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.

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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^{ϵ}-formyllysylaspartic acid (II), prepared as previously described,[5] was condensed with N^{α}-benzyloxycarbonyl-γ-benzylglutamate by means of the *p*-nitrophenyl ester method,[6] and the resulting product was subsequently hydrogenated to give glutamylhistidylphenylalanylarginyltryptophylglycylserylprolylprolyl-N^{ϵ}-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^{α}-*t*-butoxycarbonylmethionine *p*-nitrophenyl ester,[7] and the resulting product was treated with trifluoroacetic acid to give methionylglutamylhistidylphenylalanylarginyltryptophylglycylserylprolylprolyl-N^{ϵ}-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^{ϵ}-formyllysine survived the action of trifluoroacetic acid mostly unchanged.

This partially protected dodecapeptide (IV) was condensed with N^{α}-*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^{ϵ}-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^{α}-*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.

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tryptophylglycylserylprolylprolyl-N$^\epsilon$-formyllysylaspartic acid (VI. *Anal.* Calcd for $C_{88}H_{123}O_{23}N_{25}S \cdot 2CH_3CO-OH \cdot 9H_2O$: C, 49.9; H, 6.8; N, 15.8. Found: C, 50.6; H, 7.1; N, 15.0), $[\alpha]^{30}D$ −60.7° (1 *N* acetic acid); $R_f{}^3$ 0.47; amino acid ratios in an acid hydrolysate $Pro_{3.17}Tyr_{0.88}Arg_{1.82}Met_{0.94}Glu_{1.01}His_{0.97}Phe_{1.00}Gly_{0.97}$-$Ser_{0.84}Lys_{1.06}Asp_{1.00}$; (average recovery 89%).

The N-terminal protected tripeptide, N$^\alpha$-*t*-butoxycarbonyl-β-*t*-butylaspartyl-γ-*t*-butylglutamylglycine (VII, dicyclohexylamine salt. *Anal.* Calcd for $C_{24}H_{41}$-$O_{10}N_3 \cdot C_{12}H_{23}N$: C, 60.7; H, 9.0; N, 7.9. Found: C, 60.7; H, 9.3; N, 8.1), mp 128–130°, $[\alpha]^{22}D$ −15.3° in methanol, was prepared by the *p*-nitrophenyl ester method in a stepwise manner from the C-terminal glycine. This peptide (VII) was condensed with VI by means of the N-hydroxysuccinimide ester method.[16] The resulting product was then treated with trifluoroacetic acid to form aspartylglutamylglycylprolyltyrosylarginylmethionylglutamylhistidylphenylalanylarginyltryptophylglycylserylprolylprolyl-N$^\epsilon$-formyllysylaspartic acid (VIII, $[\alpha]^{30}D$ −63.6° (1 *N* acetic acid); $R_f{}^3$ 0.49; amino acid ratios in an acid hydrolysate $Asp_{1.96}Glu_{2.20}$-$Gly_{2.05}Pro_{3.10}Tyr_{0.76}Arg_{1.99}Met_{0.93}His_{1.00}Phe_{1.00}Ser_{0.98}$-$Lys_{1.06}$; average recovery 96%), which was subsequently treated with 5% aqueous hydrazine acetate at 37° for 48 hr to remove the N$^\epsilon$-formyl group from the lysine residue. The use of aqueous hydrazine acetate or hydroxylamine hydrochloride in pyridine for the removal of the formyl group from N$^\epsilon$-formyllysine has been demonstrated recently in the synthesis of α-MSH from the [11-N$^\epsilon$-formyllysine]-α-MSH derivative.[17] Care was taken to prevent possible oxidation of the methionine residue by performing the reaction in the presence of thioglycolic acid.

The purified octadecapeptide corresponding to the entire amino acid sequence of I ($[\alpha]^{30}D$ −50.7° (1 *N* acetic acid); amino acid ratios in an acid hydrolysate $Asp_{1.96}Glu_{1.97}Gly_{1.94}Pro_{3.02}Tyr_{0.79}Arg_{2.01}Met_{0.76}His_{1.08}$-$Phe_{1.06}Ser_{0.95}Lys_{1.00}$; average recovery 88%) exhibited a single spot on thin layer chromatography ($R_f{}^3$ 0.46) and behaved as a single component on paper electrophoresis in pyridine acetate buffers at two different pH values (3.5 and 6.8).

The MSH potencies (expressed as MSH units/gram) of the synthetic peptides, determined according to Shizume, *et al.*,[18] using frog skins from *Rana pipiens*, were as follows: III, 6.0 $\times 10^6$; IV, 1.9 $\times 10^6$; V, 1.8 $\times 10^8$; VI, 2.2 $\times 10^{12}$; VIII, 2.0 $\times 10^9$; and synthetic I, 2.5 $\times 10^{10}$ (lit.[19] natural monkey β-MSH, 3∼5 $\times 10^9$). It is noteworthy that the partially protected pentadecapeptide VI is nearly as active as the best preparation of natural α-MSH[20] and addition of the acidic tripeptide to this pentadecapeptide (VI) causes some detrimental effect as far as MSH activity is concerned.

The structurally related β-MSH from bovine origin was synthesized by Schwyzer, *et al.*[21] This hormone possesses the lysine residue at position 6 instead of the arginine residue present in the monkey β-MSH. This difference required a different synthetic approach to monkey β-MSH from that of Schwyzer, *et al.*, as outlined above.

During the course of this investigation, we have found that N$^\alpha$-benzyloxycarbonyl-N^G-nitroarginylmethionine methyl ester could be reduced to arginylmethionine methyl ester by catalytic hydrogenation over a palladium catalyst in the presence of boron trifluoride etherate.[22] This procedure offered an alternate synthetic approach to this hormone which possesses the particular amino acid sequence of arginylmethionine. These results will be published in the future.

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The Two-Step Polar Cycloaddition of Sulfonyl Isocyanates to Carbodiimides

Sir:

The polar 1,2-cycloaddition reaction of ketenes to vinyl ethers shows stereospecificity, thereby indicating that this reaction proceeds *via* a concerted one-step process.[1,2] In a recent article by Proskow, *et al.*,[3] evidence has been presented that in some cases the 1,2-cycloaddition reaction of 1,2-bis(trifluoromethyl)-1,2-dicyanoethylene to vinyl ethers occurs *via* an intermediate, as indicated by the loss of stereospecificity.

We wish now to report spectral and chemical evidence for a two-step polar 1,2-cycloaddition reaction of heterocarbon double-bond systems which occurs in the addition of arenesulfonyl isocyanates to dialkylcarbodiimides. For example, on addition of arenesulfonyl isocyanates to dialkylcarbodiimides in benzene or carbon tetrachloride an immediate reaction occurs, as evidenced by the appearance of two double-bond absorptions at 1869 (medium) and 1724 cm^{-1} (strong), respectively, which gradually disappear as the reaction progresses. The formation of an intermediate product can also be observed by nmr spectroscopy. On mixing *t*-butylmethylcarbodiimide and *p*-toluenesulfonyl isocyanate in carbon tetrachloride the N-methyl signal of the carbodiimide is shifted from 2.9 to 3.32 ppm, indicating attachment of the tosyl isocyanate to the less hindered nitrogen adjacent to the methyl group. The N-methyl signal at 3.32 ppm gradually decreases and new N-methyl signals appear at approximately 2.9 and 3.6 ppm.

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