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Participation of Isolated Double Bonds in the Cleavage of Peptides with N-Bromosuccinimide

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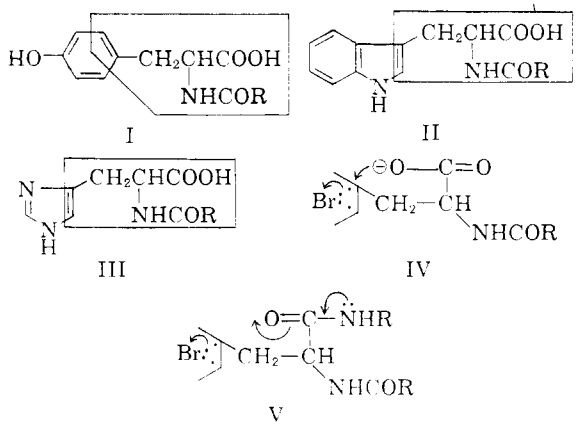
N-Acylated amides and peptides derived from DL-2-amino-4-pentenoic acid ("allylglycine"), DL-2-amino-4-methyl-4-pentenoic acid ("β-methallylglycine"), DL-baikiain and 3,4-dehydro-DL-proline have been synthesized and studied with regard to participation of the double bond in displacement reactions with N-bromosuccinimide. Three different reaction types have been observed: (i) the pentenoic acid amides and peptides released ammonia or glycine in high yield *via* isolable dibromoiminolactone and C-bromolactone intermediates, respectively; (ii) the amide of baikiain produced the N,C-dibromoiminolactone XVI, which was only slowly hydrolyzed to yield ammonia. No reaction occurred between NBS and the baikiain peptide. (iii) No participation of the β,γ-double bond in 3,4-dehydroproline was observed. However, ammonia was released at *p*H >6 from acyldehydroprolinamides probably *via* an unusually easy Hofmann rearrangement. The remarkable dicarbinolamide XXIII has been isolated from the reaction of N-carbobenzyloxydehydroprolinamide with NBS. The *p*H profiles of these cleavage reactions have been determined and are of diagnostic value in recognizing the nature and location of the double bond in peptides and amides derived from unsaturated amino acids.

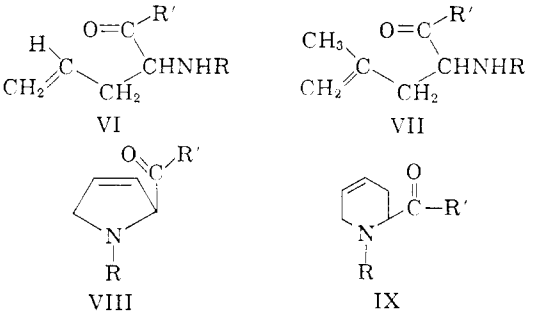
The element of 2-amino-4-pentenoic acid ("allylglycine") in N-acylated tyrosine (I),[2] tryptophan (II)[3] and histidine (III),[4] though in each case part of an aromatic phenol, indole or imidazole system, respectively, is capable of 1,5-interactions between the carboxyl group and the potential double bond. Among the electrophilic reagents that evoke such intramolecular participation reactions are bromine, N-bromosuccinimide (NBS) and N-bromoacetamide. In the case of II and



III highly labile bromonium intermediates[5] may be visualized as inviting participation of carboxylate or carboxamido groups by the general mechanism expressed in IV and V.

As an extension of these studies the participation of *isolated* double bonds in certain cyclic and acyclic amides and peptides has now been investigated in some detail, and the results are reported in this paper.

N-Acylated amides and glycine peptides of four compounds were studied: 2-amino-4-pentenoic acid ("allylglycine", VI, R = H, R′ = OH),[6,7] 2-amino-4-methyl-4-pentenoic acid ("β-methallylglycine," VII, R = H, R′ = OH),[6,7] 3,4-dehydroproline (VIII, R = H, R′ = OH),[8] and baikiain (4,5-dehydropipecolic acid IX, R = H, R′ = OH).[9] All derivatives were made from the easily accessible



synthetic DL-amino acids: The acyl groups R were benzoyl (R = Bz), carbobenzyloxy (R = Cbz) and tosyl (R = Ts). The amide components R′ were ammonia (R′ = NH_2), glycine (R′ = $NHCH_2CO_2H$) or glycinamide (R′ = $NHCH_2CONH_2$).

The action of NBS on these compounds was first studied at spectroscopic concentration, and percentage cleavage was followed by ninhydrin analysis for liberated ammonia or glycine. Reactions were done in various buffer systems to determine the influence of *p*H. The concentration of NBS was varied to find the optimal conditions for cleavage at any one *p*H. All reactions were run at room temperature. When cleavage of a particular compound was demonstrated to occur, experiments on a preparative scale were made in order to isolate the products. Mixtures of acetonitrile and water were used as solvents to obtain homogeneous reaction media.

The results fall into three clearly distinguishable patterns:

(1) Visiting Scientist of the USPHS.

(2) G. L. Schmir, L. A. Cohen and B. Witkop, *J. Am. Chem. Soc.*, **81**, 2228 (1958); E. J. Corey and L. F. Haefele, *ibid.*, **81**, 2225 (1958); G. L. Schmir and L. A. Cohen, *ibid.*, **83**, 723 (1960).

(3) A. Patchornik, W. B. Lawson and B. Witkop, *ibid.*, **80**, 4747, 4748 (1958); A. Patchornik, W. B. Lawson, Erhard Gross and B. Witkop, *ibid.*, **82**, 5923 (1960).

(4) G. L. Schmir and L. A. Cohen, unpublished results; Sh. Shaltiel and A. Patchornik, *Bull. Res. Council Israel*, **10A**, No. 1 (1961).

(5) W. B. Lawson, A. Patchornik and B. Witkop, *J. Am. Chem. Soc.*, **82**, 5918 (1960).

(6) N. F. Albertson, *ibid.*, **68**, 450 (1946). We are greatly indebted to Dr. Albertson, Sterling Research Laboratories, for the donation of samples.

(7) H. L. Goering, S. J. Cristol and K. Dittmer, *ibid.*, **70**, 3310 (1948).

(8) A. Robertson and B. Witkop, *ibid.*, **82**, 5008 (1960), and preceding paper.

(9) A. W. Burgstahler and C. E. Aiman, *J. Org. Chem.*, **25**, 489 (1960). We are greatly indebted to Dr. Burgstahler for a liberal sample of DL-baikiain.

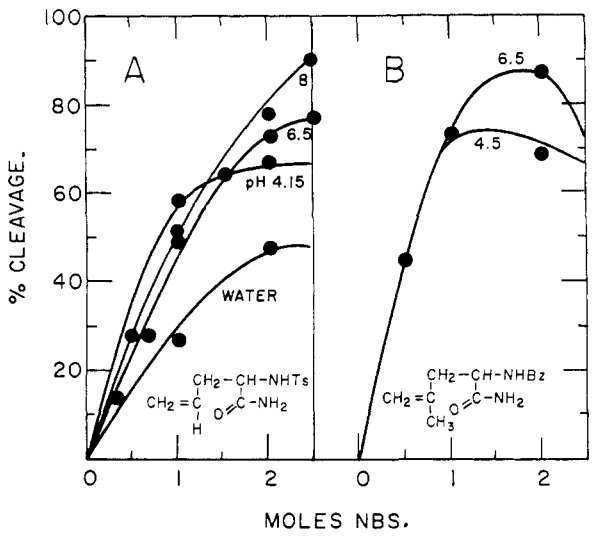


Fig. 1.—Release of ammonia from the amides of 2-N-tosylamido-4-pentenoic acid (A) and of 2-N-benzoylamido-4-methyl-4-pentenoic acid (B) by NBS as a function of *p*H.

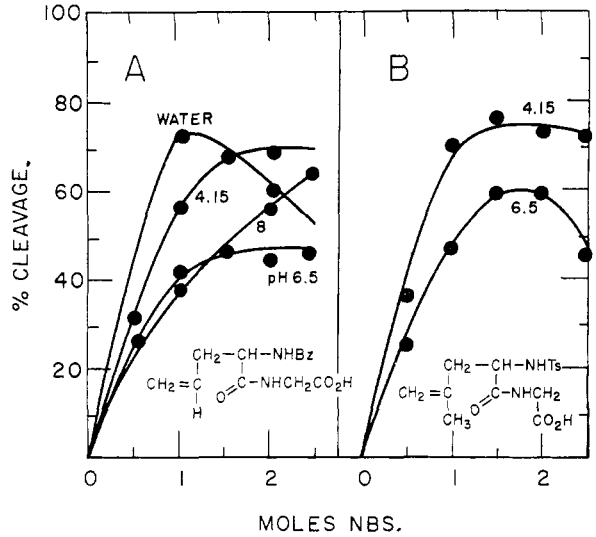
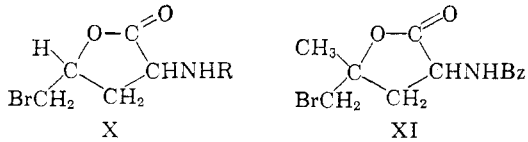


Fig. 2.—Release of glycine from 2-N-benzoylamido-4-pentenoylglycine (A) and 2-N-tosylamido-4-methyl-4-pentenoylglycine (B) as a function of the addition of NBS and of *p*H.

A. Acyclic γ,δ-Unsaturated Acid Derivatives.—All of the amide and peptide derivatives of VI and VII readily undergo 1,5-interactions and typical cleavage patterns are shown in Figs. 1 and 2. Results for compounds not illustrated are tabulated in the Experimental section. Ammonia or glycine was generally liberated in yields of 60–80% with 1–2 equivalents of NBS. The nature of the protecting N-acyl group is not significant, and the *p*H exerts little influence on the course of the reaction. When the peptide component was glycinamide instead of glycine, only minor differences in the cleavage pattern with respect to rates and *p*H were observed (Fig. 3). Calculations for Fig. 3 were based on the assumption that glycinamide was the cleavage product.

On a preparative scale, a clear difference emerged between the amides of VI and VII and their glycine peptides. The products in the case of the glycine peptides VI (R′ = $NHCH_2CO_2H$) and VII (R′ = $NHCH_2CO_2H$) are crystalline bromolactones X (R = Bz, R = Ts) and XI. The infrared absorption in X and XI shows the carbonyl peak typical



X XI

of γ-lactones. This 1,5-interaction and the formation of γ-lactones is analogous to the bromo- or iodolactonization of unsaturated acid derivatives.[2,3,10–12] An independent synthesis of X (R = Bz) was achieved by the action of NBS on the free acid VI(R = Bz, R′ = OH). The stereochemistry of these γ-lactones which contain two asymmetric centers will be discussed separately.[13]

(10) R. P. Linstead and H. N. Rydon, *J. Chem. Soc.*, 580(1933).
(11) E. E. van Tamelen and M. Shamma, *J. Am. Chem. Soc.*, **76**, 2315 (1954).
(12) P. N. Craig and I. H. Witt, *ibid.*, **72**, 4925 (1950).
(13) N. Izumiya and B. Witkop, to be published.

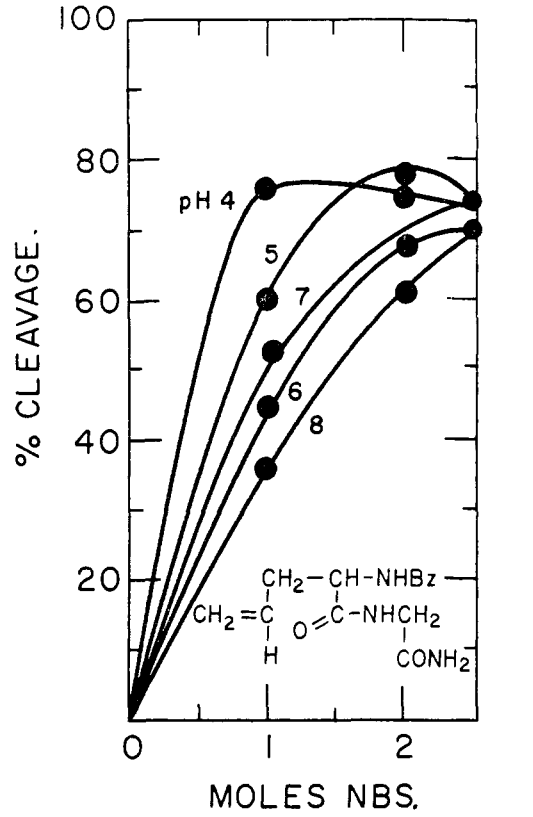


Fig. 3.—The release of glycinamide from 2-N-benzoylamido-4-pentenoylglycinamide as a function of NBS concentration and *p*H.

In contrast to the glycine peptides, the amides VI (R′ = NH_2) and VII (R′ = NH_2) yielded the crystalline N,C-dibromoiminolactones XII and XIII (R = Bz, R = Ts).

The azomethine double bond, characterized by infrared absorption near 6.0 μ, is quantitatively

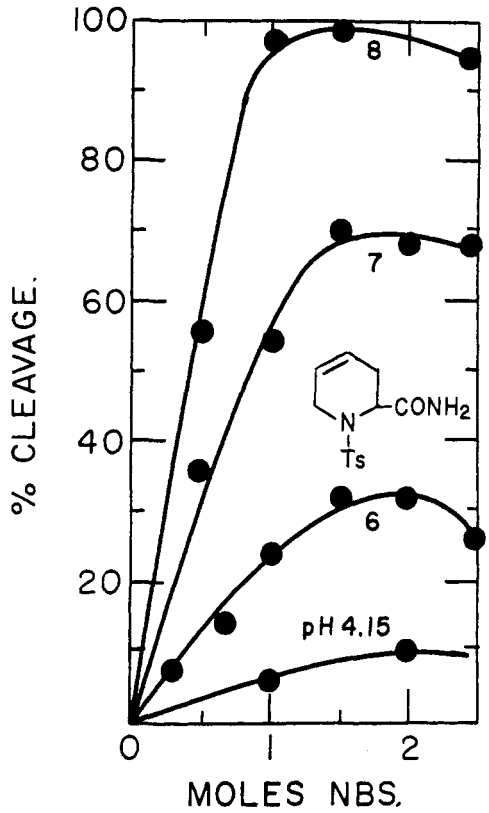
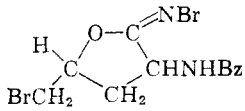
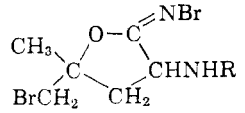


Fig. 4.—*p*H profile of the release of ammonia from N-tosyl-DL-baikiainamide with increasing amounts of NBS.

hydrolyzed under the conditions of ninhydrin assay. Thus the formation of ammonia depicted in Fig. 1 is a consequence of the analytical method, and not the direct result of the reaction with NBS. Compound XII was hydrolyzed quantitatively in boiling aqueous acetonitrile to the lactone X (R = Bz). The hydrolysis was followed by the shift in NH absorption from 3.07 to 2.91 μ; loss of C=N absorption was more difficult to observe due to the overlapping of the benzamido C=O band.



XII

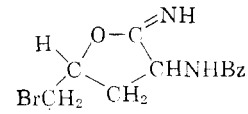


XIII

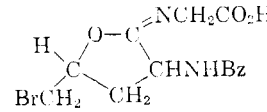
The water-soluble iminolactone hydrobromides which are formed from unsaturated acid amides and peptides by the action of bromine in chloroform[14] give a precipitate with silver nitrate. By contrast, the water-insoluble, low-melting N,C-dibromoiminolactones do not give a precipitate with silver nitrate; they contain one positive bromine atom according to iodometric analysis and, like NBS, evolve bromine during recrystallization.

The N-bromoiminolactones XII or XIII, which are formed from the iminolactones (*e.g.*, XIV) by the action of excess of NBS, precipitate from the aqueous solution. The situation is reversed in non-aqueous systems in which the iminolactone

(14) P. N. Craig, *J. Am. Chem. Soc.*, **74**, 129 (1952).



XIV

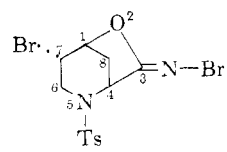


XV

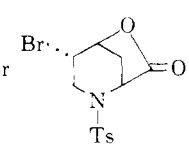
hydrobromides[14] are insoluble and precipitate. Glycine peptides lead to intermediate iminolactones (*e.g.*, XV) incapable of forming an N-bromo derivative. Instead, spontaneous hydrolysis to glycine and γ-lactone (*e.g.*, X, R = Bz) is observed.

B. Cyclic γ -Unsaturated Acid Derivatives.—The glycine peptide of N-tosylbaikiain (IX, R = Ts, R′ = $NHCH_2CO_2H$) showed no sign of cleavage on treatment with NBS over the *p*H range 4–8.

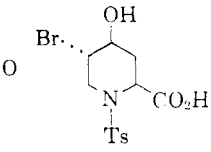
N-Tosylbaikiain amide (IX, R = Ts, R′ = NH_2) liberated ammonia, but the cleavage as estimated by ninhydrin assay was markedly dependent on *p*H, increasing from a maximum of 10% at *p*H 4 to almost 100% at *p*H 8 (Fig. 4). This result is in apparent conflict with experiments on a preparative scale, from which an 80% yield of product crystallized from the reaction mixture at *p*H 4. The compound is the N,C-dibromoiminolactone XVI; it has properties similar to the N-bromo compounds above, and it has no NH absorp-



XVI



XVII



XVIII

tion (which would be observed if it were the iminolactone hydrobromide). Integration of the n.m.r. spectrum shows a total of 14 protons contrasted with the 16 protons of the iminolactone hydrobromide.

The bicyclic lactone XVI has a much slower rate of hydrolysis than the monocyclic N-bromoiminolactones. The hydrolysis was followed by the loss of C=N absorption at 6.00 μ and the generation of γ-lactone C=O at 5.54 μ in the product XVII. Another carbonyl band also appears at 5.77 μ and this is due to the hydroxy acid XVIII. Both lactone and hydroxy acid were obtained pure and crystalline, but hydrolytic conditions have not been found which avoid the formation of the hydroxy acid.

Ninhydrin assay of the crystalline N-bromoiminolactone XVI gave only 15% of ammonia under the standard conditions (15 min. at 100°, *p*H 5 buffer), which are too mild for complete hydrolysis. When heating with the ninhydrin reagent was continued for an hour, the color yield rose only to 20%, a figure much lower than expected if the yield were only dependent on reaction time. Apparently ammonia is slowly destroyed by positive halogen present. This was proved by ninhydrin assay of XVI in the presence of formate, which showed a liberation of 22% ammonia after 15 min. and 75% after 1 hour. Formate reacts faster with positive halogen than does ammonia.

The low cleavage yield of the baikiain amide at acid *p*H (Fig. 4) is thus a consequence of the assay method and of the properties of the intermediate

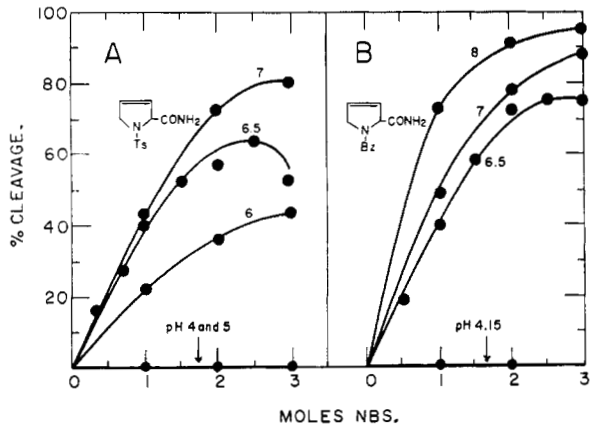


Fig. 5.—*p*H profiles of the release of ammonia from N-tosyl- (A) and from N-benzoyl-3,4-dehydro-DL-prolinamide (B) in relation to the amounts of NBS added.

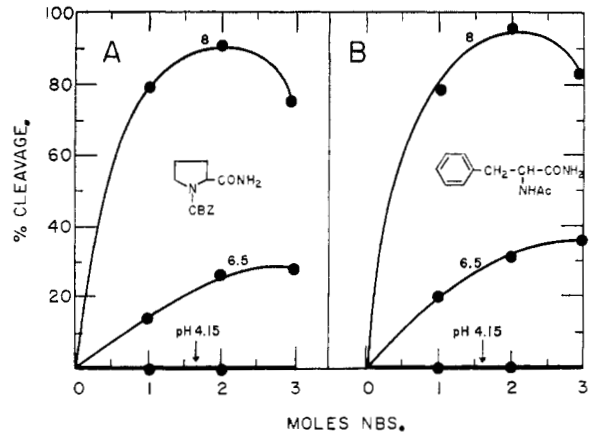
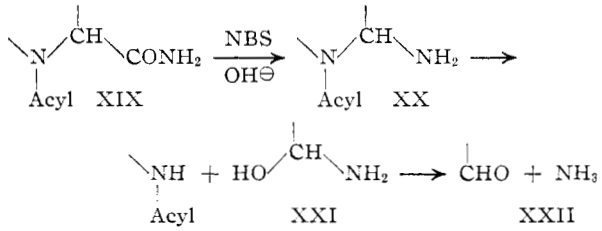


Fig. 6.—Release of ammonia from N-carbobenzyloxy-DL-prolinamide (A) and from N-acetyl-DL-phenylalaninamide (B) as a function of *p*H and of NBS concentration.

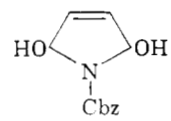
N,C-dibromoiminolactone which actually is formed in high yield. On the other hand, the marked liberation of ammonia at more alkaline *p*H is considered to be due to a Hofmann rearrangement XIX → XX under unusually mild conditions. In the breakdown of the unstable aldehyde intermediates XX and XXI ammonia is released. Evidence for this mechanism comes from the studies



on dehydroproline amides (Section C). The quantitative release of ammonia at *p*H 8 from the baikiain amide IX with *one equivalent* of NBS (Fig. 4) is also significant.

C. Cyclic β,γ-Unsaturated Acid Derivatives.—The glycine peptides of N-benzoyl-, N-tosyl- or N-carbobenzyloxydehydroproline did not cleave at all on treatment with NBS between *p*H 4–8. In this respect the N-unconjugated (allylic) double bond in a pyrroline ring behaves quite differently from the N-conjugated (vinylic) double bond in the indole ring which participates very actively in the NBS-cleavage of tryptophyl peptides.[3]

Ammonia was liberated from the three dehydroproline amides VIII (R′ = NH_2; R = Ts, Bz or Cbz), but the reaction was even more dependent on *p*H (Fig. 5) than the baikiain amide. On a preparative scale, no product could be isolated from reactions at acid *p*H, but at *p*H 7–8, a crystalline product was obtained in 60% yield from carbobenzyloxydehydroprolinamide. This compound has the remarkable dicarbinolamide formula XXIII and its preparation, structural determination and



XXIII

properties are described in detail in the succeeding paper. The loss of the amide function in the starting material and the liberation of ammonia may again be explained by the sequence XIX → XXII. A second mole of NBS is consumed in allylic bromination in position 5 and the bromine is then solvolyzed. No sign of participation of the double bond in these dehydroproline derivatives has so far been noticed, in contrast to the baikiain amide.

The extensive cleavage of dehydroproline and baikiain amides at alkaline *p*H prompted the examination of the action of NBS at *p*H 4–8 on *saturated* amides and peptides. In two cases, N-carbobenzyloxyprolinamide and N-acetylphenylalaninamide, ninhydrin assay gave *p*H cleavage profiles (Fig. 6) very similar to the dehydroprolinamides. The following compounds did not liberate ammonia: propionamide, carbobenzyloxyglycinamide, benzamide or phenylpropionamide. No release of glycine was observed from N-acetylphenylalanylglycine. Except for the Cbz-glycinamide case, these results support the possibility of a Hofmann type rearrangement, since release of ammonia would be expected only from amides derived from α-amino acids.

The *p*H profiles of these cleavage reactions are of value as a diagnostic aid in the structural determination of natural products. The application of this method to palustrine[15] led to cleavage of the amide bond and to formation of a γ-lactone, thus revealing the position of the double bond in the cyclohexene ring.[16]

A. Patchornik (personal communication) has applied this cleavage principle to phenylalanine peptides which were first reduced to di- or tetrahydro derivatives by partial reduction of the aromatic ring with lithium in methylamine at −80°. Subsequent treatment of these reduced peptides with NBS led to participation of the allylic γ,δ-double bond with formation of a γ-spirolactone and release of the peptide component in −70% yield. Markedly lower yields, as a result of steric restriction in the 1,5-participation

(15) W. Dietsche and C. H. Eugster, *Chimia*, **14**, 353 (1960).

(16) C. H. Eugster, personal communication, cf. W. Paulus and C. H. Eugster, *Angewandte Chem.*, **73**, 738 (1961).

TABLE I

LIST OF NEW AMINO ACID DERIVATIVES PREPARED

No.	Compound (formula)	Descripton of crystals and solvent of recrystn.	M.p., °C. (yield, %)	Molecular formula	Analytical results, % Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
1	DL-2-Benzamido-4-pentenoic acid (VI, R = Bz, R′ = OH)	Plates from EtAc–petr. ether	107–108 (lit.[6] 109) (86)[a]	$C_{12}H_{13}NO_3$						
2	DL-2-Benzamido-4-pentenoic acid amide (VI, R = Bz, R′ = NH_2)	Plates from MeOH–petr. ether	175 (77)[b]	$C_{12}H_{14}N_2O_2$	66.03	6.47	12.84	65.89	6.76	12.95
3	DL-2-Benzamido-4-pentenoylglycine benzyl ester (VI, R = Bz, R′ = $NHCH_2CO_2CH_2C_6H_5$)	EtAc–petr. ether	106–108 (77)[b]	$C_{21}H_{22}N_2O_4$	68.83	6.05	7.65	68.05	6.34	7.84
4	DL-2-Benzamido-4-pentenoylglycine (VI, R = Bz, R′ = $NHCH_2CO_2H$)	Acetone–petr. ether	126–129 (90)[c]	$C_{14}H_{16}N_2O_4$	60.86	5.84	10.14	60.71	6.06	10.32
5	DL-2-Benzamido-4-pentenoylglycinamide (VI, R = Bz, R′ = $NHCH_2CONH_2$)	EtOH–petr. ether	137–138 (85)[c]	$C_{14}H_{17}N_3O_3$	61.08	6.22	15.26	61.12	6.14	15.09
6	DL-2-*p*-Toluenesulfonamido-4-pentenoic acid (VI, R = Ts, R′ = OH)	Prisms from ether–petr. ether	100–101 (89)[a]	$C_{12}H_{15}NO_4S$	53.53	5.62	5.20	53.66	5.59	5.38
7	DL-2-*p*-Toluenesulfonamido-4-pentenoic acid amide (VI, R = Ts, R′ = NH_2)	Hex. prisms from MeOH–acetone	192–193 (56)[b]	$C_{12}H_{16}N_2O_3S$	53.72	6.01		53.86	6.11	
8	DL-2-Toluenesulfonamido-4-pentenoylglycine (VI, R = Ts, R′ = $NHCH_2CO_2H$)	Acetone–ether	134–136 (85)[b]	$C_{14}H_{18}N_2O_5S$	51.53	5.56	8.59	51.69	5.77	8.60
9	DL-2-Benzamido-4-methyl-4-pentenoic acid (VII, R = Bz, R′ = OH)	Plates from EtAc–petr. ether	135–136 (lit.[6] 135) (78)[a]	$C_{13}H_{15}NO_3$						
10	DL-2-Benzamido-4-Me-4-pentenoic acid amide (VII, R = Bz, R′ = NH_2)	Plates from MeOH–petr. ether	176 (78)[b]	$C_{13}H_{16}N_2O_2$	67.22	6.94	12.06	66.95	7.04	12.33
11	DL-2-Benzamido-4-Me-4-pentenoylglycine benzyl ester (VII, R = Bz, R′ = $NHCH_2CO_2CH_2C_6H_5$)	EtAc–petr. ether	123–125 (74)[b]	$C_{22}H_{24}N_2O_4$	69.45	6.36	7.36	69.15	6.65	7.58
12	DL-2-Benzamido-4-methyl-4-pentenoylglycine (VII, R = Bz, R′ = $NHCH_2CO_2H$)	EtOH–petr. ether	200 (67)[c]	$C_{15}H_{18}N_2O_4$	62.05	6.25	9.65	62.17	6.43	9.78
13	DL-2-*p*-Toluenesulfonamido-4-methyl-4-pentenoic acid (VII, R = Ts, R′ = OH)	Ether–petr. ether	112–114 (82)[a]	$C_{13}H_{17}NO_4S$	55.12	6.05	4.95	55.06	6.07	5.07
14	DL-2-*p*-Toluenesulfonamido-4-Me-4-pentenoic acid Me ester (VII, R = Ts, R′ = OCH_3)	Ether–petr. ether	81–83 (72)[b]	$C_{14}H_{19}NO_4S$	56.56	6.44	4.71	56.40	6.65	4.73
15	DL-2-*p*-Toluenesulfonamido-4-Me-4-pentenoic acid amide (VII, R = Ts, R′ = NH_2)	Prisms from MeOH–petr. ether	182–184 (48)[c]	$C_{13}H_{18}N_2O_3S$	55.31	6.43	9.92	55.56	6.60	10.03
16	DL-2-*p*-Toluenesulfonamido-4-Me-4-pentenoylglycine (VII, R = Ts, R′ = $NHCH_2CO_2H$)	EtAc–petr. ether	164–166 (74)[b]	$C_{15}H_{20}N_2O_5S$	52.93	5.92	8.23	53.43	6.15	8.24
17	Benzoyl-3,4-dehydro-DL-proline (VIII, R = Bz, R′ = OH)	Prisms from acetone–ether	137–141 (90)[a]	$C_{12}H_{11}NO_3$	66.35	5.10	6.45	66.59	5.35	6.42
18	Benzoyl-3,4-dehydro-DL-prolinamide (VIII, R = Bz, R′ = NH_2)	Plates from water	189–191 (80)[d]	$C_{12}H_{12}N_2O_2$	66.65	5.59	12.96	66.48	5.81	12.83
19	Benzoyl-3,4-dehydro-DL-prolylglycine (VIII, R = Bz, R′ = $NHCH_2CO_2H$)	EtOH–petr. ether	160–161 (60)[b]	$C_{14}H_{14}N_2O_4$	61.31	5.15	10.21	61.38	5.35	10.20
20	Carbobenzyloxy-3,4-dehydro-DL-prolinamide (VIII, R = Cbz, R′ = NH_2)	Benzene	132–133 (83)[d]	$C_{13}H_{14}N_2O_3$	63.40	5.73	11.38	63.08	5.67	11.12
21	Carbobenzyloxy-3,4-dehydro-DL-prolylglycine (VIII, R = Cbz, R′ = $NHCH_2CO_2H$)	EtOH–EtAc	168.5–169.5(60)[a]	$C_{15}H_{16}N_2O_5$	59.20	5.30	9.21	59.52	5.62	9.61

Table I (*Continued*)

No.	Compound (formula)	Description of crystals and solvent of recrystn.	M.p., °C. (yield, %)	Molecular formula	Analytical results, % Calculated C	H	N	Found C	H	N
22	Tosyl-3,4-dehydro-DL-proline (VIII, R = Ts, R′ = OH)	Prisms from methylene chloride–petr. ether	141–144 (100)[a]	$C_{12}H_{13}NO_4S$	53.93	4.90	5.24	53.82	4.86	5.28
23	Tosyl-3,4-dehydro-DL-proline Me ester (VIII, R = Ts, R′ = OCH_3)	MeOH–petr. ether	97.5–98.5 (94)[b]	$C_{13}H_{15}NO_4S$	55.51	5.38	4.98	55.79	5.51	5.10
24	Tosyl-3,4-dehydro-DL-prolinamide (VIII, R = Ts, R′ = NH_2)	Hex. prisms from MeOH	202–204 (83)[d]	$C_{12}H_{14}N_2O_3S$	54.13	5.30	10.52	54.15	5.27	10.48
25	Tosyl-3,4-dehydro-DL-prolylglycine Et ester (VIII, R = Ts, R′ = $NHCH_2CO_2C_2H_5$)	EtAc–petr. ether	84–85 (69)[b]	$C_{16}H_{20}N_2O_6S$	54.54	5.72	7.95	54.80	5.75	7.94
26	Tosyl-3,4-dehydro-DL-prolylglycine (VIII, R = Ts, R′ = $NHCH_2CO_2H$)	EtOH–ether	236–237 (70)[c]	$C_{14}H_{16}N_2O_6S$	51.85	4.96	8.64	52.11	5.22	9.10
27	Tosyl-DL-baikiain methyl ester (IX, R = Ts, R′ = OCH_3)	Hex. prisms from ether–petr. ether	76–76.5 (60)[a]	$C_{14}H_{17}NO_4S$	56.94	5.80		56.86	6.10	
28	Tosyl-DL-baikiain amide (IX, R = Ts, R′ = NH_2)	Prisms from MeOH	169–170 (42)[c]	$C_{13}H_{16}N_2O_3S$	55.70	5.75	10.00	55.92	5.73	10.28
29	Tosyl-4,5-dehydro-DL-pipecolylglycine (IX, R = Ts, R′ = NH_2)	Acetone–ether	167–168 (57)[a]	$C_{15}H_{18}N_2O_6S$	53.25	5.36	8.28	53.06	5.53	8.35
30	Acetyl-L-phenylalaninamide	Needles from EtOH–ether	182–183 (83)[c]	$C_{11}H_{14}N_2O_2$	64.06	6.84	13.58	63.78	6.75	13.20
31	Acetyl-DL-phenylalanylglycine benzyl ester	EtOH–petr. ether	143 (70)[b]	$C_{20}H_{22}N_2O_4$	67.78	6.26	7.91	67.58	6.32	7.78
32	Acetyl-DL-phenylalanylglycine	Prisms from MeOH–petr. ether	179–180 (80)[c]	$C_{13}H_{16}N_2O_4$	59.08	6.10	10.60	59.37	6.07	10.68

[a] Yield from amino acid. [b] Yield from acyl amino acid ester or acyl peptide ester. [c] Yield from acyl amino acid amide. [d] Yield from amino acid amide.

reaction, were observed when this reaction sequence was applied to the -Phe-Pro-cleavage in the cyclic decapeptide gramicidin S.

Experimental

Materials and Methods.—The derivatives of the amino acids VI-IX are listed together with analytical data in Table I. Intermediates which did not crystallize are not tabulated. Representative examples of methodology are given below for typical operations.

Carbobenzyloxylation. A. N-Carbobenzyloxy-3,4-dehydro-DL-proline.—3,4-Dehydro-DL-proline[8] (1.13 g., 10 mmoles) was dissolved in 50 ml. of water and 10.0 ml. of 1.0 *N* sodium hydroxide in a beaker fitted with the electrodes of a *p*H meter and a vibro-stirrer. The solution was cooled to its freezing point and 2 ml. of freshly prepared carbobenzoxy chloride[17] in 10 ml. of ether was added. Vibrostirring was started and 1.0 *N* sodium hydroxide was added from a buret at such a rate as to keep the *p*H at 9–10. Alkali uptake was quantitative and ceased abruptly after 40 min. The solution was extracted with ether (3 × 50 ml.) and the aqueous fraction was acidified to *p*H 2 at 0° with concentrated hydrochloric acid. The oil which precipitated was extracted into ethyl acetate (3 × 30 ml.). The organic solution was dried over magnesium sulfate and evaporated *in vacuo* leaving a colorless gum (2.40 g., 98%). Persistent attempts to induce crystallization failed both with racemic material as well as with 3,4-dehydro-L-proline.[8] The oil was used directly for the preparation of N-carbobenzyloxy-3,4-dehydroprolylglycine.

B. N-Carbobenzyloxy-3,4-dehydro-DL-prolinamide.—3,4-Dehydro-DL-prolinamide[8] (1.12 g., 10 mmoles) was dissolved in 25 ml. of 2 *N* aqueous sodium hydroxide and the solution was cooled to −10°. Carbobenzyloxy chloride (2 ml.) was added and the reaction mixture was stirred magnetically for 30 min. The crystalline product which had begun to precipitate after 5 min. was collected, dried and recrystallized from benzene to give N-carbobenzyloxy-3,4-dehydro-DL-prolinamide (2.05 g., 83%) as colorless feathery needles, m.p. 132–133°.

Benzoylations.—All amino acids were benzoylated in sodium hydroxide solution with benzoyl chloride according to the Schotten–Baumann procedure.

Tosylations. N-Tosyl-DL-baikiain.—DL-Baikiain hydrochloride (818 mg., 5 mmoles) was dissolved in 30.9 ml. of 0.3 *N* sodium hydroxide and stirred vigorously with *p*-toluenesulfonyl chloride (1 g.). Sodium hydroxide solution was added dropwise to maintain a *p*H of 9.0. After 6 hours the reaction mixture was filtered from unchanged acid chloride, acidified with hydrochloric acid and extracted 4 times with ethyl acetate. The organic layer was washed with saturated salt solution, dried over sodium sulfate and evaporated to dryness *in vacuo*. There remained 1.215 g. of colorless oil (86% yield) which could not be crystallized.

Preparation of Amides.—Solutions of the N-acylated amino acids in an organic solvent, such as freshly distilled tetrahydrofuran, were converted to the methyl esters by reaction with a slight excess of a solution of diazomethane in ether. The oily or crystalline ester in methanol was saturated with ammonia at 0°. The reaction was usually allowed to proceed for 1 to 2 days at room temperature in a tightly stoppered flask.

Preparation of Peptides.—The acylated amino acid was treated with an appropriate amino acid ester or its salt, such as glycine ethyl ester hydrochloride or glycine benzyl ester *p*-toluenesulfonate,[18] in an organic solvent such as tetrahydrofuran, chloroform or methylene chloride or a mixture thereof, with dicyclohexylcarbodiimide as activating agent and triethylamine as base. Reaction times varied from a few hours to 2 days at room temperature or 0°. In some cases, *e.g.*, in the preparation of 2-benzamido-4-pentenoylglycine benzyl ester, the mixed anhydride method with isobutyl chloroformate[19] gave almost equally good results (64% *versus* 77%). The N-acyl peptide esters, usually oils, were converted to the crystalline N-acyl peptides by the action of one equivalent of sodium hy-

(17) E. Katchalski, "Methods in Enzymology," Vol. 3, Academic Press, Inc., New York, N. Y., 1957, p. 541.

(18) N. Izumiya and S. Makisumi, *J. Chem. Soc.* (*Japan*), **78**, 130 (1957).

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

droxide in aqueous methanol at room temperature. When the consumption of alkali was not followed *in situ* in a *p*H-Stat, the saponification reaction was allowed to proceed for 12–24 hours.

Reaction of Amides and Glycine Peptide Derivatives with N-Bromosuccinimide.—In the following experiments, the amide or peptide (usually 0.2 mmole) was dissolved in 5 ml. of spectral grade acetonitrile and 5 ml. of buffer solution (see below) and diluted to 100.0 ml. with water. N-Bromosuccinimide solution was prepared in water at 10 times the molarity of the amide or peptide. Standard solutions of ammonium chloride and glycine were prepared in water at one-fifth the molarity of the amide or peptide. The Moore and Stein ninhydrin reagent was prepared by dissolving stannous chloride monohydrate (0.32 g.) in 200 ml. of citrate buffer (*p*H 5) and adding a solution of ninhydrin (8 g.) in methyl Cellosolve (200 ml.). The reagent was kept refrigerated, and was not used unless the standard solution produced a sufficiently high reading to indicate little or no decomposition. In most cases the ninhydrin solution was freshly prepared. The following procedure was then carried out: (i) 10.0 ml. of the amide or peptide solution was placed in each of eight 50-ml. volumetric flasks; (ii) to one of each of the flasks was added the following volumes of NBS solution: 0.0 ml. (blank), 0.3, 0.6, 1.0, 1.5, 2.0, 2.5 and 3.0 ml. This corresponded to the addition of 0 to 3.0 moles of NBS; (iii) after 10 min. the solutions were made up to the mark with water; (iv) 1-ml. portions of each solution were placed in separate matched colorimeter tubes, 2 × 15 cm. One-ml. portions of the standard solutions were also tested. To each tube was added 1.0 ml. of Moore–Stein ninhydrin reagent, and the tubes were capped and thoroughly mixed by shaking; (v) the tubes were heated in a *boiling* water-bath for 15 min. They were cooled, and to each was added 8 ml. of 50% aqueous 1-propanol. The tubes were again shaken to ensure uniform distribution of color, and the density value read in the colorimeter at 570 mμ.

In the first experiments, the zero reading was taken with a colorimeter tube containing 50% propanol in the light path, and all the readings calculated on the basis of this zero value. In these experiments, corrected cleavage values were obtained by subtraction of the value obtained for 0.0 mole of NBS from each of the readings. In later experiments, the solution containing no NBS was set at the zero density value, and no correction was required. The density readings of the standard solutions were taken to correspond to 100% cleavage.

For cleavage experiments at different *p*H the following buffer stock solutions were used: *p*H 4.15: Sodium formate (102 g.) and acetic acid (90.1 g.) were diluted to 1 liter (1.5 *M* solution); *p*H 6.5: $NaH_2PO_4 \cdot H_2O$ (138 g.) and $Na_2HPO_4 \cdot 7H_2O$ (134 g.) were diluted to 1 liter (1.5 *M* solution).

McIlvaine Solution.—Solution A (0.1 *M* citric acid solution) and solution B (0.2 *M* Na_2HPO_4 solution) were mixed as follows[20]

*p*H	A, ml.	B, ml.
4	12.29	7.71
5	9.70	10.30
6	7.37	12.63
7	3.53	16.47
8	0.55	19.45

The results of the cleavage experiments are summarized in Table II for those compounds not appearing in the figures. Compounds giving no cleavage reaction are not listed.

TABLE II

MAXIMUM PERCENTAGE YIELDS OF AMMONIA OR GLYCINE

Compound	*p*H 4.15	*p*H 6.5
VI, R = Bz, R′ = NH_2	70	60
VI, R = Ts, R′ = $NHCH_2CO_2H$	65	40
VII, R = Ts, R′ = NH_2	80	45
VII, R = Bz, R′ = $NHCH_2CO_2H$	65	70

Isolation of Cleavage Products. 2-Benzamido-4-hydroxy-5-bromopentanoic Acid Lactone (X, R = Bz).—To a solution of 0.276 g. (1 mmole) of DL-2-benzamido-4-pentenoylglycine in a mixture of 20 ml. of acetonitrile and 20 ml. of water was added a solution of 0.187 g. (1.05 mmoles) of NBS in 40 ml. of water. The reaction mixture was allowed to stand for 1 hour and evaporated to a volume of 10 ml. *in vacuo*. During evaporation some of the lactone crystallized and was collected. The filtrate was extracted with ethyl acetate and the aqueous layer was set aside for the isolation of glycine. The organic layer which contained more lactone was washed with dilute sodium bicarbonate solution, then water, dried over Na_2SO_4 and evaporated. The residual oil crystallized on standing. Recrystallization of the combined initial crystals and this fraction from ethyl acetate (4 ml.)–petroleum ether (12 ml.) yielded 0.125 g. (42%), m.p. 161–162°; λ_{max}^{Nujol} 5.61 μ (γ-lactone C=O), 6.00 μ (amide C=O); A sample was dried *in vacuo* at 100° for 3 hours.

Anal. Calcd. for $C_{12}H_{12}NO_3Br$: C, 48.31; H, 4.40; Br, 26.80. Found: C, 47.96; H, 4.30; Br, 26.81.

The lactone X (R = Bz) was also obtained from the interaction of NBS and DL-2-benzamido-4-pentenoic acid (0.219 g., 1 mmole) under the above conditions. The yield of recrystallized product was 0.096 g. (31%), m.p. 161–162°.

Isolation of Glycine.—The aqueous layer above containing the glycine was evaporated to dryness and the residue was washed several times with ethyl acetate and dissolved in water containing 0.5 ml. of triethylamine. The solution was evaporated and the residue was washed with cold ethanol. The yield of crude glycine was 0.041 g. (55%). In several solvent systems this material showed only a single spot identical with the R_f of authentic glycine. Recrystallization from water–ethanol yielded 0.036 g. of pure glycine, m.p. 250–252° dec. *Anal.* Calcd. for $C_2H_5NO_2$: N, 18.66. Found: N, 18.37.

2-*p*-Toluenesulfonamido-4-hydroxy-5-bromopentanoic Acid Lactone (X, R = Ts).—DL-2-*p*-Toluenesulfonamido-4-pentenoylglycine (0.326 g., 1 mmole) was treated with NBS as for the benzoyl analog above. The oily crude lactone which solidified after several days was recrystallized from ethyl acetate (2 ml.)–ether (4 ml.)–petroleum ether (12 ml.). There was obtained 0.145 g. (42%, m.p. 134–135°); λ_{max}^{Nujol} 5.60 μ (γ-lactone C=O). The sample for analysis was dried *in vacuo* at 65° for 3 hours.

Anal. Calcd. for $C_{12}H_{14}NO_4SBr$: C, 41.33; H, 4.05; Br, 22.95. Found: C, 41.62; H, 4.20; Br, 22.45.

Isolation of Glycine.—By the procedure described above there was obtained from the aqueous phase 0.042 g. (56%) of chromatographically pure glycine.

2-Benzamido-4-hydroxy-4-methyl-5-bromopentanoic Acid Lactone (XI).—DL-2-Benzamido-4-methyl-4-pentenoylglycine (0.145 g., 0.5 mmole) was cleaved with NBS as described above. The lactone crystallized in the process of concentration of the reaction mixture. After recrystallization from ethyl acetate–ether–petroleum ether there was obtained 0.058 g. (37%) of lactone, m.p. 173–174°; λ_{max}^{Nujol} 5.57 μ (γ-lactone C=O), 6.06 μ (amide C=O). The analytical sample was dried *in vacuo* at 100° for 3 hours.

Anal. Calcd. for $C_{13}H_{14}NO_3Br$: C, 50.01; H, 4.52; Br, 25.60. Found: C, 49.01; H, 4.43; Br, 25.98.

Isolation of Glycine.—Chromatographically pure glycine was obtained in a yield of 0.022 g. (59%).

2-Bromoimino-3-benzamido-5-bromomethyltetrahydrofuran (XII).—To a solution of 1 g. of DL-2-benzamido-4-pentenoic acid amide in 100 ml. of hot acetonitrile was added a solution of 1.8 g. (2.2 molar equivalents) of NBS in 400 ml. of water. Crystals began to separate after 5 min. The mixture was kept in a refrigerator for 4 hours, and the crystals were collected and washed with a mixture of ice-cold acetonitrile and water (1:4 by volume). There was obtained 1.00 g. (58%) of XII, m.p. 125–127° dec.; λ_{max}^{Nujol} 6.01 μ (C=N), 6.06 μ (amide C=O). The analytical sample was dried *in vacuo* at 65° for 3 hours.

Anal. Calcd. for $C_{12}H_{12}N_2O_2Br_2$: C, 38.33; H, 3.19; N, 7.45; Br (total), 42.51; Br^+, 21.25. Found: C, 38.18; H, 3.31; N, 7.34; Br (total), 42.65; Br^+, 20.9.

Positive bromine was analyzed by dissolving a weighed sample in glacial acetic acid, adding aqueous potassium iodide and sulfuric acid, and titrating the liberated iodine with aqueous sodium thiosulfate. Bromine was evolved when the compound was heated in methanol. A cold methanol solution did not give a precipitate with aqueous silver nitrate. The compound is moderately soluble in cold alcohols and ethyl acetate, and less in chloroform. The be-

(20) *Cf.* "Handbook of Chemistry and Physics," 36th ed., p. 1617.

havior at the melting point, which is characteristic for all the N-bromoimino compounds reported here, is a sudden change to a black char.

Hydrolysis of XII.—Aliquots of a 2×10^{-4} *M* solution of XII in acetonitrile–water (1:9) released 100% of the imino-nitrogen as ammonia under the standard conditions of ninhydrin assay.

Compound XII (100 mg.) was refluxed (80°) for 2 hours in 10 ml. of acetonitrile–water (1:1). Liberated bromine gradually evaporated. The solution was concentrated *in vacuo* and as the acetonitrile evaporated, white crystals formed. They were collected (75 mg., 95%) and their infrared spectrum (Nujol) was indistinguishable from that of authentic lactone X (R = Bz), m.p. 161–162°. Preliminary experiments showed that a reaction time of 2 hours was necessary for complete hydrolysis.

2-Bromoimino-3-benzamido-5-methyl-5-bromomethyltetrahydrofuran (XIII, R = Bz).—DL-2-Benzamido-4-methyl-4-pentenoic acid amide (0.116 g., 0.5 mmole) was treated with NBS (0.095 g., 0.525 mmole) as described above for the preparation of XII. The yield of crystalline XIII (R = Bz) collected after concentration of the reaction mixture was 0.031 g. (15%), m.p. 141–144° dec.; λ_{max}^{KBr} 6.01 μ (C=N), 6.06 μ (amide C=O). The analytical sample was dried *in vacuo* at 65° for 3 hours.

Anal. Calcd. for $C_{13}H_{14}N_2O_2Br_2$: C, 40.02; H, 3.62; Br, 40.97. Found: C, 40.59; H, 3.71; Br, 40.91.

The compound gave a positive reaction when rubbed on moist starch–iodide paper. Higher yields could undoubtedly be obtained by doubling the relative concentration of NBS.

2-Bromoimino-3-*p*-toluenesulfonamido-5-methyl-5-bromomethyltetrahydrofuran (XIII, R = Ts).—DL-2-*p*-Toluenesulfonamido-4-methyl-4-pentenoic acid amide (0.085 g., 0.3 mmole) was treated with NBS (0.057 g., 0.32 mmole) as described above; yield of XIII (R = Ts), 0.016 g. (12%), m.p. 124–126° dec., λ_{max}^{KBr} 6.05 μ (C=N). The compound gave a positive starch–iodide paper test.

Anal. Calcd. for $C_{13}H_{16}N_2O_3SBr_2$: Br, 36.47. Found: Br, 36.30.

2-Oxa-3-bromoimino-5-(tosyl)-aza-7-bromobicyclo[3,2,1]-octane (XVI). The Reaction of N-Tosylbaikiain Amide with NBS.—N-Tosyl-DL-baikiain amide (280 mg., 1 mmole) dissolved in 25 ml. of warm acetonitrile was added rapidly with shaking to a solution of NBS (400 mg., 2.2 mmoles) in 100 ml. of water. An immediate turbidity occurred and crystallization commenced within 5 minutes. The reaction mixture was kept at 0° for 2 hours, and the product was filtered off; yield of XVI, 350 mg. (80%), m.p. 142–143° dec., λ_{max}^{KBr} 6.00 μ (C=N). The analytical sample was dried *in vacuo* for 4 hours at 65°.

Anal. Calcd. for $C_{13}H_{14}N_2O_3SBr_2$: C, 35.63; H, 3.22; N, 6.39; Br (total), 36.48; Br^+, 18.24. Found: C, 35.67; H, 3.69; N, 6.52; Br (total), 35.89; Br^+, 18.7.

Area measurements of the n.m.r. spectrum (60 Mc/sec., $CDCl_3$ solvent), using the four aromatic protons as a reference area, confirm the presence of 14 hydrogen atoms. The compound is soluble in warm alcohols, ethyl acetate, chloroform and benzene. Loss of bromine was observed upon attempted recrystallization. No precipitation occurred when aqueous silver nitrate was added to a warm ethanol solution of the compound.

Hydrolysis of the Bicyclic Lactone XVI.—Aliquots of a 4×10^{-4} *M* solution of XVI in acetonitrile–water (1:4) gave a ninhydrin color yield of 15% after 15 min. at 100° and 20% after 1 hour. A 4×10^{-4} *M* solution of XVI in acetonitrile–1 *M* formate buffer, *p*H 5 (1:4), gave a color yield of 22% in 15 min. and 75% in 1 hour. Ammonium chloride solutions were treated in a similar way to obtain the reference 100% color yields.

N-Tosyl-4-hydroxy-5-bromopipecolic Acid Lactone.—When XVI (300 mg.) was refluxed for 1 hour in 10 ml. of 90% acetic acid, the liberated bromine gradually evaporated. The solvent was removed *in vacuo* and the residue was distributed between ethyl acetate and aqueous sodium bicarbonate. Evaporation of the ethyl acetate layer gave the lactone of N-tosyl-4-hydroxy-5-bromopipecolic acid (95 mg.). The analytical specimen was recrystallized twice from methanol; m.p. 179–181°, λ_{max}^{KBr} 5.54 μ (γ-lactone).

Anal. Calcd. for $C_{13}H_{14}NO_4SBr$: C, 43.34; H, 3.92; N, 3.89. Found: C, 43.37; H, 3.96; N, 3.91.

N-Tosyl-4-hydroxy-5-bromopipecolic Acid.—The bicarbonate layer was acidified with concentrated hydrochloric acid and the precipitate was extracted into ethyl acetate. Removal of the organic solvent left N-tosyl-4-hydroxy-5-bromopipecolic acid (100 mg.). The analytical specimen was recrystallized once from aqueous ethanol and once from ethyl acetate–chloroform; m.p. 193–195°, λ_{max}^{KBr} 5.75 μ (carboxyl C=O).

Anal. Calcd. for $C_{13}H_{16}NO_5SBr$: C, 41.28; H, 4.26; N, 3.70. Found: C, 41.49; H, 4.44; N, 3.63.

Preliminary experiments showed that the above reaction conditions were necessary for complete hydrolysis and also gave the maximum yield of lactone. Over 60% of XVI remained when it was treated under the conditions which effected complete hydrolysis of XII.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

Rearrangements of Dehydroproline Derivatives

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The action of N-bromosuccinimide on N-carbobenzyloxy-3,4-dehydro-DL-proline amide at *p*H 7–9 in a *remarkably easy Hofmann rearrangement* leads with loss of ammonia to a compound $C_{12}H_{13}NO_4$ which on the basis of its oxidation and reduction products, its O,O-diacetate and extensive n.m.r. data has been assigned the *unusual dicarbinolamide* structure II, formally the condensation product of maleic dialdehyde with benzyl carbamate. Whereas the action of base on esters and amides of N-benzoyl-3,4-dehydroproline fails to move the double bond into the 2,3-position, it produces the *optically active hydantoin* V from N-carbobenzyloxy-3,4-dehydro-L-proline amide and aromatizes N-tosyl-3,4-dehydro-DL-proline methyl ester to methyl pyrrole-2-carboxylate with *elimination of p-toluenesulfinate.* The betaine *3,4-dehydro-DL-stachydrine* was obtained from 3,4-dehydro-DL-proline by methylation under neutral conditions. This betaine, unlike all other dehydroproline derivatives, was unstable and rearranged easily to the isomeric *2,3-dehydrostachydrine* which was identical with the dehydration product from the two diastereoisomeric 3-hydroxystachydrines from *Courbonia virgata.*

A. The Dicarbinolamide II.—Certain aspects of the action of N-bromosuccinimide (NBS) on N-acyl-3,4-dehydroprolinamides have been discussed in the preceding paper.[1] The N-tosyl-, N-benzoyl- and N-carbobenzyloxy- derivatives all give high yields of ammonia at alkaline *p*H, but no cleavage at *p*H 4 or lower. This reaction has now been studied on a preparative scale with N-carbobenzyloxy-3,4-dehydroprolinamide (I). At *p*H 7–9 two equivalents of NBS are consumed and one equivalent of ammonia is released. A crystalline product to which the interesting structure of N-carbobenzyloxy-2,5-dihydroxy-Δ^3-pyrroline (II) has been assigned is readily isolated in 60% yield.

(1) N. Izumiya, J. E. Francis, A. V. Robertson and B. Witkop, *J. Am Chem. Soc.*, **84**, 1702 (1962).