Pyruvate Decarboxylase: a New Enzyme for the Production of Acyloins by Biotransformation

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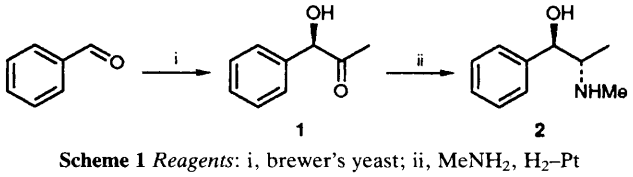
Highly purified pyruvate decarboxylase from yeast has been shown to catalyse the condensation between pyruvate and a wide range of substituted benzaldehydes to give hydroxyketones (acyloins) of the same (*R*) enantiomeric series and of high optical purity, as determined by chiral GC using novel cyclodextrin-based stationary phases.

In 1921, Neuberg and Hirsch[1] reported the formation from benzaldehyde of 3-hydroxy-3-phenylpropan-2-one (phenyl acetyl carbinol), **1**, by fermenting brewer's yeast (Scheme 1).[2] This biotransformation assumed industrial importance when it was shown that acyloin **1** could be converted by reductive methylamination into (−)-ephedrine **2** (Scheme 1). Following Neuberg's work there have been many reports of acyloin formation by yeasts.[3] The synthetic usefulness of the acyloins and of their derived diols has been amply demonstrated.[4] In spite of the interest in this system, it is only recently that convincing evidence has accumulated to show that the enzyme responsible for these acyloin condensations is pyruvate decarboxylase,[5,6] although it has been known for some time that pyruvate decarboxylase catalyses the formation from pyruvate and acetaldehyde of acetoin (3-hydroxybutan-2-one) [**3**, eqn. (1)].[7] An understanding of why this condensation should be catalysed by an enzyme, the physiological role of which is simply to decarboxylate pyruvate to acetaldehyde and

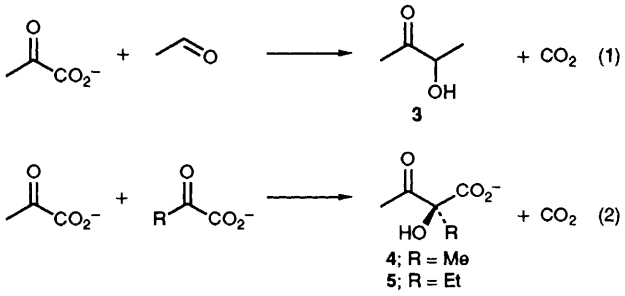


1 **2**

Scheme 1 *Reagents*: i, brewer's yeast; ii, $MeNH_2$, H_2–Pt

carbon dioxide, was recently revealed by the discovery that pyruvate decarboxylase is homologous with acetolactate synthase,[8] the enzyme that catalyses an acyloin condensation in the first common step of isoleucine-valine biosynthesis [eqn. (2)], to give α-acetolactate, **4**, or α-acetohydroxybutyrate, **5**, the precursors respectively of valine and isoleucine.

We have studied acyloin condensations between 'active acetaldehyde' generated from pyruvate, and a range of aromatic aldehydes (Scheme 2 and Table 1).† The enzyme



Ar = **a**; = Ph
b; = 2-Fluorophenyl
c; = 3-Fluorophenyl
d; = 4-Fluorophenyl
e; = 2,3-Difluorophenyl
f; = 2-Chlorophenyl
g; = 3-Chlorophenyl
h; = 4-Chlorophenyl
i; = 2,6-Difluorophenyl

Scheme 2

Table 1 Yields and relative rates of formation of acyloins **2a–h** produced from aldehydes **1a–h** and pyruvate catalysed by purified yeast pyruvate decarboxylase

Aldehyde, **6**	Acyloin, **7**	Yield[a] (%)	Yield[b] (%)	Relative initial rate
a	**a**	65	—	100
b	**b**	89	60	162
c	**c**	58	25	136
d	**d**	50	31	110
e	**e**	74	40	161
f	**f**	71	32	122
g	**g**	48	25	100
h	**h**	44	—	135

[a] As determined by GC for 7.5 h incubation. [b] Isolated yields from 70 mg starting aldehyde.

† For analytical experiments, the incubation mixture contained, in 1 ml final volume, thiamin pyrophosphate (1.5×10^{-5} mol dm^{-3}), $MgSO_4$ (3 mmol dm^{-3}), sodium pyruvate (0.1 mol dm^{-3}, pyruvate decarboxylase (7.3 U), and aldehyde (0.02 mol dm^{-3}) in potassium phosphate buffer, pH 5.9 (0.1 mol dm^{-3}). For gas chromatographic analysis, aliquots were withdrawn and extracted with ethyl acetate.

For preparative experiments, the incubation mixture was as above but in a total volume of 10 ml. The aldehydes were added portionwise, typically in three aliquots over 3 h. Fluorinated aldehydes were added to a total amount of 70 mg, chlorinated aldehydes to a total amount of 30 mg. The reaction was monitored by gas chromatography and was worked-up after 15–22 h, when most of the aldehyde had been consumed. The mixture was extracted with three portions of ethyl acetate, the extracts were dried ($MgSO_4$) and evaporated. The oily products were purified by flash chromatography with toluene: acetone 100 : 1–10 as eluent.

used was yeast pyruvate decarboxylase purified to homogeneity. The kinetics of acyloin formation were studied by GLC. The fastest reacting substrates were 2-fluorobenzaldehyde, **6b** and 2,3-difluorobenzaldehyde, **6e**. The acyloin products of these condensations, **7** (Scheme 2) were also configurationally the most stable. Chiral analysis of the acyloins **7** was carried out using capillary gas chromatography using newly developed cyclodextrin-based stationary phases.[9] For development of the chiral analytical procedures, racemic acyloins were prepared by Stetter's method.[10] As shown in Table 2, the optical purities of the acyloin products were in all cases high. Only with 3- and 4-chlorobenzaldehydes was the optical purity less than 95%.

To provide further confirmation that pyruvate decarboxylase is indeed the enzyme responsible for catalysing acyloin condensations in whole yeast, chiral analyses were carried out on the products obtained using whole yeast.‡ In all cases, the major enantiomer produced was the same as that obtained using the purified decarboxylase. However, the optical purities of the products obtained using yeast were invariably of lower optical purity than those obtained using the isolated enzyme (Table 2).

The absolute configurations of the acyloins **7**, were determined by circular dichroism (CD), using, for comparison, (*R*)-3-hydroxy-3-phenylpropan-2-one, **7a**, of unambiguously determined absolute configuration.[11,12] Typical results are shown in Fig. 1 for the series of chlorinated compounds, **7f–h** (Scheme 2). Each compound shows a negative peak near 280 nm and a positive peak of much lower amplitude near 315 nm. Similar results were obtained for the other acyloins

Table 2 Optical purities of acyloins obtained from the corresponding aldehyde and pyruvate using yeast pyruvate decarboxylase or whole yeast[a]

Acyloin, **7**	Optical purity (% e.e.)[a,b]	Optical purity (% e.e.)[c]	Stationary phase[c]
a	99	97	Lipodex A
b	99	87	D_1[d]
c	99	95	D_1
d	99	97	D_1
e	99	92	D_1
f	98	81	D_2[e]
g	99	86	Lipodex A or D_2
h	99	77	D_2
i	92	87	D_2

[a] Product obtained using purified yeast pyruvate decarboxylase. [b] e.e. = enantiomeric excess. [c] Product obtained using whole yeast (*S. cerevisiae*). [d] D_1 = 3-Acetyl-2,6-di-*O*-butyl-β-cyclodextrin. [e] D_2 = 6-*O*-Methyl-2,3-di-*O*-pentyl-γ-cyclodextrin.

‡ Whole cell transformations were carried out using baker's yeast (*Saccharomyces cerevisiae*). The yeast was suspended in an amount corresponding to 53 g wet weight l^{-1} in a medium containing (g l^{-1}) Bacto peptone (6), sodium citrate (10.5), glucose (30), brought to pH 4.5 (dil. HCl). After 1 h incubation at 30 °C on a rotary shaker the aldehyde (0.35 g) was added together with acetaldehyde (150 μl) and sucrose (2.2 g as a 15% solution). The reaction was terminated after 6 h total fermentation. Typically, the reaction was carried out in 12 flasks containing a total of 1800 ml medium. The combined fermentation medium was extracted with three aliquots of ethyl acetate, dried ($MgSO_4$) and evaporated. The oily residue was separated by flash chromatography as for the enzymatic experiments.

The yeast used in these experiments was obtained from a local supermarket (J. Sainsbury plc). This yeast had been used previously by us and deposited as NCYC.1765. Through the courtesy of Dr J. B Van der Plaat (Gist Brocades, Delft) it was confirmed by DNA fingerprinting that the yeast used in these experiments was the same as the authentic strain distributed by Gist Brocades *via* British Fermentation Products of Felixstowe to Sainsbury's supermarkets.

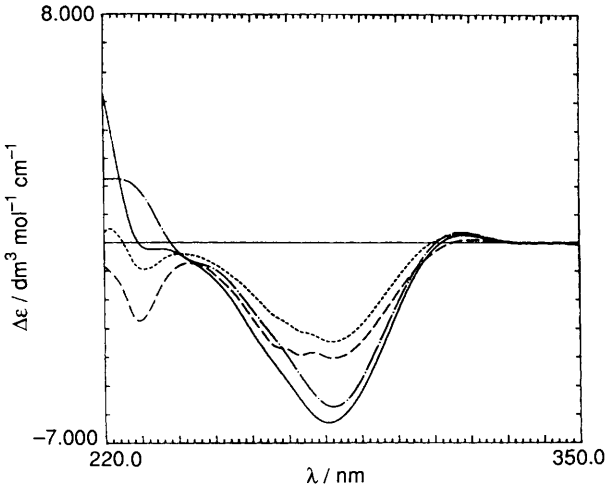


Fig. 1 CD curves: ——— 3-hydroxy-3-phenylpropan-2-one (**7a**, 77% e.e.); — — — — 3-hydroxy-3-(2-chlorophenyl)propan-2-one (**7f**, 85% e.e.); - - - - 3-hydroxy-3-(3-chlorophenyl)propan-2-one (**7g**, 76% e.e.); —·—·— 3-hydroxy-3-(4-chlorophenyl)propan-2-one (**7h**, 97% e.e.). The CD determinations were carried out using solutions in methanol. (For convenience, products from whole yeast reductions were used for these determinations.)

shown in Scheme 2. These results establish that all acyloins, whether produced by whole yeast or by pyruvate decarboxylase, belong to the (*R*) series.

Pyruvate decarboxylase has obvious advantages for producing optically active acyloins. Most important of these are the higher optical activities of the products than those obtained using whole yeast, and the absence of competing reduction of the aldehyde substrate to the corresponding alcohol under the influence of dehydrogenase(s) present in the whole organism. Ease of isolation and generally higher yields are also important factors.

From our previous studies, from the studies reported here and from other unpublished studies, it is clear that yeast pyruvate has a very strong recognition site for aromatic residues of the aldehyde acceptors of 'active acetaldehyde' attack. Acetolactate synthase also has a recognition site for aromatic systems as revealed by the tight binding of the herbicides that act by inhibiting acetolactate synthase isoenzyme II.[13] This has been attributed to the existence of a putative vestigial quinone binding site corresponding to the quinone binding site in pyruvate oxidase, a bacterial redox enzyme also homologous with acetolactate synthase.[14] It is possible that the recognition of the aromatic aldehydes by yeast pyruvate decarboxyalse is also a manifestation of a corresponding vestigial quinone binding site in this enzyme.

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