The three bullvalene molecules are orientated around the central Ag^+ ion and occupy positions about a pseudo-threefold symmetry axis through the Ag^+ ion (Figure 1). There are twelve possible bonding Ag^+–C (olefin) distances ranging from 2.43 to 3.47 A. The mode of complexation of Ag^+ to the olefinic double bonds differs among the three bullvalene molecules. In the case of molecules II and III (Figure 1), the interaction appears to be quite localized to one double bond resulting in a mean Ag^+–C distance of 2.51 A and a very much weaker interaction with one of the other double bonds resulting in a mean Ag^+–C contact of 3.35 A. In molecule I, however, complex formation involves two of the olefin bonds to an almost equal extent, corresponding to four Ag^+–C distances in the range 2.65–2.85 A. This structure represents the highest Ag^+–olefin coordination which has been studied crystallographically and indicates that a wide range of Ag^+–C (olefin) contacts are permissible, when steric and packing factors favor complexation.

There is no evidence for the presence of other tautomeric forms of bullvalene occupying the same crystallographic positions in some of the unit cells. In this complex it therefore appears that the rapid self-rearrangement of bullvalene has been effectively "frozen" in the solid state.

A more detailed discussion of the bonding and the full crystallographic results will be published at a later date.

Acknowledgment. We wish to thank Professor H. S. Gutowsky for bringing the problem to our attention and for his continued interest in the work. Dr. Adam Allerhand kindly provided us with samples of the solid complexes which he had prepared, and Mr. James McKechnie assisted at several stages of the analysis.

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The Photoexcited Triplet State of the Azulenium Cation[1]

Sir:

A number of attempts have been made to detect the lowest triplet state, T_1, of the odd-alternant aromatic hydrocarbon azulene by studying its emission properties in rigid glasses.[2] Thus far no direct evidence has been reported for this state by optical methods although indirect evidence from studies of the quenching of anthracene[2b] and naphthacene[3] triplets indicates that it probably lies between 31 and 39 kcal/mole above the ground state.[3] The difficulty in detecting the lowest triplet state arises because of a combination of short radiative and radiationless lifetimes for the first and second excited singlet states and a fast radiationless $T_1 \rightarrow S_0$ process.[4] These difficulties can be overcome in certain cases by using heavy atom solvents such as krypton or xenon to increase the rate of triplet population[4] or by forming a suitable charge-transfer complex in which the first excited charge-transfer state lies above the lowest triplet state of azulene.[5] The triplet population can be enhanced in the latter case by intersystem crossing between the excited charge-transfer state and the triplet state of azulene. However, attempts to observe such an enhancement for several different charge-transfer complexes of azulene (TCNQ, iodine, trinitrobenzene) by epr techniques were unsuccessful.[6] An alternative possibility, involving the formation of the azulenium ion (which can be considered as a strong σ-type complex), was therefore investigated, and the results are reported in this communication.

The azulenium cation was formed by dissolving small amounts of azulene in freshly distilled trifluoroacetic acid at room temperature (final concentration *ca.* 10^{-2} *M*). Under these conditions the azulene is presumed to be monoprotonated; this was confirmed by observation of the nmr spectrum which showed the pattern expected for the azulenium ion protonated in the 1 position.[7] Upon slow cooling to liquid nitrogen temperatures the solution formed a rigid glass without any visible change in color (pale yellow-brown). The glass was irradiated at low temperatures with a 1000-w PEK Hg lamp. Suitable Corning Glass filters were used to exclude all light outside the wavelength region of the first absorption band (~320 to 370 mμ) of the cation.[8]

The epr spectrum, observed under conditions of continuous illumination, consisted of a broad anisotropic signal (line width 35 gauss; signal-to-noise ratio *ca.* 30:1) at 1592 ± 5 gauss corresponding to the $\Delta m = 2$ transition of the azulenium triplet, and a much stronger signal at 3325 ± 2 gauss due to the radicals formed during the irradiation.[9] No signals corresponding to $\Delta m = 1$ transitions for the triplet were observed for the azulenium ion.[10] The $\Delta m = 2$ signal disappeared with a decay constant of 1.2 ± 0.1 sec when the light was switched off, while the radical signal persisted for a period of several hours. The intensity of the $\Delta m = 2$ signal follows the expected Curie law behavior over the temperature range 90–143°K; above 145°K the signal was not detectable above the spectrometer noise.

A detailed description of the mechanism of triplet formation in the azulenium ion requires further optical studies. However, since the only light effective in producing triplets is that with a wavelength of *ca.* 350 mμ, the simplest mechanism is one in which the cation is excited to the lowest excited singlet state S_1 and then crosses over into a low-lying triplet T_1.[11]

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[1] Work supported by the U. S. Atomic Energy Commission.

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[5] S. P. McGlynn, *Chem. Rev.*, **58**, 1113 (1958).

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[8] E. Heilbronner, "Non-Benzenoid Aromatic Compounds," Interscience Publishers, Inc., New York, N. Y., 1959, Chapter V, p 256.

[9] No triplet signal was observed for azulene in diethyl ether or EPA glasses under similar conditions. However, Smaller (private communication) has detected a weak $\Delta m = 2$ signal for azulene in EPA at 77°K using a spectrometer with somewhat better sensitivity. In view of the short lifetimes predicted for the triplet state[4] it is possible that the weak signal is due to small amounts of naphthalene impurity.

[10] Weak $\Delta m = 1$ transitions were observed in the spectrum for the photoexcited triplet of the 1:1 azulene–boron trifluoride complex. However, it is not clear whether these are due to a triplet of the complex or to the presence of azulenium ions formed by reaction with trace amounts of H_2O–BF_3.

[11] The existence of a low-lying triplet is further indicated by the

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]

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

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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.

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

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[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 $Ala_{3.93}Gly_{1.00}$.