FLAVOUR AND FRAGRANCE JOURNAL
Flavour Fragr. J. 2004; **19**: 115–120
Published online 4 February 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ffj.1326

Amino acid-catalysed retroaldol condensation: the production of natural benzaldehyde and other flavour compounds

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Received 3 January 2003
Revised 16 July 2003
Accepted 16 September 2003

ABSTRACT: The amino acid-catalysed retroaldol condensation previously described for citral has been extended to other α,β-unsaturated aldehydes. In the presence of glycine and an elevated pH, six other α,β-unsaturated aldehydes also underwent retroaldol condensation. Crotonaldehyde, as well as its proposed intermediate, aldol, were converted into acetaldehyde. Hexenal, decenal, methyl crotonaldehyde, farnesal and cinnamaldehyde were also converted into acetaldehyde plus butanal, octanal, acetone, geranyl acetone and benzaldehyde, respectively. Subsequently, the effects of temperature, pH, the buffer and substrate concentration on the conversion rate were studied, using the conversion of cinnamaldehyde into the important flavour compound benzaldehyde as a model. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: α,β-unsaturated aldehydes; retroaldol condensation; natural flavour production; benzaldehyde

Introduction

Natural flavours play an important role in the quality of food and beverages. Due to practices such as premature harvesting, extended storage and physical treatment, aromas may be lost and the addition of flavour supplements to foodstuff is often required. Furthermore, inherent levels of flavour compounds may be too low or not of the desired type. As consumers are more concerned about food quality and prefer natural food additives to chemically synthesized compounds, the 'natural' label allocated by the European and US food legislation represents a strong marketing advantage.[1] The difference in price of a natural compound and its chemically synthesized counterpart can be considerable, e.g. in 1998 the price of synthetic vanillin was approximately US$ 12/kg, whereas the price for vanillin extracted from vanilla pods was approximately US$ 4000/kg; and in 2000 the price for natural *cis*-2-hexenol was approximately US$ 1325/kg as compared to approximately US$ 67/kg for synthetic *cis*-2-hexenol.[2]

In the USA the terms 'natural flavour' or 'natural flavouring' mean the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate or any product of roasting, heating or enzymolysis, which contains the flavouring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products or fermentation products thereof, whose significant function in food is flavouring rather than nutritional (Code of Federal Regulations, Title 21, section 101, part 22; http://www.access.gpo.gov). Based on this definition, it can be concluded that products obtained from natural raw materials by mild chemical reactions (such as heating) can be considered natural.

Previously we described the retroaldol condensation of citral under mild reaction conditions.[3] Citral was efficiently converted into the aroma compounds methylheptenone and acetaldehyde. This pH-dependent reaction was catalysed by several amino acids. Furthermore, ammonium and other buffers did not catalyse the reaction. 3-Hydroxycitronellal was identified as the intermediate of this oxygen-independent reaction (Figure 1A). In this paper we explore the possibilities of extending

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Contract/grant sponsor: European Community; Contract/grant number: FAIR CT 98-3559.

this 'kitchen chemistry'-type catalysis to other α,β-unsaturated aldehyde substrates.

Materials and Methods

Materials

Acetaldehyde (ethanal), aldol, hexadienal (2,4-hexadien-1-al), hexenal (*trans*-2-hexenal) and geranylacetone (6,10-dimethyl-5,9-undecadien-2-one) were purchased from Aldrich (Steinheim, Germany). Acetone, benzaldehyde and glycine were purchased from Merck (Darmstadt, Germany). Cinnamaldehyde (*trans*-cinnamaldehyde), crotonaldehyde, decenal (*trans*-2-decenal) and decadienal (*trans*, *trans*-2,4-decadienal) were purchased from Acros (Geel, Belgium). Butanal (butyraldehyde), citral (mixture of *cis*- and *trans*-3,7-dimethyl-2,6-octadien-1-al), methyl-crotonaldehyde (3-methylcrotonaldehyde), methylheptenone (6-methyl-5-hepten-2-one) and octanal (caprylic aldehyde) were purchased from Fluka (Buchs, Switzerland). Farnesal (3,7,11-trimethyl-2,6,10-dodecatrienal) was purchased from Frinton Laboratories (Vineland, NJ, USA). All other chemicals used were of analytical grade (purity ≥ 99%).

Activity Measurements

The retroaldol condensation activity was typically determined by incubating 1 ml of reaction mixture in a 15 ml vial fitted with a Teflon Mininert valve (Supelco, Zwijndrecht, The Netherlands) in a shaking water bath (oscillating at 2.5 Hz, with an amplitude of 2 cm). Unless stated otherwise, incubation was carried out at 25 °C in 0.5 M glycine/NaOH-buffer (pH 10.0) at a substrate concentration of 2.5 mM.

The conversion of the different substrates was tested using acetaldehyde production (as determined in the headspace by GC) in time as a measure for activity. At the end of the conversion the acetaldehyde production was confirmed enzymatically and the remaining reaction mixture was extracted by an equal amount of ethyl acetate and subsequently analysed by GC and GC–MS to determine the products formed.

The effect of temperature on the retroaldol condensation of cinnamaldehyde was determined by varying the temperature during incubation and determining the acetaldehyde concentration in the headspace of the samples by GC. The effects of pH, buffer concentration and substrate concentration were studied by incubating reaction mixtures for 30 min at 70 °C. The reaction was stopped by addition of 20% 6 M HCl to the samples (to facilitate benzaldehyde extraction), after which they were extracted with ethylacetate and subsequently analysed by GC.

Analytical Methods

Benzaldehyde, geranyl acetone, methylheptenone and octanal were detected by extracting the liquid samples with ethyl acetate and subsequent GC and GC–MS analysis, as described previously,[4] except that the temperature programme was 10 min at 40 °C followed by a linear gradient of 40–160 °C (2.0 °C/min) for GC and 4 min at 50 °C; linear gradient up to 100 °C (1.5 °C/min); linear gradient up to 250 °C (10 °C/min); and 20 min at 250 °C for GC–MS.

Acetaldehyde, acetone and butanal were determined in 100 µl headspace samples, as described previously for acetaldehyde,[4] except that an isothermal oven temperature of 40 °C was used. Products were identified by comparing the retention times with those of authentic samples and by comparing the MS spectra of the products with those in the NIST spectral database (NIST Mass Spectrometry Data Center S. E. Stein, Director).

For the enzymatic acetaldehyde determination, an acetaldehyde test kit (R-Biopharm, Darmstadt, Germany) was used.

Results

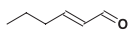
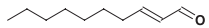
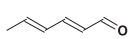
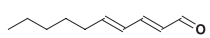
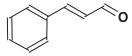
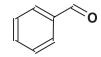
Substrate Range

It was demonstrated that, under alkaline conditions, amino acids efficiently catalyse the conversion of citral into equimolar amounts of methylheptenone and acetaldehyde.[3] Of the amino acids tested, glycine was one of the most efficient catalysts. An additional property of glycine is that it buffers strongly around pH 10, thus additional buffer or other means of pH control are not needed.

Nine different α,β-unsaturated aldehydes were tested for analogous reactions, of which seven were found to be susceptible to glycine-catalysed retroaldol condensation (Table 1). Starting with the most actively converted substrate, methyl crotonaldehyde, hexenal, crotonaldehyde, citral, decenal, farnesal and cinnamaldehyde were converted. None of the tested compounds were converted (detection limit was 0.2 µM/min) in the absence of glycine. In all instances the product predicted from the proposed pathway (Figure 1) was formed. Hexadienal and decadienal conversion could not be detected (activity < 0.2 µM/min).

The proposed intermediate of the retroaldol condensation of crotonaldehyde, aldol (Figure 1B), is commercially available and was also found to be converted into acetaldehyde. However, in contrast to 3-hydroxycitronellal (the intermediate of the citral conversion; Figure 1A[3]), aldol is also converted in the absence of glycine. Moreover, in contrast to the analogous reaction with 3-hydroxycitronellal, the reverse reaction of aldol into crotonaldehyde was not observed.

Table 1. Glycine-catalysed conversion of α,β-unsaturated aldehydes

Substrate		Acetaldehyde formation			Product formation		
Name	Structure	Activity in μM/min (SE)		Identification method	Name	Structure	Identification method
		Glycine[1]	Control[2]				
Crotonaldehyde		7.22 (0.44)[3]	<0.2	GC;[4] Enz[5]	Acetaldehyde		GC
Hexenal		5.73 (0.32)	<0.2	GC; Enz	Butanal		GC
Decenal		2.77 (0.18)	<0.2	GC[6]	Octanal		GC; GC–MS[7]
Hexadienal		<0.2	<0.2				
Decadienal		<0.2	<0.2				
Methyl-crotonaldehyde		10.81 (0.79)	<0.2	GC; Enz	Acetone		GC
Citral		2.93 (0.10)	<0.2	GC; Enz	Methylheptenone		GC; GC–MS
Farnesal		0.46 (0.03)	<0.2	GC; Enz	Geranyl acetone		GC; GC–MS
Cinnamaldehyde		0.38 (0.06)	<0.2	GC; Enz	Benzaldehyde		GC; GC–MS

Conversion-rates are determined at 25 °C and a substrate concentration of 2.5 mM. [1]**Glycine**, 0.5 M glycine/NaOH-buffer, pH 10.0. [2]**Control**, 0.5 M Na_2CO_3/$NaHCO_3$ buffer, pH 10.0. [3]One crotonaldehyde molecule yields two acetaldehyde molecules (crotonaldehyde conversion rate is 3.61 μM/min). [4]**GC**, retention time as compared to authentic sample. [5]**Enz**, acetaldehyde formation confirmed enzymatically. [6]Decenal interferes with the enzymatic acetaldehyde determination. [7]**GC–MS**, GC–MS spectrum as compared to NIST spectral database. SE, standard error.

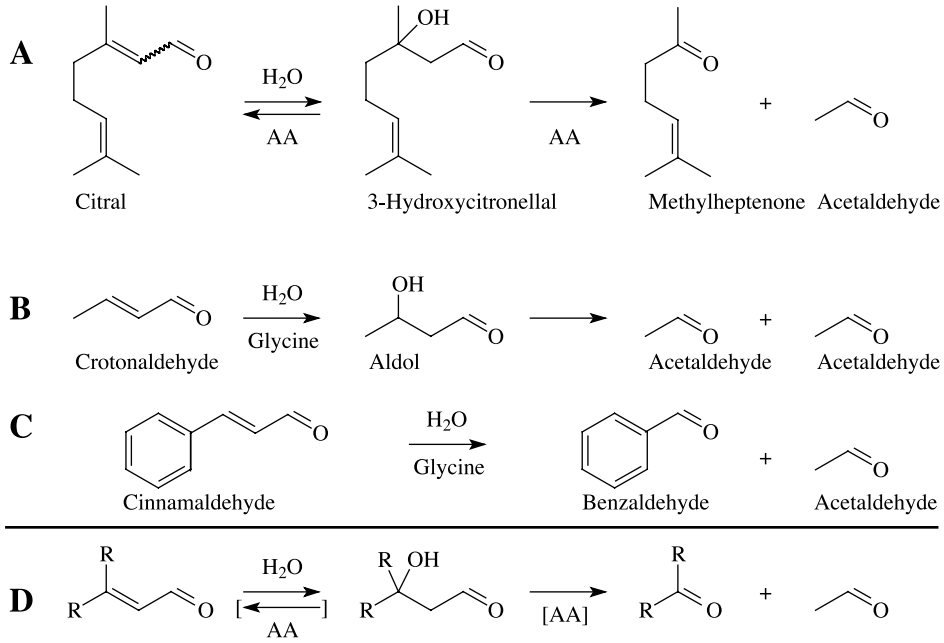


Figure 1. Amino acid-catalysed conversion of α,β-unsaturated aldehydes: Conversion of citral (A), crotonaldehyde (B) and cinnamaldehyde (C), and proposed general conversion scheme of amino acid-catalysed conversion of α,β-unsaturated aldehydes (D). AA, catalytic amino acid; Glycine, catalytic glycine; H2O, water consumed in the forward reaction (or produced in the reverse reaction)

Benzaldehyde Production

Of the reactions catalysed (Table 1), the conversion of cinnamaldehyde into benzaldehyde is the most interesting from an industrial viewpoint, as benzaldehyde is the second most utilised flavour compound worldwide.[5] Therefore, this reaction was studied in more detail.

Temperature had a pronounced effect on the activity of the conversion, which increased exponentially with the temperature (Figure 2). Raising the temperature from 25 °C to 90 °C resulted in an approximately. 290 times higher conversion activity. From the Arrhenius plot (see insert, Figure 2) an activation energy of 74.0 kJ/mol for the amino acid-catalysed cinnamaldehyde conversion was calculated.

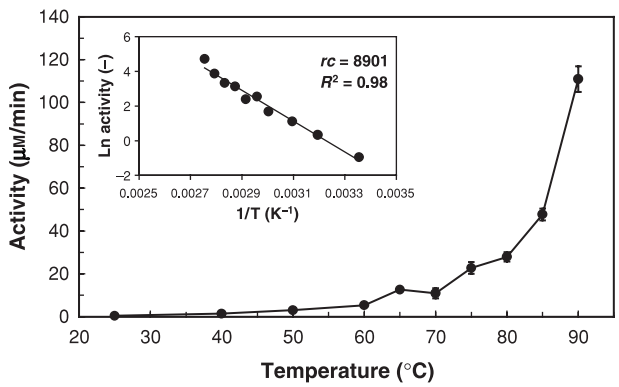


Figure 2. Temperature dependence of the glycine-catalysed conversion of cinnamaldehyde into benzaldehyde and acetaldehyde (25–90 °C, 0.5 M glycine/NaOH buffer, pH 10.0, and 2.5 mM cinnamaldehyde). Error bars represent SE of at least triplicates. Insert, Arrhenius plot

Previously, it was reported that elevating the pH in the range 6.5–10.0, had a strong positive effect on the glycine-catalysed retroaldol condensation of citral.[3] For the cinnamaldehyde conversion, the effect of pH of the glycine solution was tested throughout its buffering range (pH 8.7–10.8) and was optimal at pH 9.2 (Figure 3A). The activity of the amino acid-catalysed conversion was also dependent on the concentration of the glycine/NaOH buffer used (Figure 3B). In the absence of glycine buffer, no conversion was observed and the activity increased with the amino acid concentration. At 2 M glycine, the activity was increased to approximately 2.5-fold as compared to the normal glycine buffer concentration (0.5 M). Moreover, the effect of cinnamaldehyde concentration on the benzaldehyde formation activity was tested (Figure 3C). The activity of the conversion increased with cinnamaldehyde concentration, but levelled off somewhat at concentrations above 40 mM, probably as a result of the low solubility of cinnamaldehyde (approx. 11 mM at 25 °C[6]).

Discussion

In this report, we showed that seven different α,β-unsaturated aldehydes (Table 1) underwent

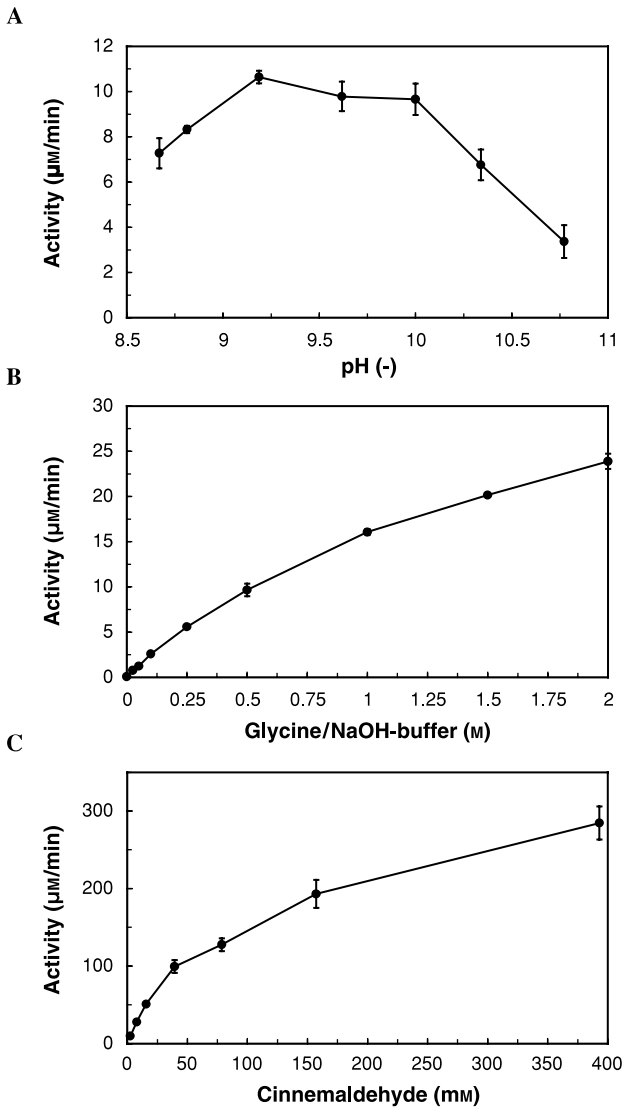


Figure 3. Benzaldehyde production-activity at 70 °C. (A) pH (0.5 M buffer, pH 8.67–10.77 and 2.5 mM cinnamaldehyde). (B) Buffer-concentration (0–2.0 M buffer, pH 10.0, and 2.5 mM cinnemaldehyde). (C) Substrate-concentration (0.5 M buffer, pH 10.0, and 2.5–393 mM cinnamaldehyde). Error bars represent the SE of triplicates

glycine-catalysed retroaldol condensation. Also the proposed intermediate of this reaction, aldol, was converted in a similar manner to the conversion of 3-hydroxycitronellal previously shown.[3] Based on these findings, a general reaction scheme is proposed (Figure 1D); first, amino acid-catalysed addition of water to the α,β-unsaturated aldehyde occurs, thus forming a 3-hydroxy intermediate. The 3-hydroxy intermediate is subsequently rearranged, resulting in acetaldehyde and the product. For catalyses of this final step, amino acids are not essential in all instances, whereas amino acids were essential for catalyses of the first step of the conversion.

All the retroaldol conversion activities were relatively low (0.38–10.8 µM/min). This low rate could be improved dramatically, as was demonstrated for the conversion of cinnamaldehyde into benzaldehyde. Although the combined effects of temperature, pH, the buffer and substrate concentration on the conversion rate were not studied in the work presented here, a rough estimation can be made. Raising the temperature during the reaction from 25 to 90 °C gave a 290-fold increase; lowering the pH from 10.0 to 9.2 gave a 1.1-fold increase; raising the buffer concentration from 0.5 to 2 M gave a 2.5-fold increase; and raising the cinnamaldehyde concentration from 2.5 to 390 mM gave a 29-fold increase. Based on the individual effects, an improvement of over 23 000-fold should be achievable. In this way it would be possible to produce natural benzaldehyde at a rate of approx. 8.9 mM/min (= 56.7 g/l/h).

Although natural benzaldehyde is not a very expensive aroma compound (it is the main constituent of bitter almond oil, which is sold for approximately US$ 400/kg, whereas synthetic benzaldehyde is sold for approximately US$ 2/kg), there is still a great deal of interest in the production of 'natural' benzaldehyde. According to Armstrong *et al.* (1994), 'natural' benzaldehyde is also commercially produced from cinnamon bark and leaf, of which cinnamaldehyde is the major constituent. However a number of firms have protested against the 'natural' status of this benzaldehyde, which is sold for approximately US$ 100/kg.[7] This is because the use of an acid or base catalyst causes concern over the 'natural' status.[8] It is likely that the method patented by Buck *et al.* in 1987[9] was used in this case, a method encompassing a reaction that takes place at pH 12.5 (obtained by addition of NaOH) at a temperature of 105 °C. This method of producing benzaldehyde (pH 12.5 and 105 °C) can easily be adapted, using the results of this paper. The resulting production method would take place at a much lower pH (9.2 or even lower if needed) and the catalyst is the natural compound glycine, rather than the organic base NaOH. Although it is not possible to calculate an exact volumetric conversion activity from the data presented in the above-mentioned patent, it can be calculated that the activity is certainly lower than 86 g/l/h (at pH 12.5 and 105 °C). This indicates that the method presented in this paper, with an activity of 57 g/l/h (at pH 9.2 and 90 °C) is certainly commercially feasible.

Besides benzaldehyde, acetaldehyde, which is produced as 'side-product', is commercially interesting. Acetaldehyde has a characteristic pungent, penetrating, ethereal odour. It is used as a flavour enhancer in orange juice to create naturalness, fruitiness and juiciness.[10] If sold as a 'natural' compound, it has a price of approximately US$ 135/kg and as a 'synthetic' compound it can still be sold at approximately US$ 47/kg.[2]

The method presented here has the potential to be applied for the production of a range of other natural

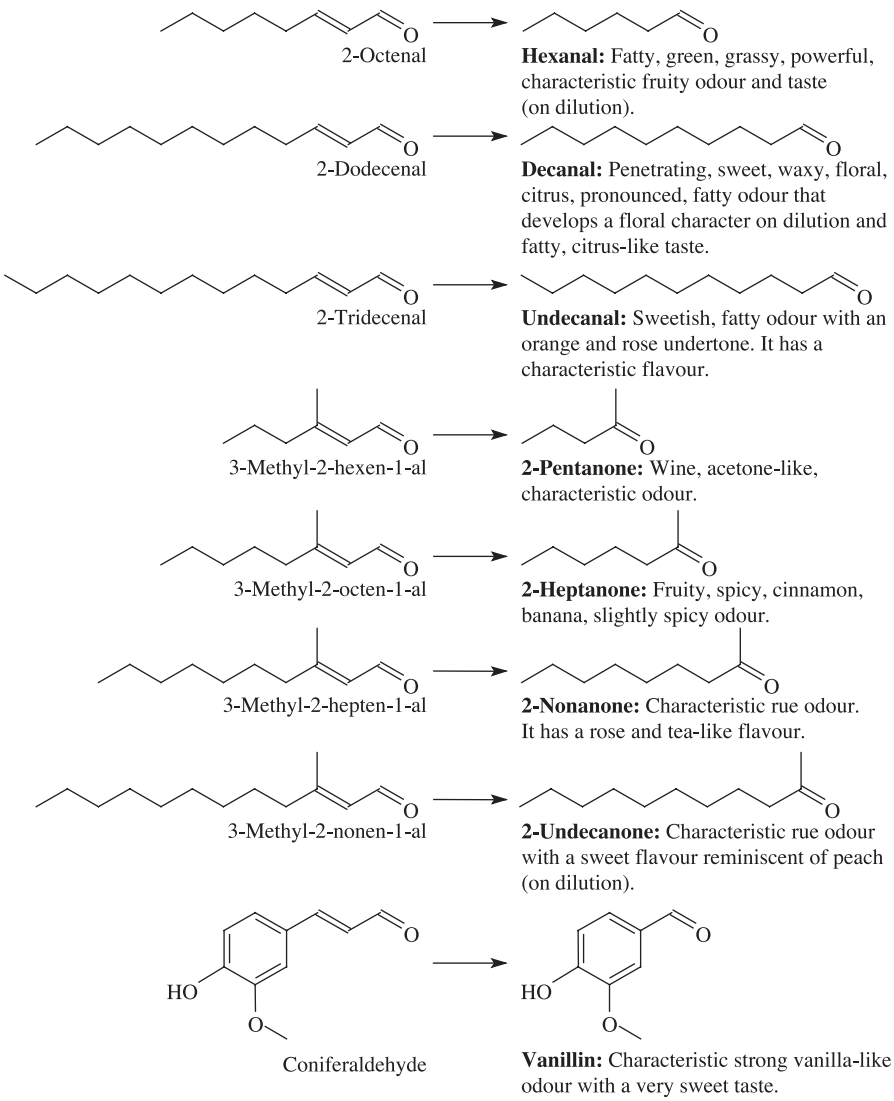


Figure 4. Possible extension of the amino acid-catalysed conversion of α,β-unsaturated aldehydes for the production of flavour compounds. Aroma descriptions are based on *Fenaroli's Handbook of Flavour Ingredients*.[10]

(aldehyde and ketone) flavour compounds (Figure 4) from α,β-unsaturated aldehydes (Table 1), e.g. the predominant aroma components of Roquefort and Camembert cheeses, 2-heptanone and 2-nonanone,[11] could be produced from the corresponding 3-methyl α,β-unsaturated aldehydes. Finally, the possibility of converting coniferaldehyde (a normal constituent of wood lignin, which is present in sandalwood and conifers[12]) into vanillin (the most important flavour compound from a commercial point of view[13]) is postulated.

Acknowledgements—This work was supported by Grant FAIR CT 98-3559 from the European Community.

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