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STUDIES ON THE METABOLISM OF DIETHYLPROPION

I. ANALYTICAL PROCEDURE

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SUMMARY

Diethylpropion is known to be metabolised by two major routes: N-dealkylation and keto reduction. A method for the analysis in urine of diethylpropion and its basic metabolites, including the diastereoisomers and enantiomers of the amino alcohol metabolites, is described.

INTRODUCTION

The metabolism of diethylpropion (I) has been investigated by various workers[1–3], but the published results are inconsistent and agree qualitatively only on two metabolic routes: N-dealkylation and keto reduction to secondary alcohols the latter route occurring for the three amino ketones (I, II and III) (Fig. 1).

The amino alcohols IV, V and VI can each exist as (+)-*erythro* (1S:2R) (−)-*erythro* (1R:2S), (+)-*threo* (1S:2S) and (−)-*threo* (1R:2R), but no information is available on the proportions of the stereoisomers formed during the metabolism of diethylpropion. Since VI represents the isomers of norephedrine and norpseudoephedrine, and V and IV represent respectively, N-ethyl- and N-diethylnorephedrine (and N-ethyl- and N-diethylnorpseudoephedrine), the biological activity of the amino alcohol metabolites will be influenced by the stereoisomeric forms present.

The present paper describes a procedure for the analysis of the amino ketones I II and III and the diastereoisomeric and enantiomeric forms of the amino alcohols IV V and VI in mixtures of some or all of these compounds.

The amino ketones I, II and III have an asymmetric centre, but racemisation will be fast during extraction under alkaline conditions, and therefore no attempt was made to investigate the proportions of these enantiomers.

EXPERIMENTAL

Apparatus

A Perkin-Elmer F11 gas chromatograph with a flame ionization detector and a Hitachi 159 recorder was used. The columns were made of stainless steel, 1/8 in. O.D

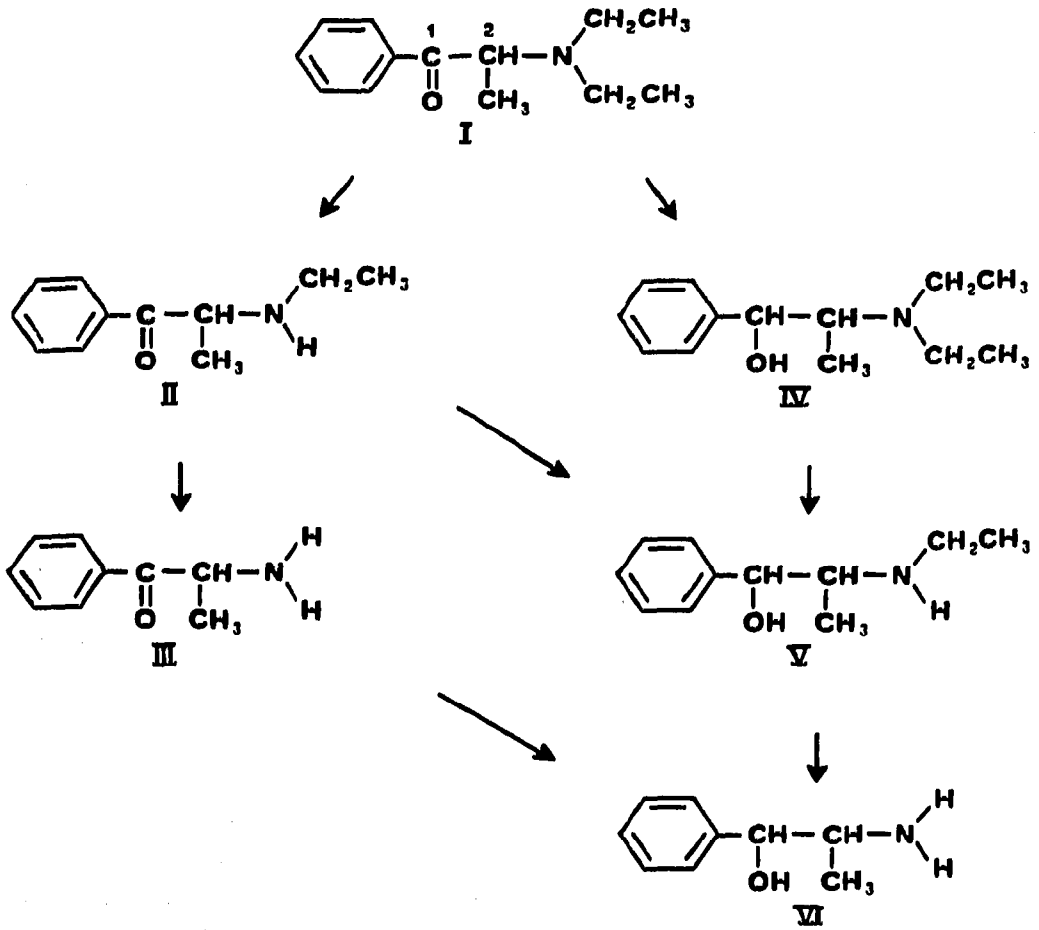


Fig. 1. Some routes of the metabolism of diethylpropion.

ments were made using a Bellingham Stanley/Bendix-Ericcson Polarmatic 62 instrument equipped with a 250 W Supersil xenon lamp with constant N_2 purging and operation at room temperature.

NMR spectra of the compounds as bases in $CDCl_3$ and as hydrochlorides in D_2O were obtained on a 60 MHz Perkin-Elmer R-10 instrument.

IR spectra were recorded on a Unicam SP 200 spectrophotometer and a Perkin-Elmer 157 G grating spectrophotometer with KCl discs.

Compounds

Compounds I to V were kindly supplied by the Wm. S. Merrell Co., Cincinnati, Ohio, U.S.A.

(−)-Norephedrine((−)-VI *erythro*) and (+)-norpseudoephedrine((+)-VI *threo*) were purchased from K & K Lab. Inc., Plainview, N. Y., U.S.A.

(+)-Norephedrine((+)-VI *erythro*) and (−)-norpseudoephedrine((−)-VI- *threo*) were kindly supplied by Wellcome Research Laboratories.

(+)- and (−)-N-ethylnorephedrine ((+)- and (−)-V *erythro*) and (+)- and (−)-N-ethylnorpseudoephedrine ((+)- and (−)-V *threo*), the preparation of these compounds has already been described[4].

(+)- and (−)-N-diethylnorephedrine ((+)- and (−)-IV *erythro*) and (+)-

follows. The corresponding primary amine base (0.005 mole) was dissolved in sodium-dried diethyl ether (*ca.* 50 ml). Acetyl chloride (0.01 mole) was added with constant stirring, and the mixture was allowed to stand for 10 min. $LiAlH_4$ (0.012 mole) was added, and the mixture refluxed for 24 h. The excess of reagent was then carefully hydrolyzed, the ethereal phase filtered, dried over anhydrous Na_2SO_4, and the ether evaporated and replaced with sodium-dried diethyl ether. The reaction products were examined by GLC on the first two columns described below. The whole synthetic process was repeated three or four times until the GLC control showed that the tertiary amine contained no primary or secondary amine. The amine hydrochloride was precipitated from a sodium-dried diethyl ether solution by the dropwise addition of ethanolic HCl, and recrystallized from an absolute ethanol–diethyl ether mixture. The identity and purity of the product were checked by GLC, IR and NMR spectroscopy and ORD.

Furfurylamphetamine acetate was kindly supplied by Laboratoires Diamant S.A., Paris.

N-Trifluoroacetyl-L-prolyl chloride (TPC) 0.1 *M* solution in $CHCl_3$ was purchased from Regis Chemical Co., Chicago, Ill., U.S.A.

AnalaR diethyl ether was freshly re-distilled.

Gas–liquid chromatography

Before GLC, the reported[5,6] general extraction procedure was used.

System A. A 1 m column packed with Chromosorb G (AW, DMCS-treated, 100–120 mesh) coated with KOH (10%), Carbowax 20M (2%) and Apiezon L (10%) was used. The following conditions were used: oven temperature, 200°; injection block temperature, *ca.* 200°; N_2 flow-rate, 33 ml/min (measured at room temperature); air and H_2 pressures, 25 and 20 p.s.i., respectively.

System B. A 2 m column packed with Chromosorb G (AW, DMCS-treated, 100–120 mesh) coated with KOH (5%) and Carbowax 20M (5%) was used. The following conditions were used: oven temperature, 180°; injection block temperature, *ca.* 200°; N_2 flow-rate, 35 ml/min (measured at room temperature); air and H_2 pressures, 25 and 20 p.s.i., respectively.

System C. A 2 m column packed with Chromosorb G (AW, DMCS-treated, 100–120 mesh) coated with SE-30 (3%) was used. The following conditions were used: oven temperature, 170°, injection block temperature, *ca.* 220°; N_2 flow-rate, 25 ml/min (measured at room temp.); air and H_2 pressures, 30 and 15 p.s.i., respectively.

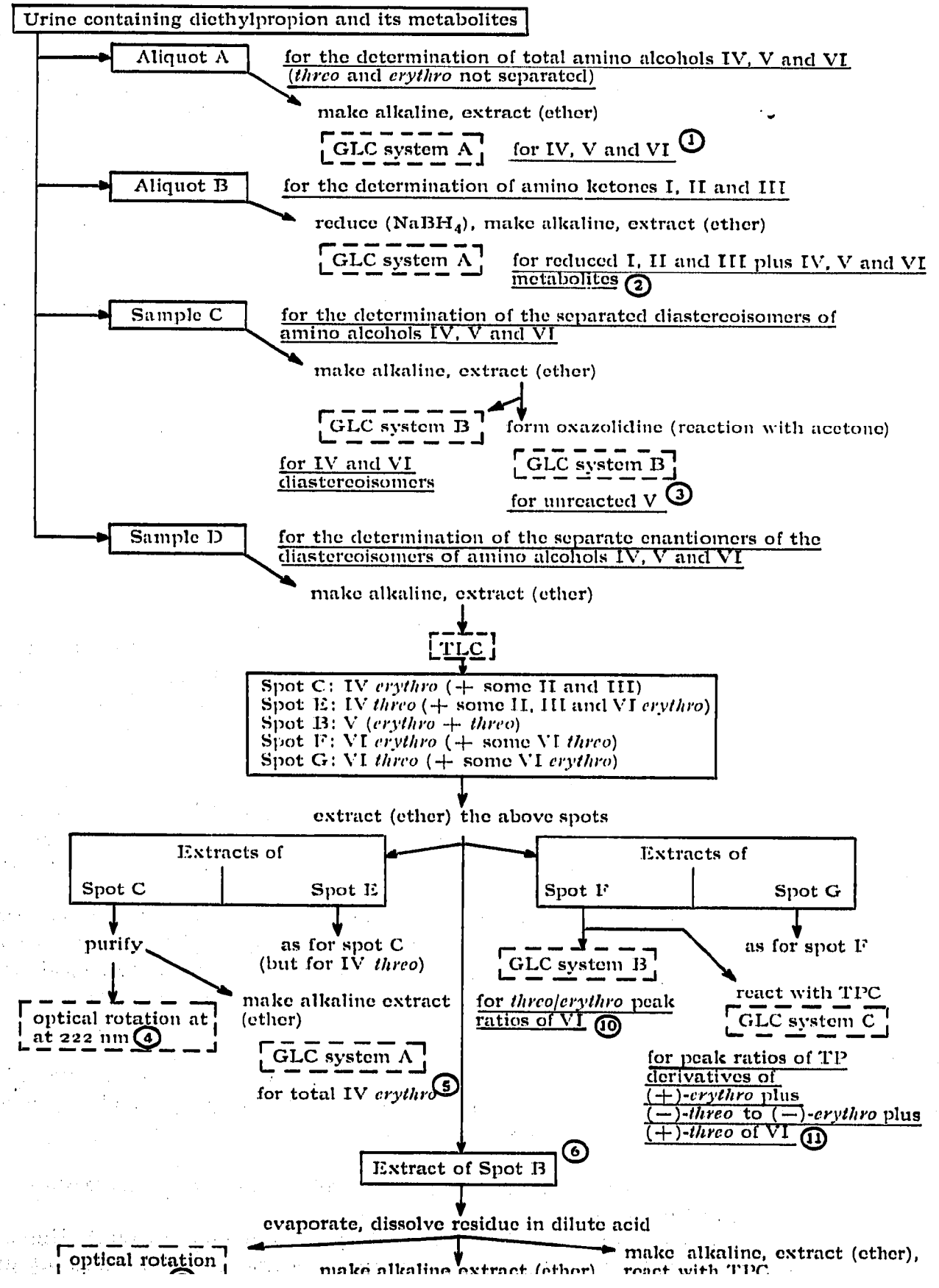
Thin-layer chromatography

For TLC, 20 × 20 cm glass plates, covered with MN Kieselgel G/254 UV, 0.5 mm thick, were activated for 1 h at 100° immediately before use. The solvent system used was acetone–methanol (88:12).

Determination of diethylpropion and its amino ketone and amino alcohol metabolites

See Fig. 2 for outline of scheme.

(*a*) *Amino alcohol metabolites* (*IV, V and VI*). To 4 ml of urine (aliquot A, Fig. 2), 1 ml of internal standard (I.S.) (furfurylamphetamine acetate, 40 μg/ml) and 0.5 ml of 5 *N* NaOH were added. The mixture was extracted with diethyl ether (4 × 3 ml).


Urine containing diethylpropion and its metabolites
Aliquot A
for the determination of total amino alcohols IV, V and VI (*threo* and *erythro* not separated)
make alkaline, extract (ether)
GLC system A
for IV, V and VI
1
Aliquot B
for the determination of amino ketones I, II and III
reduce ($NaBH_4$), make alkaline, extract (ether)
GLC system A
for reduced I, II and III plus IV, V and VI metabolites
2
Sample C
for the determination of the separated diastereoisomers of amino alcohols IV, V and VI
make alkaline, extract (ether)
GLC system B
for IV and VI diastereoisomers
form oxazolidine (reaction with acetone)
GLC system B
for unreacted V
3
Sample D
for the determination of the separate enantiomers of the diastereoisomers of amino alcohols IV, V and VI
make alkaline, extract (ether)
TLC
Spot C: IV *erythro* (+ some II and III)
Spot E: IV *threo* (+ some II, III and VI *erythro*)
Spot B: V (*erythro* + *threo*)
Spot F: VI *erythro* (+ some VI *threo*)
Spot G: VI *threo* (+ some VI *erythro*)
extract (ether) the above spots
Extracts of
Spot C
Spot E
Extracts of
Spot F
Spot G
purify
as for spot C (but for IV *threo*)
GLC system B
for *threo*/*erythro* peak ratios of VI
10
as for spot F
react with TPC
GLC system C
for peak ratios of TP derivatives of (+)-*erythro* plus (−)-*threo* to (−)-*erythro* plus (+)-*threo* of VI
11
optical rotation at 222 nm
4
make alkaline extract (ether)
GLC system A
for total IV *erythro*
5
6
Extract of Spot B
evaporate, dissolve residue in dilute acid
optical rotation
make alkaline extract (ether)
make alkaline, extract (ether), react with TPC

by GLC (system A). The concentrations of IV, V and VI in μg/ml were obtained by multiplying their peak height ratios to I.S. by the appropriate calibration factors (C.F.) (see (*c*) below). These peak height ratios were termed A_t.

(*b*) *Diethylpropion (I) and its amino ketone metabolites (II and III).* The method of BECKETT AND HOSSIE[7] was used. To 4 ml of urine (aliquot B, Fig. 2) were added 1 ml of I.S. and 0.7 ml of a phosphate buffer (52.4 g of K_2HPO_4+46.8 g of $NaH_2PO_4 \cdot 2H_2O$ in 200 ml of water). To this solution, $NaBH_4$ (*ca.* 10 mg) was added, and the mixture shaken once; this reduction process was repeated twice at 10-min intervals. Then 0.5 ml of 5 *N* NaOH was added, and the extractions and GLC analysis of the total amino alcohols IV, V and VI were carried out as described above. From the peak height ratios of IV, V and VI to I.S. (B_t), A_t values were subtracted. These differences were multiplied by the appropriate C.F. (see (*c*) below) to give the concentrations of I, II and III in μg/ml.

(*c*) *Calibration curves of I, II, III, IV, V and VI.* For these calibration curves, series of blank urine samples containing known amounts of I, II, III, IV *erythro* (or *threo* separately), V *erythro* (or *threo* separately) and VI *erythro* (or *threo* separately) were used.

Determination of the threo and erythro percentages of the amino alcohol metabolites IV, V and VI

(*a*) *Amino alcohols IV and VI.* Sample C (Fig. 2) (5–15 ml, depending on the concentration of the metabolites) was made alkaline with 5 *N* NaOH, extracted four times with diethyl ether, and the ethereal extract concentrated to 10–30 μl in the presence of AW anti-bumping granules; 2–3 μl were analysed by GLC (system B)

Fig. 2. Outline of scheme for the determination of diethylpropion and its amino ketone metabolites and amino alcohol metabolites (diastereoisomers and enantiomers).

1 Peak height ratios of IV, V and VI to internal standard (IS), furfurylamphetamine, designated as A_t.

2 Peak height ratios of reduced I, II and III plus IV, V and VI to IS designated as B_t.

3 Peak height ratios of V (*erythro* + *threo*) to IV *threo* (present as metabolite but considered as internal reference) before and after reaction with acetone to give the oxazolidine of V, designated as D and E, respectively (IV does not react).

4 The optical rotation in millidegrees is used to give G_t, concentration of optically active IV *erythro* or *threo*.

5 Peak height ratio of IV *erythro* or *threo* to IS, multiplied by the appropriate calibration factor, gives H_t, the total concentration of IV *erythro* or *threo*.

6 It is assumed that the fraction L of the *erythro* form, and the fraction $(1 - L)$ of the *threo* form of V in spot B are the same as obtained for V in the analysis of sample C.

7 The optical rotation in millidegrees is designated as K.

8 Peak height ratio of V (*erythro* + *threo*) to IS, multiplied by the appropriate CF, gives M, the total concentration of V.

9 The TP derivatives of the four isomers of V appear as two peaks; first peak (+)-*erythro* plus (−)-*threo*; second peak (−)-*erythro* plus (+)-*threo*. The ratio of the area of the first peak to the sum of the areas of both peaks (measurement of external triangles) is designated as N.

10 The ratio of the height of the *threo* peak of VI to that of the sum of the height of this peak plus the *erythro* peak of VI is termed R_1 (for spot E this ratio is termed R_2).

11 The TP derivatives of the four isomers of VI appear as two peaks (see (9)). The ratio of the area of the first peak to the sum of the areas of both peaks is termed P_1 (for spot E, the ratio of the area of the second peak to the sum is termed P_2).

In addition, the symbols m and n are used to designate the factors obtained by dividing

for IV and VI. The ratio of the *threo* peak height to the sum of *threo* and *erythro* peak heights (designated *threo* peak fraction) gave the *threo* and *erythro* percentages by comparison with calibration curves; the latter were obtained by determining the *threo* peak fraction by GLC (system B) after adding known amounts of *threo* and *erythro* forms of IV and V to blank urines.

The peak height ratio of V to IV *threo* was also measured.

(*b*) *Amino alcohol V.* From the chromatogram obtained above, the peak height ratio of V to IV *threo* was obtained (*D*).

From the remaining ethereal extract obtained in (*a*), the anti-bumping granules were removed and the ether was completely evaporated. Then 0.1 ml of a freshly prepared mixture of acetone–glacial acetic acid (100:0.25) was added to the residue, and the tube was hermetically stoppered and allowed to stand for 4 h at 46 ± 0.5° on a water-bath. The tube was cooled and the solution concentrated to a smaller volume by means of a nitrogen flow; 2–3 μl were then analysed by GLC (system B) for V and IV *threo*. The new ratio of V to IV *threo* (*E*) was determined and divided by *D* to give the fraction of unreacted V.

Blank urine samples containing known amounts of V *erythro* and V *threo* were submitted to this procedure in order to obtain a correlation between the percentage of reacted V and the *erythro* percentage of V.

Determination of the enantiomeric proportions of the diastereoisomers of IV, V and VI

Sample D (Fig. 2) (200–400 ml, depending on the concentration of the metabolites) was made acidic with 5 *N* HCl (2–4 ml) and washed twice with diethyl ether. The sample was then made alkaline with 5 *N* NaOH (10–20 ml) and extracted three times with diethyl ether. The ethereal extract was dried over anhydrous Na_2SO_4, concentrated to *ca.* 1 ml and then added as many discrete spots on one or two TLC plates. On the edges of the plates were put reference spots of authentic metabolites as controls. After developing, the spots were made visible by UV light (and by spraying with a saturated solution of iodine in CCl_4 for the reference spots).

The spots corresponding to the following metabolites were scraped off and suspended in 2–3 ml of 0.1 *N* HCl: spot C (IV *erythro*), spot E (IV *threo*), spot B (V), spot F (VI *erythro*) and spot G (VI *threo*) (see Figs. 2 and 7).

(*a*) *Amino alcohols IV erythro and IV threo.* The above separated silica gel suspensions of spots C and E were made alkaline with 5 *N* NaOH (0.5 ml) and extracted with diethyl ether (3 × 3 ml). The ethereal extracts were concentrated to *ca.* 0.5 ml. Acetic anhydride (0.02 ml) was added and the mixture allowed to stand for 30 min. Diethyl ether (1 ml) was added and the ethereal solutions were extracted with 0.1 *N* HCl (3 × 2 ml). The aqueous phases were washed with diethyl ether (2 × 2 ml) to remove any remaining amide, made alkaline and extracted with diethyl ether. These ethereal extracts were evaporated and the residues dissolved in 0.7–0.8 ml of 0.1 *N* HCl.

The optical rotations of the solution obtained from spot C and the one from spot E were measured in a 1-cm cell at 222 nm. These figures were divided by the factor obtained by dividing the optical rotation of IV *erythro* (or IV *threo*) by the concentration of pure isomer used in solutions of various concentrations; this gave the concentration G_i (in μg/ml) of optically active IV *erythro* (or IV *threo*).

solutions above was then measured by mixing 0.1 ml of each solution with 1 ml of I.S., extracting with diethyl ether and determining by GLC (system A) by comparison with calibration curves of IV *erythro* (or IV *threo*) against I.S.; the total concentration in μg/ml was designated as H_t and is equivalent to G_t plus the concentration of the (±) pair.

(*b*) *Amino alcohol V.* The silica gel suspension of spot B (Fig. 2) was extracted with diethyl ether, the extract evaporated and the residue dissolved in 0.7–0.8 ml of 0.1 *N* HCl.

The optical rotation of this solution was measured in a 1-cm cell at 222 nm and designated as *K* (in millidegrees). The factors *m* and *n* were obtained by dividing the optical rotations of various solutions (in 0.1 *N* HCl) of (+)-V *erythro* and (+)-V *threo*, respectively, by their concentrations. (The fraction L of the *erythro* form and the fraction (1 − L) of the *threo* form were assumed to be the same as already determined for V on sample C.)

The total concentration of V in this solution was measured by mixing 0.1 ml of the solution with I.S. (1 ml), extracting with diethyl ether and determining by GLC (system A) by comparison with a calibration curve of V against I.S.; the total concentration in μg/ml was designated as *M*.

The remainder of the acidic solution was made alkaline, extracted with diethyl ether, the ether was completely evaporated, and the residue dissolved in 2–3 μl of $CHCl_3$. TPC reagent (2–3 μl) was added and after 10 min this solution was analysed by GLC (system C) for the TP derivatives of (+)- and (−)-V *erythro* and (+)- and (−)-V *threo*. The fraction of (+)-V *erythro* plus (−)-V *threo* to total V (*N*) was measured by determining the ratio of the area of the first peak to the sum of the areas of this peak plus the second peak (measurements of the areas of the external triangles[4]).

(*c*) *Amino alcohols VI erythro and VI threo.* The silica gel suspensions of spot F and spot G (Fig. 2) were extracted with diethyl ether and the ethereal extracts concentrated to *ca.* 20 μl.

The extract (2–3 μl) of spot F was analysed by GLC (system B) for VI *erythro* and VI *threo* (as impurity); the fraction of VI *threo* in this solution was determined by comparison of the *threo/erythro* peak height ratio and designated as R_1.

In the same manner, the fraction of VI *threo* (VI *erythro* as impurity) in the spot G was determined, and designated as R_2.

The remaining ethereal extract of spot F was then completely evaporated, and the residue dissolved in 2–3 μl of $CHCl_3$. TPC reagent (2–3 μl) was added and after 10 min the solution was analysed by GLC (system C) for the TP derivatives of (+)- and (−)-VI *erythro* and (+)- and (−)-VI *threo*. The ratio of the area of the first peak to the sum of the areas of this peak plus the second peak ((+)-VI *erythro* plus (−)-VI *threo* to total VI) was measured and designated as P_1.

The ethereal extract of spot G was analysed in a similar manner. The ratio of the area of the second peak to the sum of the areas of this peak plus the first peak ((−)-VI *erythro* plus (+)-VI *threo* to total VI) was measured and designated as P_2.

Examination of the complete analytical scheme using mixtures of diethylpropion and its investigated metabolites in urine

The following blank urine solutions containing the various investigated

(1) solutions containing mixtures of all compounds I to VI in known concentrations;

(2) solutions containing compounds I, II and III, plus the six investigated diastereoisomers in known proportions;

(3) solutions containing compounds I, II and III, plus the enantiomers of IV *threo*, V *erythro* and *threo* and VI *erythro* and *threo* in known proportions.

These solutions were analysed as described in the scheme shown in Fig. 2 (solutions 1, as aliquots A and B; solutions 2, as samples C; solutions 3 as samples C and D).

RESULTS AND DISCUSSION

Stereochemistry of the amino alcohols IV, V and VI

The corresponding (+)-*erythro*, (−)-*erythro*, (+)-*threo* and (−)-*threo* forms of the amino-alcohols IV, V and VI were shown to have the same absolute configuration by the almost complete identity of the ORD curves of the corresponding isomers of IV, V and VI as salts in 0.1 *N* HCl and as bases in hexane (see ref. 4 for ORD curves of enantiomers of V *erythro* and V *threo*).

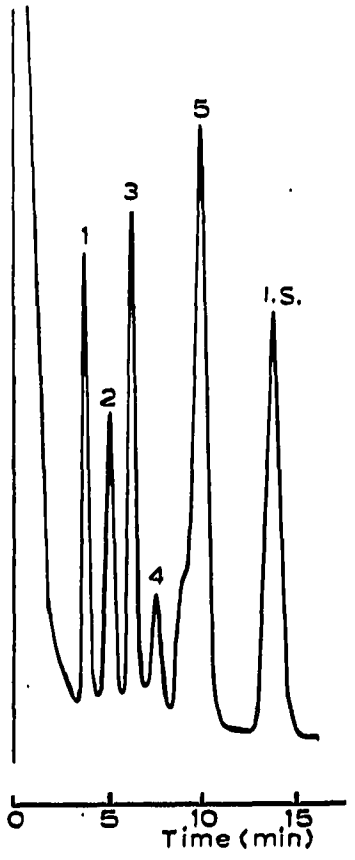


Fig. 3. Chromatogram showing the separation of diethylpropion and its metabolites. Peak 1, II + III; 2, VI; 3, V; 4, I; 5, IV; I.S., internal standard. GLC system A; 1-m stainless-steel column

Determination of diethylpropion (I) and its amino ketone (II and III) and amino alcohol (IV, V and VI) metabolites

Using GLC system A, the retention times obtained were: II + III 4.3 min; VI 5.3 min; V 6.5 min; I 7.7 min; IV 10.0 min; I.S. 13.7 min (see also Fig. 3). A number of columns and conditions were investigated, the objective being to produce a system that could give good separation of IV, V and VI with no interference from compounds I, II and III, as the latter could not be determined directly because of decomposition during extraction and on the column.

System A represented the best compromise, even though under these conditions compound I partially decomposed to give a secondary peak (retention time 9.0 min), which produced a shoulder on the peak of amino alcohol IV, but did not interfere with the peak height of IV.

In GLC system A, nicotine also gave a sharp peak (retention time 4.8 min) that partially overlapped with that of VI; hence subjects of metabolism studies with diethylpropion must refrain from smoking when the present method of analysis of Fig. 2 is used.

Calibration curves using system A under the conditions described in the EXPERIMENTAL section for amino alcohols IV, V and VI were linear over the range 0.3–10 μg/ml when the compounds were added to blank urine samples. The *threo* and *erythro* forms had exactly the same retention times and detector responses.

Although the amino ketones I, II and III underwent substantial breakdown during extraction and GLC, they could be determined by quantitative reduction with $NaBH_4$ to the corresponding amino alcohols IV, V and VI, which could be determined as such (see method of analysis of 4'-chloro-2-ethylaminopropiophenone and its metabolites[7,8]). Reduction of these ketones to the secondary alcohols as described in the experimental section, followed by GLC analysis using system A, gave straight calibration lines over the range 0.3–10 μg/ml in blank urine samples.

Although BANCI *et al.*[3] reported the determination of ketone II in solution by GLC, HOSSIE[2] was unable to determine the compounds I, II and III from aqueous solutions by GLC without substantial breakdown, even using mild conditions ($NaHCO_3$ alkalization); the present work confirmed the finding of HOSSIE.

Hence, the total of each amino alcohol IV, V and VI could be determined in one aliquot of urine (aliquot A) by direct GLC using system A. The ketones I, II and III could be determined using the same system on another aliquot of urine (aliquot B) after reduction to IV, V and VI, the peaks observed being the sum of the original amino alcohol metabolites and the same compounds produced by reduction of their corresponding ketones.

Determination of the erythro and threo percentages of the amino alcohol metabolites IV, V and VI

Using GLC system B, the retention times obtained were: II 7.4 min; I 10.1 min; III 11.5 min; IV *threo* 13.0 min; V *erythro* and *threo* 14.5 min; IV *erythro* 16.5 min; VI *threo* 18.7 min; VI *erythro* 20.0 min (see Fig. 4).

The *threo* and *erythro* forms of IV and VI were well resolved, and calibration curves of *threo* percentages against the measured *threo* peak fraction could be obtained (Fig. 5) from solutions of known amounts of *threo* and *erythro* IV and VI in blank

comparison with the above calibration curves will then allow the percentages of the *threo* and *erythro* forms of IV and VI to be determined.

Fig. 4. Chromatogram showing the separation of the amino alcohol metabolites of diethylpropion. Peak 1, II; 2, I; 3, III; 4, IV *threo*; 5, V *erythro* + *threo*; 6, IV *erythro*; 7, VI *threo*; 8, VI *erythro*. GLC system B: 2-m stainless-steel column (1/8 in. O.D.) packed with Chromosorb G (100–120 mesh, AW, DMCS-treated), coated with KOH (5%) and Carbowax 20M (5%). Oven temperature, 180°; N_2 flow-rate, 35 ml/min.

Because it was not found possible to separate the *erythro* and *threo* forms of V on a variety of columns without the peaks interfering with the peaks of other metabolites, resort was made to differences in the rates of oxazolidine formation of *erythro* and *threo* forms (*cf.*, different rates of oxazolidine formation from ephedrine and pseudoephedrine using acetone[9] or various ketones[10]). Under the conditions described in the experimental section, V *threo* reacted with acetone to give the oxazolidine much faster than did V *erythro*.

Using acidic catalysis and carefully standardized conditions (*i.e.*, acid-washed anti-bumping granules, a freshly prepared accurate mixture of acetone and acetic acid, hermetically stoppered reaction tubes, constant temperature, standardized time) followed by GLC analysis (system B), it was possible to obtain reproducible results when different mixtures of *threo* and *erythro* V were added to blank urine. The metabolically produced tertiary amine IV *threo* was used as the internal reference as it did not react with acetone. The percentage of the *erythro* form in a *threo* and *erythro* mixture of V was determined by comparison with a

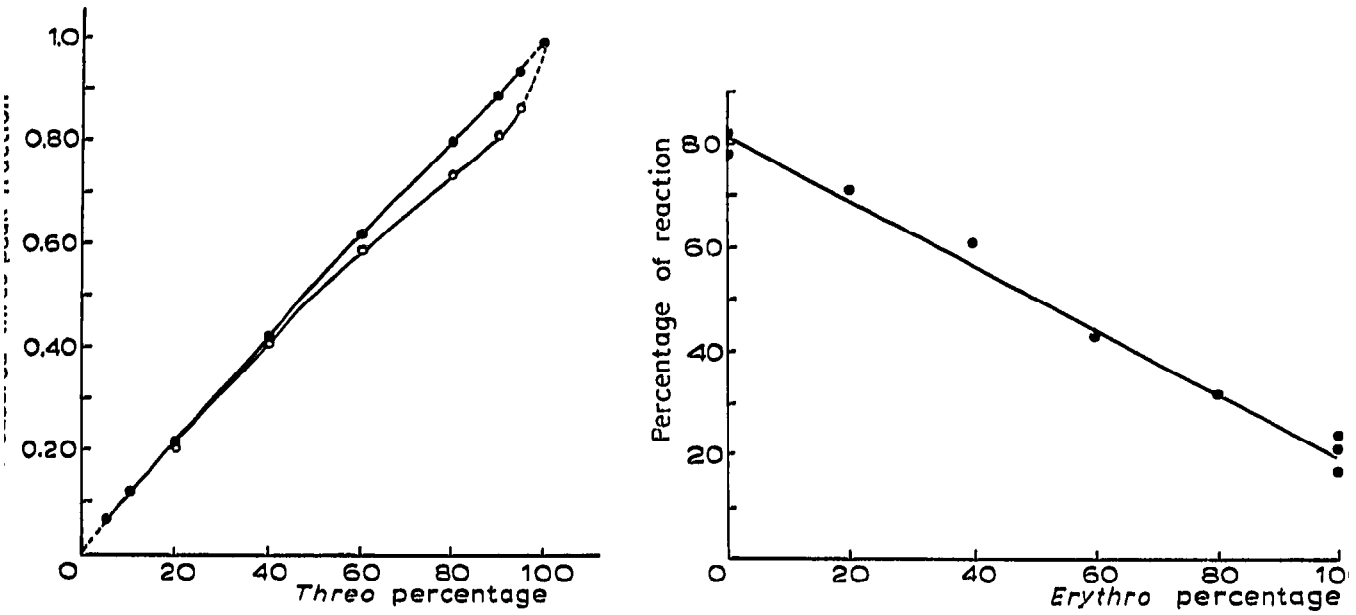


Fig. 5. Calibration curves for the determination of the *threo* percentages of IV (●) and VI (○). Conditions as in Fig. 4.

Fig. 6. Regression line correlating the percentage of *erythro* form of V and the percentage of oxazolidine formation under standardised conditions (see text). $y = -0.614x + 81.4$, $r = 0.996$.

(y) against the percentage of *erythro* form (x). This regression line was obtained from the results of GLC analysis of blank urine samples containing IV *threo* and known proportions of V *erythro* and V *threo*.

Determination of the enantiomeric percentages of the diastereoisomers of amino alcohols IV, V and VI

The application of TLC to the separation of the diastereoisomers of IV, V and VI was investigated. Of the many systems examined, the system described in the EXPERIMENTAL section proved to be the most useful. The R_F values of the pure compounds were: V *threo*, 0.13; V *erythro*, 0.20; IV *erythro*, 0.32; II + III, 0.46; IV *threo*, 0.53; VI *erythro*, 0.61; VI *threo*, 0.70; I, 0.83.

The main object of the search was to obtain of system to separate the diastereoisomers of IV, V and VI at the same time, and also to separate them from I, II and III. Hence the inability to separate II and III using the system finally adopted was not disadvantageous relative to the stated objective

Despite the separation finally effected, the spots of some of the various compounds were close to each other (Fig. 7) and therefore in metabolic studies it would not be possible to obtain a spot consisting solely of a single compound with sufficient purity to permit direct analysis of the enantiomeric percentage.

GLC analysis of the TLC spots obtained by adding all the above compounds to urine showed that spot C (R_F 0.32) consisted of IV *erythro* containing some II and III but no IV *threo*. Spot E (R_F 0.53) contained IV *threo* with some II, III and VI *erythro*, but no IV *erythro*. Spot B (R_F *ca.* 0.17) consisted of V *erythro* and *threo*. Spot F (R_F 0.61) consisted of VI *erythro* plus some VI *threo*, while spot G (R_F 0.70) consisted

Success in determining the enantiomers in the various diastereoisomers was finally achieved with the following multiple analytical procedure and calculations.

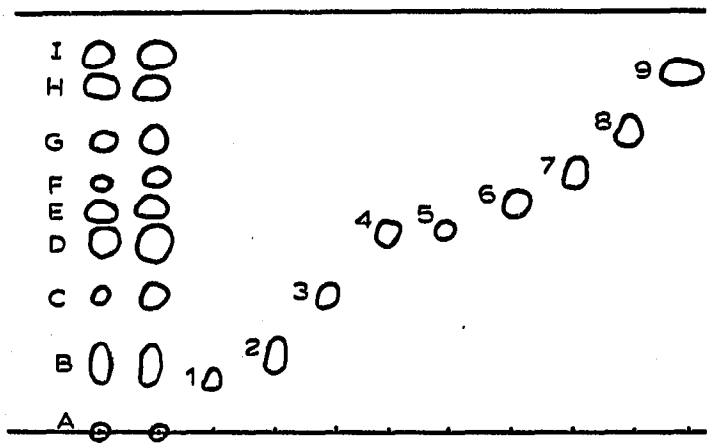


Fig. 7. Thin-layer chromatogram of diethylpropion and its metabolites. Authentic compounds: spot 1, V *threo*; 2, V *erythro*; 3, IV *erythro*; 4, II; 5, III; 6, IV *threo*; 7, VI *erythro*; 8, VI *threo*; 9, I. Spots A to I were observed from blank urine samples to which diethylpropion and its authentic metabolites were added.

(*a*) *Enantiomers of IV erythro.* The amino alcohol IV *erythro* is a tertiary amine, whereas the impurities in spot C are primary (III) and secondary (II) amines; the latter compounds were made non-basic by acetylation to form the amides.

The tertiary amine IV *erythro* was then extracted from the resultant ethereal mixture with dilute acid, and its optical rotation was determined. The purification procedure led to complete separation of IV *erythro* from III and II (less than 1% of II remained in the solution). To give a reasonable reading of the optical rotation at 222 nm, at least 100 μg of optically active IV *erythro* were required.

The two determinations carried out with this solution, namely, determination of the concentration of optically active IV *erythro* by optical rotation measurement, and the determination of the total concentration of IV *erythro* by GLC system A, allowed the percentage of the predominant enantiomer in a mixture of (+)- and (−)-IV *erythro* to be calculated by means of eqn. 1 (see later).

(*b*) *Enantiomers of IV threo.* The tertiary amine IV *threo* in spot E was contaminated with the primary amine VI *erythro* in addition to the primary III and secondary II amines. Acetylation as described for IV *erythro* ((*a*) above) was therefore adopted to separate the desired compound from its impurities in spot E.

For a reasonable reading of the optical rotation, at least 50 μg of optically active IV *threo* were required.

The determinations of the concentration of optically active IV *threo* and the determination of the concentration of total IV *threo* were carried out as described for IV *erythro*, thus permitting the use of eqn. 1 (see later) to calculate the percentage of the predominant enantiomer in a solution containing (+)- and (−)-IV *threo*.

(*c*) *Enantiomers of V erythro and V threo.* The R_F values of pure V *erythro* and V *threo* were not very different and therefore in spot B both were present as indicated by GLC analysis: no other metabolite was found in the spot.

sufficiently pure form for the following analysis. Various different determinations were necessary as described in the experimental section, each determination leading to results all of which were required for the mathematical treatment (see below) to yield the enantiomeric percentages of (+)- and (−)-V *erythro* and (+)- and (−)-V *threo*.

The required amounts of optically active V *erythro* and V *threo* for a reasonable reading of the optical rotation were the same as for IV *erythro* and *threo*.

The separation of various "ephedrines" and ' pseudoephedrines" by making TP derivatives and using GLC system C has already been described[4]. Using the analytical conditions described in the experimental section, TP derivatives of authentic V isomers showed two peaks: the first peak (retention time 114 min) consisted of (+)-V *erythro* and (−)-V *threo*; the second peak (retention time 120 min) consisted of (−)-V *erythro* and (+)-V *threo*. The separation factor of these two peaks was 0.55, thus allowing the determination of the ratio of the sum ((+)-V *erythro* plus (−)-V *threo*) to the sum ((−)-V *erythro* plus (+)-V *threo*) by measuring the areas of the external triangles of the peaks.

Using (+)- and (−)-ephedrine, we have shown[4] that the measurement of the areas of the external triangles of partially overlapping TP derivatives peaks allows a good estimation of the enantiomeric ratio.

(*d*) *Enantiomers of VI erythro and VI threo.* GLC analysis (system B) of spot F showed the presence of VI *erythro* with some VI *threo* as impurity, while spot G showed the presence of VI *threo* with some VI *erythro* as impurity. The percentage of the diastereoisomers in each spot was determined under the same GLC conditions.

Extracts of spot F and of spot G were then separately treated with TPC and subjected to GLC system C analysis. Independent analysis of the four authentic isomers of VI gave two peaks under these conditions; the first peak (retention time 63 min) consisted of (+)-VI *erythro* and (−)-VI *threo*; the second peak (retention time 70 min) consisted of (−)-VI *erythro* and (+)-VI *threo*. The separation factor was 0.93. The ratio of these two peaks was determined by measuring their areas.

The determinations carried out with the extracts of spots F and G led to results which required collective consideration as described by various equations (see below). Mathematical resolution of these equations led to eqns. 4 and 5, which allow the calculation of the enantiomeric percentages of (+)- and (−)-VI *erythro* and of (+)- and (−)-VI *threo*.

Calculations used for the determination of enantiomeric percentages

(*a*) *Enantiomers of IV erythro and IV threo.* Using G_t, the concentration of optically active amino alcohol, and H_t, the total concentration (G_t plus concentration of the (±) pair) in the solution, the percentage Z_t of the predominant enantiomer of total IV *erythro* or total IV *threo* can be obtained from eqn. 1.

$$Z_t = 100(H_t + G_t)/2H_t \qquad (1$$

(*b*) *Enantiomers of V erythro and V threo.* If a, b, c and d represent the concen

in the solution obtained from spot B, the following equations can be written:

$$m(a-b)+n(c-d)=K$$
$$(a+b)/(a+b+c+d)=L$$
$$(a+b+c+d)=M$$
$$(a+d)/(a+b+c+d)=N$$

These four equations are solved for a, b, c and d, and from the values obtained, the (+)/(−) ratio of V *erythro* ($u = a/b$), and the (+)/(−) ratio of V *threo* ($w = c/d$) can be calculated:

$$u=\frac{ML(m+n)-nM(1-2N)+K}{ML(m+n)+nM(1-2N)-K} \tag{2}$$

$$w=\frac{(2M-ML)(m+n)-M(2mN+n)+K}{M(2mN+n)-ML(m+n)-K} \tag{3}$$

The percentage U of the (+) form in total V *erythro* and the percentage W of the (+) form in total V *threo* are then calculated from

$U = 100u/(u+1)$; $W = 100w/(w+1)$.

(c) *Enantiomers of VI erythro and VI threo.* If the concentrations of the four isomers of VI ((+)- and (−)-*erythro*, (+)- and (−)-*threo*) in the extract of spot F are designated as a_1, b_1, c_1 and d_1, respectively, and a_2, b_2, c_2 and d_2 in the extract of spot G, the following equations can be written:

$$(c_1+d_1)/(a_1+b_1+c_1+d_1)=R_1$$
$$(c_2+d_2)/(a_2+b_2+c_2+d_2)=R_2$$
$$(a_1+d_1)/(a_1+b_1+c_1+d_1)=P_1$$
$$(b_2+c_2)/(a_2+b_2+c_2+d_2)=P_2$$

Then

$$s=a_1/b_1=a_2/b_2$$

and

$$t=c_1/d_1=c_2/d_2$$

where s and t are the (+)/(−) ratios of VI *erythro* and VI *threo*, respectively. Solving these equations for s and t gives:

$$t=\frac{s-sP_1+R_1-P_1}{sP_1+sR_1-s+P_1} \tag{4}$$

and

$$s^2(P_2R_1-R_2+P_1R_2)+s(2P_2R_1-R_1+2P_1R_2-R_2)+P_2R_1-R_1+P_1R_2=0 \tag{5}$$

Eqn. 5 has two solutions from the general solution of the quadratic, *viz.*, $s = -1$ and the actual chemical answer:

$$-b-\sqrt{b^2-4ac}$$

TABLE I

COMPARISON BETWEEN ADDED AND FOUND CONCENTRATIONS (IN μg BASE/ml) OF I, II, III, IV, V AND VI

Amino ketone I		*Amino ketone II*		*Amino ketone III*	
Added	*Found*	*Added*	*Found*	*Added*	*Found*
0.354	0.321	0.302	0.309	0.303	0.351
0.884	0.954	0.756	0.747	0.758	0.803
1.415	1.35	1.21	1.30	1.212	1.370
2.48	2.65	2.12	2.19	2.12	2.34
3.58	3.83	3.02	2.95	3.03	3.02
5.31	5.04	4.54	4.39	4.55	4.98
7.07	6.86	6.04	5.70	6.06	6.20
8.84	9.26	7.56	7.91	6.82	6.40
Mean difference 6.1%		*Mean difference* 4.5%		*Mean difference* 6.7%	

Amino alcohol IV		*Amino alcohol V*		*Amino alcohol VI*	
Added	*Found*	*Added*	*Found*	*Added*	*Found*
0.317	0.309	0.290	0.295	0.283	0.273
0.792	0.799	0.724	0.791	0.708	0.721
1.267	1.338	1.159	1.19	1.133	1.178
2.22	2.23	2.03	1.90	1.98	1.82
3.17	3.24	2.90	2.88	2.83	2.98
4.75	4.67	4.35	4.15	4.25	4.36
6.33	6.72	5.80	5.73	5.66	6.03
7.91	7.79	7.24	6.84	7.08	7.23
Mean difference 3.1%		*Mean difference* 4.0%		*Mean difference* 5.0%	

TABLE II

COMPARISON BETWEEN KNOWN AND DETERMINED DIASTEREOISOMERIC PERCENTAGES OF IV, V AND VI

Amino alcohol IV *Percentage of erythro form*		*Amino alcohol V* *Percentage of erythro form*		*Amino alcohol VI* *Percentage of erythro form*	
Added	*Found*	*Added*	*Found*	*Added*	*Found*
5	5.7	0	−1	5	6.0
10	10.7	20	16	10	8.0
20	19.0	40	33	20	23.3
40	40.4	60	62	40	38.0
60	60.7	80	80	60	61.3
80	80.4	100	98	80	80.0
90	89.3			90	84.0
95	94.7			95	93.7
Mean difference 0.6%		*Mean difference* 3%		*Mean difference* 3.1%	

TABLE III

COMPARISON BETWEEN KNOWN AND DETERMINED ENANTIOMERIC PERCENTAGES OF IV *threo*, V *erythro* AND *threo*, AND VI *erythro* AND *threo*

Stereoisomer		*Added* (%)	*Found* (%)	*Added* (%)	*Found* (%)
IV	(+)-*threo*	87	91	70	74
	(−)-*threo*	13	9	30	26
		100	100	100	100
V	(+)-*erythro*	5	8	10	11
	(−)-*erythro*	20	15	15	13
	(+)-*threo*	45	45	60	60
	(−)-*threo*	30	32	15	16
		100	100	100	100
VI	(+)-*erythro*	6	6	12	13
	(−)-*erythro*	53	56	47	48
	(+)-*threo*	6	10	12	15
	(−)-*threo*	35	28	29	24
		100	100	100	100

The percentage S of the (+) form in total VI *erythro* and the percentage T of the (+) form in total VI *threo* can then be calculated from

$$S = 100s/(s + 1); \qquad T = 100t/(t + 1).$$

Validation of the analytical scheme using a variety of solutions containing known amounts of compounds I, II and III, and amino alcohols IV, V and VI both in their diastereoisomeric and enantiomeric forms

The results obtained are shown in Tables I, II and III.

For Table III, the various proportions of the compounds chosen were such as to be in the range of proportions and concentrations indicated by preliminary metabolic studies of diethylpropion in man. For these reasons, the enantiomers of IV *erythro* were not considered.

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