the half wave potential is shifted to more negative voltage (Table II). At a *p*H of 7.2 there was no reduction of the oxide. 2-Azaadenine was, however, still being reduced at this *p*H.

Under electrolysis conditions, which were similar to those for the reduction of adenine 1-N-oxide, the oxide was reduced and the 2-azaadenine was identified by its ultraviolet spectra and paper chromatograms (Table I).

2,6-Diaminopurine at *p*H of 1.5 gave, as does adenine,[2] a well-defined reduction wave at −1.14 v. Its oxide showed a very small flow of current at this voltage, possibly attributable to the 2,6-diaminopurine present, but it was not otherwise reduced.

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

Synthesis of Lysine-vasopressin by the Nitrophenyl Ester Method[1]

BY MIKLOS BODANSZKY, JOHANNES MEIENHOFER AND VINCENT DU VIGNEAUD

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The protected nonapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide was prepared from ethyl glycinate by stepwise lengthening of the chain according to the nitrophenyl ester method. All the protected peptide intermediates were crystalline and the over-all yield was 50%. After removal of the protecting groups from the nonapeptide derivative and oxidation to the cyclic disulfide, lysinevasopressin was isolated and purified.

Lysine-vasopressin has already been synthesized in this Laboratory.[2,3] Recently, however, a simple and straightforward method was suggested for the preparation of long peptide chains.[4] This method, employing the *p*-nitrophenyl ester of the appropriately protected amino acid, was used for the synthesis of oxytocin[4] and has now been applied to the synthesis of lysine-vasopressin.

A new active ester, *p*-nitrophenyl N$^{\alpha}$-carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysinate (I), was prepared for this purpose. This ester was made to react with ethyl glycinate and the resulting protected dipeptide VIII was subjected to hydrogenolysis to remove the carbobenzoxy group. The free base, ethyl N$^{\epsilon}$-tosyl-L-lysylglycinate, was then brought into reaction with *p*-nitrophenyl carbobenzoxy-L-prolinate (II) to obtain the protected tripeptide IX. The protected tetrapeptide ester, ethyl S-benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinate (X), was prepared in a similar manner and transformed into the corresponding amide XI. The chain was then lengthened stepwise by successive reactions with the nitrophenyl esters of protected L-asparagine, L-glutamine, L-phenylalanine, O-benzyl-L-tyrosine and S-benzyl-L-cysteine. From the tetrapeptide through the octapeptide stage, HBr in acetic acid was used for the removal of the carbobenzoxy group. At the stage of the removal of the carbobenzoxy group from the protected octapeptide XV, the O-benzyl group of the tyrosine residue is also split off by HBr as found and previously reported in the synthesis of oxytocin by Bodanszky and du Vigneaud.[4] In the case of the protected nonapeptide, S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (XVI), all the protecting groups, including the N$^{\epsilon}$-tosyl group of the lysine residue, were removed in one operation by treatment with sodium in liquid ammonia. This reduction step was followed by aeration to form the cyclic disulfide, lysine-vasopressin. The synthesis is outlined in Chart I.

The hormone thus obtained was easily purified by ion-exchange chromatography. Electrophoresis or countercurrent distribution could be used for purification in place of the chromatography if so desired. However, we found the latter procedure to be more convenient and highly satisfactory. The efficiency of the method of synthesis presented is illustrated by the fact that yields in the eight peptide bond-forming steps were all over 80% and usually over 90%, and the over-all yield for these reactions plus the amidation of the tetrapeptide ester was approximately 50%. All the protected intermediates are crystalline. The synthesis was carried out on a scale of several grams through the preparation of the protected nonapeptide XVI, and the conversion of the latter compound to lysine-vasopressin was also carried out on a scale of more than one gram.

Experimental[5]

***p*-Nitrophenyl N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysinate (I).**—N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysine (152 g.) was dissolved in ethyl acetate (500 ml.) and *p*-nitrophenol (58.5 g.) was added. The solution was stirred and cooled to 0°. Dicyclohexylcarbodiimide (72 g.) was added. The reaction mixture was stirred for 0.5 hr. at 0° and for 1.5 hr. at room temperature. Glacial acetic acid (10 drops) was added and the N,N'-dicyclohexylurea was filtered off and washed 3 times with ethyl acetate. The solvent was evaporated *in vacuo* to a yellow oil (168 g.) which solidified after 10 minutes. The material was dissolved in hot ethanol (500 ml.) and crystallized as needles from the cooled solu-

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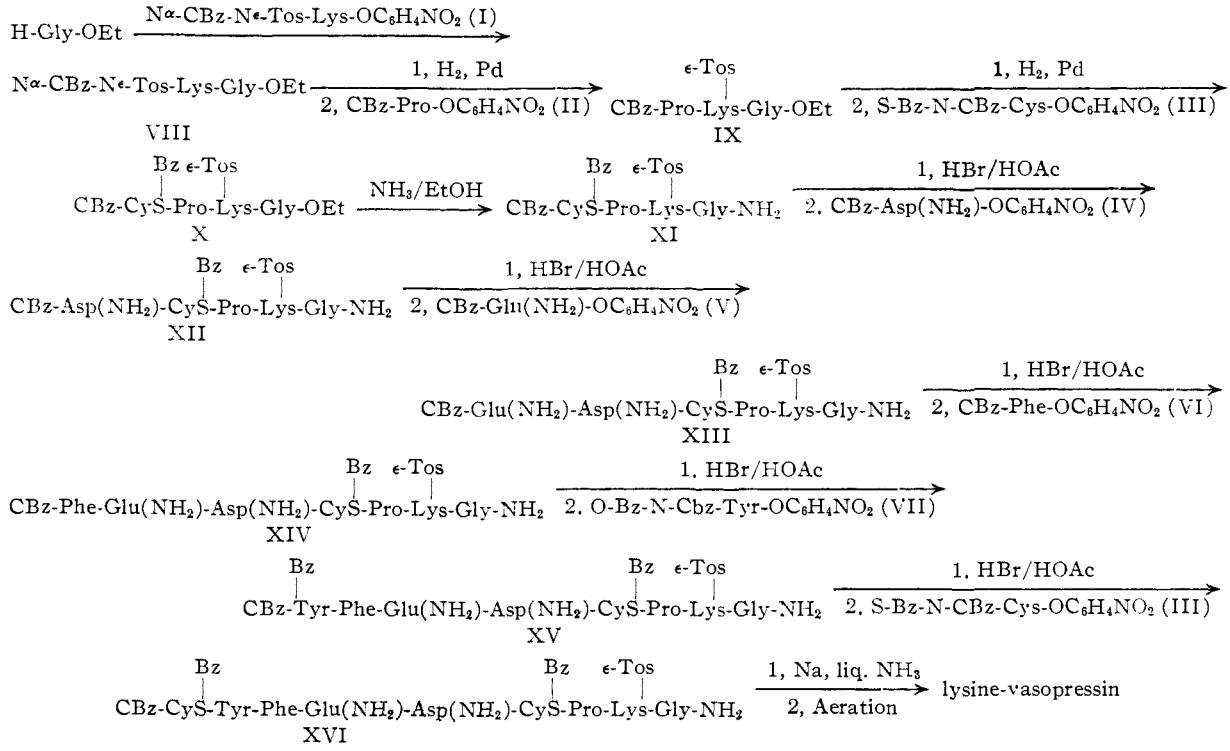
(2) M. F. Bartlett, A. Jöhl, R. Roeske, R. J. Stedman, F. H. C. Stewart, D. N. Ward and V. du Vigneaud, THIS JOURNAL, **78**, 2905 (1956); V. du Vigneaud, M. F. Bartlett and A. Jöhl, *ibid.*, **79**, 5572 (1957).

(3) J. Meienhofer and V. du Vigneaud, *ibid.*, **82**, in press (1960).

(4) M. Bodanszky and V. du Vigneaud, *Nature*, **183**, 1324 (1959); THIS JOURNAL, **81**, 5688 (1959).

(5) Capillary melting points were determined for all compounds and are corrected.

CHART I



tion. The product was filtered off, washed twice with ethanol and dried *in vacuo* over P_2O_5 and KOH; yield: 160.5 g. (82.5%), m.p. 109–110°, $[\alpha]^{20}D$ −16.5° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{27}H_{29}O_8N_3S$: C, 58.4; H, 5.26; N, 7.57. Found: C, 58.4; H, 5.44; N, 7.53.

Ethyl Nα-Carbobenzoxy-Nε-tosyl-L-lysylglycinate (VIII).—Ethyl glycinate hydrochloride (31 g.) was suspended in chloroform (300 ml.) and triethylamine (40 ml.) was added. A solution of I (111.2 g.) in chloroform (300 ml.) was added and the reaction mixture was kept at 35° for 28 hr. A part of the dipeptide derivative precipitated in crystalline form. It was filtered off and washed 3 times with cold ethanol. The mother liquor was concentrated *in vacuo* until a thick crystalline mass had precipitated. It was isolated as described for the first crop and the process repeated with the mother liquor; 98.6 g. of material was obtained in this way. Additional material could be isolated from the mother liquor after evaporation of the solvent *in vacuo* by dissolving the resulting oil in ethyl acetate and submitting the solution to extraction with 0.5 *N* ammonia (7 times), water, 1 *N* HCl and water, drying over $MgSO_4$ and concentration *in vacuo*. This material (3.5 g.) had to be recrystallized from ethanol–water before the m.p. and rotation were the same as those of the main product; yield: 101.6 g. (98%). white needles, m.p. 154–156°, $[\alpha]^{20}D$ −4.75° (*c* 2, $CHCl_3$); lit.[6]: m.p. 151.5–153.5°, $[\alpha]^{22}D$ −5.0° (*c* 1.7, $CHCl_3$).

Ethyl Carbobenzoxy-L-prolyl-Nε-tosyl-L-lysylglycinate (IX).—Compound VIII (97 g.) was suspended in methanol (1500 ml.) and palladium-black (freshly prepared from 10 g. $PdCl_2$) was added. Hydrogen was passed through while the mixture was stirred by a Vibro-Mixer.[7] After 15 minutes the protected dipeptide dissolved. After 90 minutes no more CO_2 evolved and the hydrogenation was stopped after 2 hr. The catalyst was filtered off and the solvent evaporated *in vacuo* leaving a colorless oil which was quickly dissolved in warm ethyl acetate (400 ml., 50°). Compound II (66.6 g.) was added followed by 10 drops of triethylamine. After 1 hr. the protected tripeptide ester started to crystallize and after 24 hr. at 35° the reaction mixture was cooled for 1.5 hr. in ice and the product was filtered off, washed 3 times with cold ethyl acetate and dried in air; wt.: 102 g., white needles, m.p. 152–153°, $[\alpha]^{20}D$ −56.0° (*c* 1, glacial acetic acid). From the mother liquor 2.5 g. of material with the same m.p. could be isolated; yield: 104.5 g. (94%); lit.[6]: m.p. 151–151.5°, $[\alpha]^{21}D$ −56.0 (*c* 1, glacial acetic acid).

Ethyl S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-Nε-tosyl-L-lysylglycinate (X).—Compound IX (1.23 g.) was hydrogenated in methanol (100 ml.) in the presence of palladium-black (from 0.5 g. $PdCl_2$). Evaporation of the solvent left a colorless oil which was dissolved in ethyl acetate (15 ml.). Compound III (0.933 g.) was added and the solution was kept at 35° for 2 days. It was extracted with 0.5 *N* ammonia (6 times), water, 1 *N* HCl and water and then dried over $MgSO_4$. The solution was concentrated *in vacuo* to a small volume and hexane added. The product precipitated in crystalline form; yield: 1.4 g. (86%). In a larger scale preparation (160 mmoles) in which regenerated $PdCl_2$ was used for preparing the catalyst, a yield of 105 g. (78.5%) was obtained after two recrystallizations from ethyl acetate–hexane and ethanol–water to remove colloidal palladium impurities; white needles, m.p. 108–110°, $[\alpha]^{20}D$ −25.3° (*c* 3, $CHCl_3$); lit.[6]: m.p. 106–109°, $[\alpha]^{21.5}D$ −26.0° (*c* 2.96, $CHCl_3$).

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-Nε-tosyl-L-lysylglycinamide (XI).—Compound X (38 g.) was dissolved in absolute ethanol (600 ml.). The solution was cooled to 0° and then saturated with ammonia. The flask was tightly stoppered and the solution kept at room temperature for 60 hr. The solvent was then removed *in vacuo* leaving an oil which was dissolved in warm ethyl acetate (300 ml.) and kept at 35° for 36 hr. The solvent partly evaporated during that time and the product crystallized. It was filtered off and washed with boiling ethyl acetate (500 ml.). From the mother liquors a second crop could be obtained; total yield: 33.4 g. (91%), white prisms, m.p. 130–132°, $[\alpha]^{20.5}D$ −29.0° (*c* 1, $CHCl_3$); lit.: m.p. 101–104°, $[\alpha]^{21}D$ −29.3° (*c* 1, $CHCl_3$),[6] m.p. 130–132°, $[\alpha]^{23}D$ −29.3° (*c* 1, $CHCl_3$).[8]

Carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nε-tosyl-L-lysylglycinamide (XII).—Compound XI (7.81 g.) was dissolved in acetic acid (35 ml.) and 3 *N* HBr in acetic acid (70 ml.) was added to the solution. After 1 hr. at room temperature ether (400 ml.) was added and the precipitate was washed on the filter with ether

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

(7) Vibro-Mixer, Fisher Scientific Company.

(200 ml.). The hydrobromide thus obtained was dissolved in water (650 ml.) and 0.5 *N* $KHCO_3$ (380 ml.) was added. The mixture was extracted with chloroform (250 ml. in four portions), the chloroform solution of the base was dried over $MgSO_4$ and the solvent was removed *in vacuo* leaving a syrup (7 g.) which was dissolved in dimethylformamide (20 ml.). Compound IV (4.7 g.) was added and a crystalline solid soon started to separate from the yellow solution. After 20 hr. at room temperature the semi-solid mass was triturated with ethyl acetate (300 ml.) and the product was washed on the filter with ethyl acetate (100 ml.), ethanol (100 ml.) and again with ethyl acetate (100 ml.); wt. 8.23 g. (92%), m.p. 196–199° (unchanged after recrystallization from dimethylformamide–ethanol), $[\alpha]^{20}D$ −41.6° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{42}H_{54}O_{10}N_8S_2$: C, 56.4; H, 6.08; N, 12.5. Found: C, 56.1; H, 6.12; N, 12.4.

Carbobenzoxy - L - glutaminyl - L - asparaginyl - S - benzyl - L - cysteinyl - L - prolyl - N$^{\epsilon}$ - tosyl - L - lysylglycinamide (XIII).—A suspension of XII (13.4 g.) in acetic acid (60 ml.) was treated with 4 *N* HBr in acetic acid (120 ml.). After 1 hr. at room temperature ether (600 ml.) was added to the solution and the precipitate was washed with ether (400 ml.). The hydrobromide, after being dried *in vacuo* over NaOH and $CaCl_2$ for a short time, was dissolved in dimethylformamide (75 ml.), the solution was cooled with ice water and triethylamine (7 ml.) was added, followed by V (7.2 g.). After about 24 hr. at room temperature the mixture was diluted with ethyl acetate (500 ml.) and the precipitate was washed with ethyl acetate (200 ml.) and ethanol (400 ml.). The air-dried product (15.5 g.) was dissolved in dimethylformamide (200 ml.) and precipitated in crystalline form by addition of ethyl acetate (1800 ml.). It was then washed with ethyl acetate (150 ml.) and ethanol (150 ml.) and dried *in vacuo* over P_2O_5; wt. 14.2 g. (93%), m.p. 201° (after sintering at about 187°), $[\alpha]^{20}D$ −41° (*c* 1, dimethylformamide).

For analysis, a sample was recrystallized from acetic acid–ethanol; m.p. 203° (s. 186°).

Anal. Calcd. for $C_{47}H_{62}O_{12}N_{10}S_2$: C, 55.2; H, 6.11; N, 13.7. Found: C, 54.9; H, 6.08; N, 13.6.

Carbobenzoxy - L - phenylalanyl - L - glutaminyl - L - asparaginyl - S - benzyl - L - cysteinyl - L - prolyl - N$^{\epsilon}$ - tosyl - L - lysylglycinamide (XIV).—A suspension of XIII (13.3 g.) in acetic acid (60 ml.) was treated with 4 *N* HBr in acetic acid (90 ml.). After 1 hr. at room temperature the hydrobromide was isolated and dried in the usual manner and then dissolved in dimethylformamide (60 ml.) and cooled in ice water. Triethylamine (12 ml.) was added, followed by VI (6.3 g.). After 2 days at room temperature acetic acid (6 ml.) and ethyl acetate (900 ml.) were added and the precipitate was washed with ethanol (450 ml.); wt. 15.0 g. Recrystallization from aectic acid–ethanol gave 12.5 g. (82%) of product, m.p. 205° (after sintering at 190°), $[\alpha]^{20}D$ −39° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{56}H_{71}O_{13}N_{11}S_2$: C, 57.5; H, 6.11; N, 13.2. Found: C, 57.2; H, 6.17; N, 13.1.

O-Benzyl-N-carbobenzoxy-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (XV).—A solution of XIV (4.7 g.) in acetic acid (30 ml.) was treated with 4 *N* HBr in acetic acid (30 ml.). After 1 hr. at room temperature the hydrobromide was isolated and dried. It was then dissolved in dimethylformamide (30 ml.), the solution was cooled to 0°, triethylamine (4 ml.) and VII (2.63 g.) were added and the mixture was allowed to stand at room temperature for 2 days. After the addition of acetic acid (6 ml.) the mixture was diluted with water (400 ml.) which caused the formation of a precipitate. This material was washed on the filter with water (200 ml.), ethanol (50 ml.), acetone (50 ml.) and warm ethyl acetate (100 ml.); wt. 5.7 g. The m.p. of 214–225° (after sintering at 207°) was raised by recrystallization from acetic acid–ethanol to 228–231° (s. 208°), $[\alpha]^{20}D$ −39° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{72}H_{86}O_{15}N_{12}S_2$: C, 60.7; H, 6.09; N, 11.8. Found: C, 60.4; H, 6.13; N, 11.7.

S - Benzyl - N - carbobenzoxy - L - cysteinyl - L - tyrosyl - L - phenylalanyl - L - glutaminyl - L - asparaginyl - S - benzyl - L - cysteinyl - L - prolyl - N$^{\epsilon}$ - tosyl - L - lysylglycinamide (XVI).—To a solution of XV (5.0 g.) in acetic acid 4 *N* HBr in acetic acid (30 ml.) was added, and the mixture was allowed to stand at room temperature for 1 hr. The hydrobromide was isolated, dried, dissolved in dimethylformamide (28 ml.) and cooled to 0°. Triethylamine (3.5 ml.) was added, followed by *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate (III, 1.95 g.). After 3 days at room temperature acetic acid (3 ml.) and water (500 ml.) were added. The precipitate was washed on the filter with water (250 ml.), a 1:1 mixture of acetone and ethyl acetate (50 ml.), warm ethyl acetate (200 ml.) and ethanol (50 ml.); wt. 5.3 g. (99%), m.p. 217–221° (unchanged after recrystallization from acetic acid–ethanol), $[\alpha]^{20}D$ −43° (*c* 1, dimethylformamide).[8]

Anal. Calcd. for $C_{75}H_{91}O_{16}N_{13}S_3$: C, 59.0; H, 6.01; N, 11.9. Found: C, 58.7; H, 6.02; N, 11.6.

Lysine-vasopressin.—Compound XVI (500 mg.) was dissolved in liquid ammonia (750 ml.), treated with sodium (80 mg.) and oxidized with air to the disulfide in the manner described previously.[3] The aqueous hormone solution containing 100,000 to 105,000 pressor units was desalted on an Amberlite IRC-50 (XE-64) column and 399 mg. of product with a pressor activity of 245 units per mg. was obtained.

The procedure was extended to a somewhat larger scale; 1.2 g. of protected nonapeptide was treated with sodium (190 mg.) in liquid ammonia (1 l.) and then aerated. The aqueous hormone solution contained 200,000 to 220,000 pressor units. After this solution was desalted on an XE-64 column (1.9 × 29 cm.), 958 mg. of material with a pressor activity of 220–240 units per mg. was obtained.

Purification of Lysine-vasopressin. A. By Electrophoresis on Powdered Cellulose.—The procedure followed that previously described for purification of natural lysine-vasopressin.[9] The material (120 mg., containing approximately 29,000 pressor units) was dissolved in 0.5 ml. of 0.1 *M* pyridine acetate buffer of *p*H 4 and placed 6.5 cm. from the anode end of a cellulose block[10] (46 × 10 × 1 cm.); 400 volts was applied at 0° for 66 hr. and the current of 40 mamp. at the beginning of the electrophoresis dropped to 20 at the end. From a print taken with a strip of filter paper and developed with bromophenol blue, it was found that the front of the material had traveled 22.5 cm. and the main band was 7 cm. wide. The portion of the block containing the hormone was cut into segments (1.25 cm.) and the solution pressed from each segment. The pressor activity and weight after lyophilization were determined for each segment and the values obtained are given in Table I.

TABLE I

ELECTROPHORESIS OF LYSINE-VASOPRESSIN ON CELLULOSE

Segment	Weight, mg.	Pressor activity, units/mg.
12	4.6	50
13	6.5	140
14	10.0	250
15	15.8	250
16	20.9	280
17	19.5	265
18	10.3	200

B. **By Countercurrent Distribution.**—The material possessing 245 pressor units per mg. was subjected to countercurrent distribution in 2-butanol–0.1% acetic acid.[9] The material which was isolated after 1000 transfers possessed 250–270 pressor units per mg.

C. **By Ion-exchange Chromatography.**[11]—The material (100 mg., containing approximately 24,000 pressor units)

(8) Since there was a considerable difference between the rotation of this compound and that of the corresponding ditosyl nonapeptide prepared previously (see refs. 2 and 3) by the dicyclohexylcarbodiimide method [J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955)], S-benzyl-N-tosyl-L-cysteine was coupled according to that method with the free base from XV. The resulting ditosyl nonapeptide gave, after purification, the same rotation, $[\alpha]^{22}D$ −24.5° (*c* 2, dimethylformamide), as found previously.

(9) D. N. Ward and V. du Vigneaud, *J. Biol. Chem.*, **222**, 951 (1956).

(10) Solka-Floc, SW-40-A, Brown Company, New York.

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

was dissolved in 1 ml. of 0.5 *M* ammonium acetate buffer of *p*H 6.38 (20°) and placed on an XE-64 column (1.9 × 43.5 cm.) which had been equilibrated with the same buffer. The chromatogram was developed with the buffer at room temperature with a flow rate of 3.5–4.5 ml. per hour. The volume of the fractions collected was 3.0 ml. The eluates were analyzed by determination of the absorption at 275 mμ, Folin–Lowry color reaction and pressor activity. The various fractions were combined as indicated in Table II, lyophilized 3 times to remove the ammonium acetate[11] and tested for pressor activity. The results are summarized in Table II. The recovery of pressor activity in these fractions was 80%, with 73% of the weight of the original material being present.

TABLE II

ION-EXCHANGE CHROMATOGRAPHY OF SYNTHETIC LYSINE-VASOPRESSIN

Tube no.	Weight, mg.	Pressor activity, units/mg.
47, 48	4.4	290
49	5.7	260–270
50–54	30.8	260–300
55–64	24.4	270
65–75	7.6	220

As much as 800 mg. of the material could be chromatographed on the same column without overloading it, with 81% of the pressor activity being recovered in material representing 74% of the weight placed on the column. The main fraction was desalted on an XE-64 column rather than by repeated lyophilization, which is not convenient for large amounts of material.

Paper electrophoresis[12] on Whatman No. 3 MM paper with 0.1 *M* pyridine acetate buffer of *p*H 4.0 at 400 volts showed the material containing 245 pressor units per mg. as well as the chromatographically purified preparations to travel as single spots. Paper chromatography was applied with the solvent system butanol–acetic acid–water (4:1:5) on Whatman No. 1 paper and again single spots were obtained. Starch column analysis[13] of hydrolysates showed the following amino acid content (with the ratio for glycine arbitrarily taken as 1 and the values of the material before chromatography given in parenthesis): phenylalanine 0.9 (1.0), tyrosine 0.7 (0.8), proline 0.8 (0.7), glutamic acid 1.0 (0.9), aspartic acid 1.0 (0.9), glycine 1.0, lysine 1.0 (0.8), cystine 0.9 (0.9) and ammonia 3.5 (3.0).

The specific rotation was $[\alpha]^{22}D$ −23.8° (*c* 0.5, 1 *N* acetic acid). For analysis, the compound was dried at 100° for 8 hr. over P_2O_5.

Anal. Calcd. for $C_{46}H_{65}O_{12}N_{13}S_2 \cdot C_2H_4O_2$: C, 51.6; H, 6.23; N, 16.3. Found: C, 51.9; H, 6.25; N, 16.4.

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[CONTRIBUTION FROM THE LABORATORIES OF THE DIVISION OF NUCLEOPROTEIN CHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH AND THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK 21, NEW YORK]

Physicochemical Effects of High-speed Mixing on Deoxyribonucleic Acid[1]

BY HERBERT S. ROSENKRANZ[2] AND AARON BENDICH

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Brief high-speed mixing of DNA solutions results in a decrease of the sedimentation coefficient of the sample as well as a narrowing of the distribution of sedimentation coefficients. This is interpreted as being due to scission of those DNA macromolecules above a certain size ($S > 26$). This behavior is not accompanied by significant denaturation.

Introduction

It is known that changes in macromolecular configuration occur when deoxyribonucleic acid (DNA) is subjected to a variety of physical treatments such as X-irradiation,[3–6] ultrasonic[7,8] and sonic vibrations[8–12] and shear.[13,13a] Since high-speed mixing or mincing is very frequently part of the several procedures employed in the isolation of DNA (see ref. 14 for review on methods for DNA isolation), it was considered of interest to determine whether or not such a treatment would affect the DNA.

There are reports which indicate that degradation may result when proteins are subjected to high-speed mixing.[15–17] Chargaff[18] has cautioned against the use of high-speed mixing in the preparation of DNA, and it may be deduced from the work of several laboratories[19–21] that DNA is indeed affected

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(2) Taken from the thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Medical Sciences of Cornell University, New York, N. Y., February 1959.

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