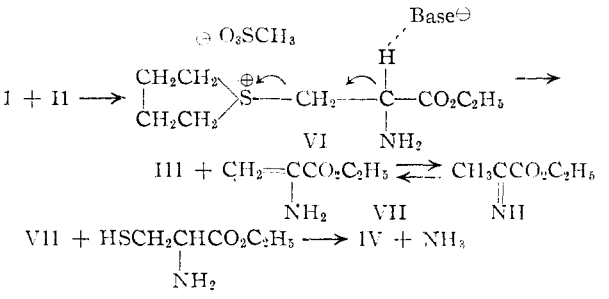

Experimental[10]

Reaction of L-Cysteine Ethyl Ester Hydrochloride with Myleran.—A solution of L-cysteine ethyl ester hydrochloride (42.7 g., 0.23 mole) in 500 ml. of absolute ethanol was added to a solution of sodium ethoxide, prepared by treating sodium (11.5 g., 0.50 mole) with 200 ml. of absolute ethanol. The resulting suspension was added rapidly to a hot solution of myleran (27.0 g., 0.11 mole) in 1700 ml. of absolute ethanol. The reaction mixture was heated at reflux for 2.5 hours and after cooling (ice-bath), the solid which precipitated was removed by filtration and washed twice with 200 ml. of ethanol. The washes and filtrate were combined, and the ethanol was removed under reduced pressure on a steam-bath. Any further precipitate which formed was removed by filtration and washed with ethanol.

Isolation of Tetrahydrothiophene.—Tetrahydrothiophene (III) was isolated from the ethanol distillate of the reaction mixture as the mercuric chloride salt.[8] A solution of mercuric chloride (16.3 g., 0.06 mole) in 200 ml. of ethanol was added to the ethanol distillate. The mixture was allowed to stand at 0° for one week. The resulting solid was collected and washed with ethanol, affording 4.0 g. (10.1%) of colorless needles melting at 128–130°. An additional 0.7 g. melting at 126–128° was obtained by concentrating the supernatant liquors to 200 ml. Recrystallization of the combined product from ethanol gave 2.5 g. (6.3%) of material which melted at 128–130°; mixture m.p. with authentic material,[8] m.p. 128–130°, was 128–130°.

Isolation of Alanine, 3-3′-(Tetramethylenedithio)-bis-, Diethyl Ester, Dihydrochloride.—Dry ether (200 ml.) was added to the residual yellow oil which remained after removal of the ethanol solvent. The ether solution was filtered to remove a gummy precipitate, and the ether was removed from the filtrate under reduced pressure on a water-bath to leave 30 g. of a yellow liquid. This product was dissolved in 100 ml. of dry ether, and saturated ethereal hydrogen chloride was added dropwise with stirring and cooling until *p*H 3 (*p*H paper) was reached. About 140 ml. of ethereal hydrogen chloride was required. The precipitate of V which formed (10.7 g., 21.8% yield, melting at 158–163° dec.) was collected, washed with ether and recrystallized from ethanol–ether to give 7.2 g., 14.5% yield, of product melting at 167–168° dec. Further recrystallization of this product from ethanol–ether gave a sample melting at 168–169° dec.

Anal. Calcd. for $C_{14}H_{30}N_2S_2Cl_2O_4$: C, 39.53; H, 7.11; N, 6.59; S, 15.07. Found: C, 39.26; H, 7.02; N, 6.47; S, 15.16.

Isolation of 2-Methyl-2,4-carbethoxythiazolidine Hydrochloride.—The ether solution from which V had been removed was treated with additional saturated ethereal hydrogen chloride, and the precipitate of IV, which formed after the mixture had stood in the refrigerator several hours, was collected and washed with ether to yield 5.1 g. (15.5%) of crystals which melted at 130–135°. Recrystallization of this product from ethanol–ether gave 2.3 g. (7.3%) of colorless crystals melting at 138–139°. A sample prepared by further recrystallization of this product from ethanol–ether melted at 138.5–140°.

Anal. Calcd. for $C_{10}H_{18}NSClO_4$: C, 42.33; H, 6.39; N, 4.94; S, 11.30. Found: C, 42.31; H, 6.44; N, 4.90; S, 11.59.

Proof of Structure of IV.—Compound IV gave a positive nitroprusside test and reacted with benzyl chloride in aqueous carbonate solution to give s-benzylcysteine.[9] A mixture of compound IV (2.3 g., 0.0081 mole), benzyl chloride (1.3 g., 0.01 mole) and potassium carbonate (5.5 g., 0.04 mole) in 20 ml. of water was heated at reflux with stirring for 5 hours. The reaction mixture was cooled to room temperature and extracted twice with 10 ml. of chloroform. The aqueous layer was cooled (ice-bath) and adjusted to *p*H 7 (*p*H paper) with 2 *N* hydrochloric acid. The product which separated was collected and recrystallized from water. The product was then washed with water and finally with ethanol; it then weighed 0.2 g. (11.8%) and melted at 209–210° dec. Further recrystallization of this material from water gave a sample melting at 210–211° dec.; reported melting point 215–216° dec., corrected.

Compound IV was shown to be 2-methyl-2,4-carbethoxythiazolidine hydrochloride by comparison of its physical properties with a sample prepared by an independent synthesis.

2-Methyl-2,4-carbethoxythiazolidine Hydrochloride.—A solution of ethyl pyruvate (14.6 g., 0.12 mole) and L-cysteine ethyl ester hydrochloride (16.8 g., 0.09 mole) in 300 ml. of ethanol was heated at reflux for 6 hours. The solvent was removed under reduced pressure on the steam-bath, and 250 ml. of dry ether was added to the residual oil. The product was cooled at 0° for two days; the crystals which formed were collected, washed with ether, and recrystallized from ethanol–ether affording 9.2 g. (36%) of colorless crystals melting at 137–140°. An additional 1.1 g. of product melting at 136–139° was recovered from the supernatant liquors by concentration and recrystallization. Further recrystallization of the combined product gave 6.0 g. (23.4%) of pure crystals melting at 139–141°, mixture m.p. with compound IV, 138.5–141°. Compound IV and the synthetic material just described had identical infrared spectra.

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(10) All melting points are uncorrected.

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

Synthesis of a Biologically Active Analog of Oxytocin, with Phenylalanine Replacing Tyrosine[1,2]

BY MIKLOS BODANSZKY AND VINCENT DU VIGNEAUD

RECEIVED JUNE 3, 1959

2-Phenylalanine oxytocin is an analog of oxytocin in which the tyrosyl residue of the hormone is replaced by phenylalanyl. The compound has been synthesized by two methods which were previously used for the synthesis of oxytocin. The analog was tested for uterine-contracting, avian depressor and milk-ejecting activity. It exhibited activity in these tests, but not to the extent shown by oxytocin. Thus the phenolic hydroxyl group contributes strongly to the potency of the hormone but is not essential qualitatively to its activity with respect to the biological properties so far tested.

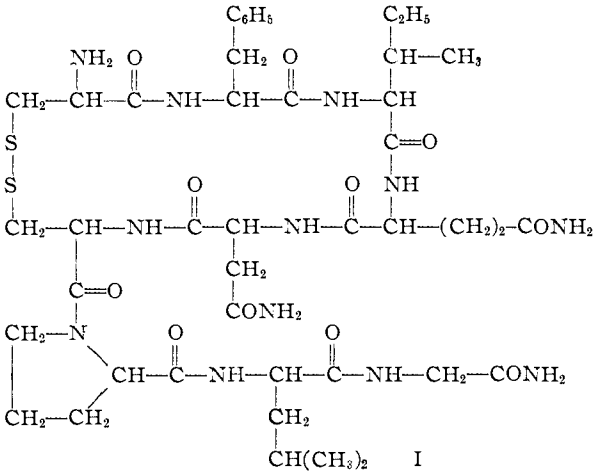
In a series of studies on the relation of structure to biological activity of oxytocin an analog of this hormone was synthesized. This analog is different from oxytocin only to the extent that the

(1) This work was supported in part by a grant from the National Heart Institute, U. S. Public Health Service, Grant No. H 1675.

(2) A preliminary report of this work has appeared [M. Bodanszky and V. du Vigneaud, THIS JOURNAL, **81**, 1258 (1959)].

tyrosyl residue is replaced by phenylalanyl. By this change it was possible to reveal the significance of the phenolic hydroxyl group, which is one of the few functional groups of the oxytocin molecule, to its biological activity. It was found that this phenolic hydroxyl group contributes to the uterine-contracting, milk-ejecting and avian depressor activities of the hormone but is not essential for these activities.[3]

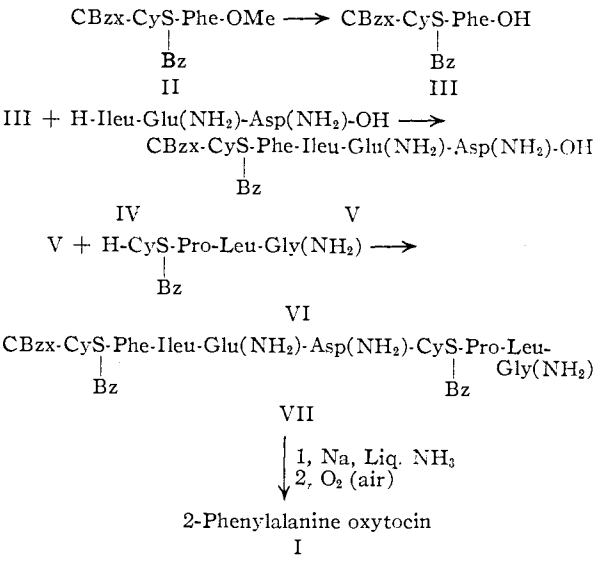
The hormone-analog mentioned above is referred to in this paper as 2-phenylalanine oxytocin[4] to indicate that the second amino acid from the N-terminal position of oxytocin is replaced by phenylalanine.



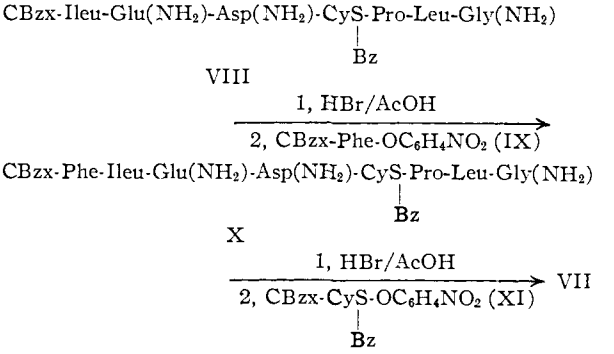
I

Synthesis of 2-phenylalanine oxytocin was accomplished along the lines of the synthesis of oxytocin reported recently by the present authors.[5] Thus a dipeptide derivative, methyl S-benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalaninate (II)[6] was prepared and saponified to the corresponding acid (III).[6] The latter was coupled to the tripeptide, L - isoleucyl - L - glutaminyl - L - asparagine (IV).[5] The protected pentapeptide, S-benzyl-N - carbobenzoxy - L - cysteinyl - L - phenylalanyl-L - isoleucyl - L - glutaminyl - L - asparagine (V), thus obtained was linked to the tetrapeptide, S-benzyl - L - cysteinyl - L - prolyl - L - leucylglycinamide (VI).[7,8] The product was the protected nonapeptide, S-benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanyl - L - isoleucyl - L - glutaminyl - L-asparaginyl - S - benzyl - L - cysteinyl - L - prolyl-L-leucylglycinamide (VII).

The same nonapeptide derivative VII was also prepared by the nitrophenyl ester method.[9]



This method was used recently for the synthesis of oxytocin by the present authors.[10] An important feature of this synthesis is the lengthening of the already existing part of a peptide chain by one protected amino acid at a time. The advantages and significance of this stepwise peptide synthesis by the nitrophenyl ester method have already been outlined earlier.[10] For the synthesis of 2-phenylalanine oxytocin the same crystalline heptapeptide derivative, N-carbobenzoxy-L-isoleucyl-L-glutaminyl - L - asparaginyl - S - benzyl - L - cysteinyl-L-prolyl-L-leucylglycinamide (VIII),[10] was used as for the synthesis of oxytocin. After removal of the carbobenzoxy group from this heptapeptide derivative the free base was allowed to react with *p*-nitrophenyl N-carbobenzoxy-L-phenylalaninate (IX) to give an excellent yield of the crystalline protected octapeptide, N-carbobenzoxy-L-phenylalanyl - L - isoleucyl - L - glutaminyl - L-asparaginyl - S - benzyl - L - cysteinyl - L - prolyl-L-leucylglycinamide (X). The same procedure was repeated, this time using *p*-nitrophenyl S-benzyl-N - carbobenzoxy - L - cysteinate (XI)[5,9,11] for the lengthening of the chain. The protected nonapeptide VII was obtained in excellent yield.



Removal of the protecting groups from the nonapeptide derivative VII by treatment with sodium

(3) The same analog of oxytocin was also prepared by P.-A. Jaquenoud and R. A. Boissonnas. *Helv. Chim. Acta*, **42**, 788 (1959). Results of pharmacological studies on their preparation were reported by H. Konzett and B. Berde, *Brit. J. Pharmacol.*, **14**, 133 (1959).

(4) In order to designate various analogs of oxytocin, the following numbering is proposed (using the reduced form)

H-CySH-Tyr-Ile-Glu(NH_2)-Asp(NH_2)-
1 2 3 4 5
CySH-Pro-Leu-Gly(NH_2)
6 7 8 9

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

(6) H. S. Bachelard and V. M. Trikojus, *J. Chem. Soc.*, 4541 (1958).

(7) C. Ressler and V. du Vigneaud, THIS JOURNAL, **76**, 3107 (1954).

(8) M. Zaoral and J. Rudinger, *Chem. Listy*, **49**, 475 (1955); *Coll. Czechoslov. Communs.*, **20**, 1183 (1955).

(9) M. Bodanszky, *Nature*, **175**, 685 (1955); *Acta Chim. Hung.*, **10**, 335 (1957). M. Bodanszky, M. Szelke, E. Tomorkeny and E. Weisz, *Chem. and Ind.*, 1517 (1955); *Acta Chim. Hung.*, **11**, 179 (1957).

(10) M. Bodanszky and V. R. du Vigneaud, *Nature*, **183**, 1324 (1959).

(11) B. Iselin, W. Rittel, P. Sieber and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).

in liquid ammonia was carried out in the manner reported in connection with oxytocin.[12] Isolation of the hormone analog also followed the methods already reported for this group of compounds.[5,12]

Experimental[13]

Methyl S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalaninate (II). A.—Methyl L-phenylalaninate hydrochloride[14] (8.6 g.) was dissolved in water (80 ml.) and $KHCO_3$ (10 g.) was added to the solution. The latter was extracted with ether (250 ml. in five portions), the ether solution was dried over $MgSO_4$ and the solvent was removed at room temperature under reduced pressure. The oily residue weighed 5.8 g. This ester (5.5 g.) and S-benzyl-N-carbobenzoxy-L-cysteine[15] (10.4 g.) were dissolved in dimethylformamide (30 ml.) and dicyclohexylcarbodiimide[16] (6.2 g.) was added to the solution at 0°. After being allowed to stand overnight at room temperature the mixture was diluted with ethyl acetate (100 ml.) and N,N'-dicyclohexylurea was filtered off. The solution was washed with 0.5 *N* HCl, 0.2 *N* $KHCO_3$ solution and water, dried over $MgSO_4$ and evaporated to dryness. The crystalline residue (15.4 g.) was recrystallized from methanol (30 ml.) and then washed with cold methanol (60 ml.); wt. 9.1 g. (60%), m.p. 105–106°, $[\alpha]^{20}D$ −37° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{28}H_{30}O_5N_2S$: C, 66.4; H, 5.97; N, 5.53. Found: C, 66.2; H, 6.08; N, 5.63.

B.—Methyl L-phenylalaninate (9.0 g.) and *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate[5] (14 g.) were dissolved in ethyl acetate (30 ml.). The mixture was allowed to stand overnight at room temperature, additional ethyl acetate (100 ml.) was added and the solution was washed and the product isolated and recrystallized as described in Section A; wt. 12.8 g. (84%), m.p. 104–105°, $[\alpha]^{20}D$ −37° (*c* 2, dimethylformamide). Another recrystallization raised the m.p. to 106–107°, and the specific rotation was unchanged; lit.[6] m.p. 105–105.5°.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanine (III).[6]—II (9.1 g.) was dissolved in acetone (36 ml.) and *N* NaOH (20 ml.) was added with stirring. After 30 minutes at room temperature 2 *N* HCl (12 ml.) and water (180 ml.) were added, and the precipitated acid was filtered off and washed with water; wt. 8.7 g. (98%), m.p. 157–158°, $[\alpha]^{20}D$ −22° (*c* 2, pyridine). This product (8.5 g.) was recrystallized from ethanol (100 ml.) with the addition of water (50 ml.); 7.7 g. was recovered with unchanged m.p. and specific rotation. The compound (4 g.) was also recrystallized from absolute ethanol (42 ml.); wt. 2.05 g., m.p. 158–159°, specific rotation unchanged; lit.[6] m.p. 155.5°.

Anal. Calcd. for $C_{27}H_{28}O_5N_2S$: C, 65.8; H, 5.73; N, 5.69. Found: C, 65.9; H, 5.92; N, 5.76.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparagine (V).—A solution of III (3.45 g.) in tetrahydrofuran (70 ml.) and triethylamine (1 ml.) was cooled to −20°. Isobutyl chloroformate (0.96 g.) was added and after 13 minutes a solution of L-isoleucyl-L-glutaminyl-L-asparagine monohydrate (IV)[5] (2.8 g.) in a mixture of water (14 ml.) and triethylamine (1 ml.) was added with stirring. The mixture was allowed to come to room temperature and stand for 1 hr. Water (300 ml.) and 2 *N* HCl (7 ml.) were added and the precipitate was filtered and washed with water. After being dried at room temperature a product (5.5 g.), m.p. 225–228°, was obtained, which in turn was extracted with several portions of hot ethanol (a total of 160 ml.) and dried; wt. 3.3 g. (56%), m.p. 234–236°, $[\alpha]^{20}D$ −27° (*c* 5, dimethylformamide). This product was used for the preparation of the nonapeptide derivative VII.

For analysis a sample (0.5 g.) was dissolved in hot 80% ethanol (150 ml.) and when the solution was cooled a solid separated, which was collected and washed with ethanol; wt. 0.43 g., m.p. 235–238° (placed in bath at 220°).

Anal. Calcd. for $C_{42}H_{53}O_{10}N_7S$: C, 59.5; H, 6.30; N, 11.6. Found: C, 59.7; H, 6.30; N, 11.6.

***p*-Nitrophenyl N-carbobenzoxy-L-phenylalaninate (IX).**—N-Carbobenzoxy-L-phenylalanine (3.6 g.) and *p*-nitrophenol (2.06 g.) were dissolved in ethyl acetate (24 ml.) and dicyclohexylcarbodiimide (2.5 g.) was added to the solution at 0°. After 30 minutes at 0° and 2 hr. at room temperature the N,N'-dicyclohexylurea was filtered off and washed with ethyl acetate (40 ml.). The combined mother liquor and washings were evaporated to dryness *in vacuo* and the residue was recrystallized from hot ethanol; wt. 3.8 g. (75%), m.p. 126–126.5°, $[\alpha]^{20}D$ −24.7° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{23}H_{20}O_6N_2$: C, 65.7; H, 4.80; N, 6.66. Found: C, 65.8; H, 4.86; N, 6.61.

N-Carbobenzoxy-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (X).—To a solution of N-carbobenzoxy-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VIII)[10] (3.9 g.) in acetic acid (20 ml.), 3 *N* HBr in acetic acid (40 ml.) was added. After about 1.5 hr. at room temperature dry ether (400 ml.) was added to the clear solution. The precipitated hydrobromide was filtered off and washed with ether. After a short period of drying *in vacuo* the hydrobromide was dissolved in dimethylformamide (32 ml.) and triethylamine (3.8 ml.) was added to the solution with cooling. IX (2 g.) was added to the reaction mixture; crystallization of the product started in a short time. After 20 hr. at room temperature ethyl acetate (400 ml.) was added and the precipitate was washed with ethyl acetate (100 ml.); wt. 4.15 g. (93%), m.p. 251–253°, $[\alpha]^{22}D$ −41° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{55}H_{75}O_{12}N_{11}S$: C, 59.3; H, 6.79; N, 13.8. Found: C, 59.0; H, 6.85; N, 13.7.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VII). A.—V (3.4 g.) and S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide sesquihydrate (VI) (2.2 g.) were dissolved in dimethylformamide (75 ml.) and dicyclohexylcarbodiimide (2.5 g.) was added at 0°. After 20 hr. at room temperature the mixture was heated to 40° and acetic acid (1 ml.) was added. The precipitate was filtered off and washed with dimethylformamide (5 ml.). Water (400 ml.) was added to the filtrate with cooling. The precipitate was washed with water (400 ml.) and dried. After extraction of the product (5.8 g.) with hot methanol (250 ml.), 2.45 g. (47%), m.p. 243–245° dec. remained. This material was used in the next step. For analysis a sample (0.50 g.) was dissolved in dimethylformamide (20 ml.) at 45° and water (200 ml.) and acetic acid (1 ml.) were added to the filtered solution. The precipitate was washed with water. The material (0.50 g.) was extracted with hot methanol (50 ml.) and dried (0.30 g.), m.p. 247–248° dec., $[\alpha]^{22}D$ −52° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{65}H_{86}O_{13}N_{12}S_2$: C, 59.7; H, 6.63; N, 12.9. Found: C, 59.6; H, 6.77; N, 12.7.

B.—To a suspension of the protected octapeptide derivative X (3.4 g.) in acetic acid (20 ml.) 3 *N* HBr in acetic acid (40 ml.) was added. After an hour at room temperature the hydrobromide was precipitated with ether (360 ml.) and washed with ether (240 ml.). This hydrobromide after a short period of drying *in vacuo* was dissolved in dimethylformamide (30 ml.) and treated with triethylamine (3.8 ml.) at 0°. *p*-Nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate (X)[5,9,11] (1.68 g.) was added to the reaction mixture. A solid soon began to separate. After 22 hr. at room temperature the product was isolated in the manner described for compound X; wt. 3.75 g. (95%), m.p. 250–253° dec., $[\alpha]^{20}D$ −48.3° (*c* 1, dimethylformamide).

Anal. Found: C, 59.5; H, 6.69; N, 13.0.

2-Phenylalanine Oxytocin (I).—A solution of VII (1.3 g.) in liquid ammonia (500 ml.) was treated with sodium until a blue color persisted (0.25 g. of sodium). Ammonium chloride (0.55 g.) was added and the ammonia was allowed to evaporate. The residue was dissolved in water (1300 ml.) and the *p*H was adjusted to 6.5 with acetic acid. After aeration for 5 hr. the nitroprusside reaction for the mercapto group was negative. Assay of the solution for avian depressor activity showed a total of 23,000 units. The solvent was removed *in vacuo* below room temperature and the residue was extracted with ethanol (35 ml.). Ethyl acetate (500 ml.) was added and the precipitate was filtered off and

(12) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, THIS JOURNAL, **76**, 3115 (1954).

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

(14) R. A. Boissonnas, St. Guttmann, P.-A. Jaquenoud and J. P. Waller, *Helv. Chim. Acta*, **39**, 1421 (1956).

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

(16) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

washed with ethyl acetate. It was then dried, extracted with pyridine (20 ml.) and reprecipitated with ethyl acetate (100 ml.). The dried product weighing 0.50 g. represented 13,000 units of avian depressor activity. For further purification this solid was dissolved in 50 ml. of the lower phase of the solvent system butanol–ethanol–0.05% acetic acid (4:1:5) and placed in the first five tubes of a 200-tube countercurrent distribution apparatus. After 310 transfers a peak with a distribution coefficient of approximately 0.7 emerged and this contained all of the avian depressor activity. With the exception of tubes No. 110 to 150 the apparatus was emptied and refilled with fresh solvents. The distribution was continued by recycling. After a total of 580 transfers all the avian depressor activity was in tubes no. 10–60 with a maximum at tube no. 35. The theoretical curve calculated for the K value 0.68 was in excellent agreement both with the curve obtained by plotting the Folin color determinations[17] and with that obtained by using the biological assay values.[18] The contents of tubes no. 20 to 50 were pooled and the solvent was removed *in vacuo*. The residue was dissolved in ethanol (16 ml.) and ethyl acetate (1 l.) was added to the solution. The precipitate was collected and washed with ethyl acetate. (Considerable amounts of activity were found in the mother liquor.) The product (50 mg.) possessed approximately 60 units/mg. of avian depressor activity[18] and approximately 30 units/mg. of rat uterine-contracting activity.[19,20] Professor H. B. van Dyke of the College of Physicians and Surgeons has found that 2-phenylalanine oxytocin shows milk-ejecting activity of about 60 units per mg.

Anal. Calcd. for $C_{43}H_{66}O_{11}N_{12}S_2$: C, 52.1; H, 6.71; N, 17.0; mol. wt.,[21] 991. Found: C, 52.1; H, 6.83; N, 16.9; mol. wt.,[21] 940.

Acknowledgments.—The authors wish to thank Mr. Joseph Albert for the microanalyses reported herein, Mr. David De Peter for the determination of the molecular weight, Miss Dade Tull and Miss Maureen O'Connell for the biological assays and Mr. David N. Reifsnyder for technical assistance.

(17) O. H. Lowry, N. V. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **183**, 265 (1951).

(18) J. M. Coon, *Arch. Intern. Pharmacodynamie*, **62**, 79 (1939).

(19) J. H. Burn, D. J. Finney, L. G. Goodwin, "Biological Standardization," Oxford University Press, 1950.

(20) In this test results were obtained several times, which were different from the average reported here. This result might be explained by the different dose–response relationship for oxytocin and 2-phenylalanine oxytocin.

(21) E. V. Baldes, *Biodynamica*, **46**, 1 (1939).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Degradation of Adenosine-5′-triphosphoric Acid (ATP) by Means of Aqueous Barium Hydroxide[1]

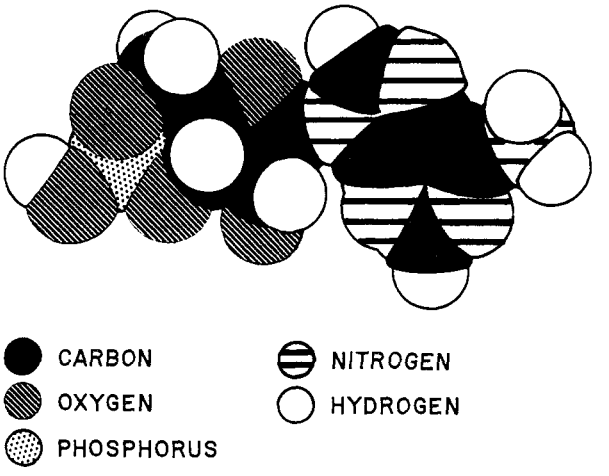
BY DAVID LIPKIN, ROY MARKHAM[2] AND WILLIAM H. COOK[3]

RECEIVED MAY 6, 1959

Degradation of ATP by means of aqueous barium hydroxide at 100° was shown to lead to the formation of nine adenine-containing compounds. Of these, the most interesting are adenosine-2′ (and 3′)-phosphoric acid, adenosine-3′:5′-phosphoric acid and 2′,5′ (and 3′,5′)-diphosphoryladenosine. Mechanisms for the degradation of ATP are suggested which account for the formation of the observed products.

The fact that adenosine-5′-triphosphoric acid (ATP) yields adenosine-5′-phosphoric acid (A-5′-P) and inorganic pyrophosphate on hydrolysis in barium hydroxide solution has been known for some years.[4] In a recent study of the alkaline degradation of ATP, Hock and Huber[5] showed that the reaction is more complex than previous investigators had indicated. They demonstrated that in the degradation of ATP by 1N sodium hydroxide solution at 100° there also is formed adenine and an unidentified phosphorus-containing substance which has a smaller R_f value than ATP on paper chromatography with 1-propanol–ammonia as the developing solvent. Hock and Huber also showed that degradation of ATP at 100° and pH *ca.* 9–10 in the presence of barium ion leads to the formation of a substance which they designated as "NF." They indicated that this was an "adenosine diphosphate." These investigators noted, in addition, the marked catalytic effect of barium ion on the degradation of ATP in alkaline solution.

Additional complexities in the alkaline degradation of ATP already have been reported briefly by Cook, Lipkin and Markham.[6] A more detailed study shows that the following adenine compounds are formed in the degradation of ATP at 100° in the presence of aqueous barium hydroxide: adenine, adenosine, A-5′-P, adenosine-2′-phosphoric acid (A-2′-P), adenosine-3′-phosphoric acid (A-



(1) Presented at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April, 1958.

(2) On leave from the Agricultural Research Council Virus Research Unit, Cambridge, England, and who would like to thank the Wellcome Foundation for a travel grant.

(3) Universal Match Co. Fellow, 1958–1959.

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