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Gas-liquid chromatographic separation of the optical isomers of some "ephedrines" and "pseudoephedrines"

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Gas-liquid chromatographic separation of the optical isomers of norephedrine, norpseudoephedrine, ephedrine, pseudoephedrine, *N*-ethylnorephedrine and *N*-ethylnorpseudoephedrine by means of their *N*-trifluoroacetyl-L-prolyl derivatives has been investigated. A quantitative determination of the enantiomeric percentages was possible for the primary amines, and for all secondary amines except pseudoephedrine. The method is suitable for metabolic studies.

The separation of optically active amines by gas-liquid chromatography (g.l.c.) can be achieved by using either an optically active stationary phase, after making derivatives with a suitable optically inactive reagent (e.g. Corbin & Rogers, 1970) or an optically active reagent to form diastereoisomers followed by chromatography on an optically inactive stationary phase.

N-Trifluoroacetyl-L-prolyl chloride (TPC) is such an optically active reagent. It has been used for the resolution of numerous asymmetric amines, including ephedrine (Halpern & Westley, 1966; Karger, Stern & others, 1969; Westley & Halpern, 1969; Westley, Halpern & Karger, 1970), but the purpose of those studies was mainly to correlate stereochemical features of the asymmetric compounds to the relative retention times of their diastereoisomeric derivatives. Neither the separation factors (see below) nor the suitability of the method for quantitative work (i.e. determination of the enantiomeric percentages) were investigated.

The latter approach was followed for amphetamine by Gordis (1966); Gunne (1967) investigated the urinary output of both (+)- and (−)-amphetamine in man after intake of racemic amphetamine and methylamphetamine.

Numerous metabolic studies involving ephedrine-type compounds have been published. However, the determination of the enantiomeric percentages of minute quantities of the excreted products has not been possible, due to the lack of a sensitive analytical technique.

We now report investigations designed to provide a method for the separation and quantitative determination of the optical isomers of some "ephedrines" and "pseudoephedrines" encountered in metabolic studies.

MATERIALS AND METHODS

Compounds

TPC. *N*-Trifluoroacetyl-L-prolyl chloride 0·1M in $CHCl_3$ (Regis Chemical Co., Chicago, Ill.).

(+)- *and* (−)-*Norephedrine* (*NE*), (+)- *and* (−)-*norpseudoephedrine* (*NPE*) were kindly supplied by Welcome Research Laboratories.

(+)- *and* (−)-*Ephedrine* (*E*), (+)- *and* (−)-*pseudoephedrine* (*PE*) were kindly supplied by Prof. J. B. LaPidus, Ohio State University.

(+)- *and* (−)-N-*Ethylnorephedrine* (*ENE*), (+)- *and* (−)-N-*ethylnorpseudoephedrine* (*ENPE*). The appropriate primary amines (0·005 mol) were dissolved in a cold solution of $NaHCO_3$ (0·02 mol) in water (25 ml) and acetic anhydride (0·012 mol) was added with constant stirring. After 15 min, 5N HCl was added to pH 5, and the solution was extracted with ether. The ethereal solution was washed with 2% acetic acid (2 × 20 ml) to remove the unreacted primary amine (its absence in the ether solution was then checked by g.l.c.). The ether solution was dried (Na_2SO_4 anhyd.), evaporated to dryness, and the residue dissolved in Na-dried ether, $LiAlH_4$ (0·015 mol) was added, and the mixture refluxed for 24 h. After destruction of the excess $LiAlH_4$ with wet ether and water, the reaction mixture was extracted with ether. The ether in this solution was then replaced by Na-dried ether as previously described, and the hydrochloride of the amine was precipitated by ethanolic HCl. The product was recrystallized from absolute ethanol–ether, and analysed by g.l.c. (Beckett, Tucker & Moffat, 1967), infrared (0·5% KCl discs) and nmr (base in $CDCl_3$).

Optical rotatory dispersion. Measurements* were made using a Bellingham Stanley/Bendix Ericsson Polarmatic 62 equipped with a 250 W Supersil Xenon Lamp with constant N_2 purging (1·0 cm cell) at room temperature (20°). The solvent used was 0·1N HCl.

Preparation of TP derivatives. TPC solution (5–10 μl) was added to the amine in chloroformic solution (10–50 μl). After 10–15 min, the solution (1–5 μl) was injected in the chromatograph.

G.l.c. conditions. The column was of stainless steel, 2 metres long, ⅛ in o.d., packed with Chromosorb G (AW, DMCS treated, 100–120 mesh) coated with SE 30 3%; oven temperature 170°, injection block temperature *ca* 220°, nitrogen flow rate 25 ml min^{-1} (measured at room temperature), air and hydrogen pressures 30 and 15 p.s.i. respectively.

Determination of the enantiomeric percentages of norephedrine and ephedrine. For these determinations, series of blank urine samples containing norephedrine and ephedrine in known enantiomeric percentages were prepared. These solutions were made alkaline and extracted with ether. The separated ether extract was completely evaporated, and the dry residue dissolved in chloroform before reacting with TPC.

Separation factor. The separation factor R was calculated from the formula (Pattison, 1969):

$$R = 2\,\overline{NQ}/(\overline{AB} + \overline{CD})$$ (see Fig. 1A)

*(+)-*Ethylnorephedrine:* Concentration 5·6 × 10^{-3}M; $[\Phi]_{500}$ + 60, $[\Phi]_{400}$ + 95, $[\Phi]_{350}$ + 140, $[\Phi]_{300}$ + 185, $[\Phi]_{270}$ + 165, $[\Phi]_{267}$ + 48, $[\Phi]_{264}$ + 280, $[\Phi]_{259}$ + 230, $[\Phi]_{256}$ + 430, $[\Phi]_{253}$ + 490, $[\Phi]_{250}$ + 600, $[\Phi]_{233}$ + 790, $[\Phi]_{222}$ + 1200.

(−)-*Ethylnorephedrine:* Concentration 4·4 × 10^{-3}M; $[\Phi]_{500}$ − 76, $[\Phi]_{400}$ − 120, $[\Phi]_{350}$ − 180, $[\Phi]_{300}$ − 260, $[\Phi]_{270}$ − 260, $[\Phi]_{267}$ − 60, $[\Phi]_{264}$ − 410, $[\Phi]_{259}$ − 300, $[\Phi]_{256}$ − 620, $[\Phi]_{253}$ − 600, $[\Phi]_{250}$ − 780, $[\Phi]_{233}$ − 1000, $[\Phi]_{222}$ − 1450.

(+)-*Ethylnorpseudoephedrine:* Concentration 4·5 × 10^{-3}M; $[\Phi]_{500}$ + 145, $[\Phi]_{400}$ + 290, $[\Phi]_{350}$ + 440, $[\Phi]_{300}$ + 700, $[\Phi]_{270}$ + 1000, $[\Phi]_{267}$ + 1000, $[\Phi]_{264}$ + 1180, $[\Phi]_{259}$ + 1180, $[\Phi]_{256}$ + 1400, $[\Phi]_{250}$ + 1600, $[\Phi]_{233}$ + 2450, $[\Phi]_{22}{}^{5}$ + 3600.

(−)-*Ethylnorpseudoephedrine:* Concentration 2·3 × 10^{-3}M; $[\Phi]_{500}$ − 190, $[\Phi]_{400}$ − 300, $[\Phi]_{350}$ − 460,]$\Phi[_{300}$ − 690, $[\Phi]_{270}$ − 970, $[\Phi]_{267}$ − 970, $[\Phi]_{264}$ − 850, $[\Phi]_{259}$ − 830, $[\Phi]_{256}$ − 1300, $[\Phi]_{250}$ − 1500, $[\Phi]_{233}$ − 2200, $[\Phi]_{222}$ − 3100.

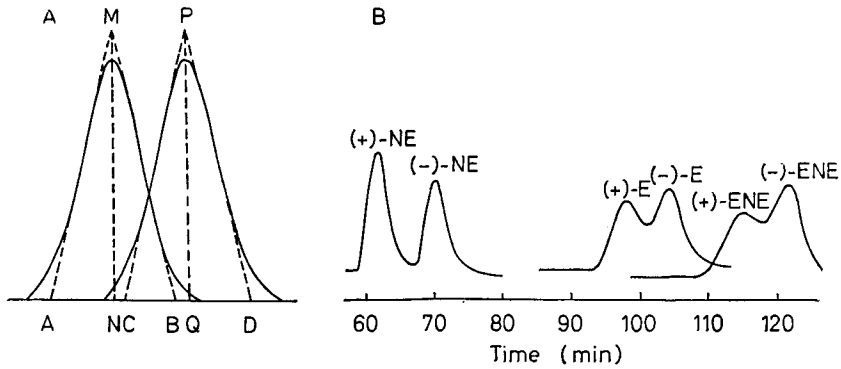

FIG. 1A. Theoretical chromatograms: AM, BM, CP and DP are inflection tangents; AB and CD are bases of triangles.

B. Graphical combination of three chromatograms, each of them showing the separation of one enantiomeric pair: norephedrine (NE), ephedrine (E) and *N*-ethylnorephedrine (ENE).

RESULTS AND DISCUSSION

Configuration

The synthetic method for the above *N*-ethyl compounds is not expected to produce any configurational change. Nearly complete identity of the O.R.D. curves of (+)-norephedrine, (+)-ephedrine and (+)-ethylnorephedrine gives further evidence for identical configuration, i.e. (1*S*:2*R*). So does comparison of the O.R.D. curves of the other trios: (−)-erythro (1*R*:2*S*), (+)-threo (1*S*:*S*), (−)-threo (1*R*:2*R*). (For the O.R.D. curves of ephedrines, see Mitscher, Kautz & Lapidus, 1969).

G.l.c. separation of optical isomers

From the various columns tried, SE 30 on Chromosorb G AW, DMCS treated, 100–120 mesh, gave the best resolution. The best concentration of stationary phase was found to be 3%. Decreasing oven temperature and carrier gas flow rate increased resolution.

The retention times of the TP derivatives of the compounds studied are shown in Table 1.

The separation is quantitatively expressed by R, the separation factor. Three cases can be distinguished. For the primary amines, the separation of enantiomers is complete ($R > 1$), and the triangles ABM and CDP (see Fig. 1) do not overlap. For the secondary amines, the separation is not complete ($R < 1$), and the two triangles ABM and CDP do overlap. But for all these secondary amines except pseudoephedrine, $R > 0{\cdot}5$, which means there is no overlap in the external triangles ANM and QDP. For pseudoephedrine, the external triangles ANM and QDP overlap ($R < 0{\cdot}5$).

Examples of the separation obtained are given in Fig. 1B, which is a graphical combination of three chromatograms, each of them showing the resolution of one enantiomeric pair: (+)- and (−)-norephedrine, (+)- and (−)-ephedrine and (+)- and (−)-ethylnorephedrine.

The above method does not separate diastereoisomers with the same configuration at the carbon atom α to the nitrogen (carbon 2).

Table 1. *Separation of the TP derivatives of some "ephedrines" and "pseudoephedrines".*

	Retention time in min	Width of triangle base in min*	Separation factor R	Comment
(+)-Norephedrine	62	6		Complete
(−)-Norephedrine	70	6	1·33	resolution
(+)-Norpseudoephedrine ..	71	6		Complete
(−)-Norpseudoephedrine ..	64	6	1·15	resolution
(+)-Ephedrine	98	9		Usable
(−)-Ephedrine	105	9	0·78	resolution
(+)-Pseudoephedrine	105	9		Poor
(−)-Pseudoephedrine	101	9	0·45	resolution
(+)-Ethylnorephedrine	114	9		Usable
(−)-Ethylnorephedrine	121	9	0·78	resolution
(+)-Ethylnorpseudoephedrine ..	118	9		Usable
(−)-Ethylnorpseudoephedrine ..	113	9	0·56	resolution

Column: Chromosorb G, AW, DMCS treated, 100–120 mesh, coated with 3% SE 30, packed in a 2 metre stainless steel tube $\frac{1}{8}$ in o.d.

Conditions: Oven temperature 170°, injection block temperature *ca* 220°, nitrogen flow rate 25 ml min^{-1}, air pressure 30 p.s.i., H^2 pressure 15 p.s.i.

* See Fig. 1A.

Quantitative assessment of the g.l.c. separation

When $R > 1$, as is the case for norephedrine and norpseudoephedrine, the triangles ABM and CDP do not overlap, and their areas are expected to be proportional to the enantiomeric percentages of the amine. To prove this point, norephedrine was taken as a model compound, and urine solutions containing various enantiomeric percentages were prepared and analysed. The actual and found percentages are given in Table 2; a comparison between the two sets of values shows that the method is suitable for the determination of enantiomeric percentages.

Table 2. *Determination of enantiomeric percentages for prepared mixtures of (+)- and (−)-norephedrine and (−)-ephedrine by g.l.c. of TP derivatives, followed by measurements of the triangle areas for norephedrine and of the external triangle areas for ephedrine.*

Norephedrine		Ephedrine		
Prepared isomeric mixtures [(+)/(−)]	Found isomeric % (2 determinations)	Prepared isomeric mixtures [(+)/(−)]	Found isomeric % (2 determinations)	
100/0	100/0*	100/0	100/0*	
90/10	85/15	90/10	†	
80/20	77/23	80/20	83/17	Suggested limits of usefulness of the analytical method
60/40	57/43	60/40	65/35	
50/50	49/51	50/50	51/49	
40/60	39/61	40/60	45/55	
20/80	21/79	20/80	28/72	
10/90	14/86	10/90	13/87	
0/100	6/94‡	0/100	<5/>95‡	

* Some slight tailing possibly due to some enantiomeric impurity.

† (−) as a shoulder because the isomer with the longer retention time is in much smaller concentration in the mixture.

‡ This could either be due to the enantiomeric impurity, or to the diastereoisomeric impurity (−)-ephedrine-trifluoroacetyl-D-prolyl amide.

Column and conditions as stated in Table 1.

However, when $R < 1$, this method is not usable. But, provided the peaks shape is symmetrical and $R > 0{\cdot}5$, the enantiomeric percentages can be obtained by the percentages of the areas of the external triangles ANM and QDP. Ephedrine was taken as a model compound for the case $0{\cdot}5 < R < 1$. The results (Table 2) show that the measurement of the areas of the external triangles is suitable for a determination of enantiomeric percentages. Only when the isomeric mixtures are such that the peak with the longer retention time represents less than one quarter of the other peak, the former appears only as a shoulder, and no quantitative determination is possible; when the smaller peak is the one with the shorter retention time resolution is good, and quantitative assessment is practicable.

When $R < 0{\cdot}5$, measurement of the areas of external triangles is precluded by their overlap.

Suitability of the method for biological studies

Approximately 50 μg of an ephedrine-type compound was the minimum quantity needed for an adequate determination to be made out. This amount is sufficiently small to allow the method to be used in metabolic studies.

No interfering peaks were detected when blank urine samples from various subjects were submitted to the analytical procedure.

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