The following full text is a publisher's version.

For additional information about this publication click this link. http://hdl.handle.net/2066/142600

Please be advised that this information was generated on 2017-06-16 and may be subject to change.
DEUTERIUM ISOTOPE EFFECTS IN THE METABOLISM OF
N-ALKYLSUBSTITUTED AMPHETAMINES IN MAN

T. B. VREE, J. P. M. C. GORGELS, A. TH. J. M. MUSKENS AND J. M. VAN ROSSUM
Catholic University of Nijmegen, Department of Pharmacology,
Geert Groote Plein N 21, Nijmegen (The Netherlands)

SUMMARY

Deuterium isotope effects in the metabolism of deuterated amphetamine and
N-substituted deuterated amphetamines were studied in man. The isotope effect is
large when in the elimination process of the amphetamine from the body, the metaboli-
c processes, deamination and N-dealkylation are the major pathways and the renal
excretion is the minor one. It appeared to be probable, that in the deamination and
N-dealkylation process the tertiary hydrogen atom is of importance.

INTRODUCTION

Within a series of N-alkyl substituted amphetamines it has been observed that
the larger alkyl groups are more easily removed (N-dealkylation) than the smaller
groups; i.e. isopropyl > ethyl > methyl. As a result of this dealkylation, amphetamine
itself becomes the metabolite. Examination of the ratio of unaltered compound/
amphetamine metabolite, when the excretion is maximal, indicates a fixed ratio
between the two substances. These ratios are: 6 for methylamphetamine, 2 for ethyl-
amphetamine and 0.6 for isopropylamphetamine. Within the same series, we are
dealing with the energy difference of the C-H bond between the primary H of the
methyl group, secondary H of the ethyl group and tertiary H of the isopropyl group.
Whereas the dextro-isomers show the above decrease in ratio of compound/metabolite
excreted, the levo-isomers show no change from one system to another, with all com-
ounds exhibiting the same fixed ratio of 10:1. The physico-chemical parameters
(e.g pKa, lipid solubility) of both isomers (d-l) show only small differences, whereas
the levo-isomer show no stimulant properties and are metabolized to a much lesser
extent, indicating a sterical preference both for the stimulus receptor system and to the
metabolizing enzyme system.12

Metabolic clearance of amphetamine derivatives involves three pathways:
parahydroxylation, deamination and N-dealkylation. Dring7 has reported that, in
man, the parahydroxylation is about 1-2% when the urinary excretion rate of the
amphetamine is maximal. In view of the preceding statement, it may be assumed that
the metabolic clearance due to parahydroxylation is negligible and that the overall
elimination of amphetamines is due to excretion of unaltered compound, deamination

and dealkylation. We may therefore assume that the sum of the unaltered amphetamine excreted, plus deaminated and dealkylated product is equivalent to 100% of the ingested dose.

In this manner, the percentage of deaminated amphetamine, found after excretion of 70% unaltered compound, is 30%. (see Fig. 1). Similar results are found when amphetamine and phentermine are compared. When these compounds are simultaneously administered to the same person, 100% of the phentermine and 70% of the amphetamine is excreted unaltered. The total amount of amphetamine excreted is dependent upon the ratio of metabolic and renal clearance constants, km resp. kr which in turn are dependent upon the physico-chemical parameters of both the amphetamine and the enzyme system. Since from each amount of amphetamine absorbed or metabolically formed, 70% is excreted unaltered when amphetamine is a metabolite of some administered compound, the percentages of excretion, deamination and dealkylation of the original compound may be calculated (see Table I).

Phenylethylamine is excreted only 1–5% unaltered. Comparison of this result to that of amphetamine plus its derivatives suggests that the deaminating enzyme is hindered when the aliphatic side chain is branched and/or that the deamination requires a hydrogen atom. If the deamination did not require the presence of a proton,

### Table I

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Deamination</th>
<th>% Dealkylation</th>
<th>% Excretion*</th>
<th>kr &gt; km</th>
</tr>
</thead>
<tbody>
<tr>
<td>+amphetamine</td>
<td>30</td>
<td>70</td>
<td>100</td>
<td>km &gt; km</td>
</tr>
<tr>
<td>+methylamphetamine</td>
<td>20</td>
<td>15</td>
<td>65</td>
<td>km &gt; km</td>
</tr>
<tr>
<td>+ethylamphetamine</td>
<td>30</td>
<td>30</td>
<td>40</td>
<td>km = km</td>
</tr>
<tr>
<td>+isopropylamphetamine</td>
<td>45</td>
<td>45</td>
<td>10</td>
<td>km &gt; km</td>
</tr>
<tr>
<td>+dimethylamphetamine</td>
<td>50</td>
<td>50</td>
<td>40</td>
<td>km &lt; km</td>
</tr>
<tr>
<td>+amphetamine</td>
<td>10</td>
<td>90</td>
<td>10</td>
<td>km &lt; km</td>
</tr>
<tr>
<td>-methylamphetamine</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td>km &gt; km</td>
</tr>
<tr>
<td>-ethylamphetamine</td>
<td>0</td>
<td>10</td>
<td>85</td>
<td>km &gt; km</td>
</tr>
<tr>
<td>-isopropylamphetamine</td>
<td>0</td>
<td>15</td>
<td>85</td>
<td>km &gt; km</td>
</tr>
<tr>
<td>-dimethylamphetamine</td>
<td>10</td>
<td>20</td>
<td>80</td>
<td>km &gt; km</td>
</tr>
</tbody>
</table>

* % of ingested dose, kr renal clearance constant, km metabolic clearance constant.

then we would have expected some deamination of the phentermine. Since the latter
is excreted essentially unchanged, it may initially be assumed that the hydrogen atom
is of importance. The purpose of this investigation therefore was to determine the
importance of the alkyl hydrogen with respect to dealkylation and deamination and
whether its abstraction was the rate limiting step.

When proton abstraction (C–H bond rupture) is believed to be the rate limiting
step in the reaction sequence between a particular molecule and an enzyme system,
the use of deuterium in place of hydrogen at the suspected position of proton ab-
straction may demonstrate the validity of the hypothesis; that is to say, if a deuterium
isotope effect can be demonstrated, the corresponding C–H bond exerts a determining
role with respect to the rate limiting step\(^1,4,10,11\). In accordance with this, the hydrogen
atom in question was replaced by a deuterium atom. The occurrence of a primary
deuterium effect indicates a slower rate of C–D bond rupture compared to C–H bond
rupture.

**RESULTS**

The results obtained give clear evidence as to the involvement of the tertiary
hydrogen in both the N-dealkylating and deaminating sequence. It should be recog-
nized that, in the human body, several elimination mechanisms may mask a small
deuterium effect.

The excretion rate, of the unaltered compound, which is maintained constant
and maximal by maintaining the urine acidic, can be of importance. For example,
when the hydrogen compound is excreted 50% unaltered and the deuterium com-
pound is excreted 50.5% unaltered, the conclusions resulting from the cumulative
excretion, may be that there is no isotope effect, whereas many observations may show
a statistical significant effect. Other factors, such as the partition volume and the
excretion rate of the deuterium and hydrogen compound may be different. The partition
volumes and the excretion rates both depend upon the \(pK_a\) value of the com-
pound and this value may differ maximal 0.05 between the 2 compounds\(^5\) Table II.
The deuterated compounds are more ionized than the hydrogen compounds at the pH
of the blood (7.40) Since the nonionized form ofamphetamine is reabsorbed in the
renal tubulus, the deuterated compounds are, or can be, more rapidly excreted than
the hydrogen compounds. If the latter is true then the difference in excretion rate
must be constant during the whole period of excretion and the deuterium content
of the urine samples must gradually decrease.

**TABLE II**

<table>
<thead>
<tr>
<th>Drug</th>
<th>(pK_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>9.83 ± 0.01</td>
</tr>
<tr>
<td>Deuteroamphetamine</td>
<td>9.88 ± 0.01</td>
</tr>
<tr>
<td>Methylandamphetamine</td>
<td>9.90 ± 0.02</td>
</tr>
<tr>
<td>Trideuteroamphetaamine</td>
<td>9.95 ± 0.02</td>
</tr>
<tr>
<td>Isopropylamphetamine</td>
<td>10.31 ± 0.01</td>
</tr>
<tr>
<td>Deuteroisopropylbenzphetamine</td>
<td>10.36 ± 0.01</td>
</tr>
<tr>
<td>Deuterodeuteroisopropyldeuterophosphate</td>
<td>10.38 ± 0.01</td>
</tr>
<tr>
<td>Dimethylamphetamine</td>
<td>9.97 ± 0.01</td>
</tr>
<tr>
<td>Di-trideuteromethylamphetamine</td>
<td>10.08 ± 0.01</td>
</tr>
</tbody>
</table>

The retention time of the compounds on the Apiezon-KOH column reflects the lipid solubility of the compounds. The deuterium compounds tend to decrease the retention time and due to this observation it is unlikely that the lipid solubility and the related partition volume have increased.

When one is dealing with a deuterium effect in the metabolism, (N-deamination or the N-dealkylation), the enzymatic process must be slower for the deuterium compound due to the higher force constant of the C-D bond. Therefore, the gradual increase in the deuterium content to the hydrogen content in the urine at subsequent time intervals will be less, or even a decrease may be found.

In our study a continuous increase of the percentage deuterium in the samples was found indicating a necessary manifestation of the deuterium effect in the metabolism. The observation that the deuterated compound is excreted totally to a higher extent is secondary and is caused by factors other than the difference in metabolism. The increase of the percentage deuterium of the samples may be a large one, for instance with deuteroisopropylamphetamine or a small one, as with deuteroamphetamine.

\((+)-\text{deuterated amphetamine} \text{ vs. } (+)-\text{amphetamine}\) (Table III) (Fig. 2).

Observation of the deuterium percentage of amphetamine in the first urine sample after ingestion of both compounds (50\% H, 50\% D), and the deuterium content in one of the last urine samples (48 h) indicates an appropriate increase of 15\% (Accuracy of the determination is ± 2\%).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D dosis</th>
<th>%D start</th>
<th>%D end</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>% excr.</th>
<th>% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.G.</td>
<td>12 mg</td>
<td>50.8</td>
<td>45</td>
<td>60</td>
<td>79</td>
<td>68.9</td>
<td>10.1</td>
<td>15</td>
</tr>
<tr>
<td>F.H.</td>
<td>10 mg</td>
<td>50.1</td>
<td>50</td>
<td>68</td>
<td>71.9</td>
<td>90</td>
<td>18.1</td>
<td>18</td>
</tr>
<tr>
<td>F.H.</td>
<td>12 mg</td>
<td>50.4</td>
<td>53</td>
<td>62</td>
<td>70.7</td>
<td>88.4</td>
<td>17.7</td>
<td>9</td>
</tr>
<tr>
<td>H.F.</td>
<td>12 mg</td>
<td>50.0</td>
<td>54</td>
<td>61</td>
<td>54</td>
<td>75.3</td>
<td>20.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

From this data it can be concluded that there is a small deuterium effect. The composition of deuterium and hydrogen compound in the first urine samples determines the total cumulative excretion data. Foreman* has shown with deuterated amphetamine a distinct deuterium effect with respect to metabolism by homogenized rabbit liver. The rabbit and human metabolize amphetamine in the same way, but from Table I it can be seen that when the renal excretion \(k_r\) is more important than the metabolic clearance \(k_m\), the deuterium effect measured is small.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D dosis</th>
<th>%D start</th>
<th>%D end</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>% excr.</th>
<th>% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.V.</td>
<td>7.7 mg</td>
<td>50.1</td>
<td>54</td>
<td>54</td>
<td>69</td>
<td>88</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>L.S.</td>
<td>7.0 mg</td>
<td>50.0</td>
<td>55</td>
<td>55</td>
<td>74</td>
<td>90</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>J.G.</td>
<td>7.5 mg</td>
<td>50.2</td>
<td>56</td>
<td>56</td>
<td>61.3</td>
<td>75.7</td>
<td>14.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Renal excretion rate, urine pH, urine production and cumulative excretion of dextro-amphetamine and dextro-deuteroamphetamine. The deuterium content of the urine samples shifted from 50% in the first urine samples to 60% in the last ones (Table III). The difference in excretion rate between D- and H-amphetamine is small, due to the fact that the renal clearance of the unaltered compound is much more important than the metabolic clearance (Table I).

\[ (+) \text{ trideuteromethylamphetamine vs. } (+) \text{ methylamphetamine} \] (Table IV)

With methylamphetamine, the deuterium content of the urine samples varies between 52 and 56% throughout the total period and essentially there is no shift in the percentage deuterium. The total amount of trideuteromethylamphetamine excreted is higher than the amount of methylamphetamine excreted.
Fig. 3. Renal excretion rate, urine pH, urine production and cumulative renal excretion of dextro-deuteroisopropylampheta mine and dextro-isopropylamphetamine. There is a remarkable deuterium effect, which begins 10 h after ingestion of the compounds.

\((-\text{)}\) di-trideuteromethylamphetamine vs. \((\text{+})\) dimethylamphetamine (Table V)

Dimethylamphetamine does not show a deuterium effect in the $N$-dealkylation of the first and second methyl group. The deuterium content of the urine samples varies but no deuterium shift is observed.

TABLE V
DIFFERENCES IN METABOLISM AND EXCRETION BETWEEN (+) DIMETHYLAMPHETAMINE AND (+) DITRI-
DEUTEROMETHYLAMPHETAMINE

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D</th>
<th>%H excr.</th>
<th>%D excr.</th>
<th>Δ% excr.</th>
<th>Δ% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.G.</td>
<td>20 mg</td>
<td>50</td>
<td>66</td>
<td>18</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>J.B.</td>
<td>21 mg</td>
<td>50</td>
<td>61</td>
<td>18</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>J.H.</td>
<td>14.6 mg</td>
<td>50</td>
<td>57</td>
<td>6</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>M.H.</td>
<td>13.8 mg</td>
<td>50</td>
<td>60</td>
<td>17</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

(−) dimethylamphetamine and (−) ditrideuteromethylamphetamine.

M.H. 15 mg 50.0

The accuracy in %D by means of massfragmentometry is ±5%.

TABLE VI
DIFFERENCES IN METABOLISM AND EXCRETION BETWEEN (+) ISOPROPYLAMPHETAMINE AND (+) DEUTERO-
ISOPROPYLAMPHETAMINE

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D</th>
<th>%H</th>
<th>%D excr.</th>
<th>Δ% excr.</th>
<th>Δ% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.V.</td>
<td>13 mg</td>
<td>50</td>
<td>48</td>
<td>90</td>
<td>13.7</td>
<td>14.2</td>
</tr>
<tr>
<td>J.G.</td>
<td>15 mg</td>
<td>49.5</td>
<td>48</td>
<td>89</td>
<td>8.35</td>
<td>9.90</td>
</tr>
<tr>
<td>J.S.</td>
<td>15.1 mg</td>
<td>49.5</td>
<td>48</td>
<td>82</td>
<td>8.75</td>
<td>10.14</td>
</tr>
<tr>
<td>J.G.</td>
<td>15 mg</td>
<td>50.0</td>
<td>48</td>
<td>90</td>
<td>5.0</td>
<td>6.50</td>
</tr>
<tr>
<td>C.V.</td>
<td>15 mg</td>
<td>50.0</td>
<td>48</td>
<td>80</td>
<td>12.1</td>
<td>12.4</td>
</tr>
</tbody>
</table>

(−) isopropylamphetamine and (−) deuteroisopropylamphetamine.

J.S. 15 mg 50.0

P.R. 16 mg 52.1

J.G. 17 mg 52.0

TABLE VII
DIFFERENCES IN METABOLISM AND EXCRETION BETWEEN (+) ISOPROPYLAMPHETAMINE AND DEUTERO-
ISOPROPYL-DEUTEROAMPHETAMINE

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosis</th>
<th>%D</th>
<th>%H</th>
<th>%D excr.</th>
<th>Δ% excr.</th>
<th>Δ% pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.V.</td>
<td>44.1 mg</td>
<td>49.5</td>
<td>50</td>
<td>80</td>
<td>7.95</td>
<td>6.02</td>
</tr>
<tr>
<td>T.V.</td>
<td>43.0 mg</td>
<td>50.0</td>
<td>45</td>
<td>75</td>
<td>3.14</td>
<td>2.04</td>
</tr>
<tr>
<td>J.B.</td>
<td>42.3 mg</td>
<td>51.3</td>
<td>40</td>
<td>77</td>
<td>1.70</td>
<td>1.25</td>
</tr>
</tbody>
</table>

(−) isopropylamphetamine and (−) deuteroisopropyldeuteroamphetamine.

F.H. 41.6 mg 50.0

H.H. 40.4 mg 50.1

(+) deuteroisopropylamphetamine vs. (+) isopropylamphetamine (Table VI) (Fig. 3)

This compound elicits a rather large difference between the hydrogen and deuterium compound. The deuterium content increases from 48% to 90% in all the subjects, but in some subjects the deuterium shift begins or is measurable only 10 h after ingestion. When the urine is acidic, (pH 5) the total amount of isopropylamphetamine excreted in the first 10 h determined the total cumulative excretion and for this reason the differences between deuterium and hydrogen cumulative excretion are very small, in spite of the great deuterium shift. The dextro-isomer shows this effect, but the levo-isomer does not. From Table I it can be seen that for the dextro-isomer the metabolic processes of elimination are much more important than the renal excretion and that with levo-isopropylamphetamine the relationship between renal and metabolic clearance is reversed.

Fig. 4. Renal excretion rate, urine pH, urine production and cumulative renal excretion of dextro deuteroisopropyleuteroamphetamine and dextroisopropylamphetamine. There is a deuterium-effect of the same magnitude as mentioned in Fig. 3.

According to the results with deuteroisopropylamphetamine it was expected that the halflife time of deuteroisopropyldeuteroamphetamine would be doubled. The experiments, on the contrary, do not show a further increase in T 1/2 of the dideuteroisopropylamphetamine. The reason for this may possibly be the following:

The purpose of the experiments was to prove that both tertiary hydrogen atoms in isopropylamphetamine are chemically identical for the enzyme. Therefore they were replaced by deuterium atoms in order to show that the hydrogen atoms in question are involved and are rate limiting. We have compared H-isopropyl-with D-isopropylamphetamine and found an increase in T 1/2 from 2 h to 4 h for the deuterium compound. From above experiments 2 hypotheses may follow:

Hypothesis I. The enzymes deaminate and dealkylate the H- and D-isopropylamphetamine with the same affinity, thus, the difference in excretion rate and T 1/2 is caused by the C-D bond rupture.

Hypothesis II. The enzyme can perform one metabolic action with the compound and after that action the compound leaves the enzyme action site.

If both dealkylation and deamination occurred at the same time, no amphetamine would be found as metabolite. When two different enzymes are involved, then the dideuterated isopropylamphetamine must be metabolized at a slower rate than the monodeuterated isopropylamphetamine. When we are comparing isopropylamphetamine with the dideuteroisopropylamphetamine and take into account hypothesis II, then it does not matter whether the C-D bond of the amphetamine moiety is broken or the C-D bond of the isopropyl moiety is broken. When the isopropyl group has been removed, the metabolite shows the normal deuterium effect.

Experiments

Synthesis

Deuterated dextro- and levo-amphetamine were synthesized according to Foreman. The rotations of the d- and l-deuterated amphetamine found were: (a)_{D}^{25} = +29.0° C = 1.995 in water (d-tartrate salt) and (a)_{D}^{25} = −28.0° C = 2.005 in water (l-tartrate salt).

Di-trideuteromethylamphetamine. 0.01 mole of d-amphetamine was treated with 0.1 mole deuterated formic acid and 0.05 mole of trideuteroformaldehyde. The reaction was followed by means of gas chromatography-mass spectrometry. Completion of reaction was evidenced by disappearance of the basepeak at 72 (H-dimethylamphetamine) and the appearance of the base peak 78 (D-dimethylamphetamine). The levo isomer was prepared in a similar manner.

Trideuteromethylamphetamine. 500 mg of the di-trideuteromethylamphetamine was oxidized by a tenfold excess of an alkaline permanganate solution and the reaction was followed by GLC-MS until the ditrideuteromethylamphetamine disappeared.

Deuteroisopropylamphetamine. 2 g of d-amphetamine were refluxed with 150 ml of acetone and 5 g of anhydrous magnesium sulphate. The reaction was followed gaschromatographically. The acetone was removed by vacuum and to the concentrated Schiff base 3 ml of D_4O and sufficient sodiumborodeuteride were added in small portions under reflux. The reduction was followed continuously by GLC-MS and stopped when the base peak of the Schiff base in the mass spectrum (m = 84) had dissappeared.
The mass spectrum of the deuteroisopropylamphetamine showed a base peak at 

\[ m = 87 \]. The levo isomer was prepared in the same way.

*Deuteroisopropyl-deuteroamphetamine.* 1 g of \( \alpha \)-deuteroamphetamine is refluxed 

with 150 ml of acetone and 5 g of anhydrous magnesiumsulphate. Due to the magne-

siumsulphate the yield of the reaction is quantitative.

The excess of acetone is removed under vacuum and sodiumborodeuteride and 

\( \text{D}_2\text{O} \) was added in small portions. The reduction was followed by GLC-mass spec-

 trometry.

Full details of the reactions will be published elsewhere\(^1\).

**METHODS**

**Apparatus**

Gaschromatograph H P 402 with flame ionisation detection. Glass column, 1.80 

m, 3 mm diameter, filled with 20% Apiezon L 5% KOH Diatoport-S-60-80 mesh. Oven 

temperature 160°, temperature injection block 200° and temperature detector block 

220°. Nitrogen flow 30 ml/min, hydrogen flow 40 ml/min and air flow 300 ml/min.

Gaschromatograph-massspectrometer LKB 9000.

Glass column 1.20 m, 3 mm diameter, filled with 20% Apiezon L 5% KOH 

Diatop-S-60-80 mesh. Oven temperature 140°, injection block 200° separator 220° 

and ion source temperature 250°.

Ionisation energy 70 eV, trapcurrent 60 mA, accelerating voltage 3.5 kV.

**Detection methods**

The concentration of the amphetamines in the urine of the subjects was 

measured by means of the gaschromatographic analysis with the internal standard 

method. The same samples were injected into the LKB 9000 system in order to de-

termine the percentage of deuterium of the sample.

**Determinations**

Mixtures of equal amounts of amphetamine and deuterated amphetamine were 

ingested as HCl salts by male subjects (Table III-VII).

The urine was kept acidic, \( \text{pH} 5.0 \pm 0.2 \), by the ingestion of 1.6 g of ammonium-

chloride four times a day. Each amount of urine was collected for 60 h or until such 

time that the excretion rate was 1% of the maximum excretion rate. The \( \text{pH} \) of the 

urine sample was measured upon receipt with a Copenhagen Radiometer. The average 

urine production was measured and the curves of average renal excretion rate were 

constructed. To 10 ml of the urine, 10 \( \mu \text{g} \) of the internal standard \( \text{N} \)-methyl benzyl-

amine was added and the solution made alkaline to \text{pH} 13 with KOH pellets. Then 

the alkaline solution was extracted twice with 10 ml of freshly distilled ether, the 

combined ether layers extracted with 5 ml 2 \( \text{N} \) HCl. The acidic layer was made alka-

line to \text{pH} 13 and extracted twice with 10 ml of ether. The combined ether layers 

were evaporated under a mild stream of dry air until 100 \( \mu \text{l} \) remained. 5–8 \( \mu \text{l} \) was 

injected into the gaschromatograph HP 402 and into the LKB 9000.

**Detection methods**

In order to be able to determine the ratio of deuterated–non deuterated amphet-
amine in a mixture of these compounds, we used the mass spectrum of this mixture, taking the ratio of the base peaks as a measure for the ratio of concentrations in the mixture. A calibration curve was made using mixtures of D- and H-amphetamines of known composition. The masses examined were: amphetamine-deuteroamphetamine, line intensities m/e+44-45 methylamphetamine-trideuteromethylamphetamine, line intensities m/e+ 58-61 isopropylamphetamine-deuteroisopropylamphetamine, line intensities m/e+ 86-87 isopropylamphetamine-deuteroisopropyldeuteroamphetamine, line intensities m/e+ 86-88 dimethylamphetamine-ditrideuteromethylamphetamine, mass fragmentometry ratio of peak areas of the masses 72 and 78.

For the calibration curves, line intensities at various concentrations in the gaschromatographic peak were measured. The average ratio of the line intensities was used. This method is allowed when the retention times of the H- and D-amphetamine are equal. When there is a difference in retention time, as is the case with dimethylamphetamine, the method of mass fragmentometry9 must be followed. For the other compounds both methods are followed but give the same calibration curve. The accuracy of the estimation of the percentage deuterium in the samples is ±2%.

An example of a calibration curve is given in Fig. 5.

![Calibration curve of percentage deuterium isopropylamphetamine and the ratio of line intensities of the base peaks m/e+ = 86 and m/e+ = 87.](image)

**Fig. 5.** Calibration curve of percentage deuterium isopropylamphetamine and the ratio of line intensities of the base peaks m/e+ = 86 and m/e+ = 87.

**Determination of pKa value of some amines by means of second differential titration curves**

pH measurements were carried out with Radiometer equipment, consisting of a pH meter type PHM 26, equipped with a scale expander, a glass electrode type G 202 B combined with a calomel electrode, type K 401. The system was calibrated using standard buffers: for iso-pH-adjustment: 0.025 M KH$_2$PO$_4$ (3.40225 g/l) and 0.025 M Na$_2$HPO$_4$ · H$_2$O 12 (8.95210 g/l) yieldings: 15° pH 6.900

20° pH 6.881

25° pH 6.865

for electrode sensitivity adjustment: 0.05 M KHptalate (10.21150 g/l) with
pH = 3.999 at 15°
4.002 at 20°
4.008 at 25°
The titration vessel was thermostated at 22° and contained about 4 ml solution.
The titrant was carbonate free KOH 0.100 N (Titrisol, Merck). A microburette (max.
volume 5 ml) was connected with a fine capillary, dipping into the solution. The plunger
type burette had a accuracy of about 0.1% per 1 ml titrant. The solution was stirred
with a magnetic stirrer and kept under nitrogen. 0.1 mmole of the amine HCl salt
was dissolved. The end point of the titration was determined by differentiating the
pH curve and taking d\(\text{pH}/dV\) in which V is the added volume titrant. The pKa
value was determined by interpolating in the pH curve.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. P. J. van Gemert and Mr. J. Bormans for syn-
thesizing the deuterated amphetamines, Mr. F. Bos for the measurement of the pKa
values and Mr H. W. A. Hoeben and Dr. P. Doukas (Temple University, Philadelphia)
for their critical remarks.

The authors wish to thank the various students who have taken and carefully
excreted the deuterated compounds.

This study was supported by grants from the Netherlands Organization for
Pure Scientific Research through its Fundamental Medical Research Organization.

REFERENCES

1 H. Aebi, Kinetic isotope effects in metabolic studies with deuterated and tritiated compounds,
International Conference on Radioactive Isotopes in Pharmacology. Wiley-Interience, 1967,
p. 305.
2 A. H. Beckett, L. G. Brookes and E. V. B. Shenov, Urinary excretion of the drug and its
main metabolite in man, after the administration of (+), (−) and (−) ethylamphetamine,
4 C. J. Collins, Some aspects of deuterium isotope effects in mechanism studies, Ann. New
York Acad. Sci., 84 (1960) 603.
5 E. Elison, H. Rapoport, R. Laursen and H. W. Elliot, Effect of deuteration of N-CH3
6 L. G. Dring, R. L. Smith and R. T. Williams, The fate of amphetamine in man and other
7 Ibid, Patterns of metabolism of \(\alpha\)-phenylisopropylamines in man and other species, Amphetamine
8 R. L. Foreman, F. P. Siegel and R. G. Mrtek, Synthesis of deuterio-\(l\)-amphetamine d4 sul-
9 B. Holmstedt, C. G. Hammar and R. Ryhage, Identification of chlorpromazine and its meta-
10 I. A. Ross, The use of kinetic isotope effects in the study of metabolic control, J. Biol. Chem.,
236 (1961) 603.
12 T. B. Vree and J. M. van Rossum, Kinetics of metabolism and excretion of amphetamines
in man, Amphetamine and Related compounds. Proceedings of the Mario Negri Institute for
Pharmacological Research, Milan, E. Costa and S. Garattini (Eds.), Raven Press, New York,
1970, p. 156.