The much longer life-time of the triplet state in the cation, as compared with azulene, is presumably due to a greater energy separation between the S_1 and T_1 states in the cation. Although a number of resonance forms are possible for the azulenium triplet the low value for the zero-field splitting parameter (D^* = 0.0674 cm^{-1})[13] indicates that any contribution from a form in which the two unpaired electrons are localized on the carbon in the 1 position (*i.e.*, a cyclopentadienylidene derivative of tropylium) is negligible. In general D^* values for π triplets are less than 0.2 cm^{-1} [14] while the values for cyclic carbenes (cyclopentadienylidene, indenylidene) are appreciably higher, *ca.* 0.4 cm^{-1}.[15]

observation of a green phosphorescence for the irradiated cation at 77°K.[12] This green phosphorescence disappears within several seconds after the light is turned off.

(12) A green phosphorescence has been reported recently for the azulene in H_2SO_4: E. Sawicki and H. Johnson, *Microchem. J.*, **8**, 85 (1964).

(13) Calculated from $D^* = (D^2 + 3E^2)^{1/2} = [^3/_4(h\nu)^2 - 3(g\beta H_{min})]^{1/2}$.

(14) B. Smaller, *J. Chem. Phys.*, **37**, 1578 (1962).

(15) E. Wasserman, L. Barash, A. M. Trozollo, R. W. Murray, and W. A. Yager, *J. Am. Chem. Soc.*, **86**, 2304 (1964).

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The Controlled Synthesis of Peptides in Aqueous Medium. I. The Use of α-Amino Acid N-Carboxyanhydrides

Sir:

We wish to report that we have successfully used α-amino acid N-carboxyanhydrides (NCA's) in the controlled and sequential synthesis of peptides in aqueous medium. This result, which differs from previous conclusions,[1-4] was achieved by adding the NCA directly to an aqueous solution of an amino acid or peptide with close control of temperature and pH and rapid mixing of reactants, thus minimizing side reactions.

We have used all of the standard 20 amino acids in peptide syntheses. Protection of the additional functional group was required only with the NCA's of serine, threonine, lysine, histidine, and cysteine. In the nucleophilic component only lysine and cysteine required protection. In a number of cases we have carried out sequentially several steps of a polypeptide synthesis without isolation of the intermediate peptide by decarboxylating the product carbamate and repeating the cycle with an additional NCA. The progress of each individual step was readily assessed by thin layer chromatography, permitting periodic interruption of a sequential synthesis for purification of an intermediate peptide. This sequential synthesis of peptides, which is rapid, was followed by conventional-type purification including precipitation with acid or ammonium sulfate and chromatography on Sephadex or silica gel.

We have found no evidence for racemization in the NCA-peptide syntheses on analysis by tlc or by treatment of our peptides with leucine aminopeptidase (LAP). Confirmation of this conclusion was also obtained by using what we believe to be a new, highly sensitive method for determining racemization in peptide synthesis: measurement of tritium incorporation during reactions carried out in tritiated water. By this method the synthesis of tyrosylserine from serine and the NCA of tyrosine was found to proceed with less than 0.004% racemization even after correction for an independently determined tritium isotope effect of 4.6.

We have successfully used the NCA's of the seven simple difunctional amino acids as well as those of tryptophan, methionine, ε-carbobenzoxylysine, S-benzylcysteine, and *im*-N-benzylhistidine. We have also found that the NCA of glutamic acid[5] can be used in peptide synthesis in aqueous medium, yielding exclusively the α-linked glutamyl peptide in high yield. The usefulness of this NCA is noteworthy in view of the ease with which it undergoes intramolecular rearrangement.[6] We have also prepared the crystalline NCA of aspartic acid[7,8] and found it to yield only α-aspartyl peptides. We also prepared the novel NCA's of asparagine and of glutamine *via* the carbobenzoxy derivatives and used them to prepare glutaminyl and asparaginyl peptides in good yield. Surprisingly, intramolecular rearrangements[9] did not interfere with the usefulness of these two NCA's.

The O-unprotected NCA's of serine and threonine could not be employed in peptide synthesis because of intramolecular rearrangement.[10] We prepared the novel O-trimethylsilyl derivatives and used them successfully in peptide syntheses. The trimethylsilyl protecting group is hydrolyzed during the peptide syntheses, giving directly the desired O-unprotected peptides. The NCA of arginine hydrochloride has been prepared in an impure but usable form. We have also synthesized the NCA of an O-dihydropyranyl ether of tyrosine, and found it to give better yields of peptides than tyrosine NCA.

The following examples illustrate the general method. Phenylalanine in aqueous potassium borate buffer at pH 10.2 and 0° was allowed to react in a Waring Blendor with a 5% excess of the NCA of proline for 2 min to give the peptide carbamate. As soon as the pH had been adjusted to 5 with sulfuric acid to effect decarboxylation, the pure product began to crystallize from the reaction mixture (90% yield). Similarly, glycine at pH 10 was treated with the theoretical amount of the NCA of alanine. After decarboxylation and removal of CO_2 in a stream of N_2 (pH 3, 0°, 15 min), the pH was readjusted to 10 and the cycle was repeated three more times. Acidification precipitated the crystalline Ala_4-Gly,[11] $[\alpha]_{589}$ −89.8° (*c* 1%, 6 *N* HCl). The yield was 64% over-all.

(1) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, p 871.

(2) P. D. Bartlett and R. H. Jones, *J. Am. Chem. Soc.*, **79**, 2153 (1957); P. D. Bartlett and D. C. Dittmer, *ibid.*, **79**, 2159 (1957).

(3) For a recent discussion, see N. H. Grant and H. E. Alburn, *ibid.*, **86**, 3870 (1964), and references cited therein.

(4) The controlled synthesis of small peptide esters in anhydrous medium has been described by J. L. Bailey, *J. Chem. Soc.*, 3461 (1950).

(5) A. Berger, J. Kurtz, T. Sadeh, and A. Yaron, *Bull. Res. Council Israel*, **7A**, 98 (1958).

(6) J. Kovacs, H. N. Kovacs, and R. Ballina, *J. Am. Chem. Soc.*, **85**, 1839 (1963).

(7) All new NCA's are analytically pure. All amino acids, NCA's, and peptides referred to, except glycine, have the L configuration.

(8) We are indebted to Mr. Richard N. Boos and his associates for analyses. The amino acid ratios were determined by Mr. Robert Redfield. NCA's were assayed by Mr. Alan White.

(9) See, *e.g.*, J. Rudinger, *Record Chem. Progr.*, **23**, 3 (1962).

(10) T. Saito, *Bull. Chem. Soc. Japan*, **37**, 624 (1964).

(11) Purity was confirmed by paper strip chromatography and by elemental analysis. A solubility analysis indicated a purity of 99 ± 1%. Acid hydrolysis gave an amino acid ratio of $Ala_{3.98}Gly_{1.00}$.

A more critical test of the method was the synthesis of leucylalanylglycylprolylphenylalanylarginine from crystalline phenylalanylarginine prepared by the NCA method. The crude reaction mixture was purified on silica gel H to give a 60% yield of hexapeptide which moved as a single component by tlc, $[\alpha]_{589}$ −62.4° (*c* 1%, 6 *N* HCl), and which showed the following amino acid ratio in its acid hydrolysate: $Leu_{0.99}Ala_{1.01}Gly_{0.98}Pro_{1.00}Phe_{0.98}Arg_{1.01}$. Sequential treatment of the purified hexapeptide with a 5–8% excess of the NCA's of glutamic acid, isoleucine, and proline, respectively, gave a product which was purified on Sephadex to give a 46% yield (based on hexapeptide) of the nonapeptide, $[\alpha]_{589}$ −86.7° (*c* 1%, 6 *N* HCl). The amino acid ratio after acid hydrolysis was: $Pro_{2.04}Ileu_{0.95}Glu_{0.95}Leu_{0.97}Ala_{0.98}Gly_{0.98}Phe_{1.00}Arg_{1.00}$.

Phenylalaninamide, prepared from the NCA by treatment with NH_3, was treated sequentially with the NCA's of aspartic acid, methionine, and tryptophan in a Waring Blendor. The crude product was precipitated at pH 7 and purified on silica gel H to give a 30% yield of the C-terminal[12] tetrapeptide sequence of gastrin characterized as the crystalline hydrochloride, $[\alpha]_{589}$ −17.5° (*c* 2%, methanol), −31.6° (*c* 1%, DMF). The amino acid composition, as determined by acid and LAP hydrolyses, was $Try_{0.95}Met_{1.00}Asp_{1.00}Phe_{1.01}NH_4^+{}_{0.95}$ and $Try_{1.03}Met_{1.05}Asp_{0.94}Phe_{0.99}$, respectively. The LAP cleavage left no peptide fragments detectable by tlc. This tetrapeptide synthesis required a total of about 1 hr and the isolation procedures and crystallization about 3 days.

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(12) J. C. Anderson, *et al.*, *Nature*, 204, 933 (1964).

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Use of Polymers as Chemical Reagents. I. Preparation of Peptides

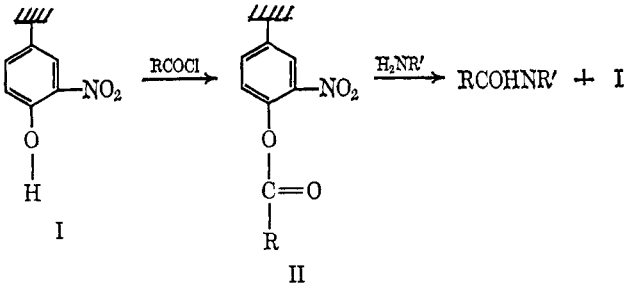
Sir:

Polymers of the type Ⓟ–A containing covalently bound groups A, which readily react with a low molecular weight reagent B, can be used to synthesize compound A–B according to eq 1. To increase yields

$$Ⓟ\text{–}A + B \longrightarrow A\text{–}B + Ⓟ$$

and to facilitate the synthetic procedure insoluble polymers of the above type may be added in large excess to a solution of B in a suitable solvent. At the end of the reaction the insoluble polymer can be removed by filtration or centrifugation. The filtrate which is devoid of A should thus contain only A–B and unreacted B. The most suitable polymers Ⓟ–A to be used as chemical reagents should contain a relatively large amount of A, should show high stability on storage, and should possess the suitable mechanical properties.

To test the possible use of chemically reactive polymers in acylation reactions we prepared insoluble, high molecular weight active polyesters of acetic acid (IIa) and benzoic acid (IIb) by allowing the corresponding chlorides to react with poly-4-hydroxy-3-nitrostyrene cross-linked with 4% divinylbenzene (I) in dimethylformamide (DMF), in the presence of pyridine. The high molecular weight active esters (II) contained approximately 5 mmoles of acyl residues/g of insoluble polymer and could be stored at room temperature in powder form without decomposition. Treating IIa or IIb (1.0 g) in suspension in DMF (15 ml) with 0.5 mmole of tri-L-alanyl-*p*-nitrobenzyl ester (L-Ala_3-PNB) was carried out at room temperature for 4–5 hr. The polymer was removed by centrifugation and the supernatants evaporated to dryness *in vacuo*. The solid residues were found to contain a practically quantitative yield of the corresponding acyl derivatives: acetyl-L-Ala_3-PNB, mp 226°, $[\alpha]^{25}D$ −28.9° (*c* 0.58, DMF); benzoyl-L-Ala_3-PNB, mp 216–218°, $[\alpha]^{25}D$ +2.9° (*c* 0.70, DMF).



II: esters of: acetic acid (IIa); benzoic acid (IIb); Z-L-Phe (IIc); Z-L-Ileu (IId); Z-L-Pro (IIe); N,S-Di-Z-L-Cys (IIf); N-Z-α,-OBz-L-Glu (IIg), where Z = C_7H_7OCO and Bz = C_7H_7

The successful preparation of active esters of N-blocked amino acids of type II enabled their utilization in peptide synthesis. Thus peptides with N- and C-blocked terminal groups were obtained on coupling the insoluble active esters IIc to IIg with desired soluble amino acid or peptide esters containing a free α-amino group. Preferential removal of the N-blocking group from the newly formed peptide enabled the repetition of the coupling reaction with an insoluble active ester of another N-blocked amino acid. Further repetitions of this set of reactions lead obviously to the elongation of the peptide chain and formation of a peptide with a predetermined amino acid sequence.

The insoluble active esters IIc–g were derived from the following corresponding benzyloxycarbonyl amino acid derivatives: benzyloxycarbonyl-L-phenylalanine, benzyloxycarbonyl-L-isoleucine, benzyloxycarbonyl-L-proline, N,S-dibenzyloxycarbonyl-L-cysteine, and N-benzyloxycarbonyl-α-benzyl-L-glutamate, by their coupling with polymer I in DMF by the DCC method.[1,2] The insoluble polymers IIc to IIg contained per gram approximately 1.0–1.5 mmoles of amino acid and could be stored at room temperature without decomposition, similarly to IIa and IIb. In suspension in inert organic solvents polymers IIc to IIg showed chemical behavior similar to that of the cor-

(1) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, 77, 1067 (1955); M. Rothe and F. W. Kunitz, *Ann.*, **609**, 88 (1957); D. F. Elliott and D. W. Russell, *Biochem. J.*, 66, 499 (1957).
(2) M. Fridkin, A. Patchornik, and E. Katchalski, *J. Am. Chem. Soc.*, 87, 4646 (1965).