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Isotopic substitution experiments in the hydrogenation of mandelonitrile over a carbon supported Pd catalyst: A nuclear magnetic resonance study

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ABSTRACT

A mechanistic exploration of the liquid phase hydrogenation of the aromatic cyanohydrin mandelonitrile ($C_6H_5CH(OH)CH_2CN$) over a carbon supported Pd catalyst to produce the primary amine, phenethylamine ($C_6H_5CH_2CH_2NH_2$) is conducted. Prior examination showed the reaction to involve the production of the ketone intermediate 2-aminoacetophenone ($C_6H_5C(O)CH_2NH_2$), formed as a consequence of the presence of an acid catalysed tautomeric side reaction. The corresponding deuteration reaction, reported here and analysed by multinuclear NMR spectroscopy and mass spectrometry, is employed to further investigate accessible pathways. Examination of the resultant product distribution of the deuteration, and the location of deuterium incorporation establishes the role of a hydroxy-imine species as a key reaction intermediate. In addition, the acid catalysed tautomerism to the ketone is shown to be a reversible side reaction, but also a contributor to desired product formation. Moreover, an order for the three critical hydrogen consuming steps in phenethylamine formation is established. Hydrogenation of the nitrile functionality to afford the hydroxy-imine precedes hydrogenolysis of the hydroxyl group, with the final step being hydrogenation of the imine to form the target product, phenethylamine.

1. Introduction

The heterogeneously catalysed hydrogenation of nitriles represents an effective and efficient method of affording primary amines [1,2]. Primary amines are valuable feedstocks for numerous industrial applications, including aspects of the agrichemical sector [3–5], where this study finds direct application. Nevertheless, whilst simple aliphatic nitriles and, more recently, aromatic nitriles such as benzonitrile, have been extensively studied, chemical systems of greater complexity have not been examined in as much detail [3,4,6–8].

In the synthesis of some of the more complex materials, where there is the presence of additional functionality, a simple hydrogenation method for reducing the nitrile group is often not sufficient. There are frequently cases where the route to the desired product requires the hydrogenolytic cleavage of one functional group, but simultaneous preservation of another. This is found to be the case for the hydrogenation of the cyanohydrin mandelonitrile (MN: $C_6H_5CH(OH)CH_2CN$), selected as a suitable probe substrate. In this instance, removal of the hydroxy-group, but maintenance of the amine functionality is required in order for the desired primary amine, phenethylamine (PEA: $C_6H_5CH_2CH_2NH_2$), to be selectively produced. What makes this reaction system so complex is not the nitrile reduction reaction step, but instead, the accompanying reactions that occur alongside this process which also need to be managed. Indeed, it is noted that achieving a high selectivity towards the target amine product is a significant challenge in nitrile hydrogenation reactions [9].

Heterogenous catalyst technology is widespread, and in many instances highly successful. There is, however, still opportunity to improve on factors such as efficiency, activity and selectivity [10]. Further, mechanistic awareness of a process can lead to the rational design of catalysts such that improvements in areas such as activity and selectivity can be made [10,11]. For example, E.D. Goodman et al. report the use of the mechanistic understanding of sintering to develop a new stable catalyst which supress specific sintering processes [12].

As a consequence of the aforementioned requirements, and bearing in mind the advances in catalyst design, catalyst choice for nitrile reductions has moved away from the skeletal metals traditionally used, towards milder supported palladium catalysts [2,13–18]. Consequently, a carbon supported palladium catalyst has been selected here for application in the hydrogenation of mandelonitrile.

The liquid phase hydrogenation of mandelonitrile over a 5 % Pd/C catalyst has recently been studied by McAllister et al. [19], revealing a

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degree of mechanistic complexity. As such, a number of possible pathways to the desired product, phenethylamine, were postulated. The preliminary analysis of this substrate system identified that the molecule, 2-amino-1-phenylethanol (2-APE: $C_6H_5CH(OH)CH_2NH_2$) was building-up as a by-product in the reaction mixture due to the slow kinetics of its transformation to phenethylamine. Whilst this finding narrowed down the potential pathways to the desired product, it was found that a degree of mechanistic uncertainty, which could not be fully elucidated by the chromatographic analysis procedures adopted in that study, existed [19].

In this study, deuteration experiments analysed primarily by NMR spectroscopy are utilised to provide greater awareness regarding the complexity of the transformation of mandelonitrile to phenethylamine in a stirred autoclave over a Pd/C catalyst. Hydrogen/deuterium isotopic labelling experiments can be beneficial in mechanistic studies, allowing complex detail regarding reaction pathways and their links to selectivity for catalytic systems to be elucidated [20–22]. A particular example of this is found in the hydrogenation of (*E*)-2-methyl-2-butenoic acid over a cinchonidine modified Pd/C catalyst reported by Sugimura and co-workers. In this instance, the combination of isotopic labelling experiments and chiral analysis reveal that the configuration of the double bond migration and the hydrogenation process may be controlled by the interaction of the reagent ((*E*)-2-methyl-2-butenoic acid) with the cinchonidine adsorbed to the Pd surface [23].

Reaction schemes, accounting for the experimental observations for the hydrogenation and deuteration reactions of mandelonitrile, and the subsequent analysis, are presented. It is important to note that all analysis was conducted on the phenethylamine hydrogen sulphate salt isolated from either the hydrogenation or the deuteration of mandelonitrile. Salt formation was incurred as a consequence of the sulphuric acid additive presence, found to be essential for successful mandelonitrile conversion [19]. This analysis, however, relates only to phenethylamine production. Though this provides useful insight into the production of this molecule, a more global consideration of the reaction system must not be overlooked.

2. Experimental

2.1. Materials

A commercial grade 5 % Pd/C catalyst (Sigma Aldrich, code number: 205680) was exclusively used. This catalyst has been used previously in a preliminary study of the mandelonitrile hydrogenation reaction and, consequently, a detailed characterisation of the catalyst has been reported prior by the authors [19]. Briefly, the percentage metal loading was determined by both atomic absorption spectroscopy and Inductively Coupled Plasma – Optical Emission Spectrometry, and found to be 3.66 % and 4.12 % respectively. From pulsed carbon monoxide chemisorption measurements a Pd dispersion of 39 % and a mean particle size of 2.77 nm was obtained. In agreement with CO chemisorption data, TEM analysis reveals a reasonably narrow particle size distribution with the majority of particles ranging from 2 to 3 nm in diameter. Nitrogen physisorption revealed a specific surface area of $775 \pm 19\,m^2\,g^{-1}$.

Mandelonitrile (Tokyo Chemical Industry, > 97 % purity) and sulphuric acid (Fischer Scientific, nominally 95–98 % H_2SO_4) are used, as received, without further purification. In all instances methanol (Riedel-de Haën, 99.9 % purity) was utilised as the reaction solvent.

An automated gas flow controller (BPC 1202) allowed delivery of inert (N_2, BOC, 99.9 % purity) and active (H_2, BOC, ≥99.8 % purity/ D_2, BOC, ≥99.8 % purity) gases to be delivered to the reactor via a gas reservoir. Helium gas (BOC, 99.9 % purity) was also employed for regent degassing.

2.2. Hydrogenation/deuteration reaction procedure

The hydrogenation/deuteration reactions were carried out in a 500 mL stirred autoclave (Büchi Glas Uster). The reactor was first charged with catalyst (300 mg) and solvent (310 mL methanol) prior to being purged with inert gas (nitrogen). The catalyst/solvent mixture was heated and stirred at approximately 300 rpm under hydrogen/deuterium for one hour in order to reduce the catalyst. The reaction vessel was heated by silicon oil passed around the reactor via a heating circulator (Julabo, F25). In a separate vessel, the substrate (11 mmol mandelonitrile) was combined with two molar equivalents of sulphuric acid, dissolved in 40 mL reaction solvent and degassed under helium.

Once the desired temperature (40 °C) was obtained, substrate addition was made, the system was sealed, and the hydrogen/deuterium pressure elevated. When the reaction pressure was attained, agitation was commenced at 1050 rpm with the reaction start time occurring once the pressure of the system stabilised at the chosen value. This agitation speed is found to be on the plateau region of the rate versus agitation plot for the consumption of mandelonitrile, signifying the reaction to be under kinetic control for this transformation [24].

2.3. Analysis: nuclear magnetic resonance spectroscopy

For the hydrogenation of mandelonitrile, ^1H and ^{13}C NMR spectroscopies were conducted using a Bruker AVI 400 MHz spectrometer. For the deuteration of mandelonitrile both 1D NMR (^1H, ^2H and ^{13}C) and 2D (HSQC – Heteronuclear Single Quantum Coherence Spectroscopy) data were acquired. The ^2H analysis was run, without dilution by a deuterated solvent, on a Bruker AVIII 500 MHz spectrometer. Both ^1H and ^{13}C were obtained using a Bruker AVIII 600 MHz with 5 mm TCI cryoprobe (303 K). In order to achieve a quantitative ^{13}C NMR spectra (deuteration reaction) two parameter changes, from standard, were required. Firstly, as the various components of the reaction mixture may have different relaxation times, the relaxation delay (D1) was increased to 30 s. This action satisfies system relaxation requirements. Secondly, the decoupling power used during the relaxation delay was reduced to prevent potential build-up of the Nuclear Overhauser Effect (NOE). ^1H-^{13}C correlation HSQC for the deuterated sample was also undertaken. These data were collected using a DEPT (Distortionless Enhancement by Polarisation Transfer) edited sequence resulting in a different phase for CH/CH_3 and CH_2 signals. To obtain high resolution, this spectrum was run with a reduced ^{13}C sweep width (10 ppm) centred between the signals of interest [25]. Additionally, for the HSQC experiment the sample was diluted due to the high ionic strength. In all instances, methanol-d_4 was utilised as the deuterated solvent for analysis. Subsequent data handling was undertaken using the MestReNova program.

2.4. Analysis: mass spectrometry

Measurements were performed using a Bruker microTOFq High Resolution Mass Spectrometer. An ESI ion source was used and analysis was performed in positive ionisation mode.

3. Results

3.1. The catalytic deuteration of mandelonitrile

A previous study undertaken on this reaction system utilising high performance liquid chromatography (HPLC) as an analytical tool provided useful, but rudimentary, information on the reaction scheme [19]. Despite the insight gleaned, the analytical method did not allow for the identification of short-lived intermediates or surface species, such as imines and enamines. As such, the mechanistic understanding was restricted. Consequently, a strategy to overcome the aforementioned limitations is proposed. Herewith, the deuteration of

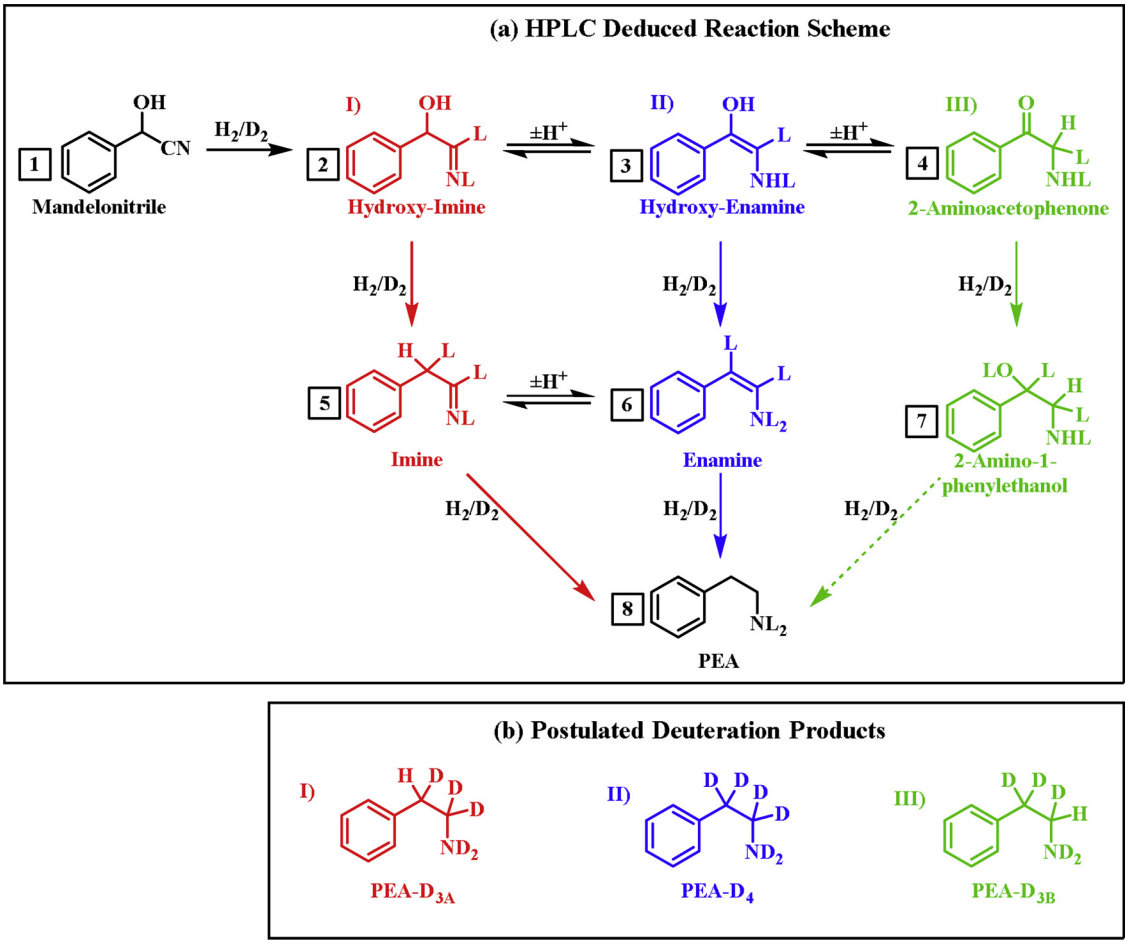


Fig. 1. (a) Postulated reaction scheme based on HPLC analysis [19] and (b) the postulated deuteration of phenethylamine based on the three potential forward reaction pathways (I–III) from reagent to product. Pathways I–III have been coloured red, blue and green respectively for lucidity. Numbers have been associated with each species for clarity in later analyses. Subscripts 'A' and 'B' have been used to highlight positioning of H atom in the deuterated products. In this instance **L** denotes either H or D, and is dependent on the active gas used. The presented scheme has been adapted from reference [19]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mandelonitrile is invoked to supplement the corresponding hydrogenation study. Further, the use of isotopic labelling as a means of following the reaction pathway provides a potential method of distinguishing the postulated pathways and minimise the residual mechanistic uncertainty.

With focus on the preferred product, phenethylamine, three potential forward pathways that afford this molecule, labelled I–III in Fig. 1, have been identified [19]. Each pathway must incur two hydrogenation steps *and* hydrogenolysis of the hydroxyl group in order for phenethylamine to be produced. The presence of a tautomeric pathway, activated as a consequence of the acid additive, necessary to prevent catalyst deactivation [19], opens up the possibility for three molecules to undergo the hydrogenolysis step to ultimately afford phenethylamine. Moreover, it is identified that the molecule undergoing hydrogenolysis is dependent on the pathway taken: the hydroxy-imine (**2**), the hydroxy-enamine (**3**) or, the hydroxy-amine (**7**) for Pathways I, II and III respectively. Also highlighted in Fig. 1 are the proposed outcomes of the deuterium incorporation in the major product for each of the presented pathways. This outcome assumes fast exchange of ND for NH in the presence of acid. Further, analysis focuses primarily on the nature of the C—L bonds defined in Fig. 1. A full schematic representation for each pathway leading to the corresponding anticipated product, showing deuterium incorporation, can be found in the Supplementary material section (Section A: Table S1 and Schemes S1–S8).

The slow reaction kinetics of the conversion of 2-amino-1-phenylethanol to phenethylamine, reported previously [19], has provided an indication that Pathway III (green) is not in occurrence [19]. For completeness, it should be additionally be noted that, in order to afford 2-amino-1-phenylethanol, the tautomeric pathway, originating at the hydroxy-imine (**2**), and proceeding via both the hydroxy-enamine (**3**) and 2-aminoacetophenone (**4**), may be followed. Alternatively, a direct step from either the hydroxy-imine or the hydroxy-enamine may also yield 2-amino-1-phenylethanol [19]. This study therefore aims to confirm that Pathway III is not operational in the formation of phenethylamine within the considered time frame (2 h), but also, and more importantly, to differentiate between Pathways I and II in order to identify which is the active route.

3.2. NMR analysis indicates deuterium incorporation within phenethylamine

Upon completion of both the hydrogenation and the deuteration reactions, NMR spectroscopy of the resultant reaction mixtures was conducted. Preliminary examination of the ^{13}C spectra indicated that, as expected, peaks associated with both 2-amino-1-phenylethanol and phenethylamine were observed (Supplementary material Section B: Fig. S1). Multiplicity observed at a specific chemical shift provides an indication that deuterium has been incorporated at the corresponding carbon atom [26,27]. Deuterium incorporation, as evidenced by peak splitting in the ^{13}C NMR spectrum, was observed at both aliphatic carbons associated with phenethylamine (denoted A and B, and defined in Fig. 2). However, with reference to Fig. 1, where it is shown that at

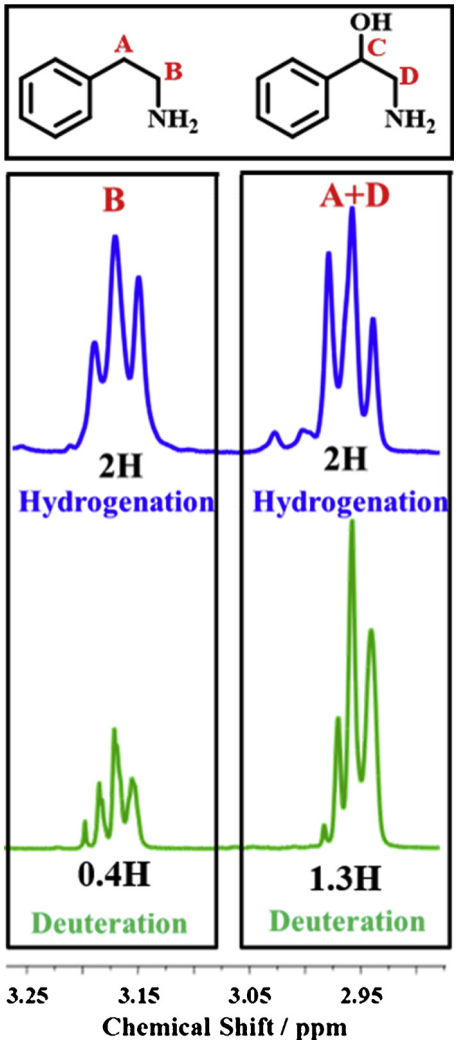


Fig. 2. 400 MHz ^{1}H spectra for the hydrogenation (blue) and deuteration (green) of mandelonitrile from 2.90 to 3.25 ppm. Letters A, B and C, D correspond to aliphatic carbon positions on, respectively, phenethylamine and 2-amino-1-phenylethanol. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

least one deuterium atom is to be expected at both aliphatic positions regardless of the route taken, this finding does not, in itself, provide a great deal of mechanistic insight. Nonetheless, the successful deuteration of mandelonitrile is confirmed.

Further examination of the reaction mixture by ^{1}H NMR spectroscopy does, however, provide some insight. Whilst the main focus of the study is to examine the route from mandelonitrile to phenethylamine, the presence of the by-product 2-amino-1-phenylethanol, and its potential to complicate the resultant spectra, cannot be overlooked. As such, reference spectra taken under hydrogenation conditions were used to determine the possible interference arising from a product mixture. In the aliphatic region peak overlap, resulting from protons associated with carbons A and D, was found to occur at 2.96 ppm (Fig. 2). Although this means that the spectra must be interpreted with care and an awareness of the associated error (typically ± 5 % error for the integrations associated with routinely acquired ^{1}H spectra), the proportion of 2-amino-1-phenylethanol, representing around 5 % of the total (as determined by ^{1}H NMR), can be considered as negligible. Indeed, for the hydrogenation reaction, integration of the aliphatic protons yields a value of 2H at both positions (A and B), as would be expected in the absence of 2-amino-1-phenylethanol.

Also seen in Fig. 2 is the ^{1}H NMR analysis of the deuterium reaction. Here it is observed that integration of the aliphatic protons no longer gives integrations of 2H. Instead, due to a differing degree of deuteration at each carbon, integrations of 1.3H and 0.4H, at carbons A and B respectively, were observed. Thus carbon A is found to be only partially deuterated (*ca.* 35 % D), whereas carbon B is highly deuterated (*ca.* 80 % D). Though not quantitative, this trend was confirmed by deuterium NMR where the peak associated with carbon B was clearly more intense than the peak representing carbon A (Supplementary material Section C: Fig. S2).

The ^{1}H and ^{2}H spectral analysis has allowed the degree of deuteration at both aliphatic positions to be estimated. The detected percentage composition most closely matches the anticipated product of Pathway I according to Fig. 1, where the expected deuteration at carbons A and B is 50 % (experimental *ca.* 35 %) and 100 % (experimental *ca.* 80 %) respectively. Though this result indicates that it is likely that Pathway I is favoured, the hydrogen contribution is shown to be slightly greater than expected. This indicates partial scrambling of H/D within the molecule and, hence the presence of a mixture of isotopologues. In order to further understand the reaction, a more in-depth analysis of the exact product distribution is required.

3.3. Isotopologue distribution for mandelonitrile deuteration as determined by mass spectroscopy

To achieve a more comprehensive understanding, mass spectrometry of the deuterated product mixture yielded from the mandelonitrile deuteration reaction was undertaken, with sampling occuring at reaction completion (t = 2 h). The resultant spectra, presented in Fig. 3, shows peaks representing the two main fragments of phenethylamine [m/z = 122 $(C_6H_5CH_2CH_2NH_3)^+$ and m/z = 105 $(C_6H_5CH_2CH_2)^+$]. The by-product of the reaction, 2-amino-1-phenylethanol, would be expected to exhibit $[MH]^+$ at m/z = 138 and a $[MN–H_2O]^+$ fragment at m/z = 120. Thus, in the deuteration experiment, ions for this fragment may be observed in the m/z = 120–123 range, depending on the level of deuteration. Therefore, overlap of phenethylamine and 2-amino-1-phenethanol in the m/z = 122–126 range is a possibility. The phenethylamine fragment at m/z = 122 was thus excluded from the subsequent analysis. The m/z = 105 fragement, representitive of phenethylamine minus the amine group, is used for analysis. The accompanying peaks (m/z = 106–109) are therefore representitive of the isotopic pattern.

It is proposed in Section 3.2 that the deuterated sample does not contain a single species, but instead, a mixture of different molecules containing an unknown combination of 0–4 deuterium atoms. For a given mixture, a mass spectrum can then be simulated from the linear combination of each of the phenethylamine-D_0–D_4 molecules. The resulting composition is given in Table 1, and further details can be found

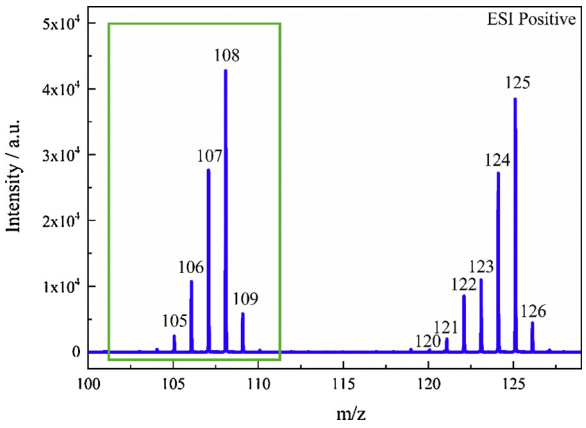


Fig. 3. Mass spectrometric (ESI^+) analysis of the product mixture of deuterated mandelonitrile.

Table 1
Percentage composition of each deuterated species in the reaction mixture as determined by mass spectrometry conducted on the deuterated product mixture at reaction completion (t = 2 h).

Deuterium	D_0	D_1	D_2	D_3	D_4
Mole Percent/%	3.0	11.8	33.7	49.2	2.3

in the Supplementary material section (Section D: Fig. S3).

From the istopic distribution calculated by mass spectrometry, it is deduced that the average hydrogen content of carbons A and B is 1.64 H (*cf. ca.* 1.7 H by ^{1}H NMR, Section 3.2). The results obtained by mass spectrometry are therefore in good agreement with the NMR analysis.

The aforementioned analysis, allowing the exact composition of the mixture to be determined, provides valuable mechanistic information. It is shown in Table 1 that the deuterated sample is composed predominantly of phenethylamine-D_3 species (49.2 %), as would be expected from Pathway I. Moreover, both the phenethylamine-D_0 and the phenethylamine-D_4 contribution have been found to be ≤3 %, and hence can be considered as negligible. It is the absence of phenethylamine-D_4 that enables Pathway II via the hydroxy-enamine to be eliminated. Despite this evidence, which, again suggests that Pathway I is favoured, there is significant production of both phenethylamine-D_1 (11.8 %) and phenethylamine-D_2 (33.7 %). This finding further complicates analysis as these products cannot be rationalised by Pathways I–III.

3.4. Reverse tautomeric reactions rationalise the increased hydrogen content observed

Postulated Paths I–III (Fig. 1) do not account for the occurence of the phenethylamine-D_1 and the phenethylamine-D_2 species detected by mass spectrometry (Section 3.3). Thus, the presence of more than one aliphatic hydrogen, as is implied by the presence of both phenethylamine-D_1 and phenethylamine-D_2, cannot easily be explained. The origin of the observed hydrogen enrichment must therefore be considered further. It should be noted that under the presented reaction conditions in the presence of catalyst, phenethylamine-H_4 does not undergo deuterium scrambling with deuterium gas or deuterated methanol. Therefore, any H/D exchange observed *must* occur at the steps incurred prior to product formation. As such, the increased hydrogen contribution is rationalised by a H/D exchange process with either the solvent (MeOH) or the acid additive (H_2SO_4) on the intermediate species.

It has previously been established that formation of the intermediate species of this reaction, 2-aminoacetophenone (2-AAP: $C_6H_5C(O)CH_2NH_2$), occurs via an acid catalysed tautomeric pathway originating at the hydroxy-imine species [19]. It is therefore suggested that, alongside forward Pathway I, there are other pathways in operation which involve the tautomeric equilibria responsible for 2-aminoacetophenone formation. It is proposed that the acid catalysed reverse reaction, from the 2-aminoacetopheneone to the hydroxy-enamine, and subsequently, the hydroxy-imine results in the increased H incorporation observed.

Scheme 1 shows the first step in the abovementioned reverse tautomeric reaction from 2-aminoacetophenone (**4**) to the hydroxy-enamine (**3**). Here it is shown that tautomerisation of 2-aminoacetopheneone can yield two different hydroxy-enamine species based on whether it is a H or a D atom which is lost from position B, as assigned in Scheme 1. The same principle can also be applied to the second equilibria back to the hydroxy-imine from the hydroxy-enamine (not shown). The subsequent hydrogenation of either of the hydroxy-imine or the hydroxy-enamine isotopologues may then afford a wider product distribution which includes phenethylamine-D_1 and phenethylamie-D_2. The equilibria between species (**2**), (**3**) and (**4**), as defined in Fig. 1, thus provides a route for the enhancement of hydrogen content at position B. In the same manner it is believed that the acid catalysed tautomerisation of the imine intermediate (**5**) into the enamine (**6**) (Fig. 1), allows further hydrogen enhancement at position A.

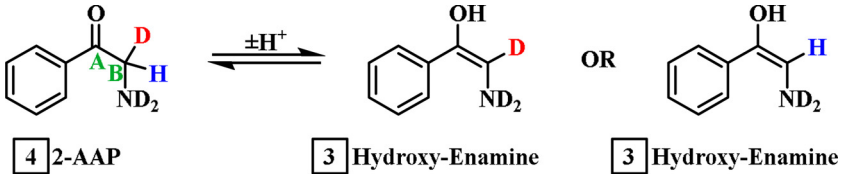
Tautomerisation along the hydrogen enriching equilibria has been shown to be operational, as evidenced by the production of intermediate 2-aminoacetophenone, however, this process takes a secondary role to direct Pathway I which leads to phenethylamine-D_3 as evidenced by the deuterated product distribution determined by mass spectrometry (Table 1). If the equilibrium reactions were faster than the direct reduction (Pathway I), complete scrambling of the deuterium in the molecule would occur resulting in the dominance of either phenethylamine-D_1 or phenethylamine-D_2, depending on the final step. As determined by mass spectrometry this is not the case (Table 1), it can hence be understood that the forward (Pathway I) and the reverse reactions occur concurrently, with the tautomerisation being a reversible side reaction.

3.5. Six isotopologues identified in the ^{13}C NMR mandelonitrile deuteration spectra

In total, there are nine isotopologues which can be produced from the deuteration of mandelonitrile ranging from phenethylamine-D_0 to phenethylamine-D_4. All nine potential isotopologues are presented in Fig. 4. From the mass spectrometry analysis (Section 3.3), it is determined that there are only minimal contributions from phenethylamine-D_0 and phenethylamine-D_4. In addition, Table 1 shows phenethylamine-D_3 to be the major product. Phenethylamine-D_3 may manifest itself as two different isotopologues (phenethylamine-D_{3A} or phenethylamine-D_{3B}, as defined in Fig. 1) depending on the positioning of the H atom. The complementary NMR studies (Section 3.2), however, have indicated that the greater degree of deuteration is on carbon B. This would thus suggest that phenethylamine-D_{3A} is the dominant phenethylamine-D_3 isotopologue.

Adding a further layer of detail regarding the isotopologues present in the reaction mixture, analysis of the splitting pattern for the ^{13}C spectra for the deuteration of mandelonitrile reveals the presence of six out of the possible nine isotopologues. The detection of multiple isotopologues reflects the complex nature of this reaction system. The ^{13}C spectra for both aliphatic carbon atoms are presented in Fig. 5.

For ^{13}C NMR, whilst the protons have been decoupled from the spectra the deuteriums have not. Consequently, different splitting patterns are to be expected based on the number of deuterium atoms present. Peak assignment for each carbon can therefore be primarily achieved using the predicted splitting pattern associated with each species at that location (singlet/triplet/quintet corresponding to CH_2/CHD/CD_2). Moreover, an additional factor to consider is that the presence of deuterium induces an upfield chemical shift on the carbon atoms. This is usually between 0.2–1.5 ppm for a one-bond shift, and around 0.1 ppm for a two-bond shift [28,29]. Consequently, each isotopologue present in the mixture will be expected to have its own set of



Scheme 1. Reverse tautomeric reaction from the ketone, 2-aminoacetopheneone (2-AAP), showing the two possible hydroxy-enamine species.

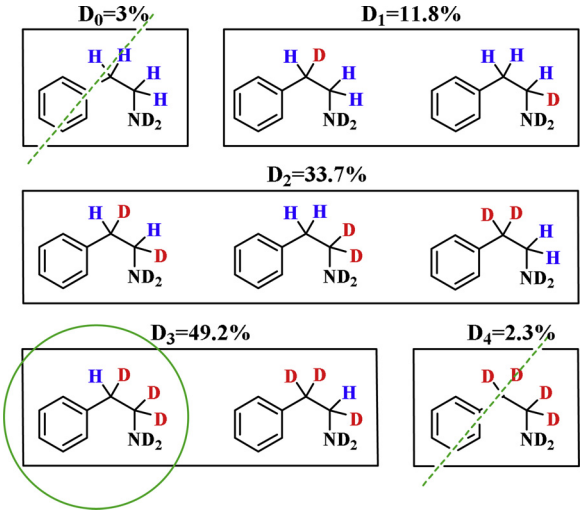


Fig. 4. The nine possible isotopologues from the deuteration of mandelonitrile. The values for the percentage composition of each of the deuterated species (D_0–D_4) originate from Table 1. Highlighted by the dashed green lines is the minimal contribution of phenethylamine-D_0 and phenethylamine-D_4, as determined by the mass spectrometry analysis. Further, the green circle highlights the favoured isotopologue of phethylamine-D_3 as determined by ^{1}H NMR spectroscopy of the deuterated sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

multiplets.

An expansion of the splitting pattern for peak A, which corresponds to the benzylic carbon, can be observed in Fig. 5, left. The spectra can be interpreted as a combination of six isotopologues comprised of three triplets (CHD, δ = 33.74/33.04/34.14 ppm) denoted **a**, **b** and **c**, and three singlets (CH_2, δ = 34.28/34.38/34.47 ppm) designated **d**, **e** and **f** in Fig. 5. Due to the absence of quintets the possibility of D_2 at position A can be eliminated. Therefore, it can be deduced that the composition of carbon A is either CHD or CH_2. The presence of the third peak for triplet **c** is confirmed by 2D DEPT edited HSQC NMR (see Supplementary material, Section E: Figs. S4 and S5).

A similar analysis on carbon B (Fig. 5, right) indicates a different splitting pattern, and thus, it is found that the composition of carbon B is not identical to carbon A. The splitting pattern of carbon B is comprised of two quintets (CD_2, δ = 41.39/41.45 ppm), two triplets (CHD, δ = 41.69/41.75 ppm) and two singlets (CH_2, δ = 41.98/42.03 ppm). The three signals corresponding to the three unique compositions of

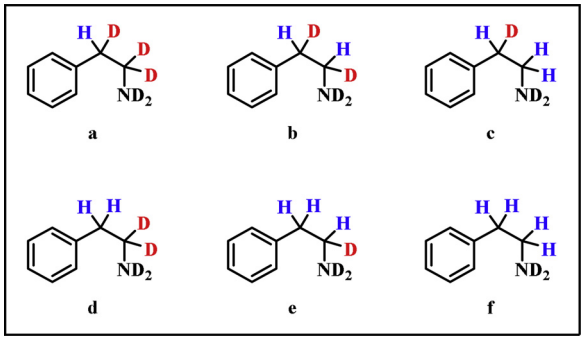


Fig. 6. The six deuterated species identified by ^{13}C NMR. Letter assignments correspond to the associated peaks observed in Fig. 5.

carbon B (CD_2, CHD or CH_2) are each split in two with a chemical shift separation of 0.05 ppm. This separation is a direct result of the isotope effect incurred due to the two isotopic options available at carbon A (CH_2/CHD). Identification of all six isotopologues, based on this effect and the splitting patterns of both aliphatic carbon atoms, are presented in Fig. 6. It should be noted that phenethylamine-D_4, detected by mass spectrometry in negligible quantities, was not detectable by NMR. Interestingly, however, phenethylamine-D_0, detectable in similar quantities by mass spectrometry to phenethylamine-D_4 was observed by NMR. If the isotopic composition of phenethylamine-D_4, namely the absence of aliphatic protons, is considered it becomes clear why this molecule is not detectable by ^{13}C NMR spectroscopy. The deuterium presence greatly reduces the intensity of the signals on the ^{13}C spectra as a consequence of peak splitting (carbons bonded to deuterium are split by residual C—D coupling), making detection harder. Further, an increase in the ^{13}C T_1 arises due to the fact that it is the interaction with protons which provides the main relaxation mechanism for the ^{13}C spins. Whilst an increased relaxation delay (30 s) was employed to ensure full relaxation for all deuterated species produced in the reaction, it is possible that this delay was not sufficient for the deuterated species which contained a higher deuterium content [30]. Therefore, the presence of isotopologues with a high deuterium content, such as phenethylamine-D_4, will be underestimated when compared to isotopologues which have a low deuterium content, for example phenethylamine-D_0.

3.6. Isotopologue distribution for mandelonitrile deuteration as determined by ^{13}C NMR spectroscopy

To compliment the mass spectrometry study (Section 3.3),

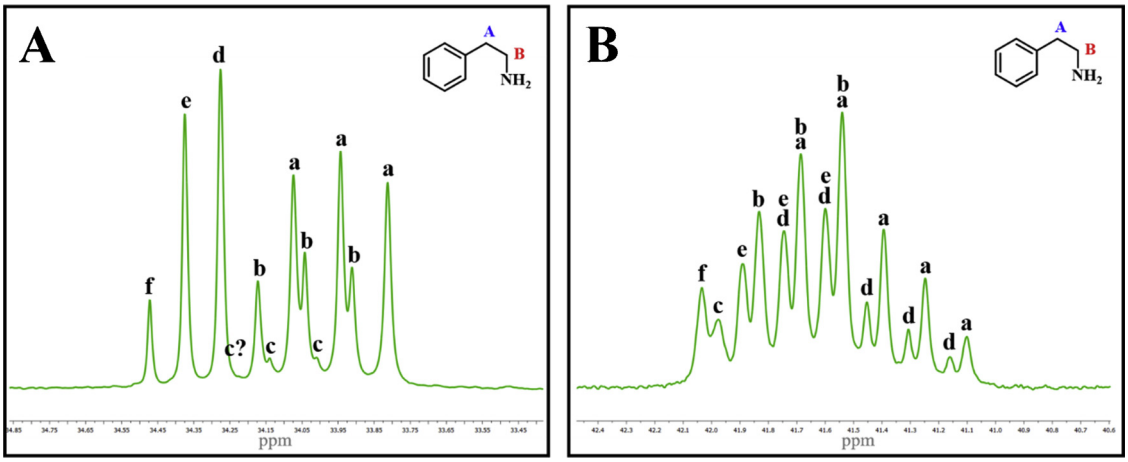


Fig. 5. Expansion of the ^{13}C NMR splitting pattern observed for aliphatic peak A (left) and peak B (right), assigned against phenethylamine, inset. Assignments **a**–**f** denote the splitting pattern associated with each isotopologue present in the reaction mixture.

Table 2
Percentage of each of the six phenethylamine isotopologues in the reaction mixture as determined by quantitiative ^{13}C NMR.

Isotopologue	a	b	c	d	e	f
Composition	HD DD	HD HD	HD HH	HH DD	HH HD	HH HH
Percentage / %	39.5	22.6	4.2	16.0	13.7	4.0

quantitative ^{13}C NMR spectroscopy on the deuterated product mixture was conducted. Again, peak overlap is a significant concern during spectral analysis. Referring to Fig. 5, where the splitting patterns associated with aliphatic Carbons A and B are considered, it is observed that there is a greater degree of peak overlap at Carbon B. Whilst analysis of both splitting patterns should yield the same result, Carbon A is selected for quantitative analysis due to the lesser extent of peak overlap at this position. Subtraction of known peaks at overlap regions can be used to determine peak integration of the components of a mixed peak. This method, however, is less reliable than integration of a lone peak and can decrease overall accuracy. Applying this method, a percentage of each identified isotopologue produced in the reaction (Fig. 6) can be calculated. The results of which are presented in Table 2.

Combining the percentages of isotopologues **b** and **d**, and isotopologues **c** and **e** gives the full component of phenethylamine-D_2 and phenethylamine-D_1 respectively. The results can then be compared to the quantitative mass spectrometry findings presented in Section 3.3 (Fig. 7).

Fig. 7 displays that the quantitative mass spectrometry and NMR spectroscopy studies show the same general trend and are largely comparable. Despite this, there are some notable differences between the two techniques. Specifically, this relates to the number of deuterium atoms present. It is proposed, with reference to Section 3.5, that as isotopologues with a higher deuterium content (phenethylamine-D_3 and phenethylamine-D_4) are harder to detect by NMR spectroscopy, these isotopologues are under-represented using this technique when compared to mass spectrometry. The opposite is true for isotopologues with a low deuterium content.

3.7. Mechanistic refinement

Analysis by mass spectrometry (Section 3.3) and NMR spectroscopy (Section 3.5) has indicated the presence of a mixture of phenethylamine isotopologues ranging from phenethylamine-D_0 to phenethylamine-D_3. If this outcome is then linked back to the reaction scheme (Fig. 1), insight may be gleaned.

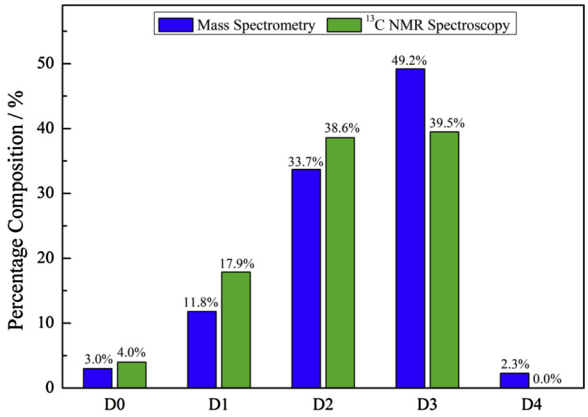


Fig. 7. Percentage composition of each deuterated species in the reaction mixture at reaction completion (t = 2 h) as determined by mass spectrometry (blue) and ^{13}C NMR spectroscopy (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

To a first approximation, the negligible quantities of phenethylamine-D_4 detected by both mass spectrometry and NMR spectroscopy indicate that forward Pathway II, via the hydroxy-enamine, can be eliminated. Excluding Pathway III on kinetic grounds [19], this would then imply that the remaining pathway, Pathway I, is the active route. Nevertheless, due to the presence of a complex reaction mixture containing six isotopologues of phenethylamine and the occurrence of a H/D scrambling process (Sections 3.3 and 3.4), a number of contributing pathways must be additionally considered.

Whilst Pathway I is identified as a definite contributor, as evidenced by the detection of phenethylamine-D_{3A} as the major product (49.2 % by mass spectrometry, Table 1), the occurrence of reverse acid catalysed tautomeric pathways, resulting in the hydrogen enrichment observed in the isotopologue distribution, must also be acknowledged. With reference to Fig. 1a, hydrogen scrambling during the deuteration reaction may *only* occur at two reaction steps and affect *only* one carbon at a time. The equilibria between molecules (**3**) and (**4**) will enrich carbon B whilst the equilibria between molecules (**5**) and (**6**) will enrich carbon A. Moreover, it is essential to note that the remaining equilibria in the system, between molecules (**2**) and (**3**), is not involved in the H/D scrambling process as, whilst a H is removed, it is also replaced, hence rendering it enrichment neutral.

In order to establish what is possible mechanistically within the mandelonitrile reaction system, the position of the deuterium and the degree of deuteration within the molecule must be followed. The expected deuterated product from each potential pathway can then be compared to the known isotopologue distribution (Fig. 6) to determine if a pathway is feasible or not.

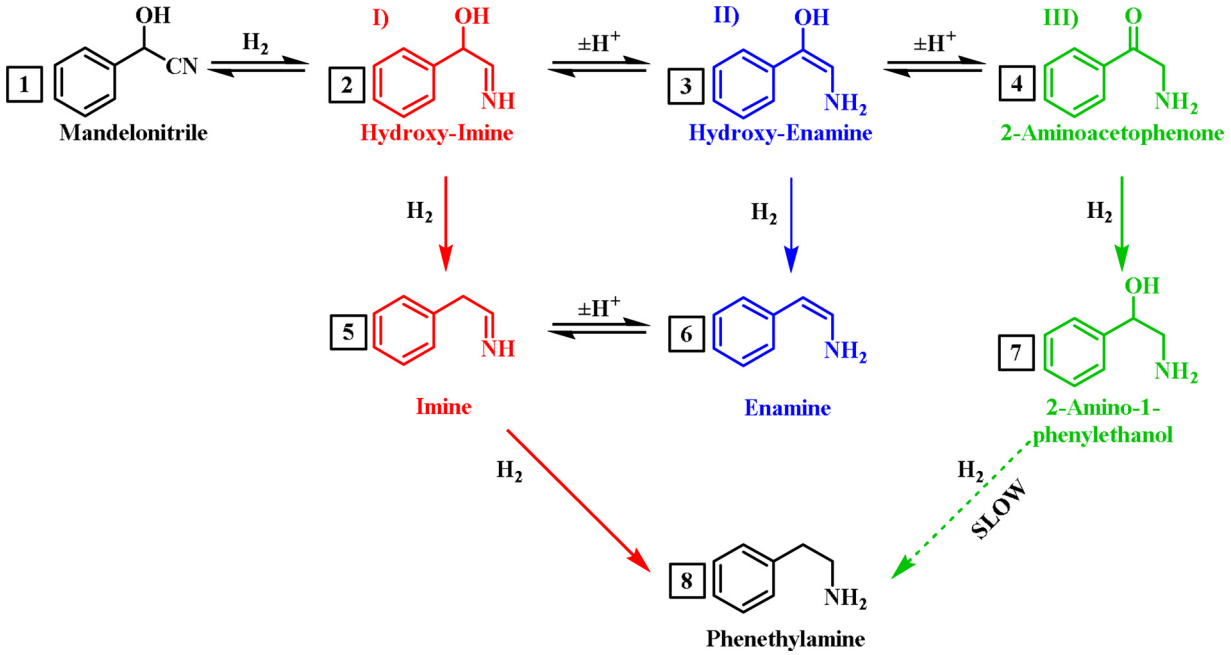
From the observed isotopologue distribution it is clear that the equilibria involved in the aforementioned hydrogen enrichment of both carbons A and B are operational. It can also be deduced that the final step in this reaction cannot be the hydrogenation of enamine (**6**) to phenethylamine (**8**). If direct Pathway II is followed it should yield phenethylamine-D_4 (experimentally determined by mass spectrometry as 2.3 %). However, if scrambling is invoked then either phenethylamine-D_{3B} (experimental 0 %), or the pathway unspecific product phenethylamine-D_2 (experimentally determined by mass spectrometry as 33.7 %) is expected. Quantitative NMR studies (Fig. 7) confirm the general trends of the above mass spectrometry findings. Consequently, these finding allow the number of active pathways to be narrowed down.

Accordingly, three active pathways are proposed. With reference to Scheme 2, where all involved species are numbered for clarity, the following pathways can be defined with the sequence of numbered species defining the individual steps:

(i) **1,2,5,8**
(ii) **1,2,3,4,3,2,5,8**
(iii) **1,2,3,4,3,6,5,8**

Here path (i) proceeds exclusively in the forward direction to yield phenethylamine-D_3 and constitutes the major pathway. In contrast, both paths (ii) and (iii) invoke the reverse tautomeric reactions upon formation of 2-aminoacetophenone (**4**). The reformation of the hydroxy-enamine (**3**) represents the branching point between paths (ii) and (iii). Path (ii) tautomerises further, back to the hydroxy-imine (**2**) prior to undergoing hydrogenation to phenethylamine (**8**) via hydrogenolysis to the imine (**5**) as was observed in path (i). Path (iii), however, undergoes hydrogenolysis to the enamine (**6**) before tautomerisation back to the imine (**5**) occurs, from imine (**5**) the final hydrogenation stage to phenethylamine (**8**) can be completed.

Upon examination of the contributing pathways similarities can be identified. Namely, all three hydrogen requiring processes in the reaction (two hydrogenation steps and one hydrogenolysis) occur in a specified order. The first step of the reaction is hydrogenation of the nitrile moiety of mandelonitrile to furnish the hydroxy-imine (**1**→**2**).



Scheme 2. Expanded reaction scheme for the hydrogenation of mandelonitrile. The presented scheme has been adapted from reference [15].

Secondly, a hydrogenolysis step resulting in cleavage of the hydroxyl group will occur (**2**→**5** or **3**→**6**). Finally, the concluding step involves the hydrogenation of the imine to phenethylamine (**5**→**8**). Adding a degree of complexity to this process, the presence of the acid catalysed tautomerisation pathway means that the hydrogenolysis step may occur on either the hydroxy-imine (**2**) or the hydroxy-enamine (**3**). Examination of the energetics associated with the hydrogenolytic cleavage of the hydroxyl group to determine which molecule (hydroxy-imine (**2**) or hydroxy-enamine (**3**)) the hydrogenolysis is likely to occur on represents future work. However, it is chemically likely that both molecules undergo this process. As such, the reaction scheme for the hydrogenation of mandelonitrile over a carbon supported Pd catalyst (Fig. 1) has been adapted to include the presented findings (Scheme 2). Nevertheless, as a static scheme, some of the more dynamic aspects of this reaction process are not fully apparent. Scheme 2 therefore cannot be considered in isolation, with the accompanying descriptions relating to the full mechanistic complexity necessary for a comprehensive understanding.

4. Conclusions

Mechanistic aspects of the liquid phase heterogeneously catalysed hydrogenation of mandelonitrile (aromatic cyanohydrin) over a Pd/C catalyst to afford phenethylamine (primary amine) have been investigated. It was initially proposed that further mechanistic studies, as presented here, could be used to provide an enhanced understanding regarding the pathway taken from reagent to product. The reported study aimed to differentiate between pathways I and II, which could not be achieved by the chromatographic means previously employed, and further, to confirm the inactivity of path III. It was found that this was an overly simplistic and blinkered view of the reaction scheme. Instead, the following, more detailed mechanistic conclusions have been drawn:

- NMR analysis of the reaction mixture confirms the successful deuteration of the product and provides information on the location of deuterium incorporation within phenethylamine.
- Mass spectrometry and quantitative ^{13}C NMR spectroscopy reveal a similar isotopologue distribution. The detection of phenethylamine-D_3 as the major product is used to propose that Pathway I via the hydroxy-imine is favoured. Nevertheless, the significant contribution from phenethylamine-D_1 and phenethylamine-D_2 in the reaction mixture highlights the presence of a hydrogen enrichment process occurring alongside the main pathway.
- To rationalise the increased hydrogen content, the significance of the reverse tautomeric reactions along the acid catalysed equilibria are highlighted.
- Examination of the multiplicity observed in the ^{13}C spectra for the deuteration of mandelonitrile indicates the presence of six isotopologues.
- Analysis of the deuterated product distribution allows refinement of the mechanism. The three hydrogen requiring processes involved in the conversion of mandelonitrile to phenethylamine are found to occur in a specified order. Hydrogenation of mandelonitrile to the hydroxy-imine precedes hydrogenolysis of the hydroxyl group with the final step being the hydrogenation of the double bond of the imine to phenethylamine.

CRediT authorship contribution statement

Mairi I. McAllister: Conceptualization, Methodology, Investigation, Validation, Writing - original draft. **Cédric Boulho:** Methodology, Investigation. **Colin Brennan:** Conceptualization, Investigation, Writing - review & editing, Supervision, Funding acquisition. **Philip J. Sidebottom:** Investigation, Validation. **David Lennon:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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spectrometry on the deuterated sample.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mcat.2019.110720.

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