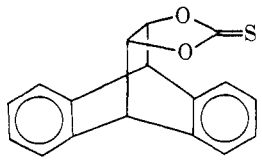

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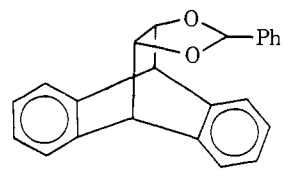

8

and **8** were synthesized by an alternate route from anthracene. The known thionocarbonate,[5b] **7**, and **8** were prepared by treatment of 9,10-dihydro-9,10-ethanoanthracene-11,12-diol[6a] with *N*,*N*-thionocarbonyldiimidazole and benzaldehyde, respectively. In both instances the compounds prepared by the alternate route were identical with the adducts obtained directly from the Diels–Alder route.

2-Phenyl-1,3-dioxol-4-ene (**6**), in addition to its utility as an acetylene equivalent, will also be of value in the synthesis of 1,2-diols by the Diels–Alder route. The adduct obtained with **6** can be readily converted to the diol upon mild acid treatment. In this regard the use of **6** complements the use of vinylene carbonate; the adduct formed using this latter dienophile is converted to the diol upon base treatment.

Further work is underway to more completely define the dienophilicity of **4** and **6** and to use this general route to prepare other potentially useful dienophiles (*e.g.*, 2-methyl-2-carboxy-1,3-dioxol-4-ene, an oxirene equivalent[11]) *via* the intermediacy of **2**.

Acknowledgment. This work was supported in part by General Research Support Grant No. G002-B from the National Institutes of Health.

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Determination of the Amino Acid Sequence of the C-Terminal Cyanogen Bromide Fragment of Actin by Computer-Assisted Gas Chromatography–Mass Spectrometry

Sir:

Recently we have described a general method aimed at the determination of the amino acid sequence of proteins by computer-assisted gas chromatography–mass spectrometry (GC–MS–computer) of complex mixtures of oligopeptides.[1] Conditions for the generation of optimally suited mixtures from primary degradation peptides of proteins, by acid or enzymatic hydrolysis, were evaluated. Esterification, acetylation, and reduction by $LiAlD_4$ converted these oligopeptides to the corresponding polyamino alcohols[2,3] which were then *O*-trimethylsilylated. These derivatives, now well suited for gas chromatographic separation, also produce mass spectra with abundant sequence determining ions.[1] This simplicity of the mass spectra, which are easily interpretable even if due to minor components of the mixture, and the general applicability (Arg, His, Trp, and sulfur containing amino acids can be handled without modification) certainly outweigh the more involved derivatization procedure, compared with unreduced derivatives. A detailed account of the chemical steps involved as well as the gas chromatographic and mass spectrometric properties of the resulting derivatives is in preparation.[4,5] Although no report on the handling of such complex mixtures by other mass spectrometric techniques is available in the literature, the gas chromatographic properties of the silylated polyamino alcohols make them the most promising candidates for a highly automated, generally applicable sequencing technique.

We have used this approach to determine the amino acid sequence of the C-terminal cyanogen bromide fragment of rabbit skeletal muscle actin. This peptide contains 20 amino acid residues and has the following amino acid composition:[6] Lys 2, His 1, Arg 1, Asp 1, Thr 1, Ser 1, Glu 2, Gln 1, Pro 1, Gly 1, Ala 1, Val 1, Ile 2, Tyr 1, Phe 1, Trp 1, AEtCys (aminoethylcysteine) 1.

The peptide (0.75 μmol) was hydrolyzed with 6 *N* HCl at 110° for 20 min. The resulting mixture of oligopeptides was then derivatized as outlined above and the resulting mixture of *O*-trimethylsilylated polyamino alcohols[7] was analyzed by a GC–MS–computer system.[8,9] The total ionization plot (*i.e.*, computer-generated gas chromatogram) obtained in this experiment is shown in Figure 1 together with the results of the interpretation of the data in terms of the sequences of the oligopeptides present in the mixture prior to derivatization.

The identification of the *O*-trimethylsilylated polyamino alcohols was based on three sets of data generated by the computer in the course of this GC–MS experiment. (1) Mass spectra. As in the free polyamino alcohols[2,3] cleavage of the carbon–carbon bonds of the ethylenediamine backbone units leads to abundant sequence determining ions from which the sequence of the side chains and thus that of the amino acids in the original oligopeptide can easily be deduced. *O*-Silylation not only overcomes the previous obstacle encountered with polyfunctional amino acids[3] but also enhances the abundance of the C-terminal fragment ions.[1,10] (2) Mass chromatograms[11] (plots of ions *vs.* time). Coincidence of maxima of mass chromatograms of the various sequence determining ions may be used efficiently to locate peptide derivatives as well as to resolve gas chromatographic fractions of incompletely separated peptide derivatives. (3) Retention indices[12] were automatically assigned to all gas chro-

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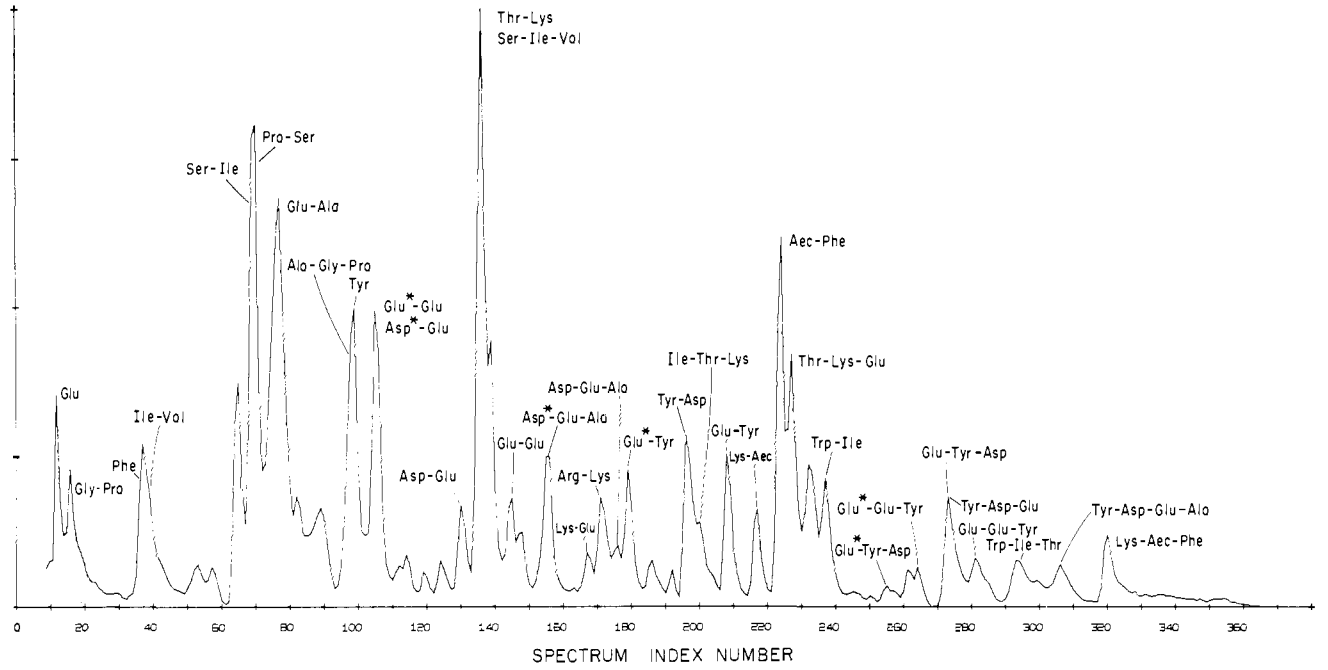

Figure 1. Total ionization plot of *O*-trimethylsilylated polyamino alcohols obtained by derivatization of an acid hydrolysate (6 *N* HCl, 110°, 20 min) of the C-terminal cyanogen bromide fragment (an eicosapeptide) of actin. Derivatives formed by elimination of trimethylsilanol from the corresponding trimethylsilylated polyamino alcohols are marked with an asterisk on the affected amino acid.

Table I. Oligopeptide Fragments Identified as *O*-Trimethylsilylated Polyamino Alcohols by GC–MS–Computer Analysis of a Derivatized Acid Hydrolysate (6 *N* HCl, 110°, 20 min) and Reassembled Sequence of the C-Terminal Cyanogen Bromide Fragment of Actin (A)

Trp-Ile
Trp-Ile-Thr
Ile-Thr-Lys
Thr-Lys
Thr-Lys-Glu
Lys-Glu
Glu-Glu
Glu-Glu-Tyr
Glu-Tyr
Glu-Tyr-Asp
Tyr-Asp
Tyr-Asp-Glu
Tyr-Asp-Glu-Ala
Asp-Glu
Asp-Glu-Ala
Glu-Ala
Ala-Gly-Pro
Gly-Pro
Pro-Ser
Ser-Ile
Ser-Ile-Val
Ser-Ile-Val-His[a]
Ile-Val
Arg-Lys
Lys-AEtCys
Lys-AEtCys-Phe
AEtCys-Phe

5 10 15 20

A: Trp-Ile-Thr-Lys-Glx-Glx-Tyr-Asp-Glx-Ala-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-AEtCys-Phe

[a] Identified by fractional vaporization of an aliquot of the derivatized sample mixture directly into the ion source of the mass spectrometer.

matographic peaks corresponding to the trimethylsilylated oligopeptides[13] (hydrocarbons were coinjected with the sample as internal standards) and then compared with the values which have been predicted[4] from the amino acid composition of the original oligopeptides.

More complex histidine containing peptide derivatives are not amenable to analysis by GC–MS,[4] and these are identified by fractional vaporization of another aliquot of the derivatized mixture directly into the ion source of the mass spectrometer (which is scanned continuously as for a GC–MS experiment). These less volatile derivatives vaporize after the bulk of those that are sufficiently volatile for gas chromatography. The trimeth-

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ylsilylated polyamino alcohol corresponding to Ser-Ile-Val-His was identified in this manner by mass chromatograms of sequence determining ions.

All together 27 peptide derivatives were identified (Figure 1), which can only be reassembled to two separate partial sequences indicating that one overlap was missing (Table I). However, identification of Trp as the N-terminal residue (as the trimethylsilylated phenylthiohydantoin by gas chromatography[14] and mass spectrometry[15]) permits reassembly of sequence A shown in Table I both manually and by a computer program[10] (Glx may be either glutamic acid or glutamine).

Due to the lability of the primary amide group of glutamine under the conditions of acid hydrolysis, the position of the single glutamine residue remained to be determined. The partial sequence -Lys[4]-Glx[5]-Glx[6]- suggested a simple conventional experiment to solve this problem. A sample of the eicosapeptide was digested with trypsin which was expected to produce, among others, a peptide with the N-terminal sequence Glx-Glx- . . .. The digest was then subjected to a one-step Edman degradation.[16] The presence of Gln> PhNCS in the resulting mixture of phenylthiohydantoins as demonstrated by thin layer chromatography[16] and high resolution mass spectrometry places Gln at position 5. The latter technique was found to be an excellent method for the analysis of relatively complex mixtures of phenylthiohydantoins due to selectivity, high sensitivity and dynamic range.

Thus, the structure of this cyanogen bromide fragment of actin was unambiguously determined as

5 10
Trp-Ile-Thr-Lys-Gln-Glu-Tyr-Asp-Glu-Ala-Gly-Pro-Ser-Ile-
15 20
Val-His-Arg-Lys-AEtCys-Phe

The same structure has been deduced independently by conventional techniques.[17,18]

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(18) Since it was one of the objectives of this investigation to test the reliability of this technique on a protein derived peptide of realistic size and amino acid composition, communication concerning the sequence was assiduously avoided between Dr. Elzinga's and our own laboratories before completion of the work described here.

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Quenching of Aromatic Hydrocarbon Excited Singlet States by Wurster's Blue Cation Radical

Sir:

The quenching of aromatic hydrocarbon excited singlet states ($^1R^*$) by doublet-state molecules such as nitric oxide,[1] nitroxyl radical,[2] and di-*tert*-butyl nitroxide (DTBN)[3] has been reported. Electron-exchange enhanced intersystem crossing

$$^1R^* + {}^2Q \longrightarrow {}^3R^* + {}^2Q \qquad (1)$$

has beeen implicated, both theoretically[4] and experimentally, as a possible mechanism for these singlet quenching reactions. In addition to the mechanistic and theoretical interest in the interaction of doublet-state species with excited singlet states, it is necessary to understand these processes in order to formulate a complete description of electrogenerated chemiluminescence (ECL). In ECL excited singlet states are generated by an anion radical–cation radical annihilation reaction in a region of space cooccupied by excess radical ions. The extent of this excess is controlled by the lifetime of the excited singlet state and the kinetics of the anion–cation radical annihilation reaction. Although it has been demonstrated that radical cations can act as triplet quenchers,[5] the extent to which they act as singlet quenchers has not been studied except in a preliminary way.[6] In this communication we report that the tetramethyl-*p*-phenylenediamine cation radical (Wurster's blue, $^2TMPD\cdot^+$) is an extemely effective quencher of aromatic hydrocarbon excited singlets.

The bimolecular rate constants for fluorescence quenching, k_q, by $^2TMPD\cdot^+$ were obtained from a Stern–Volmer analysis (eq 2) of aromatic hydrocarbon

$$\tau_F^0/\tau_F = 1 + k_q[^2TMPD\cdot^+]\tau_F^0 \qquad (2)$$

fluorescence lifetimes, τ_F, measured by the time-correlated single photon timing technique.[7] The fluorescence lifetime approach for monitoring quenching was chosen because the lifetime is not dependent on complications such as competitive absorption by the quencher or radiative (trivial) energy transfer; both of which are present in the systems studied here. Fluorescence quenching samples were prepared by exhaustive electrooxidation at +0.20 V *vs.* sce of thoroughly deoxygenated acetonitrile solutions containing 10^{-4} *M* aromatic hydrocarbon, 0.1 *M* tetrabutylammonium perchlorate (TBAP), and the appropriate concentration of TMPD neutral. The free amine was prepared and purified as described by Bard, *et al.*[8] Resultant $^2TMPD\cdot^+$ concentrations were determined both coulometrically and spectrophotometrically at 568 nm using ϵ_{568} 1.26×10^4 M^{-1} cm^{-1}. Uv–visible absorption spectra and fluorescence emission and excitation spectra were recorded for all samples. The absorption spectra could be reproduced exactly by summing the absorption spectra of the individual components. No

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