TABLE III

CHANGING ISOMER RATIO WITH ISOMERIZATION OF L-XYLOASCORBIC ACID

Time, hr.	L-XAA/L-AAA	Time, hr.	L-XAA/L-AAA
0	100/0	8	70/30
1	91/9	12	63/37
4	77/23	16	60/40
6	74/26	24	57/43
		28	57/43

TABLE IV

ROTATIONAL CHANGES OF D-ARABOASCORBIC ACID IN BASE

Time, hr.	$[\alpha]^{27}D$	Time, hr.	$[\alpha]^{27}D$
0	24.72	11	6.00
1	22.16	13	4.41
2	19.59	15	3.18
5	13.95	16.75	2.43
7	11.25	24.5	−0.02
9	8.17	31	−1.09
		33	−1.42

with 95 g. (100% excess) of Amberlite IR-120 (H^+). The resin was removed and the filtrate concentrated *in vacuo* to remove the methanol. Lyophilization of the residual aqueous solution yielded an amorphous yellow solid which, on crystallization from acetonitrile, yielded three crops of recovered L-xyloascorbic acid and finally a fourth crop of crystals (3.9 g.). Infrared and ultraviolet spectra, m.p. 166–170° dec., and $[\alpha]^{27}D$ +13° identified the final material to be L-araboascorbic acid.[15]

(15) T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta*, **17**, 510 (1934).

TABLE V

CHANGING ISOMER RATIO WITH ISOMERIZATION OF D-ARABOASCORBIC ACID

Time, hr.	D-AAA/D-XAA	Time, hr.	D-AAA/D-XAA
0	100/0	9	58/42
1	90/10	11	56/44
3	83/17	17	48/52
5	74/26	24	47/53

Isomerization of D-Araboascorbic Acid.—Similar treatment of D-araboascorbic acid in an identical system yielded the optical rotation measurements shown in Table IV. T.l.c. here indicated formation of a less mobile species, R_f 0.26, comparable to that of a xyloascorbic acid, with equilibration near completion between 17 and 24 hr., with a ratio of about 1.0:1.1 of araboascorbic–xyloascorbic isomers (Table V).

A similar processing of the reaction mixture after 15 hr. of refluxing produced a crude yellow solid which, on fractional crystallization from acetonitrile, yielded a first crop (5.8 g.) of colorless crystals. Recrystallization from the same solvent gave material melting at 188–190° dec. with infrared and ultraviolet spectra identical with those for L-xyloascorbic acid. A mixture of this material with an equal amount of authentic L-xyloascorbic acid depressed the melting point to 170°, the value reported for the DL pair.[16] The optical rotation of the isolated material was levoratotory ($[\alpha]^{27}D$ −16°) thus identifying it as D-xyloascorbic acid.[16,17]

(16) T. Reichstein, A. Grüssner, and R. Oppenauer, *ibid.*, **16**, 1019 (1933).

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Selective Cleavage of Ornithyl and Diaminobutyryl Peptides[1]

MELVIN A. LIPSON[2] AND ERNEST SONDHEIMER

Department of Chemistry, State University College of Forestry, Syracuse University, Syracuse, New York

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The selective cleavage of the N-α-naphthylamides of L-ornithylglycylglycine, L-2,4-diaminobutyrylglycylglycine, and L-ornithyl-L-leucylglycine leading to the lactams and dipeptide naphthylamides was studied. Although alkaline or acidic aqueous systems did not yield satisfactory results, selective cleavage could be demonstrated in absolute ethanol in the presence of triethylamine at 65°. Since glycylglycyl units are particularly prone to alkaline hydrolysis, the presence of this moiety in a peptide affords a very severe test for the selectivity of the cleavage. The scission of the leucylglycine derivative was slow but highly specific, a result attributable to steric hindrance.

Intramolecular nucleophilic attack of an amino group on a peptide can lead to lactam formation and scission of an amide bond under exceptionally mild conditions. Thus, Holley and Holley[3] found that if an aqueous solution of N-(2-amino-4-carbomethoxyphenyl)glycylglycylglycine is held at 25° for 5 hr. or at 70° for 15 min., the dihydroquinoxalone derivative and glycylglycine are obtained in very high yields. Another example of this type of facile peptide cleavage is the removal of the chloroacetyl group from N-chloroacetyl peptides with *o*-phenylenediamine, a reaction that is completed in aqueous solution at 100° after 1 hr.[4] Since in the above cases the attacking group is an aromatic amino group, it seemed of interest to ascertain whether lactam formation could also lead to selective peptide bond cleavage with aliphatic amines. An earlier study by Barrass and Elmore[5] on the cleavage of α-N-tosyl-DL-ornithylglycine and α-N-tosyl-L-2,4-diaminobutyrylglycine to yield the lactams and glycine gave no direct information on this point. With those substances there is no way of ascertaining the degree of cleavage specificity, nor do their results lend themselves readily to quantitative interpretation.

Most of the work reported here deals with the cleavage of the N-α-naphthylamides of L-ornithylglycylglycine, L-2,4-diaminobutyrylglycylglycine, and L-ornithyl-L-leucylglycine. The presence of the glycylglycine moiety affords a particularly stringent test for the specificity of the cleavage procedure since studies by Levene, *et al.*,[6] and by Synge[7] have shown this grouping to be the most susceptible to base- or acid-catalyzed hydrolysis. Information on the effects of steric hindrance was obtained from the leucylglycine derivative. The presence of the α-naphthylamide group permits

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(6) P. A. Levene, R. C. Steiger, and A. Rothen, *J. Biol. Chem.*, **97**, 717 (1932).

(7) R. L. M. Synge, *Biochem. J.*, **39**, 351 (1945).

TABLE I

CLEAVAGE OF PEPTIDE α-NAPHTHYLAMIDES[a]

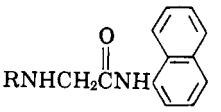
Peptide naphthylamides	Time, hr.	Amount unchanged, %	Dipeptide naphthylamide, %	Glycine naphthylamide, %
L-Orn-Gly-Gly-	36	15	72	5
	48	8	73	11
	72	3	80	17
L-Dab-Gly-Gly-	36	49	42	5
L-Orn-L-Leu-Gly-	36	71	24	0

[a] In 0.05 *M* triethylamine in ethanol at 65°.

solutions in absolute ethanol containing 0.05 *M* triethylamine at 65°. Data on the cleavage of the naphthylamides are summarized in Table I. In addition to the listed products, the expected lactams were qualitatively detected with ninhydrin sprays. In no case could the presence of ornithine, diaminobutyric acid, or free naphthylamine be demonstrated. It is seen that although peptide bond cleavage through lactam formation with aliphatic amino groups is much slower than with *o*-phenylenediamine derivatives, it nevertheless

TABLE II

PHYSICAL CONSTANTS AND ANALYSES OF THE GLYCINE-N-α-NAPHTHYLAMIDES



R	Yield, %	M.p., °C.	$[\alpha]^{25}D$	R_f	Color with ninhydrin	—Carbon, %— Calcd.	Found	—Hydrogen, %— Calcd.	Found	—Nitrogen, %— Calcd.	Found	—Chlorine, %— Calcd.	Found
Cbz-[a]	43	150–152				71.84	71.78	5.42	5.32	8.38	8.56		
Cbz-Gly-[b]	47	199–200				67.51	67.07	5.41	5.79	10.74	10.75		
Cbz-L-Leu-[c]	50	162–164	−5.3[d]			69.76	69.59	6.53	6.59	9.39	9.56		
DiCbz-L-Dab-Gly-[e]	49	194–196	−38.6[f]			65.27	64.59	5.64	5.79	11.19	11.31		
DiCbz-L-Orn-Gly-[g]	60	189–191	−8.4[f]			65.71	65.65	5.83	5.94	10.95	11.12		
DiCbz-L-Orn-L-Leu-[h]	65	172–175	−13.2[f]			67.32	67.28	6.52	6.88	10.07	10.13		
H·HOAc[i,j]	74	165–166 dec.		0.73	Brown	64.60	64.92	6.20	6.06	10.76	10.68		
Gly-·HCl[i,k]	88	246–248 dec.		0.62	Yellow	57.24	56.83	5.49	5.69	14.30	14.48	12.07	12.25
L-Leu-·HCl[i]	47	205–207	+39.5[l]	0.85	Blue	61.79	61.95	6.91	7.04	12.01	11.77	10.14	10.83
L-Dab-Gly-·2HCl[i,m]	76		−25.8[l]	0.33	Brown	50.24	49.49	5.86	6.25	16.28	16.11	16.48	16.98
L-Orn-Gly-·2HCl[i,m]	75		+26.1[l]	0.28	Purple	51.35	51.47	6.13	6.38	15.76	14.97	15.96	16.05
L-Orn-L-Leu-·2HCl[i,m,n]	80		−8.6[l]	0.60	Blue	55.20	54.45	7.05	7.20	14.00	13.90	14.17	14.02

[a] Coupling product of carbobenzoxyglycine and α-naphthylamine. [b] Coupling product of carbobenzoxyglycylglycine and α-naphthylamine. [c] Coupling product of carbobenzoxy-L-leucylglycine and α-naphthylamine. [d] *c* 0.4% in dimethylformamide. [e] Coupling product of dicarbobenzoxy-L-diaminobutyric acid [S. Wilkinson, *J. Chem. Soc.*, 104 (1951)] and α-naphthylamine. [f] *c* 1% in dimethylformamide. [g] Coupling product of dicarbobenzoxy-L-ornithylglycylglycine and α-naphthylamine. Recrystallized from 90% ethanol. [h] Coupling product of dicarbobenzoxy-L-ornithine [R. L. M. Synge, *Biochem. J.*, **42**, 99 (1948)] and L-leucylglycinenaphthylamide hydrochloride. Product forms a gel that crystallizes very slowly from aqueous ethanol at room temperature. [i] Hydrogenolysis of the carbobenzoxy derivative was carried out at room temperature and 1 atm. using 50–75 mg. of palladium black/mmole in ethanol containing an excess of acetic acid. Hydrogen was passed through the mixture with mechanical shaking until carbon dioxide evolution ceased. The filtered solution was evaporated under reduced pressure and the residue was treated with a slight excess of 0.5 *N* hydrochloric acid, evaporated, and crystallized from appropriate solvents. [j] The carbon analysis for glycine-N-α-naphthylamide hydrochloride, m.p. 220–225 dec., was low. Calcd. for $C_{12}H_{12}N_2O$·HCl: C, 60.89; H, 5.53; Cl, 14.98; N, 11.84. Found: C, 59.08; H, 5.79; Cl, 14.96; N, 12.15. [k] Neut. equiv.: calcd., 290; found, 294. [l] *c* 1% in 0.5 *N* hydrochloric acid. [m] The hydrochloride was obtained as a gel or glass that could not be crystallized owing to hygroscopic properties. Purification was achieved by precipitation from alcoholic solution with ether. The compound was dried to constant weight at 80° prior to analysis. The acetate was also noncrystalline. [n] Treatment of the dicarbobenzoxy derivative with 30% hydrogen bromide in acetic acid did not yield a crystalline dihydrobromide.

quantitative spectral determination of the cleavage products and prevents side reactions at the C-terminals.

In preliminary studies it was found that cyclization was too slow to be of value unless the pH was raised to the point at which a significant concentration of the free amino groups was present. The tendency toward lactam formation under a variety of conditions decreased from L-ornithine methyl ester to L-ornithinamide to L-ornithylglycylglycine. Storage of ornithinamide for 24 hr. at 25° in 0.5 *M* phosphate buffer of pH 8.9 gave complete conversion to the lactam, conditions under which the tripeptide was recovered unchanged. Further trials with L-ornithylglycylglycine in aqueous alkaline solutions either at higher temperatures or higher pH values gave unsatisfactory results due to nonspecific cleavage. However, nonaqueous systems provided a more favorable reaction medium. Optimum results were obtained with approximately 0.01 *M* peptide proceeds faster than the cleavage of the highly susceptible glycylglycine bond. The time study with L-ornithylglycylglycine-α-naphthylamide shows that the longer the reaction time the lower the selectivity of the cleavage. Presumably, the nonspecific cleavage is due to base-catalyzed alcoholysis. Lactam formation with L-2,4-diaminobutyrylglycylglycine-α-naphthylamide was somewhat slower than for the ornithine analog, resulting in a decrease in the specificity of the cleavage. The cleavage of L-ornithyl-L-leucylglycine-α-naphthylamide proceeded with the highest selectivity and at the slowest rate. Both of these results are attributable to steric hindrance. It is, therefore, concluded that the selectivity with which ornithine and 2,4-diaminobutyric acid containing peptides can be cleaved by the procedure outlined here depends on the amino acid sequence of the peptide chains. Completely specific scission would probably be encountered only

rarely, but, since lactam formation has been shown to be faster than glycylglycyl cleavage, a significant degree of selectivity should be obtainable in all cases.

Experimental[8]

Dicarbobenzoxy-L-ornithine Methyl Ester.—This compound was prepared from dicarbobenzoxy-L-ornithine[9] and ethereal diazomethane, m.p. 71–72° from chloroform–ligroin.

Anal. Calcd. for $C_{22}H_{26}N_2O_6$: C, 63.75; H, 6.32; N, 6.76. Found: C, 63.83; H, 6.37; N, 6.04.

Dicarbobenzoxy-L-ornithinamide.—Dicarbobenzoxy-L-ornithine methyl ester, 1 g., was stored overnight at 0° in 10 ml. of methanolic ammonia. Filtration yielded 600 mg. of amide, 62%, m.p. 168.

Anal. Calcd. for $C_{21}H_{25}N_3O_5$: C, 63.14; H, 6.31; N, 10.52. Found: C, 62.93; H, 6.34; N, 9.84.

L-Ornithylglycylglycine was prepared by a modification of the method used by Goldschmidt and Rosculet.[10] Dicarbobenzoxy-L-ornithylglycylglycine was synthesized by direct coupling of dicarbobenzoxy-L-ornithine[9] and sodium glycylglycinate with ethyl chloroformate[11]; m.p. 169–171° from ethyl acetate–ligroin; Goldschmidt and Rosculet[10] reported m.p. 125–126°. Because of discrepancy in melting point, our preparation was analyzed.

Anal. Calcd. for $C_{25}H_{30}N_4O_8$: C, 58.35; H, 5.88; N, 10.89; neut. equiv., 514. Found: C, 58.18; H, 5.89; N, 11.31; neut. equiv., 513.

Hydrogenolysis at room temperature and 1 atm. in the presence of palladium black, 75 mg./mmole, in 80% aqueous acetic acid containing a slight excess of hydrochloric acid yielded noncrystalline L-ornithylglycylglycine hydrochloride, R_f 0.08, purple color with ninhydrin; $[\alpha]^{25}D$ +22.0° (*c* 1%, 0.5 *N* HCl); Goldschmidt and Rosculet[10] reported $[\alpha]^{25}D$ +25.9° (*c* 2%, 0.5 *N* HCl).

Anal. Calcd. for $C_9H_{18}N_4O_4 \cdot HCl$: C, 38.23; H, 6.77; Cl, 12.54; N, 19.82. Found: C, 38.39; H, 6.80; Cl, 12.90; N, 19.70.

The naphthylamide derivatives were prepared by the coupling procedure of Vaughan and Osato.[11] Physical constants and analytical data for these compounds are listed in Table II. In addition to the chromatographic data recorded in Table II, the R_f values and ninhydrin colors of the compounds listed below were obtained: ornithinamide prepared by hydrogenolysis of the dicarbobenzoxy derivative, 0.04, purple; L-ornithine hydrochloride, 0.08, purple; ornithine methyl ester dihydrochloride,[12] 0.10, purple; 2,4-diaminobutyric acid hydrochloride, 0.12, purple; glycylglycine, 0.12, yellow; glycine, 0.18, brown; 3-amino-2-pyrrolidone hydrochloride,[13] 0.24, yellow; 3-amino-2-piperidone hydrochloride,[12] 0.28, yellow; and α-naphthylamine, 0.91, tan.

Cleavage Procedures.—Peptides at concentrations of 2 mg./ml. were allowed to react at controlled temperatures between 25 and 85° in 2-ml. sealed glass ampoules, and the reaction was terminated by cooling or mild acidification. Aliquots (10 μl.) were spotted in triplicates on Whatman No. 1 filter paper and chromatographed at room temperature using *n*-butyl alcohol–acetic acid–water, 4:1:5 (v./v.) as the mobile phase, and the products were detected qualitatively either by ninhydrin sprays or with a Mineralite, Model SL 2537 hand lamp. The sections containing the ultraviolet-absorbing compounds were cut out, the substances were eluted with water, and the eluates were made up to 4 ml. Under the elution conditions used, variable amounts of ultraviolet-absorbing impurities were detected which we could not remove satisfactorily. However, the interference of these compounds could be minimized by using aqueous extracts from the sections of the chromatograms adjacent to the naphthylamide spots as spectrophotometric blanks and taking readings at 292 mμ. The choice of this wave length rather than the absorption maximum, 281 mμ, represents a compromise between minimum interference by the impurities and loss of sensitivity of the detection procedure. Corrections for losses during chromatography and elution of the compounds were made through the use of calibration curves. The average molar extinction coefficient, ε, of the six naphthylamide salts in aqueous solutions at 292 mμ was 5190 ± 4%, and at 281 mμ it was 6100 ± 4%. The average molar extinction coefficient for the carbobenzoxynaphthylamides in 95% ethanol at 290 mμ, their absorption maximum, was 7050 ± 1%.

(8) All melting points were determined with a Mel-Temp heating block in capillary tubes and are uncorrected. Analyses are by George Robertson, Florham Park, N. J. R_f values were obtained on Whatman No. 1 filter paper with *n*-butyl alcohol–acetic-acid–water, 4:1:5 (v./v.), descending.

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A Carbon-by-Carbon Degradation of Carbon-14-Labeled Nicotinic Acid[1]

DAVID R. CHRISTMAN, RAY F. DAWSON, AND KARIN I. C. KARLSTROM

Departments of Chemistry and Biology, Brookhaven National Laboratory, Upton, New York, and the Department of Botany, Columbia University, New York

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A carbon-by-carbon chemical degradation of carbon-14-labeled nicotinic acid is presented which is capable of giving the specific activity of each carbon atom in the molecule directly. With reasonably good counting equipment, the method can be applied to nicotinic acid containing 5 μc. of activity in 2–3 mmoles. Randomly labeled acid containing this level of activity would produce carbon dioxide in the final stages with a specific activity of about 0.1 mμc./mg. of carbon.

The carbon-by-carbon degradation of the pyridine ring of nicotinic acid (I) has become an increasingly necessary step in the elucidation of some biochemical pathways by C^{14} tracer methods. No complete degradation scheme applicable directly to I has been available so far, although several partial degradations of the pyridine ring of I and of ricinine have been published which have made the specific activity of each of the ring carbon atoms potentially available.[2] Starting from I, these would require total amounts of activity of the order of 0.2–0.5 mc., however.[2f] A scheme is presented here which is capable of yielding the specific activity of each of the pyridine ring carbon atoms of labeled I unambiguously, in essentially a single sequence of reactions. This method can easily be applied to an amount of I containing 5 μc. of total C^{14} activity if reasonably good counting equipment is available[3] (low level liquid scintillation, or, preferably, low level gas

(1) Research performed under the auspices of the U. S. Atomic Energy Commission at Brookhaven National Laboratory and under Grants G12855 and GB1129 from the National Science Foundation at Columbia University.

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