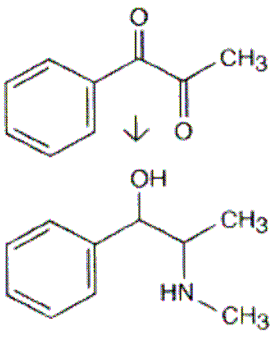
Ephedrine from 1-Phenyl-1,2-propanedione

by Wilfrid Klavehn

15 grams of phenylpropanedione are dissolved in 250 cc. of an ethereal solution of methylamine containing 3.4 grams of the latter. While cooling, 32 grams of activated aluminium are introduced. In the course of 3-5 hours, 30 grams of water are allowed to drop in, whereby the ether is kept gently boiling. When the evolution of hydrogen has ceased, the whole is filtered from the aluminium hydroxide which has been formed and the colourless ethereal solution, after repeated extraction of the residue, is shaken with diluted hydrochloric acid, extract the base, or the later may be precipitated in the form of the hydrochloride by introducing gaseous hydrochloric acid. By crystallization from alcohol, the hydrochloride of phenylmethylamino propanol (*l*-ephedrine) of melting point 185-186° C. is obtained in very good yield. Source: Kalvekhn (1929) Ephedrine can be obtained by reducing phenylpropanedione with iron filings (Sugino 1953).

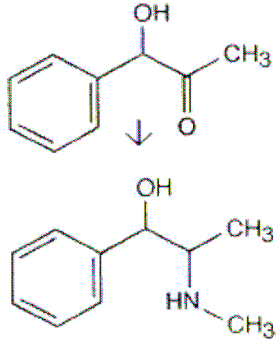


Ephedrine from 1-Phenylpropan-1-ol-2-one

(1-Phenylpropanol-1-one-2); (Phenylpropanolone)
(Phenylacetylcarbinol), (L-PAC)

by Gustav Hildebrandt and Wilfrid Klavdhn

120 grams of the fermentation product containing phenylpropanolone obtained by extraction with ether (see page 150) are allowed to run, without further purification, in the course of about two hours into a solution of 10 grams of methylamine in 500 cc. of ether in presence of 20 grams of activated aluminium whilst stirring. Simultaneously 20-30 grams of water are added, drop by drop. The vigorous reaction which at once sets in is moderated by periodical cooling. When the reaction is complete the ethereal solution is filtered and the optically active base which has been formed is extracted from the filtrate by means of dilute hydrochloric acid. The product is worked up in the usual manner.



There is obtained the hydrochloride of levo-1-phenyl-2-methylamino-propanol-1 (ephedrine) having a melting point of 214° C. and having the optical rotation given in the literature. The yield amounts to 25-45 grams of the hydrochloride depending upon the nature of the parent material. Source: Hildebrandt 1931, 1934 Ref.: Neuberg 1922

Preparation of Mercuric Bichloride
Caution: mercury containing chemicals are poisonous!

Mercury bichloride can be prepared by several different reactions.

A mixture of 12 grams of mercury are boiled with 18 grams of sulfuric acid on a sand bath until a dry white residue remains. This white residue is mercury sulfate. The residue of mercury sulfate is ground with 9 grams of sodium chloride (salt) in an earthenware mortar. The powder is then placed in a sublimation apparatus. A vacuum is drawn on the apparatus and the stopcock is closed to keep the vacuum. The sublimation apparatus is gradually heated to sublime the mixture which condenses mercury bichloride on the walls of the apparatus.

Mercury bichloride can also be produced by dissolving mercuric oxide (red) in hydrochloric acid and evaporating the solution to leave a residue of mercury bichloride.

Mercury bichloride can also be produced by the direct reaction of chloride gas with mercury vapor.

Methylamine Hydrochloride
Preparation

$$2HCHO+NH_4Cl \rightarrow CH_3NH_2\,HCl+HCO_2H$$

Prepared by C. S. Marvel and R. L. Jenkins.
Checked by J. B. Conant and F. C. Whidden.

1. Procedure

IN a 5-L. round-bottom flask fitted with a stopper holding a condenser set for downward distillation and a thermometer which will extend well into the liquid, are placed 4000 g. of technical formaldehyde (35-40 per cent; sp. gr. 1.078 at 20°) and 2000 g. of technical ammonium chloride. The mixture is heated on the steam bath until no more distillate comes over and then over a flame until the temperature of the

1-Phenylpropan-1-ol-2-one (L-PAC) Preparation from Benzaldehyde

by Andrew Smallridge, Maurice Trewhella and Margaret Del Guidice, Robert Coughlin, Wafaa Mahmoud; A. Halim El-Sayed and John W. Rothrock

Ephedrine (*alpha*-[1-(methylamino)ethyl]benzene-methanol), originally isolated from plants of the genus *Ephedra*, occurs as the naturally-occurring isomers *l*-ephedrine and *d*-pseudoephedrine, and other pharmacologically active isomers include *d*-ephedrine and *l*-pseudoephedrine. These compounds are adrenergic sympathomimetic agents and have antihistamine activity; *l*-ephedrine is widely used as a bronchodilator, while *d*-pseudoephedrine is widely used as a decongestant. Compounds of these groups are present in a very wide range of prescription and over-the-counter pharmaceutical formulations.

The production of l-phenylacetylcarbinol, a precursor of *l*-ephedrine, by catalysis using whole baker's yeast cells in aqueous medium was one of the first microbial biotransformation processes to be used commercially Neuberg (1921); see also Hildebrandt (1934). This reaction involves the yeast-induced condensation of benzaldehyde with acetyl-coenzyme A. The reaction has been widely investigated, and has been shown to be mediated by the enzyme pyruvate decarboxylase Groger (1966). It has also been shown that the reaction has a relatively broad specificity for the substrate, enabling a variety of substituted aromatic aldehydes to be converted to the corresponding substituted optically-active phenylacetylcarbinols Long (1989)...

We have now surprisingly found that yeast-mediated acyloin condensation of benzaldehyde can be achieved in an organic solvent using non-fermenting yeast, and that addition of a small proportion of ethanol to the reaction mixture suppresses formation of undesired side-products. Even more surprisingly, by performing the reaction at reduced temperature, an even greater reduction of side-reactions can be achieved, without loss of catalytic activity. The effect of reduction in temperature appears to be generally applicable to both aqueous and non-aqueous systems utilizing a non-fermenting yeast...

Any yeast capable of effecting reduction may be used. It is economically advantageous to use the cheapest yeast available, and ordinary baker's yeast, *Saccharomryces cerevisiae*, is preferred. Strains of yeast adapted to other purposes, including brewing yeast and wine or sherry yeasts could also be employed. Strains specifically adapted to an organic solvent environment or for enhanced reduction efficiency may be used; such strains include conventionally-selected and genetically modified strains. For maximum efficiency of reaction, it is advisable to present the maximum surface area of yeast for contact with the reactants. This can be effected by using "active" dried yeast, which is readily commercially available as "instant dry yeast", and may be stored at room temperature. Alternatively, well-pulverised dry baker's yeast may be used. Other yeasts, such as those described in Leuenberger (1988), or fungi such as those disclosed in Chenevert (1992) may also be used. The person skilled in the art will readily be able to test whether any specific organism will function for the purposes of the invention, using the methods described herein.

Preferably the aliphatic alcohol or aliphatic aldehyde is ethanol or acetaldehyde, suitably 0.1 mL per gram yeast. This results in a significant increase in the yield of carbinol, and reduces the amount of aromatic alcohols produced as a side-reaction. Ethanol is preferred, since this results in superior conversion of the aromatic aldehydes to the desired carbinol, and lower yield of undesired reduction product. Without wishing to be bound by any particular theory, it is believed that the ethanol or acetaldehyde provides an alternative substrate for the reductase enzymes thus inhibiting the formation of side products such as benzyl alcohol. Therefore, it is predicted that other aliphatic alcohols or aliphatic aldehydes could perform the same function.

Although the reaction can be performed at ambient temperature, suitably 16-24° C., preferably 20° C., we have surprisingly found that significantly better results are obtained at lower temperatures, in the range 0-5° C. The reason for the improved performance and further reduction of side-reactions which is observed is not presently understood; however, we have observed that the activity of the yeast at these reduced temperatures is comparable to that at ambient temperature. This result is particularly surprising, because it would normally be expected that a yeast-mediated reaction would demonstrate a temperature optimum at ambient or slightly elevated temperature, although Shiu (1996), have shown that isolated pyruvate decarboxylase, the enzyme involved in the acyloin condensation reaction, exhibits increased activity at 4° C.

It is known that in order to preserve functioning of an enzyme in an organic solvent environment it is necessary for the enzyme to be fully hydrated by being surrounded by a few layers of water molecules. This requirement is satisfied by providing a ratio of 0.6 to 1.2 mL water/g of yeast, preferably 1.0 mL water/g of yeast. This results in a single phase organic system as all of the water is absorbed into the yeast. A two-phase system reduces the yield of the product and makes isolation of the product considerably more difficult.

Once the yeast-mediated reaction has been completed, the yeast can readily be separated from the reaction mixture by filtration and washing. The reaction mixture, comprising product, unreacted starting material, solvent and minor impurities, is subjected to conventional purification, for example by flash distillation, to yield the purified product. Optionally the yeast can be extracted with an organic solvent such as ethyl acetate to yield a marginal amount of further product.

While the ratio of yeast to substrate will vary depending on the individual system, and is readily determined experimentally using routine trial and error methods, we have found that for the conversion of benzaldehyde to phenylacetylcarbinol the optimum ratio is 5 grams yeast/mmol benzaldehyde; increasing the amount of yeast results in only a small increase in conversion, and lower amounts of yeast provide lower conversion.

Similarly, the optimum reaction time may readily be determined, and for the benzaldehyde-phenylacetyl-carbinol system we have investigated reaction times from 12 to 72 hours, and have found that when the reaction is continued for longer than 24 hours there is very little improvement in conversion, and that there is an increase in production of by-products.

In a particularly preferred embodiment, production of undesired side-products is reduced by performing the catalysis reaction at below ambient temperature. Preferably the temperature is 0-5° C.
Source: Smallridge (2001)

Saccharomyces cerevisiae accession number ATCC 834 of the American Type Culture Collection, 12301 Parkland Drive, Rockville, Md., 20852 USA. was maintained on a medium that contained (per liter): 10 g of yeast extract, 10 grams of malt extract, 4 grams of dextrose, 20 grams of agar. The liquid medium used for growing the yeast cells and for production of L-PAC contained (per liter): 6 grams of yeast extract, 4 grams of NH_4SO_4, 0.6 grams of $MgSO_4$, 1 g of KH_2PO_4, 100 grams of dextrose, with the balance water. For sterilization, a solution of dextrose and a solution containing the other ingredients were autoclaved separately, then mixed and the pH adjusted to 6.2. The yeast was grown for 24 hrs on the liquid medium at 28°-30° C., centrifuged, re-suspended in sterile water then re-centrifuged.

The washed and centrifuged cells were re-suspended in a solution containing 3% (w/v) of sodium alginate (Aldrich Chemical Company). The latter cell suspension was extruded as drops into a 2% calcium chloride solution thereby forming beads which were kept in the CaCl2 solution for 1 hour before filtering and washing them on a Buchner funnel. These beads of immobilized yeast were then used to inoculate the medium in the shake flasks so that the cell dose was 2.8 grams (cell wet weight) per 100 mL of medium in 250 mL Erlenmeyer flasks. After shaking under aerobic conditions for 1 hour, shaking was continued under anaerobic conditions. Pre-purified (by distillation) benzaldehyde was added in four equal aliquots at 1 hour intervals during the anaerobic shaking. The total amount of benzaldehyde added was 6 g per liter of medium. Thereafter, with continued shaking under anaerobic conditions, small samples were withdrawn over the ensuing 24-hour period and assayed for L-PAC by the method of Groger (1966). The maximum liter of L-PAC was 5.5 g/L. Source: Coughlin (1992)

A 3,4-substituted benzaldehyde compound, wherein the 3 and 4 substitutes are hydroxy, alkoxy, or when taken together an alkylenedioxy (e.g. methylenedioxy) substitute, is contacted with a growing culture of an acyloin-producing microoranism at a temperature of abut 25-30° C. and the L-acyloin produced is isolated from the fermentation broth by extraction, chromatography and crystallization.

The acyloin-producing microorganisms utilized is the process of my invention are preferably commercially available yeast strains ordinarily employed as "wet compressed yeast" is the baking and brewing industries, in particular selected strains of *Saccharomyces* cerevisiae. In addition, selected strains of microorganisms belonging to the *Schizomysetes*, *Myxomycetes*, and *Eumycetes* are effective in carrying out the desired stereospecific conversion to the L-acyloin.

For any given species of microorganisms it is necessary to select acyloin-producing strains of microorganisms, by a simple test. This test includes growing the selected microorganism in contact with a 3,4-disubstituted-benzaldehyde, and filtering and extracting the fermentation broth with ethyl acetate. A portion of the extract is then spotted on filter paper and chromatographed using n-butanol saturated with 3% aqueous ammonium hydroxide solution. A positive blue tetrazolium spot test indicates the presence of the desired acyloin and the strains showing a positive tetrazolium test are then utilized in the fermentation process...

A suitable sterile nutrient medium containing assimilable sources of carbon and nitrogen are inoculated with a selected strain of microorganism and aerated and agitated until substantial growth of the microorganism has occurred. Generally, a period of 1-7 days is required. Typical media utilized in the process of my invention are indicated in the following tables.

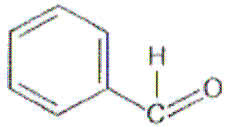
Medium I

Molasses ..... 6 grams
Water: sufficient to make 100 mL.
pH adjusted to 5.5 with phosphoric acid.

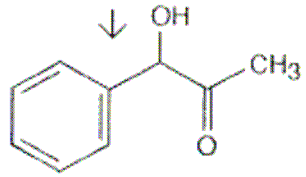
Medium II

	Grams
Commercial yeast extract	20
Dextrose	20
$MgSO_4 \cdot 7H_2O$	0.5
KH_2PO_4	0.2
$NaHPO_4$	0.2

Water ..... (sufficient to make 1.0 liter).
pH adjusted to 7.



Benzaldehyde
+ Molasses
+ Yeast
+Dextrose



1-Phenylpropan-1-ol-2-one

Following the growth period, the selected 3,4-disubstituted-benzaldehyde is added to the medium in a concentration of approximately 1-10 grams of the compound per liter of medium, and the medium containing the test compound incubated with agitation at about 28° C. for a period of from 1-10 hours.

Following the incubation period, the fermented broth containing the desired compound along with other products of metabolism is extracted with a water-immiscible solvent for the acyloin such as an ester of a lower aliphatic acid, preferably ethyl acetate.

The solvent extract contains, in addition to the desired acyloin, unreacted aldehyde as well as the corresponding substituted benzyl alcohol and the substituted benzoic acid. The desired acyloin compound is separated from the related compounds by processes including partition chromatography, by fractional crystallization and by extraction with aqueous sodium bisulfate solution.