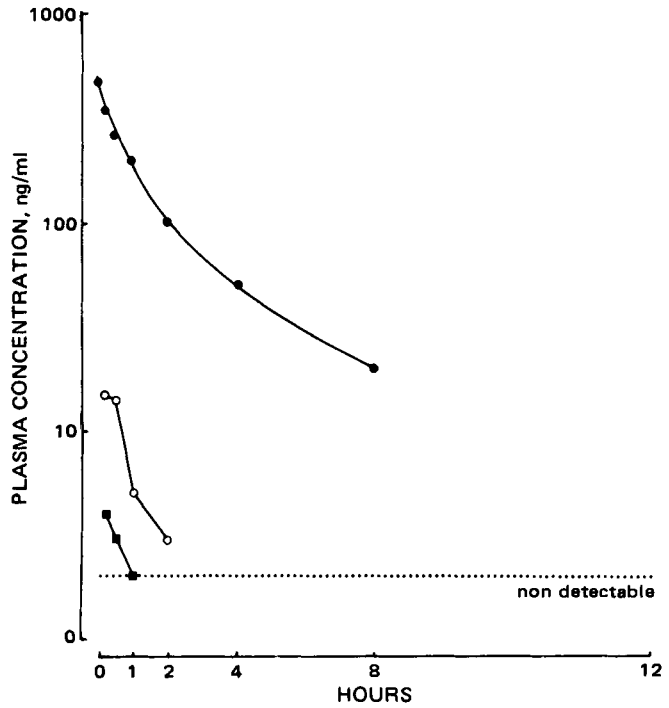

Figure 3—*Plasma concentration–time curve in the dog given a 1 mg/kg iv dose of dexamethasone. Key: (●), dexamethasone; (○), hydrocortisone; (■), corticosterone.*

efficient, precise, sensitive, and selective. It also allows the examination of the effects of dexamethasone on circulating hydrocortisone and corticosterone concentrations.

The selectivity of the extraction procedure and chromatographic system is demonstrated by the chromatogram from the blank plasma (Fig. 1*a*). Analysis of the figure indicates that there is no significant interference from endogenous compounds. It should be noted that one source of interference was encountered when the methylene chloride layer was recovered by filtration on phase-separating paper. One large interfering peak eluted at a retention time of 5 min and was labeled as an endogenous biological sample.

This chromatographic procedure differs from those previously described (11–19), as a new radial compression separation system was used. In this system a strong radial compression is applied to a soft column, thus diminishing the dead volume and the formation of preferential ways inside the column. This results in higher efficacy, with a lower pressure and increased flow rate. Moreover, it is possible to attain the same separation in 2 min with a flow rate of 6 ml/min and with only a 10% decrease in response.

Responses were considered significant when the signal to noise ratio was >1.5. In such conditions, using a detector sensitivity of 0.005 aufs, levels as low as 2 ng/ml, giving 0.5-cm peaks, could be quantitated.

In conclusion, the described method offers a simple, rapid, and reliable determination of corticosteroids for use in pharmacokinetic studies.

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NOTES

Rapid Enzymatic Preparation of [^{14}C]D-Leucine from [^{14}C]DL-Leucine

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Received July 6, 1981, from the *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Kentucky, Lexington, KY 40506.* Accepted for publication September 25, 1981.

Abstract □ A rapid enzymatic method for the preparation of [1-^{14}C]D-leucine is described. [1-^{14}C]D-Leucine was obtained from [1-^{14}C]DL-leucine by oxidative deamination of the L-isomer using immobilized L-amino acid oxidase. The total preparation (including ion exchange purification) was accomplished in 40 min with an 83% yield. The methodology is applicable to the production of [1-^{11}C]D-leucine, a potential imaging agent for tumor localization.

Keyphrases □ [^{14}C]D-leucine—rapid enzymatic preparation for tumor localization □ Enzymatic preparation—D-leucine isomers, [^{11}C]D-leucine production for tumor localization

Amino acids labeled with gamma-emitting radionuclides (carbon 11 or nitrogen 13) have been investigated for tumor localization, pancreatic scanning, and the study of normal physiological processes. Several ^{13}N-labeled L-amino acids have been synthesized enzymatically and their tissue distribution and pancreatic uptake studied in animals

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(1–3). Transplanted tumors in rats have been detected by external scintigraphy using [^{11}C]carboxyl-labeled 1-aminocyclopentanecarboxylic acid (4). A modified synthesis[1] has been successfully utilized for the production of [^{11}C]carboxyl-labeled amino acids (5, 6). On the basis of data obtained from animal experiments and studies with humans it has been suggested that [^{11}C]carboxyl-labeled DL-tryptophan (7, 8) and DL-valine (9), when used in conjunction with positron computerized transaxial tomography, have significant potential as clinical pancreas-imaging agents.

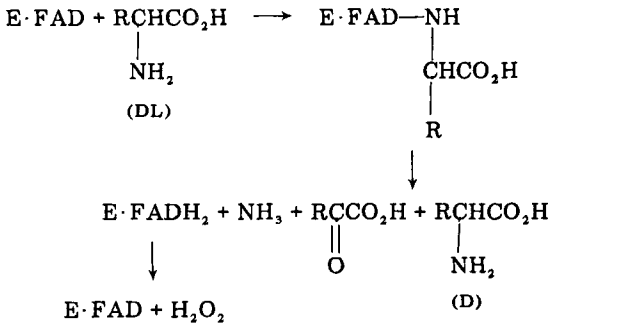
Recent studies suggest that optically active isomers of ^{11}C-labeled amino acids would be biologically more interesting than the racemic amino acids. For example, it has been previously demonstrated (10) that ^{14}C-labeled D-isomers of some amino acids showed greater affinity for tumors in mice than did the L-isomers. More specifically, ^{14}C-labeled D-leucine has been shown to exhibit a preferential incorporation into tumor cells of nude mice bearing human colon and thyroid cancer[2].

The present report describes the methodology for the rapid preparation of D-leucine which makes possible the availability of this amino acid in its ^{11}C-labeled form as a potential diagnostic reagent for tumors.

EXPERIMENTAL

Immobilization of L-Amino Acid Oxidase—Snake venom L-amino acid oxidase[3] (50 mg) was dissolved in 30 ml of 0.1 *M* sodium pyrophosphate buffer, pH 8.3. Cyanogen bromide-activated sepharose-4B beads[3] (2.5 g) were washed and swollen by treating with 400 ml of 1×10^{-3} *N* HCl. The beads were recovered by filtration using a coarse sintered glass funnel and were immediately added to the buffered enzyme solution in a 50-ml round-bottom flask. The mixture was stoppered and gently mixed with a wrist action shaker at 4–5° for 30 hr. After immobilization, the beads were washed several times on a coarse sintered glass funnel using 0.1 *M* sodium pyrophosphate, pH 8.3, to remove the soluble enzyme. Subsequently, the immobilized enzyme was alternately washed twice with 0.1 *M* pyrophosphate buffer (pH 8.3) and 0.1 *M* acetate buffer (pH 5.6) and finally twice more with 0.1 *M* pyrophosphate buffer (pH 8.3). The resulting L-amino acid oxidase on sepharose-4B was stored at 5–7° in 50–100 ml of 0.1 *M* pyrophosphate buffer at pH 8.3.

Preparation of [1-^{14}C]D-Leucine—[1-^{14}C]DL-Leucine[4] (1.25 μCi) (specific activity: 59 mCi/mmole; concentration: 50 μCi/ml) and 2.5 g sepharose-immobilized L-amino acid oxidase were added to a 50-ml solution of 1×10^{-3} *M* DL-leucine[3] in 0.1 *M* sodium phosphate buffer (pH 8.3). The preparation was described previously. The resulting mixture was gently agitated on a gyratory shaker bath at 37° for 10 min with concomitant passage of oxygen through the reaction mixture at the rate of 0.5 liters/min using a gas dispersion tube.



Scheme I—*Resolution of DL-leucine by oxidative deamination. FAD = flavin adenine dinucleotide, E = immobilized L-amino acid oxidase (L-AAO), and R = $CH_2CH(CH_3)_2$.*

The immobilized enzyme was removed from the reaction mixture by use of a sintered glass filter funnel and an aspirator vacuum. The filtrate was acidified with 1 ml of concentrated HCl and added to a cation exchange column (10 × 15 mm)[5]. The column was washed with two 50-ml portions of distilled water. (Insignificant amounts of radioactivity were found in the second wash.) The total radioactivity in these washings was 0.54 μCi. To recover the D-leucine the cation exchange column was eluted with 100 ml of 0.2 *N* NaOH which resulted in 0.52 μCi of radioactivity. The pH of the eluted [1-^{14}C]D-leucine solution was adjusted to 7.0, the resulting solution was concentrated to 1 ml, and an aliquot was applied to a silica gel TLC plate[6] (20 × 20 cm).

After two-dimensional chromatography [butanol[7]–acetic acid[8]–water (4:1:2) followed by phenol[9]–water, (75:25)] the plate was monitored by autoradiography[10]. The respective R_f values were 0.55 and 0.74. The position of the radioactive spot corresponded to the colored spot obtained prior to X-ray film exposure upon spraying with ninhydrin[3]. This spot corresponded to that of authentic leucine.

Optical Rotary Dispersion Spectropolarimetric Determination of the Production of D-Leucine—A solution of nonradioactive DL-leucine (1×10^{-3} *M*) was incubated with immobilized L-amino acid oxidase as described previously. The pH of the amino acid solution in 100 ml of 0.2 *N* NaOH recovered from the cation exchange column was adjusted to 7.0 and taken to dryness at 40° using a rotary evaporator. The residue was reconstituted into a 15-ml aqueous HCl solution (final pH 1.0). The optical rotary dispersion curve was determined in a 5-cm cell using an optical rotary dispersion spectropolarimeter[11]. The optical rotary dispersion spectrum was scanned in the wavelength region of 300 nm to 200 nm at a sensitivity setting of 350 millidegrees. The maximum absorbance was found at 224 nm. For comparison of the optical rotary dispersion spectrum to that of authentic D-leucine[3], a 0.25 m*M* solution of D-leucine in 0.2 *N* NaOH was prepared, dried, and reconstituted as for the previous solution. Also, a solution of D-leucine (0.5 m*M*) in 50 ml of 0.1*M* sodium pyrophosphate buffer was subsequently treated as the enzymatically produced D-leucine except for the enzyme exposure and the optical rotary dispersion was run.

Keto Acid Assay—The enzyme reaction was carried out as described previously using nonradioactive DL-leucine. 2,4-Dinitrophenylhydrazine[12] (0.5 ml, 0.1%) dissolved in 2 *N* HCl was added to vials containing 0.5 ml of the 50-ml filtrate. The mixtures were allowed to stand at 25° for 10 min, followed by the addition of 5 ml of 1.25 *N* NaOH. The optical densities at 440 nm using a spectrophotometer[13] were determined. A standard calibration curve was plotted and found to be linear at concentrations of keto acid (4-methyl-2-oxopentanoic acid[14]) ranging from 0.0625×10^{-3} *M* to 0.5×10^{-3} *M*.

RESULTS AND DISCUSSION

Resolution of [1-^{14}C]DL-leucine was accomplished by oxidative deamination as outlined in Scheme I. [1-^{14}C]D-Leucine was obtained from [1-^{14}C]DL-leucine by destruction of the L-isomer by the action of sepharose-immobilized L-amino acid oxidase at pH 8.3. When the reaction was complete (10 min) the mixture was acidified and passed through a cation exchange column to remove the keto acid. The column was washed with water until the eluate was practically free of radioactivity. Of the original activity in the starting [^{14}C]DL-leucine, 43% was found in these washings in the form of 4-methyl-2-oxopentanoic acid. This 43% recovery represents a yield of 86% for the keto acid. The [1-^{14}C]D-leucine was eluted from the column with 0.2 *N* NaOH in an 83.2% yield (41.6% of original radioactivity). These yields are an average of five experiments. An additional 11% of the unaccounted radioactivity (15%) was recovered by washing the enzyme two times with 50 ml of water. Two-dimensional TLC of the leucine recovered from the cation exchange column revealed that the product was pure and it chromatographed identically to authentic leucine as determined by spraying the plate with ninhydrin and finding that the ninhydrin positive spot coincided with the position of

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[2] O. Tamemasa, R. Goto, A. Takeda, and S. Maruo, manuscript in preparation.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Amersham Corporation, Arlington Heights, Ill.
[5] The resin (AG 50 W-X2, 50–100 mesh) and column were obtained from Bio-Rad Laboratories, Richmond, Calif.
[6] Quantum Industries, Fairfield, N.J.
[7] Aldrich Chemical Co., Milwaukee, Wis.
[8] Fisher Scientific Co., Cincinnati, Oh.
[9] Mallinckrodt Chemical Works, St. Louis, Mo.
[10] Packard Model 7201 Radiochromatogram Scanner, Packard Instrument Co., Downers Grove, Ill.
[11] JASCO Model UV-5.
[12] Eastman Chemical Co., Rochester, N.Y.
[13] Cary Model 118, Varian Associates, Instrument Division, Palo Alto, Calif.
[14] Tridem-Fluka, Inc., Hauppauge, N.Y.

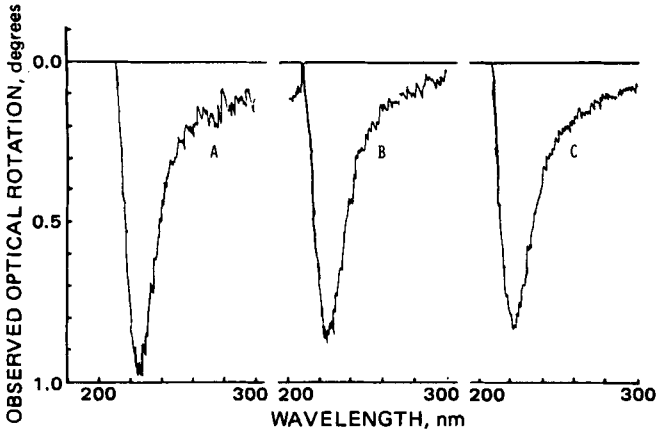

Figure 1—*Optical rotary dispersion curves for authentic D-leucine (A), authentic D-leucine recovered from cation exchange column (B), and enzymatically produced D-leucine after purification by cation exchange column chromatography (C).*

the radioactivity detected by autoradiography. The total resolution time including the ion exchange purification step was 40 min.

A nonradioactive resolution was analysed by optical rotary dispersion polarimetry in the range of 300–200 nm (Fig. 1). The optical rotary dispersion curve (Curve C) of the resolved D-leucine gave an optical rotation at 224 nm practically identical to that (Curve B) obtained by starting with half the amount of authentic D-leucine relative to the weight of DL-leucine used in the resolution and submitting it to the same ion exchange and reconstitution treatment applied to the resolved product.

The oxidative deamination of L-leucine in DL-leucine to the corresponding 4-methyl-2-oxopentanoic acid also was confirmed in the developmental work using nonradioactive DL-leucine by reacting aliquots of the reaction mixture with 2,4-dinitrophenylhydrazine yielding the 2,4-dinitrophenylhydrazone derivative which was monitored colorimetrically (440 nm) against standard 2,4-dinitrophenylhydrazone solutions. The keto acid yield was 84%. It was shown in this keto acid assay that the amino acid does not interfere with the analysis for the keto acid, making it unnecessary to separate the keto acid from the D-leucine.

Advantages of the oxidative deamination method for resolution are that it requires simple materials, is easily adaptable to hot cell conditions, and produces each of the enantiomers depending on the amino acid oxidase used.

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Correction of Perfusate Concentration for Sample Removal

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Abstract □ Repeated sampling of a drug solution that is recirculated through a perfused body increases the rate of drug disappearance from the perfusate. When the volume of the drug solution (V_T) is maintained constant by addition of drug-free perfusate after sampling, the measured drug concentration (C_i) can be corrected for drug removed in previous samples by using the equation $C_i' = C_i V_T C_{i-1}'/(V_T - V_S)C_{i-1}$, where C_i' is the corrected drug concentration in the ith sample, V_S is the volume of the sample, and $C_1' = C_1$. An error in any particular C_i is not transmitted to a subsequent C_i' value. The method can be used when the time interval between samples and when V_S vary from sample to sample, but return of the drug from the perfused body to the perfusate after sampling may cause C_i' to be overestimated.

Keyphrases □ Perfusates—correction of perfusate concentration for sample removal □ Drug concentration—correction of perfusate concentration for sample removal □ Correction methods—perfusate concentrations for sample removal

Several experimental techniques involve the perfusion of a tissue, an organ, or an entire organism with a drug solution (perfusate); *e.g.*, muscle (1), kidney (2), placenta (3), liver (4), intestine (5), and fish (6). Samples of the perfusate are periodically removed for determination of drug concentration. One experimental approach involves sampling the perfusate after it is passed once through the perfused body. An alternative approach is to recirculate the perfusate, usually by pumping it from a reservoir, through the perfused body, and back to the reservoir. Using the once-through approach, correction for sampling is unnecessary. When samples are removed repeatedly from recirculated perfusate, however, the concentration of drug is reduced, as a result of sample removal, in all samples but the first. Sample removal thereby biases the concentration–time relationship and confounds a kinetic analysis of the data.

If the ratio of the total sample volume to the perfusate volume is small, the bias is small and may be ignored. This ratio can be reduced by reducing the sample volume, reducing the number of samples, or increasing the volume

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