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Manufacturing by-products from, and stereochemical outcomes of the biotransformation of benzaldehyde used in the synthesis of methamphetamine

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ABSTRACT

Clandestine synthesis of methamphetamine in Australia has predominantly started from pseudoephedrine extracted from over the counter cold and flu medications. However, recently introduced restrictions on the sale of these products have made pseudoephedrine much more difficult to obtain. As a result clandestine chemists have had to resort to other means of obtaining the necessary chemical precursors. A recent drug raid (Adelaide, January 2008) resulted in the seizure of an unusual reaction mixture that indicated a novel approach involving the fermentation of glucose by yeast in the presence of benzaldehyde to give 1-hydroxy-1-phenylpropanone, also known as *l*-phenylacetylcarbinol (*l*-PAC), a known precursor to ephedrine and pseudoephedrine and hence methamphetamine. A study was undertaken into this process with the aim of determining the characteristic reaction by-products associated with methamphetamine made in this way. The study also looked at the stereochemical selectivity of the fermentation reaction and the stereochemistry of the subsequent reaction products, ephedrine and pseudoephedrine, and the final methamphetamine.

1. Introduction

Methamphetamine **1** is a highly addictive psychostimulant drug [1]. It is one of the most frequently abused illicit drugs, with approximately 35 million regular users worldwide [2,3]. In Australia, it is the second most commonly abused illicit drug and the main amphetamine-type stimulant (ATS) confiscated by law enforcement agencies [4]. The main supply of methamphetamine is from domestic clandestine laboratories [5], though most crystal methamphetamine ('ice') is imported from south-east Asia [6].

Domestic clandestine production has predominantly started from pseudoephedrine **2**, extracted from commercial cold and flu medications [7]. The hypophosphorous acid method is one of a number of methods commonly used to convert ephedrine **3** or pseudoephedrine **2** to methamphetamine **1** [8,9]. However, recent changes in Australian Government regulations have restricted the availability of pseudoephedrine **2** based cold and flu medications, and so have reduced the availability of this common precursor to methamphetamine **1**. As a result clandestine chemists have been forced to look for alternative starting materials requiring more novel approaches. During a police drug raid in Adelaide in January 2008 an unusual fermentation reaction was confiscated. GC–MS analysis of an extract of a fermentation 'broth' detected benzaldehyde **4** and most interestingly, *l*-PAC **5**, a known precursor of ephedrine **3** and pseudoephedrine **2**, and consequently methamphetamine **1**, as illustrated in Scheme 1. Though there have been several publications describing this biotransformation of benzaldehyde **4** to *l*-PAC **5** [10–15], up until this case, there have been no local clandestine drug laboratories seized that have used this approach.

Determining the synthetic route associated with a methamphetamine seizure can provide useful intelligence to assist in criminal investigations to establish relationships between drug seizures, drug sources and drug trafficking routes. The ability to identify the manufacturing route also gives an insight into current synthetic trends that may lead to the control of the starting materials and chemical reagents, so potentially reducing production, trafficking and use of the drug [16,17]. This 'impurity profiling' can be achieved through the identification of characteristic impurities found in the drug that arise from the specific starting materials, synthetic route, reaction conditions and purification techniques used [16,18]. The most commonly used method for detecting these by-products is gas chromatography–mass spectrometry (GC–MS). This particular method is used because of its ability to separate complex mixtures and detect trace amounts of compounds [17], and because the mass spectrum provides a highly characteristic 'fingerprint' of the compounds

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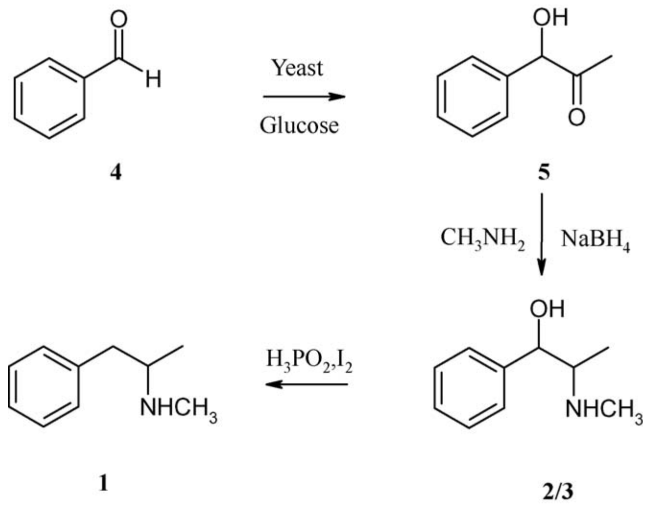

Scheme 1. Biotransformation of benzaldehyde to *l*-PAC leading to methamphetamine.

detected that can be used to help identify them by MS library database matching or by structural analysis from the fragmentation pattern.

GC–MS analysis of the seized fermentation mixture (Fig. 1) confirmed the presence of *l*-PAC **5** and also identified a number of interesting by-products. Two of these, 1-phenyl-propan-1,2-dione **6** and 2-hydroxy-1-phenyl-propan-1-one **7** were of particular interest as they were thought to be indicative of the biotransformation, and as they contain the ketone functional group, both compounds were expected to undergo reductive amination along with *l*-PAC **5** and carry on through the subsequent step to the final methamphetamine **1**, and so yield potential characteristic by-products.

Hence the aim of this study was to determine and fully characterise forensically useful chemical impurities derived from the biotransformation of benzaldehyde **4** that carry through the subsequent steps into the final methamphetamine **1**. To achieve this, *l*-PAC **5** from the biotransformation of benzaldehyde **4** was converted to ephedrine **3** and then to methamphetamine **1**. GC–MS analysis at the end of each step was used to monitor the impurities through to the final methamphetamine **1**. Characterisation of the impurities was achieved by matching their retention times and mass spectra to those of chemical standards that were independently synthesised and their structures confirmed by NMR analysis. This approach has been used recently in the determination of chemical markers from the oxidation of safrole and isosafrole in the clandestine manufacture of MDMA [19,20]. In addition to this, the study aimed to determine the stereoselectivity of *l*-PAC **5** formed in the initial fermentation process and the stereochemistry of the subsequent reaction products through to the final methamphetamine **1**.

2. Experimental procedures

2.1. Chemicals and reagents

Premium Bakers' Yeast (Lowan Whole Foods) was purchased from a local supermarket. Celite (acid washed), sodium borohydride, propiophenone, (*S*)-(−)-*N*-(trifluoroacetyl)prolyl chloride (TPC) and 1-phenyl-1,2-propandione were obtained from Sigma–Aldrich, Sydney, Australia. Glucose (analytical reagent), benzaldehyde and methylamine (44% in water) were obtained from AJAX Fine Chemical, Sydney Australia. Methylamine (33% in ethanol) was obtained from BDH Chemicals, Poole, England. Pentafluoropropionic anhydride (PFAA) was obtained from Pierce Protein Research Products, a Division of Thermo Fisher Scientific, Rockford, IL, USA. Ephedrine, pseudoephedrine and methamphetamine were in house standards calibrated against primary reference standards obtained from the National Measurement Institute, Sydney, Australia. All solvents and other reagents used in this work were of analytical reagent grade and were purchased from Sigma–Aldrich, Sydney, Australia.

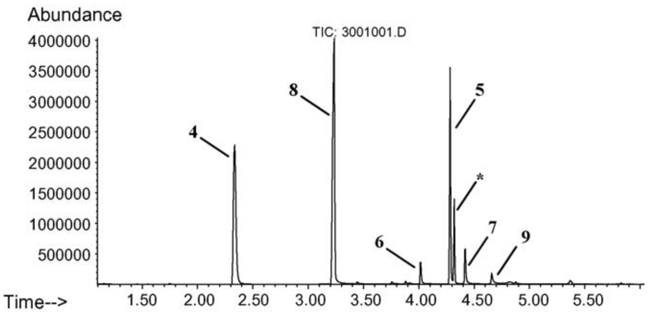

Fig. 1. Gas chromatogram of the seized fermentation of benzaldehyde to *l*-PAC.

2.2. Instrumentation

GC–MS analyses were performed on a Hewlett Packard 6890 plus gas chromatograph using an Agilent HP-1MS capillary column (15 m × 0.25 mm × 0.25 μm) fitted with a Hewlett Packard 5973 mass selective detector. The carrier gas used was helium. The injector temperature was set to 300 °C, with an initial oven temperature of 60 °C, then ramped at 45 °C/min to 300 °C and held there for 4 min. The mass selective detector operated between m/z = 40 and 500 in electron impact (EI) mode with an ionization energy of 70 eV.

Chiral analyses were performed on a Perkin Elmer Clarus 500 GC–MS using a Cyclosil-B 112–6632 chiral column (30 m × 0.25 mm × 0.25 μm). The carrier gas used was helium. The injector temperature was set to 250 °C with an initial oven temperature of 90 °C, then ramped at 5 °C/min to 250 °C and held there for 10 min. The mass spectrometer operated from m/z = 40 to 500 in EI mode with an ionization energy of 70 eV.

Accurate masses were obtained using an Agilent 6510 Q-TOF LC–MS (quadrupole-time-of-flight liquid chromatography–mass spectrometry) operated in positive ion mode with a mass range of m/z = 50–3200. Samples were directly infused into the dual ESI (electrospray ionization) source at 250 μL/h. Nitrogen was used as the drying gas at 350 °C with a flow rate of 5 L/min. The acquisition rate was 1.02 spectra/s and acquisition time was 976.1 ms/spectrum. Capillary voltage (VCap) was set at 3500 V. The nebuliser pressure was set at 15 psig.

Proton nuclear magnetic resonance (NMR) spectra were obtained using a Varian Gemini 200 MHz or Varian 600 MHz spectrometer as specified. The solvent used was deuterochloroform ($CDCl_3$), with tetramethylsilane (TMS) as the reference compound. Chemical shifts were recorded in parts per million (ppm) relative to TMS and the coupling constants (*J*) in hertz (Hz).

2.3. Extraction of the seized fermentation mixture

The seized fermentation mixture (500 mL) was extracted with ethyl acetate (2× 500 mL). The ethyl acetate extracts were combined, dried over anhydrous Na_2SO_4, filtered and evaporated to yield a pale yellow oil (1.78 g) that gave six major constituents by GC–MS (refer to Fig. 1).

GC–MS (t_r): 2.35 min (benzaldehyde **4**), 3.25 min (benzyl alcohol **8**), 4.05 min (1-phenylpropan-1,2-dione **6**), 4.30 min (*l*-PAC **5**), 4.45 min (2-hydroxy-1-phenylpropan-1-one **7**), 4.65 min (1-phenylpropan-1,2-diol **9**).

Note these structural assignments were based on MS library data matching and/or GC–MS comparison with in house standards [17].

2.4. Synthetic procedures—synthesis of manufacturing by-products

2.4.1. N-Methylbenzylamine

A solution of 33% methylamine in ethanol (2.87 mL) and ethanol (11.2 mL) was added to benzaldehyde (0.508 g, 4.78 mmol) and allowed to sit at room temperature for 30 min. The solution was cooled to 0 °C and then sodium borohydride (0.550 g, 14.5 mol) was added to the mixture and allowed to sit for 3 h after which additional sodium borohydride (0.189 g, 5.0 mmol) and a solution of 10% hydrochloric acid (1.5 mL) was added. A solution of 10% sodium hydroxide (10 mL) was then added to the mixture and extracted with CH_2Cl_2 (3× 5 mL). The CH_2Cl_2 extracts were combined and extracted with a solution of 10% hydrochloric acid (10 mL). The aqueous layer was then basified and extracted with CH_2Cl_2 (3× 5 mL). The CH_2Cl_2 extracts were combined, dried (anhydrous Na_2SO_4), filtered and evaporated to yield a pale yellow oil (0.237 g) consisting of *N*-methylbenzylamine **11**.

2.4.2. N^1,N^2-Dimethyl-1-phenylpropan-1,2-diamine

Methylamine 40% in water (1.175 mL) and ethanol (0.445 mL) was added to 1-phenyl-1,2-propandione (0.2101 g, 1.42 mmol) and allowed to sit at room temperature for 30 min. The solution was cooled to 0 °C and then sodium borohydride (0.155 g, 4.11 mmol) was added to the mixture and allowed to sit for 1 h. A solution of 10% hydrochloric acid (5 mL) was added to the mixture and washed with CH_2Cl_2 (2× 5 mL). The aqueous layer was then basified with a solution of 10% sodium hydroxide (5 mL) and extracted with CH_2Cl_2 (2× 5 mL). The CH_2Cl_2 extracts were combined, dried (anhydrous Na_2SO_4), filtered and evaporated to yield an oil (0.1736 g) consisting of N^1,N^2-dimethyl-1-phenyl-propan-1,2-diamine **12**.

^{1}H NMR ($CDCl_3$, 600 MHz)[1] δ 0.96 (d, 3H, –CHC*H*$_3$, 6.45 Hz), 1.6–1.8 (s, 2H, –N*H*CH$_3$), 2.29 (s, 3H, –NHC*H*$_3$), 2.41 (s, 3H, –PhCHNHC*H*$_3$), 2.73 (dq, 1H, –C*H*CH$_3$, 4.7, 6.45), 3.45 (d, 1H, CHC*H*NHCH$_3$, 4.7), 7.2–7.36 (m, 5H, Ar–*H*).

2.4.3. 1-(Methylamino)-1-phenyl-propan-2-ol

2.4.3.1. 2-Bromopropiophenone. Bromine (7.17 g, 44.8 mmol) was added drop wise to a stirred solution of propiophenone (5 g, 36.2 mmol) and ether (25 mL) at 0 °C. The solution was then stirred at room temperature until a colour change was observed. Saturated sodium thiosulphate (20 mL) was then added to the mixture and it was extracted with ethyl acetate (2× 30 mL). The ethyl acetate extracts were combined, dried (anhydrous Na_2SO_4), filtered and evaporated to yield a yellow-brown oil (7.67 g) consisting of 2-bromopropiophenone.

^{1}H NMR data ($CDCl_3$, 200 MHz): δ 1.90 (d, 3H, –CHBrC*H*$_3$, 6.44 Hz), 5.30 (q, 1H, –C*H*BrCH$_3$, 6.44), 7.50 (m, 3H, Ar–*H*), 8.05 (m, 2H, Ar–*H*).

2.4.3.2. Conversion of 2-bromopropiophenone to 2-hydroxy-1-phenylpropan-1-one. Acetone (125 mL) was added to a stirred solution of 2-bromopropiophenone (8.41 g, 37.7 mmol) and water (85 mL) at 0 °C until homogenous. Sodium hydroxide (1.53 g, 38.1 mmol) was added to the mixture and allowed to stir at 0 °C for 15 min. Water (675 mL) was added to the mixture which was then extracted with ethyl acetate (2× 120 mL). The ethyl acetate extracts were combined, dried (anhydrous Na_2SO_4), filtered and evaporated to yield a pale yellow oil (3.30 g) consisting of 2-hydroxy-1-phenyl-propan-1-one **7**.

^{1}H NMR data ($CDCl_3$, 200 MHz): δ 1.45 (d, 3H, –CHOHC*H*$_3$, 6.44 Hz), 3.65 (s, 1H, –O*H*) 5.20 (q, 1H, –C*H*OHCH$_3$, 6.44), 7.50 (m, 3H, Ar–*H*), 7.95 (m, 2H, Ar–*H*).

2.4.3.3. Conversion of 2-hydroxy-1-phenylpropan-1-one to 1-(methylamino)-1-phenyl-propan-2-ol. A solution of 33% methylamine in ethanol (11.7 mL) and ethanol (6.5 mL) was added to 2-hydroxy-1-phenylpropan-1-one (2.90 g) obtained from Section 2.4.3.2 and allowed to sit at room temperature for 30 min. The solution was cooled to 0 °C and then sodium borohydride (2.16 g, 58.0 mmol) was added to the mixture and allowed to sit for 1 h. A solution of 10% sodium hydroxide (20 mL) was added to the mixture and extracted with CH_2Cl_2 (3× 10 mL). The combined CH_2Cl_2 layers were extracted with a solution of 10% hydrochloric acid (20 mL). The aqueous layer was basified with a solution of 10% sodium hydroxide (20 mL) and extracted with CH_2Cl_2 (3× 20 mL). The CH_2Cl_2 extracts were combined, dried (Na_2SO_4), filtered and evaporated to yield a pale yellow oil (0.691 g) consisting of 1-(methylamino)-1-phenyl-propan-2-ol **13**.

^{1}H NMR ($CDCl_3$, 600 MHz):

> *Major diastereomer:* δ 1.00 (d, 3H, –CHOHC*H*$_3$, 6.44 Hz), 2.30 (s, 3H, –NHC*H*$_3$), 3.48 (d, 1H, –C*H*NHCH$_3$, 4.10), 4.00 (m, 1H, –C*H*OHCH$_3$), 7.10–7.40 (m, 5H, Ar–*H*).
>
> *Minor diastereomer:* δ 0.97 (d, 3H, –CHOHC*H*$_3$, 6.44 Hz), 2.20 (s, 3H, –NHC*H*$_3$), 3.12 (d, 1H, –C*H*NHCH$_3$, 8.78), 3.72 (m, 1H, –C*H*OHCH$_3$), 7.10–7.40 (m, 5H, Ar–*H*).

2.4.4. Synthesis of N-methyl-1-phenylpropan-1-amine

A solution of 33% methylamine in ethanol (4.53 mL) and ethanol (2.23 mL) was added to propiophenone (1.01 g, 7.31 mmol) and allowed to sit at room temperature for 24 h. The solution was cooled to 0 °C and then sodium borohydride (0.877 g, 23.2 mmol) was added to the mixture and allowed to sit for 2 h. A solution of 10% sodium hydroxide (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3× 10 mL). The combined CH_2Cl_2 layers were extracted with a solution of 10% hydrochloric acid (10 mL). The aqueous layer was basified with a solution of 10% sodium hydroxide (15 mL) and extracted with CH_2Cl_2 (3× 10 mL). The CH_2Cl_2 extracts were combined, dried (anhydrous Na_2SO_4), filtered and evaporated to yield a pale yellow oil (0.240 g) consisting of *N*-methyl-1-phenylpropan-1-amine **15**.

^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.80 (t, 3H, –CH$_2$C*H*$_\mathbf{3}$, 7.4 Hz), 1.5–1.7 (s, 1H, –N*H*), 1.68 (m, 2H, –CHC*H*$_a$*H*$_b$CH$_3$), 2.25 (s, 3H, –NHC*H*$_3$), 3.42 (dd, 1H, –CH$_2$C*H*NHCH$_3$, 5.85, 7.85), 7.2–7.4 (m, 5H, Ar–*H*).

2.5. Chiral studies

2.5.1. Sodium borohydride reduction of 1-phenylpropan-1,2-dione

Sodium borohydride (3.40 mg, 0.09 mmol) was added to 1-phenyl-1,2-propan-dione (0.107 g, 0.72 mmol) dissolved in methanol (0.50 mL) and allowed to sit at room temperature for 10 min.

2.5.2. Procedure for the PFAA derivatisation of ephedrine and pseudoephedrine

A small sample of ephedrine/pseudoephedrine (~1 mg) was added to a gas chromatography vial containing CH_2Cl_2 (0.5 mL). Pentafluoropropionic anhydride (PFAA) (20 μL) was added to the vial and allowed to stand for 10 min. The reaction was quenched with water (50 μL) and the aqueous layer removed immediately prior to GC–MS analysis.

2.5.3. Procedure for the TPC derivatisation of methamphetamine

A small sample of methamphetamine (~1 mg) was added to a gas chromatography vial containing iso-octane (0.5 mL). (*S*)-(−)-*N*-(trifluoroacetyl)prolyl chloride (TPC) (50 μL) was added to the vial and allowed to stand for 10 min prior to GC–MS analysis.

3. Results and discussion

3.1. Biotransformation of benzaldehyde to l-PAC

The pathway involved in the formation of *l*-PAC **5**, via the fermentation of glucose in the presence of benzaldehyde **4**, and the major by-products associated with the reaction are shown in Scheme 2 [12]. Formation of *l*-PAC **5** is catalysed by the enzyme pyruvate decarboxylase (PDC), where thiamine pyrophosphate (TPP) and magnesium ions are required as cofactors [11]. The reaction involves condensation of benzaldehyde with an active "acetaldehyde" arising from the decarboxylation of pyruvic acid, a metabolite of glucose [11,12]. Alcohol dehydrogenase (ADH), the enzyme normally responsible for the reduction of acetaldehyde to ethanol, reduces benzaldehyde to benzyl alcohol **8** and *l*-PAC **5** to 1-phenylpropan-1,2-diol **9** both of which were detected in the gas chromatogram. A more detailed description of this process can be found in Tripathi et al. [14].

In the present study, the biotransformation of benzaldehyde **4** to *l*-PAC **5** using bakers' yeast was carried out following a slight modification of the procedure described by Ohta et al. [13]. A typical gas chromatogram of an extract of the fermentation is shown in Fig. 2. Peaks can be seen due to benzaldehyde **4**, benzyl alcohol **8**, *l*-PAC **5** and 1-phenylpropan-1,2-diol **9**. Closer analysis (see expansion of the boxed region) reveals peaks corresponding to benzoic acid **10** and the two by-products of interest 1-phenyl-propan-1,2-dione **6**, and 2-hydroxy-1-phenyl-propan-1-one **7**. These structural assignments were made based on mass spectral

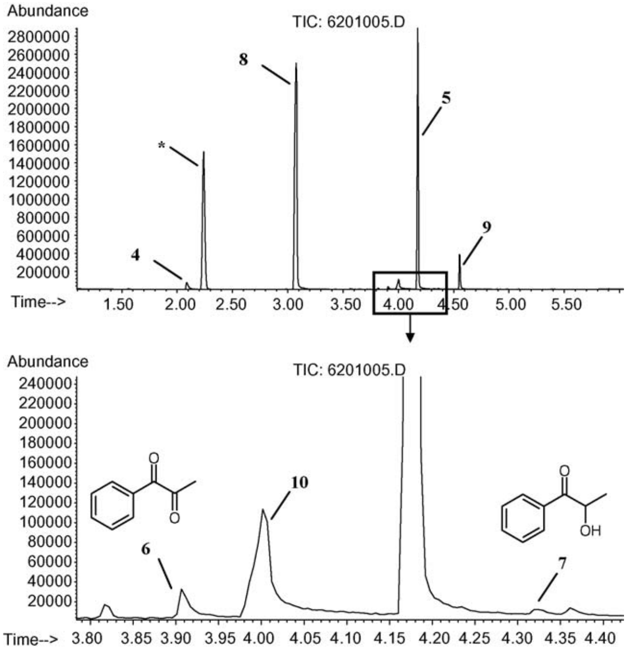

Fig. 2. Gas chromatogram of the fermentation of benzaldehyde to *l*-PAC. *Propylbenzene internal standard.

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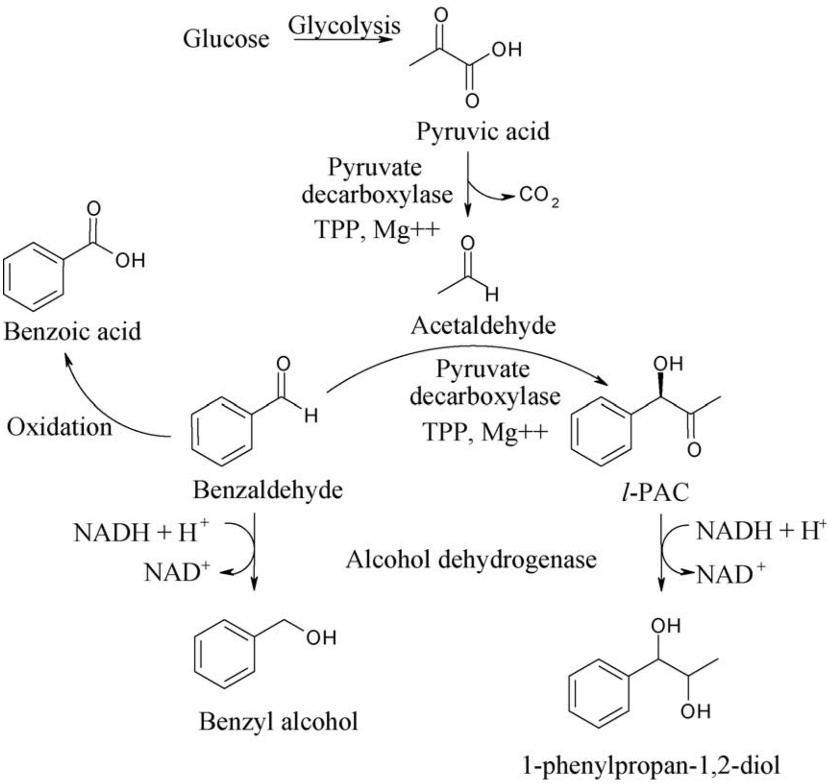
[1] ^{1}H NMR data for major diastereomer only.



Scheme 2. Pathway involved in the formation of *l*-PAC and various by-products.

library database matching, or comparison to the retention time and mass spectra of laboratory standards. This gas chromatogram is comparable to the one from the seized fermentation mixture shown in Section 1 (Fig. 1).

3.2. Reductive amination of l-PAC

The crude *l*-PAC **5** from the biotransformation of benzaldehyde **4** was treated with methylamine (CH_3NH_2), and sodium borohydride ($NaBH_4$) under reductive amination conditions following the method of Shukla et al. [12]. Fig. 3 shows a gas chromatogram of an extract of the result of this reaction. The peak at a retention time of 4.85 min indicated the formation of a significant amount of ephedrine **3**/pseudoephedrine **2** that, under our chromatographic conditions, co-eluted**.** However, the major peak was due to benzyl alcohol **8**, one of the major by-products resulting from the initial fermentation. Closer inspection (see expansion of the boxed region) identified a peak at t_r = 3.43 min that was thought to be due to *N*-methylbenzylamine **11**, that most likely arose from the reductive amination of residual benzaldehyde **4**. Its structure was confirmed by comparison to the retention time and mass spectrum of an independently synthesised sample of *N*-methylbenzylamine **11** (see Section 2.4.1).

Peaks due to the two by-products of interest, 1-phenylpropan-1,2-dione **6** or 2-hydroxy-1-phenylpropan-1-one **7**, were not found in this chromatogram. However, since both contain the ketone functional group, it was expected that they would undergo analogous reductive amination to their corresponding amines to give N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12** and 1-(methylamino)-1-phenylpropan-2-ol **13** respectively, as shown in Scheme 3. Peaks with mass spectra consistent with these two compounds were not immediately obvious in the chromatogram (Fig. 3), however, fragmentation by way of α-cleavage at the benzylic position would be expected to give a common fragment ion of m/z = 120 as shown in Scheme 3.

Reprocessing of the chromatographic data, by extracting ion m/z = 120, resulted in the four peaks shown in Fig. 4(a). Peaks ‘a’ and ‘b’ gave essentially the same mass spectrum (Fig. 4(b)), as did peaks ‘c’ and ‘d’ (Fig. 4(c)). Four peaks were expected since both N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12** and 1-(methylamino)-1-phenylpropan-2-ol **13** have two chiral centres and therefore, each could exist as a pair of diastereomers with different retention times but similar mass spectra.

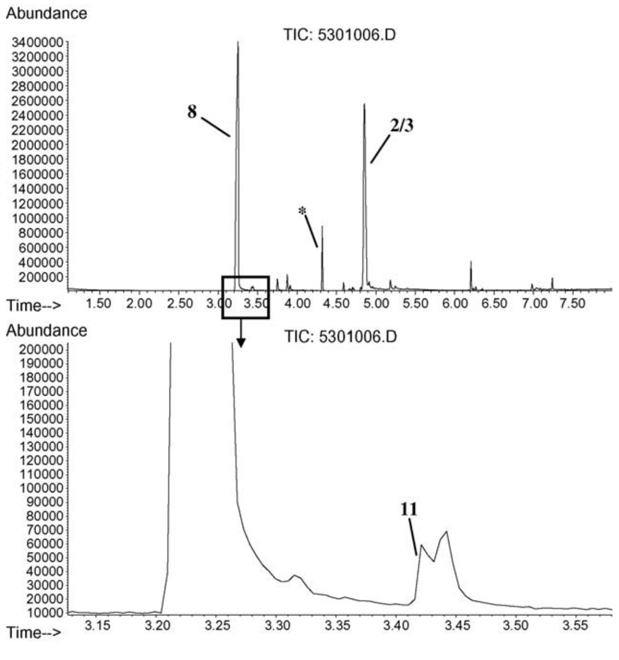
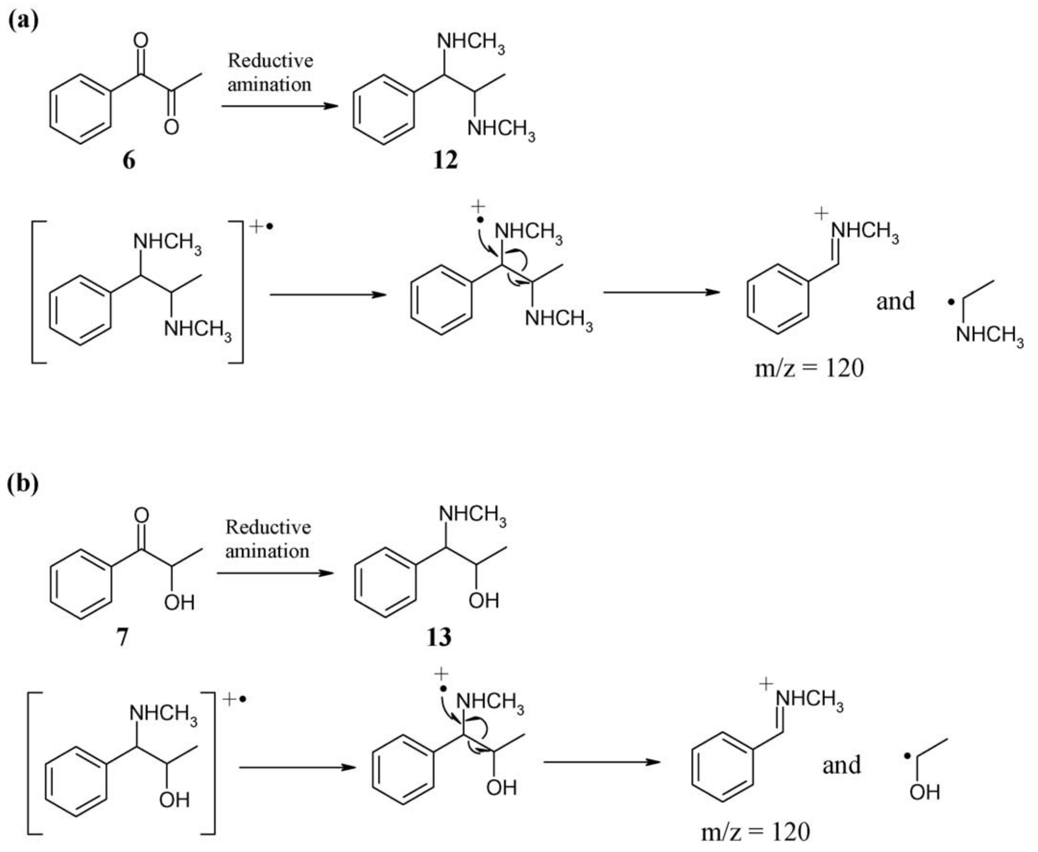


Fig. 3. Gas chromatogram of the reductive amination of *l*-PAC extracted from a sample of the seized fermentation mixture. *Dodecane internal standard.



Scheme 3. Formation and expected mass spectral fragmentation of (a) N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine and (b) 1-(methylamino)-1-phenylpropan-2-ol.

Fragmentation of N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12** was also expected to give a fragment ion at $m/z = 58$ for $C_3H_8N^+$. The mass spectra for peaks 'c' and 'd' (Fig. 4(c)) contained this ion so was consistent with N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12**. To confirm this it was independently synthesised as described in Section 2.4.2, and its structure was confirmed by NMR analysis (also see Section 2.4.2), which also showed the presence of two diastereomers. This structure was further supported by accurate mass measurement, where the experimental mass obtained for the molecular ion was $m/z = 178.1471$ while the calculated mass for $C_{11}H_{18}N_2$ was $m/z = 178.1470$. GC–MS of the synthesised sample resulted in two peaks with the same retention times and same mass spectra as peaks 'c' and 'd'. Consequently, these two peaks were assigned as the diastereomers of N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12**.

Peaks 'a' and 'b' were thought to be due to 1-(methylamino)-1-phenylpropan-2-ol **13**. To confirm this, a sample of 1-(methylamino)-1-phenylpropan-2-ol **13** was also synthesised, as described in Section 2.4.3. Again, NMR analysis (also see 2.4.3), showed the presence of two diastereomers and confirmed the structure. This was further supported by accurate mass measurement, where the mass obtained was $m/z = 165.1154$, identical to the calculated mass for $C_{10}H_{15}NO$, $m/z = 165.1154$. GC–MS of the synthesised sample gave two peaks eluting at the same retention times and having the same mass spectra as peaks 'a' and 'b', confirming that these two peaks were due to the diastereomers of 1-(methylamino)-1-phenylpropan-2-ol **13**. Hence the two by-products of interest, 1-phenyl-propan-1,2-dione **6** and 2-hydroxy-1-phenylpropan-1-one **7** formed in the initial biotransformation had, as expected, undergone reductive amination to their corresponding amines.

3.3. Synthesis of methamphetamine from ephedrine/pseudoephedrine

The hypophosphorous acid or 'hypo cook' method, using hypophosphorous acid and iodine to generate hydroiodic acid (HI) *in situ*, was used to convert the ephedrine **3**/pseudoephedrine **2,** obtained from the reductive amination of *l*-PAC **5,** to methamphetamine **1** [21]. As illustrated by the chromatogram of the product shown in Fig. 5, the major product is the expected methamphetamine **1** along with some unreacted ephedrine **3**/ pseudoephedrine **2**.

Though not immediately obvious in this chromatogram (Fig. 5), the two by-products of interest, N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12** and 1-(methylamino)-1-phenylpropan-2-ol **13** were detected in the product mixture. When the raw GC–MS data was reprocessed using the extracted ion $m/z = 120$ the chromatogram showed four peaks with identical retention times and mass spectra to the four peaks shown in Fig. 4(a) for N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12** and 1-(methylamino)-1-phenylpropan-2-ol **13**. Hence these two compounds may be considered as characteristic manufacturing by-products and their detection in seized methamphetamine may be indicative of a synthetic approach that began with the biotransformation of benzaldehyde **4**.

There also appeared to be several other *N*-benzylated compounds in the final crude product, and several of these have been identified. A number were believed to result from the *N*-benzylation of N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12**, however, only one will be discussed in detail as it relates specifically to the aims of this study. In this reaction mixture a small peak eluted at $t_r = 4.02$ min (Fig. 5, not indicated in the chromatogram) that gave a mass spectrum which was essentially identical to the mass spectrum of 1-(methylamino)-1-phenylpro-

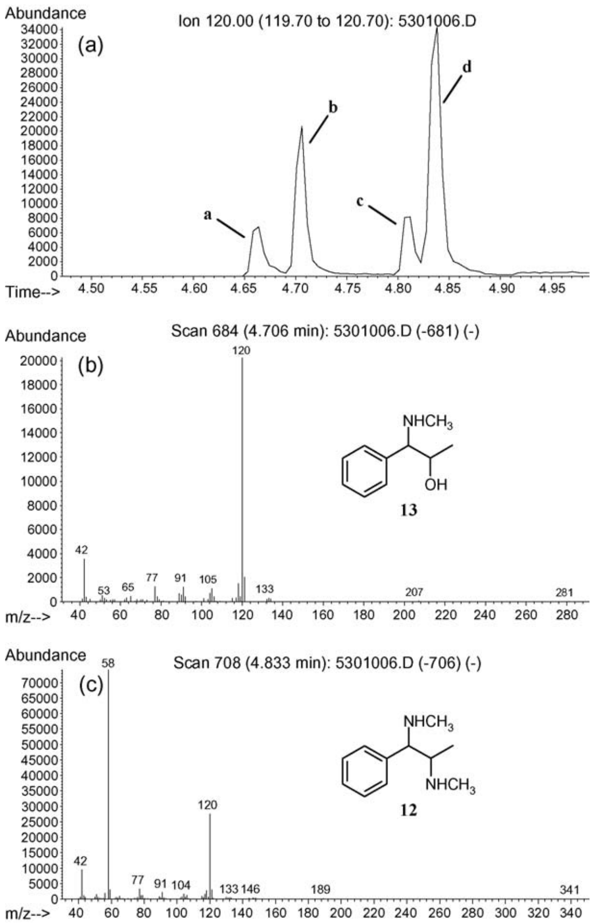


Fig. 4. (a) Partial extracted ion chromatogram resulting from $m/z = 120$, (b) mass spectra of peak 'b' and (c) mass spectra of peak 'd'.

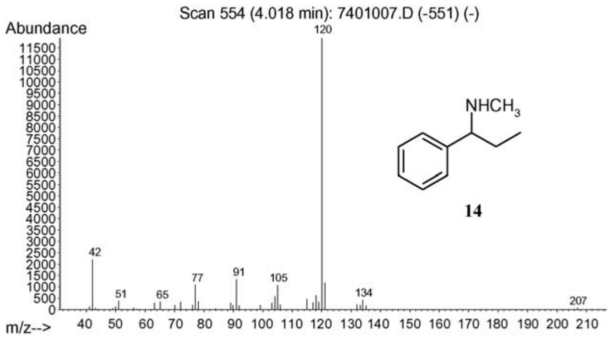


Fig. 6. Mass spectrum of peak $t_r = 4.02$ min, thought to be *N*-methyl-1-phenylpropan-1-amine.

pan-2-ol **13**, shown in Fig. 4(b). However, the peak could not be due to this compound as it had a different retention time. This highlights the fact that the structure of a compound cannot be unambiguously assigned through interpretation of its mass spectrum alone. Instead, this peak was thought to be due to *N*-methyl-1-phenylpropan-1-amine **14,** from the reaction of 1-(methylamino)-1-phenylpropan-2-ol **13** with HI. To confirm this, *N*-methyl-1-phenylpropan-1-amine **14** was synthesised as described in Section 2.4.4 and its structure confirmed by NMR analysis. GC–MS of this synthesised compound resulted in a single peak that eluted at a $t_r = 4.02$ min and gave the same mass spectrum to the one shown in Fig. 6. Consequently, the compound that eluted at 4.02 min in the reaction product extract was determined to be *N*-methyl-1-phenylpropan-1-amine **14**. To further confirm this, a sample of 1-(methylamino)-1-phenylpropan-2-ol **13** was treated under the same 'hypo cook' conditions and this also resulted in the formation of *N*-methyl-1-phenylpropan-1-amine **14**. This compound was of considerable interest as it was shown to be derived from 1-(methylamino)-1-phenylpropan-2-ol **13** and consequently from 2-hydroxy-1-phenyl-propan-1-one **7** and so may be characteristic of the manufacturing process.

In summary then it has been shown that a number of by-products found in the final crude methamphetamine **1** were formed from by-products of the original biotransformation of benzaldehyde **4**. In particular, N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine **12** and 1-(methylamino)-1-phenylpropan-2-ol **13,** the reductive amination products of 1-phenyl-propan-1,2-dione **6** and 2-hydroxy-1-phenyl-propan-1-one **7** were detected along with *N*-methyl-1-phenylpropan-1-amine **14**, from the reaction of 1-(methylamino)-1-phenylpropan-2-ol **13** with HI. These three products appear to be indicative of the manufacture of methamphetamine **1** starting from the biotransformation of benzaldehyde **4**, and may be characteristic of this process.

3.4. Chiral analyses

It has been reported by several authors [10–12,15] that the biotransformation of benzaldehyde **4** leads to enantiomerically pure *l*-PAC **5**. To confirm this, an extract of *l*-PAC **5** was analysed by GC–MS on a Cyclosil-B 112-6632 chiral stationary phase column. The resultant gas chromatogram, shown in Fig. 7, contained two peaks **5** and **15** that had identical mass spectra and were consistent with PAC. Integration of these two peaks gave 99% of one enantiomer, assigned to *l*-PAC **5** and 1% of the other enantiomer, *d*-PAC **15**. To confirm that these two peaks were due to PAC, a sample of 1-phenylpropan-1,2-dione **6** was partially reduced with sodium borohydride, as described in Section 2.5.1. Chiral analysis

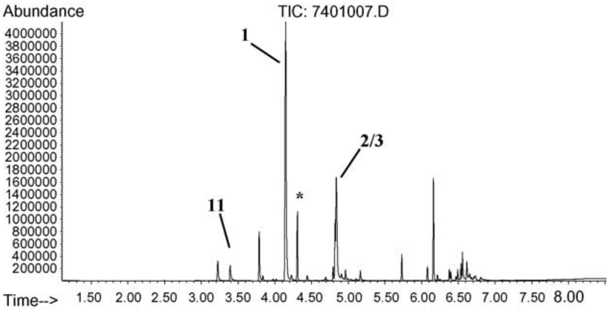


Fig. 5. Gas chromatogram of methamphetamine synthesised in the presence of small amounts of benzyl alcohol. *Dodecane internal standard.

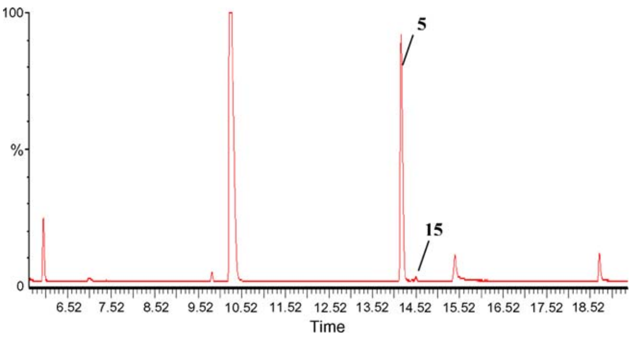


Fig. 7. Gas chromatogram resulting from the analysis of *l*-PAC using a Cyclosil-B 112–6632 chiral stationary phase column.

of the product gave two peaks that had the same retention times and mass spectra as the proposed *l*- and *d*-PAC from the fermentation extract.

As discussed previously, when this crude product was reductively aminated, the chromatogram of the resultant mixture (Fig. 4) gave a presumed co-eluting peak for ephedrine **3**/pseudoephedrine **2**. The two diastereomers should arise due to the formation of a second, new stereocenter at the initially achiral carbonyl carbon, while retaining the configuration (*R* for *l*-PAC) at the chiral benzylic carbon bearing the alcohol. Consequently, this reaction should give the diastereomer (1*R*,2*S*)-ephedrine **3** and (1*R*,2*R*)-pseudoephedrine **2**.

To confirm that the reductive amination of *l*-PAC **5** resulted in the formation of two diastereomers a sample of this reaction product was analysed on the Cyclosil-B 112-6632 chiral column. Unfortunately this was unsuccessful and resulted in broad unresolved peaks unsuitable for our requirements. Hence a sample was derivatised using pentafluoropropionic anhydride (PFAA) as described in Section 2.5.2. The product was analysed on a standard achiral column resulting in the chromatogram shown in Fig. 8(a), containing four peaks. Peaks 'a' and 'b' gave identical mass spectra (Fig. 8(b)) consistent with doubly derivatised ephedrine **3** and pseudoephedrine **2**. Similarly, peaks 'c' and 'd' also gave identical mass spectra (Fig. 8(c)) consistent with singularly *N*-derivatised ephedrine **3** and pseudoephedrine **2**. To determine which peaks corresponded to (1*R*,2*S*)-ephedrine **3**, a reference sample was derivatised and analysed under identical conditions. This resulted in two peaks that had the same retention times and mass spectra as peaks 'a' and 'c', confirming that peak 'a' was due to the double derivative, and that peak 'c' was the single derivative of (1*R*,2*S*)-ephedrine **3**. This was repeated with a reference sample of (1*S*,2*S*)-pseudoephedrine **2** that confirmed that peaks 'b' and 'd' were due to the double and single derivatives of (1*R*,2*R*)-pseudoephedrine **2** respectively.

Integration of the peaks shown in Fig. 8(a), showed that 74% of (1*R*,2*S*)-ephedrine **3** and 26% of (1*R*,2*R*)-pseudoephedrine **2** resulted from the reductive amination of the 99% *l*-PAC **5** and 1% *d*-PAC **15**. The derivatisation of these standards also confirmed that no epimerisation of ephedrine **3** to pseudoephedrine **2** and *vice versa* occurred in this reaction.

This reaction is similar to reduction reactions involving chiral carbonyl compounds where a new stereocenter is formed giving rise to diastereomers. In such cases the product distribution is governed by steric considerations associated with the preferred conformation of the carbonyl group (here an imine) and the preferred direction of attack of the hydride. In such cases, the two diastereomers would be expected to form in unequal amounts and *Cram's rule* [22] can be used to predict the major diastereomer. Scheme 4 shows the mechanism of the reduction of the imine in Newman projection form. The preferred conformation of the imine is between the two least bulky groups (S for small and M for medium) attached to the existing stereocenter. The hydride (H^-) then preferentially attacks from the side of the molecule that contains the small group, the least hindered side. Hence, the major diastereomer is predicted to be (1*R*,2*S*)-ephedrine **3** which is consistent with our experimental result.

Previous attempts at the 'hypo cook' conversion of ephedrine **3** and pseudoephedrine **2** to methamphetamine **1** required long reaction times (many hours) to get complete conversion. However, this is not the case for the hydrochloride salts of ephedrine and pseudoephedrine, which converted completely to methamphetamine in 3 h. The resultant product was expected to be a mixture of *d*-(*S*)- and *l*-(*R*)-methamphetamine **1**. Being enantiomers, they could not be separated on our standard achiral column. So a sample

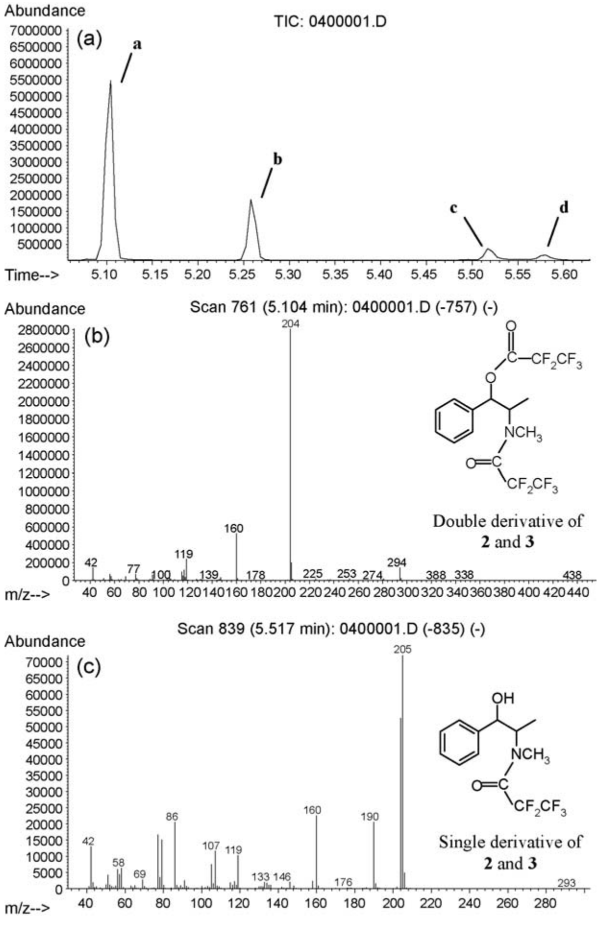
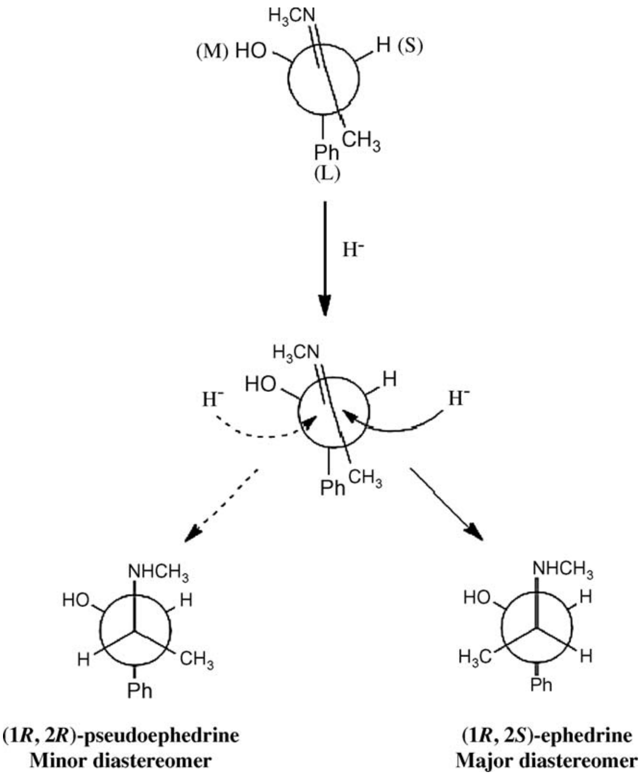


Fig. 8. (a) Gas chromatogram showing peaks corresponding to the double and single derivative of ephedrine and pseudoephedrine, (b) mass spectra for peak 'a' and (c) mass spectra for peak 'c'.



Scheme 4. Mechanism of the reduction of *l*-PAC.

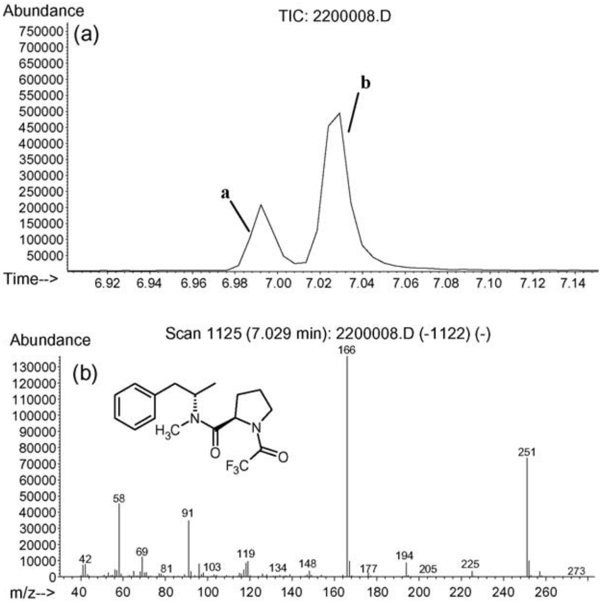


Fig. 9. (a) Gas chromatogram showing peaks for TPC derivatised *d* and *l*-methamphetamine originating from the biotransformation of benzaldehyde and (b) mass spectra of peak ‘b’.

of the crude methamphetamine was derivatised using the chiral derivatising reagent (*S*)-(−)-*N*-(trifluoroacetyl)prolyl chloride (TPC) according to Section 2.5.3 and the resultant product analysed on our standard achiral column. Part of this gas chromatogram appears in Fig. 9(a) showing two peaks that had very similar mass spectra (Fig. 9(b)) and were consistent with TPC derivatised methamphetamine. As expected, the derivatisation gave two diastereomers, from the two enantiomers of methamphetamine **1** that had different retention times but similar mass spectra.

To determine which peak corresponded to *d*-(*S*)-methamphetamine, a reference standard was derivatised and analysed under identical conditions. This resulted in a single peak at the same retention time and with the same mass spectrum as peak ‘b’, confirming that peak ‘b’ was due to the TPC derivative of *d*-(*S*)-methamphetamine and therefore peak ‘a’ was due to the TPC derivative of *l*-(*R*)-methamphetamine. Integration gave 74% of *d*-(*S*)-methamphetamine and 26% of *l*-(*R*)-methamphetamine, the same stereochemical distribution found in ephedrine **3**/pseudoephedrine **2**. Hence methamphetamine originating from the biotransformation of benzaldehyde **4** yielded predominantly the potent *d*-(*S*)-methamphetamine **1**.

4. Conclusion

The biotransformation of benzaldehyde resulted in the formation of a significant amount of *l*-PAC. From this reaction, two by-products, 1-phenyl-propan-1,2-dione and 2-hydroxy-1-phenyl-propan-1-one were monitored through the subsequent stages involved in the synthesis of methamphetamine. Reductive amination of *l*-PAC gave the expected ephedrine and pseudoephedrine. The two by-products, 1-phenyl-propan-1,2-dione and 2-hydroxy-1-phenyl-propan-1-one were also found to undergo analogous reductive aminations to give N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine and 1-(methylamino)-1-phenylpropan-2-ol respectively. Treatment of ephedrine and pseudoephedrine, and in particular their hydrochloride salts, with iodine and hypophosphorous acid resulted in the formation of methamphetamine. N^1,N^2-dimethyl-1-phenylpropan-1,2-diamine and 1-(methylamino)-1-phenylpropan-2-ol were also found to be present in this final product, along with *N*-methyl-1-phenylpropan-1-amine, from the reaction of 1-(methylamino)-1-phenylpropan-2-ol with HI. The presence of these three compounds in a sample of methamphetamine appears to be indicative of its manufacture starting with the biotransformation of benzaldehyde.

Chiral analysis determined that the biotransformation of benzaldehyde resulted in the formation of PAC that was 99% *l*-PAC and 1% of *d*-PAC. Reductive amination of this crude product using methylamine and sodium borohydride gave the desired product that was 74% (1*R*,2*S*)-ephedrine and 26% (1*R*,2*R*)-pseudoephedrine. Treatment of the hydrochloride salts of these two diastereomers with hypophosphorous acid and iodine yielded methamphetamine that was shown to be 74% of the potent *d*-(*S*)-methamphetamine and 26% of the less potent *l*-(*R*)-methamphetamine making this a viable route to this illicit drug.

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