[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Preparation of Lysine-vasopressin through a Crystalline Protected Nonapeptide Intermediate and Purification of the Hormone by Chromatography[1]

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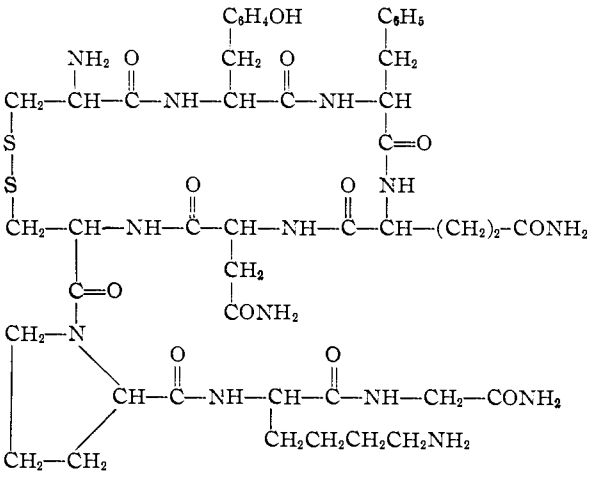
The protected nonapeptide intermediate used in the synthesis of lysine-vasopressin, namely S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide, has been obtained in crystalline form. It was prepared by the coupling of the pentapeptide S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine with the tetrapeptide S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide according to the N,N′-dicyclohexylcarbodiimide method. Various improvements in the synthesis of these two peptides have been presented. The synthetic lysine-vasopressin obtained by removal of the protecting groups from this intermediate and closure to the disulfide ring possessed a pressor activity closely approximating that of natural lysine-vasopressin. Further purification was accomplished by use of ion-exchange chromatography. The procedures presented for the synthesis of lysine-vasopressin, particularly with the obtaining of the protected nonapeptide in crystalline form, and for the purification of the hormone provide a means for the preparation of highly active lysine-vasopressin in relatively large amounts.

In an earlier communication from this Laboratory[2] a synthesis of lysine-vasopressin (I) was presented in which the intermediate protected nonapeptide S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide



I

was obtained in amorphous form by the coupling of the pentapeptide S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine[2] with the tetrapeptide S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide[3] according to the N,N′-dicyclohexylcarbodiimide method.[4] After cleavage of the protecting groups with sodium in liquid ammonia and conversion of the sulfhydryl nonapeptide to the disulfide form by aeration, the aqueous hormone solution was concentrated and lyophilized to give crude salt-containing material. The laborious purification procedure involved countercurrent distribution and electrophoresis. Based on the pressor activity obtained initially following aeration, the losses incurred in purification of the material ranged from 55 to 75%, and the scale employed for the electrophoresis yielded 50–60 mg. of purified material from any one experiment. We have restudied the synthesis and purification of lysine-vasopressin with the aim of finding methods which would give higher yields and allow the isolation of the hormone on a larger scale.

In the preparation of the pentapeptide, which followed essentially the procedure previously described,[2] we found that the use of S-benzyl-N-tosyl-L-cysteinyl-L-tyrosine methyl ester was preferable to that of the corresponding ethyl ester since the former compound was more easily crystallized and purified. In the synthesis of the protected tetrapeptide, S-benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (V),[3] the overall yield was increased from 47 to 68% (based on the protected dipeptide N$^{\alpha}$-carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysylglycine ethyl ester (II)) by using hydrogenation in the presence of palladium-black for the removal of the carbobenzoxy groups rather than hydrogen bromide in glacial acetic acid and by preparing the tripeptide intermediate III by the mixed anhydride procedure with isobutyl chloroformate[5] and the protected tetrapeptide V by the nitrophenyl ester method.[6] In addition, the conversion to the amide was performed at the tripeptide stage and the protected tripeptide amide IV, obtained in high yield and crystalline form (prisms), was then decarbobenzoxylated and used directly in the coupling reaction to prepare V. The series of reactions which was found to be most satisfactory for the synthesis of lysine-vasopressin (I) is given in Chart I.

The protected nonapeptide VI was prepared by the dicyclohexylcarbodiimide method in 90% tetrahydrofuran–water.[2,7] We found that an amorphous sample of this nonapeptide could be purified by repeated precipitations from dimethylformamide–ethyl acetate and then crystallized from dimethylformamide–formic acid. In the present experiments the amorphous nonapeptide amide was crystallized directly from dimethylformamide–

(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 to the Fulbright Commission in Germany (Bad Godesberg) for a Fulbright Travel Grant.

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

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

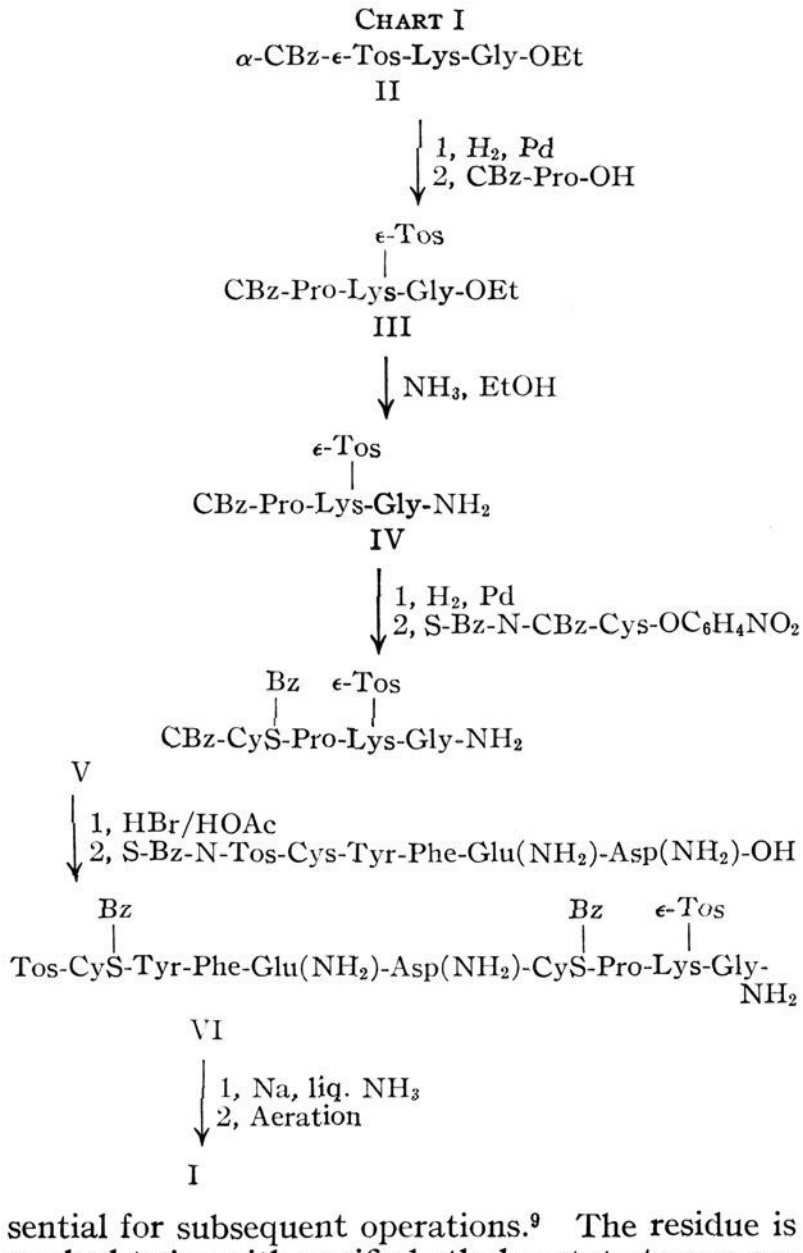
(5) J. R. Vaughan, Jr., and J. A. Eichler, *ibid.*, **75**, 5556 (1953).

(6) M. Bodanszky, *Nature*, **175**, 685 (1955); M. Bodanszky, M. Szelke, E. Tömörkeny and E. Weisz, *Chemistry and Industry*, 1517 (1955).

(7) Couplings with the use of *o*-phenylene chlorophosphite or carbodiimide in dimethylformamide gave lower yields.

Fig. 1.—Crystals of the protected nonapeptide, S-benzyl-*N*-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-glycinamide (VI) (×380).

CHART I



formic acid. It forms long needles as shown in Fig. 1. The yield in the coupling reaction forming the nonapeptide was raised from 39% of amorphous product[2] to 56% of crystalline material by increasing the amount of carbodiimide up to three equivalents, applying longer reaction times (74 hr.) and allowing the nonapeptide to crystallize slowly from the dimethylformamide–formic acid solution of the amorphous material.

Crystallization of this intermediate proved to be of great value in the synthesis of the hormone, since the yield and purity of the lysine-vasopressin obtained after cleavage of the protecting groups and aeration were found to depend greatly on the degree of purity of the protected nonapeptide. Recrystallized material yielded, for each mg., approximately 200 units of pressor activity.[8]

For the preparation of a highly active vasopressin from the nonapeptide in high yield, it is essential to take several precautions. The liquid ammonia should be as anhydrous as possible and is distilled from sodium. The sodium for the cleavage reaction should be added in small quantities over a period of 20 to 40 minutes. After the cleavage most of the ammonia is removed by evaporation under atmospheric pressure and the rest is then evaporated from the frozen state under reduced pressure. In this manner the resulting dry residue is obtained as a loose, porous powder, which is essential for subsequent operations.[9] The residue is washed twice with purified ethyl acetate to remove the thiocresol formed during the cleavage. The product is then dissolved as quickly as possible in oxygen-free redistilled water at 0°, to give a 0.05–0.1% solution which is oxidized with air after adjustment of the *p*H to 6.5–6.8. Approximately 100,000 units of pressor activity was obtained from 500 mg. of VI. The hormone was adsorbed on an Amberlite IRC-50 (XE-64) column for desalting.[10] It was eluted with pyridine acetate and lyophilized to give, from 500 mg. of VI, about 400 mg. of a salt-free material with a pressor activity of approximately 250 units per mg. at this early stage of purification. This potency compares favorably with that of highly purified natural lysine-vasopressin.[11] In several experiments the recovery of activity in this step was between 95 and 100%.

Further purification was performed by ion-exchange chromatography on IRC-50 (XE-64) with 0.5 *M* ammonium acetate buffer.[11] Material having 275–300 pressor units per mg. was obtained. The recovery of activity from such a chromatographic purification in several experiments was approximately 80% and the recovery in terms of weight, 73%. The coincidence of the curves plot-

(8) All assay values reported herein are based on the U. S. Pharmacopeia Posterior Pituitary Standard Powder, as outlined in "The Pharmacopeia of the United States of America," 15th revision, second supplement, Mack Printing Co., Easton, Pa., 1959, p. 8.

(9) If the residue is not in a loose form, the bringing of the material into solution requires a long time (5 to 10 minutes) and a considerable amount of insoluble material is formed with a consequent marked loss in yield.

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

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

ted from the Folin–Lowry color, ultraviolet absorption and pressor assay values determined on the chromatographic fractions indicated that the material was a single compound. The homogeneity of the purified lysine-vasopressin was further checked by paper chromatography and paper electrophoresis, and starch column and elementary analyses gave the expected values.

Experimental[12,13]

S-Benzyl-N-tosyl-L-cysteinyl-L-tyrosine Methyl Ester.—S-Benzyl-N-tosyl-L-cysteine[2] (51 g.) and methyl L-tyrosinate[14] (28.5 g.) were dissolved in peroxide-free tetrahydrofuran (300 ml.). The solution was cooled in ice and N,N′-dicyclohexylcarbodiimide (28.5 g.) was added with stirring. Stirring was continued overnight at 5°. The N,N′-dicyclohexylurea was then filtered off, the filtrate was concentrated *in vacuo* and the residual oil was dissolved in ethyl acetate. This solution was washed successively with 5% sodium bicarbonate, water, 1 *N* hydrochloric acid and water and then dried over magnesium sulfate. After the solution was concentrated to a small volume the product crystallized as needles. It was filtered and washed with cold ethyl acetate; wt. 65 g. From the mother liquor an additional 1.3 g. was obtained by the addition of hexane. Recrystallization from methanol–water gave 64 g. (84%) of white needles, m.p. 139–140°, $[\alpha]^{19.5}D$ +7.8° (*c* 2.5, ethanol).

Anal. Calcd. for $C_{27}H_{30}O_6N_2S_2$: C, 59.8; H, 5.57; N, 5.17; S, 11.8. Found: C, 59.8; H, 5.57; N, 5.22; S, 11.8.

N$^{\epsilon}$-Tosyl-L-lysine Copper Complex.—The copper complex was prepared following essentially the procedure described by Roeske, *et al.*[3] With longer reaction times (15–20 hr.) higher yields (78–82%) were obtained. The blue powder could be isolated in analytically pure form, m.p. 241–243° dec., lit.,[3] m.p. 238–240° dec.

Anal. Calcd. for $C_{26}H_{38}O_8N_4S_2Cu$: C, 47.2; H, 5.78; N, 8.46; S, 9.68; CuO, 12.0. Found: C, 46.9; H, 5.81; N, 8.46; S, 9.52; CuO (residue), 12.2.

Carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycine Ethyl Ester (III).—N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysylglycine ethyl ester[3] (II, 30 g.) was suspended in methanol (1 l.) and palladium-black (freshly prepared from 3 g. $PdCl_2$) was added. Hydrogen was passed through the solution which was rapidly stirred by a Vibro-Mixer.[15] After 30 minutes the carbobenzoxy compound had been dissolved completely. After 2.5 hr. more palladium-black (from 1 g. $PdCl_2$) was added. After 5 hr. the hydrogenation was completed and the catalyst filtered off. The solvent was evaporated *in vacuo* leaving the free base as an oil, which often crystallized in needles. It was immediately dissolved in peroxide-free tetrahydrofuran (100 ml.) and used directly for the preparation of the tripeptide. A solution of carbobenzoxy-L-proline (14.5 g.) in peroxide-free tetrahydrofuran (100 ml.) was cooled to −10°. Triethylamine (8.05 ml.) and isobutyl chloroformate (7.95 g.) were added, and the mixture was stirred at −15° for 15 minutes. The ice-cold solution of the dipeptide ester was then added over a period of 2 minutes and the mixture was stirred at −10° for 10 minutes when the reaction product crystallized out. After 15 hr. at room temperature the almost solid mixture was poured into water (2 l.), and the precipitate was filtered off and washed successively with water, 5% sodium bicarbonate, water, 1 *N* hydrochloric acid and water and dried over P_2O_5 and KOH; wt. 34.5 g. The crude product was recrystallized from ethanol–water; yield 33 g. (92.5%, based on the protected dipeptide), m.p. 152–153°. Recrystallization from ethyl acetate raised the melting point to 153–154°, $[\alpha]^{20.5}D$ −57° (*c* 1, glacial acetic acid); lit.,[3] m.p. 151–151.5°, $[\alpha]^{21}D$ −56° (*c* 1, glacial acetic acid).

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

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

(14) E. Fischer, *Ber.*, **41**, 855 (1908).

(15) Vibro-Mixer, Fisher Scientific Company.

Carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (IV).—This amide was prepared by the method of Roeske, *et al.*,[3] and obtained as white prisms in 87% yield, m.p. 184–185°, $[\alpha]^{20.5}D$ −33.0° (*c* 1, dimethylformamide); lit.,[3] m.p. 183–185°.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (V). A. By Nitrophenyl Ester Method.—The protected tripeptide amide IV (4.7 g.) was dissolved in methanol (200 ml.) and palladium-black (freshly prepared from 1 g. $PdCl_2$) was added. Hydrogen was passed through while the mixture was stirred with a Vibro-Mixer. After 2 hr. the catalyst was filtered off and the solvent was evaporated *in vacuo* leaving the free base as a colorless oil which was immediately dissolved in peroxide-free tetrahydrofuran (35 ml.). *p*-Nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate[16] (3.73 g.) was added. After 3 days at room temperature the solvent was evaporated *in vacuo*. The residual oil was dissolved in ethyl acetate and the solution was washed successively with 5% ammonia (6 times), water, 1 *N* hydrochloric acid and water and dried over magnesium sulfate. The solvent was allowed to evaporate slowly at 35° and the reaction product crystallized. It was purified by several washings with boiling ethyl acetate (100 ml.); yield 5.25 g. (84.5%, based on the protected tripeptide IV), m.p. 130–132°, $[\alpha]^{23}D$ −29.3° (*c* 1, chloroform); lit.,[3] m.p. 101–104°, $[\alpha]^{21}D$ −29.3° (*c* 1, chloroform).

Anal. Calcd. for $C_{38}H_{48}O_8N_6S_2$: C, 58.5; H, 6.19; N, 10.8; S, 8.21. Found: C, 58.4; H, 6.34; N, 10.7; S, 8.13.

B. By Mixed Anhydride Method.—The free base was prepared by hydrogenation of IV (18 g.) as described in section A and dissolved in peroxide-free tetrahydrofuran (100 ml.). A solution of S-benzyl-N-carbobenzoxy-L-cysteine[17] (12.9 g.) in peroxide-free tetrahydrofuran (50 ml.) was cooled to −10°. Triethylamine (5.2 ml.) and isobutyl chloroformate (5.12 g.) were added and the mixture was stirred at −12° for 15 minutes. The ice-cold solution of the tripeptide amide was then added over a period of 2 minutes and the mixture was stirred at −10° for 10 minutes. After 15 hr. at room temperature the triethylammonium chloride was filtered off and the solvent was evaporated *in vacuo*. The resulting colorless oil was dissolved in ethyl acetate and washed successively with 5% sodium bicarbonate, water, 1 *N* hydrochloric acid and water, and then dried over magnesium sulfate. The product was crystallized and purified as described in section A; yield 16.5 g. (69%, based on IV), m.p. 127–130°.

S-Benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide (VI).—S-Benzyl-L-cysteinyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysylglycinamide[3] was prepared from V (1.72 g.). The resulting oil (1.3 g.) was dissolved along with S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine[2] (1.85 g.) in peroxide-free 90% tetrahydrofuran–water (45 ml.) with slight warming. The solution was cooled to 0° and N,N′-dicyclohexylcarbodiimide (1.2 g.) was added. The reaction mixture was stirred under cooling with ice for 4 hr. and at room temperature for 70 hr. Acetic acid (0.3 ml.) was added to the thick mass followed by ethyl acetate (400 ml.). The precipitate was filtered off, washed successively with ethyl acetate (100 ml.) and methanol (200 ml.) and dried in air; yield of amorphous product: 1.96 g. (63.5%), m.p. 225–226° dec.

For crystallization the material was dissolved in 15 ml. of purified dimethylformamide containing 1% formic acid–water (90%) and kept in an open crystallizing dish at room temperature for several days. The crystalline material was filtered off, washed twice with cold dimethylformamide and then with ethyl acetate and dried over P_2O_5 and KOH; yield: 1.73 g. (56%), m.p. 228–230°, dec. at 233°, $[\alpha]^{20.5}D$ −24.0° (*c* 2, dimethylformamide).

Recrystallization from dimethylformamide–formic acid (99:1) gave, in 98% recovery, material with m.p. 231–232°, $[\alpha]^{22}D$ −24.5° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{74}H_{91}O_{16}N_{13}S_4$: C, 57.5; H, 5.93; N, 11.8; S, 8.29. Found: C, 57.5; H, 6.01; N, 11.7; S, 8.25.

(16) M. Bodanszky and V. du Vigneaud, THIS JOURNAL, **81**, 2504 (1959).

(17) C. R. Harington and T. H. Mead, *Biochem. J.*, **30**, 1598 (1936).

TABLE I

ION-EXCHANGE CHROMATOGRAPHY OF SYNTHETIC LYSINE-VASOPRESSIN

Tube no.	Act. before lyophil.	Wt. after lyophil., mg.	Pressor act. after lyophil., units/mg.	Total pressor act. in fract., units
41, 42	2,200	9.5	245	2,300
43–46	20,500	68.0	275	18,500
47–50	14,500	50.0	280–300	14,500
51–60	11,500	43.5	290	12,500
61–70	2,500	11.5	250	2,900
Recovery		182.5		50,700

Lysine-vasopressin.—The recrystallized protected nonapeptide VI (500 mg.) was dissolved in liquid ammonia (500 ml.) which had been distilled from sodium. Sodium was added in small quantities over a period of 20 to 40 minutes until a permanent blue color remained for 3 minutes, approximately 50 mg. of sodium being necessary. Glacial acetic acid (0.25 ml.) was added and the ammonia was then evaporated to a small volume (20–30 ml.). The rest of the ammonia was evaporated from the frozen state on a water pump with a KOH drying jar between pump and flask. This left the solid material in a loose and porous form. In order to remove last traces of ammonia the flask was kept under reduced pressure for another 1–2 hr. and then dry nitrogen was admitted. To remove most of the thiocresol the residue was washed twice with ethyl acetate (250 ml.) which had been freshly distilled from calcium chloride. The residue was then quickly dissolved in 1 l. of oxygen-free, redistilled water at 0°. The *p*H of the solution (4.5–5.0) was adjusted to 6.5–6.8 with dilute ammonia and air was then passed through the solution for 4 hr. The solution contained approximately 100,000 pressor units.[8] The *p*H was adjusted to 4.0 with acetic acid and the solution was passed through an IRC-50 (XE-64) column (1.9 × 23.5 cm.) in the H^+ form for desalting.[10] The column was washed with 0.25% acetic acid (400 ml.) until the *p*H of the effluent reached approximately 3.0 and then with water (25 ml.). The hormone was eluted with a 30% pyridine–4% acetic acid solution. The eluate (about 20 ml.) was lyophilized to a product (401 mg.) with a pressor activity of approximately 250 units per mg., or a total of about 100,000 pressor units.

Purification of Lysine-vasopressin by Ion-exchange Chromatography.—The above material (250 mg., containing approximately 62,500 pressor units) was dissolved in 1 ml. of 0.5 *M* ammonium acetate buffer, *p*H 6.38 (20°) and placed on an IRC-50 (XE-64) column (1.9 × 43.5 cm.) which had been equilibrated with the buffer. The chromatogram was developed with the same buffer at room temperature with a flow rate of 4 ml. per hour. The volume per fraction was 3.2 ml. The eluates were analyzed by determination of ultraviolet absorption (275 mμ), Folin–Lowry color reaction (700 mμ) and pressor activity. One single sharp peak was obtained in each case. The curves for the three determinations coincided closely, indicating that the material was a single compound. The contents of the peak tubes Nos. 41 + 42, 43–46, 47–50, 51–60, and 61–70 were pooled and lyophilized three times to remove the ammonium acetate. The pressor activities of the fractions are summarized in Table I. The recovery of activity in these fractions was 81 % and the recovery in terms of weight, 73%.

Tests of Purity of Synthetic Lysine-vasopressin.—The material was subjected to paper electrophoresis[18] on Whatman No. 3 MM paper with 0.1 *M* pyridine–acetate buffer of *p*H 4.0 at 400 volts and was found to travel as a single spot (15.5 cm. in 13 hr). Paper chromatography with the system butanol–acetic acid–water (4:1:5) showed the chromatographically purified material to travel as a single spot (R_F 0.12–0.16), whereas the material before chromatography contained a small amount of slow-moving impurities (R_F 0.02).[19] Amino acid analysis, on the starch column,[20] of hydrolysates of the synthetic product before and after ion-exchange chromatography showed the following amino acid content, expressed in molar ratios (with the ratio for glycine arbitrarily taken as 1 and the ratios for the product before chromatography given in parentheses): phenylalanine 1.0 (0.9), tyrosine 1.0 (0.9), proline 1.0 (0.8), glutamic acid 1.1 (0.9), aspartic acid 1.2 (1.0), glycine 1.0, lysine 1.0 (1.0), cystine 0.9 (0.9) and ammonia 3.4 (2.8).

The specific rotation of the synthetic material in 1 *N* acetic acid was $[\alpha]^{21.5}D$ −23.0° (*c* 0.5). It was found that 1 *N* acetic acid was a more satisfactory solvent than water, which had hitherto been used,[2] for determining the rotation of lysine-vasopressin.

A sample of the purified material was dried at 100° over P_2O_5 for 8 hr. for analysis.

Anal. Calcd. for $C_{46}H_{65}N_{13}O_{12}S_2 \cdot C_2H_4O_2$: C, 51.7; H, 6.23; N, 16.4. Found: C, 51.5; H, 6.35; N, 16.5.

(18) S. P. Taylor, Jr., V. du Vigneaud and H. G. Kunkel, *J. Biol. Chem.*, **205**, 45 (1953).

(19) Sometimes material remains at the origin. This is due to secondary reactions during the drying of the vasopressin solution on the paper. The difficulty can be overcome by using buffered paper and applying the hormone solution just before the solvent front moves over the starting line.

(20) S. Moore and W. H. Stein, *J. Biol. Chem.*, **178**, 53 (1949).

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, THE UNIVERSITY OF NEW MEXICO, AND THE BIOMEDICAL RESEARCH GROUP,[3] LOS ALAMOS SCIENTIFIC LABORATORY, UNIVERSITY OF CALIFORNIA]

Liquid Scintillators. XI. 2-(2-Fluorenyl)-5-aryl-substituted Oxazoles and 2-(2-Fluorenyl)-5-phenyl-1,3,4-oxadiazole[1]

BY MARTIN D. BARNETT,[2] GUIDO H. DAUB, F. NEWTON HAYES AND DONALD G. OTT

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Fluorene-2-carboxylic acid (I) has been prepared, either by treatment of 2-acetylfluorene with one equivalent of iodine in excess pyridine followed by basic cleavage of the intermediate pyridinium salt (II), or by direct carboxylation of fluorene with oxalyl chloride. The reaction of fluorene-2-carbonyl chloride (III) with the appropriate α-aminoketone salt (IV) gave 1-(2-fluorenyl)-4-aryl-2-aza-1,4-butanediones (V) which were cyclized to the respective oxazoles (VI) with phosphorus oxychloride. 2-(2-Fluorenyl)-5-phenyl-1,3,4-oxadiazole (X) was prepared by cyclization of 1-benzoyl-2-(fluorene-2-carbonyl)-hydrazine (IX), obtained by the reaction of III with benzoylhydrazine. The oxazoles and oxadiazole were evaluated as primary liquid scintillation solutes. In addition, 5-(4-biphenylyl)-2-(2-fluorenyl)-oxazole (VIb), 2-(2-fluorenyl)-5-(1-naphthyl)-oxazole (VIc) and 2-(2-fluorenyl)-5-(2-naphthyl)-oxazole (VId) were screened as potential secondary solutes. The compounds exhibited excellent scintillation characteristics.

The 2,5-diaryl-substituted oxazole nucleus (VI) has received much attention[4–8] in the study of organic compounds as solutes in scintillation detector systems. Comparison of a 2,5-diaryloxazole with a similarly electronically constituted

(1) From the dissertation presented by Martin D. Barnett to the graduate faculty of The University of New Mexico in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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