

# Synthesis of Peptides of 1-Aminocyclopentanecarboxylic Acid

S. SHANKMAN, S. HIGA, F. LEW, AND M. E. ROBERTS

*Pasadena Foundation for Medical Research, Pasadena, California*

*Revised Manuscript Received July 3, 1961*

Twenty-three carbobenzoxy dipeptide esters, carbobenzoxy peptides, and dipeptides of 1-aminocyclopentanecarboxylic acid were synthesized. Rotation,  $R_f$ , and electrophoretic migrations are given. The ability to inhibit Sarcoma-180 in mice varied significantly with structure.

Cycloaliphatic amino acids have been investigated for their antitumor properties by Ross,<sup>1</sup> Connors,<sup>2</sup> and Martel<sup>3</sup> and their collaborators, with the finding that 1-aminocyclopentanecarboxylic acid (NSC-1026)<sup>4</sup> (I) is effective on Carcinoma-755 and Sarcoma-180 in mice, and on Walker (rat) Carcinoma-256. Preliminary clinical trials have been reported.<sup>5</sup>

Connors, *et al.*,<sup>2</sup> have evaluated the effect of substitution on the cyclopentane ring and the amino and carboxyl groups, and conclude that limited substitution markedly diminishes antitumor activity. Both C-terminal and N-terminal glycine peptides retained activity in rats, but the phenylalanyl-1-aminocyclopentanecarboxylic acid peptide was inactive.

This Laboratory has been concerned with the synthesis of antibacterial and potential antitumor peptides.<sup>6-9</sup> Here we report the synthesis of the L-alanyl, L-valyl, L-leucyl, L-arginyl, L-histidyl, DL-methionyl, and L-phenylalanyl peptides of 1-aminocyclopentane-

(1) R. B. Ross, C. I. Noll, W. C. J. Ross, M. V. Nadkarni, B. H. Morrison, and H. W. Bond, *J. Med. Pharm. Chem.*, **3**, 1 (1961).

(2) T. A. Connors, L. A. Elson, A. Haddow, and W. J. C. Ross, *Biochem. Pharmacol.*, **5**, 108 (1960).

(3) F. Martel and L. Berlinguet, *Can. J. Biochem. Physiol.*, **37**, 433 (1959).

(4) Consecutive test compound number of the Cancer Chemotherapy National Service Center.

(5) W. W. Benefiel, J. T. Helsper, and G. S. Sharp, *Cancer Chemotherapy Rep.*, **9**, 21 (1960).

(6) S. Shankman and Y. Schvo, *J. Am. Chem. Soc.*, **80**, 1164 (1958).

(7) S. Shankman, S. Higa, H. A. Florsheim, Y. Schvo, and V. Gold, *Arch. Biochem. Biophys.*, **86**, 204 (1960).

(8) S. Shankman, S. Higa, and V. Gold, *J. Am. Chem. Soc.*, **82**, 990 (1960).

(9) S. Shankman, S. Higa, and V. Gold, *Texas Rep. Biol. Med.*, **19**, 358 (1961).

carboxylic acid (I), its dipeptide, and a diketopiperazine for evaluation in mouse tumors. Preliminary data indicate variation in antitumor activity with peptide structure. Larionov<sup>10</sup> has found a similar variation in his studies of peptides of the cytotoxic amino acid, sarcosylsin.

The dipeptides were made by coupling the carbobenzoxy amino acids with the 1-aminocyclopentanecarboxylic acid methyl ester by the mixed anhydride<sup>11</sup> or the dicyclohexylcarbodiimide (DCC) procedure.<sup>12</sup> The benzyl ester was used for two peptides. Coupling and saponification of the peptides proceeded smoothly. Prolonged hydrogenolysis with palladium black was necessary in some cases to obtain the free peptides. The methionyl peptide was obtained in low yield (20% based on starting material; 50% corrected for recovery) due to poisoning of the catalyst. Analytical data, yields, and melting points of the carbobenzoxy (Z) peptide esters, Z peptides, and peptides are given in Tables I-III.

Preliminary biologic data on mouse Sarcoma-180 gave  $T/T_c$ <sup>13</sup> of 0.06, 0.17, and 0.20 for compound (XV), and 0.24 and 0.05 for (XVI), both at 100 mg./kg. At 90 mg./kg., 1-aminocyclopentanecarboxylic acid (I) gave  $T/T_c$  of 0.67, and at 60 mg./kg. gave  $T/T_c$  of 0.63.<sup>14</sup> These respective levels of (I) have the same content of (I) as 100 mg. of (XV) and (XVI). Peptides (XVII) and (XX) were substantially without effect on this tumor. Complete biologic data will be published elsewhere.

## Experimental

a.—Molecular weights were determined by titration with perchloric acid in glacial acetic acid, with mercuric acetate used (when necessary) to sharpen end-points. Leucine and valine were determined microbiologically with *Lactobacillus plantarum* (ATCC 8014). DL-Methionine was determined with *Lactobacillus fermenti* (ATCC 9338). Arginine was determined with *Lactobacillus casei* (ATCC 7469) and by the Sakaguchi colorimetric procedure. Histidine, alanine, and phenylalanine were determined with *Pediococcus cerevisiae* (ATCC 8081). Although both D and L isomers of alanine are reported equally active for this culture,<sup>15</sup> the DL-alanine, which was employed at first for reference standard, was

(10) L. F. Larionov, *Cancer Research*, **21**, 99 (1961).

(11) J. R. Vaughan and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

(12) J. C. Sheehan and G. P. Hess, *Ibid.*, **77**, 1067 (1955).

(13)  $T/T_c$  = average weight of treated tumor/average weight of control tumor.

(14) J. Leiter and M. A. Schneiderman, *Cancer Research*, **19**, #3, Part 2, p. 163 (1959).

(15) H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.*, **177**, 545 (1949).

TABLE I  
CARBOBENZOXY PEPTIDE ESTERS OF 1-AMINOCYCLOPENTANECARBOXYLIC ACID (I)

	Compound	Formula	Yield, %	M.p., °C. <sup>a</sup>	Calcd., %			Found, %		
					C	H	N	C	H	N
II	Carbobenzoxy-1-aminocyclopentanecarboxyl-1-aminocyclopentanecarboxyl methyl ester	$C_{21}H_{28}N_2O_6$	63	136-138 <sup>b</sup>	65.0	7.22	7.21	64.7	7.20	7.08
III	Carbobenzoxy-1-alanyl-1-aminocyclopentanecarboxyl methyl ester	$C_{18}H_{24}N_2O_5$	81	Oil						
IV	Carbobenzoxy-1-valyl-1-aminocyclopentanecarboxyl methyl ester	$C_{20}H_{26}N_2O_5$	74	137-138 <sup>c</sup>	63.8	7.48	7.45	63.5	7.41	7.38
V	Carbobenzoxy-1-leucyl-1-aminocyclopentanecarboxyl methyl ester	$C_{21}H_{28}N_2O_5$	70	102.5-104.5 <sup>c</sup>						
VI	Tricarbobenzoxy-1-arginyl-1-aminocyclopentanecarboxyl benzyl ester	$C_{42}H_{57}N_5O_9$	85	80-83 <sup>d</sup>	66.5	6.04	9.22	65.5	6.10	9.2
VII	Dicarbobenzoxy-1-histidyl-1-aminocyclopentanecarboxyl benzyl ester	$C_{32}H_{44}N_4O_7$		Oil						
VIII	Carbobenzoxy-DL-methionyl-1-aminocyclopentanecarboxyl methyl ester	$C_{20}H_{28}N_2O_5S$	82 <sup>c</sup>	86-87 <sup>b</sup>	58.7	6.86	6.85	58.0	6.70	6.80
IX	Carbobenzoxy-1-phenylalanyl-1-aminocyclopentanecarboxyl methyl ester	$C_{22}H_{28}N_2O_5$	62	99-101 <sup>b</sup>	68.0	6.61	6.60	68.1	6.71	6.53

<sup>a</sup> Capillary, uncorrected. <sup>b</sup> Recrystallized from ethyl acetate-petroleum ether. <sup>c</sup> Recrystallized from chloroform-petroleum ether. <sup>d</sup> Recrystallized from hot methanol. <sup>e</sup> By carbodiimide condensation.

more active in promoting growth than the L-alanine; therefore, L-alanine was used as reference standard. The Keston photoelectric polarimeter was employed for rotations. Electrophoretic migrations were carried out with a Spinco Model R electrophoresis apparatus, Durrum type. The NaOCl-KI-starch test<sup>16</sup> was very useful in detecting peptide spots on chromatography as distinct from amino acid spots. All peptides gave single spots on chromatography before hydrolysis, and the correct two spots after hydrolysis with HCl. The homogeneity of the free peptides was also demonstrated by electrophoresis.

**b.**—Carbobenzoxy-1-aminocyclopentanecarboxylic acid was prepared from carbobenzoxy chloride and (I) in toluene. It was recrystallized from chloroform-petroleum ether, m.p. 95–98°.

*Anal.* Calcd. for  $C_{14}H_{17}NO_4$ : C, 63.85; H, 6.45; N, 5.32. Found: C, 63.78; H, 6.42; N, 5.42.

**c.**—Compounds (II), (III), (V), (VI), and (IX) were prepared by the mixed anhydride method<sup>11</sup> in dry chloroform. (IV) was made by way of the mixed anhydride in dry ethyl ether. (VII) and (VIII) were prepared by DCC<sup>12</sup> condensation in chloroform and methylene chloride, respectively. The tricarbobenzoxy-arginine intermediate of Zervas<sup>17</sup> was purchased from Aldrich Chemical Company. The dicarbobenzoxyhistidine intermediate was made as described by Sakiyama *et al.*<sup>18</sup>

**d.**—The benzyl ester of (I), *p*-toluenesulfonate salt, was made by the method of Zervas<sup>19</sup> for glycine benzyl ester. The salt was precipitated with ethyl ether, and recrystallized from methanol-ether, m.p. 189–191°.

*Anal.* Calcd. for  $C_{20}H_{25}NO_6S$ : C, 61.37; H, 6.38; N, 3.58. Found: C, 61.36; H, 6.66; N, 3.50.

**e.**—Palladium black was generally used as catalyst for hydrogenolysis in methanol plus acetic or hydrochloric acid. For (XIX), 10% palladium on charcoal in methanol containing 10% acetic acid was used. For (XXI), the catalyst was one-half the compound weight. For (XV) and (XX), decarbobenzoxylation by 12 *N* hydrochloric acid was used,<sup>20</sup> then hydrogenation without isolation for (XX). Other accepted practice was used, as indicated for the valyl and histidyl compounds below.

**f. Compound (IV).**—To a mixture of 20 mM. of carbobenzoxy-L-valine and 20 mM. of triethylamine in dry ethyl ether at –5 to –10°, 20 mM. of ethyl chloroformate was added dropwise, maintaining the temperature at –5 to –10°. After 25 minutes, 22 mM. of a cold solution of the methyl ester of (I) in methylene chloride-ether was added at once. The reaction was maintained at –5° for 30 minutes, allowed to reach 25°, and left overnight. The solvents were removed and the dry residue was dissolved in methylene chloride and washed with water, 0.1 *N* hydrochloric acid, water, 5% potassium carbonate solution, and water, and

(16) S. C. Pan and J. D. Dutcher, *Anal. Chem.*, **28**, 836 (1956).

(17) L. Zervas, T. T. Otani, M. Winitz, and J. P. Greenstein, *J. Am. Chem. Soc.*, **81**, 2878 (1959).

(18) F. Sakiyama, K. Okawa, T. Yamakawa, and S. Akabori, *Bull. Chem. Soc. Japan*, **31**, 926 (1958).

(19) L. Zervas, M. Winitz, and J. P. Greenstein, *J. Org. Chem.*, **22**, 1515 (1957).

(20) R. B. Merrifield and D. W. Woolley, *J. Am. Chem. Soc.*, **78**, 4646 (1956).

CARBOBENZYL ESTERS OF 1-AMINOCYCLOPENTANECARBOXYLIC ACID (I)

Compound	Formula	Yield, %	M.p., °C.	Calcd., %			Found, %		
Carbobenzoxy-				C	H	N	C	H	N
I 1-Aminocyclopentanecarboxyl-1-amino-									
cyclopentanecarboxylic acid	$C_{20}H_{30}N_2O_6$	70	184-185	64.2	6.96	7.5	64.2	6.64	7.3
XI L-Alanyl-1-aminocyclopentanecarboxylic acid	$C_{17}H_{22}N_2O_6$	67	144.5-145 <sup>a</sup>	...	...	8.40	...	...	8.35
XII L-Valyl-1-aminocyclopentanecarboxylic acid	$C_{19}H_{24}N_2O_6$	68	140-141 <sup>b</sup>	63.2	7.20	7.74	62.0	7.26	7.72
XIII DL-Methionyl-1-aminocyclopentanecar-									
boxylic acid	$C_{19}H_{28}N_2O_6S$	72	127-127.5 <sup>b</sup>	57.9	6.60	7.10	56.9	6.49	7.09
XIV L-Phenylalanyl-1-aminocyclopentanecar-									
boxylic acid	$C_{23}H_{26}N_2O_6$	80	129-130 <sup>c</sup>	67.4	6.35	6.82	66.7	6.51	7.04

<sup>a</sup> Recrystallized from ethyl acetate. <sup>b</sup> Recrystallized from ethanol-water. <sup>c</sup> Recrystallized from chloroform-petroleum ether.

<sup>a</sup> Recrystallized from ethyl acetate. <sup>b</sup> Recrystallized from ethanol-water. <sup>c</sup> Recrystallized from chloroform-petroleum ether.

TABLE III

## DIPEPTIDES OF 1-AMINOCYCLOPENTANECARBOXYLIC ACID (I)

	Compound	Formula	Yield, %	M.p., °C.	Calcd., %			Found, %		
					C	H	N	C	H	N
XV	(A) 1-Aminocyclopentanecarboxyl-1-aminocyclopentanecarboxylic acid·HCl·H <sub>2</sub> O	C <sub>12</sub> H <sub>22</sub> ClN <sub>2</sub> O <sub>4</sub>	70 <sup>a</sup>	..... <sup>b</sup>	49.2	7.85	9.50	49.2	7.89	9.50
	(B) 1-Aminocyclopentanecarboxyl-1-aminocyclopentanecarboxylic acid·HCl <sup>c</sup>	C <sub>12</sub> H <sub>21</sub> ClN <sub>2</sub> O <sub>3</sub>	..	.....	..	..	10.1	..	..	9.80
XVI	L-Alanyl-1-aminocyclopentanecarboxylic acid·H <sub>2</sub> O	C <sub>9</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	50 <sup>d</sup>	290-291 <sup>e</sup>	49.5	8.26	12.8	48.0	8.41	12.3
XVII	L-Valyl-1-aminocyclopentanecarboxylic acid·H <sub>2</sub> O	C <sub>11</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	60	280-282 <sup>e</sup>	53.5	8.93	11.35	53.5	9.08	11.25
XVIII	L-Leucyl-1-aminocyclopentanecarboxylic acid·HCl	C <sub>12</sub> H <sub>23</sub> ClN <sub>2</sub> O <sub>3</sub>	50	<sup>f,g</sup>	51.7	8.25	10.02	51.2	8.32	10.05
XIX	L-Arginyl-1-aminocyclopentanecarboxylic acid acetate	C <sub>14</sub> H <sub>27</sub> N <sub>5</sub> O <sub>5</sub>	95	195-197 <sup>h</sup>	48.8	7.82	20.3	48.5	8.02	20.1

[illegible]

<sup>a</sup> Z removal by 12 N HCl. <sup>b</sup> Liquefies at 120°, solidifies at 170°, decomposes at 195°. <sup>c</sup> Recrystallization from methanol-ether. <sup>d</sup> HBr in dioxane to remove Z. <sup>e</sup> Recrystallization from water-ethanol. <sup>f</sup> Recrystallization from ethanol-ether. <sup>g</sup> Liquefies at 125°, solidifies at 195°, melts at 265–270°. <sup>h</sup> Recrystallization from acetic acid-ethanol. <sup>i</sup> Over-all yield. <sup>k</sup> Butanol:water:acetic acid, 8:10:1 descending. Detection of spots by ninhydrin and NaOCl-starch-KI, applied consecutively to the same chromatogram. The NaOCl decolorizes the ninhydrin spots. <sup>l</sup> pH 8.6, diethyl barbiturate buffer ionic strength 0.075, 2.5 ma., 18 hr. <sup>m</sup> Calcd.: Cl, 12.1. Found: Cl, 11.97. <sup>n</sup> Calcd.: Cl, 12.83. Found: Cl, 13.00. Both compounds had the same melting behavior. <sup>o</sup> No ninhydrin spot on paper. Diethyl barbiturate buffer interfered with NaOCl-starch-KI test.<sup>14</sup> <sup>p</sup> Assayed against an L-alanine standard with *P. cerevisiae* (ATCC#9081). <sup>q</sup> Calcd.: Cl, 13.11. Found: Cl, 12.74. <sup>r</sup> In 1 N HCl, C = 2; others in water, C = 2. <sup>s</sup> Colorimetric, Sakaguchi. <sup>t</sup> Bacterimetric, *L. casei*. <sup>u</sup> Absence of demethylation was proven by both single spot chromatography and by the inability of homocysteine to promote growth of *L. fermenti* on a methionine-free medium. <sup>v</sup> In 1 N HCl, C = 1. <sup>w</sup> L-Valine. <sup>z</sup> In glacial acetic acid, C = 2.

dried over sodium sulfate. The solvent was reduced to small volume *in vacuo*, and petroleum ether (30–60°) was added to incipient cloudiness. After standing cold, the peptide ester precipitated; yield, 74%; m.p. after recrystallization, 137–138°.

**g. Compound (XII).**—To 18.2 mM. of (IV) in methanol was added 10 ml. of 2 *N* methanolic sodium hydroxide and the mixture was allowed to stand for 16 hr. Addition of water precipitated unsaponified peptide ester, which was filtered off. The filtrate was freed of methanol *in vacuo*. The solution was extracted with methylene chloride, treated with charcoal, filtered, then acidified with hydrochloric acid to congo red. The oily layer was extracted into methylene chloride, dried over sodium sulfate, and precipitated with petroleum ether. The material, m.p. 110–118°, was recrystallized once from chloroform–petroleum ether and once from ethanol–water: recrystallized yield, 4.4 g., (66%), m.p. 140–141°; unreacted ester (1.6 g.) was recovered.

**h. Compound (XVII).**—A solution of 6.3 g. of (XII) in methanol plus the equivalent of HCl was hydrogenated, employing 2.1 g. of palladium black as catalyst. After CO<sub>2</sub> evolution stopped, completion was checked with a small amount of fresh catalyst. The catalyst was removed by filtration, and the filtrate taken to dryness with repeated flushing with ethyl ether. The peptide was dissolved in water and shaken with Amberlite IR-45 (OH form) to remove chloride. The volume was reduced and the peptide precipitated with ethanol. After recrystallization, 2.5 g. of (XVII) was obtained, m.p. 280–282°.

**i. Compounds (VII) and (XX).**—To a solution of 5.3 g. of benzyl ester from (d), 2.2 ml. of triethylamine, and 3.0 g. of DCC in 40 ml. of chloroform was added 6.3 g. of dicarbobenzoxyhistidine alcoholate in 60 ml. of chloroform. All solutions were pre-cooled to 10°. The reaction proceeded one-half hr. at this temperature and for 16 hr. at 20°. The dicyclohexylurea was removed by filtration and 1 ml. of acetic acid was added to decompose excess DCC. After filtration, the filtrate was washed with 0.2 *N* hydrochloric acid, 1% sodium bicarbonate solution, and water and dried over sodium sulfate. The solvent was removed *in vacuo*. The residue was an oil which gave one spot by paper chromatography. The oil was suspended in 30 ml. of concentrated hydrochloric acid and held at 37° for 80 min. The HCl was removed *in vacuo* and the compound dissolved in methanol and hydrogenated over palladium black for 1.5 hr. After filtration, the filtrate was taken to dryness, dissolved in ethanol and 2 equivalents of triethylamine added. After cooling overnight, the peptide precipitated. It was recrystallized from water–ethanol.

**j. Compound (XXIII), valyl-1-aminocyclopentanecarboxylic acid anhydride (3-isopropyl-6-(1,4-tetramethylene)diketopiperazine).**—The *Z* valyl peptide (XII) was decarbobenzoxyated by hydrogen bromide in dioxane, the dioxane was removed *in vacuo*, and the solids were washed repeatedly with ethyl ether. The gum was dissolved in ethanol, and an equivalent of tributylamine was added. No precipitation occurred initially, but on standing two to three weeks at 4°, crystallization took place. The product did not have a free amino group by ninhydrin test or by perchloric acid titration. It was washed with hot water until the nitrogen analysis was constant. It was insoluble in 6 *N* hydrochloric acid and 6 *N* sodium hydroxide, and slightly soluble in ethanol.

**Acknowledgments.**—We wish to thank S. Makineni, Mrs. V. Gold, and T. Giudici for able assistance. Microanalyses for carbon and hydrogen were by Drs. Weiler and Strauss of Oxford, England. This work was supported by grants CY-3609 and CY-4318 from the National Cancer Institute.

---

## Pyridoxal Hydrazine Derivatives for Cancer Chemotherapy

RICHARD H. WILEY AND GETHER IRICK

*Department of Chemistry, College of Arts and Sciences, University of Louisville, Louisville 8, Kentucky*

*Received June 24, 1961*

A series of pyridoxal and pyridoxal phosphate derivatives have been prepared for cancer chemotherapy evaluation. The dimethylhydrazone and methylhydrazone are relatively toxic and the latter has a borderline tumor growth retardation activity against Sarcoma 180. Catalytic reduction cleaves the hydrazone linkage to form pyridoxamine.

Interest in the tumor growth retardation characteristics of a series of methyl and dimethylhydrazones<sup>1,2</sup> has prompted the study of similar derivatives of pyridoxal. These derivatives were prepared from pyridoxal hydrochloride as such or in solution obtained on manganese dioxide<sup>3</sup> oxidation of pyridoxine. The pyridoxal methylhydrazone could not be isolated from the crude pyridoxine oxidation mixture and had to be made from the isolated pyridoxal hydrochloride. Pyridoxal phosphate was obtained by manganese dioxide oxidation of pyridoxamine phosphate<sup>4</sup> and was converted to derivatives without isolation. Catalytic reduction of pyridoxal dimethylhydrazone in methanol, ethanol, dioxane, or acetic acid over platinum oxide or in ethanol with added hydrochloric acid gives pyridoxamine.

(1) R. H. Wiley and G. Irick, *J. Org. Chem.*, **24**, 1925 (1959).

(2) R. H. Wiley, S. C. Slaymaker, and H. Kraus, *ibid.* **22**, 204 (1957).

(3) E. E. Snell, *J. Am. Chem. Soc.*, **66**, 2082 (1944).

(4) E. A. Peterson and H. A. Sober, *ibid.*, **76**, 169 (1954).