4), 309 (30), 296 (2), 123 (8); exact mass m/e 352.1782 (calcd for $C_{21}H_{24}O_3N_2$ m/e 352.1787).

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Registry No. (±)-**1a**, 89240-74-4; (±)-**1b**, 120385-49-1; (±)-**1c**, 120385-50-4; (±)-**1d**, 120385-54-8; (±)-**2**, 120444-79-3; (±)-**3**, 120385-45-7; (±)-**4a**, 120385-46-8; (±)-**4b**, 120411-03-2; (±)-**5b**, 120385-47-9; (±)-**5c**, 120411-04-3; (±)-**5d**, 120385-51-5; (±)-**5e**, 120385-53-7; (±)-**6**, 120385-48-0; (±)-**7**, 120385-52-6; CH_2=$CHCO_2Me$, 96-33-3; CH_2=$CHSO_2C_6H_5$, 5535-48-8.

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Reverse Micelles, an Alternative to Aqueous Medium for Microbial Reactions: Yeast-Mediated Resolution of α-Amino Acids in Reverse Micelles†

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The use of enzymes and microbial cells in organic synthesis although fast increasing[1-3] is limited by the reactant solubility in aqueous medium. The problem of using organic solvents for these reactions can be occasionally solved by using a two-phase enzyme in aqueous substrate in water-immiscible organic solvent system[4,5] or more gen-

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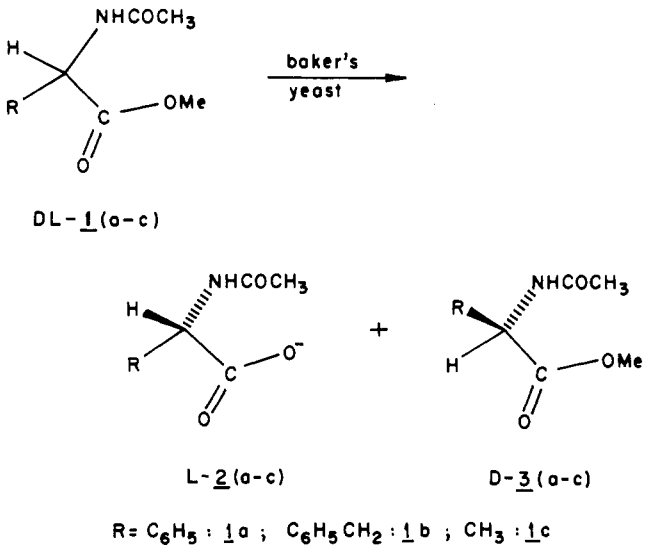
Scheme I



erally by using reverse micelles.[6-9] Although many studies have been made on enzymes solubilized in reverse micelles, very little is known about microbial cells in the medium. Luisi and co-workers have reported solubilization of bacterial cells[10,11] in reverse micelles. However, these studies are mainly concerned with the fundamental aspects of solubilization. Recently, Fadnavis and Luisi have developed a methodology of using gelentrapped enzymes in reverse micelles.[12] This approach combines the advantages of immobilized enzymes (enzyme stability, recovery, and recycle) and reverse micelles (improved solubility of hydrophobic substances). One could visualize a microbial cell as a system consisting of an enzyme immobilized by entrapment or attachment to the cell wall and, in principle, it should be possible to use the microbial cells in reverse micelles for a practical purpose. In this paper we demonstrate this by using bakers' yeast (*Saccharomyces cerevisiae*) as the microbe and enantioselective hydrolysis of methyl esters of racemic α-*N*-acetylamino acids as a model reaction (Scheme I).

Fermenting[13] and lyophilized yeast cells[14] have been used for asymmetric hydrolysis of racemic esters. For our experiments we have used yeast cells cross-linked with glutaraldehyde[15] to minimize the possibility of protein (enzyme) extraction by the reverse micelles[16] and to simplify the experimental procedure. Also, messy emulsions that appear during workup of aqueous reactions using free cells are avoided.

Table I. Enantioselective Hydrolysis of Methyl Esters of Racemic *N*-Acetyl-α-amino Acids by Bakers' Yeast in Aqueous and Reverse Micellar Suspensions[a]

		time of reactn, h		isoltd yield, %		ee, %	
substr	prod	aq	rev mic	aq	rev mic	aq	rev mic
1a	2a	72	48	64	60	96[b]	98[b]
	3a			60	46	>99[c]	>99[c]
1b	2b	48	24	57	53	98[b]	98[b]
	3b			55	48	>99[c]	>99[c]
1c	2c	60	32	72	65	97[b]	96[b]
	3c			52	40	>99[c]	>99[c]

[a] Reactions were carried out at room temperature (~30 °C) using substrate (5 mmol) in solvent (100 mL) and yeast cells (5 g, wet). [b] Determined by comparison of the optical rotation value with authentic samples and literature data. [c] Determined by ^{1}H NMR spectroscopy using [Eu(tfc)$_3$].

A priori, it was observed that in aqueous suspension the cross-linked cells catalyze the enantioselective hydrolysis of **1**, yielding optically pure **2** and **3** (Table I). No reaction was observed when a water-immisicible solvent like chloroform or ethyl acetate was added. Apparently in such a system the substrate is preferentially partitioned in the organic phase and is not available for the reaction.

First experiments with a suspension of yeast cells in a reverse micellar system consisting of 50 mM hexadecyltrimethylammonium bromide (CTAB) in 1:1 (v/v) isooctane–chloroform and 2% aqueous buffer (0.1 M phosphate, pH 8.2) were encouraging but not satisfactory. The reactions did not go to completion (ee <60%) due to reduction of pH of the medium by liberated acid. To overcome this problem reactions were carried out in reverse micelles of bis(2-ethylhexyl) sulfosuccinate, sodium salt in chloroform–isooctane (1:9, v/v). Acid **2** liberated due to yeast-mediated hydrolysis was periodically removed from the micellar medium by decanting the micellar solution and gently stirring with aqueous buffer (0.2 M phosphate, 1 M KCl; pH 8.2). The acid was thus extracted in the aqueous phase. The micellar solution containing the unreacted ester was returned to yeast cells. The results were now highly satisfactory. Unreacted D ester **3** and the L acid **2** were present in two different media and were isolated at the end of reaction (monitored by HPLC). The enantioselectivity of the reaction is as high as in water, yielding products with ee 98 ± 2% (determined by ^{1}H NMR using [Eu(tfc)$_3$] for the esters and measuring optical rotations and comparing with literature values[17-22] for the acids). The chemical yield of the isolated products is 40 to 60%. D esters isolated after the reaction were again treated with fresh yeast cells and the hydrolysis reaction was allowed to proceed for 12 h both in water and reverse micellar suspension. No hydrolysis reaction could be detected for the D esters, emphasizing the enantioselectivity of the yeast cells. Self hydrolysis of the esters was less than 1 μmol/h, i.e., 1.5% in 72 h in aqueous buffer, which is

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even more suppressed in reverse micelles.

Preliminary experiments indicate that the hydrolysis proceeds more efficiently in reverse micelles than in water (see Table I). This is more apparent when the substrate is not completely dissolved in water, e.g., when the reaction was carried out by using **1a** (5 g) in aqueous buffer (200 mL) and reverse micelles of bis(2-ethylhexyl) sulfosuccinate, sodium salt (200 mL) using yeast cells (20 g, wet) in each solvent system; after 72 h the unreacted ester in aqueous medium showed an ee of only 30% while that in reverse micelles was 75%. This reflects the limitation of substrate solubility in aqueous medium. The use of reverse micelles eliminates this solubility limitation. Also the product can be continuously removed from the reaction medium, minimizing the product inhibition, a clear advantage over the aqueous-phase reaction.

Our experiments thus demonstrate that it is indeed possible to use microbial cells in reverse micelles and with a proper choice of surfactant system and experimental setup, bench-scale reactions can be easily carried out. Further studies are in progress to determine general applications of this novel methodology.

Experimental Section

All solvents were analytical grade obtained from Spectrochem, India, and were used as received. Tris[3-[(trifluoromethyl)-hydroxymethylene]-*d*-camphorato]europium(III), [Eu(tfc)$_3$], was obtained from Aldrich. Amino acids and other reagents were obtained from Loba Chemie, India. Hexadecyltrimethylammonium bromide (CTAB) was first extracted with ethyl acetate in a Soxhlet extractor for 24 h and then recrystallized from methanol–water (4:1). Bis(2-ethylhexyl) sulfosuccinate, sodium salt was purified by Menger's method[23] and lyophilized. *N*-Acetylamino acids and their methyl esters were prepared by a standard method.[17] Optical rotations were measured on a Jasco DIP-360 polarimeter. HPLC analyses were performed on a Du Pont Zorbax C_8 reversed phase column (46 mm × 15 cm) using acetonitrile–water as eluent (UV detection at 254 nm). ^{1}H NMR spectra were recorded on a Bruker 300-MHz NMR spectrometer. Melting points were recorded on a Mettler FP5 melting point apparatus.

Preparation of Cross-Linked Yeast. Yeast cells (*S. cerevisiae* NCIM 3044) were grown in a culture medium consisting of peptone (1%), yeast extract (0.5%), sodium chloride (0.1%), glucose (2%), and magnesium sulfate (0.05%). After 72 h of growth the cells were collected, washed with distilled water, and then stirred with a 10% glucose solution for 2 h. The cells were then centrifuged, washed with buffer solution (0.1 M phosphate, pH 6.8), and resuspended in same buffer (10 g wet cells in 100 mL). Glutaraldehyde was added to make a 5% aldehyde solution, the suspension was shaken for 4 h at room temperature and centrifuged, and the cells were washed several times with phosphate buffer to remove excess glutaraldehyde. The pellet was stored at −20 °C.

Enantioselective Hydrolysis by Bakers' Yeast. The resolution of DL-α-(acetylamino)benzeneacetic acid, methyl ester (**1a**) is described below as a typical example.

Hydrolysis in Water. Cross-linked yeast cells (5 g, wet) were suspended in phosphate buffer (0.2 M, 1 M KCl, pH 8.2, 100 mL). The substrate ester (1.03 g, 5 mmol) in dimethyl sulfoxide (1 mL) was added with stirring. The suspension was then stirred at room temperature (~30 °C). The pH of the solution was intermitently adjusted to 8.2 with 1 N NaOH. The reaction was monitored by reversed phase HPLC using acetonitrile–water as the eluent. At the end of reaction there was no change in the peak heights of the ester and the acid (66 h). The reaction was continued for a further 6 h. The suspension was then filtered through a sintered glass funnel. The yeast cells were stirred with methanol and filtered (2 × 25 mL). The filtrate was evaporated, and the residue was mixed with the aqueous filtrate and extracted with dichloromethane (3 × 50 mL). The organic layer was dried and evaporated. The unreacted ester thus obtained was chromatographed over a silica gel column using a chloroform–methanol mixture (9:1) as the eluent (R_f = 0.68); yield 300 mg, 60% of theoretical yield. The *N*-acetyl-L-amino acid present in the aqueous portion was collected by acidifying to pH 1 with HCl, lyophilizing, and extracting the residue with acetone in a Soxhlet extractor. The acetone extract yielded the L acid, 320 mg, 64% yield.

Enantioselective Hydrolysis in Bis(2-ethylhexyl) Sulfosuccinate, Sodium Salt Reverse Micelles. A solution of bis(2-ethylhexyl) sulfosuccinate, sodium salt in isooctane–chloroform (9:1, v/v; 56 mM, 200 mL) was gently stirred with phosphate buffer (0.2 M, 1 M KCl, pH 8.2; 50 mL) for 1 h, taking care that the two layers did not mix. Any water-soluble impurity in the surfactant was thus removed. The aqueous layer was discarded and the surfactant solution was reequilibrated with fresh buffer for 30 min. Aqueous and organic (reverse micellar) layers were separated. Yeast cells (5 g, wet) were suspended in reequilibrated reverse micellar solution (25 mL) and stirred for 2 min. Cells were allowed to settle down and the turbid supernatant was discarded. The cells were stirred with fresh solution (25 mL). This was repeated until the suspension was clear (two to three times). The yeast cells were transferred to a flask containing the reverse micellar solution (90 mL) and the substrate ester (1.03 g, 5 mmol) in 1 mL of chloroform was added to the suspension with stirring. Isooctane (9 mL) was added and the suspension was stirred at room temperature. At intervals of 1 h stirring was stopped, yeast cells were allowed to settle down, and the supernatant was transferred to the flask containing aqueous buffer (50 mL). The aqueous layer was gently stirred for 10 min to extract *N*-acetyl-L-amino acid. The organic layer was transferred back to yeast cells. The reaction was monitored by following the appearance of acid in the aqueous layer by HPLC (42 h). The reaction was allowed to proceed for 6 h more. The acid was then collected from the aqueous extract as described earlier, 300 mg, 60% yield. Unreacted D ester was recovered by filtering the organic layer, evaporating the solvent, and chromatographing the residue on a silica gel column using chloroform–methanol (9:1) as the eluent, 230 mg, 46%. After elution of the ester the surfactant was eluted with 95% ethanol. On removal of ethanol and drying, it could be reused.

DL-α-(Acetylamino)benzeneacetic Acid, Methyl Ester (1a). This was prepared from DL-α-aminobenzeneacetic acid by acetylation with acetic anhydride followed by esterification with methanol/hydrochloric acid;[17] mp 76.8 °C (lit.[18] mp 76–77 °C): ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 7.35 (s, 5 H, Ar), 6.48 (br s, 1 H, NH), 5.59 (d, 1 H, CH, J = 7.3 Hz), 3.73 (s, 3 H, OCH_3), 2.04 (s, 3 H, $COCH_3$).

L-α-(Acetylamino)benzeneacetic Acid (2a). This was obtained by reaction of **1a** (1.03 g, 5 mmol) with yeast cells (5 g, wet). Isolated yield of crude after acetone extraction 330 mg for reaction in water, 64%, and 310 mg for reaction in reverse micelles, 60%: mp 189–190 °C (lit.[17] mp 194–195 °C); $[\alpha]^{25}_D$ +189° (c = 1, 1 N NaOH) (lit.[17] $[\alpha]^{25}_D$ +191°); ^{1}H NMR (300 MHz, $CDCl_3$, DMSO-d_6, Me_4Si) δ 9.81 (br s, 1 H, COOH), 7.43 to 7.29 (m, 5 H, Ar), 5.50 (d, 1 H, CH, J = 7.3 Hz), 2.03 (s, 3 H, $COCH_3$).

D-α-(Acetylamino)benzeneacetic Acid, Methyl Ester (3a). Isolated yield of crude 311 mg, 60%, for reaction in water, and 235 mg, 46%, for reaction in reverse micelles: mp 114.7 °C (lit.[18] mp 116–117 °C); $[\alpha]^{25}_D$ −174° (c = 1, ethanol) (lit.[18] $[\alpha]^{25}_D$ −176.2°); ee >99% as determined by ^{1}H NMR using [Eu(tfc)$_3$]; TLC, chloroform–methanol (9:1) R_f = 0.68.

***N*-Acetyl-DL-phenylalanine methyl ester (1b)** was prepared from DL-phenylalanine as described for **1a**: mp 63.2 °C (lit.[19] mp 62–63 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.08 (m, 5 H, Ar), 5.92 (br s, 1 H, NH), 4.89 (dt, 1 H, CH, J = 6.0 and 2.0 Hz), 3.73 (s, 3 H, OCH_3), 3.15 (d, 2 H, CH_2, J = 6.0 Hz), 1.99 (s, 3 H, $COCH_3$).

***N*-Acetyl-L-phenylalanine (2b)** was obtained by reaction of **1b** (1.1 g, 5 mmol) with yeast cells (5 g, wet). Isolated yield of crude, 314 mg, 57%, for reaction in water and 290 mg, 53%, for reaction in reverse micelles: mp 168 °C (lit.[19,20] mp 171–172 °C); $[\alpha]^{25}_D$ +51.2° (c = 1, ethanol) (lit.[19,20] $[\alpha]^{25}_D$ +50.4°); ^{1}H NMR (300 MHz, $CDCl_3$, DMSO-d_6) δ 9.73 (br s, 1 H, COOH), 7.35–7.10 (m, 5 H, Ar), 6.89 (br s, 1 H, NH), 4.27 (m, 1 H, CH, J = 7.5 Hz), 3.11 (d, 2 H, CH_2, J = 6.0 Hz), 1.90 (s, 3 H, $COCH_3$).

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N-Acetyl-D-phenylalanine Methyl Ester (3b). Isolated yield of crude, 302 mg, 55%, for reaction in water and 264 mg, 48%, for reaction in reverse micelles: mp 88.2 °C (lit.[20] mp 89.5–90 °C); $[\alpha]^{25}_D$ −19.3° (c = 1, methanol) (lit.[20] $[\alpha]^{25}_D$ −19°); ee >99% as determined by ^1H NMR using [Eu(tfc)$_3$]; TLC, chloroform–methanol (9:1), R_f = 0.66.

N-Acetyl-DL-alanine methyl ester (1c) was prepared from DL-alanine as described for **1a**: bp 76 °C (0.2 mm); ^1H NMR (300 MHz $CDCl_3$) δ 6.11 (br s, 1 H, NH), 4.58–4.65 (q, 1 H, CH, J = 7.27 Hz), 3.76 (s, 3 H, OCH_3), 2.02 (s, 3 H, $COCH_3$), 1.41 (d, 3 H, CH_3 J = 7.2 Hz). Anal. Calcd for $C_6H_{11}NO_3$: C, 49.65; H, 7.59; N, 9.65. Found: C, 49.62; H, 7.67; N, 9.56.

N-Acetyl-L-alanine (2c) was obtained by reaction of **1c** (1.45 g, 10 mmol) with yeast cells (10 g, wet) in 100 mL of solvent. Isolated yield of crude, 522 mg, 72%, for reaction in water and 470 mg, 65%, for reaction in reverse micelles: mp 122–123 °C (lit.[21] mp 115–128 °C); $[\alpha]^{25}_D$ −57.3° (c = 1, water) (lit.[21] $[\alpha]^{25}_D$ −59.7°); ^1H NMR (300 MHz, $CDCl_3$, DMSO-d_6) δ 7.16 (br s, 1 H, NH), 4.36–4.55 (q, 1 H, CH, J = 7.2 Hz), 1.98 (s, 3 H, $COCH_3$), 1.39 (d, 3 H, CH_3 J = 7.2 Hz).

N-Acetyl-D-alanine Methyl Ester (3c). Isolated yield of crude, 378 mg, 52%, for reaction in water and 290 mg, 40%, for reaction in reverse micelles: $[\alpha]^{25}_D$ +90.1° (c = 1, water) (lit.[22] $[\alpha]^{25}_D$ +89.4°); ee >99% as determined by ^1H NMR using [Eu(tfc)$_3$]; TLC, chloroform–methanol (9:1) R_f = 0.55.

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Registry No. **1a**, 36061-00-4; **1b**, 62436-70-8; **1c**, 26629-33-4; **2a**, 42429-20-9; **2b**, 2018-61-3; **2c**, 97-69-8; **3a**, 36060-85-2; **3b**, 21156-62-7; **3c**, 19914-36-4; bis(2-ethylhexyl) sulfosuccinate sodium salt, 577-11-7.

Synthesis of the Pheromone Components of the Grape Borer *Xylotrechus pyrrhoderus* by Microbiological Reduction of an α-Diketone†

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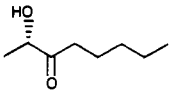
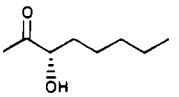
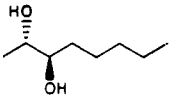
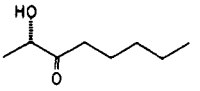
In a continuation of our investigation on the preparation of chiral hydroxy synthons by microbial reduction of the corresponding mono- or diketones,[1-3] we have carried out a study on the reduction of α-diketones. Enzymatic or microbiological reductions of α-diketones have already been studied, mostly on substituted benzils or benzoins[4-7] and α,β-diketodithianes,[8] and α-hydroxy ketones have been reduced in the same manner to obtain optically active α-diols,[9-11] but to our knowledge, no systematic investigation on the microbiological reduction of acyclic α-diones has been published. We started such a study on various acyclic α-diones. Resting cells able to reduce these compounds were screened from our stock cultures of bacteria, yeasts, and fungi, and none of them failed. Either or both carbonyl groups can be reduced, and thus various isomers and stereoisomers of α-hydroxy ketones and α-diols were obtained, as will be described elsewhere.

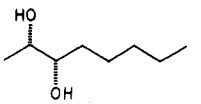
This paper reports on the microbiological reduction of one particular α-diketone, octane-2,3-dione, that enabled us to obtain the two components of a natural pheromone in an efficient two-step chemoenzymatic synthesis. The male sex pheromone of an important pest of Japanese vineyards, the grape borer *Xylotrechus pyrrhoderus*, has been identified as a mixture of (+)-(2S)-2-hydroxyoctan-3-one (**1**) and (−)-(2S,3S)-octane-2,3-diol (**2**) in a ratio of 20:80 to 5:95.[12]

Table I. Microbiological Reduction of Octane-2,3-dione (3) by Bakers' Yeast

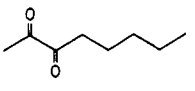
reduced compd	conversn yield,[a] %	absolute confign	$[\alpha]^{25}_J$, deg	ee, %
 **1**	71	2S	+61	92
 **4**	22			
 **5**	7	2S,3R	+19	99

[a] After 1 h of incubation time, as shown by GC analysis.


1


2

A number of methods have been reported for the synthesis of these two compounds, leading to either racemic[13] or chiral molecules.[14] Optically active α-ketol **1** and diol **2** have been obtained by Mori et al.[14b] by chemical methods from 1-octen-3-ol after seven and six steps, respectively. Our chemoenzymatic synthetic route, the microbiological reduction of octane-2,3-dione (**3**), is much shorter, and the starting α-diketone is easily obtained from 2-octanone by hydrolysis of the corresponding oxime.[15]


3

Hydroxy ketone **1**, the minor component of the pheromone, is formed in all cases but rapidly transformed into

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