Decomposition of Glycyl-L-histidyl-L-serine (VI) in Water.—A solution of 1 mg. of glycyl-L-histidyl-L-serine (VI) in 0.1 ml. of water was heated on a steam-bath for 3 hours, giving a mixture of unchanged VI and two decomposition products, one Pauly-negative and ninhydrin-positive, the other Pauly-positive and ninhydrin-negative. The former compound was identical in behavior with an authentic sample of L-serine on paper chromatography in BPAW, BAW and 1-butanol-butanone–water–diethylamine (10:10:5:1), and on electrophoresis. The latter compound was identical in behavior to X on paper chromatography in BPAW and BAW, and on electrophoresis.

Catalysis of the Hydrolysis of *p*-Nitrophenyl Acetate by L-Histidine, Glycyl-L-histidyl-L-serine (VI) and cyclo-Glycyl-L-histidyl-L-serylglycyl-L-histidyl-L-seryl (I).—The conditions of Katchalski, *et al.*,[23] were employed. At zero time, 0.500 ml. of a solution of 0.001587 g. of *p*-nitrophenyl acetate[28] in 10.0 ml. of purified, peroxide-free dioxane was mixed thoroughly with 15.0 ml. of 0.20 *M* phosphate buffer, *p*H 7.76, at 28.0 ± 0.5°, containing the desired histidine-containing compound. The changes in optical density at 400 mμ were followed on a Coleman Junior spectrophotometer. Readings were taken at 1–5 minute intervals for the first 50% of the reaction, then an infinite time reading was taken after 10 half-lives. The first-order rate constant, k_1, was calculated from eq. 1. It was found that k_1, in all

$$k_1 = \frac{1}{t} \ln \frac{OD_t}{OD - OD_t} \qquad (1)$$

cases (including the absence of a histidine-containing compound), dropped rapidly during the first 15% of reaction, then became constant within experimental error (probably a very gradual decrease in k_1). The values reported for k_1 were calculated from the data obtained between 20% and 40% reaction. The second-order rate constant, k_2, was calculated from eq. 2, where c = molarity of histidine residues and k_w = the first-order rate constant in the absence of a

$$k_2 = (k_1 - k_w)/c \qquad (2)$$

histidine-containing compound. The results are summarized in Table I, together with some of the data from Katchalski, *et al.*,[23] for comparison.

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

Synthesis of D-Leucine-oxytocin, a Biologically Active Diastereoisomer of Oxytocin, and Demonstration of its Separability from Oxytocin upon Countercurrent Distribution[1]

BY CONRAD H. SCHNEIDER AND VINCENT DU VIGNEAUD

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The synthesis of the D-leucine isomer of oxytocin is described. The required nonapeptide intermediate, S-benzyl-N-carbobenzoxy - L - cysteinyl - L - tyrosyl - L - isoleucyl - L - glutaminyl - L - asparaginyl - S - benzyl - L - cysteinyl - L - prolyl-D-leucylglycinamide, was obtained by coupling S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine with S-benzyl-L-cysteinyl-L-prolyl-D-leucylglycinamide by means of dicyclohexylcarbodiimide. The protected nonapeptide so formed was reduced with sodium in liquid ammonia and subsequently oxidized to the octapeptide amide, D-leucine-oxytocin, which was purified by countercurrent distribution. The D-leucine-oxytocin was found to possess oxytocic and avian depressor activities of approximately 20 units per mg. and a milk-ejecting activity of at least 50 units per mg. The separability of this diastereoisomer from oxytocin upon countercurrent distribution was demonstrated by subjection of a mixture of the D-leucine-oxytocin and tritium-labeled oxytocin to this procedure.

A recent communication from this Laboratory[2] described the synthesis of tritium-labeled oxytocin from L-leucine labeled with a crude preparation of leucine of high specific activity prepared by the catalytic reduction of dehydro-L-leucine with tritium gas. It was hoped that radioactivity not due to L-leucine would be eliminated during the crystallizations of the intermediates in some of the steps of the synthesis as well as during the purification of the hormone by countercurrent distribution. In fact, the countercurrent distribution of the final radioactive hormone preparation gave no evidence of the presence of impurities. However, there was a possibility that the tritium-labeled leucine used in the radioactive synthesis may have contained some racemic leucine. If this were true, it is conceivable that any resulting diastereoisomer may not have been eliminated completely in the course of the purification of the crystalline protected peptide intermediates, carbobenzoxy-L-prolyl-L-leucine, carbobenzoxy-L-prolyl-L-leucylglycinamide and S-benzyl-N-carbobenzoxy-L-cysteinyl - L - prolyl - L - leucylglycinamide. Furthermore, any radioactive D-leucine diastereoisomer which might have been carried through the protected nonapeptide stage would then be present in the radioactive oxytocin as radioactive D-leucine-oxytocin and might not have been separated from the oxytocin during countercurrent distribution.

It therefore seemed desirable to synthesize the non-radioactive D-leucine isomer of oxytocin and study its separability from oxytocin upon countercurrent distribution under the conditions previously employed for the purification of the radioactive hormone. If the two diastereoisomers were separable under these conditions, it would appear unlikely that the final radioactive oxytocin preparation had been contaminated with the D-leucine isomer.

The D-leucine isomer of oxytocin was also of interest in its own right, since a study of its properties would make a basic contribution to the general question of the relationship of diastereoisomerism to the biological activity and other properties of the hormone. Investigations along these lines were initiated a few years ago in this Laboratory and are being continued at the present time.

For the synthesis of the D-leucine-oxytocin the same route was employed as for the synthesis of the tritium-labeled oxytocin.[2] Thus, the C-termi-

(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) V. du Vigneaud, C. H. Schneider, J. E. Stouffer, V. V. S. Murti, J. P. Aroskar and G. Winestock, *J. Am. Chem. Soc.*, **84**, 409 (1962).

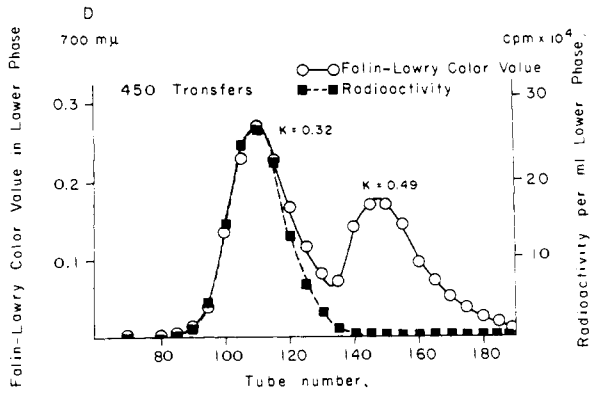

Fig. 1.—Countercurrent distribution of a mixture of tritium-labeled oxytocin (K = 0.32) and D-leucine-oxytocin (K = 0.49) in butanol–propanol–0.05% acetic acid at 4°.

nal tripeptide sequence carbobenzoxy-L-prolyl-D-leucylglycinamide was prepared from D-leucine following essentially the method of Cash[3] for the L,L isomer with certain variations as given in the Experimental section. Coupling of the decarbobenzoxylated tripeptide with *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate and subsequent decarbobenzoxylation of the product yielded the tetrapeptide S - benzyl - L - cysteinyl - L - prolyl - D-leucylglycinamide which was coupled by means of dicyclohexylcarbodiimide with the pentapeptide S - benzyl - N - carbobenzoxy - L - cysteinyl - L-tyrosyl - L - isoleucyl - L - glutaminyl - L - asparagine[4] to give the required protected nonapeptide intermediate S - benzyl - N - carbobenzoxy - L-cysteinyl - L - tyrosyl - L - isoleucyl - L - glutaminyl-L - asparaginyl - S - benzyl - L - cysteinyl - L - prolyl-D-leucylglycinamide. D-Leucine-oxytocin was prepared from the protected nonapeptide by reduction with sodium in liquid ammonia followed by oxidation of the reduced material in aqueous solution. The analog was isolated by countercurrent distribution (800 transfers) in the system butanol–propanol–0.05% acetic acid (2:1:3) at 4° followed by concentration and lyophilization.

A mixture containing roughly equal amounts of D-leucine-oxytocin and oxytocin, to which a small amount of radioactive oxytocin had been added, was subjected to countercurrent distribution in the system butanol–propanol–0.05% acetic acid (2:1:3) at 4°. After 450 transfers D-leucine-oxytocin was found to be separated from the labeled oxytocin. The oxytocin and radioactive oxytocin exhibited a partition coefficient (K) of 0.32, whereas the D-leucine-oxytocin possessed a K value of 0.49 in this system, as shown in Fig. 1. This demonstration of the separability of oxytocin from D-leucine-oxytocin by countercurrent distribution gives confidence that the biological potencies reported below for the D-leucine-oxytocin purified by 800 transfers represent inherent activities of the compound and are not due to contamination with oxytocin which could conceivably have arisen from a slight degree of racemization in the coupling of the carbobenzoxy-L-prolyl-D-leucine with glycinamide. Moreover these results can be taken as an indication of the feasibility of synthesis of isotopically labeled oxytocin starting from DL-leucine labeled with any isotope or combination of isotopes desired.

It is to be noted that the curves in Fig. 1 for radioactivity and for Folin–Lowry color values for the oxytocin peak coincide and further that no radioactivity was present in the tubes containing the D-leucine-oxytocin. Thus, the result demonstrates that the radioactive oxytocin previously reported[2] which was used in this experiment does not contain detectable D-leucine-oxytocin, for otherwise radioactivity would have appeared in the D-leucine-oxytocin separated in this experiment.

The D-leucine-oxytocin was assayed against the U.S.P. Standard Powder for certain of the biological activities associated with oxytocin. The avian depressor activity[5] of this diastereoisomer of oxytocin was approximately 20 units per mg., and the oxytocic activity[6] was also in this range. On the other hand, oxytocin possesses approximately 500 units per mg. of each of these activities. The milk-ejecting activity[7] of the D-leucine-oxytocin was higher than the other two, preliminary assays indicating a minimum value of 50 units per mg. No pressor effect[8] was noted in rats with doses up to 250 μg., and in fact it was found that the compound exerted a weak inhibitory effect on the pressor response to the Standard. The compound also was found to exert an extremely weak antidiuretic effect in the rat.[9] Thus the substitution of D-leucine for the naturally occurring L-leucine in the penultimate position of the side chain of oxytocin exerts a pronounced effect upon the biological activities. A more detailed study of the response of D-leucine-oxytocin in the various assay procedures is under way and will be published elsewhere.

Experimental[10]

Carbobenzoxy-L-prolyl-D-leucine.—Triethylamine (6.40 ml.) was added to carbobenzoxy-L-proline (10.9 g.) in tetrahydrofuran (65 ml.), and the solution was cooled to −10°. Isobutyl chloroformate (6.0 g.) was added and the solution was stirred at −5° for 20 minutes. A solution of D-leucine (5.75 g.) and triethylamine (8.8 ml.) in water (45 ml.) was then added and stirring was continued for 100 minutes without further cooling. The reaction mixture was acidified with 6 *N* HCl until the pH reached 3 and the tetrahydrofuran was removed *in vacuo*. A colorless oil separated from the residual aqueous solution and crystallized upon being allowed to stand overnight in the refrigerator. The solid was filtered off and washed with a small volume of *N* HCl and water. It was dissolved in glacial acetic acid (30 ml.) and crystallized by the slow addition of water (450 ml.). The dried material was again recrystallized from approximately 300 ml. of chloroform–hexane (1:4); wt. 11.0 g., m.p. 133–135°, $[\alpha]^{25}D$ −66.5° (*c* 6, chloroform).

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(10) All melting points were determined in capillary tubes and are corrected.

Anal. Calcd. for $C_{19}H_{28}O_5N_2$: C, 63.0; H, 7.23; N, 7.73. Found: C, 62.9; H, 7.21; N, 7.77.

Carbobenzoxy-L-prolyl-D-leucylglycinamide.—This material was prepared in two equal batches. Triethylamine (2.17 ml.) was added to carbobenzoxy-L-prolyl-D-leucine (5.45 g.) in tetrahydrofuran (65 ml.), and the solution was cooled to −15°. Isobutyl chloroformate (2.06 g.) was added, and the solution was stirred at −10° for 10 minutes. Glycinamide hydrochloride (1.83 g.) in a mixture of water (15 ml.) and triethylamine (25 ml.) was then added to the reaction mixture at −20° and stirring was continued for 10 minutes at −10° and then for 80 minutes at room temperature. The pH of the solution was adjusted to 3 by the addition of *N* HCl and the tetrahydrofuran was removed *in vacuo*. The crystalline product which separated from the residual aqueous solution was washed with water and then dissolved in dichloromethane (90 ml.). The dichloromethane solution was extracted with 30 ml. of 0.5 *N* HCl, 15 ml. of water, 30 ml. of 0.5 *M* $KHCO_3$ and three times with 20 ml. of water. It was then stored in a calcium chloride desiccator for several hours. The dry solvent was removed *in vacuo* and the residue was crystallized from 300 ml. of chloroform–hexane (1:6); wt. 4.5 g., m.p. 162–165°.

Material with this melting point (4.3 g.) was recrystallized from hot water; wt. 4.1 g., m.p. 167–168°, $[\alpha]^{20}D$ −10.5° (*c* 2, 95% ethanol).

Anal. Calcd. for $C_{21}H_{30}O_5N_4$: C, 60.3; H, 7.23; N, 13.4. Found: C, 59.9; H, 7.14; N, 13.3.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-D-leucylglycinamide.—Carbobenzoxy-L-prolyl-D-leucylglycinamide (4.1 g.) was dissolved in 2 *N* HBr in glacial acetic acid (12 ml.), and the solution was stirred for 100 minutes at room temperature and then concentrated to a syrup *in vacuo*. Dry ether (30 ml.) was added and the precipitate which formed was washed with ether and dissolved in methanol (30 ml.). Amberlite IRA-400 in the OH form was added to the methanol solution until the reaction for bromide ion became negative. After the resin had been removed by filtration, the solution was evaporated *in vacuo* to a partially crystalline residue of the desired L-prolyl-D-leucylglycinamide. This material was dissolved in dimethylformamide (4 ml.) and *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate (4.5 g.) was added to the solution. The first crystallization of the product was induced by seeding with a trace of crystalline S-benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-L-leucylglycinamide after the reaction mixture had been allowed to stand for several hours with no sign of crystallization. Two days later ethyl acetate (75 ml.) was added with stirring. After being allowed to stand in the refrigerator for 2 more days, the crystalline material was filtered off and washed with ethyl acetate (50 ml.); wt. 3.1 g., m.p. 167–169°. A second batch on the same scale gave 3.0 g. of product with the same melting point. The material was further purified by two recrystallizations from methanol–water; 3 g. of the crystals was dissolved in 35 ml. of methanol and boiled for 2 minutes in the presence of charcoal (1 g.); the charcoal was filtered off and the product was crystallized from the warm filtrate by the slow addition of the same volume of water. In the second recrystallization no charcoal was used. This purification raised the melting point of the product to 171–172°, with approximately a 10% loss in weight; $[\alpha]^{22}D$ −13.0° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{31}H_{41}O_6N_5S$: C, 60.9; H, 6.75; N, 11.5. Found: C, 60.8; H, 6.74; N, 11.5.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-D-leucylglycinamide.—The carbobenzoxy group was removed from the protected tetrapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-prolyl-D-leucylglycinamide by treatment with HBr in acetic acid as already described for the preparation of L-prolyl-D-leucylglycinamide. From 2.0 g. of protected tetrapeptide 1.5 g. of the free tetrapeptide S-benzyl-L-cysteinyl-L-prolyl-D-leucylglycinamide was obtained as an amorphous solid. An aliquot of the material (0.1 mg.) was subjected to descending chromatography on Whatman No. 1 paper in the solvent system butanol–acetic acid–water (4:1:5). A single sharp ninhydrin spot (R_f 0.76) was obtained.

The free tetrapeptide (1.27 g.) and the pentapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine[4] (2.0 g.) were dissolved in 11.5 ml. of dimethylformamide. To this solution 0.96 g. of dicyclohexylcarbodiimide (100% excess) was added at 0°. The reaction mixture was stirred at 0° for 30 minutes and at room temperature for 5 hr. After storage overnight in the refrigerator, the solidified material was mixed with glacial acetic acid (1 ml.) and ice-cold water (120 ml.). The precipitate was filtered off, washed with liberal amounts of water and dried over P_2O_5 *in vacuo*. Thus 3.3 g. of a white amorphous solid was obtained. The bulk of this material (3.0 g.) was extracted with methanol, precipitated with water from a dimethylformamide solution and again extracted with hot methanol. The substance was dried to constant weight over P_2O_5 *in vacuo;* wt. 0.75 g., m.p. 237–239° dec., $[\alpha]^{19}D$ −27.5° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{65}H_{86}O_{14}N_{12}S_2$: C, 59.0; H, 6.55; N, 12.7. Found: C, 59.2; H, 6.63; N, 12.4.

D-Leucine-oxytocin.—The procedure for the reduction of the protected nonapeptide intermediate with sodium in liquid ammonia was essentially the one described in the synthesis of tritium-labeled oxytocin.[2] The reduced material was aerated in aqueous solution at pH 6.8 at a concentration of 0.5 mg. per ml. for approximately 2 hr., whereupon oxidation was completed by the addition of potassium ferricyanide.[11,12] After removal from the solution of ferrocyanide and ferricyanide ions by means of the resin AG3-X4 in the chloride form, the pH was adjusted to 4 and the solution was concentrated *in vacuo* below room temperature.

Such a concentrate (30 ml.) resulting from the reduction of 200 mg. of the protected nonapeptide intermediate which was found to contain a total of approximately 1400 units of avian depressor activity was placed in the first 10 tubes of a 6 ml. 400-tube Craig countercurrent machine and subjected to a total of 800 transfers in the solvent system butanol–propanol–0.05% acetic acid (2:1:3) at 4°. After 400 transfers a separation into two peaks with *K* values of 0.2 and 0.5 had been accomplished, as detected by the Folin–Lowry[13] color reaction. The material of the slow-moving peak did not possess avian depressor activity, and no activity was found in the tubes between the two peaks. The peak resulting from the D-leucine-oxytocin (*K* 0.50) was in good agreement with a calculated curve except for a small shoulder on the leading edge. The avian depressor activity was associated with the material of this peak and its distribution followed closely the theoretical curve. After 800 transfers the impurity detected on the leading edge of the D-leucine-oxytocin peak was separated from the main peak. The solvent phases from Tubes 259–284, containing the D-leucine-oxytocin from the central portion of the peak were combined, concentrated to a small volume and lyophilized to give approximately 35 mg. of the desired analog. From a second, similar run, in which 350 mg. of the protected nonapeptide was used, another portion of about 60 mg. of lyophilized D-leucine-oxytocin was obtained agreeing in properties with the other preparation. In this experiment the distribution of D-leucine-oxytocin after 800 transfers was analyzed by measurement of Folin–Lowry color as well as by determination of the residual weights in the lower phase. The two curves obtained by plotting these values were in good agreement with a calculated curve. For elementary analysis a sample was dried at 100° over P_2O_5 *in vacuo* for 5 hr. with a loss in weight of 7%; $[\alpha]^{20}D$ +12° (*c* 0.5, *N* acetic acid).

Anal. Calcd. for $C_{43}H_{66}O_{12}N_{12}S_2 \cdot C_2H_4O_2$: C, 50.6; H, 6.61; N, 15.8. Found: (corrected for 0.70% ash): C, 50.8; H, 6.68; N, 15.7.

An amino acid analysis of the lyophilized material was performed on a Beckman–Spinco amino acid analyzer according to the procedure of Spackman, Stein and Moore.[14] The following amino acid molar ratios (with the ratio for leucine taken as 1) were obtained: aspartic acid 1.0, glutamic acid 0.9, proline 1.1, glycine 1.0, cystine 1.0, isoleucine 0.9, leucine 1.0, tyrosine 0.75, ammonia 3.1.

D-Leucine-oxytocin traveled somewhat faster than oxytocin when they were subjected to paper chromatography in

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the following manner. Oxytocin (100 μg.) and D-leucine-oxytocin (100 μg.) as well as a mixture of 100 μg. of each of the two peptides were applied separately to a strip of Whatman No. 1 paper and chromatographed for 16 hr. at room temperature with the solvent system butanol–acetic acid–water (4:1:5) (descending). The R_f values of the ninhydrin spots obtained were 0.67 for oxytocin and 0.73 for D-leucine-oxytocin. The mixture of the two peptides had been separated into two distinct spots according to the R_f values reported.

Countercurrent Distribution of a Mixture of Oxytocin and D-Leucine-oxytocin.—A mixture of oxytocin (10 mg.), D-leucine-oxytocin (9 mg.) and synthetic tritium-labeled oxytocin[2] (1 mg.) was introduced into the first 10 tubes of the 6-ml. 200-tube Craig countercurrent machine and submitted to 450 transfers in the solvent system butanol–propanol–0.05% acetic acid (2:1:3) at 4°. The resulting distribution of the material was analyzed by measurement of the Folin–Lowry color and of the radioactivity by liquid scintillation counting.[15] Oxytocin and radioactive oxytocin, exhibiting a K value of 0.32, were separated from D-leucine-oxytocin with a K value of 0.49, as shown in Fig. 1.

Acknowledgments.—The authors wish to thank Mr. Joseph Albert for the microanalyses, Mrs. Lorraine Abrash for the amino acid analyses, and Miss Maureen O'Connell, Miss Catharine Smith and Mr. Hans Holzhauser for the bioassays.

(15) Aliquots of the lower phase (0.1 ml.) were counted in a Tracerlab scintillation counter with the dioxane–xylene solution of the phosphor as used by H. J. Jacobson, G. N. Gupta, C. Fernandez, S. Hennix and E. V. Jensen, *Arch. Biochem. Biophys.*, **86**, 89 (1960).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZ.]

Potential Purine Antagonists. XXXIV. The Synthesis of 3-Methylguanine and a Study of the Structure and Chemical Reactivity of Certain 3-Methylpurines[1]

BY LEROY B. TOWNSEND AND ROLAND K. ROBINS

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The synthesis of 3-methylguanine (I) has been achieved. A study of the chemical properties of I and a number of related 2-amino-3-methyl-6-substituted purines has revealed that the classical, fixed double-bond type structure which can be written for these compounds does not account for the observed chemical reactivity toward nucleophilic substitution. Evidence is presented which suggests that these compounds possess a high degree of aromaticity with an increased electron density in the imidazole ring and an over-all decrease of electron density in the pyrimidine ring.

The superior antitumor activity of 2-amino-1-methyl-6-purinethione[2] over that of 2-amino-6-purinethione (6-thioguanine) against *Adenocarcinoma* 755 prompted us to investigate the synthesis of additional N-methyl derivatives of 6-thioguanine. The preparation of the 9-methyl[3] and 7-methyl[4] derivatives of 2-amino-6-purinethione have already been reported by thiation of the corresponding N-methylguanine with phosphorus pentasulfide in pyridine. Since the required 3-methylguanine (I) has not been reported previously, the synthesis of I was undertaken in our laboratory.

The study of 3-N-substituted purines is presently of considerable interest in view of the work of Leonard and Deyrup[5] who have shown that the naturally-occurring alkaloid, triacanthine, is 6-amino-3-(γ,γ-dimethylallyl)-purine. Also it has been shown[6] recently that uric acid-3-riboside occurs in beef blood.

It is of interest that a previous unsuccessful attempt to prepare 3-methylguanine has been recorded.[7] Brookes and Lawley[8] have recently noted that no 3-substituted guanines are known. The synthesis of 3-methylguanine (I) *via* the classical Traube synthesis was investigated. The condensation of methylguanidine and ethyl cyanoacetate has been described by Roth, Smith and Hultquist[9] as yielding 4-amino-6-hydroxy-2-methylaminopyrimidine and a ring N-methylated isomer which was assigned the structure 2,4-diamino-1-methyl-6-pyrimidone. This structural assignment was re-investigated by Boon and Bratt[10] and Curran and Angier[11] who established the structure as 2,4-diamino-3-methyl-6-pyrimidone. Nitrosation and reduction[9] provided 3-methyl-2,4,5-triamino-6-pyrimidone (IV) sulfate. Ring closure to 3-methylguanine (I) proceeded readily with boiling formamide.[12]

All known N-methylguanine derivatives were compared with I utilizing ultraviolet absorption spectra and paper chromatography and were shown to be different from I. A rigorous structure proof of 3-methylguanine was sought, however, in view of several recorded rearrangements[13,14] of various N-methylpyrimidines. Treatment of 3-methylguanine with mineral acid and sodium nitrite under standard conditions for converting guanine to xanthine gave only unreacted I. Refluxing 6 N hydrochloric acid did not change 3-methylguanine after 3 hours. Refluxing 2 N sodium hydroxide, however, converted I to 3-methylxanthine (II) in good yield. An authentic sample of II was prepared from 2-mercapto-3-methyl-6-purinone[15]

(1) Supported by Research Grant No. T-181 from the American Cancer Society.

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