ratios identical with those of guanylic acid under similar conditions. Further analyses are listed in the table.

COMPOSITION OF FRACTION III

Analyses are on a total eluate concentrated to 50 ml.

Component	mmol./liter
Guanine	0.41
Total phosphate	0.82
Labile phosphate	0.39
Pentose	0.39
Total amino acids	0.35
Acid labile amino acids	0.38

It is apparent that guanine, pentose and amino acids (ninhydrin on a hydrolysate with 8 *N* HCl for 24 hr. at 105°) were present in a 1:1:1 ratio. Total phosphate was in a ratio of 2:1 with the other components but half was released by hydrolysis with 1 *N* HCl for 30 min. Guanosine diphosphate was the major nucleotide found in an alkaline hydrolysate with a formate column.[5] The arrangement of amino acids cannot be as a protein or as a single peptide; hydrolysis with 1 *N* HCl for 60 min. at 100° led to the release of the amino acids (acid labile amino acids). Thus the amino acids must be combined in some type of labile linkage with guanine nucleotides.

These results support the concept that polynucleotide, non-protein materials have catalytic activity in the hydrolysis of peptides. Uridine nucleotides and hexosamine, previously reported as minor components of an active fraction from Dowex columns, have not been found in this material; otherwise, the composition is much as was reported.[6]

(5) R. B. Hurlbert, H. Schmitz, A. F. Brumm and V. R. Potter, *J. Biol. Chem.*, **209**, 23 (1954).

(6) F. Binkley, C. K. Olson and C. Torres, Abstracts, 128th Meeting, American Chemical Society, Minneapolis, 1955.

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RECEIVED DECEMBER 8, 1958

SYNTHESIS OF A BIOLOGICALLY ACTIVE ANALOG OF OXYTOCIN, WITH PHENYLALANINE REPLACING TYROSINE[1]

Sir:

We have studied the significance of one of the few free functional groups of oxytocin, the phenolic hydroxyl group of the tyrosyl residue, by synthesis of an analog of oxytocin with tyrosine replaced by phenylalanine. This analog, 2-phenylalanine oxytocin,[2] was prepared by a method recently used for the synthesis of oxytocin.[3]

Methyl S-benzyl-N-carbobenzoxy-L-cyteinyl-L-phenylalaninate (I), m.p. 106–107°, $[\alpha]^{20}D$ −37° (*c* 2, 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), was prepared by coupling S-benzyl-N-carbobenzoxy-L-cysteine with methyl L-phenylalaninate by the dicyclohexylcarbodiimide procedure[4] and also by the reaction of *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate[3,5,6] with methyl L-phenylalaninate. I was hydrolyzed to give S-benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanine (II), m.p. 157–158°, $[\alpha]^{20}D$ −22° (*c* 2, pyridine), (*Anal.* Calcd. for $C_{27}H_{28}O_5N_2S$: C, 65.8; H, 5.73; N. 5.69; neut. equiv., 492.6. Found: C, 65.9; H, 5.92; N, 5.76; neut. equiv., 491). II was converted to a mixed anhydride[7] by the action of isobutyl chloroformate and brought into reaction with L-isoleucyl-L-glutaminyl-L-asparagine (III).[3] The protected pentapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparagine (IV), m.p. 235–238°, $[\alpha]^{20}D$ −27° (*c* 0.5, dimethylformamide), (*Anal.* Calcd. for $C_{42}H_{53}O_{10}N_7S$: C, 59.5; H, 6.30; N, 11.6; neut. equiv., 848. Found: C, 59.7; H, 63.0; N, 11.6; neut. equiv., 848), thus obtained was linked[4] to S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[3,8,9] (V) to give 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 (VI), m.p. 247–248°, $[\alpha]^{22}D$ −52° (*c* 1, dimethylformamide), (*Anal.* Calcd. for $C_{65}N_{86}N_{12}O_{13}S_2$: C, 59.7; H, 6.63; N, 12.9. Found: C, 59.6; H, 6.77; N, 12.7). The protecting groups were removed from VI with sodium in liquid ammonia and the resulting nonapeptide oxidized by aeration to form the cyclic disulfide (VII), an octapeptide, the 2-phenylalanine analog of oxytocin. One milligram of VII gave in this procedure 18 units of avian depressor activity[10] and 0.08 unit of pressor activity in the rat.[11] VII was purified by extraction with alcohol and precipitation by ethyl acetate followed by extraction with pyridine and reprecipitation by ethyl acetate. The solid thus obtained assayed about 25 units/mg. of avian depressor activity and was further purified by countercurrent distribution in a solvent system of butanol–ethanol–0.05% acetic acid (4:1:5). The distribution curve obtained by Folin color assay[12] was in excellent agreement with the curve showing biological activity and also with a curve calculated for the *K* value 0.68. This agreement indicates the presence of a single compound. After this purification VII showed an avian depressor activity[10] of about 60–70 units per mg. and about 30 units per mg. of oxytocic activity when assayed on the isolated rat uterus.[13] No pressor activity[11] was found in the rat at a total

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

CySH-Tyr-Ileu-Glu(NH₂)-Asp(NH₂)-CySH-Pro-Leu-Gly(NH₂).
1 2 3 4 5 6 7 8 9

(3) M. Bodanszky and V. du Vigneaud: in press.

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

(5) 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).

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(7) J. P. Vaughan, Jr., THIS JOURNAL, **74**, 6137 (1952).

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(10) J. M. Coon, *Arch. Intern. Pharmacol.*, **62**, 79 (1939).

(11) J. Dekanski, *Brit. J. Pharmacol.*, **7**, 567 (1952).

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

(13) J. H. Burn, D. J. Finney and L. G. Goodwin, "Biological Standardization," Oxford University Press, New York, N. Y., 1950.

dose of 60γ. (*Anal.* Calcd. for $C_{43}H_{66}N_{12}O_{11}S_2$: C, 52.1; H, 6.71; N, 17.0; mol.wt., 991. Found: C, 52.1; H, 6.83; N, 16.9 ; mol. wt.[14] 940).

From these data we can conclude that the phenolic hydroxyl group of oxytocin contributes strongly to the activity of the hormone but is not essential for biological activity.[15,16]

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(14) E. V. Baldes, *Biodynamica* **No. 46**, 1 (1939).

(15) As the experiments reported here were completed, we learned at a lecture delivered by Dr. R. A. Boissonnas that the same analog of oxytocin was prepared and studied in his laboratory simultaneously but independently from our work.

(16) After this communication was submitted for publication, Professor H. B. van Dyke found that 2-phenylalanine oxytocin shows milk ejecting activity of about 60 units per mg.

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RECEIVED JANUARY 12, 1959

EVIDENCE OF A 1,4-METHYL MIGRATION DURING A FISCHER REACTION

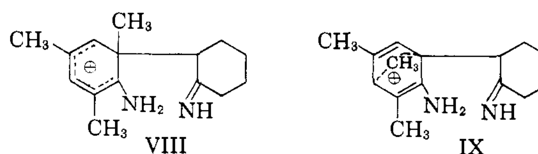
Sir:

An investigation of the structure of a compound isolated some years ago[1] as its picrate from the product of the action of boiling acetic acid on cyclohexanone mesitylhydrazone (I) has now disclosed that the compound is not "1,2,3,4-tetrahydro-6,8,12-trimethylisocarbazole" (III),[1] but is instead 6,7,8-trimethyl-1,2,3,4-tetrahydrocarbazole (II).

Mesitylhydrazine, m.p. 60–61° (N_2), was prepared by a new synthetic method based on the addition of mesitylene to ethyl azodicarboxylate.[2] The adduct, m.p. 159–160° (Found: C, 61.67; H, 7.61; N, 9.57[3]) was converted to mesitylhydrazine by boiling ethanolic potassium hydroxide. I, m.p. 45–47° (N_2), from mesitylhydrazine and cyclohexanone in the absence of solvent, was too unstable to analyze. Under nitrogen, boiling acetic acid converted I to II, isolated as its picrate, m.p. 171–172° (d.), as reported.[1] (Found: C, 57.61; H, 5.00; N, 12.87.) The tetrahydrocarbazole, from the picrate and sodium hydroxide, was extremely air-sensitive, m.p. 92–98° (N_2), insufficiently stable to analyze. Chloranil in xylene under nitrogen converted II to 1,2,3-trimethylcarbazole, m.p. 127.5–128.5°. (Found: C, 85.32; H, 7.37; N, 6.85); infrared and ultraviolet curves very similar to those of carbazole. Exposure in ether solution of II to air produced 11-hydroperoxy-6,7,8-trimethyl-1,2,3,4-tetrahydrocarbazolenine IV, m.p. 134° (d.). (Found: C, 72.46; H, 7.81; N, 5.77), which isomerized in ethanol solution to 9,10,11-trimethyl-1-benzazonidine-2,7-dione V, m.p. 171–172°. (Found: C, 73.08; H, 7.90; N, 5.55). The latter was converted by acid hydrolysis to δ-(2-amino-3,4,5-trimethylbenzoyl)-valeric acid VI, m.p. 146-149°. (Found: C, 68.56; H, 7.84; N, 5.50), and by alkali to 2,3-trimethylene-6,7,8-trimethyl-4-quinolone VII, darkens above 300°. (Found: C, 80.90; H, 7.64; N, 5.86). These transformations of II through IV and V to VI or VII parallel a precisely analogous series of reactions starting with tetrahydrocarbazole.[4] The ultraviolet and infrared spectra of V–VII are notably similar to those of the lower homologs derived from tetrahydrocarbazole.

The location of the methyl groups in the structure II and those of its derivatives was proven by synthesis of II from hemimellitene. The latter afforded an adduct with ethyl azodicarboxylate, m.p. 151–152° (Found: C, 60.10; H, 7.62; N, 9.60), which was converted to 2,3,4-trimethylphenylhydrazone, m.p. 105–106° (N_2), too unstable to analyze. The structure of this compound was demonstrated by its hydrogenation over Raney nickel to 2,3,4-trimethylaniline, characterized as its N-acetyl derivative. Cyclohexanone 2,3,4-trimethylphenylhydrazone, oily solid, too unstable to analyze, when boiled in acetic acid under nitrogen, afforded II, isolated as its picrate, m.p. and mixed m.p. 171–172°(d.). From this picrate II itself and from the latter 1,2,3-trimethylcarbazole and IV, V, VI and VII were prepared. Their m.pts. mixed m.pts. and spectroscopic properties identified them with samples obtained from I as starting material.

Although it is possible to rationalize the formation of II from I by means of a series of three consecutive 1,2 methyl shifts, along with the required accompanying reactions, a single 1,4 shift of methyl, although to our knowledge unprecedented, seems to us to offer a superior explanation. In either instance the shift would start with an intermediate VIII, a homolog of one for which evidence has been offered previously.[5] A single 1,4 shift of methyl, through transition state IX, would yield a second intermediate from which II could be formed by previously suggested routes.[6]



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RECEIVED DECEMBER 24, 1958

AMINO ACID INCORPORATION INTO LIPOIDAL MATERIAL BY CELL-FREE LIVER PREPARATIONS

Sir:

The incorporation of amino acids into cellular constituents via adenosine triphosphate–amino acid activation has been studied by many workers,