Stereochemistry of Metabolism of Amphetamines: Use of (−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl Chloride for GLC Resolution of Chiral Amines

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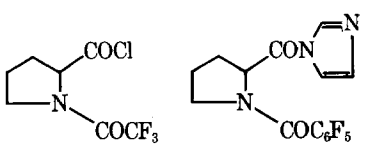
Abstract □ Amphetamine and eight related compounds were reacted with the chiral acylating reagent (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride to obtain, in each case, two diastereomeric amide derivatives. GLC conditions were found for the separation of the diastereomers in seven of the nine cases studied. This procedure may be suitable for the resolution of amino acid enantiomers *via* the amide derivatives of the methyl esters. Secondary amines were not derivatized under the reaction conditions used. A correlation between absolute configuration and elution order of the diastereomeric amides was observed for five compounds. The chiral acylating reagent was used in the determination of the enantiomeric composition of 2,5-dimethoxy-4-methylamphetamine excreted in rat urine after intraperitoneal drug administration. The chiral acylating reagent is suitable for the determination of the optical composition of compounds extracted from biological fluids.

Keyphrases □ Amphetamine and related compounds—reacted with chiral acylating reagent, GLC separation of diastereomeric amide derivatives □ Chiral acylating reagents—(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, reacted with amphetamine and related compounds, GLC separation of diastereomeric amide derivatives □ GLC—separation of diastereomeric amide derivatives of amphetamine and related compounds after reaction with chiral acylating reagent □ Stereochemistry—amphetamine and related compounds, reacted with chiral acylating reagent, GLC separation of diastereomeric amide derivatives □ Stimulants—amphetamine and related compounds, reacted with chiral acylating reagent, GLC separation of diastereomeric amide derivatives

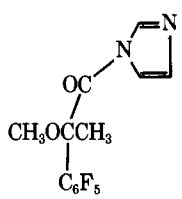
While studying the stereochemistry of the metabolism of amphetamine (1-phenyl-2-aminopropane) derivatives, it was necessary to determine the enantiomeric compositions of several members of this class of compounds extracted in small amounts from biological fluids. A literature survey revealed that derivatization of an amphetamine with chiral acylating reagent I (1), II (2), or III (3), followed by GLC separation of the resulting diastereomeric amides, provides a sensitive analytical method for the determination of the enantiomeric composition of the parent amine extracted from a biological medium.

However, none of these compounds was deemed entirely satisfactory for this study. Compound I, an *l*-proline derivative, is easily racemized (1, 4), and commercial samples are contaminated with about 6–10% of the *d*-form. Attempts to achieve the resolution of *p*-methoxyamphetamine by derivatization with I failed, since five GLC bands were obtained when the reaction product was injected into the gas chromatograph. Others also reported (4) difficulties with I, although the reagent has been successfully used (5, 6).

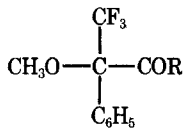


I

II



III



IV: R = OH
V: R = Cl

Compound II is highly suitable for electron-capture GLC due to the pentafluorophenyl group and has been employed with good results in the determination of the optical composition of a ring-substituted amphetamine derivative in metabolic studies (7). However, II provides poor GLC separation of the diastereomeric amides derived from amphetamine (2). Finally, III also appeared impractical since it must be synthesized *via* an elaborate route and its precursor carboxylic acid must be resolved (3). For these reasons, an alternative chiral reagent was sought.

Dale *et al.* (8) first reported the synthesis of α-methoxy-α-(trifluoromethyl)phenylacetic acid (IV) and its application to the determination of the enantiomeric purity of amines and alcohols by fluorine and proton NMR. Nichols *et al.* (4) extended the use of IV to the GLC determination of the enantiomeric purity of amphetamine derivatives prepared *via* a new asymmetric synthesis. Acid IV, lacking α-hydrogens, is highly resistant to racemization (8) and is commercially available in its resolved form[1]. In this paper, the applicability of IV to the GLC resolution of several amines of pharmacological interest is examined, and the suitability of this reagent for the determination of the enantiomeric composition of an amphetamine derivative excreted in rat urine is demonstrated.

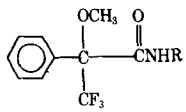
EXPERIMENTAL

Materials—(*R,S*)- and (*S*)-Amphetamines[2] (VI), (*R,S*)- and (*R*)-*p*-hydroxyamphetamine[2] hydrobromides, (*R,S*)- and (*R*)-phenylalanine methyl ester[3] (VII) hydrochlorides, (*R,S*)- and (*S*)-α-methylbenzylamines[1] (VIII), (*R,S*)-*p*-chloroamphetamine[4] (IX) hydrochloride, (*R,S*)-β-phenylisopropylhydrazine[4] (X) hydrochloride, (*R,S*)-tranylcypromine[4] (XI) hydrochloride, and (*R,S*)-*p*-chlorophenylalanine[4] were obtained from commercial sources. (*R,S*)-*p*-Chlorophenylalanine methyl ester (XII) was prepared from the amino acid by the method of Brenner and Huber (9), using methanolic thionyl chloride. The treatment of (*R,S*)- and (*R*)-*p*-hydroxyamphetamines with diazomethane gave the corresponding *p*-methoxy compounds, (*R,S*)- and (*R*)-XIII. Samples of (*R,S*)- and (*R*)-2,5-dimethoxy-4-methylamphetamines (XIV) were donated[5].

The preparation (8) of V was carried out by refluxing for 50 hr a mixture of IV (1.0 g) and freshly distilled thionyl chloride (3 ml). The excess thionyl chloride was removed under reduced pressure (~30 mm), and the crude V was dissolved in 10 ml of 1,2-dichloroethane (XV). The solution

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Smith Kline and French Laboratories, Philadelphia, Pa.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Regis Chemical Co., Morton Grove, Ill.
[5] Dr. Neal Castagnoli, Jr., School of Pharmacy, University of California, San Francisco, CA 94143.

Table I—GLC Data on Amides Derived from (—)-IV and Various Amines



Amine Precursor	Retention Times of Diastereomers, min	Column Temperature	Resolution Factor[b]
VI	12.4[c], 14.0[d]	185°	1.4
XIII	12.5[c], 14.1[d]	210°	1.6
XIV	11.4[c], 13.6[d]	220°	2.3
IX	5.3, 6.0	210°	1.5
VII	11.6[d], 12.8[c]	206°	1.4
XII	8.4, 9.5	215°	1.5
VIII	13.4[c], 14.9[d]	175°	1.4

[a] See *Experimental* for GLC conditions. [b] See Ref. 11. Note that when the resolution factor = 1.0, the peak resolution is about 98% complete; when the resolution factor = 1.5, baseline separation (99.7%) is achieved. [c] From (*R*)-amine. [d] From (*S*)-amine.

was stored in a rubber-septum-capped bottle in a refrigerator. This reagent solution was used in the derivatizations.

Organic solvents used in the analytical procedures were of spectral quality grade.

GLC Analysis—GLC was carried out on two gas chromatographs[6,7], both equipped with flame-ionization detectors. System I[6], used to obtain the data in Table I and Fig. 1, consisted of a U-shaped glass column, 1.8 m × 0.2 cm i.d., packed with 3.0% phenyl methyl silicone[8] coated on acid-washed, dimethylchlorosilane-treated diatomite support[9]. The column temperatures used are given in Table I; the injector temperature was 250°, the detector temperature was 280°, and the nitrogen flow rate was 30 ml/min.

GLC System II[7], used for the analysis of XIV extracted from urine and derivatized with V, was performed using a coiled glass column of the same dimensions and containing the same packing as the column of System I. The nitrogen carrier gas rate was 20 ml/min. The ratios of diastereomeric amide derivatives of XIV were calculated from the area percent data of each peak provided by the instrument.

Mass Spectrometry—A quadrupole GLC–mass spectrometry system[10] was used with the GLC conditions given for GLC System II, except helium carrier gas was used at a flow rate of 50 ml/min.

Animal Procedures—Male Sprague–Dawley rats, 250–300 g, were given XIV hydrochloride (5 mg/kg ip) in saline or saline only. The animals were housed in individual metabolic cages with free access to food and water. Urine was collected for 24 hr.

Extraction, Purification, and Derivatization of XIV—To 4 ml of urine in a 20-ml screw-capped test tube were added 0.4 ml of 5 *N* NaOH and 6 ml of heptane. The mixture was shaken mechanically[11] for 15 min. After centrifugation for 10 min, 5 ml of heptane was transferred to a 12-ml conical screw-capped centrifuge tube containing 0.6 ml of 1 *N* HCl. The tubes were shaken for 10 min and centrifuged for 5 min. The heptane was aspirated and discarded.

One 500-μl aliquot of the acidic lower phase was removed with a micropipet and transferred to another conical centrifuge tube containing 1.2 ml of benzene and 0.2 ml of 5 *N* NaOH. After shaking for 15 min and centrifuging for 5 min, 1.0 ml of benzene was transferred to a 3-ml microcentrifuge tube[12], and the benzene was evaporated in a stream of nitrogen. To the residue were added 50 μl of the reagent solution of V and 10 μl of pyridine, and the mixture was swirled on a mixer[13] for 10 sec. The tube was then capped and heated at 70° for 30 min, followed by cooling in ice for 5 min.

The reaction mixture was treated with 1 ml of 1 *N* HCl, and the tube was shaken for 5 min and centrifuged for 5 min. The acidic upper layer was aspirated off and discarded, and 0.4 ml of 15% Na_2CO_3 was added to the tube. The tube was then shaken for 10 min and centrifuged for 10 min, followed by GLC analysis of the lower organic layer as described.

[6] Life Sciences gas chromatograph 2100, Varian Associates, Palo Alto, Calif.
[7] Gas chromatograph 5830, Hewlett-Packard, Palo Alto, Calif.
[8] OV-17, Applied Sciences Laboratories, State College, Pa.
[9] Gas Chrom Q, Applied Science Laboratories, State College, Pa.
[10] Model 5981A, Hewlett-Packard, Palo Alto, Calif.
[11] International bottle shaker, International Equipment Co., Boston, Mass.
[12] Microflex tube, Kontes Glass Co., Vineland, N.J.
[13] Vortex Genie, Scientific Industries, Springfield, Mass.

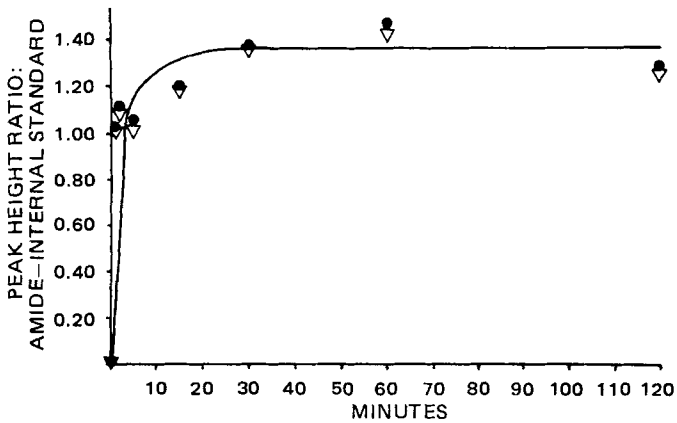


Figure 1—*Rates of formation of amides from V and (R)-VI (●) and (S)-VI (▽). See* Experimental *for reaction conditions and analytical procedure.*

Derivatization of Pure Compounds—The amine or amine salt (0.1–0.2 mg) in a conical screw-capped tube was treated with XV (50 μl), V reagent solution (50 μl), and pyridine (20 μl). The tube was heated in an oil bath at 70° for 30 min and then cooled in an ice bath. Hydrochloric acid (1 *N*, 1 ml) was added, and the tube was shaken mechanically for 5 min and centrifuged at 3000 rpm for 10 min. The aqueous acid layer was aspirated off, and 15% sodium carbonate solution (0.5 ml) and XV (100 μl) were added. The tube was shaken for 5 min and centrifuged for 10 min. The organic phase was then analyzed by GLC.

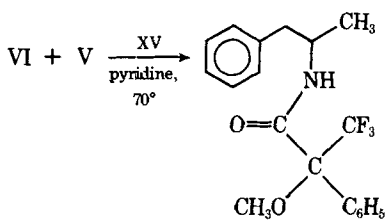
Rate of Formation of Derivatives—To a solution of (*R,S*)-amphetamine (2.0 mg) in 100 μl of XV was added 40 μl of dry pyridine. The solution was heated to 70°, 100 μl of the V reagent solution was added, and the stopwatch was started. At the appropriate times, a 25-μl aliquot was withdrawn and added to an ice-cold mixture containing 100 μl of XV, 1.0 ml of 1 *N* HCl, and 100 μl of a 10 m*M* solution of the internal standard, 1-methoxymethyl-3-methylphenobarbital (10).

The mixture was swirled for 15 sec and then frozen in dry ice–acetone until all of the time-point samples had been collected. The tubes were then shaken for 5 min and centrifuged for 10 min. The aqueous layer was aspirated off, 100 μl of 15% Na_2CO_3 solution was added, and the mixture was shaken for 5 min and centrifuged for 10 min. The organic layer was then analyzed by GLC.

RESULTS AND DISCUSSION

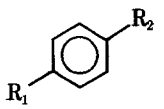
Acid IV was converted to V, its acid chloride (8), which could be used to acylate amines. The reaction (Scheme I) of V with racemic amphetamine at 70°, catalyzed by pyridine, was studied as a function of time (Fig. 1). The reaction was essentially complete in 30 min, and the product amides appeared to be stable under the reaction conditions. This short reaction time allows the use of the reagent in routine procedures where overnight reaction times (4) would be cumbersome.

Several amines (VI, VIII, IX, XI, XIII, and XIV), a hydrazine derivative (X), and two amino acid esters (VII and XII) were derivatized with V. The free base or a salt (*e.g.*, hydrochloride or sulfate) could be used in the acylation reaction with identical results. With two exceptions, the resulting diastereomeric amides[14] were well resolved by GLC (Table I). The identity of the product amides in Table I was confirmed by GLC–mass spectrometry. All amides gave a molecular ion[15].

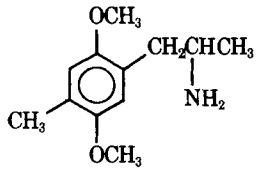


Scheme I

[14] All of the amides prepared were derived from (−)-IV.
[15] A detailed study of the mass spectral fragmentation of the amides prepared will be published.



VI: R_1 = H, R_2 = $CH_2CH(NH_2)CH_3$
VII: R_1 = H, R_2 = $CH_2CH(NH_2)COOCH_3$
VIII: R_1 = H, R_2 = $CH(CH_3)NH_2$
IX: R_1 = Cl, R_2 = $CH_2CH(NH_2)CH_3$
X: R_1 = H, R_2 = $CH_2CH(NHNH_2)CH_3$
XI: R_1 = H, R_2 = *trans*-cyclo-$(C_3H_4)NH_2$
XII: R_1 = Cl, R_2 = $CH_2CH(NH_2)COOCH_3$
XIII: R_1 = OCH_3, R_2 = $CH_2CH(NH_2)CH_3$



XIV

The successful resolution of the enantiomers of VII and XII indicates that V may be suitable for the resolution of amino acids (as their methyl esters), an area of considerable current interest (12). Two compounds, X and XI, gave diastereomeric amides that were not sufficiently resolved under the GLC conditions used, since only a slight "fork" at the top of the peak was observed in each case.

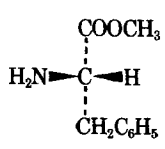
Attempts to derivatize secondary amines, *e.g.*, *N*-methylamphetamine, showed that the reaction of these amines with V was considerably slower than the reaction of primary amines. Under the reaction conditions used, secondary amines remained essentially underivatized.

Compounds VI–VIII, XIII, and XIV were available in their resolved form and, since their absolute configurations are known, the relationship between the elution order of their diastereomeric amide derivatives and absolute configuration could be examined. As seen from Table I, with one exception, the amides derived from the (*R*)-amines had shorter retention times than those from the corresponding (*S*)-amines, in agreement with the findings of Nichols *et al.* (4). The exception was VII, where the amide from (*S*)-VII eluted before the amide from (*R*)-VII. This finding is stereochemically logical, since (*S*)-VII and (*R*)-amphetamines have corresponding configurations, even though the configurational notations are opposite.

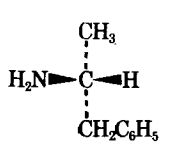
Among the desirable characteristics of a chiral reagent suitable for routine use in biological studies are: (*a*) ready availability in resolved form, (*b*) chemical stability and resistance to racemization, (*c*) simple derivatization procedure and workup, (*d*) good GLC resolution of the diastereomeric derivatives without unduly long retention times, and (*e*) high response of the derivatives to the GLC detector. While no chiral reagent has yet been developed that is superior in all of these considerations, the results described indicate that V offers distinct advantages over reagents I–III. In addition to the earlier mentioned ready availability and stability of V, this reagent was superior to I–III in providing better GLC resolution in cases where comparison could be made. For example, VI (5, 6), VII (13), and XIII (14) were derivatized with reagent I; VI, VIII, and XIV were resolved with II (2) and III (3). In these cases, V tended to give better resolution and/or shorter retention times. However, II and III gave derivatives with excellent electron-capture properties, most likely not equaled by those of V, which lacks the pentafluorophenyl group. This disadvantage of the derivatives of V may be partially overcome by using GLC–mass spectrometry in the selected-ion-monitoring mode, which provides extremely high sensitivity (15). Experiments in this direction are in progress.

By using a flame-ionization detector, the minimum detectable amount of V-derivatized (*R*)- or (*S*)-amphetamine was of the order of 1×10^{-13} mole/sec or 5 ng of sample injected.

The applicability of V to metabolic studies was tested by examining an aspect of the stereochemistry of the *in vivo* metabolism of XIV. The



(*S*)-VII



(*R*)-amphetamine

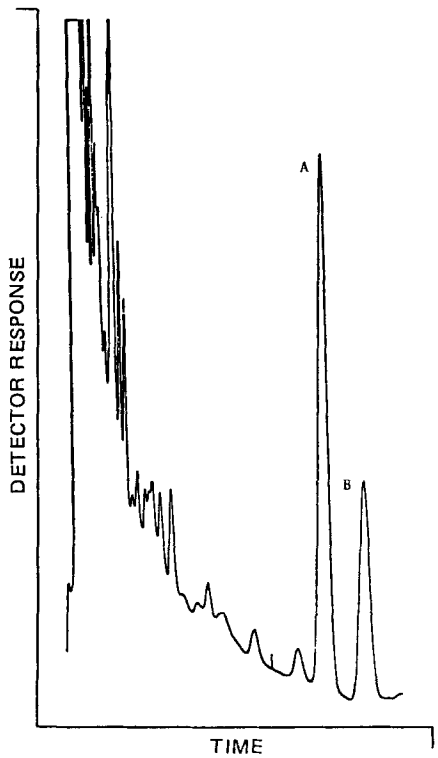


Figure 2—*Gas chromatogram of V-derivatized XIV extracted from rat urine. Key: A, derivative from* (R)*-XIV; and B, derivative from* (S)*-XIV. See* Experimental *for GLC conditions.*

metabolic fate of this compound, a potent psychotomimetic agent (16, 17), was studied in the rabbit (7, 18) and rat (19). Since the psychotomimetic activity of XIV resides in the (*R*)-enantiomer but not in the (*S*)-enantiomer (20), some investigators have paid special attention to the stereochemical aspects of its metabolism (7, 18). Thus, it was found (7) that the (*R*)/(*S*) ratio of unchanged XIV excreted in the 24-hr urine of rabbits administered racemic XIV was dose dependent and varied between 1.0 and 1.7.

Since the metabolism of XIV in the rabbit differs from that in the rat (7, 19), it seemed of interest to examine the stereochemistry of unchanged XIV excreted in the urine of rats. Racemic XIV hydrochloride (5 mg/kg ip) in saline was given to rats, and the urine was collected for 24 hr. Unchanged XIV was extracted from urine using a multistep procedure (21), which purifies and concentrates the amine before derivatization. Reaction with V was followed by GLC analysis. No peaks attributable to the amide derivatives of XIV were observed when the urine of the two animals given saline only was analyzed. The (*R*)/(*S*) ratio of XIV in the urine of three animals given the drug was 2.0, 2.1, and 1.3, respectively. Figure 2 shows a typical gas chromatogram of V-derivatized XIV extracted from rat urine.

The amount of unchanged XIV excreted was not determined, although it was reported (19) that 5% of a 5-mg/kg ip dose of racemic hydrochloride appeared as unchanged XIV in the 24-hr urine of rats. The magnitude of the (*R*)/(*S*) ratio of XIV found in rat urine is in the same direction as that observed in rabbits (7). However, at the 5-mg/kg dose level, the (*R*)/(*S*) ratio in rabbits was 1.0 and reached 1.7 only at 21 mg/kg (7).

Differences in pharmacological activity and in disposition between enantiomers of amphetamines and of related compounds are receiving considerable attention (7, 17, 22–24). Resolution of the optical isomers by GLC of diastereomeric derivatives may be useful in studying such differences. Compound V is an important addition to the list of chiral acylating agents (3, 25, 26) used for this purpose.

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Use of Microcapsules as Timed-Release Parenteral Dosage Form: Application as Radiopharmaceutical Imaging Agent

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Abstract □ The development of a new type of parenteral dosage form is described. A system of microencapsulation was formulated which produced microcapsules containing a water-soluble core material. The basic microencapsulation system could be altered to produce microcapsules with varied timed-release characteristics. Tracer methodology was employed as a sensitive and versatile analytical tool for the development and evaluation of the microencapsulation system. The core material was labeled by neutron activation after microcapsule formulation, which eliminated the radiation hazard and contamination problems that could occur during formulation with a labeled core material. Both *in vitro* and *in vivo* testing showed that the release patterns of labeled core material could be altered and detected. The microcapsules developed have potential as a timed-release parenteral dosage form and as an organ-imaging radiopharmaceutical.

Keyphrases □ Microcapsules—formulated, potential as timed-release parenteral dosage form and organ-imaging radiopharmaceutical evaluated *in vitro* and *in vivo*, mice □ Dosage forms, parenteral—timed-release microcapsules formulated, evaluated *in vitro* and *in vivo*, mice □ Radiopharmaceuticals—timed-release microcapsules formulated, potential use in organ imaging evaluated, mice

A dependable parenteral timed-release dosage form would eliminate many problems associated with oral administration. The process of microencapsulation may hold the key to the ideal timed-release dosage form because of the smallness of the produced microcapsules and the various properties instilled into the microcapsules. However, such a dosage form must contain microcapsules with very precise properties.

The main objective of this investigation was to develop a microencapsulating system that utilized a water-soluble polymer as the chief capsule wall component and allowed for the encapsulation of a water-soluble material. Other desired characteristics included microcapsules from 1 to 20 μm in diameter and formulation flexibility allowing alterations in core release rates. Tracer methodology was selected as the analytical tool for evaluation of the microcapsules. A second objective was to produce radioactivity in the core material by neutron activation after formulation. *In vitro* and *in vivo* test procedures were derived to evaluate the microcapsules as a timed-release parenteral dosage form and as a radiopharmaceutical imaging agent.

EXPERIMENTAL

Basic Procedure—Coacervation was selected as the microencapsulation method after a review of other methods (1–16). The following general procedure (Table I) was used in preparing all microcapsules.

Forty milliliters of dioxane[1], analytical grade, was added to a 50-ml beaker. A 1.25-cm magnetic stirrer bar was placed in the beaker, which

[1] Mallinckrodt Chemical Works, St. Louis, Mo.