Inhibitors of Polyamine Biosynthesis V: *In Vivo* Effects of α-Methyl-(±)-ornithine and α-Methyl-(±)-ornithine *tert*-Butyl Ester

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Abstract □ The effects of α-methyl-(±)-ornithine and its *tert*-butyl ester on the survival time and the levels of polyamines in spleen tissue of mice inoculated intraperitoneally with L-1210 lymphoid leukemic cells were studied *in vivo*. These compounds were administered at doses of 100–300 mg/kg from Day 1 of tumor inoculation until death. This treatment neither significantly increased survival time nor altered the increases in polyamine levels normally observed during tumor growth. A method was developed to determine polyamine levels in spleen tissue. The polyamines in the tissue extracts were converted to their 5-(dimethylamino)-1-naphthalenesulfonyl derivatives, and these derivatives were separated by TLC followed by high-pressure liquid chromatography on a bonded phase microparticle column. The *in vivo* metabolism of 1-^{14}C-α-methyl-(±)-ornithine was studied and compared to that of 1-^{14}C-(±)-ornithine. 1-^{14}C-α-Methyl-(±)-ornithine was rapidly excreted after administration to mice inoculated with L-1210 leukemic cells. Within 24 hr after injection, over 70% of the radioactive dose was found in the urine, primarily as the unchanged compound. Less than 0.1% of the administered dose appeared as ^{14}C-labeled carbon dioxide during the 24 hr following injection. In contrast, 34% of the administered dose of 1-^{14}C-(±)-ornithine was metabolized to labeled carbon dioxide and 28% was excreted in urine during the 24 hr following injection.

Keyphrases □ Polyamines—effects of α-methyl-(±)-ornithine and its *tert*-butyl ester on biosynthesis in leukemic mice, TLC–high-pressure liquid chromatographic analysis of tissue extracts □ α-Methyl-(±)-ornithine—radiochemical study of metabolism in mice, effects on polyamine biosynthesis in leukemic mice, *tert*-butyl ester synthesized □ TLC–high-pressure liquid chromatography—analysis, polyamines in tissue extracts, mice □ Radiochemistry—study of metabolism of α-methyl-(±)-ornithine in mice

In spite of the many biological roles attributed to the polyamines, their exact physiological function is still not known. The multitude of effects elicited by the polyamines in cell free systems complicates definition of their true physiological function. However, recent evidence strongly implies that their primary function is the regulation of growth processes (1).

Previously, the synthesis and evaluation of inhibitors of polyamine biosynthesis were described (2–5). In particular, inhibitors of ornithine decarboxylase were studied because this enzyme appears to catalyze the rate-limiting step in the overall polyamine biosynthetic pathway. α-Alkyl- and aralkyl-(±)-ornithine and (±)-5-amino-2-hydrazino-2-methylpentanoic acid were synthesized and were potent reversible inhibitors of mammalian ornithine decarboxylase *in vitro*. It was also shown that α-methyl-(±)-ornithine (I) did not produce its inhibitory effects by acting as an alternative substrate for ornithine decarboxylase *in vitro* (3). A recent study found that I decreased the cellular levels of putrescine and spermidine in L-1210 leukemic cells of mice grown in culture. This inhibition of polyamine biosynthesis was not accompanied by inhibition of growth of these cells (5).

To elucidate the function of the polyamines, a study of the effects of I on the survival time and polyamine levels in mice inoculated with L-1210 lymphoid leukemia cells in *vivo* was undertaken. The results are reported in the present paper. The synthesis and *in vivo* evaluation of α-methyl-(±)-ornithine *tert*-butyl ester (V) also are described. This compound was prepared in an attempt to provide an inhibitor with prolonged *in vivo* activity. Furthermore, a method for the determination of polyamines in tissue is described, and preliminary studies of the metabolic fate of I in mice are discussed.

EXPERIMENTAL[1]

Melting points were determined in open capillary tubes and are uncorrected. NMR spectra were taken in deuterochloroform or deuterium oxide with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard, respectively. Mass spectral analyses were performed at 70 ev with a 200° chamber temperature.

Metabolic studies with radiolabeled compounds were performed using enclosed environment glass metabolic cages[2]. Autoradiographic data were obtained using medical X-ray film[3] developed by a rapid process developer[4]. TLC plates for the separation of biological samples were silica gel GF and Avicel F[5], 250 μm, 20 × 20 cm. High-pressure liquid chromatography (HPLC) was performed on a bonded phase column[6]. Eluted compounds were detected by monitoring absorbance at 254 nm.

(±) - N^2,N^5-Bis(benzyloxycarbonyl)-2,5-diamino-2-methylpentanoic Acid (III)—The procedure was a modification of the method of Synge (6). To a solution of I monohydrochloride monohydrate (4.387 g, 0.02 mole) in 2 *N* NaOH (30 ml) at 0° were added alternately four portions of 2 *N* NaOH (20 ml) and benzyl chloroformate (II) (5 ml) (Scheme I). Each addition pair was stirred for 5–10 min, and the final reaction mixture was stirred for 0.5 hr. Concentrated hydrochloric acid (25 ml) was then added, and the resulting oily mixture was extracted with ether (3 × 50 ml).

The ether layer was then extracted with 7% sodium bicarbonate (4 × 200 ml), and the aqueous layer was acidified with concentrated hydrochloric acid (110 ml) and extracted with ether containing 10% ethyl acetate (3 × 250 ml). The organic layer was dried (anhydrous calcium sulfate) and then evaporated to dryness. Crystallization from toluene–petroleum ether (bp 60–70°) gave III (5.15 g, 41% yield), mp 112–114°; IR (KBr): 3370, 3320, 1715, 1680, 1520, 1250, 740, and 685 cm^{-1}; NMR (deuterochloroform): δ 1.41 (broad m, 2H), 1.56 (s, 3H), 1.90 (broad m, 2H), 3.1 (t, 2H), 5.08 (s, 4H), and 7.32 (s, 10H) ppm.

Anal.—Calc. for $C_{22}H_{26}N_2O_6$: C, 63.76; H, 6.32; N, 6.76. Found C, 63.79; H, 6.32; N, 6.57.

(±) -N^2,N^5-Bis(benzyloxycarbonyl)-2,5-diamino-2-methylpentanoic Acid *tert*-Butyl Ester (IV)—Preparation of IV was accomplished by modifications of the method by Pandit[7]. To a suspension of III (3.70 g, 0.0089 mole) in methylene chloride (250 ml) containing 0.5 ml of sulfuric acid was added isobutylene gas (about 40–50 ml) until no further volume change occurred (Scheme I). The reaction mixture was capped and stirred for 72 hr at room temperature. Then ammonia gas was added to bring the pH to 8, and excess isobutylene was removed with a nitrogen stream.

[1] Instruments used were: a Thomas-Hoover melting-point apparatus; a Perkin-Elmer 237 or Beckman IR-9 IR spectrophotometer; a Varian A-60 D NMR spectrometer; an AEI MS-30 mass spectrometer; a Beckman LS-150 liquid scintillation counter; a model U6K injector and a model 6000 solvent delivery system, Waters Associates, Bedford, Mass.; an LDC UV-monitor, model 1285; and a Hewlett-Packard 9100A calculator. Elemental analysis was performed by MHW Laboratories, Garden City, Mich., and Galbraith Laboratories, Knoxville, Tenn.

[2] Colman-Delmar, Newark, Del.

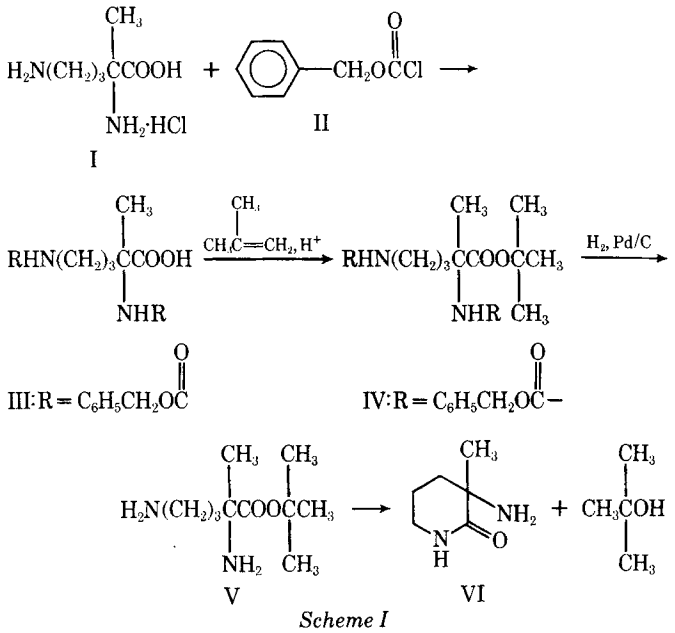
[3] Kodak RP/R-54.

[4] Kodak X-OMAT.

[5] Analtech, Newark, Del.

[6] Micropak CN-10 (25 cm × 2.2 mm i.d.), Varian Aerograph.

[7] Dr. U. Pandit, Laboratorium Voor Organische Scheikunde Der Universitat Van Amsterdam, Amsterdam, The Netherlands, personal communication.



Scheme I

The reaction mixture was washed with water, dried over anhydrous calcium sulfate, and evaporated to provide crude IV as an oil (3.59 g, 86% yield); IR (neat): 3420, 3350, 2975, 2940, 1720, 1520, 1500, 1450, 1365, 1250, 735, and 690 cm^{-1}; NMR (deuterochloroform): δ 1.43 (s, 9H), 1.51 (s, 3H), 1.86 (broad m, 4H), 3.17 (t, 2H), 5.08 (d, 4H), and 7.33 (s, 10H) ppm.

Anal.—Calc. for $C_{26}H_{34}N_2O_6$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.23; H, 7.30; N, 5.81.

After prolonged storage at 0°, the oil crystallized from ethanol–water, mp 85–86.5°. All spectral data established identity of the solid with the oil (IV).

(±)-2,5-Diamino-2-methylpentanoic Acid *tert*-Butyl Ester Dihydrochloride (V Dihydrochloride)—Palladium catalyst (10% on carbon, 100 mg) was added to a solution of IV (3.59 g, 0.0076 mole) in ethanol (75 ml), and the reaction mixture was hydrogenolyzed with 2.2 kg/cm^2 of hydrogen for 24 hr at room temperature (Scheme I). The slurry was then filtered through a diatomaceous earth[8] bed, rinsed with ethanol, and carefully evaporated to dryness. The oil was quickly dissolved in cold absolute ethanol, and then ether saturated with hydrogen chloride was added slowly, with stirring, to the chilled solution.

The precipitated solid was filtered and crystallized from ethanol–ether to give V dihydrochloride (1.69 g, 81% yield), mp >90° dec.; IR (KBr): 2000, 1730, 1595, 1515, 1365, 1250, 1150, 830, and 735 cm^{-1}; NMR (deuterium oxide): δ 1.55 (s, 9H), 1.58 (s, 3H), 1.91 (m, 4H), and 3.04 (t, 2H) ppm.

Anal.—Calc. for $C_{10}H_{24}Cl_2N_2O_2$: C, 43.66; H, 8.79; N, 10.18. Found: C, 43.67; H, 8.70; N, 9.87.

Anal.—Calc. for $C_{20}H_{50}Cl_4N_4O_5$: C, 42.26; H, 8.87; N, 9.86. Found: C, 42.26; H, 8.83; N, 10.04.

Animals—Adult male DBA/2 mice[9], 15–20 g, and adult male BDF_1 mice[10], 18–20 g, were allowed food[11] and water *ad libitum*.

Materials and Solutions—^{14}C-Toluene (4.0×10^5 dpm/ml), 1-^{14}C-DL-ornithine (specific activity 7.66 mCi/mmole), and sodium ^{14}C-bicarbonate (specific activity 8.4 mCi/mmole) were obtained commercially[12]. 1-^{14}C-Compound I (specific activity 0.53 mCi/mmole) was synthesized as described previously (3). The counting cocktail was either 5.5 g of fluor[13] dissolved in 1 liter of toluene–methanol (1:2) or 5.5 g of fluor dissolved in 1 liter of nonionic surfactant[14]–toluene (1:2). Liquid chromatographic grade solvents[15] were used.

Tumor Inoculation—The L-1210 lymphoid leukemia was obtained as a frozen cell suspension. The tumor was carried in DBA/2 male mice by weekly intraperitoneal passages. A 0.1-ml volume of a suspension (in Eagle's 1-X balanced media) containing $1.0–2.0 \times 10^6$ L-1210 ascites leukemic cells/ml, which were obtained on Day 7 from DBA/2 mice, was inoculated intraperitoneally into each recipient BDF_1 test mouse. Each test group was inoculated with cells obtained from the same DBA/2 mouse.

Drug Regimen—A daily intraperitoneal injection of 0.1 ml of the test compounds, from Day 1 until death, was given to leukemic BDF_1 mice inoculated on Day 0 with tumor cells as described. All test compounds were dissolved in sterile 0.9% NaCl solution, and the solutions were prepared on Days 1 and 5 of tumor growth. A 0.1-ml volume of a 0.9% NaCl solution was injected into each control group. Compounds I at 300 and 100 mg/kg and V at 100 mg/kg were used in the survival time study and in the polyamine metabolism study. 1-^{14}C-Compound I was administered at 300 mg/kg once on Day 3 of tumor growth to study I metabolism.

Survival Time Test—Protocol 1.100 (7) of the National Cancer Institute was followed. Eight mice were used in each test group, and 18 mice were used in the control group. A suspension of 1.2×10^5 L-1210 cells was inoculated into each BDF_1 mouse on Day 0.

Preparation of Spleen Extracts for Polyamine Determination—Two animals from each test group and two animals from the control group were sacrificed by cervical dislocation on Days 0 (control only), 3, 6, and 8 after tumor inoculation. The spleens were removed quickly, excised from surrounding fatty tissue, rinsed in 0.9% NaCl, blotted dry, and frozen immediately in a liquid fluorocarbon[16]. The spleens were weighed and then quickly homogenized in 10 volumes of chilled 0.4 *N* perchloric acid homogenization solution. The homogenate was centrifuged at 5000 rpm for 20 min, and the supernate was removed and stored at −70° until analysis.

Determination of Polyamines in Spleen Extracts—Spleen extracts were dansylated according to the method of Seiler and Wiechmann (8), but the following modifications were necessary to obtain optimal conditions. A 100-μl portion of the spleen tissue extract was shaken overnight in a stoppered tube in a reaction mixture containing 600 μl of a solution of 1-dimethylaminonaphthalene-5-sulfonyl (dansyl) chloride (12 mg/ml) in acetone, 200 μl of a saturated sodium carbonate solution, and 50 μl of 80.0 μM 1,3-propanediamine in water. Duplicate samples were analyzed. In a similar fashion, polyamine standard solutions were dansylated. Volumes of 10, 25, 50, 75, and 100 μl of an aqueous solution containing 0.0454 m*M* putrescine, 0.333 m*M* spermidine, and 0.320 m*M* spermine were reacted. A 100-μl portion of 0.4 *N* perchloric acid was added to the standards along with 50 μl of the 1,3-propanediamine solution. Standards were run in duplicate or triplicate.

After a 16-hr reaction period, 300 μl of an aqueous proline solution (300 m*M*) was added to each sample and the mixtures were shaken for 2 hr to eliminate excess dansyl chloride. Acetone in the reaction mixture was evaporated at 40° under a nitrogen stream. Water (0.5 ml) was added to the evaporated mixture, and the dansylated polyamines were extracted into benzene (5 ml). The benzene layer was evaporated to dryness at 40° under a nitrogen stream, and the resulting residue was dissolved in a small portion of chloroform. The chloroform solution was applied to silica gel GF TLC plates, and the plates were developed for 15 cm with cyclohexane–ether (1:9).

The dansylated polyamine standards were developed concurrently with the tissue extracts; areas corresponding to the polyamines and to 1,3-propanediamine (UV visualization) were scraped, using a glass recovery tube[17], and eluted with 5 ml of triethylamine–2-propanol (1:1). The eluant was evaporated to dryness at 40° under a nitrogen stream. The residue was dissolved in methylene chloride (50 or 100 μl), and 5–10-μl aliquots were used for separation by HPLC. The solvent system used for the HPLC separation was cyclohexane–methylene chloride–2-propanol (88.5:7.5:4) at a flow rate of 1.0 ml/min.

Monitoring of the eluted dansylated polyamines was accomplished by a UV detector at 254 nm. Baseline separation of all amines was achieved, and the total time per sample was under 25 min (Fig. 1). To ensure chromatographic purity, the eluants from the HPLC column corresponding to each amine were collected separately during two individual separations, evaporated to dryness, and then rechromatographed two dimensionally (15 cm each direction) on silica gel GF TLC plates, first with chloroform–triethylamine (10:1) and then with cyclohexane–ethyl acetate (1:1).

Each chromatogram yielded only one fluorescent spot that coincided exactly to the respective dansylated polyamine standard. The ratio of the area under the peaks of the polyamines to that of 1,3-propanediamine

[8] Celite.
[9] Jackson Laboratories, Bar Harbor, Me.
[10] ARS Sprague-Dawley, Madison, Wis.
[11] Purina chow.
[12] New England Nuclear, Boston, Mass.
[13] Permablend III, Packard Instruments, Downers Grove, Ill.
[14] Triton X-100, Packard Instruments, Downers Grove, Ill.
[15] Burdick and Jackson Laboratories, Muskegon, Mich.
[16] Freon.
[17] Chromaflex recovery tube, Kontes Glass Co., Vineland, N.J.

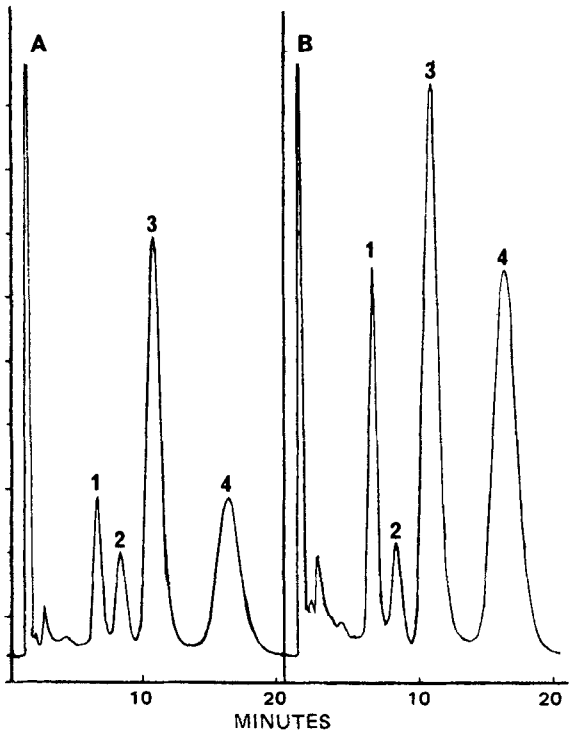


Figure 1—*HPLC separation of a standard solution of the dansyl derivatives of the polyamines (A) and of the dansyl derivatives of the polyamines obtained from the spleens of mice inoculated with L-1210 leukemic cells (B). The compounds tested were the dansyl derivatives of 1,3-propanediamine (internal standard) (1), putrescine (2), spermidine (3), and spermine (4).*

was calculated from the HPLC charts by the triangulation method. Standard curves for each amine were obtained by linear regression analysis of area ratios. Values of the polyamine content in spleen tissue were obtained from each standard curve.

Determination of 1-^{14}C-I Metabolism—Two adult male BDF_1 mice, 19–20 g, were inoculated with a suspension of 1.7×10^5 L-1210 leukemic ascites cells on Day 0. On Day 3 of tumor growth, each mouse was injected with 0.1 ml (0.1035 g) of a solution containing 0.0392 g (0.195 mmole) of 1-^{14}C-I and 0.0797 g of unlabeled I dissolved in 2.00 ml of 0.9% NaCl. The animals were then placed into a glass-enclosed environment metabolism cage, designed to separate the urine and feces and to allow carbon dioxide collection.

Air was pulled through the cage at the rate of 150–200 cm^3/min. The air entry tube contained granulated sodium hydroxide[18], which removed all atmospheric carbon dioxide. Expired carbon dioxide was collected at 4 hr and from 4 to 24 hr in separate traps containing 75 ml of 10 *N* NaOH. A backup trap of 10 *N* NaOH (200 ml) was used during the entire experiment. The urine was collected at 4 hr and from 4 to 24 hr, and the metabolic cage was rinsed carefully with water at each period. The diluted urine solutions were filtered through sintered-glass funnels and then frozen until analysis.

Previously, a standard curve was prepared to determine the quenching effect of urine on the counting efficiency. Various dilutions of urine obtained from male BDF_1 mice and a constant amount of 1-^{14}C-I were mixed in water to a final volume of 1.0 ml. Then the toluene–nonionic surfactant cocktail (10 ml) was added to each sample, and the counts per minute of the samples were measured. Examination of the standard quench curve showed that urine diluted between 1:10 and 1:100 had no effect on the counting efficiency in the scintillation system used.

^{14}C-Toluene and 1-^{14}C-DL-ornithine standards were used to determine the cocktail counting efficiency, which was calculated to be 86.7%. Triplicate samples of each urine collection were analyzed. For each sample, a 1.0-ml volume of the diluted urine solution was added to 10.0 ml of the cocktail. The percent of the administered dose of 1-^{14}C-I found in the urine was calculated by converting all values obtained by liquid scintillation counting to disintegrations per minute.

[18] Ascarite.

Table I—Effects of I and V on the Survival Time of Male BDF_1 Mice Inoculated Intraperitoneally with L-1210 Leukemic Cells of Mice

Group	Test Compound	Dose, mg/kg	Number of Animals	Mean Survival Time, days[a]
1	I hydrochloride	100	8	7.7
2	I hydrochloride	300	8	7.4
3	V dihydrochloride	100	8	8.1
4	Control (0.9% NaCl)	—	18	7.4 (±0.55)

[a] Mean survival time was calculated according to Protocol 11 in Ref. 7.

The ^{14}C-labeled carbon dioxide collected during the metabolism study was measured as follows. Duplicate samples of 1.0, 2.0, and 5.0 ml of each collection time period were pipetted into separate 25-ml conical flasks. The flasks were sealed with a rubber stopper, which held a polypropylene center well containing 200 μl of ethanolamine. The labeled carbon dioxide was liberated by the injection of similar volumes of concentrated hydrochloric acid into the reaction flasks. The flasks stood for 20–24 hr to ensure complete absorption of the carbon dioxide by the ethanolamine. Then the polypropylene cups were carefully cut and placed into a counting vial containing 10.0 ml of methanol–toluene cocktail.

The efficiency of the carbon dioxide collection was determined by comparison of values obtained from a standard sodium ^{14}C-bicarbonate solution using the described method to values obtained by a direct count of an identical volume (0.01 ml) of the sodium ^{14}C-bicarbonate solution in the cocktail. Collection efficiency was calculated to be 93.2 ± 0.8%. The counting efficiency of the cocktail, determined by use of ^{14}C-toluene and 1-^{14}C-DL-ornithine, was calculated to be 73%. All final cocktail mixtures contained 10.0 ml of the methanol–toluene cocktail, 0.2 ml of ethanolamine, and 0.01 ml of water.

Portions of the urine samples were freeze dried and then applied to cellulose TLC plates. The plates were developed two dimensionally (15 cm in each direction) by combinations of the following systems: A, methanol–pyridine–1.2 *N* HCl (8:1:2); B, methanol–pyridine–water–acetic acid (6:6:4:1); and C, 1-butanol–water–acetic acid (3:1:1). Each developed chromatogram was coated with a saturated solution of anthracene (in benzene), dried, placed into a metal cassette containing X-ray film, and then exposed for 48 hr in a dry ice chamber. The autoradiogram was developed and fixed.

Authentic I (2–3 mg) was added to the freeze-dried solutions prior to the TLC application. The same TLC plates that were used for autoradiography were afterward sprayed with ninhydrin (1% in ethanol) and then heated. The ninhydrin-positive spots that appeared were correlated to the autoradiograms. Authentic 1-^{14}C-I was run separately in each TLC system, autoradiographed, and visualized with ninhydrin as described. Results were correlated to urine samples.

1-^{14}C-DL-Ornithine was diluted appropriately, and 0.1 ml of this solution (0.0993 g) was injected on Day 3 into two BDF_1 male mice; these mice had been inoculated on Day 0 with 1.02×10^5 L-1210 cells. The mice were housed in the metabolism cages, and the urine and ^{14}C-labeled carbon dioxide were collected and measured as described. The specific activity of the stock 1-^{14}C-DL-ornithine solution was determined using a toluene–nonionic surfactant cocktail.

The animals were not fed throughout both metabolic studies, but water was given *ad libitum*.

RESULTS AND DISCUSSION

The synthesis of I and ^{14}C-I were reported previously (2, 3). Compound V was very susceptible to intramolecular ring closure and gave the lactam VI and *tert*-butanol (Scheme I). The dihydrochloride salt of V was not susceptible to this decomposition. However, V dihydrochloride must be prepared with ice-cold dilute ethereal hydrogen chloride to prevent the acid hydrolysis of the *tert*-butyl ester moiety. Compound V dihydrochloride was very hygroscopic and formed a stable hemihydrate upon exposure to air. Periodic proton magnetic resonance examination of dried samples of V dihydrochloride stored at 0° under vacuum showed that no formation of *tert*-butanol had occurred.

Compound V was prepared to act as a prodrug that may have better *in vivo* transport and distribution characteristics than I. *In vivo*, V would hydrolyze to liberate the active decarboxylase inhibitor I.

Compounds I and V produced no increase in the survival time of mice (Table I). Russell (9) reported similar results using I (10); however, no details of dosage regimen or test conditions were given.

The effects of I and V on the changes in the polyamine levels during

Table II—Effects of I on the Polyamine Levels in Spleen of BDF_1 Mice Inoculated with L-1210 Leukemic Cells of Mice

Polyamine	Day[a]	Levels, μmoles/g (Range)[b] Group 1[c]	Group 2[c]	Group 3[c]	Group 4[c]
Putrescine	3	0.19 (0.16–0.22)	0.11 (0.10–0.12)	0.19 (0.13–0.26)	0.18 (0.14–0.21)
	6	0.31 (0.29–0.34)	0.28 (0.35–0.32)	0.29 (0.22–0.36)	0.32 (0.30–0.33)
	8	0.30	0.23	0.38	0.26
Spermidine	3	1.64 (1.30–1.97)	1.21 (1.13–1.28)	1.70 (1.46–1.96)	1.59 (1.18–2.01)
	6	2.14 (2.09–2.19)	2.00 (1.95–2.05)	2.05 (1.72–2.38)	2.06 (1.66–2.45)
	8	1.99	1.82	2.10	1.70
Spermine	3	1.06 (0.92–1.20)	0.84 (0.77–0.90)	1.00 (0.86–1.14)	1.00 (0.79–1.21)
	6	1.15 (1.03–1.27)	1.02 (0.99–1.05)	1.05 (0.86–1.23)	1.02 (0.95–1.09)
	8	0.77	0.78	0.84	0.72

[a] Days after inoculation of mice with 1.2×10^5 L-1210 ascites tumor cells. [b] Ranges between animals within each test group. [c] Compounds were administered by intraperitoneal injection from Days 1 through 7 to the mice in the following groups: Group 1, 100 mg/kg of I hydrochloride monohydrate; Group 2, 300 mg/kg of I hydrochloride monohydrate; Group 3, 100 mg/kg of V dihydrochloride; and Group 4, 0.9% NaCl.

tumor growth were studied using spleen tissue obtained from BDF_1 mice inoculated with L-1210 leukemic cells. The basal polyamine levels on Day 0 were 0.17, 1.5, and 0.9 μmoles/g of tissue for putrescine, spermidine, and spermine, respectively. These levels progressively increased to the maxima on Day 6. Although the animals were of the same age, weight, and sex, the levels of each polyamine varied widely from animal to animal within each test group. In general, the mean values for the accumulation of the polyamines during L-1210 tumor growth were consistent in magnitude with values reported by others (11, 12). One notable exception was that the putrescine levels were slightly higher than those reported previously (11). Daily injections of I at doses of 100 and 300 mg/kg or V at a dose of 100 mg/kg had no significant effect on the polyamine level changes occurring during L-1210 tumor growth (Table II).

Standard solutions of each polyamine were used to obtain standard curves. A linear relationship between the amount of amine added and the ratio of the area of its peak to that of the internal standard was observed. The correlation coefficients, calculated by regression analysis, were 0.995 for putrescine, 0.996 for spermidine, and 0.996 for spermine. The polyamine concentrations in the spleen extracts were obtained by entering the ratio of the peak areas of the polyamine to that of 1,3-propanediamine onto the standard curve. Duplicate samples from each spleen extract were within ±10%.

To delineate the factors that may have contributed to the lack of *in vivo* activity of I, the fate of the 1-^{14}C-labeled compound was studied in mice. The fate of (±)-ornithine (VII) was also studied to monitor the metabolic changes of the substrate L-ornithine. Less than 0.1% of the injected dose of I was recovered as ^{14}C-labeled carbon dioxide during 24 hr (Table III). This value correlated well with results obtained by others with α-methyl-substituted amino acid analogs that inhibit decarboxylase enzymes (13–15). It is also in agreement with results of studies on the decarboxylation of I *in vitro* (3). Compound I was eliminated relatively rapidly in urine. Four hours after injection, 38% of the injected dose of I was recovered in the urine. During the 4–24-hr period, another 32% of the dose was excreted in urine. The rapid elimination of I *via* the kidney after intraperitoneal injection may be responsible for the lack of *in vivo* activity.

During a 24-hr period, 34% of the administered dose of ^{14}C-VII was metabolized to ^{14}C-labeled carbon dioxide and 28% was excreted in urine. The pattern of the major elimination pathway changed dramatically over the 24 hr. During the 0–4-hr interval after administration, decarboxylation occurred to the extent of 28% while urinary excretion accounted for only 0.1% of the injected dose. It is assumed that only the L-form of the injected ^{14}C-VII was decarboxylated. Thus, over 50% of the natural substrate was rapidly decarboxylated. From 4 to 24 hr, metabolism *via* labeled carbon dioxide loss amounted to only 6% whereas urinary excretion represented 28% of the injected dose. The radioactivity excreted in the urine was probably due solely to the D-enantiomer since this enantiomer cannot be decarboxylated (16) and probably cannot be reabsorbed by kidney tubules.

The urine samples collected after intraperitoneal injection of 1-^{14}C-I were examined for possible metabolites. In all chromatographic systems examined, the radioactivity was located entirely in ninhydrin-positive areas and these spots were coincident with authentic 1-^{14}C-I. These results indicate that I was excreted in urine primarily unchanged.

Table III—Elimination of ^{14}C-I and ^{14}C-VII after Intraperitoneal Injection in Mice

Compound[a]	Hours[b]	Percent of Dose Excreted in Urine	Percent of Dose Excreted as Labeled Carbon Dioxide
^{14}C-I	0–4	38.20	0.04
	4–24	32.43	0.03
^{14}C-VII	0–4	0.10	27.50
	4–24	28.01	6.00

[a] Compounds were injected intraperitoneally in BDF_1 mice on Day 3 after inoculation with 1.71×10^5 L-1210 cells. [b] Hours after intraperitoneal injection of the test compounds.

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