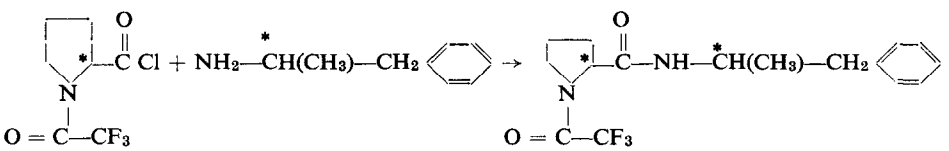
3. R. W. SCHAYER, *Fedn Proc.* **24**, 1295 (1965).
4. J. E. FISCHER et S. H. SNYDER, *Fedn Proc.* **24**, 1334 (1965).
5. D. A. BRODIE, R. W. MARSHALL et O. M. MORENO, *Gastroenterology* **43**, 675 (1962).
6. G. ROSSI, S. BONFILS, F. LIEFFOGH et A. LAMBLING, *C.r. Séanc Soc. Biol.* **150**, 2124 (1956).
7. Y. KOBAYASHI, *Analyt. Biochem.* **5**, 284 (1963).
8. K. S. KIM et P. A. SHORE, *J. Pharmac.* **141**, 321 (1963).
9. R. J. LEVINE, *Fedn Proc.* **24**, 1331 (1965).
10. W. R. THAYER, A. H. TOFFLER, G. CHAPO et H. M. SPIRO, *Yale J. Biol. Med.* **38**, 257 (1965).

Biochemical Pharmacology, 1966, Vol. 15, pp. 2124–2126. Pergamon Press Ltd., Printed in Great Britain.

Gas-chromatographic resolution of optical isomers in microgram samples of amphetamine*

(*Received* 21 *May* 1966; *accepted* 2 *August* 1966)

DURING the past five years, several enantiomeric pairs have been resolved by gas-liquid chromatography: *d,l*-camphor,[1] *d,l*-amino acids,[2–4] and *d,l*-2-alkanols.[4] The resolving agent, trifluoroacetyl-*l*-prolyl chloride (hereafter abbreviated TPC), was introduced by Halpern and Westley[3] for the gas-chromatographic resolution of amino acids. With amino acids, TPC forms diastereoisomeric trifluoroacetyl-*l*-prolyl peptides which are soluble in organic solvents and sufficiently volatile. This report describes the gas-chromatographic resolution of the optical isomers of amphetamine as the diastereoisomeric pair, *l*-(N-trifluoroacetyl)-prolyl-*d,l*-amphetamine.



* Asymmetric carbon.

MATERIALS

The resolving agent was prepared by the method of Weygand *et al.*:[5] 1·1 g *l*-proline, dried by heating 15 min at 130° under vacuum, was dissolved in 1·6 ml trifluoroacetic anhydride at −15°, then warmed briefly at 30°. The trifluoroacetic acid and anhydride were evaporated under vacuum. The residue was refluxed 30 min in thionyl chloride and the thionyl chloride evaporated. The product was dissolved in benzene and used without further purification. It has remained in satisfactory condition several months, stored in a desiccator at 4°.

METHODS AND RESULTS

A benzene solution of amphetamine was mixed with the solution of the resolving agent, warmed 1 min on a water bath, and neutralized with tributylamine. The benzene solution was washed with

* Presented in part at the 50th annual meeting of the Federation of American Societies for Experimental Biology, April 1966. Abstracted in *Fedn Proc.* **25**, 690 (1966). Supported in part by U.S. Department of Health, Education and Welfare, AM 03963-06; and in part by Health Research Council of the City of New York, Grant U-1501.

water, dried with sodium sulfate, and injected into the column. The isomers were resolved by chromatography on a 9-foot column of 3% SE-30 siloxane polymer on Gas-Chrom P at 175°. (Column packings were obtained from Applied Science Laboratories, State College, Pa.) Argon, delivered at 15 lb/in^2 was the carrier gas, and effluents were detected with a strontium-90 ionization detector.

Figure 1 shows the results obtained by gas chromatography of TPC alone, and of the diastereoisomers derived from the individual isomers and from the racemic mixture. The lowermost tracing shows the resolution of a mixture of the separately prepared diastereoisomers. Five microgram samples were easily resolved.

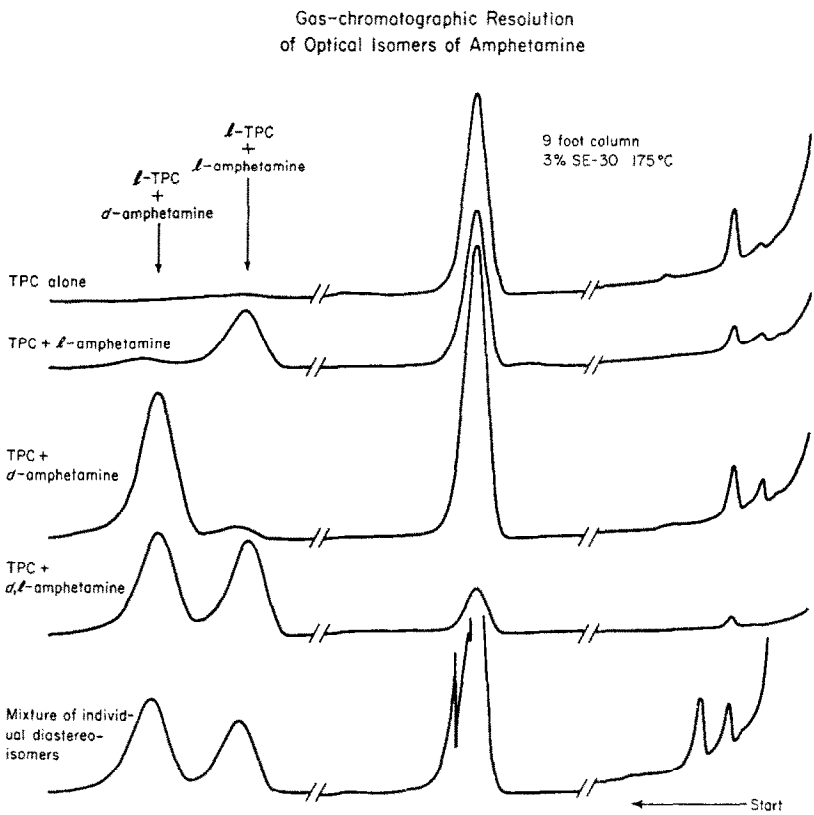


FIG. 1. Resolution of optical isomers of amphetamine by gas chromatography. The resolving agent, *l*-trifluoroacetyl-prolyl chloride (TPC), is the large peak seen in the middle of each tracing. It is followed by the derivatives it forms with *l*-amphetamine and *d*-amphetamine. (The small peaks seen at the start of each run are unidentified impurities in the TPC solution.) Conditions for gas chromatography are described in the text.

For routine analyses, an alternative to this procedure, which is both simpler and more economical of reagent, is to form the diastereoisomer directly in the column. When benzene solutions of amphetamine and TPC are mixed in the same syringe and injected into the column, the same resolution is obtained, since the diastereoisomers are formed instantly in the hot vapors of the injection port.

To compare the results of gas-chromatographic resolution with the measurements of optical rotation, 4% (w/v) aqueous solutions of amphetamine sulfate were prepared from commercial samples labeled "*l*-amphetamine sulfate" and "*d*-amphetamine sulfate" and from three mixtures of the two. (Walker Chemicals Inc., Mount Vernon, N.Y.) The mixtures contained the "*l*-amphetamine sulfate" and the "*d*-amphetamine sulfate" in the proportions ·25/·75, ·50/·50, and ·75/·25. Optical rotations were measured. The solutions were then alkalinized, the amphetamine extracted into benzene, and

the proportions of isomers in the five samples determined by the gas-chromatographic procedure described. Chromatography showed that the material labeled "*d*-amphetamine sulfate" contained only a trace of *l*-amphetamine, but that the material labeled "*l*-amphetamine sulfate" contained 13·4 per cent of the *d*-isomer. The graph in Fig. 2 correlates the gas-chromatographic measurements

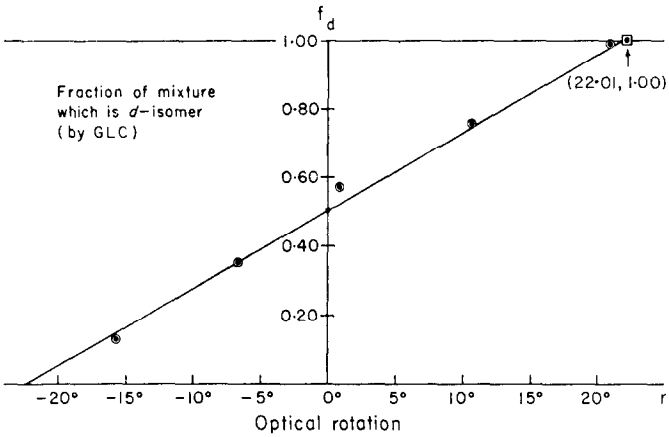


FIG. 2. Correlation of gas-chromatographic resolution of amphetamine enantiomers with measurements of optical rotation; 4% aqueous solutions (w/v) of amphetamine sulfate were prepared from commercial samples labeled "*l*-amphetamine sulfate" and "*d*-amphetamine sulfate", and from three mixtures of the two (see text). Each point marked ⊙ represents two observations: the optical rotation r, and the fraction of the mixture determined to be the *d*-isomer by gas chromatography (f_d). A straight line was fitted to these five points and to the point (0, 0·5) (see text). The value of r at the point of intersection ⊡ of this line with the line $f_d = 1{\cdot}00$ is $[\alpha]_D^{25°}$ for amphetamine sulfate, 22·01°.

with the measurements of optical rotation. The horizontal coordinate (r) represents the optical rotation of the solution, and the vertical coordinate (f_d) represents the fraction of the mixture which is the *d*-isomer, determined by gas chromatography. A straight line was fitted to these five points and to the point (0, 0·5) by the method of least squares. (The line must pass through the point (0, 0·5), since by definition, a racemic mixture has zero rotation.) The specific rotation $[\alpha]_D^{25°}$ of amphetamine sulfate was determined from the value of r at the point of intersection of the fitted line and the line $f_d = 1{\cdot}00$. This value was 22·01°. The value given in the *Handbook of Chemistry and Physics*[6] is 22°.

With this analytic technique, we have found that after the administration of either isomer to man and to the rat, no racemization takes place. Only the isomer administered is found in the urine of man, and in the urine and tissues of the rat.

The Rockefeller University,
New York, N.Y., U.S.A.

ENOCH GORDIS

REFERENCES

1. J. CASANOVA, JR. and E. J. COREY, *Chem. and Ind.* 1664 (1961).
2. E. GIL-AV, R. CHARLES-SIGLER and G. FISCHER, *J. Chromatog.* **17**, 408 (1965).
3. B. HALPERN and J. W. WESTLEY, *Biochem. biophys. Res. Commun.* **19**, 361 (1965).
4. E. GIL-AV, R. CHARLES-SIGLER, G. FISCHER and D. NUROK, *J. Gas Chromatog.* **4**, 51 (1966).
5. F. WEYGAND, P. KLINKE and I. EIGEN, *Chem. Ber.* **90**, 1896 (1957).
6. *Handbook of Chemistry and Physics*, 46th ed. Chemical Rubber Co., Cleveland, Ohio (1965).