102-07-8; methyl iodide, 74-88-4; *p*-nitroaniline, 100-01-6; urea, 57-13-6; *p*-nitrophenyl isocyanate, 100-28-7; *N*-*p*-nitrophenyl-*N'*-*p*-methoxyphenylurea, 40387-34-6; *p*-anisidine, 104-94-9; *N*,*N'*-di(*p*-methoxyphenyl)-urea, 1227-44-7; *p*-methoxyphenyl isocyanate, 5416-93-3; phenylurea, 64-10-8; *N*-ethyl-*N*-*p*-nitrophenylcarbamoyl chloride, 34208-12-3; diethylamine, 109-89-7; *N*-ethyl-*N*-methoxycarbamoyl chloride, 40387-35-7.

Protection of Tryptophan with the Formyl Group in Peptide Synthesis[1]

DONALD YAMASHIRO AND CHOH HAO LI*

Hormone Research Laboratory, University of California, San Francisco, California 94122

Received March 15, 1973

N^{α}-*tert*-Butyloxycarbonyl-N^{i}-formyltryptophan has been synthesized and used for the solid-phase synthesis of the heptapeptide Gly-Ala-Arg-Gly-Ala-(formyl)Trp-Gly which was isolated in high yield. Removal of the formyl group in 0.01 *M* ammonium bicarbonate buffer of pH 9 was accompanied by an unexpected side reaction, but this could be greatly diminished by use of 1 *M* buffer. The overall yield of the deprotected heptapeptide was substantially higher than in a parallel synthesis where tryptophan was not protected. The formyl group can also be effectively removed with little side reaction in liquid ammonia containing hydroxylamine hydrochloride.

Synthesis of tryptophan-containing peptides has been handicapped by lack of a protecting group for the indole side chain. Destruction of tryptophan in synthesis has generally been regarded to occur during the acid treatments for removal of protecting groups. Butylation of the indole moiety during this step constitutes a serious danger.[2] Use of HCl–acetic acid together with mercaptoethanol as scavenger[3] have been recommended as deprotecting agent for removal of the N^{α}-Boc group,[4] but it has been reported recently that these tactics are ineffective in solid-phase peptide synthesis and lead to a heterogeneous product.[5] On the other hand, use of HCl–formic acid as deprotecting agent gave a nearly homogeneous peptide.[5] Since reversible modification of tryptophan with the formyl group has already been described,[6] the conclusion was reached that this protection might be suitable in peptide synthesis. We wished to explore this possibility under our synthetic conditions as part of efforts to develop a complete set of side-chain protecting groups for use with N^{α}-Boc protection in solid-phase synthesis of peptides.[7] We have now synthesized the model heptapeptide Gly-Ala-Arg-Gly-Ala-Trp-Gly (I) with and without formyl protection of tryptophan and have found the protection to be well suited for peptide synthesis. Removal of the formyl group led to unexpected side reaction but conditions were established to reduce this to a minimum.

One of the most reliable means for removal of the N^{α}-Boc group in solid-phase synthesis has been 50% trifluoroacetic acid in dichloromethane.[8,9] The reagent was not recommended for tryptophan-containing peptides.[3] We decided to synthesize peptide I with use of this reagent to ascertain the extent of the problem. Boc-Glycyl resin was prepared by a modified Loffet procedure.[10,11] N^{α}-Boc protection was used along with N^{G}-tosyl protection of arginine. Removal[12] of protecting groups and the solid support in HF gave a product which proved to be heterogeneous on gel filtration on Sephadex G-10 (Figure 1a). The fast-moving side-product (Figure 1a) was purified in carboxymethylcellulose[13] and gave an ultraviolet spectrum similar to that reported for peptides containing an altered tryptophan residue.[5] Peptide I required further purification on carboxymethylcellulose and by partition chromatography[14] on Sephadex G-25 before its isolation in highly purified form[15] (yield *ca.* 23% based on starting Boc-glycyl resin.).

It has been shown that N^{i}-formyltryptophan or a suitable derivative is stable in solution under the acidic and basic conditions used for solid-phase synthesis including treatment with HF.[5] We therefore decided to attempt synthesis and isolation of the formyl derivative of I, namely Gly-Ala-Arg-Gly-Ala-(formyl)Trp-Gly (II). For this purpose N^{α}-Boc-N^{i}-formyltryptophan was required. This derivative was prepared from N^{i}-formyltryptophan[5] by the dimethyl sulfoxide procedure[16] and isolated as its crystalline dicyclohexylamine salt. Its ultraviolet spectrum was in agreement with that expected for a derivative of N^{i}-formyltryptophan. Synthesis of II was carried out by procedures entirely analogous to those used for synthesis of I, including treatment of the finished peptide resin with HF. Gel filtration of the product on Sephadex G-10 gave a single peak (Figure 1b). Further chromatography on carboxymethylcellulose in which only a single peak was detected gave peptide II in highly purified

(1) This work was supported in part by the American Cancer Society, the Allen Foundation, and the Geffen Foundation.

(2) Yu. B. Alakhov, A. A. Kiryushkin, V. M. Lipkin, and G. W. A. Milne, *J. Chem. Soc. D*, 406 (1970).

(3) G. R. Marshall in "Pharmacology of Hormonal Polypeptides and Proteins," N. Back, L. Martini, and R. Paoletti, Ed., Plenum Press, New York, N. Y., 1968, p 48.

(4) Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **11**, 1726 (1972).

(5) M. Ohno, S. Tsukamoto, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Jap.*, **45**, 2852 (1972).

(6) A. Previero, M. A. Coletti-Previero, and J.-C. Cavadore, *Biochim. Biophys. Acta*, **147**, 453 (1967).

(7) D. Yamashiro, R. L. Noble, and C. H. Li in "Chemistry and Biology of Peptides, Proceedings of the Third American Peptide Symposium," J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 197.

(8) B. Gutte and R. B. Merrifield, *J. Amer. Chem. Soc.*, **91**, 501 (1969).

(9) S. Karlsson, G. Lindeberg, J. Porath, and U. Ragnarsson, *Acta Chem. Scand.*, **24**, 1010 (1970).

(10) A. Loffet, *Int. J. Protein Res.*, **3**, 297 (1971).

(11) D. Yamashiro and C. H. Li, *J. Amer. Chem. Soc.*, **95**, 1310 (1973).

(12) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jap.*, **40**, 2164 (1967); J. Lenard and A. B. Robinson, *J. Amer. Chem. Soc.*, **89**, 181 (1967); R. H. Mazur and G. Plum, *Experientia*, **24**, 661 (1968).

(13) E. A. Peterson and H. A. Sober, *J. Amer. Chem. Soc.*, **78**, 751 (1956).

(14) D. Yamashiro, *Nature (London)*, **201**, 76 (1964).

(15) When peptide I was synthesized with use of 1,2-ethanedithiol in the deprotecting agent the results were comparable to those indicated in Figure 1a.

(16) J. M. Stewart and J. D. Young, "Solid-Phase Peptide Synthesis," W. F. Freeman, San Francisco, Calif., 1969, p 29.

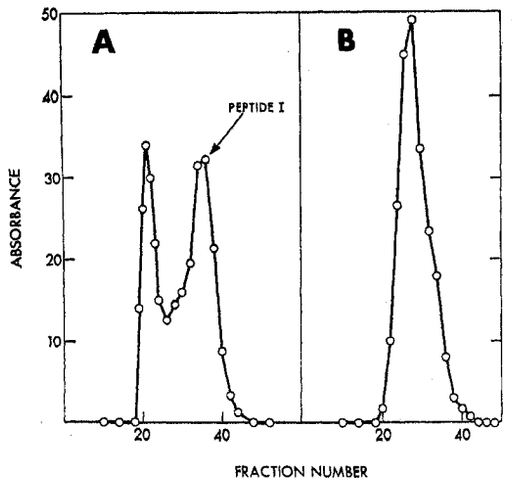


Figure 1.—Gel filtration on Sephadex G-10 in 1 *N* acetic acid: A, crude peptide I (280 nm); B, crude peptide II (300 nm). Column size, 1.37 × 42.3 cm.

form with a yield of about 88% (based on starting Boc-glycyl resin), substantially higher than in the synthesis of I. Its ultraviolet spectrum is characteristic of that of N^{i}-formyltryptophan and its derivatives.

Partition chromatography on Sephadex G-25 proved to be an effective means for the separation of peptides I and II, as shown in Figure 2. Since chromatography of II alone by this procedure gave only a single peak with no detectable trace of peptide I as contaminant, we concluded that the synthesis of II was achieved without loss of the formyl protecting group. Furthermore, the good yield and high degree of purity in which peptide II was secured show that the protected indole moiety was stable under the synthetic conditions employed.

The formyl group is removed under basic conditions and its course is conveniently followed[6] by disappearance of the strong 300 nm absorption. When peptide II was treated in 0.01 *M* NH_4HCO_3 buffer of pH 9 for 8 hr, partition chromatography of the product gave two peaks of similar size. In addition to the desired peptide I, a substantial side product was observed. Although this side product appeared on the chromatogram in the same position as peptide II, its ultraviolet spectrum was characteristic of tryptophan and it was clearly a new peptide. The amino acid composition of a toluenesulfonic acid hydrolysate[17] was identical with I. Its electrophoretic behavior was that of a substance less basic than either I or II. It gave positive reactions to Ehrlich and Sakaguchi reagents, but it was negative to ninhydrin. These results taken together indicated that the side product was identical with peptide I with the exception that the amino group was blocked. This would suggest that in the treatment of II at pH 9 the amino group can serve as nucleophile for removal of the formyl group.

Although it is evident that removal of the formyl protection might be effected without side reaction if the amino group were protected, we directed our efforts toward finding conditions for deprotection in the presence of the amino group. When peptide II was treated in 1 *M* NH_4HCO_3 buffer of pH 9 for 24 hr, partition chromatography of the product (Figure 3) showed

(17) T.-Y. Liu and Y. H. Chang, *J. Biol. Chem.*, **246**, 2842 (1971).

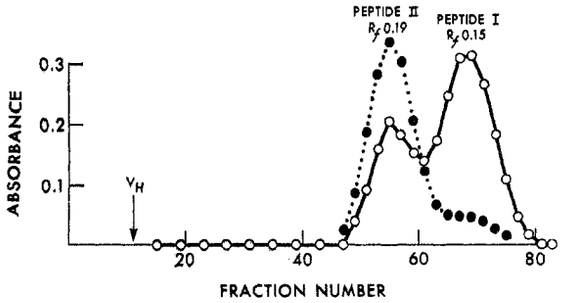


Figure 2.—Partition chromatography on Sephadex G-25 of a mixture of peptides I (1.87 mg) and II (2.00 mg) in solvent system B (see Experimental Section): 280 nm, O—O—O; 300 nm, ●···●···●; V_H = hold-up volume. Column size, 1.89 × 37 cm.

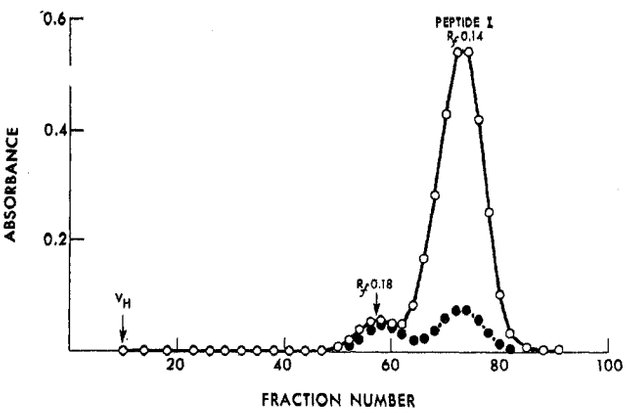


Figure 3.—Partition chromatography on Sephadex G-25 of peptide II after treatment with 1 *M* NH_4HCO_3 buffer of pH 9 for 24 hr: 280 nm, O—O—O; 300 nm, ●···●···●; V_H = hold-up volume; solvent system B. Column size, 1.89 × 37 cm.

very little side product (less than 8.5%) and the major product of deprotection was found to be identical with peptide I synthesized without protection of tryptophan. The overall yield of I through protection and deprotection of tryptophan was about 70% (based on starting Boc-glycyl resin), considerably higher than the yield where tryptophan was unprotected.

The formyl group can also be removed by treatment with liquid ammonia. However, peptide II again gave the aforementioned side product (*ca.* 19%) in addition to peptide I as the major product. When the reaction was carried out in the presence of added hydroxylamine hydrochloride the extent of side product was reduced to about 5%.

Experimental Section

Melting points were determined on a Fisher-Johns block and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. Thin layer chromatography was run on silica gel in 1-butanol–pyridine–acetic acid–water, 30:20:6:24 (BPAW). Chloromethylated styrene–divinyl benzene resin (Bio-Beads S-X-1, 200–400 mesh, 0.69 mmol/g, Bio-Rad Laboratories) served as starting material for synthesis.

Partition chromatography on Sephadex G-25 (100–200 mesh block polymerisate) was performed on a 1.89 × 37 cm column by procedures previously described.[14] Solvent systems used were 1-butanol–ethanol–0.2 *M* aqueous ammonium acetate (3:1:4, pH 7.6) (solvent system A) and the same system with pH adjusted to 6.2 with glacial acetic acid (solvent system B). Fractions of about 2.8–3.0 ml were collected and were appropriately diluted with 50% ethanol to measure absorbance at 280 or 300 nm. Isolation of peptide material was performed in

the usual manner with the exception that repeated lyophilization was required to remove ammonium acetate.

Carboxymethylcellulose chromatography was performed in a 1.23 × 44 cm column with an initial buffer of 0.01 *M* ammonium acetate of pH 5. After elution with 100 ml, a gradient with respect to pH and salt concentration was started by introducing 0.1 *M* ammonium acetate through a 500-ml mixing chamber containing starting buffer. In procedure A the 0.1 *M* buffer had pH 7.1, while in procedure B this buffer was adjusted to pH 6.3 with glacial acetic acid. Peptides were detected spectrophotometrically at either 280 or 300 nm.

N^{α}-*tert*-Butyloxycarbonyl-N^{i}-formyltryptophan Dicyclohexylamine Salt.—A mixture of N^{i}-formyltryptophan hydrochloride[5] (2.7 g, 10 mmol), diisopropylethylamine (6.0 ml, 35 mmol), and Boc-azide (15 mmol) was stirred in dimethyl sulfoxide (35 ml) overnight at room temperature. The resulting solution was diluted with cold water (175 ml) and washed with two 100-ml portions of ether. The aqueous layer was acidified with citric acid (15 g) with cooling, and the product was extracted with two 100-ml portions of ethyl acetate. The combined ethyl acetate layers were washed with three 50-ml portions of water and dried over anhydrous $MgSO_4$. Removal of drying agent and solvent gave 3.5 g of oil. Conversion to the crystalline salt was effected at 4° in anhydrous ether (30 ml) with dicyclohexylamine (2.25 ml), yield 4.1 g. For recrystallization a sample (1.03 g) was dissolved in $CHCl_3$ (4 ml) and evaporated *in vacuo* to an oil which was immediately taken up in anhydrous ether (10 ml) and stored at 4°; the product was collected and the process was repeated to give 0.78 g (60% yield), mp 121–124° dec, $[\alpha]^{24}D$ +35.5° (*c* 2, absolute ethanol).

Anal. Calcd for $C_{29}H_{43}N_3O_5$ (513.68): C, 67.81; H, 8.44; N, 8.18. Found: C, 67.57; H, 8.28; N, 8.20.

The ultraviolet absorption spectrum, taken at 270–330 nm in absolute ethanol, showed the characteristics of N^{i}-formyltryptophan and its derivatives.[5,6] The ratio of the absorbance at 300 nm to that at 280 nm was 1.59. For use in synthesis the free acid was obtained from the salt by standard procedures.

Solid-Phase Peptide Synthesis Procedures.—Boc-glycyl resin was prepared by a modification[11] of the Loffet method.[10] A sample of the resin deprotected and neutralized gave an amine content[18] of 0.40 mmol/g.

For synthesis of peptide I, Boc-glycyl resin (0.75 g, 0.30 mmol) was carried through the same schedule of operations recently described.[11] N^{α}-Boc protection was used throughout along with tosyl protection for the side chain of arginine. For the coupling of Boc-tryptophan, 5% DMF in CH_2Cl_2 was used as solvent. Yield of protected peptide I polymer was 0.97 g.

For synthesis of peptide II, Boc-glycyl resin (1.01 g, 0.40 mmol) was carried through the same aforementioned procedures with the exception that the coupling of Boc(formyl)tryptophan could be effected in CH_2Cl_2 alone. Yield of protected peptide II polymer was 1.34 g.

Peptide I.—Protected peptide I resin (505 mg) was treated with 15 ml of liquid HF for 30 min at 0° in the presence of anisole (0.5 ml). After removal of the HF the dried resin mixture was extracted with trifluoroacetic acid (10 ml) and filtered. The filtrate was evaporated *in vacuo* and after the addition of glacial acetic acid (10 ml) further evaporation gave an oily residue which was taken up in 1 *N* acetic acid (5 ml). The aqueous solution was washed with two 5-ml portions of ether, concentrated *in vacuo* to a smaller volume, and applied to a 1.37 × 42.3 cm Sephadex G-10 (40–74 μ) column. Elution with 1 *N* acetic acid and the collection of 1.53-ml fractions gave the results shown in Figure 1a. Isolation by lyophilization of the material represented by the slower moving peak (fractions 31–42) gave 63.2 mg. This material was submitted to chromatography on carboxymethylcellulose (procedure A); a large peak was detected (130 ml after buffer change) along with two minor peaks. Isolation of material represented by the large peak gave 35.5 mg. This material was resubmitted to chromatography on carboxymethylcellulose (procedure A) to give 32.1 mg. An aliquot (25.9 mg) of this material was subjected to partition chromatography on Sephadex G-25 in solvent system A to give a major peak with R_f 0.15 along with a minor peak with R_f 0.50. Isolation of material represented by the major peak gave 19.2 mg of highly purified peptide I (*ca.* 23% yield based on starting Boc-glycyl resin): tlc (BPAW) R_f 0.32; $[\alpha]^{24}D$ −59.5° (*c* 0.40, 0.5 *N* acetic acid).

Paper electrophoresis in pyridine acetate buffer (pH 3.7, 400 V, 4 hr) showed a single spot (ninhydrin and Ehrlich reagents) with R_f 0.56 relative to lysine. The ultraviolet spectrum, taken at 240–320 nm in 0.5 *N* acetic acid, showed the characteristics of a tryptophan spectrum. Amino acid analyses of a toluenesulfonic acid hydrolysate[17] and a leucine amino peptidase digest (pH 8, 24 hr, 37°) gave $Trp_{1.1}Arg_{0.9}Gly_{3.2}Ala_{2.0}$ for both.

Material represented by the faster moving peak in Figure 1a (fractions 19–25: isolated material, 29.1 mg) was submitted to chromatography on carboxymethylcellulose (procedure A). After elution with 300 ml of the 0.1 *M* buffer the gradient was increased by use of 0.2 *M* ammonium acetate. The major peak appeared 120 ml after this buffer change. The isolated peptide (17.1 mg) gave an ultraviolet spectrum, taken at 240–320 nm in 0.5 *N* acetic acid, with absorbance maximum at 284 nm, a minimum at 255 nm, and similar in appearance to that previously reported for peptides containing an altered tryptophan residue.[5]

Peptide II.—Protected peptide II resin (503 mg) was treated with HF and worked up in the same manner as just described for peptide I. Gel filtration of the product on Sephadex G-10 in the same manner gave a peak (fraction 26) with an apparent shoulder. All the detectable peptide material (300 nm) was isolated and resubmitted to gel filtration (Figure 1b) to give 111.3 mg (fractions 22–36). An aliquot (60.1 mg) of this material was subjected to chromatography on carboxymethylcellulose (procedure B) to give only one peak (135 ml after the buffer change) and isolation of material represented by this peak gave 49.7 mg of highly purified peptide II (*ca.* 88% yield based on starting Boc-glycyl resin): tlc (BPAW) R_f 0.32; $[\alpha]^{24}D$ −50.5° (*c* 0.46, 0.5 *N* acetic acid).

Paper electrophoresis in pyridine acetate buffer (pH 3.7, 400 V, 4 hr) showed a single spot (ninhydrin positive and Ehrlich negative) with R_f 0.52 relative to lysine. Partition chromatography of a sample (2.05 mg) on Sephadex G-25 in solvent system B gave one peak with R_f 0.19 and no detectable trace of material corresponding to the position of peptide I (R_f 0.15). The ultraviolet spectrum of II, taken at 270–330 nm in 0.5 *N* acetic acid, showed the characteristics of N^{i}-formyltryptophan and its derivatives. The ratio of the absorbance at 300 nm to that at 280 nm was 1.57. Amino acid analysis of a toluenesulfonic acid hydrolysate gave $Trp_{0.9}Arg_{1.0}Gly_{3.2}Ala_{2.2}$; removal of the formyl group under strong acidic aqueous conditions is known (see ref 6).

Conversion of Peptide II to Peptide I. Deprotection of Tryptophan.—A sample (31.4 mg) of peptide II from the Sephadex G-10 gel filtration step was dissolved in 9.0 ml of 1 *M* NH_4HCO_3 buffer of pH 9.1 and allowed to stand at 24° for 24 hr. The material was lyophilized three times to remove volatile salts and subjected to partition chromatography on Sephadex G-25 in solvent system B as shown in Figure 3. The side product with R_f 0.18, which appeared to be contaminated with a small amount of unreacted starting peptide II, represented about 8.5% of the total detectable material in the chromatogram. The R_f value of 0.14 for the large peak is in close agreement with the known position of peptide I (see Figure 2) and isolation of material corresponding to this peak (fractions 65–80) gave 19.9 mg of I (*ca.* 70% yield based on starting Boc-glycyl resin). This material was identical with that from the preparation of I described above on thin layer chromatography, paper electrophoresis, ultraviolet spectral analysis, and optical rotation. Amino acid analysis of a leucine amino peptidase digest gave $Trp_{1.0}Arg_{0.9}Gly_{3.1}Ala_{1.9}$.

Treatment of II on Dilute Buffer.—A sample (8.75 mg) of peptide II was treated in 10 ml of 0.01 *M* NH_4HCO_3 buffer of pH 9 until the absorbance at 300 nm had declined to 15% of its starting value (8 hr). The solution was lyophilized and subjected to partition chromatography on Sephadex G-25 in solvent system A. Two well-separated peaks of similar size were observed at 280 nm with R_f values of 0.19 and 0.15, the latter corresponding to peptide 1. Isolation of material corresponding to these peaks gave 3.3 mg for each.

The side product, which had R_f 0.19, exhibited the typical ultraviolet spectrum of tryptophan and gave positive responses to both Ehrlich and Sakaguchi reagents but negative to ninhydrin. Paper electrophoresis run under the same conditions as previously mentioned gave a major spot (Ehrlich) with R_f 0.2 relative to lysine and travelling slower than either I or II. Thin layer chromatography gave a major spot with R_f 0.42 (Ehrlich) and travelling slightly faster than either I or II. Amino acid analysis of a toluenesulfonic acid hydrolysate gave $Trp_{0.9}Arg_{0.9}Gly_{3.0}Ala_{2.0}$.

(18) B. F. Gisin, *Anal. Chim. Acta*, **58**, 248 (1972).

Treatment of II in Liquid Ammonia.—A sample (3.3 mg) of II was stirred in *ca.* 20 ml of liquid ammonia for 2 hr at −60°, after which time the solution was allowed to evaporate to dryness at the boiling point. Residual ammonia was removed *in vacuo* and the product was subjected to partition chromatography on Sephadex G-25 in solvent system B. Two peaks were detected at 280 and 300 nm with R_f values of 0.19 and 0.15, the latter being the major peak and corresponding to peptide I. The smaller peak with R_f 0.19 was apparently the same aforementioned side product and represented about 19% of the total detectable material on the chromatogram.

In a second run a sample (1.9 mg) of II was treated in the same manner with the exception that hydroxylamine hydrochloride (16.7 mg) was present. Partition chromatography in the same manner gave a major peak with R_f 0.15 corresponding to peptide I and a very small peak with R_f 0.19 corresponding to side product. The latter represented about 5% of the total detectable material in the two peaks.

Registry No.—N^{α}-*tert*-Butyloxycarbonyl-N^{i}-formyltryptophan dicyclohexylamine salt, 40463-72-7; N^{i}-formyltryptophan hydrochloride, 38023-86-8; peptide I, 40463-74-9; peptide II, 40463-75-0.

Acknowledgment.—We thank Mr. Kenway Hoey and Mr. W. F. Hain for their skilled technical assistance.

In Vitro Decomposition of *S*-Methylmethioninesulfonium Salts

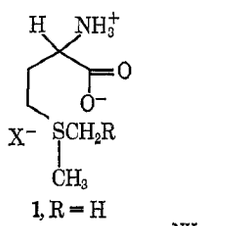
Fausto Ramirez,* Jeffrey L. Finnan, and Merle Carlson

Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11790

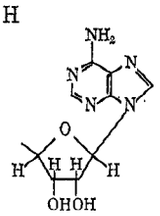
Received March 19, 1973

The *in vitro* decomposition of *S*-methylmethioninesulfonium salts (SMM) was studied in neutral, basic, and acidic aqueous solutions. The previously reported formation of dimethyl sulfide and homoserine (*via* its lactone) was verified. A new mode of self-destruction of SMM was discovered, *i.e.*, a nucleophilic attack by the dimethyl sulfide on one of the methyl groups of SMM with formation of trimethylsulfonium salt and methionine. The intermolecular demethylation of SMM was favored over the intramolecular decomposition to homoserine lactone with increasing acidity of the medium. Sodium thiosulfate effectively demethylates SMM in aqueous solution.

The *S*-methylmethioninesulfonium salts (SMM, **1**), the analogs of "active methionine" or *S*-adenosylmethioninesulfonium salts[1,2] (SAM, **2**), are of considerable interest biologically and medicinally. SMM is enzymatically synthesized from SAM and methionine in jack bean roots,[3] and can in turn be utilized as substrate by several methyl transferases.[4,5] SMM is widely distributed in nature and has been reported as a constituent of milk,[6] potatoes,[7] sweet corn,[8] soybean,[9] asparagus,[10] and cabbage.[11] Several reports have implicated SMM (vitamin U) in the prevention of ulcers of shay in rats,[12] of ulcers and of certain hepatic disorders in humans,[13] and of dietary hypercholesterolemia in rabbits.[14]



1, R = H



2, R =

In vitro syntheses of various SMM salts have been described,[15−17] and the pK values of the chloride have been measured.[18] McRorie, *et al.*,[11] reported the formation of homoserine (**5**, Scheme I) and of its lactone **3** (as hydroiodides) when an aqueous solution of SMM iodide was heated for 12 hr in an autoclave at unspecified temperatures. Challenger and Hayward[10] studied the decomposition of SMM in hot aqueous alkaline solution and reported the formation of dimethyl sulfide, homoserine, and methionine sulfoxide, which they regarded as the result of an oxidation of methionine. These authors[10] assumed that SMM decomposed by two paths: (1) formation of dimethyl sulfide and homoserine; (2) formation of methanol and methionine. Subsequently, Witkop and his coworkers[19] provided evidence for the initial formation of homoserine lactone (**3**) in the decomposition of SMM.

This paper describes an investigation of the behavior of SMM salts in aqueous solutions at neutral, basic, and acidic pH's, as a necessary first step in the elucidation of the much more complex behavior of SAM.

(1) S. K. Shapiro and F. Schlenk, "Transmethylation and Methionine Biosyntheses," The University of Chicago Press, Chicago, Ill., 1965.

(2) (a) H. R. Mahler and E. H. Cordes, "Biological Chemistry," 2nd ed, Harper and Row, New York, N. Y., 1971, pp 724, 806, 834; (b) p 366.

(3) R. C. Greene and N. B. Davis, *Biochim. Biophys. Acta*, **43**, 360 (1960).

(4) J. E. Turner and S. K. Shapiro, *Biochim. Biophys. Acta*, **51**, 585 (1961).

(5) S. H. Mudd, W. A. Klee, and P. D. Ross, *Biochemistry*, **5**, 1653 (1966).

(6) T. W. Keenan and R. C. Linsay, *J. Dairy Sci.*, **51**, 112 (1968); *Chem. Abstr.*, **68**, 38277n (1968).

(7) G. Werner, R. Hossli, and H. Neukom, *Lebensm.-Wiss. Technol.*, **2**, 145 (1969); *Chem. Abstr.*, **72**, 99338n (1970).

(8) D. D. Bills and T. W. Keenan, *J. Agr. Food Chem.*, **16**, 643 (1968); *Chem. Abstr.*, **69**, 85542a (1968).

(9) T. Hino, A. Kimizuka, K. Ito, and T. Ogasawara, *Nippon Nagei Kugaku Kaishi*, **36**, 314 (1967); *Chem. Abstr.*, **56**, 10889c (1961).

(10) F. Challenger and B. J. Hayward, *Chem. Ind. (London)*, 729 (1959).

(11) R. A. McRorie, G. L. Sutherland, M. S. Lewis, A. D. Barton, M. R. Glazener, and W. Shive, *J. Amer. Chem. Soc.*, **76**, 115 (1954).

(12) G. G. Vinci, *Boll. Soc. Ital. Biol. Sper.*, **35**, 1672 (1959); *Chem. Abstr.*, **54**, 11293c (1960).

(13) B. Colombo, *Minerva Med.*, 2944 (1960); *Chem. Abstr.*, **54**, 4881b (1960).

(14) (a) K. Nakamura and H. Ariyama, Tohoku *J. Agr. Res.*, **11**, 273 (1960); *Chem. Abstr.*, **55**, 7676d (1961); (b) *J. Agr. Res.*, **12**, 49 (1961); *Chem. Abstr.*, **55**, 27657d (1961); (c) *J. Arg. Res.*, **12**, 383 (1961); *Chem. Abstr.*, **57**, 5271b (1962).

(15) G. Toennies and J. J. Kolb, *J. Amer. Chem. Soc.*, **67**, 849 (1945).

(16) R. O. Atkinson and F. Poppelsdorf, *J. Chem. Soc.*, 1378 (1951).

(17) T. F. Lavine, N. F. Floyd, and M. S. Cammaroti, *J. Biol. Chem.*, **207**, 107 (1954).

(18) W. A. Klee and S. H. Mudd, *Biochemistry*, **6**, 989 (1967).

(19) W. B. Lawson, E. Gross, C. M. Foltz, and B. Witkop, *J. Amer. Chem. Soc.*, **84**, 1715 (1962).