addition of four volumes of benzene precipitated DNA from dimethylsulfoxide. Measured physical constants in buffer solution were $[\alpha]^{25}D + 135°$ and $[\alpha]^{25}_{436} + 270°$.

Pea microsomal RNA was prepared according to the method described previously.[8] Immediately after preparation it was quick-frozen in buffer solution and stored at $-50°$ until needed. Solutions in the two organic solvents could be prepared directly from the precipitated RNA (isolated from buffer solution by the addition of three volumes of cold ethanol and washed with absolute ethanol). Isolation from formamide solution was carried out by precipitation with two volumes of buffer and six volumes of ethanol at ice temperature. Last traces of solvent could be removed by repeated washing with absolute ethanol or ethyl ether or by redissolving the precipitate in buffer and separating it a second time by alcohol dilution. The addition of two parts of benzene and two parts of ethanol (ethyl ether may be substituted for benzene) brought about precipitation of RNA from dimethylsulfoxide. Measured physical constants were $[\alpha]^{25}D + 170°$ and $[\alpha]^{25}_{436} + 370°$.

The organic solvents were 99% formamide (Eastman White Label) and practical grade dimethylsulfoxide (Eastman) with a melting point of about 18°. The aqueous solvent was an 0.1 *M* acetate buffer at *p*H 5.5 containing 0.1 *M* sodium chloride and 0.001 *M* magnesium chloride.

Instrumentation and Methods of Analysis.—Measurements of optical rotations were made on a Rudolph Model 200S polarimeter with oscillating polarizer and xenon and mercury arc lamps. The polarimeter tubes were of unitized glass construction with water jacket, center-fill device and quartz windows. The windows were sealed to the optically ground tube ends with an epoxy resin, Epocast 502. Although some problem of strain birefringence may have been inherent in the system, it was possible to reproduce zero readings at various temperatures with pure solvents in the polarimeter tubes. In most of the measurements it was possible to duplicate results on the instrument to better than ± 2%.

Optical density measurements were made with a Beckman DK-2 ultraviolet spectrophotometer fitted with a modified temperature control device. Quartz cells were fitted with 20 mm. immersion, standard taper thermometers for direct reading of solution temperatures. The cell compartment cover was adapted to pass the calibrated portion of the thermometer.

Analysis for the concentrations of nucleic acids was made indirectly by the colorimetric phosphate determination described by Allen,[10] employing a Beckman DU spectrophotometer. Direct perchloric acid digestions were carried out only on the aqueous and formamide solutions. The thymus DNA was calculated to contain 9.30% phosphorus[11] and the microsomal RNA 9.09%, determined from the base-ratio analysis in our Laboratory.

Analytical ultracentrifugation was performed in the Model E ultracentrifuge, Spinco Division, Beckman, Inc., with phase-plate schlieren optics and within ultraviolet absorption optics. The instrument was provided with a temperature control system. The absorption patterns were taken on commercial film with a 20 second exposure time on solutions of O.D.^{260} of 1.0 in 12 mm. Kel-F cells. The photometric records were within the linear range of the characteristic curve of the film as shown by the trace of exponential aperture[12] in the counter balance cell in each run. The films were traced with a Double-Beam Recording Microdensitometer, Joyce Lobel Co., Newcastle upon Tyne, England. The direction of sedimentation of all the patterns is from left to right.

(10) R. J. L. Allen, *Biochem. J.*, **34**, 858 (1940).

(11) E. Chargaff in "Nucleic Acids," Vol. II, Chargaff and Davidson, Academic Press, Inc., New York, N. Y., 1955, p. 335.

(12) E. Robkin, M. Meselson and J. Vinograd, THIS JOURNAL, **81**, 1305 (1959).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK, N. Y.]

Synthesis of a Lysine-vasopressin Derivative with a Methyl Substituent on the Imino Nitrogen of the Peptide Bond between Lysine and Glycinamide (9-Sarcosine Lysine-vasopressin)[1]

BY JOHANNES MEIENHOFER AND VINCENT DU VIGNEAUD

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An analog of lysine-vasopressin has been synthesized in which the glycinamide residue in the side-chain is replaced by a sarcosinamide residue. The protected nonapeptide intermediate, which was reduced with sodium in liquid ammonia and then aerated to obtain the 9-sarcosine lysine-vasopressin, was itself prepared by the coupling of the appropriately protected pentapeptide and tetrapeptide derivatives with the use of dicyclohexylcarbodiimide. The nitrophenyl ester method was employed for the synthesis of the protected tetrapeptide used in the preparation of the nonapeptide intermediate. The 9-sarcosine lysine-vasopressin possessed 0.4–0.5 unit of pressor activity per mg. in contrast to the approximately 290 units per mg. of pressor activity exhibited by lysine-vasopressin. Moreover, the 9-sarcosine analog showed less than 0.01 unit per mg. of avian depressor activity and no detectable rat uterine-contracting activity. Thus it would appear that these pharmacological effects of lysine-vasopressin are either markedly decreased or eliminated entirely by the substitution of a methyl group for the hydrogen of the imino group of the terminal peptide bond in the side-chain of this hormone.

In the course of studies on the relation between structure and the biological activities of oxytocin and the vasopressins, an analog of lysine-vasopressin has been synthesized in which the glycinamide residue in position 9 is replaced by a sarcosinamide residue. The 9-sarcosine lysine-vasopressin (I) differs from lysine-vasopressin in the replacement of the hydrogen on the nitrogen of the terminal peptide bond of the side-chain by a methyl group.

H-CyS-Tyr-Phe-Glu(NH)$_2$-Asp(NH$_2$)-CyS-Pro-Lys-Sar-NH$_2$
(1) (2) (3) (4) (5) (6) (7) (8) (9)

9-Sarcosine Lysine-vasopressin

I

The synthesis of the protected nonapeptide intermediate for 9-sarcosine lysine-vasopressin, namely S-benzyl-N-tosyl-L-cysteinyl-L- tyrosyl - L - phenyl - alanyl-L-glutaminyl-L-asparaginyl-S-benzyl - L - cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylsarcosinamide, was carried out by coupling S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L- phenylalanyl - L - glutaminyl - L - asparagine[2] with S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylsarcosinamide by the use of dicyclohexylcarbodiimide[3] in 90% tetrahydrofuran–water.

(1) This work was supported in part by a grant (H-1675) from the National Heart Institute, U. S. Public Health Service. One of the authors (J.M.) is indebted to the Conference Board of Associated Research Councils (Washington) and the Fulbright Commission (Bad Godesberg, Germany) for a Fulbright Travel Grant.

(2) V. du Vigneaud, M. F. Bartlett and A. Jöhl, THIS JOURNAL, **79**, 5572 (1957).

(3) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).

Several approaches were investigated for the synthesis of the required protected tetrapeptide intermediate, S-benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylsarcosinamide, starting from sarcosine ethyl ester and involving conversion to the amide at various stages. The use of the nitrophenyl ester method involving stepwise lengthening of the chain[4] starting from carbobenzoxysarcosinamide (II) was found to be the most satisfactory. At each stage the carbobenzoxy group was removed by hydrogenation in the presence of palladium. Finally, the protected tetrapeptide was treated with hydrogen bromide in acetic acid and coupled with the protected pentapeptide. The resulting crude protected nonapeptide was subjected to reduction with sodium in liquid ammonia for removal of the tosyl and benzyl groups and to ring closure through the formation of the disulfide bond of the cystine residue by oxidation with air to obtain the 9-sarcosine lysine-vasopressin. A salt-free material was obtained after desalting of the crude product on an ion-exchange column (XE-64) in the H^+ form[5] according to the procedure described for the synthesis of lysine-vasopressin.[6]

The salt-free material was then chromatographed on an ion-exchange column of IRC-50 (XE-64) at *p*H 6.38 and the 9-sarcosine lysine-vasopressin was isolated from the main peak. The product gave a single spot upon electrophoresis on paper in pyridine–acetate buffer at *p*H 4.0 and a single symmetrical peak on analytical ion-exchange chromatography. It also appeared as a single spot on paper chromatography in butanol–acetic acid–water (4:1:5) and gave the expected results on elementary analysis and amino acid analysis on the starch column.

Assay of the 9-sarcosine lysine-vasopressin for pressor activity in the rat[7] showed it to possess 0.4–0.5 unit per mg., in contrast to the approximately 290 units per mg. of pressor activity exhibited by lysine-vasopressin. In the assay for avian depressor activity[8] less than 0.01 unit per mg. was obtained. The material showed no appreciable uterine-contracting activity on the isolated rat uterus.[9] Thus the replacement of the hydrogen on the imino group between the lysine and glycinamide residues in lysine-vasopressin by a methyl group produces a remarkable decrease in the pressor activity and eliminates the avian depressor and oxytocic effects. This profound effect is undoubtedly of considerable significance to the question of the relation of structure to the biological activity of lysine-vasopressin. The nature of the effect may become more apparent when information becomes available on the role that hydrogen bonding may play in the conformation of lysine-vasopressin and when the mechanism of action of the vasopressins is elucidated.

A marked loss in biological activity has also been observed when the hydrogen at the corresponding position in the oxytocin molecule is substituted by a methyl group.[10]

Experimental[11,12]

Carbobenzoxysarcosinamide (II).[10]—A solution of carbobenzoxysarcosine[13] (13.5 g.) in peroxide-free tetrahydrofuran (50 ml.) was cooled to −10°. Triethylamine (8.6 ml.) and isobutylchloroformate (8.45 g.) were added and the mixture was stirred at −10° for 15 minutes. Concentrated ammonia (6.7 ml.) was then added. After 15 hr. at room temperature the solvent was evaporated *in vacuo*. The substance was crystallized from ethanol–water; wt. 9.4 g. (70%), white needles, m.p. 82–83°.

N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysylsarcosinamide (III).—Carbobenzoxysarcosinamide (II, 13.1 g.) was dissolved in methanol (100 ml.) and palladium-black (freshly prepared from 3.0 g. of $PdCl_2$) was added. Hydrogen was passed through the solution which was rapidly stirred by a Vibro-Mixer.[14] After 3 hr. the catalyst was filtered off and the solvent was evaporated *in vacuo* leaving the free base as an oil which was dissolved in ethyl acetate (100 ml.). *p*-Nitrophenyl N$^{\alpha}$-carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysinate[15] (22.0 g.) was added. After 36 hr. at 35° the almost solid crystalline mass was filtered off and washed with boiling ethyl acetate. Recrystallization from ethanol (1 l.) gave 18.5 g. (92%), m.p. 172°, $[\alpha]^{20}D$ +4.5° (*c* 1, dimethylformamide); $[\alpha]^{20.5}D$ +10.0° (*c* 1, acetic acid).

Anal. Calcd. for $C_{24}H_{32}O_6N_4S$: C, 57.1; H, 6.38; N, 11.1; S, 6.36. Found: C, 56.9; H, 6.41; N, 11.0; S, 6.33.

Compound III was also prepared from the corresponding dipeptide ethyl ester, described in a subsequent section, by treatment with ammonia in ethanol for 48 hr. After recrystallization from ethanol III was obtained in 62% yield, m.p. 170–172°, $[\alpha]^{20.5}D$ +10.0 (*c* 1, acetic acid).

Carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylsarcosinamide (IV).—The free dipeptide base was prepared from III (10.1 g.) by hydrogenation as described in the preceding section. The resulting oil was dissolved in 5 ml. of dimethylformamide and 100 ml. of ethyl acetate. *p*-Nitrophenyl carbobenzoxy-L-prolinate[16] (7.41 g.) was added. After 36 hr. at 35° the solution was washed successively with 1 *N* ammonia (6 times), water, 1 *N* hydrochloric acid and water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* leaving an oil (12 g.) which could be crystallized only in small yield from ethyl acetate–hexane; wt. 4.9 g. (41%), m.p. 139–140°. The rest remained as an oil. A sample recrystallized from ethyl acetate melted at 140–141°, $[\alpha]^{20}D$ −35.5° (*c* 1, chloroform).

Anal. Calcd. for $C_{29}H_{39}O_7N_5S$: C, 57.9; H, 6.53; N, 11.6; S, 5.32. Found: C, 57.8; H, 6.67; N, 11.7; S, 5.34.

(4) M. Bodanszky, *Nature*, **175**, 685 (1955); M. Bodanszky, M. Szelke, E. Tömörkeny and E. Weiss, *Chem. and Ind. (London)*, 1517 (1955); M. Bodanszky and V. du Vigneaud, *Nature*, **183**, 1324 (1959).

(5) H. B. F. Dixon and M. P. Stack-Dunne, *Biochem. J.*, **61**, 483 (1955).

(6) J. Meienhofer and V. du Vigneaud, THIS JOURNAL, **82**, 2279 (1960).

(7) The pressor activity was determined by comparison with the U.S.P. Posterior Pituitary Powder ("The Pharmacopeia of the United States of America," 16th Revision, Mack Printing Co., Easton, Pa., 1960, p. 546). An assay using a 4 point design was employed (C. W. Emmens, "Hormone Assay," Academic Press, Inc., New York, N. Y., 1950, p. 18).

(8) The avian depressor assay was carried out by the method of Coon [*Arch. Intern. Pharmacodynamie*, **62**, 79 (1939)], as modified by Munsick, Sawyer and van Dyke [*Endocrinology*, **66**, 860 (1960)].

(9) The uterine-contracting activity was determined according to the method of Holton [*Brit. J. Pharmacol.*, **3**, 328 (1948)], as modified by Munsick [*Endocrinology*, **66**, 451 (1960)].

(10) W. D. Cash, D. E. Nettleton and V. du Vigneaud, unpublished experiments.

(11) Capillary melting points were determined and are corrected.

(12) The authors are indebted to Mr. Joseph Albert of this Laboratory for carrying out the elementary analyses, Mrs. Lorraine S. Abrash for performing the starch column chromatography and Miss Maureen O'Connell and Miss Dade W. Tull for the assay of the samples for biological activity.

(13) D. Ben-Ishai and E. Katchalski, THIS JOURNAL, **74**, 3688 (1952).

(14) Vibro-Mixer, Fisher Scientific Company.

(15) M. Bodanszky, J. Meienhofer and V. du Vigneaud, THIS JOURNAL, **82**, 3195 (1960).

(16) M. Bodanszky and V. du Vigneaud, *ibid.*, **81**, 5688 (1959).

Compound IV also was prepared from carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysine, described in a subsequent section, and sarcosinamide (prepared from II by treatment with 2 *N* hydrogen bromide in acetic acid) by the mixed anhydride method using isobutylchloroformate[17]; yield of crystalline material, 39%, m.p. 140–141°.

Compound IV was also prepared from the correponding tripeptide ethyl ester, described in a subsequent section, by treatment with ammonia in ethanol for 48 hr. Evaporation of the solvent left IV as an oil which could be crystallized from ethyl acetate–hexane in 31% yield, m.p. 136–138°.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylsarcosinamide (V). A. By Nitrophenyl Ester Method.—The free tripeptide base was prepared by hydrogenation of IV (3.3 g.) in the manner described for similar treatment of II. The resulting oil was dissolved in dimethylformamide (15 ml.) and *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate[18] (2.57 g.) was added. After 50 hr. at 35° ethyl acetate (300 ml.) was added and the solution successively with 1 *N* ammonia (5 times), water, 1 *N* hydrochloric acid and water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* leaving an oil which was crystallized from toluene; wt. 3.98 g. (93%), white needles, m.p. 117–118°, $[\alpha]^{22.5}D$ −20.0° (*c* 1, chloroform). After recrystallization of a sample from toluene, the m.p. and rotation remained unchanged.

Anal. Calcd. for $C_{39}H_{50}O_8N_6S_2$: C, 58.9; H, 6.33; N, 10.6; S, 8.06. Found: C, 58.6; H, 6.41; N, 10.5; S, 8.08.

B. By Mixed Anhydride Method.[17]—A solution of S-benzyl-N-carbobenzoxy-L-cysteine (790 mg.) in peroxide-free tetrahydrofuran (25 ml.) was cooled to −10°. Triethylamine (0.32 ml.) and isobutyl chloroformate (312 mg.) were added, and the mixture was stirred at −10° for 15 minutes. An ice-cold solution of the tripeptide base, which had been prepared from IV (1.3 g.) by hydrogenation according to the procedure already described, in tetrahydrofuran (15 ml.) was added over a period of 2 minutes. After 20 hr. at room temperature the triethylammonium chloride was filtered off and the solvent was evaporated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed successively with 5% sodium bicarbonate, water, 1 *N* hydrochloric acid and water and dried over magnesium sulfate. The solvent was evaporated *in vacuo* leaving an oil which was crystallized from toluene; wt. 1.38 g. (80%), m.p. 116–118°.

Carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysine.[19]—A solution of carbobenzoxy-L-proline (7.5 g.) in peroxide-free tetrahydrofuran (50 ml.) was cooled to −10°. Triethylamine (4.16 ml.) and isobutyl chloroformate (4.1 g.) were added and the mixture was stirred at −10° for 15 minutes. A solution of N$^{\epsilon}$-tosyl-L-lysine[19] (9.65 g.) and triethylamine (12.4 ml.) in water (30 ml.), cooled to the freezing point, was then slowly added to the mixed anhydride solution. After 15 minutes at −10° and 24 hr. at room temperature the solvent was evaporated *in vacuo* and the residue dissolved in chloroform. The solution was washed successively with 1 *N* hydrochloric acid and water and dried over magnesium sulfate. On evaporation of the solvent the product was obtained as a colorless oil which was crystallized from ethyl acetate–hexane; wt: 11.35 g. (78%), m.p. 110–113°. A sample for analysis was recrystallized from sodium bicarbonate solution by acidification with 2 *N* hydrochloric acid; m.p. 114–116°. $[\alpha]^{22}D$ −22.4° (*c* 1, ethanol); lit.[19] $[\alpha]^{21}D$ −22.6° (*c* 1, chloroform).

Anal. Calcd. for $C_{26}H_{33}O_7N_3S$: C, 58.7; H, 6.26; N, 7.91; S, 6.03. Found: C, 58.7; H, 6.30; N, 7.88; S, 5.99.

N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysylsarcosine Ethyl Ester.—A solution of N$^{\alpha}$-carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysine[19] (1.5 g.) in peroxide-free tetrahydrofuran (20 ml.) was cooled to −10°. Triethylamine (0.48 ml.) and isobutylchloroformate (0.47 g.) were added and the mixture was stirred at −10° for 15 minutes. A solution of ethyl sarcosinate in tetrahydrofuran (15 ml.) was prepared from the ester hydrochloride[20] (0.53 g.) and triethylamine (0.48 ml.) and was then added to the mixed anhydride. After 15 minutes at −10° and 3 hr. at room temperature the solvent was evaporated *in vacuo*. The resulting oil was dissolved in ethyl acetate. The solution was washed successively with 5% sodium bicarbonate, water, 1 *N* hydrochloric acid and water and dried over magnesium sulfate. On evaporation of the solvent the product was obtained as a colorless oil (1.2 g.). Compound III was prepared by amidation of this product as already described.

Carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylsarcosine Ethyl Ester.—A solution of carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysine (8.5 g.) in peroxide-free tetrahydrofuran (75 ml.) was cooled to −10°. Triethylamine (4.45 ml.) and isobutyl chloroformate (2.17 g.) were added and the mixture was stirred at −10° for 15 minutes. A solution of ethyl sarcosinate in tetrahydrofuran (25 ml.) was prepared from the ester hydrochloride (2.72 g.) and triethylamine (2.4 ml.) and was then slowly added to the mixed anhydride solution. The mixture was stirred for 30 minutes at −10° and for 5 hr. at room temperature. After the mixture was allowed to stand in the refrigerator for 40 hr., the product was isolated as a colorless oil (10.0 g.) by the procedure already described for the preparation of compound V. Compound IV was prepared by amidation of this product as already described.

Preparation of the Protected Nonapeptide Intermediate and Conversion to 9-Sarcosine Lysine-vasopressin (I).—The protected tetrapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylsarcosinamide (V, 1.75 g.) was treated for 1 hr. with 2 *N* hydrogen bromide in acetic acid at room temperature. The resulting hydrobromide was dissolved in water, potassium carbonate was added and the solution was washed 3 times with chloroform. The chloroform solution was dried over magnesium sulfate and the solvent was then evaporated *in vacuo* leaving an oil (1.3 g.) which was dissolved along with S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine[2] (1.85 g.) in 90% peroxide-free tetrahydrofuran–water (40 ml.) with slight warming. The solution was cooled to 0° and dicyclohexylcarbodiimide (1.2 g.) was added. The reaction mixture was stirred at 0° for 4 hr. and at room temperature for 80 hr. Acetic acid (0.3 ml.) was added to the thick mass which was then filtered off and the precipitate was washed twice with 90% tetrahydrofuran–water (20 ml.) and dried. From the mother liquor more material could be isolated after evaporation of the solvent by dissolving the residue in dimethylformamide and adding ethyl acetate. The combined crude product was washed thoroughly with ethanol in order to remove the N,N′-dicyclohexylurea. It was then purified by repeated precipitations from dimethylformamide–ethyl acetate and from acetic acid–ethanol with variable results in different preparations. In one experiment 1.05 g. of a white amorphous powder was obtainable with m.p. 211–215°.

This material (275 mg.) was dissolved in liquid ammonia (500 ml.) which had been distilled from sodium. Sodium (approximately 30 mg.) was added until a blue color persisted for 3 minutes. Acetic acid (0.3 ml.) was added and the ammonia was evaporated to 25 ml. The rest of the ammonia was removed by lyophilization on a water pump with a KOH drying jar between pump and flask. After 2 hr. the residue was washed with freshly distilled ethyl acetate (250 ml.) and then dissolved in 500 ml. of oxygen-free redistilled water at 0°. The *p*H of the resulting solution was 6.2. Air was passed through the solution for 2.5 hr. The *p*H was adjusted with acetic acid to 4.5 and the solution was passed through an Amberlite IRC-50 (XE-64) column (1.9 × 13.5 cm.) in the H^+ form for desalting.[5] After the column was washed with 0.25% acetic acid (400 ml.) and water (20 ml.) the peptide was eluted with a 30% pyridine–4% acetic acid solution. The eluate was lyophilized to a salt-free material (198 mg.) which possessed about 0.24 pressor unit per mg.

The material was purified by ion-exchange chromatography according to the procedure described previously.[6,21] A portion of the salt-free material (109 mg.) was dissolved in 0.5 *M* ammonium acetate buffer of *p*H 6.38 (18°) and

(17) J. R. Vaughan, Jr., and J. A. Eichler, THIS JOURNAL **75**, 5556 (1953).

(18) M. Bodanszky and V. du Vigneaud, *ibid.*, **81**, 2504 (1959).

(19) R. Roeske, F. H. C. Stewart, R. J. Stedman and V. du Vigneaud, *ibid.*, **78**, 5883 (1956).

(20) S. M. McElvain and P. M. Laughton, *ibid.*, **73**, 448 (1951).

(21) A. Light, R. Acher and V. du Vigneaud, *J. Biol. Chem.*, **228**, 633 (1957).

was placed on an IRC-50 (XE-64) column (1.9 × 43.3 cm.). The chromatogram was developed with the same buffer at room temperature with a flow rate of 5.5 ml. per hour. The tubes were analyzed by determination of the absorption at 275 mμ and the Folin–Lowry color reaction.[22] The contents of the tubes of the main peak were pooled and the solution was lyophilized three times to remove the ammonium acetate. From this peak, 38 mg. of 9-sarcosine lysine-vasopressin was obtained (35% based on the weight of material placed on the column). In another chromatogram the recovery in terms of weight was approximately 40%. The pressor activity of the purified 9-sarcosine lysine-vasopressin was 0.4–0.5 unit per mg.

Paper electrophoresis was performed with 0.1 *M* pyridine acetate buffer of *p*H 4.0 at 400 V. on Whatman No. 3 MM paper.[23] 9-Sarcosine lysine-vasopressin traveled as a single spot in the cathode direction at the same rate as lysine-vasopressin. Analytical ion exchange chromatography (5.99 mg., 0.9 × 12.7 cm. column of XE-64, 0.5 *M* ammonium acetate buffer of *p*H 6.38) gave one symmetrical peak. Paper chromatography with the solvent system butanol–acetic acid–water (4:1:5) showed the compound to travel as a single spot with the same R_F value (0.2) as lysine-vasopressin. Amino acid analysis of a hydrolysate on the starch column[24] gave the following amino acid content expressed in molar ratios (with the ratio of phenylalanine arbitrarily taken as 1): phenylalanine 1.0, tyrosine 1.0, proline 0.7, glutamic acid 1.0, aspartic acid + sarcosine 1.9,[25] lysine 0.7, cystine 0.8, ammonia 3.2.

A sample for analysis was dried at 100° for 8 hr. over P_2O_5.

Anal. Calcd. for $C_{47}H_{67}O_{13}N_{12}S_2 \cdot C_2H_4O_2$: C, 52.1; H, 6.33; N, 16.1. Found: C, 52.4; H, 6.32; N, 16.0.

(22) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

(23) S. P. Taylor, Jr., V. du Vigneaud and H. G. Kunkel, *ibid.*, **205**, 45 (1953).

(24) S. Moore and W. H. Stein, *ibid.*, **178**, 53 (1949).

(25) Aspartic acid and sarcosine emerge from a starch column as one single peak. The color yields of both amino acids are approximately the same.

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE, NEW YORK 21, N. Y.]

Synthesis of Structures Related to Bacitracin A

BY W. STOFFEL AND L. C. CRAIG

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When all the available evidence for the structure of bacitracin A is considered, the most probable one is that shown in Fig. 1.[1–4] It would seem most likely that a linear dodecapeptide is first synthesized by the organism and that two rings are then formed as shown in Fig. 2. The thiazoline ring is now well established,[3–6] but direct evidence for the linkage connecting the aspartyl-β-carboxyl group and the ε-amino-nitrogen of the lysine has not been obtained. However, if our earlier interpretation[7] of peptide 17 is the correct one, it is a tripeptide, His·Asp·Lys. Since a pentapeptide (Asp_2, Lys, Orn, Ileu) also was isolated and since the Lys·Orn and Ileu-α-amino-Lys linkages have been well established, there seems little doubt but that the second aspartic acid is attached to the first. It is the one found by Lockhart and Abraham[8] to be more easily split off on acid hydrolysis and to be dextrorotatory. The reduction experiments of Swallow and Abraham[9] further show that this aspartic acid carries the single amide group of bacitracin on its α-carboxyl.

Recently in this Laboratory[10] partial hydrazinolysis experiments to be reported soon have shown that the larger ring is easily split to liberate one of the amino groups of lysine. Since all of the amino acids originally present in bacitracin A still appear to be connected, the linkage split must be the ε-amino group of lysine known to be attached[8] to an aspartic acid. The β-carboxyl of an aspartic acid might be expected to be more easily split by hydrazine than the other amide linkages in bacitracin.

Interesting as these unusual linkages are they are no more so than the ones directly concerned with the thiazoline ring. Here it seemed wise to confirm certain of the proposed structures by synthesis. This paper will report the synthesis of a number of thiazoline derivatives and the required intermediates for these and still other thiazoline peptides. Since the thiazoline ring system has been postulated to be present in proteins[11] but not proved to be in any protein as yet, it seemed of considerable interest apart from the bacitracin problem to learn more of the requirements as regards substitution which would permit cysteine peptides to cyclize to the thiazoline ring and yet have sufficient stability to remain in this form.

The compounds synthesized are (I) 2-methylthiazoline and its methyl iodide derivative, (II) 2-(1-acetamino-2-methyl-propyl)-thiazoline, (III) ethyl-2-(1-acetamino-2-methyl-propyl)-4-carboxythiazoline, the dipeptides and their derivatives given in Table I and the tripeptides, pentapeptides and their derivatives given in Table II.

In the tables and the Experimental part which follow, the standard amino acid abbreviations are used. Other abbreviations used are: Z = carbobenzoxy, Bz = benzyl, Me = methyl, DCC = dicyclohexylcarbodiimide, Ac = acetyl.

The typical absorption spectrum of the thiazoline ring is well known[1,3] and is shown by bacitracin A in Fig. 3a. The latter has a similar extinction co-

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