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, 6.0×10^6; IV, 1.9×10^6; V, 1.8×10^8; VI, 2.2×10^{12}; VIII, 2.0×10^9; and synthetic I, 2.5×10^{10} (lit.[19] natural monkey β-MSH, $3 \sim 5 \times 10^9$). 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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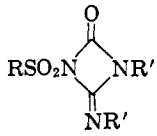
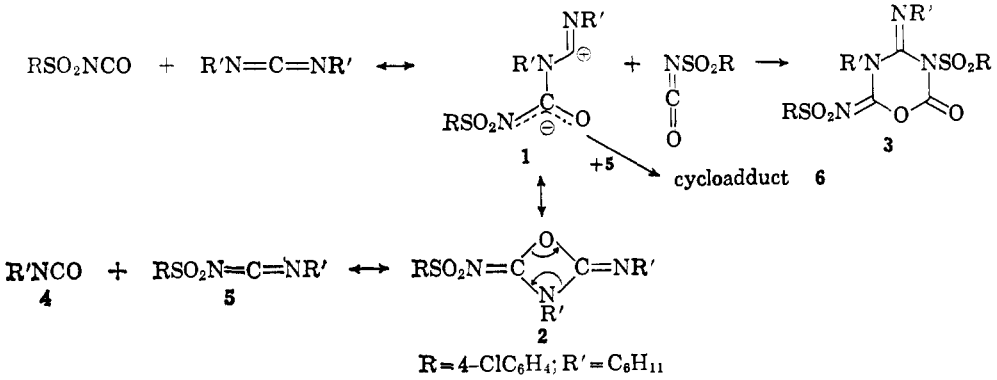
The chemical composition of the observed intermediate is most likely that of a 1:1 adduct of both reagents, *i.e.*, **1** or **2** or the isomeric cycloadduct



Singer and Bartlett[4] observed a C=N absorption at 1736 cm^{-1} for cycloadducts of type **2**, and a C=O absorption at 1754 cm^{-1} has been reported for the isomeric cycloadduct.[5] Therefore, it is indicated that one of the observed absorptions (1869 cm^{-1}) may in fact be caused by the linear adduct **1**.

Scheme I



After the reaction is completed (disappearance of the N=C=O band at 2227 cm^{-1}) the solvent is evaporated and the less soluble 2:1 cycloadducts **3** are precipitated by the addition of diethyl ether. If the reaction is conducted in carbon tetrachloride, the cycloadducts **3** precipitate on standing. For example, on addition of 4-chlorobenzenesulfonyl isocyanate to dicyclohexylcarbodiimide, the 2:1 cycloadduct **3** (R = 4-chlorobenzenesulfonyl; R′ = cyclohexyl) (mp 150–153°; infrared: C=O and C=N absorptions at 1754, 1721, and 1672 cm^{-1}; *Anal.* Calcd for $C_{27}H_{30}Cl_2N_4O_6S_2$: C, 50.55; H, 4.68; N, 8.74. Found: C, 50.49; H, 5.11; N, 8.60) is obtained in 22.7% yield (based on carbodiimide). From the reaction mixture a second cycloadduct (mp 163–165°, infrared: C=O and C=N absorptions at 1718, 1672, and 1658 cm^{-1}) is obtained in 20.6% yield (based on sulfonyl isocyanate). *Anal.* Calcd for $C_{33}H_{41}Cl_2N_5O_5S_2$: C, 55.03; H, 5.73; N, 9.07. Found: C, 55.18; H, 6.17; N, 9.11.

The formation of the 2:1 cycloadduct **3** is readily explained by 1,4 cycloaddition of **1** across the C=N double bond of a second molecule of arenesulfonyl isocyanate. The possibility of trapping is enhanced if the mode of addition is reversed. Thus, 57.8% **3** is obtained when dicyclohexylcarbodiimide is added slowly to 2 equiv of 4-chlorobenzenesulfonyl isocyanate.

While the reaction progresses an exchange process *via* the four-membered ring 1:1 cycloadduct **2** gives rise to the formation of alkyl isocyanate (**4**) and arenesulfonylcarbodiimide (**5**), and **5** competes with arenesulfonyl isocyanate for the dipolar intermediate **1**, thereby forming the second isolated cycloadduct (**6**) (Scheme I).

The six-membered-ring cycloadducts **3** and **6** are stable at room temperature. However, above 100° thermolysis occurs with formation of the expected fragments. Therefore, heating of the total reaction mixture in *o*-dichlorobenzene and constant removal of the lowest boiling component (cyclohexyl isocyanate) by distillation shift the equilibria in the direction of formation of **4** and **5**.

The structure of the 2:1 cycloadduct **3** is evident from chemical and spectral data. For example, thermolysis in *o*-dichlorobenzene affords cyclohexyl isocyanate (2257 cm^{-1}), 4-chlorobenzenesulfonyl isocyanate (2227 cm^{-1}), and 4-chlorobenzenesulfonylcyclohexylcarbodiimide (2165 cm^{-1}). Although four different structures can be written for this adduct (cycloaddition across either one of the negative sites in **1** and addition across the C=N or the C=O bond in the arenesulfonyl isocyanate), the infrared data are more in line with the indicated structure.[6]

Thermolysis of cycloadduct **6** in *o*-dichlorobenzene affords cyclohexyl isocyanate and 4-chlorobenzenesulfonylcyclohexylcarbodiimide. Since four different structures can be envisaged for **6** and the infrared and nmr data are not conclusive, further work is underway to establish their structure unequivocally.

The stepwise cycloaddition of arenesulfonyl isocyanates to carbodiimides is a general reaction, and the detailed information will be described in a forthcoming publication. The formation of 2:1 adducts, which has been encountered quite often in cycloaddition reactions of heterocumulenes, could be a convenient diagnostic indicator for a stepwise mechanism. Most likely trapping of the linear intermediates by more reactive dipolarophiles may often be possible and could lead to the synthesis of a great variety of six-membered-ring compounds which are not easily obtainable by other methods. The exchange reaction which has served in our examples to synthesize N-sulfonylcarbodiimides likewise is an exceedingly useful synthetic method for the interconversion of hetero double-bond systems.

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(5) W. Neumann and P. Fischer, *Angew. Chem. Intern. Ed. Engl.*, **1**, 621 (1962).
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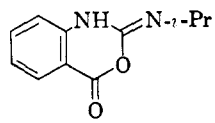

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