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BIOTRANSFORMATION OF ACTIVE METHYLENE COMPOUNDS BY *Saccharomyces cerevisiae*

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The biotransformation of several activated CH-acid compounds was examined using Saccharomyces cerevisiae *as a biocatalyst. Alkylation–hydroxylation of 1,3-dicarbonyl compounds at the α-position was achieved. Investigation of the reaction of these compounds was extended to external aldehydes subjected to the action of this biocatalyst. Aromatic aldehydes afforded condensation products, but aliphatic aldehydes were not involved in the reaction. The structures of the products were characterized by IR, EI-MS, ^{1}H NMR, ^{13}C NMR, and elemental analysis. Finally, the structure of compound* ***2a*** *was confirmed unambiguously by single-crystal X-ray analysis.*

Keywords: biotransformation, *Saccharomyces cerevisiae*, dicarbonyl compounds, knoevenagel, alkylation, condensation.

Recently, the use of enzymatic or microbial systems in particular yeast-mediated transformations in organic synthesis has gained much attention as a tool for the preparation of optically active compounds [1–4]. The asymmetric reduction catalyzed by *Saccharomyces cerevisiae* (baker′s yeast), known to be relative simple and inexpensive and using a very easy to handle catalyst, is one of the most practical methods for establishing chiral centers in organic compounds [5, 6]. Because of the advantages mentioned above, this biocatalyst has been employed in enantioselective reduction of various prochiral compounds with good chemical yield [7–9]. In addition, the reduction of cyclic β-dicarbonyl compounds with baker′s yeast has been investigated. This biocatalyst has been found to be an active microorganism in the reduction of these compounds with substituents at the α-position to ketols with excellent enantiomeric excesses [10, 11]. Many other different organic reactions have been successfully carried out in the presence of this biocatalyst, such as acyloin type condensations [12, 13], C–C bond formation [14], stereospecific reduction of activated carbon–carbon double bonds [15], oxidative coupling of thiols to disulfides [16], bioreduction of PEG–acetoacetate [17], and the Hantzsch reaction [18, 19].

With regards to the ability of baker′s yeast to reduce carbonyl and carbon–carbon double bonds, the subject of this study was the Knoevenagel condensation of 1,3-cyclic dicarbonyl compounds with acetaldehyde (formed by baker′s yeast). Then the enantioselective reduction of the carbon–carbon double bond and carbonyl group resulting in α,β-unsaturated carbonyl compounds was also expected of this microorganism. Therefore, 5,5-dimethylcyclohexane-1,3-dione (dimedone, **1a**) was added to dry baker′s yeast, but the obtained result was different from previously reported results in the literature [10, 11]. In fact, the hydroxylation of α,β-unsaturated compounds resulting from Knoevenagel condensation (**2a**) was achived by baker′s yeast, and the reduced product (**3a**) was not detected at all (Scheme 1).

As shown in Scheme 1, dimedone (**1a**) was transformed to 2-ethyl-2-hydroxy-5,5-dimethylcyclohexane-1,3-dione (**2a**) by fermenting yeast in water and in the presence of DMSO as co-solvent. The ^{1}H NMR spectrum of **2a** consisted of two singlets for $C(CH_3)_2$ (δ 0.81 and 1.24 ppm), a quartet for the methylene group of CH_2CH_3 (δ 1.97 ppm, $^{3}J_{HH}$ = 7.34 Hz), a triplet for the methyl of CH_2CH_3 at 0.90 ($^{3}J_{HH}$ = 7.34 Hz), and two doublets for methylene protons (δ 2.47 and 2.89 ppm, $^{2}J_{HH}$ = 14 Hz, H-4, 6). The ^{13}C NMR spectrum of **2a** showed eight distinct resonances, in agreement with the proposed structure.

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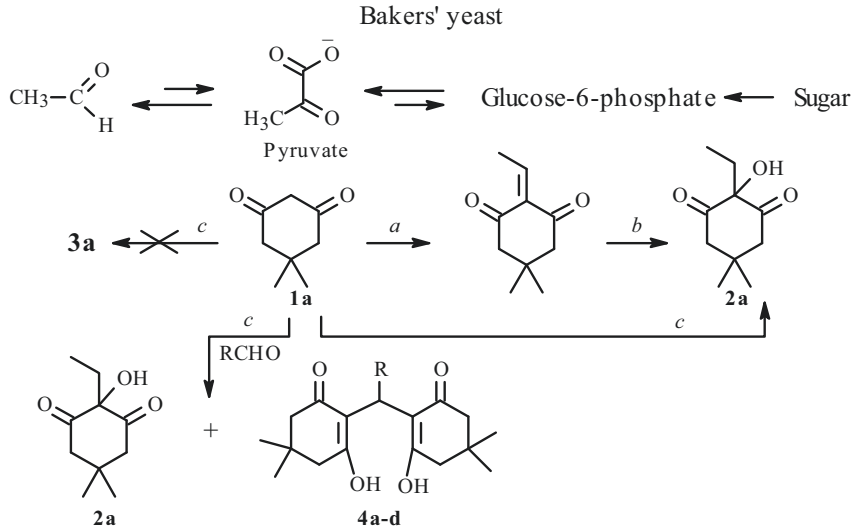


R = 4-Cl-C_6H_4 (**1**); 4-F-C_6H_4 (**2**); C_6H_5 (**3**); 4-NO_2-C_6H_4 (**4**); CH_3CH_2 (**5**); $CH_3CH_2CH_2$ (**6**)

a. acetaldehyde, 30–35°C, 24 h; *b*. hydroxylation; *c*. bakers' yeast, 30–35°C, 24 h

Scheme 1

The assignment of these resonances is given in the experimental section. The mass spectrum of this compound displayed a molecular ion peak at the appropriate *m/z* value. Finally, the structure of the product **2a** was confirmed by single-crystal X-ray analysis (Fig. 1).

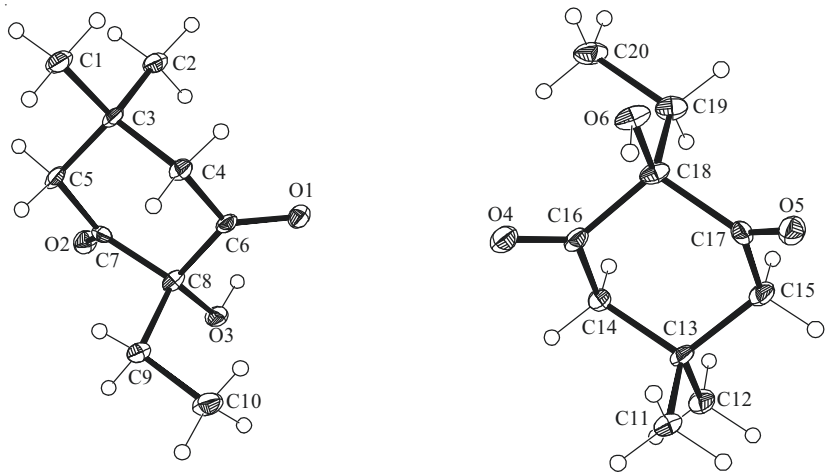
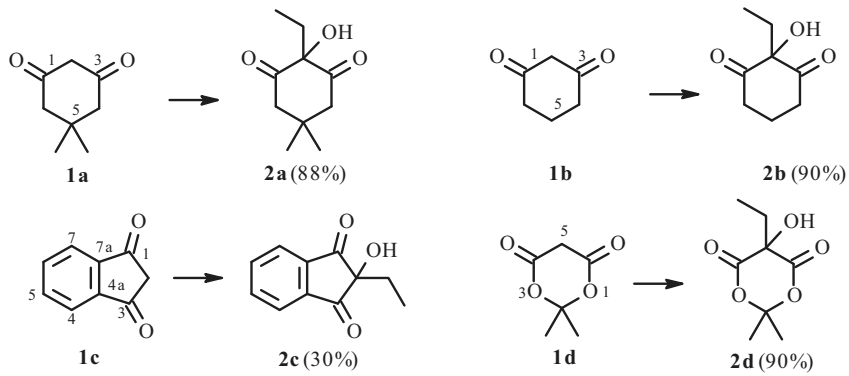


Fig. 1. ORTEP diagram of asymmetric unit of **2a**. Thermal ellipsoids are at 30% probability level.

The reaction was carried out in an aqueous solution containing carbohydrate with shaking. This biocatalyst is able to metabolize the common sugars, such as D-glucose, D-mannose, sucrose, and maltose. The metabolism of the carbohydrate is accompanied by the production of carbon dioxide, acetaldehyde, ethanol, and other smelly by-products. Although the mechanism of this reaction has not been established experimentally, the formation of these compounds can be rationalized by initial Knoevenagel condensation of the acetaldehyde (generated *in situ* from fermenting baker′s yeast) with dicarbonyl compounds, which leads to the formation of α,β-unsaturated compounds [18, 20]. The resulting α,β-unsaturated derivatives seemed to be subject to further transformation, and subsequent hydroxylation at their α-position resulted in alkylated–hydroxylated products (Scheme 2).



Scheme 2

To explore the scope and limitations of this reaction and create a library, studies were extended to other active methylene compounds. As indicated in Scheme 1, the reactions proceeded efficiently in relative good yields with substrates **1a** to **1d**, and all the reactions produced the corresponding alkylated–hydroxylated products. However, dihydro-1,3-dimethylpyrimidine-4,6(1*H*,5*H*)-dione, dihydropyrimidine-4,6(1*H*,5*H*)-dione, and 1,3-linear dicarbonyl compounds showed no reaction.

Introduction of Different Alkyl and Aryl Groups. In order to study the effect of external aldehydes on the above-mentioned reaction, the biotransformation of **1a** in the presence of various aldehydes such as propionaldehyde, butyraldehyde, and aromatic aldehydes was carried out. When active aryl aldehydes were added to yeast under fermentation conditions, only trace amounts of 2-ethyl-2-hydroxy-5,5-dimethylcyclohexane-1,3-dione (**2a**) was obtained under the mentioned reaction conditions, and instead dimedone–aldehyde derivatives (**4a–4d**) were obtained as major products in excellent yields. In the presence of aliphatic aldehydes such as propionaldehyde and butyraldehyde, 2-ethyl-2-hydroxy-5,5-dimethylcyclohexane-1,3-dione (**2a**) was obtained in low yield. In fact, in the presence of aliphatic aldehydes, which are typically poor substrates, none of the dimedone–aldehyde derivatives were obtained. Instead the acetaldehyde generated *in situ* by fermenting baker′s yeast was condensed with dimedone, and alkylated–hydroxylated product (**2a**) was obtained in low yield (Scheme 1).

Next, the alkylated–hydroxylated product (**2a**) was examined for reduction of the carbonyl group by fermenting yeast prepared under the above described condition, but no reduction was observed.

In order to study the catalytic efficiency of baker′s yeast, a control reaction was carried out. In the absence of catalyst, no product was detected in the blank experiment. The control experiment clearly indicated that the catalytic effect of baker′s yeast was responsible for alkylation–hydroxylation of active methylene compounds.

Since baker's yeast is a versatile and inexpensive catalyst, the transformations of various classes of organic molecules was examined using it by organic synthetic chemists. We have developed an efficient, inexpensive, nontoxic and environmentally friendly method for the conversion of the active methylene compounds **1a–d** into **2a–d** by baker′s yeast.

EXPERIMENTAL

Chemicals and Instruments. All melting points were measured on an Electrothermal 9100 apparatus and are uncorrected. Direct electron ionization mass spectrum (EI-MS) was recorded on an Agilent 5973 at 70 eV. IR spectra were recorded on a Shimadzu IR-470 spectrometer. ^{1}H and ^{13}C NMR spectra were run on a Bruker AVANCE 300 (Bruker Biospin, Rheinstetten, Germany) spectrometer at 300.13 and 75.47 MHz, respectively. Preparative TLC was performed on silica gel 60 mesh GF_{254} plates (20 × 20 cm), and observation of plates was carried out under UV. The chemicals used were purchased from Merck and Fluka. Organic extracts were dried over anhydrous sodium sulfate. Baker′s yeast was obtained from a local store. Elemental analyses were performed using a Heracus CHN-O-Rapid analyzer.

Elemental analyses of all compounds agreed with those calculated.

General Procedure for Biotransformation of 1a–d. One gramm of dry yeast was added to a solution of 4.6 g of sucrose and 0.030 g of disodium hydrogen phosphate in 20.0 mL of water, (35°C). The mixture was shaken at 35–40°C for 30 min (CO_2 was evolved). Substrate (1 mmol) in 1 mL DMSO was added slowly to the fermenting solution. The mixture was shaken in an orbital shaker at 30°C and 150 rpm for 24 h and then extracted with chloroform.

Typical Procedure for Preparation of Products 4a–4d. Dimedone (0.140 g, 1 mmol) and aldehyde (1 mmol) were dissolved in 1 mL DMSO and added to the fermenting yeast prepared under the above described condition. The reaction mixture was shaken at 30°C and 150 rpm for 24 h.

Purification and Structural Analysis of Products. The reaction mixture was extracted with chloroform (three times). The organic layer was dried and concentrated under vacuum to give a crude product. The residue was purified by preparative thin-layer chromatography on silica gel using *n*-hexane–ethyl acetate as the solvent.

2-Ethyl-2-hydroxy-5,5-dimethylcyclohexane-1,3-dione (2a). $C_{10}H_{16}O_3$. Colorless needle crystals, yield 0.123 g (88%), mp 90–93°C. MS (*m/z*, %): 184 (M^+, 5%), 168 (44), 167 (22), 129 (40), 128 (34), 83 (100), 57 (56). IR (KBr, ν, cm^{-1}): 3458, 2964, 2928, 1727, 1691. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.82 (1H, br.s, OH), 2.89 (2H, d, J = 14, H-4, 6), 2.47 (2H, d, J = 14, H-4, 6), 1.97 (2H, q, J = 7.34, $\underline{CH_2}CH_3$), 1.24 (3H, s, CH_3), 0.90 (3H, t, J = 7.34, $CH_2\underline{CH_3}$), 0.81 (3H, s, CH_3). ^{13}C NMR (75.47 MHz, $CDCl_3$, δ, ppm): 206.12 (C-1, 3), 89.42 (C-2), 50.89 (C-4, 6), 33.06 ($\underline{CH_2}CH_3$), 31.47 (C-5), 30.56 (CH_3), 26.28 (CH_3), 7.32 ($CH_2\underline{CH_3}$). X-Ray data: $C_{10}H_{16}O_3$, M = 184.23, monoclinic system, space group $P2_1/c$; a = 11.965 (2), b = 10.202 (2), c = 16.722 (3) Å, β = 103.56 (3)°, V = 1984.3 (7) $Å^3$; Z = 8; D_{calc} = 1.233 g·cm^{-3}; μ (Mo–Kα) = 0.090 mm^{-1}; T = 120 (2) K; crystal size $0.37 \times 0.35 \times 0.2$ mm^3. The X-ray diffraction measurement was made on a STOE IPDS-2T diffractometer with graphite monochromated Mo-Kα radiation. The structure was solved using SHELXS. The structure refinement and data reduction were carried out with SHELXL using the X-STEP32 suite of programs [21]. The non-hydrogen atoms were refined anisotropically by full matrix least-squares on F^2 values to final R_1 = 0.0959, wR_2 = 0.2261, and S = 1.042 with 249 parameters using 3491 independent reflections. Hydrogen atoms attached to oxygen atoms were located in a difference Fourier map and refined isotropically. All other hydrogen atoms were added in idealized positions. The crystallographic information file has been deposited with the Cambridge Data Centre, CCDC 920547.

2-Ethyl-2-hydroxycyclohexane-1,3-dione (2b). $C_8H_{12}O_3$. Light yellow crystals, yield 0.14 g (90%), mp 158–162°C. MS (*m/z*, %): 156 (M^+, 5%), 115 (29), 100 (39), 57 (100). IR (KBr, ν, cm^{-1}): 3428, 2968, 2927, 2875, 1710, 1643, 1570. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.28 (1H, br.s, OH), 2.36 (4H, br.t, H-4, 6), 2.21 (2H, q, J = 7.34, $\underline{CH_2}CH_3$), 1.89 (2H, quintet, J = 6.34, H-5), 0.86 (3H, t, J = 7.34, $CH_2\underline{CH_3}$). ^{13}C NMR (75.47 MHz, $CDCl_3$, δ, ppm): 207.87 (C-1, 3), 91.00 (C-2), 32.34 (C-4, 6), 20.60 ($\underline{CH_2}CH_3$), 14.51 (C-5), 12.16 ($CH_2\underline{CH_3}$).

2-Ethyl-2-hydroxy-2*H*-indene-1,3-dione (2c). $C_{11}H_{10}O_3$. Yellow oil, yield 0.057 g (30%). MS (m/z, %): 190 (M^+, 26%), 174 (6), 149 (30), 133 (47), 104 (63), 76 (81), 57 (100). IR (KBr, ν, cm^{-1}): 3436, 2923, 2857, 1714, 1593. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.02 (2H, m, ArH). 7.93 (2H, m, ArH), 2.18 (br.s, OH), 1.93 (2H, q, J = 7.22, $\underline{CH_2}CH_3$), 0.94 (3H, t, J = 7.22, $CH_2\underline{CH_3}$). ^{13}C NMR (75.47 MHz, $CDCl_3$, δ, ppm): 199.79 (C-1, 3), 140.31 (C-4a, 7a), 136.37 (C-5, 6), 123.92 (C-4, 7), 79.4 (C-2), 29.70 ($\underline{CH_2}CH_3$), 7.66 ($CH_2\underline{CH_3}$).

5-Ethyl-5-hydroxy-2,2-dimethyl-1,3-dioxane-4,6-dione (2d). $C_8H_{12}O_5$. Colorless needle crystals, yield 0.169 g (90%), mp 107–108°C. MS (*m/z*, %): 188 (M^+, 1%), 172 (11), 170 (5), 150 (15), 115 (22), 105 (100), 104 (10). IR (KBr, ν, cm^{-1}): 3429, 2985, 2919, 2892, 1785, 1739, 1401, 1314. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.52 (1H, t, J = 4.5, OH), 2.19 (2H, dq, J = 7, 4.5, $\underline{CH_2}CH_3$), 1.80 (3H, s, CH_3), 1.78 (3H, s, CH_3), 1.07 (3H, t, J = 7.2, $CH_2\underline{CH_3}$). ^{13}C NMR (75.47 MHz, $CDCl_3$, δ, ppm): 165.5 (C-4, 6), 104.8 (C-5), 47.1 (C-2), 29.7 ($\underline{CH_2}CH_3$), 26.9 (CH_3), 20.0 (CH_3), 10.7 ($CH_2\underline{CH_3}$).

4-Chlorophenyl-2,2′-methylene-bis(3-hydroxy-5,5-dimethyl-2-cyclohexen-1-one) (4a). Yield 0.37 g (92%), mp 139–142°C; lit. mp [22] 139°C. IR (KBr, ν, cm^{-1}): 3427, 2958, 1590, 1489, 1376. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.89 (1H, br.s, OH), 10.09 (1H, br.s, OH), 7.25 (2H, d, J = 7.9, ArH), 7.03 (2H, d, J = 7.9, ArH), 5.49 (1H, s, CH), 2.51–2.29 (8H, m, CH_2), 1.23 (6H, s, CH_3), 1.12 (6H, s, CH_3).

4-Fluorophenyl-2,2′-methylene-bis(3-hydroxy-5,5-dimethyl-2-cyclohexen-1-one) (4b). Yield 0.33 g (85%), mp 183–185°C; lit [23] mp 185–187°C. IR (KBr, ν, cm^{-1}): 3410, 2961, 2934, 1594, 1526. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.90 (1H, br.s, OH), 11.56 (1H, br.s, OH), 6.93–7.07 (4H, m, ArH), 5.49 (1H, s, CH), 2.35–2.44 (8H, m, CH_2), 1.27 (6H, s, CH_3), 1.11 (6H, s, CH_3).

Phenyl-2,2′-methylene-bis(3-hydroxy-5,5-dimethyl-2-cyclohexen-1-one) (4c). Yield 0.313 g (81%), mp 189–190°C, lit. [22] mp 190–191°C. IR (KBr, ν, cm^{-1}): 3412, 2953, 2870, 1650, 1582. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz); 11.65 (1H, br.s, OH), 9.98 (1H, br.s, OH), 6.54–6.83 (5H, m, ArH), 5.48 (1H, s, CH), 2.32–2.45 (8H, m, CH_2), 1.18 (6H, s, CH_3), 1.09 (6H, s, CH_3).

4-Nitrophenyl-2,2′-methylene-bis(3-hydroxy-5,5-dimethyl-2-cyclohexen-1-one) (4d). 0.363 g (88%), mp 188–190°C, lit. [22] mp 189°C. IR (KBr, ν, cm^{-1}): 2950, 1591, 1510, 1376. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.73 (2H, br.s, OH), 8.09 (2H, d, J = 7.7, ArH), 7.23 (2H, d, J = 7.7, ArH), 5.52 (1H, s, CH), 2.43 (8H, m, CH_2), 1.19 (6H, s, CH_3), 1.11 (6H, s, CH_3).

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