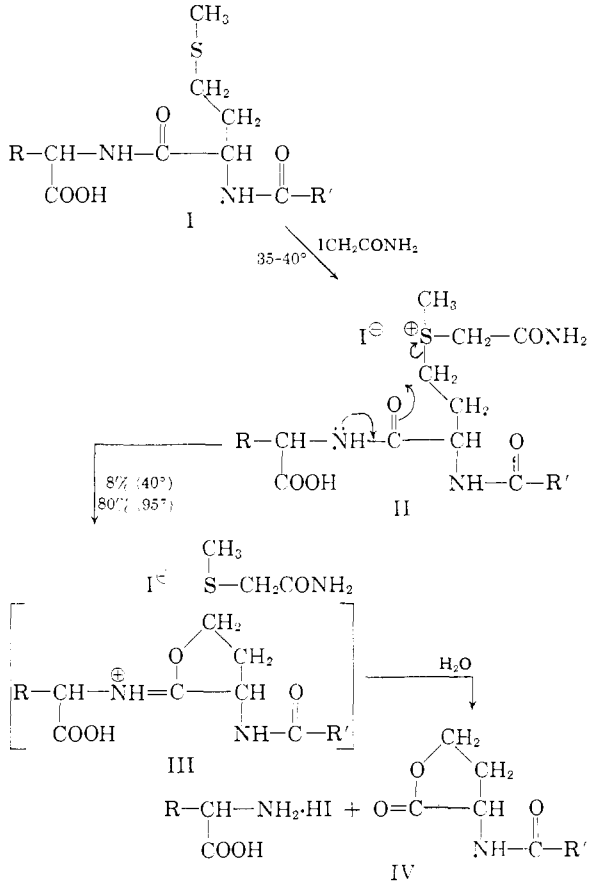

cleavage of ethyl N-acetylmethionylglycinate depends on the nature of the alkylating agent. Carbamylmethylsulfonium salts gave the highest yield (~50%).

TABLE II

ALKYLATION AND CLEAVAGE OF METHIONYL PEPTIDES

Peptide	Time of alkylation, hr.	Degree of alkylation by titration, %	Per cent. of peptide cleavage
Carbobenzoxy-L-methionyl-L-glutamic acid[a,b]	68[c]	92	8.1[d]
	135[e]	91	80[f]
	135[e]	91	85[g]
	135[e]	91	81[h]
Benzoyl-DL-methionylglycine[a,b]	68[c]	99	8.6[d]
Benzoyl-DL-methionylglycine ethyl ester[a,b]	135[e]	93	54[f]
	135[e]	93	65[g]
	135[e]	93	62[h]
Carbobenzoxy-L-methionyl-L-tyrosine[a,b]	68[c]	92	7.9[d]
	65[c]	84	84[g]

[a] Concentration in ethanol-water (1:1) was 2×10^{-2} *M*. [b] Three equivalents of alkylating agent were used. [c] Temperature of alkylation was 40°. [d] Mixture was fractionated on columns of Amberlite IR 120 and the cleaved amino acid determined with automatic recording equipment.[6] [e] Temperature of alkylation was 35–40°. [f] Reaction mixture was analyzed directly by ninhydrin method.[4] [g] Reaction mixture was heated for 1 hr. at 95° before analysis.[4] [h] Reaction mixture was extracted with ether, heated for 1 hr. at 95° and analyzed.[4]

The procedure was applied to additional dipeptides and the course of the alkylation at 35–40° followed by argentometric titration.[5] With over 90% formation of carbamylmethylsulfonium salts only 8% of peptide cleavage occurred at 40°; however, on brief heating at 95° the yields of cleaved amino acid increased to 54–85% (Table II). Paper chromatography and paper electrophoresis of such reaction mixtures showed that in each case the cleaved amino acid was the only ninhydrin-positive substance present.

Since in peptides and proteins alkyl halides at *p*H 2.8 react only with the sulfur of methionine,[3,7] this procedure permits specific chemical cleavage of methionyl peptide bonds. The application of an improved methionine peptide cleavage to the selective splitting of ribonuclease is described in a following communication.

(5) G. Toennies and J. J. Kolb, THIS JOURNAL, **67**, 849 (1945).

(6) D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(7) P. T. Vithayathil and F. M. Richards, *J. Biol. Chem.*, **235**, 2343 (1960).

(8) Max-Planck Institut für Biochemie, München, Germany.

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RECEIVED SEPTEMBER 24, 1960

SELECTIVE CLEAVAGE OF THE METHIONYL PEPTIDE BONDS IN RIBONUCLEASE WITH CYANOGEN BROMIDE[1]

Sir:

Cyanogen bromide reacts with ethyl N-benzoyl-DL-methionylglycinate in alcohol–water *at room temperature* to yield, *via* the unstable cyanosulfonium salt, (i) 70% N-benzoyl-DL-homoserine lactone (m.p. 143°, rep. 142°),[2a] (ii) methyl thiocyanate, assayed by gas chromatography and infrared absorption, and (iii) 75–90% of ethyl glycinate. By contrast, the von Braun cyanogen bromide cleavage of dialkyl thioethers in which there is no intramolecular assistance by functional groups requires *elevated temperatures* for the formation of alkylthiocyanate and alkyl bromide.[2b]

The usefulness and selectivity of this improved method for the cleavage of methionyl peptide bonds[3] was demonstrated with ribonuclease, which in a chain of 124 amino acids contains four methionines.[4] Bovine pancreatic ribonuclease[5] in 0.1–0.3 *N* HCl solution reacted with up to 30 equivalents of cyanogen bromide at 20° for 24 hours. After removal of solvent, excess reagent and methyl thiocyanate by lyophilization the residue in aliquots of 1.5–2.0 mg. was subjected to paper electrophoresis for 4 hours at 1100 v., 60 m A. and *p*H 6.5 in a pyridine–acetate buffer system. In order

(1) Presented in part at the Annual Meeting of the Chemical Society of Japan (NIHON KAGAKU KAI NENKAI), April 1–4, 1961, in Tokyo.

(2) (a) E. Fischer and H. Blumenthal, *Ber.*, **40**, 106 (1910); (b) J. von Braun *et al.*, *Ber.*, **56**, 1573 (1923); **59**, 1202 (1926); *Ann.*, **490**, 189 (1931).

(3) *Cf.* W. B. Lawson, E. Gross, C. M. Foltz and B. Witkop, *J. Am. Chem. Soc.*, **83**, 1509 (1961).

(4) C. H. W. Hirs, S. Moore and W. H. Stein, *J. Biol. Chem.*, **235**, 633 (1960); C. H. W. Hirs, *Ann. N. Y. Acad. Sci.*, **88**, 611 (1960).

(5) Sigma Chemical Company, St. Louis, Lot R60B-069.

of increasing electromobility these four major fractions were identified: (I) *free* homoserine resulting from *free* homoserine lactone; (II) "core" material together with ribonuclease (possibly containing methionine sulfoxide which is resistant to NCBr); (III) 25% (in terms of whole protein methionine) (theor.) of isolated *chemical tail peptide*, presumably a heptadecapeptide (in contrast to the enzymatically produced 20-residue S-peptide[6]), containing the original NH_2-terminal lysine and a C-terminal homoserine (lactone), (IV) *free* homoserine lactone, by cleavage of the methionyl-methionyl-lysine (29–30–31) sequence; its yield together with the *free* homoserine with which it equilibrates in aqueous solution approximates 50% (in terms of whole protein methionine.)

TABLE I

SURVEY OF THE NH_2-TERMINAL RESIDUES INVOLVED IN THE CLEAVAGE OF RIBONUCLEASE BY CYANOGEN BROMIDE

Fraction	Dinitrophenylation and Hydrolysis Products	NH_2-term. resid. Found[a]	Calc.	Numerical Position and Sequence of Methionine(s) Involved in Cleavage
Original mixture of reaction of cyanogen bromide with ribonuclease	Di-DNP-Lys	0.90	2.0	Met-Lys (Lys) 30 31 (1)
	DNP-Ser	0.85	2.0	Met-Ser 17 18 79 80
	DNP-HomoSer, DNP-HomoSer lactone	0.50	1.0	Met-*Met*-Lys 29 30 31
Electrophoretic fraction II: ribonuclease (METH-O) and modified "Core"	Di-DNP-Lys	0.75	2.0	Met-Lys (Lys) 30 31 (1)
	DNP-Ser	0.65	2.0	Met-Ser 17 18 79 80
Electrophoretic fraction III: chemical tail peptide (1–17)	Di-DNP-Lys	0.55[b]	1.0	(Orig. NH_2-terminal Lys)

[a] Corrections for losses during hydrolysis were made with authentic DNP-amino acids which were taken concomitantly through the entire procedure. [b] Hydrolysis time was 18 hours compared with 8 hours and 90% Di-DNP-Lys, *cf.* C. B. Anfinsen, *et al.*, *J. Biol. Chem.*, **207**, 201 (1954).

At this preliminary stage the question of glutamic acid occurring either in positions 18[4] or 11 (Fig. 1)[7] seems to be answered for *native* ribonuclease in favor of position 11[7] (i) by the absence of DNP-Glu,[8] (ii) by the presence of 0.65–0.85 of 2.0 DNP-Ser (Table I) and (iii) by the consistent ratio of Glu:Ala:Phe of 3:3:1 in the amino acid composition of Fraction III.[9]

A study of the reaction of cyanogen bromide in 0.3 *N* HCl with the common amino acids showed that, besides methionine, only cysteine,[10] but neither cystine, tyrosine nor tryptophan, reacted. Cleavage of the α-chain of human hemoglobin containing two methionines[11] has been observed and led to three fractions on a sephadex-G25 column.[12,13]

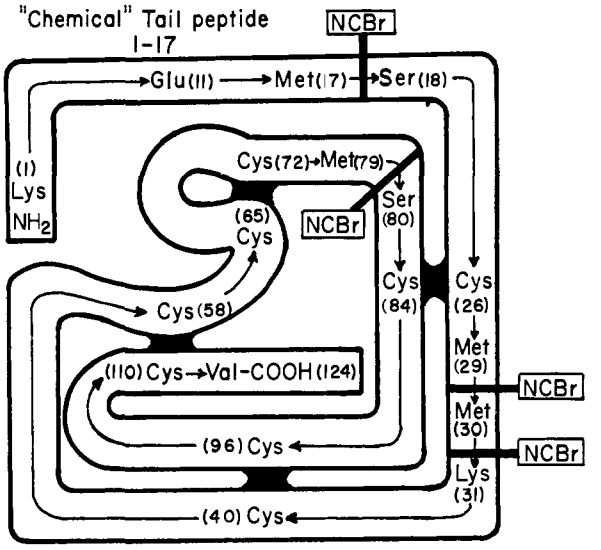

Fig. 1.—Topography of the cyanogen bromide cleavages of native ribonuclease [simplified diagrammatic "approximation" of D. H. Spackman, W. H. Stein and S. Moore, *J. Biol. Chem.*, **235**, 656 (1960)], with Glu (11) and Ser (18) arranged according to C. B. Anfinsen and R. R. Redfield, *Adv. in Protein Chem.*, **14**, 255 (1959).

(6) *Cf.* P. J. Vithayathil and F. M. Richards, *J. Biol. Chem.*, **235**, 2343 (1960).

(7) R. R. Redfield and C. B. Anfinsen, *J. Biol. Chem.*, **221**, 385 (1956); C. B. Afinsen, M. Sela and H. Tritch, *Arch. Biochem. Biophys.*, **65**, 156 (1956).

(8) Up to 0.3 of 1 residue of DNP-Asp is found in the original cleavage mixture and in Fraction II. An inherently labile aspartyl or asparaginyl residue in our opinion is rearranged and its amino group liberated concomitant with, or subsequent to, NCBr cleavage.

(9) The supplementary NCBr cleavage of the 20-residue S-peptide (ref. 6), is in progress.

(10) *Cf.* J. M. Swan, "Current Trends in Heterocyclic Chemistry," Editors A. Albert, G. M. Badger, C. W. Shoppee, Academic Press, Inc., New York, N. Y., 1958, p. 65.

(11) R. J. Hill and W. Konigsberg, *J. Biol. Chem.*, **235**, PC21 (1960); *cf.* G. Braunitzer *et. al.*, *Hoppe-Seyler's Z. physiol Chem.*, **320**, 283 (1960).

(12) The reaction of hemoglobin with cyanogen chloride has been studied previously in another connection without suspicion of cleavage: W. N. Aldridge, *Biochem. J.*, **48**, 271 (1950).

(13) W. Konigsberg and R. J. Hill, personal communication.

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RECEIVED DECEMBER 15, 1960

α- AND β-VERBESINOL. SESQUITERPENE ALCOHOLS OF THE *cis*-DECALIN SERIES

Sir:

Continuous petroleum ether extraction of the freeze-dried root of *Verbesina virginica* L. ("ice plant" or "crownbeard") affords up to 4% of a mixture of two isomeric sesquiterpene esters (I), m.p. 116–122°, $[\alpha]^{16}D$ +49°, λ_{max}^{EtOH} 230, 314 mμ (ϵ 8,300, 18,200), $\lambda_{max}^{0.1N\ NaOH}$ 313, 367 mμ (ϵ 2,500, 26,000).[1] The methyl ethers of I, formed readily by treatment of I with dimethyl sulfate, gave rise to *p*-hydroxycinnamic acid under pyrolytic conditions. Dehydrogenation (Pd–C) afforded eudalene and β-(*p*-methoxyphenyl)-propionic acid.

(1) All compounds described gave satisfactory analytical data. Optical rotations were measured using chloroform as the solvent.