Synthesis of Deuterio-*l*-amphetamine, d_1 Sulfate

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Abstract □ In order to investigate the isotope effect on the enzymatic deamination of amphetamine, deuterio-*l*-amphetamine, d_1 sulfate was synthesized by the selective reduction of phenyl-2-propanone oxime with lithium aluminum deuteride, followed by resolution of the racemic product. The yield of racemic amphetamine was 46% when approximately equimolar quantities of the oxime and $LiAlD_4$ were employed. The identity of the deuterated amphetamine base was confirmed by determination of the physical properties of the racemic mixture. From NMR data, deuterium was found exclusively in the methine hydrogen position at a purity of greater than 99 atom percent. The optical isomers of deuterioamphetamine were resolved through repeated fractional crystallization of the *d*-amphetamine-*d*-bitartrate and *l*-amphetamine-*l*-bitartrate diastereomers. The isomers were found to possess 97 to 99% optical purity, based on values for pure isomers of protioamphetamine. *In vitro* metabolism studies indicate that a significant deuterium isotope effect operates in the oxidative deamination of deuterio-*l*-amphetamine. Under specified conditions, the ratios of apparent rate constants (k_H/k_D) based on initial velocities, yield a value of 2.0 ± 0.3.

Keyphrases □ Deuterio-*l*-amphetamine, d_1 SO_4—synthesis □ Deamination, deuterio-*l*-amphetamine, d_1 SO_4—isotope effect □ Metabolism, *in vitro*—deuterio-*l*-amphetamine, d_1 SO_4 □ Protio and deuterio-*l*-amphetamine, d_1 SO_4—comparison □ NMR spectroscopy—structure □ Specific rotation—purity determination

The deuterium-isotope effect has been applied with significant success to the study of experimental pharmacology. Belleau, in a recent monograph (1), has reviewed the applications of the isotope effect and has proposed a mechanism for certain bioreceptor-substrate interactions which has importance in the studies of drug response and metabolism. The concept of a pseudo-primary isotope effect has been developed to explain the large isotope effect observed in some systems which involve a Michaelis complex formation. The existence of a deuterium-isotope effect in the metabolism of various therapeutic agents has led these investigators to consider the possibility that such an effect exists in the oxidative deamination of amphetamine.

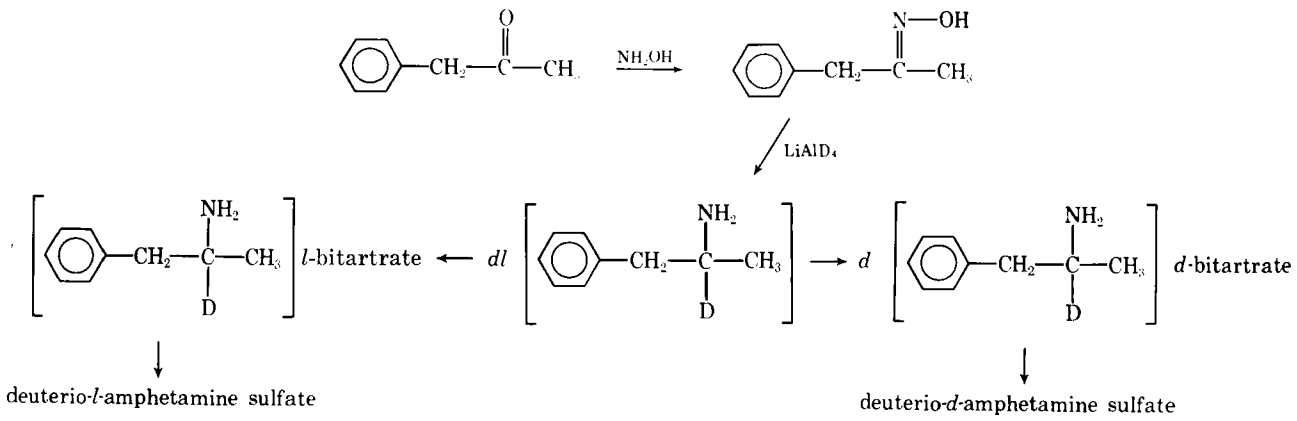
Although sustained retention of a drug does not necessarily mean an increased duration of action, the correlation has frequently been observed. Beckett *et al.* (2) have reported an increased duration of wakefulness in human volunteers receiving *d*-amphetamine, which could be correlated with a depressed excretion rate of administered drug. While various paths of excretion and tissue storage are prime considerations in the fate of a drug, the existence and extent of metabolic activity toward such a drug can be a major factor in the total elimination process. Quinn *et al.* (3) have demonstrated that a correlation exists between depressed microsomal enzymatic activity and extended hexobarbital sleeping time.

Dring *et al.* (4) have presented a comprehensive report on the fate of amphetamine in man and other species. Deamination appeared to be the major metabolic reaction in the rabbit, dog, and man. Unaltered amphetamine was a major excretory product in man and in the dog; whereas a low recovery (4% in 24 hr.) of unmetabolized amphetamine was found in the rabbit. An interesting stereospecific effect relating to the metabolism of amphetamine has been noted by several investigators. Alles and Wisegarver (5) isolated amphetamine from the urine of a human volunteer. Determination of the optical activity of the isolated drug indicated the presence of more *l*-amphetamine than *d*-amphetamine. These workers concluded that *d*-amphetamine is metabolized to a greater degree than is the *l*-form, when racemic amphetamine is administered. Moreover in rabbits, the *l*-form has been found to be metabolized more extensively than the *d*-isomer. Axelrod (6) has located a deaminating enzyme in the microsomal fraction of rabbit liver homogenate which shows such stereospecificity.

A similar microsomal preparation has been found to effect the *N*-demethylation of morphine. Ellison *et al.* (7) studied the pharmacological properties and kinetics of *N*-demethylation of morphine which has been substituted with deuterium in the *N*-methyl position. Deuterium substitution effected a slower rate of demethylation and a significant reduction in pharmacologic potency. Belleau (1) explained that *N*-demethylation must occur at the receptor site as a requisite to the biological response to morphine. Should this reaction be subject to an isotope effect, the respective response would be similarly affected.

Substitution of deuterium into compounds by reduction has been facilitated by the availability of lithium aluminum deuteride. The reduction of the appropriate precursor with lithium aluminum deuteride eliminates the more tedious aspects of catalytic hydrogenation (D_2 gas) procedures. Although several different chemical syntheses involving various precursors, might result in substitution of deuterium in the α-position of amphetamine, the reduction of phenyl-2-propanone oxime reported by Larsson (8) was chosen for this study. Resolution of the product, racemic deuterio-amphetamine, was effected using the method of Blackburn and Burghard (9). The cyclic crystallization of *d*-amphetamine-*d*-bitartrate and *l*-amphetamine-*l*-bitartrate diastereomers is a convenient procedure for obtaining maximum optical purity and recovery.

It is apparent from a consideration of the substrate structure and that of the proposed metabolite, benzyl methyl ketone, that the C—D bond of α-deuterioamphetamine (see Scheme I) will be broken sometime during an enzymatic oxidative deamination. If this event is involved in the rate-limiting process, a significant deuterium isotope effect might well be anticipated. The relevance of such an effect to amphetamine therapy



Scheme I—Synthesis of deuterio-l-amphetamine, d_1 sulfate.

is interesting. The normally low excretion rate of amphetamine is significantly depressed under conditions of high urinary pH (see *Reference 2*). Under these conditions, the duration of certain pharmacological responses to this drug could be determined by the rate at which it is metabolized.

The purpose of the present investigation was to synthesize deuterio-*l*-amphetamine, d_1 sulfate which possesses maximum optical and isotopic purity, and to determine the existence of a deuterium-isotope effect in the oxidative deamination of this drug.

EXPERIMENTAL

Phenyl-2-propanone Oxime—The microsynthetic procedure of Wilson (10) was modified to prepare phenyl-2-propanone oxime by the addition-dehydration reaction between phenyl-2-propanone and hydroxylamine (Scheme I). The product of the reaction, phenyl-2-propanone oxime, was extracted into ether, washed twice with saturated calcium chloride solution, and dried over anhydrous magnesium sulfate. The solvent was removed at reduced pressure using a rotary evaporator, and the residue was distilled, giving a colorless viscous liquid boiling at 81–84° (0.15 mm. Hg)[1] [reported (11) b.p. 99° (2 mm. Hg)]. The crude oxime was recrystallized from *n*-hexane to a constant melting point, 69.0° [reported (12) m.p. 70.0°]. The yield of recrystallized oxime was 85%.

Anal.—Calcd. for $C_9H_{11}NO$: C, 72.45; H, 7.43; N, 9.39. Found: C, 72.27; H, 7.51; N, 9.58.

NMR spectra of phenyl-2-propanone oxime were determined in deuterochloroform at 60 Mc.p.s.,[2] at probe temperature, using an internal TMS standard.

Deuterio-dl-amphetamine, d_1—The methods of Larsson (8) and Smith *et al.* (13) were used to reduce the phenyl-2-propanone oxime. Into a three-necked flask fitted with a condenser, dropping funnel, and a mechanical stirrer, was placed 100 ml. of dry ether. A solution of 3.40 g. (81 mmoles) of lithium aluminum deuteride[3] was prepared by adding the compound to the ether and stirring until it had dissolved. Then a solution of 10.95 g. (74 mmoles) of the oxime in 50 ml. of dry ether was added at a rate sufficient to maintain gentle refluxing. After all of the oxime had been added, the reaction mixture was refluxed for about 7 hr. After cooling, the excess hydride was decomposed by the dropwise addition of water (*ca.* 4 ml.). The complex was hydrolyzed by the addition of 7 ml. of 30% sodium hydroxide solution. The mixture was stirred at room temperature for 30 min. and then an additional 15 ml. of water was added. The ether layer was then decanted from the granular residue of metal hydroxides. The residue was washed several times with ether, and all washings were combined with the original ether fraction. The combined ether fraction was first dried over sodium hydroxide and then the solvent was removed with a rotary evaporator. The residual oil was distilled twice, under vacuum, through a short-path distillation apparatus to yield 4.62 g. (45.9%) of deuterio-*dl*-amphetamine, d_1, a colorless liquid, b.p. 43–45° (0.15 mm. Hg); n_D^{26} 1.517 [reported (14) n_D^{26} 1.518]. The boiling point at atmospheric pressure was determined[4]; b.p. 205.5° [reported (15) b.p. 205.0°].

The hydrochloride derivative of deuterio-*dl*-amphetamine was prepared by the dropwise addition of 2 ml. of HCl-saturated absolute ethanol to a cooled flask (5°) containing 0.30 g. of deuterio-*dl*-amphetamine in 75 ml. of anhydrous ether. The crystals were filtered with suction, washed several times with dry ether, and dried over P_2O_5. The melting points of deuterio-*dl*-amphetamine HCl and an authentic protio-*dl*-amphetamine HCl sample were compared: deuterio-*dl*-amphetamine HCl m.p. 146–148°; protio-*dl*-amphetamine HCl, m.p. 146–148° [reported (15) m.p. 145–147°].

NMR spectra of deuterio-*dl*-amphetamine were determined (neat) on a spectrometer at probe temperature using an internal TMS standard, in order to establish the position and extent of deuterium substitution. Spectra were also determined in deuterochloroform and carbon tetrachloride, using an internal TMS standard.

Resolution of Deuterio-dl-amphetamine—All determinations of optical purity were based on the observed specific rotations of authentic samples of the appropriate protio-amphetamine-bitartrate.

Resolution of 10.5 g. (77 mmoles) of racemic deuterio-amphetamine, accumulated during three separate runs, was achieved by the cyclic crystallization of the bitartrate diastereomers. A portion of the racemic deuterio-amphetamine (5.56 g.; 41 mmoles) was dissolved in 70 ml. of 91% isopropanol. The solution was heated to 80°, 6.38 g. (42.5 mmoles) of *l*-tartaric acid was added, and the temperature was maintained at 80° until the acid dissolved. The solution was allowed to cool slowly (*ca.* 1 hr.) to 60° and maintained at that temperature for 24 hr., whereupon crystals formed. After decantation of the mother liquor, the crystalline *l*-deuterio-amphetamine-*l*-bitartrate was filtered and washed with 13 ml. of 91% isopropanol at 60°. Optical purity of the crystalline deuterio-*l*-amphetamine-*l*-bitartrate (3.84 g.) was approximately 90%. After one recrystallization at 60° from 91% isopropanol, 2.14 g. of the diastereomer was obtained with an optical purity of 98% (equivalent to 37% of the *l*-isomer present in 5.56 g. of racemic deuterio-amphetamine). The mother liquor, which was rich in *d*-deuterio-amphetamine, and all rinses were combined and the solvent was removed *in vacuo*. The salt was dissolved in 15 ml. of water, made strongly alkaline with 25% sodium hydroxide, and extracted with 300 ml. of ether. Approximately 5.0 g. of *d*-tartaric acid was dissolved in a 91% isopropanol solution of the *d*-rich deuterio-amphetamine base which had been recovered from the *d*-rich deuterio-amphetamine-*l*-bitartrate salt. Crystallization of *d*-amphetamine-*d*-bitartrate was effected at 60° in a manner identical to that used for the *l*-bitartrate diastereomer. A yield of 3.1 g. of deuterio-*d*-amphetamine-*d*-bitartrate was obtained with an optical

[1] All melting and boiling points are uncorrected. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill.
[2] Varian A60 spectrometer.
[3] Purchased from Metal Hydrides, Inc., Beverly, Mass.
[4] Mettler FP-1 apparatus.

Table I—Effect of Lithium Aluminum Hydride to Oxime Ratio on Yield of Amphetamine

Ratio, LAH/Oxime	Yield, %	Ratio, moles Amphetamine/ moles LAH
2.20:1	75.0	0.34
1.65:1	67.0	0.41
1.10:1	48.5	0.44

purity of 98%. This crystallization cycle was repeated three times, adding the remainder of racemic deuterio-amphetamine (4.94 g.) after the third crystallization. The combined deuterio-*l*-amphetamine-*l*-bitartrate fractions yielded 4.94 g. of the diastereomer, $[\alpha]_D^{25}$ −29.5° (2%, H_2O), equivalent to 45% of the *l*-isomer present in 10.5 g. of racemic deuterio-amphetamine base. The yield of deuterio-*d*-amphetamine-*d*-bitartrate was 5.91 g. (54%), $[\alpha]_D^{25}$ +29.8° (2%, H_2O). The synthesized isomers possess an optical purity of at least 98% when compared to authentic samples of each diastereomer. Specific rotation of the standard samples was −30.3° and +30.5° for the *l*- and *d*- forms, respectively.

The diastereomer salt of each isomer was dissolved in 15 ml. of water, made strongly alkaline with 25% sodium hydroxide, and extracted with 300 ml. of ether. The solvent was removed *in vacuo*. The sulfate salt of each isomer was prepared by the addition of an equivalent amount of 50% sulfuric acid to a solution of the respective deuterio-amphetamine base in 91% isopropanol. The crystals of each isomer were filtered with suction and dried over P_2O_5. A recrystallized sample of deuterio-*l*-amphetamine d_1 sulfate was submitted for elemental analysis.

Anal.—Calcd. for $C_{18}D_2H_{26}N_2 \cdot H_2SO_4$: C, 58.35; H, 8.16; N, 7.56. Found: C, 58.15; H, 8.04; N, 7.57.

In Vitro Metabolism Studies—The preparation of liver homogenate was based on the method of Axelrod (6). Six-month-old Dutch rabbits were sacrificed by a blow to the head, the liver was removed, rinsed in cold isotonic KCl solution, and then homogenized in two volumes of 0.01 *M* phosphate buffer (pH 7.0). The 9,000 × *g* supernatant fraction obtained contains the microsomal enzymes and those cofactors present in the soluble portion of the homogenate.

Substrates were incubated in open 125-ml. conical flasks at 37°, using a magnetic stirring bar. The composition of the reaction mixture follows:

Substrate	1.5×10^{-4} *M*
NADP	1.0×10^{-4} *M*
Glucose-6-phosphate	4.0×10^{-3} *M*
Nicotinamide	1.0×10^{-2} *M*
$MgCl_2$	5.0×10^{-3} *M*
Phosphate buffer (pH 7.4)	2.4×10^{-2} *M*
Homogenate	25 mg. liver/ml.
Total volume	40.0 ml.

The oxidative deamination of protio-*l*-amphetamine was followed by the determination of substrate concentration at various time intervals, using the method of Axelrod (16). Absorbance of the methyl orange-amphetamine complex was determined at 540 mμ in a spectrophotometer.[5]

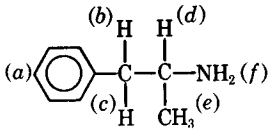
Under these conditions, the reaction was demonstrated to be linear with respect to time for at least 30 min. The relative rates of metabolism of protio-*l*-amphetamine and deuterio-*l*-amphetamine were determined by performing simultaneous incubations of the two substrates under identical conditions. Samples for analysis were drawn at zero time and 20-min. incubation.

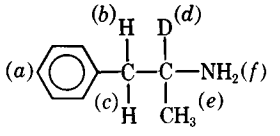
Protein Assay—Protein was determined with the Folin phenol reagent after an alkaline copper treatment, by the method of Lowdry *et al.* (17). Bovine serum albumin served as a standard for protein determinations. Enzyme activity is expressed in units of micromoles substrate metabolized per hour per gram of protein (μmole/hr./g.).

RESULTS AND DISCUSSION

Preliminary experiments using lithium aluminum hydride indicated that the most favorable ratio of reducing agent to oxime was 1.65:1, based on the oxime precursor (see Table I). However, the most favorable ratio based on the reducing agent was determined to be 1.1:1. This ratio was chosen for the preparation of deuterio-*dl*-amphetamine. No significant difference in yield of amphetamine was observed when lithium aluminum deuteride was substituted for lithium aluminum hydride in the preparation of the deuterio-product.

Table II—Summary of Chemical Shifts (δ, p.p.m. from TMS)





Protio-*dl*-amphetamine			Deuterio-*dl*-amphetamine		
Proton	δ	Integral and (Multiplicity)[a]	Proton	δ	Integral and (Multiplicity)
(*a*)	7.22	5 (s)	(*a*)	7.22	5 (s)
(*b* or *c*)	2.58	1 (s)	(b, *c*)	2.55	2 (s)
(*c* or *b*)	2.48	1 (d)			
(*d*)	2.97	1 (m)	(*d*)	no signal	0
(*e*)	1.01	3 (d)	(*e*)	1.00	3 (s)
(*f*)	1.10	2 (s)	(*f*)	1.06	2 (s)

[a] Multiplicity: (s), singlet; (b), doublet; (m), multiplet. All spectra were obtained in (neat) solution at 60 MHz.

Identity of the product as deuterio-amphetamine was confirmed by the determination of several physical constants. These determinations were found to be in close agreement with literature values for protio-amphetamine. As expected, the values for deuterio-amphetamine (mol. wt. 136.21) are not greatly different from the constants for authentic protio-amphetamine (mol. wt. 135.21). Resolution of deuterio-*dl*-amphetamine yielded isomers which exhibited optical activity similar to that of the protio-amphetamine isomers.

A most important aspect of this study concerned the positional isotope analysis of deuterio-*dl*-amphetamine. The NMR spectra of deuterio-*dl*-amphetamine were the basis for this analysis. Table II contains a summary of the chemical shifts for deuterio-*dl*-amphetamine and protio-*dl*-amphetamine. As expected, the methyl signal of deuterio-*dl*-amphetamine is reduced to a singlet and the methine signal of protio-*dl*-amphetamine is absent from a single scan of deuterio-*dl*-amphetamine. Spectra of a (neat) sample of deuterio-*dl*-amphetamine in the region of 2.5–3.0 p.p.m. (methine), obtained at high-spectrum amplitude, confirm the replacement of the proton by deuterium at this molecular position. The methine signal was totally absent, in spectra obtained for deuterio-*dl*-amphetamine. The identity of deuterio-*dl*-amphetamine, represented in Scheme I, has been assumed to be established. Isotopic purity at the α-position appears to be greater than 99%.

An apparent solvent effect on the methylene signal of deuterio-*dl*-amphetamine was observed, illustrating the nonequivalence of the methylene protons. The methylene signal of deuterio-*dl*-amphetamine appears as a singlet in a neat sample, a partially split broad peak in carbon tetrachloride, and a doublet in deuterochloroform. Similar solvent effects have been observed with protio-amphetamine (18).

Phenyl-2-propanone oxime is most commonly recovered as a viscous oil, but crystallizes on standing. The melting point reported for this compound is 70° (12). Spin-splitting analysis of phenyl-2-propanone oxime supports evidence, first reported by Lustig (19), for the simultaneous existence of *syn*- and *anti*-isomers in the oil. Lustig suggests that, "nonlinearity of the C=NO— group gives rise to this isomerism," which locates the methylene protons of the isomers in a nonequivalent magnetic environment. The nonequivalent methylene protons are represented by single peaks at 3.50 p.p.m. (1.5 H) and 3.75 p.p.m. (0.5 H). Integration of the signals at 3.50 and 3.75 p.p.m. indicates that the oil consists of a nonequilibrium mixture of two isomers (*syn* and *anti*), present in a ratio of approximately three to one. Lustig further suggests that the crystalline phenyl-2-propanone oxime (m.p. 70°) gives rise to the stronger component (3.50 p.p.m.) of the methylene signal. Isolation of the second isomer has not been reported.

[5] Beckman DU, Beckman Instruments, Inc., Fullerton, Calif.

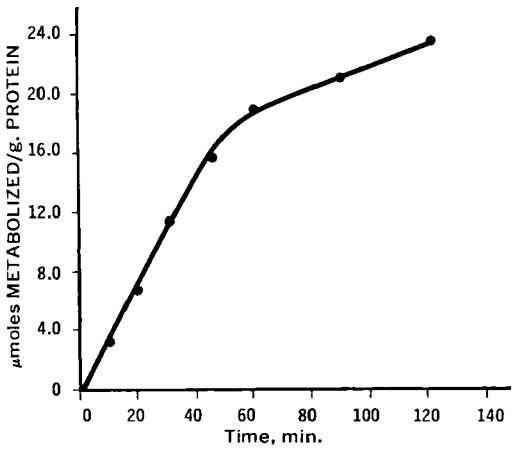


Figure 1—*Rate of metabolism of protio-*l*-ampethamine. Protio-*l*-amphetamine (1.5 × 10⁻⁴M) was incubated with the 9,000 × g supernatant fraction from rabbit liver under the standard conditions.*

Metabolic Studies—The initial experiments of this study were effected using a dialyzed homogenate and all cofactors except glucose-6-phosphate. It was found that effectively dialyzed homogenates possess little or no enzymatic activity, presumably due to the removal of soluble essential cofactors. Full activity could be restored in a dialyzed homogenate by the addition of reduced NADP (1×10^{-3} *M*). The reaction studied was found to proceed linearly only over the first 30 to 40 min., after which the rate of protio-substrate metabolism decreased rapidly (see Figs. 1 and 2). Specific activity values, based on substrate metabolism during the first 30 min., are 21–27 µmoles/hr./g. protein or approximately 0.22–0.29 µmole/hr./100 mg. liver. These values are in close agreement with those of Fouts (20) who used a 1-hr. sampling time. Determination of specific activity based on substrate concentration sampled after 2-hr. incubation will necessarily reflect the relative decrease in activity which occurs after 40-min. incubation. Axelrod (6) reports an activity of 0.25 µmole/2 hr./100 mg. liver, which is significantly lower than values obtained in this study.

The metabolism of deuterio-*l*-amphetamine was also demonstrated to proceed linearly for at least 30 min. Experiments indicate that a significant deuterium-isotope effect operates in the oxidative deamination of deuterio-*l*-amphetamine. At a substrate concentration of 1.5×10^{-4}*M*, the ratios of apparent rate constants (k_H/k_D), based on the initial velocities, yield a value of 2.0 ± 0.3 (mean apparent rate constant ratio ± standard deviation; $N = 10$). Figure 2 indicates a close agreement between the ratio of the slopes of the lines for protio-*l*- and deuterio-*l*-amphetamine and the rate constant ratio derived from single-point determinations. It is reasonable to assume that this ratio will be affected by changes in substrate concentration in a manner similar to the results observed for the *N*-demethylation of deuteriomorphine (21). Further investigation of this enzymatic reaction to determine the Michaelis constants of deuterio-*l*-amphetamine and deuterio-α-amphetamine, as well as the protio-amphetamine isomers, is now in progress.

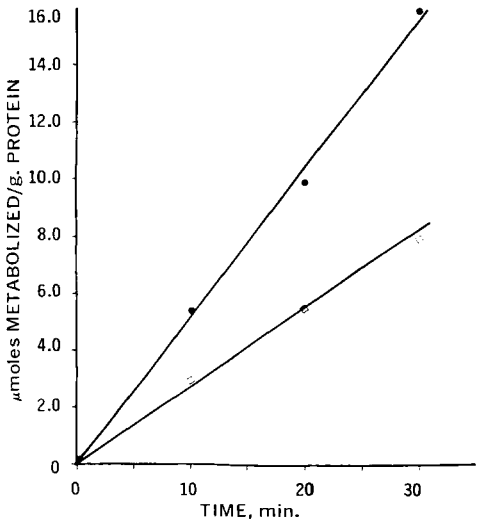


Figure 2—*Rate of metabolism of protio-*l*-amphetamine (—●—) and deuterio-*l*-amphetamine(—□—). Substrates (1 × 10⁻⁴M) were incubated with the 9,000 × g supernatant fraction from rabbit liver under the standard conditions. The calculated slope ratio* (k_H/k_D) = *1.9.*

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