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Antineoplastic Agents. 2. Structure–Activity Studies on N-Protected Vinyl, 1,2-Dibromoethyl, and Cyanomethyl Esters of Several Amino Acids

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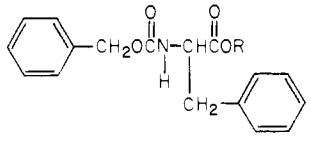
Previously reported work on N-protected activated esters of phenylalanine has been extended to include N-protected vinyl, dibromoethyl, and cyanomethyl esters of several other amino acids. These compounds have been synthesized and evaluated in Ehrlich ascites carcinoma, Walker 256 carcinosarcoma, and P388 lymphocytic leukemia tests. Among compounds tested were derivatives of tyrosine, tryptophan, glycine, leucine, proline, aspartic acid, glutamic acid, 4-aminobutyric acid, and 6-aminocaproic acid. Compounds of greatest potential interest from this study are *N*-carbobenzoxyglycine 1,2-dibromoethyl ester and *N*-carbobenzoxy-L-leucine 1,2-dibromoethyl ester. Both compounds were highly active in Ehrlich ascites test systems (33 mg/kg/day). The glycine derivative was also active in the Walker 256 test (2.5 mg/kg/day). Values for LD_{50}'s in mice were 148 mg/kg (0.37 mmol/kg) and 225 mg/kg (0.50 mmol/kg) for glycine and leucine derivatives, respectively; therefore, these compounds do not appear to be toxic at effective dose levels.

Our interest in certain N-protected amino acid active esters as potential antineoplastic agents has been discussed previously.[1] In the course of structure–activity studies described there, several active compounds were discovered, among them the vinyl and 1,2-dibromoethyl esters of *N*-carbobenzoxy-L-phenylalanine (Ia and Ib, respectively).



Ia, R = $-CH=CH_2$
b, R = $-CHBrCH_2Br$

These compounds were found to be active vs. Ehrlich ascites carcinoma (33 mg/kg/day) and Walker 256 carcinosarcoma (2.5 mg/kg/day). Of particular interest was the fact that these dose levels are considerably below toxic levels as indicated by acute toxicity studies in mice. The LD_{50} values for Ia and Ib were >2000 mg/kg (>6.15 mmol/kg) and 74 mg/kg (0.15 mmol/kg), respectively.

These previous studies on phenylalanine derivatives have now been extended to include vinyl, 1,2-dibromomethyl, and cyanomethyl esters of a number of other amino acids.

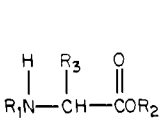
Chemistry. Vinyl and 1,2-dibromoethyl esters of N-protected derivatives of tyrosine, tryptophan, glycine, leucine, proline, aspartic acid, glutamic acid, 4-aminobutyric acid, and 6-aminocaproic acid were prepared from the corresponding acids by methods previously described.[1] Yields and pertinent physical information for new compounds are included in Table I. Cyanomethyl and allyl esters were prepared in a straightforward manner from the corresponding acids according to literature methods involving the reaction of the acid with chloroacetonitrile or allyl bromide and the base, triethylamine.[1,2] Propargyl esters were prepared in a similar manner utilizing propargyl bromide.[1,3] New compounds are listed in Table I. All compounds appeared to be stable for a period of at least several months when stored in a dry atmosphere, as judged by TLC, melting point, and optical data.

Biological Testing. Compounds were evaluated in three in vivo antitumor protocols: Ehrlich ascites carcinoma, Walker 256 ascites carcinosarcoma, and lymphocytic leukemia P388 (see Tables II–IV). In addition, acute toxicity testing was completed in mice in order to determine LD_{50} values for all compounds (Table II). The details of all of these test systems have been described previously.[1]

Results and Discussion

For purposes of comparison with new compounds, results of Ehrlich ascites testing on several previously reported phenylalanine derivatives have been included in Table II (**31–37**). Initially, derivatives of two other aromatic L-amino acids, tyrosine and tryptophan, were prepared and tested. *N*-Carbobenzoxy (Cbz) vinyl and cyanomethyl esters of both of these amino acids were

Table I. Physical Properties of N-Protected L-Amino Acid Vinyl Esters, Dibromoethyl Esters, Cyanomethyl Esters, and Propargyl Esters



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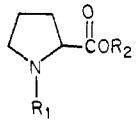

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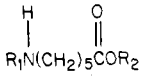
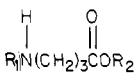


23-25



26-30

No.	R_1	R_2	R_3	Mp, °C	Crystn solvent	Yield, %	$[\alpha]^{24}D$, deg	Formula	Analyses
				L-Tyrosine Derivatives					
1	Tos	-CH=CH$_2$	*p*-HO-C$_6$H$_4$-CH$_2$-	112	CHCl$_3$-ligroine	15			C, H, N, S
2	Tos	-CH$_2$CN	*p*-HO-C$_6$H$_4$-CH$_2$-	93-95	CHCl$_3$-ligroine	50	−15.5[b]	$C_{18}H_{18}N_2O_5S\cdot{}^1/_3H_2O$	C, H, N, S
3	Tos	-CH$_2$CN	*p*-(*p*-Tos)O-C$_6$H$_4$-CH$_2$-	127-128	CHCl$_3$-ligroine	15		$C_{25}H_{24}N_2O_2S_2$	C, H, N, S
4	Cbz[d]	-CH=CH$_2$	*p*-(*p*-Tos)O-C$_6$H$_4$-CH$_2$-	83	CHCl$_3$-ligroine	30	−26.9[c]	$C_{19}H_{19}NO_5$	C, H, N
5	Cbz	-CH$_2$CN	*p*-(*p*-Tos)O-C$_6$H$_4$-CH$_2$-	84	CHCl$_3$-ligroine	30	+3.9[c]	$C_{19}H_{18}N_2O_5$	C, H, N
				L-Tryptophan Derivatives					
6	Cbz	-CH=CH$_2$	3-Indolylmethyl	*a*	Oil	52		$C_{21}H_{20}N_2O_4\cdot 0.5H_2O$	C, H, N
7	Cbz	-CH$_2$CN	3-Indolylmethyl	*a*	Oil	50		$C_{21}H_{19}N_3O_4\cdot H_2O$	C, H, N
				Glycine Derivatives					
8	Cbz	-CH=CH$_2$	H	*a*	Oil	45		$C_{12}H_{13}NO_4$	C, H, N
9	Cbz	-CHBrCH$_2$Br	H	*a*	Oil	75		$C_{12}H_{13}Br_2NO_4$	C, H, N, Br
10	Cbz	-CH$_2$C≡CH	H	54-55	CHCl$_3$-ligroine	65		$C_{13}H_{13}NO_4$	C, H, N
11	Cbz	-CH$_2$CN	H	69-70 (lit. 70)[e]		70			
				L-Leucine Derivatives					
12	Cbz	-CH=CH$_2$	-CH(CH$_3$)$_2$	*a*	Oil	70	+10.0[c]	$C_{16}H_{21}NO_4$	C, H, N
13	Cbz	-CHBrCH$_2$Br	-CH(CH$_3$)$_2$	61	EtOAc-ligroine	70	−128.0[c]	$C_{16}H_{21}Br_2NO_4$	C, H, N, Br
14	Cbz	-CH$_2$CN		83-84 (lit. 84-85)[e]					
15	Cbz	-CH$_2$C≡CH	-CH(CH$_3$)$_2$	*a*	Oil	41	−15.8[c]	$C_{17}H_{21}NO_4$	C, H, N
				L-Glutamic Acid Derivatives					
16	Cbz	-CH=CH$_2$	-CH$_2$C(=O)OCH=CH$_2$	46	CHCl$_3$-ligroine	75	+6.1[c]	$C_{17}H_{19}NO_6$	C, H, N
17	Cbz	-CH$_2$CN	-CH$_2$C(=O)OCH$_2$CN	*a*		30		$C_{17}H_{17}N_3O_6\cdot H_2O$	C, H, N
18	Cbz	-CH$_2$CN	-CH$_2$C(=O)NH$_2$	130-132 (135)[e]		80			
				L-Aspartic Acid Derivatives					
19	Cbz	-CH$_2$C≡CH	-CH$_2$C(=O)OCH$_2$C≡CH	*a*	Oil	25		$C_{18}H_{17}NO_6$	C, H, N
20	Cbz	-CH$_2$CN	-CH$_2$C(=O)OCH$_2$CN	*a*	Oil	80		$C_{16}H_{15}N_3O_6\cdot H_2O$	C, H, N
				L-Proline Derivatives					
21	Cbz	-CH=CH$_2$		*a*	Oil	60-65	−54.9[c]	$C_{15}H_{17}NO_4$	C, H, N
22	Cbz	-CHBrCH$_2$Br		*a*	Oil	70	−28.6[c]	$C_{15}H_{17}Br_2NO_4$	C, H, N, Br
				6-Aminocaproic Acid Derivatives					
23	Cbz	-CH=CH$_2$		*a*		70		$C_{16}H_{21}NO_4\cdot 1.5H_2O$	C, H, N
24	Cbz	-CHBrCH$_2$Br		*a*		60-65		$C_{16}H_{21}Br_2NO_2$	C, H, N, Br
25	Cbz	-CH$_2$CN		*a*	Oil	70-75		$C_{16}H_{20}N_2O_4$	C, H, N
				4-Aminobutyric Acid Derivatives					
26	Cbz	-OH		65-65.5		92		$C_{12}H_{15}NO_4$	C, H, N
27	Cbz	-CH=CH$_2$		*a*		85		$C_{14}H_{17}NO_4$	C, H, N
28	Cbz	-CHBrCH$_2$Br		*a*		72		$C_{14}H_{17}Br_2NO_4$	C, H, N, Br
29	Cbz	-CH$_2$C≡CH		*a*		70		$C_{15}H_{17}NO_4$	C, H, N
30	Cbz	-CH$_2$CN		*a*		87		$C_{14}H_{16}N_2O_4$	C, H, N

[a] Noncrystalline. [b] $[\alpha]^{30}D$. [c] $[\alpha]^{25}D$. [d] Cbz = carbobenzoxy. [e] See ref 14.

considerably less active than corresponding analogues of L-phenylalanine (**4–7**). Corresponding *N*-tosyl derivatives of tyrosine (**1–3**) were also of lower activity. After these initial observations, *N*-Cbz derivatives of several other amino acids, starting with the simplest, glycine, were converted to vinyl, 1,2-dibromoethyl, cyanomethyl, and propargyl esters in turn. Evaluation of each (compounds **8–30**) in the Ehrlich test resulted in certain observations. These conclusions are tentative, however, because of the variable nature of the test system involved. In general, the 1,2-dibromoethyl esters as a group possessed the highest antitumor activity, as reflected by results with such derivatives of *N*-carbobenzoxyglycine (**9**), *N*-carbobenzoxy-L-leucine (**13**), *N*-carbobenzoxy-L-proline (**22**), and

Table II. Effect of N-Protected Amino Acids and Their Activated Esters on Ehrlich Ascites Tumor Growth in Mice. Acute Toxicity Evaluation (LD_{50}) in Mice

No.	Compd[a]	N[c]	Survival at 7th day	Ascrit (packed cell volume)	Ascites vol, mL	% inhibn	Acute toxicity evaln, LD_{50}, mmol/kg
	0.05% Tween 80	78	77/78	32.75 ± 7.87[b]	4.1 ± 1.24[b]		
Phenylalanine reference compounds[g]							
31	*N*-Tos-L-Phe-OCH=CH_2	6	5/6	19.2	3.8	56.8	
32	*N*-Benzoyl-L-Phe-OCH=CH_2	6	6/6	9.8	2.1	56.8	
33	*N*-Cbz-L-Phe-OCH=CH_2	6	6/6	0.4	0.1	99.9	>6.15
34	*N*-Cbz-L-Phe-OCHBrCH_2Br	6	6/6	1.4	0.0	100.0	0.15
35	*N*-Tos-L-Phe-OCH_2CN	6	4/6	3.4	0.4	97.0	0.73
36	*N*-Benzoyl-L-Phe-OCH_2CN	6	5/6	13.2	1.8	47.0	0.36
37	*N*-Cbz-L-Phe-OCH_2CN	6	6/6	0.1	0.2	99.9	0.44
New compounds							
1	*N*-Tos-L-Tyr-OCH=CH_2	6	6/6	17.5	1.9	75.0	
2	*N*-Tos-L-Tyr-OCH_2CN	6	5/6	15.8	3.8	56.0	0.22
3	*N,O*-(Tos)$_2$-L-Tyr-OCH_2CN	6	6/6	6.5	0.8	53.0	
4	*N*-Cbz-L-Tyr-OCH=CH_2	6	6/6	29.7	3.9	16.0	
5	*N*-Cbz-L-Tyr-OCH_2CN	6	6/6	31.1	4.5	0.0	
38	*N*-Cbz-L-Try-OH	6	6/6	41.1	1.4	55.0	
6	*N*-Cbz-L-Try-OCH=CH_2	6	6/6	14.3	1.3	87.0	
7	*N*-Cbz-L-Try-OCH_2CN	6	6/6	19.7	4.3	38.0	
39	*N*-Cbz-Gly-OH	7	5/7	22.0	0.2	97.0	
8	*N*-Cbz-Gly-OCH=CH_2	6	6/6	34.0	1.5	63.0	2.13
9	*N*-Cbz-Gly-OCHBrCH_2Br	6	6/6	0.0	0.0	100.0	0.37
10	*N*-Cbz-Gly-OCH_2C≡CH	6	6/6	27.0	5.2	0.0	
11	*N*-Cbz-Gly-OCH_2CN	6	6/6	44.7	2.2	29.0	
12	*N*-Cbz-L-Leu-OCH=CH_2	6	6/6	38.0	0.9	48.0	
13	*N*-Cbz-L-Leu-OCHBrCH_2Br	6	6/6	14.0	0.002	99.0	0.50
14	*N*-Cbz-L-Leu-OCH_2CN	6	6/6	38.0	2.7	27.0	
15	*N*-Cbz-L-Leu-OCH_2C≡CH	6	6/6	23.0	0.5	85.0	1.65
40	*N*-Cbz-L-Glu-OH	6	6/6	28.0	0.7	81.0	1.78
16	*N*-Cbz-L-glutamyl divinyl ester	6	6/6	37.0	0.9	69.0	1.50
17	*N*-Cbz-L-Gln-OCH_2CN	6	5/6	40.0	2.0	51.0	
18	*N*-Cbz-L-glutamyl dicyanomethyl ester	6	6/6	32.0	3.2	35.0	
41	*N*-Cbz-L-Asp-OH	6	6/6	21.0	3.7	31.0	
19	*N*-Cbz-L-Asp dicyanomethyl ester	6	5/6	33.0	4.7	7.0	
20	*N*-Cbz-L-Asp dipropargyl ester	6	6/6	21.0	3.7	31.0	
42	*N*-Cbz-L-Pro-OH	6	6/6	32.0	1.9	53.0	
21	*N*-Cbz-L-Pro-OCH=CH_2	6	5/6	35.0	1.0	73.0	1.82
22	*N*-Cbz-L-Pro-CHBrCH_2Br	6	6/6	10.0	2.1	84.0	0.19
43	6-(*N*-Cbz)aminocaproic acid	6	6/6	33.0	1.0	63.0	
23	6-(*N*-Cbz)aminocaproic acid vinyl ester	6	6/6	36.0	0.6	75.0	
24	6-(*N*-Cbz)aminocaproic acid 1,2-dibromoethyl ester	6	5/6	30.0	1.7	50.0	
25	6-(*N*-Cbz)aminocaproic acid cyanomethyl ester	6	6/6	41.0	1.0	54.0	
26	4-(*N*-Cbz)aminobutyric acid	6	6/6	32.3	0.8	71.0	
27	4-(*N*-Cbz)aminobutyric acid vinyl ester	6	6/6	39.5	3.2	6.0	
28	4-(*N*-Cbz)aminobutyric acid 1,2-dibromoethyl ester	6	4/6	2.0	0.6	98.7	
29	4-(*N*-Cbz)aminobutyric acid propargyl ester	6	6/6	34.5	2.3	20.0	
30	4-(*N*-Cbz)aminobutyric acid cyanomethyl ester	6	4/6	48.8	0.6	71.0	
	Acetaldehyde	6	5/6	56.0	2.0	43.0	
	Benzaldehyde	6	6/6	49.0	0.8	69.0	
	TPCK[d]	6	5/6	0.05	0.01	100.0	
	Melphalan[e]	6	6/6	3	0.1	99	
	6-MP[d]	6	6/6	0.3	0.7	99.6	
	Uracil mustard						0.015[f]
	Chlorambucil						0.061

[a] See Table I for structural equivalent. [b] Mean and standard deviation on the control value for volume was 4.12 ± 1.24 and ascrit (total packed cell volume) was 32.75 ± 7.87 at 7 days. [c] *N* is the number of animals per group. [d] Sigma Chemical Co. [e] Wellcome Research Laboratories, Research Triangle Park, N.C. [f] Merck Index, 8th ed. [g] See ref 1.

Table III. Effect of Antitumor Agents on Walker 256 Ascites Tumor Growth

No.	Compd	N[c]	Days survived	T/C[a]
	0.05% Tween 80	6	7.5 ± 0.5	
	Reference compounds			
	Melphalan[b]	5	23.0	305
28	*N*-Cbz-L-Phe-OCH=CH_2	6	18.1	226
29	*N*-Cbz-L-Phe-OCHBrCH_2Br	6	23.0	305
	New compounds			
6	*N*-Cbz-L-Try-OCH=CH_2	6	8.0	106
9	*N*-Cbz-Gly-OCHBrCH_2Br	6	10.1	135
13	*N*-Cbz-L-Leu-OCHBrCH_2Br	6	8.5	113

[a] T/C > 125 value denotes minimum significant activity. [b] Wellcome Research Laboratories, Research Triangle Park, N.C. [c] *N* is the number of animals per group.

Table IV. Effect of Antitumor Agents on P388 Lymphocytic Leukemia Growth

No.	Compd	N[b]	Days survived	T/C[a]
	0.05% Tween 80	6	8.1	100
	Reference compounds			
	5-FU[c]	6	11.0	136
	6-MP[c]	6	12.0	148
28	*N*-Cbz-L-Phe-OCH=CH_2	6	8.1	100
29	*N*-Cbz-L-Phe-OCHBrCH_2Br	6	6.7	83
	New compounds			
9	*N*-Cbz-Gly-OCHBrCH_2Br	6	2.2	28
15	*N*-Cbz-L-Leu-OCH_2C≡CH	6	9.1	112
13	*N*-Cbz-L-Leu-OCHBrCH_2Br	6	9.1	112

[a] T/C > 125 value denotes minimum significant activity. [b] *N* is the number of animals per group. [c] Sigma Chemical Co.

N-carbobenzoxy-4-aminobutyric acid (**28**). The dibromoethyl ester of 6-aminocaproic acid (**24**) appeared to be an exception. *N*-Carbobenzoxy vinyl esters of the three natural amino acids (**8**, **12**, and **21**) as well as those of glutamic acid (**16**), 4-aminobutyric acid (**27**), and 6-aminocaproic acid (**23**) all appeared to be considerably less active than the brominated counterparts tested. As a group, cyanomethyl esters of *N*-Cbz derivatives of glycine (**11**), leucine (**14**), aspartic acid (**19**), glutamine (**17**), glutamic acid (**18**), 4-aminobutyric acid (**30**), and 6-aminocaproic acid (**25**) appeared to be still less potent. This order of activity might also have been observed with L-phenylalanine derivatives **33**, **34**, and **37**, all of which gave 100% inhibition at doses tested, if the dose had been lowered so that separation of activity might be observed. *N*-Carbobenzoxypropargyl esters (**10**, **15**, **20**, and **29**) elicited a variable response in the test. Of special interest is the observation that the free acid forms of *N*-Cbz derivatives alone appeared to exhibit some activity depending upon the particular derivative involved (**26** and **38–43**). This fact had also been observed previously with *N*-carbobenzoxyphenylalanine and may be related to the possible metabolic formation of benzaldehyde from such a compound. Benzaldehyde itself exhibits some activity in this test system (see Table II). Antitumor results of two compounds, one proteolytic inhibitor (TPCK) and two standard antineoplastic agents [6-mercaptopurine (6-MP) and melphalan], have been included in Table II for purposes of comparison.

Obvious omissions from the list of compounds prepared (Table I) are derivatives of basic amino acids, arginine and lysine, and the sulfur-containing amino acids, methionine and cysteine. As anticipated, synthetic and stability problems have been encountered in these cases because of the presence of two reactive groupings within the same molecule. Under the right conditions, however, such derivatives should be preparable as evidenced by reported success with compounds such as tosyllysyl chloromethyl ketone (TLCK) and *p*-nitrocarbobenzoxy-L-arginine chloromethyl ketone.[4,5] Work on such derivatives is continuing.

Compounds **6**, **9**, **13**, and **15** were selected and evaluated along with phenylalanine derivatives **33** and **34** in Walker 256 carcinosarcoma tests (Table III) and P388 lymphocytic leukemia tests (Table IV). Of the four new compounds chosen, only the *N*-carbobenzoxyglycine 1,2-dibromoethyl ester **9** exhibited significant activity and then only against Walker 256 carcinosarcoma.

Acute toxicity studies were carried out on a number of selected derivatives, particularly those which were most active in the Ehrlich procedure. All compounds were relatively nontoxic, with LD_{50} values considerably higher than effective dose figures.

The glycine derivative, *N*-carbobenzoxyglycine 1,2-dibromoethyl ester **9**, active in Ehrlich and Walker tests and with an LD_{50} of 148 mg/kg (0.37 mmol/kg), has been selected as the subject for further biological evaluation in tests thought relevant to its possible mechanism of action.

Experimental Section

Chemistry. Methods utilized in the synthesis of vinyl, 1,2-dibromoethyl, cyanomethyl, and propargyl esters of *N*-carbobenzoxyamino acids described here have been described in detail previously for phenylalanine derivatives.[1] Vinyl esters were purified by column chromatography on silica gel and 1,2-dibromoethyl, cyanomethyl, and propargyl esters by recrystallization from ethyl acetate or chloroform–ligroine. Amino acids were purchased from Eastman Organic Chemicals and converted to *N*-Cbz derivatives by well-known methods, and properties were compared with those reported in the literature for L-tyrosine,[6] L-tyrptophan,[7] glycine,[6] L-leucine,[8] L-proline,[9] L-aspartic acid,[6] L-glutamic acid,[6] L-glutamine,[10] and 6-aminocaproic acid.[11] The *N*-Cbz derivative of 4-aminobutyric acid was previously unreported and prepared according to literature procedures:[6] mp 90–92 °C (EtOAc). Anal. C, H, N. The following tosyl derivatives of L-tyrosine were prepared according to literature descriptions: *N*-tosyl-L-tyrosine[12] and *N*,*O*-ditosyl-L-tyrosine.[13]

Biological Testing. All methods utilized here have been described previously[1] or in the original reference cited above.

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Nitramino Acids. Synthesis and Biological Evaluation of 1-Nitroproline, 1-Nitropipecolic Acid, and *N*-Nitrosarcosine

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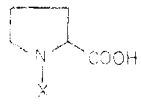
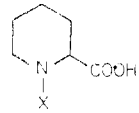
The *N*-nitro derivatives of secondary α-amino acids, viz., 1-nitroproline (**1a**) (L and D), 1-nitro-DL-pipecolic acid (**2a**), and *N*-nitrosarcosine (**3a**), were prepared by the oxidation of the corresponding nitrosamino acids with peroxytrifluoroacetic acid. These nitramino acids (**1a**–**3a**) were not active against *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, or *Mycobacterium smegmatis*, and **1a** and **2a** did not show mutagenic activity in a *Salmonella typhimurium* TA-100 system, with or without added rat liver 9000g supernatant fraction. The marginal mutagenicity of **3a** in this system suggests that additional work should be done to assess its carcinogenic–mutagenic potential.

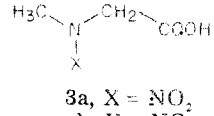
Although the *N*-nitroso derivatives of the naturally occurring cyclic or secondary α-amino acids such as proline and sarcosine, e.g., 1-nitrosoproline (**1b**) and *N*-nitrososarcosine (**3b**), have been known for some time,[1] the corresponding *N*-nitro-*sec*-α-amino acids, e.g., **1a** and **3a**, have not been described. The nitramino acids (**1a**–**3a**) are chemically related to the nitrosamino acids (**1b**–**3b**) and differ only in the oxidation state of the exocyclic nitrogen.



1a, X = NO_2
b, X = NO

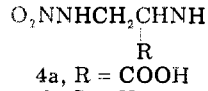


2a, X = NO_2
b, X = NO



3a, X = NO_2
b, X = NO

Contemporary interest in the nitrosamino acids derives from the possibility of their formation in processed meat and other foodstuff that have been preserved with nitrite,[2] their decarboxylation to *N*-nitroso-*sec*-amines under normal cooking conditions,[3] and the implications of carcinogenic hazards thereby.[4] Nitrosamines such as dimethylnitrosamine and 1-nitrosopyrrolidine are highly carcinogenic[5] and have also been shown to be mutagenic.[6] Biochemical rationale for the formation in food of nitramino acids or their decarboxylation products, the nitramines themselves, has not been advanced. However, the natural occurrence of β-nitraminoalanine (**4a**), the *N*-nitro derivative of β-aminoalanine, and its decarboxylated product, *N*-nitroethylenediamine (**4b**), in mushrooms (*Agaricus silvaticus*) appears to be well documented[7] and the production of *N*-nitroglycine (**5**) by *Streptomyces norsei* has also been described.[8]



4a, R = COOH
b, R = H

5

Whether these nitramino acids can be reduced in vivo to the corresponding nitrosamino acids, e.g., **1a** to **1b**, by mammalian enzymes is not known. It appears possible that bacterial systems might effect such reductions on account of the ubiquitous presence of nitro,[9] nitrate, and nitrite reductases[10] in bacteria. In vivo decarboxylation of the nitramino acids to secondary nitramines should also be considered. The recent report that dimethylnitramine is carcinogenic to rodents[11] suggests that such decarboxylations may yield highly toxic products. Moreover, many *C*-nitro heterocyclic compounds, while possessing potent antibacterial, antiprotozoal, and anthelminthic properties,[12] also elicit disturbing degrees of mutagenic activities which are correlative with their carcinogenicities.[13] These considerations prompted us to examine the titled nitramino acids for (a) growth inhibition against a cross section of clinically endemic bacteria and fungi in culture and (b) mutagenicity in the *Salmonella typhimurium* system of Ames et al.[14] The preparation of the nitramino acids, 1-nitro-L- and -D-proline (L- and D-**1a**), 1-nitro-DL-pipecolic acid (**2a**), and *N*-nitrosarcosine (**3a**), is presented herewith, together with an evaluation of their biological properties.

Chemistry. All attempts to N-nitrate proline directly with nitronium tetrafluoroborate[15] in the presence of pyridine in the manner used to N-nitrosate this cyclic amino acid with nitrosyl tetrafluoroborate[16] were unsuccessful, possibly due to competitive ring-opening reactions undergone by pyridine with this reagent.[15] However, use of other amines such as tri-*n*-butylamine or dimethylaniline in highly polar solvents such as nitromethane or sulfolane did not give **1** nor did the use of the more soluble nitronium hexafluorophosphate (NO_2PF_6).[17] Direct nitration of L-proline with HNO_3–H_2SO_4 or HNO_3–Ac_2O was also unsuccessful.

The nitramino acids (**1a**–**3a**) were successfully prepared by oxidation of the corresponding nitrosamino acids (**1b**–**3b**, respectively)[16] with peroxytrifluoroacetic acid. We were unable to prepare 1-nitro-L-azetidine-2-carboxylic acid by this method due to the instability of the 1-nitroso-L-azetidine-2-carboxylic acid under these strongly acid conditions. The Emmons procedure[18] for the oxidation of nitrosamines to nitramines required some modification (procedure B) due to the water solubility of these nitramino acids. For the more lipophilic 1-nitropipecolic acid (**3a**), a standard workup procedure (procedure A) gave satisfactory yields (Table I). Use of less potent oxidizing agents such as *m*-chloroperoxybenzoic acid or peroxyacetic acid gave only low yields of the desired products.

The electron-ionization mass spectra (EI–MS) of these nitramino acids gave molecular ions ($M^{+\cdot}$) of only feeble and sometimes undiscernible intensities, but all gave characteristic (and therefore diagnostic) fragment ions at M – 45 $[M - CO_2H]^+$, M – 75 $[M - CO_2H - NO]^{+\cdot}$, and M – 91 $[M - CO_2H - NO_2]^{+\cdot}$ (Table I). These fragmentation paths were verified by the presence of appropriate metastable peaks. In addition, trace amounts of fragment ions