ceeded smoothly to afford 4-amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIb) in 83% yield: mp 260°, λ_{max}^{EtOH} 278 (ε 15,100) and 229 mμ (ε 8200); $[\alpha]^{26}D$ −45.7 ± 1.9° (*c* 1.0, 0.1 *N* HCl).[27] A comparison of chromatographic mobilities, ultraviolet absorption, and infrared spectra of VIb with those of an authentic sample of sangivamycin[27] established that the samples were identical. Additional evidence for the structural assignment of sangivamycin and toyocamycin was furnished by the conversion of sangivamycic acid to the related pyrrolo[2,3-*d*]pyrimidine antibiotic tubercidin since the structure of tubercidin has previously been unequivocally established.[28] Sangivamycic acid (VIc) has been previously prepared[8] as an intermediate but was never characterized. Sangivamycic acid hydrochloride was prepared in our laboratory by heating toyocamycin at reflux temperature in 3 *N* hydrochloric acid in a nitrogen atmosphere for 12 hr to obtain a 55% yield of VIc: mp 238° dec; λ_{max}^{EtOH} 279 (ε 13,500) and 231 mμ (ε 7600). Decarboxylation was accomplished by immersion of VIc in a preheated oil bath (238°) for approximately 10 sec; this gave a dark amber melt. An aqueous ethanol extract of this melt furnished tubercidin (VId, 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine) in 13% yield. The identity of VId was confirmed by rigorous comparison with authentic tubercidin.[29]

(27) The authors wish to thank Dr. K. V. Rao for an authentic sample of sangivamycin hydrochloride which showed an optical rotation of $[\alpha]^{26}D$ −42.2 ± 1.9° under similar conditions.

(28) Y. Mizuno, M. Ikehara, K. A. Watanabe, S. Suzaki, and T. Itoh, *J. Org. Chem.*, 28, 3329 (1963).

(29) The authors wish to thank Dr. Saburo Suzuki for an authentic sample of tubercidin

(30) National Aeronautics and Space Administration Fellow, 1965–1967.

Richard L. Tolman,[30] Roland K. Robins
Leroy B. Townsend
Department of Chemistry, University of Utah
Salt Lake City, Utah 84112
Received September 21, 1967

The Rearrangement of N,N′-Dimethylhydrazobenzene[1]

Sir:

Although the benzidine rearrangement has been the subject of many investigations,[2] its intimate mechanism continues to be the subject of considerable discussion and disagreement, with some[3] preferring a concerted mechanism in which the new bonds found in the products are formed as the nitrogen–nitrogen bond in the hydrazoaromatic is broken, and others[4] favoring a process in which bond scission and formation occur sequentially. Even among those holding the latter view there are differences regarding the nature of the intermediate, some[4a] supporting a π complex and others[4b] arguing for a solvent-caged cation–radical pair.

Most of the product and kinetic data for the benzidine rearrangement can be equally well explained by either a concerted or a stepwise mechanism. However, *p*-semidine (*p*-aminodiphenylamine) formation is difficult to interpret in terms of the polar transition-state mechanism (the most recent proposal for the concerted process) since bond formation over a distance of about 5.3 Å would be required.[5] Indeed, *p*-semidine-type products have seldom been isolated from benzidine rearrangements[6] and in most cases in which they have been obtained the conditions were unusual. These facts have led to the suggestion[3] that *p*-semidines are not formed in the normal benzidine rearrangement. This conclusion has been used to support the polar transition state.[3] On the other hand, the formation of a *p*-semidine would be a logical outcome of the π complex or cation–radical mechanisms since the fragments resulting from nitrogen–nitrogen bond breaking would be able to assume a variety of positions with respect to each other.

A *p*-semidine-like compound has been detected among the products resulting from rearrangement of N,N′-dimethylhydrazobenzene at 25° in 25% aqueous methanol using 0.01 *M* hydrochloric acid as catalyst. Analysis was accomplished by the isotope dilution procedure. The following compounds were observed: 50.7% N,N′-dimethyl-4,4′-diaminobiphenyl (benzidine), 20.3% N,N′-dimethyl-2,4′-daminobiphenyl (diphenyline), 0.9% N,N′-dimethyl-2,2′-diaminobiphenyl (*o*-benzidine); 15.5% N,N′-dimethyl-*o*-aminodiphenylamine (*o*-semidine); 3.0% N,N′-dimethyl-*p*-aminodiphenylamine (*p*-semidine), and 11.2% N-methylaniline (disproportionation product). The benzidine and diphenyline were isolated as the N,N′-dibenzoyl derivatives, the semidines as monobenzamides, and the disproportionation product as the acetamide. These derivatives were purified by chromatography followed by several recrystallizations (often from two different solvents). From the material balance, it is obvious that essentially all of the products have been accounted for in this analysis.

The kinetics of acid-catalyzed rearrangement of N,N′-dimethylhydrazobenzene in 25% aqueous methanol at 25° were also investigated. The experimental results conform to an expression of the following type: rate = $k_2(H^+)$(substrate). The rates of isomerization of the 4,4′-dichloro and 4,4′-dimethyl derivatives of N,N′-dimethylhydrazobenzene were also determined, and the second-order rate constants for the three hydrazo compounds were correlated by σ^+ constants and a ρ value of −11.85.

The rearrangement of N,N′-dimethylhydrazobenzene is similar to the isomerization of hydrazobenzene in that the major products are the benzidine and the diphenyline.[7] The ratio of these two products is about the same in both reactions, about 2.3–2.5. However, the two rearrangements differ in that sizable amounts of both the *o*- and *p*-semidine products result from the reaction involving N,N′-dimethylhydrazobenzene. The formation of a *p*-semidine is particularly interesting since it cannot arise from con-

(1) Research supported by National Science Foundation Grant GP-1970.

(2) See H. J. Shine, "Aromatic Rearrangements," Elsevier Publishing Company, New York, N. Y., 1967, pp 126–179, for an excellent review of this subject.

(3) D. V. Banthorpe, E. D. Hughes, and C. K. Ingold, *J. Chem. Soc.*, 2864 (1964).

(4) (a) M. J. S. Dewar and A. P. Marchand, *Ann. Rev. Phys. Chem.*, **16**, 338 (1965); M. J. S. Dewar in "Molecular Rearrangements," Vol. 1, P. de Mayo, Ed., Interscience Publishers, Inc., New York, N. Y., 1963; (b) G. S. Hammond and J. S. Clovis, *J. Org. Chem.*, 28, 3283, 3290 (1963).

(5) The intermolecular distance in the very weakly bonded molecular complexes (2.5–3.5 Å) is less.

(6) M. Vecera, J. Petranek, and J. Gasparic, *Collection Czech. Chem. Commun.*, **22**, 1603 (1957); P. Jacobson, *Ann.*, **428**, 76 (1922).

(7) R. B. Carlin, R. G. Nelb, and R. C. Odioso, *J. Am. Chem. Soc.*, **73**, 1002 (1951).

certed bond breaking and making in the transition state. Thus, the rearrangement of hydrazoaromatics must involve nitrogen–nitrogen bond cleavage to form an intermediate which then rebonds to yield the products.

The effect of substituents on the rate of reaction also argues against a one-step concerted process. Most reactions of this type are only weakly affected by substituents.[8] The large substituent effect observed is entirely consistent with a process in which an extranuclear bond breaks in the rate-determining step generating a center which can relieve its electron deficiency by resonance with the ring and the substituent.[9] Therefore, the stepwise mechanism for the benzidine rearrangement is again indicated.

In conclusion, product and substituent effect data obtained in a study of the rearrangement of N,N′-dimethylhydrazobenzene strongly favor a mechanism for the benzidine rearrangement in which the nitrogen–nitrogen bond of the hydrazo compound is broken before the carbon–carbon or carbon–nitrogen bonds in the products are formed.

(8) See, for example, C. H. DePuy and C. A. Bishop, *J. Am. Chem. Soc.*, 82, 2532 (1960); C. H. DePuy and R. E. Leary, *ibid.*, 79, 3705 (1957); S. C. J. Olivier and A. P. Weber, *Rec. Trav. Chim.*, 53, 869 (1934); W. N. White, C. D. Slater, and W. K. Fife, *J. Org. Chem.*, 26, 627 (1961).

(9) See for comparison Y. Okamoto, T. Inukai, and H. C. Brown, *J. Am. Chem. Soc.*, 80, 4972 (1958); J. F. Norris and C. Banta, *ibid.*, 50, 1804 (1928); N. C. Deno and W. L. Evans, *ibid.*, 79, 5804 (1957).

William N. White, Edgar E. Moore
Department of Chemistry, University of Vermont
Burlington, Vermont 05401
Received November 13, 1967

Studies on Peptides. XX. Synthesis of the Octadecapeptide Corresponding to the Entire Amino Acid Sequence of Monkey β-Melanocyte-Stimulating Hormone[1,2]

Sir:

The structure of β-melanocyte-stimulating hormone (I) from monkey pituitary glands was elucidated by Lee, *et al.*,[3] in 1961. We wish to report the synthesis of the octadecapeptide which embodies the entire amino acid sequence of this hormone.

H-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH

Choice of the protecting groups for the α-amino and guanidino group of arginine which is adjacent to the methionine residue and the ε-amino group of the lysine residue at position 17 determined the strategy toward the total synthesis of this peptide hormone.

The formyl group was selected for the protection of the ε-amino group of lysine.[4] Thus, histidylphenylalanylarginyltryptophylglycylserylprolylprolyl-N$^{\epsilon}$-formyllysylaspartic acid (II), prepared as previously described,[5] was condensed with N$^{\alpha}$-benzyloxycarbonyl-γ-benzylglutamate by means of the *p*-nitrophenyl ester method,[6] and the resulting product was subsequently hydrogenated to give glutamylhistidylphenylalanylarginyltryptophylglycylserylprolylprolyl-N$^{\epsilon}$-formyllysylaspartic acid (III, monoacetate octahydrate. *Anal.* Calcd for $C_{63}H_{86}O_{18}N_{18}\cdot CH_3COOH\cdot 8H_2O$: C, 49.2; H, 6.7; N, 15.9. Found: C, 49.3; H, 7.1; N, 15.6), $[\alpha]^{20}D$ −73.6° (water); R_f^1 0.20, R_f^2 0.28; amino acid ratios in an acid hydrolysate $Glu_{1.00}His_{0.92}Phe_{0.87}Arg_{0.87}Gly_{1.03}Ser_{1.01}Pro_{1.98}Lys_{1.12}Asp_{1.04}$; average recovery 100%. This partially protected undecapeptide (III) was allowed to react with N$^{\alpha}$-*t*-butoxycarbonylmethionine *p*-nitrophenyl ester,[7] and the resulting product was treated with trifluoroacetic acid to give methionylglutamylhistidylphenylalanylarginyltryptophylglycylserylprolylprolyl-N$^{\epsilon}$-formyllysylaspartic acid (IV) (*Anal.* Calcd for $C_{68}H_{95}O_{19}N_{19}S\cdot CH_3COOH\cdot 6H_2O$: C, 50.0; H, 6.6; N, 15.8. Found: C, 50.1; H, 7.2; N, 15.5), $[\alpha]^{20}D$ −66,6° (water); R_f^1 0.11, R_f^2 0.25; amino acid ratios in an acid hydrolysate $Met_{1.05}Glu_{1.01}His_{1.04}Phe_{0.97}Arg_{0.92}Gly_{1.00}Ser_{0.97}Pro_{1.97}Lys_{1.14}Asp_{1.02}$; average recovery 100%. It was confirmed in a preliminary experiment that N$^{\epsilon}$-formyllysine survived the action of trifluoroacetic acid mostly unchanged.

This partially protected dodecapeptide (IV) was condensed with N$^{\alpha}$-*t*-butoxycarbonyl-N^{G}-nitroarginine[8] by the mixed anhydride procedure.[9,10] The resulting product was treated with anhydrous hydrogen fluoride according to Sakakibara and Shimonishi[11] to remove the *t*-butoxycarbonyl group and the nitro group from the arginine residue.[12,13] It is known that the formyl group on model peptides is not affected by this treatment.[13] The desired partially protected tridecapeptide, arginylmethionylglutamylhistidylphenylalanylarginyltryptophylglycylserylprolylprolyl-N$^{\epsilon}$-formyllysylaspartic acid (V, $[\alpha]^{30}D$ −36.7° (1 *N* acetic acid), R_f^3 0.17, contaminated with a trace amount of the side reaction product from the mixed anhydride reaction, presumably a ethoxycarbonyl derivative[14,15] of IV, R_f^3 0.40), was then allowed to react with N$^{\alpha}$-*t*-butoxycarbonylprolyltyrosine azide derived from the corresponding hydrazide (mp 174–176°. *Anal.* Calcd for $C_{19}H_{28}O_5N_4$: C, 58.2; H, 7.2; N, 14.3. Found: C, 57.6; H, 7.3; N, 14.3), and the resulting product was subsequently treated with trifluoroacetic acid to give the partially protected pentadecapeptide, prolyltyrosylarginylmethionylglutamylhistidylphenylalanylarginyl-

(1) Peptides and their derivatives mentioned in this communication are of the L configuration. R_f^1 values refer to the system 1-butanol–acetic acid–water, 4:1:5 on paper chromatography. R_f^2 and R_f^3 values refer to the system of 1-butanol–pyridine–acetic acid–water, 4:1:1:2 and 15:10:3:12, respectively, on thin layer chromatography (Kieselgel G, Merck). Column chromatography on carboxymethylcellulose was used extensively for purification of these peptides with ammonium acetate or pyridine acetate buffers as eluent.

(2) Part XIX of this series: H. Yajima and K. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), in press.

(3) T. H. Lee, A. B. Lerner, and V. B.-Janusch, *J. Biol. Chem.*, 236, 1390 (1961).

(4) K. Hofmann, E. Stutz, G. Spühler, H. Yajima, and E. T. Schwartz, *J. Am. Chem. Soc.*, 82, 3727 (1960).

(5) H. Yajima, Y. Okada, Y. Kinomura, and E. Seto, *Chem. Pharm. Bull.* (Tokyo), 15, 270 (1967).

(6) M. Bodanszky, *Nature*, 175, 685 (1955); *Acta Chim. Hung.*, 10, 335 (1957).

(7) K. Hofmann, W. Haas, M. J. Smithers, and G. Zanetti, *J. Am. Chem. Soc.*, 87, 631 (1965).

(8) K. Hofmann, W. Haas, M. J. Smithers, R. W. Wells, Y. Wolman, N. Yanaihara, and G. Zanetti, *ibid.*, 87, 620 (1965).

(9) Th. Wieland and H. Bernhard, *Ann.*, 572, 190 (1951).

(10) J. R. Vaughan, Jr., and R. L. Osato, *J. Am. Chem. Soc.*, 73, 3547 (1951).

(11) S. Sakakibara and S. Shimonishi, *Bull. Chem. Soc. Japan*, 38, 1412 (1965). The authors wish to express their sincere appreciation to Dr. S. Sakakibara for offering his equipment for this experiment.

(12) J. Leonard and A. B. Robinson, *J. Am. Chem. Soc.*, 89, 181 (1967).

(13) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, 40, 2164 (1967).

(14) A. R. Emery and V. Gold, *J. Chem. Soc.*, 1443, 1447 (1950).

(15) Th. Wieland, B. Heinke, K. Vogeler, and H. Morimoto, *Ann.*, 655, 189 (1962).