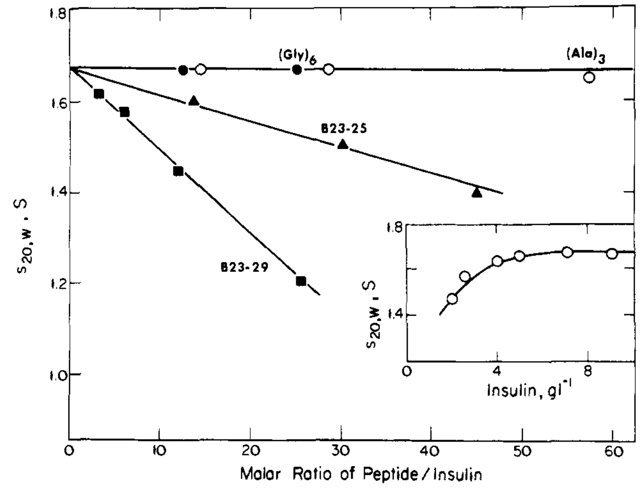

Figure 2. Effect of oligopeptides on the sedimentation coefficient of 0.38% insulin in glycine-HCl-NaCl buffer (pH 2; ionic strength 0.1) at 20 °C. Inset: concentration dependence of the s value of insulin in the same buffer.

indicated that these three compounds had little or a small effect on the MGC of deoxyHb S.[5] It seems that the morphology of red cells can be altered by agents which are without effect on in vitro gelation.

Insulin: For insulin dimerization, the extended C-terminal residues of the B chains:

$$\overset{23}{\cdots\text{Gly}}\ \text{Phe Phe Tyr Thr}\ \overset{29}{\text{Pro}}\ \text{Lys Ala}$$

run antiparallel to each other. This makes possible the antiparallel β-form between B24 and B29 which contains four hydrogen bonds between two monomers (B24 of one molecule to B26 of the other).[6,7] Figure 2 shows the effect of several oligopeptides on the dissociation of insulin dimer in glycine-HCl-NaCl buffer (pH 2; $I = 0.1$). The concentration of the protein was kept at 0.38% (6.6×10^{-4} M (monomer)), since the sedimentation coefficient, s, of insulin began to drop below this concentration (see inset; also ref 8). Addition of a heptapeptide B23-29 (prepared by trypsin digestion of insulin)[9] reduces the s value linearly from about 1.7 S (mostly due to the dimer)[8] toward the monomer (about 1.2 S at infinite dilution of the protein solution).[10,11] Even a tripeptide B23-25 appears to shift the dimer-monomer equilibrium, although it is less effective than the heptapeptide B23-29. On the other hand, hexaglycine and (Ala)$_3$ have no effect on the sedimentation coefficient. These results again suggest that oligopeptides having the same sequence as a portion of the C-terminal residues of the B chain might interfere with the dimerization of insulin.

Segments of the peptide chain in a protein molecule are fixed in a right conformation, but isolated fragments such as the oligopeptides are largely random in solution. The binding, if any, of these compounds to proteins would result in a loss of configurational entropy, which must be compensated by a decrease in enthalpy through hydrogen bonding or hydrophobic interaction or both. Therefore, in general, the equilibrium association constant K_p is expected to be much larger than the equilibrium binding constant K_A in reaction 1. The standard free energy change, $\Delta G°$, for the equilibrium involving PA would determine the effectiveness of these antiaggregation agents. Raising their concentrations could help shift the equilibrium toward the monomeric PA. In this respect, the work of Eisinger et al. on tRNA$^{\text{Phe}}$ may have some bearing on our findings.[12] A codon trinucleotide does not bind with the complementary anticodon trinucleotide. However, a weak, but detectable binding does occur when the anticodon triplet is fixed in the tRNA$^{\text{Phe}}$ molecule. The binding is the strongest when the triplets are parts of tRNA and mRNA molecules, respectively. Our protein-oligopeptide interaction may represent a similar intermediate case.

Our initial objective is to seek an antisickling agent for deoxyHb S gel without introducing any chemical modification of the intact native proteins (an effective antisickling compound is simply one that raises the MGC so high that deoxyHb S would not gel under physiological conditions).[1] The idea that is implicit in reaction 1 can equally well be applied to other biological aggregates if the binding of the antiaggregation agent to a biopolymer is energetically favorable. This leads us to study the insulin dimerization. Although the evidence is not yet conclusive, the proposed working hypothesis merits further investigations of other biological systems.

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Interpretation of Mass Spectroscopic Fragmentation by State Correlation Diagrams

Sir:

State correlation diagrams are useful in the interpretation of photochemical mechanisms.[1] Here we apply such diagrams to a fundamental mass spectroscopic reaction, the fragmentation (or α-cleavage) of ketone positive radical-ions.[2,3] The departure of the alkyl fragment is assumed to occur in a coplanar fashion. It is then possible to correlate all the low-lying states of reactant ion with the states of primary products. The coplanar path is not necessarily the best pathway for a given state (specific geometry reorganization occurs in each state), but it reveals correlations which control the dynamical behavior of even noncoplanar pathways.[1] We illustrate the process with the α-cleavage of the acetaldehyde radical-cation, whose mass spectrum[3b] shows peaks for HCO^+ (abundance 100) and CH_3^+ (abundance 32)—corresponding to CC bond cleavage—as well as a peak for CH_3CO^+ (abundance 42), corre-

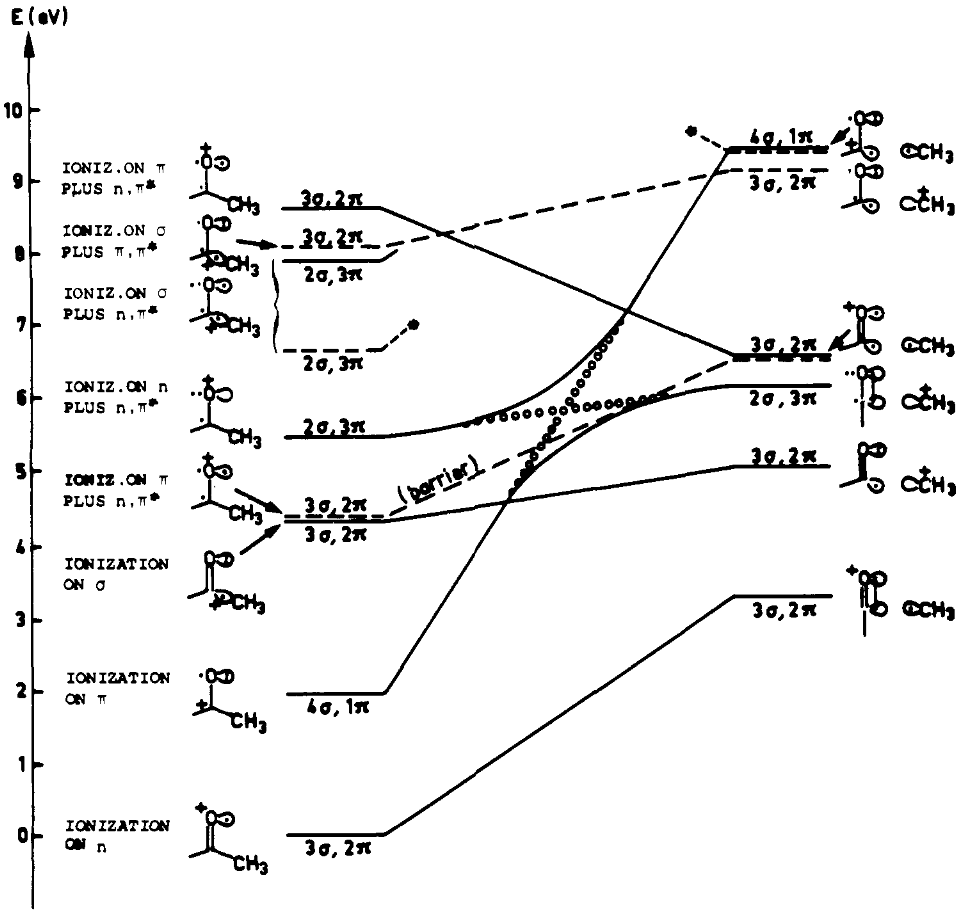


Figure 1. Correlation diagram for α-cleavage of the acetaldehyde radical–cation. Stars indicate states which correlate via an avoided crossing. Doublet states are indicated in full lines, quartet states in dotted lines. Ionization from the σ orbital is schematically localized in the CC bond, although the (9a′)σ orbital of acetaldehyde is delocalized.

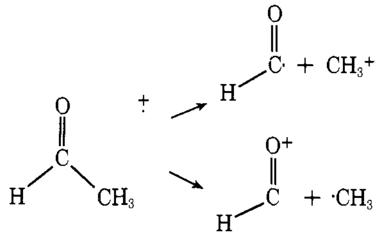
Table I. Energies of the Electronic States of the Acetaldehyde Radical–Cation. (Energies *Relative* to the $^2A'$ Ground State Are Used in the Figure)

Nature of state relative to parent molecule	Symmetry	Electron count relative to reaction plane	Energy (au)
Ionization on n	$^2A'$	$3\sigma, 2\pi$	−150.606
Ionization on π	$^2A''$	$4\sigma, 1\pi$	−150.533
Ionization on σ	$^2A'$	$3\sigma, 2\pi$	−150.446
Ionization on n, plus n,π^*	$^2A''$	$2\sigma, 3\pi$	−150.404
Ionization on π, plus n,π^*	$^4A'$	$3\sigma, 2\pi$	−150.444
Ionization on n, plus π,π^*	$^2A'$		−150.286
Ionization on σ, plus n,π^*	$^4A''$	$2\sigma, 3\pi$	−150.359
	$^2A''$		−150.313
Ionization on π, plus π,π^*	$^2A''$	$4\sigma, 1\pi$	−150.178[a]
Ionization on σ, plus π,π^*	$^4A'$	$3\sigma, 2\pi$	−150.306
	$^2A'$		−150.086[a]

[a] These high-energy states are not included in the correlation diagram.

sponding to the less facile CH bond cleavage. The correlation diagram reveals the nature of the states leading respectively to HCO^+ and to CH_3^+.

Formally the primary step for carbon–carbon bond cleavage can be written as shown below. The establishment of a state



correlation diagram requires careful consideration of the electronic states of both reactant and products. For CH_3CHO^+ we have considered the states arising respectively from (1) ionization from the n orbital of acetaldehyde; (2) ionization from the π orbital of acetaldehyde; (3) ionization from the highest σ orbital of acetaldehyde below the n orbital (this highly delocalized orbital has simultaneous oxygen 2s lone pair character, CO antibonding character, and CC bonding character; the latter should rapidly predominate as the CC bond length increases); (4), (5), (6) the three same ionizations coupled with n,π^* excitation; (7), (8) the same ionizations coupled with π,π^* excitation.[4] The energies were calculated[5] respectively for (1), (2), (3) by application of Koopmans' theorem to the closed-shell SCF energy; (4)–(8) in all other cases, by Koopmans' theorem applied to the energy of the appropriate open-shell calculated by Nesbet's restricted SCF method.[6] The results are given in Table I, where we have also specified the electron count[1] (including π, σ_{CC}, and n electrons) in each state. The calculated energies of the first three states relative to acetaldehyde (9.17, 11.16, 13.52 eV) compare reasonably with the observed ionization potentials (10.19, 13.09, 13.93 eV).[7]

The energies of the CH_3^+ and HCO^+ ions were calculated by the restricted closed-shell (or open-shell[6] for excited HCO^+) self-consistent methods. The energies of the $\cdot CH_3$ and $\cdot HCO$ *radicals* were calculated by the *unrestricted* self-consistent field method.[8] Since this technique includes a fair amount of electron correlation, primary product state energies will be

Table II. Energies of the Electronic States of the Formyl Cation HCO^{+}[c]

Symmetry Linear → bent	Major configurations	Electron count relative to reaction plane	Geometry	(geometry of corresponding state of HCN[13])	Energy (au)
$^1\Sigma^+$	$\pi_x{}^2\pi_y{}^2$	$2\sigma, 2\pi$	Linear	(linear)	−111.505
$(^3\Sigma^+) \rightarrow {}^3A'$	$\pi^2\sigma\sigma^*$	$2\sigma, 2\pi$	Bent	(bent)	−111.383
$^3\Delta \nearrow (^3A'')$	$\sigma^2\pi\sigma^*$	$3\sigma, 1\pi$	Bent	(bent)	−111.277
$\searrow (^3A')$	$\pi_x{}^2\pi_y\pi_y{}^*, \pi_y{}^2\pi_x\pi_x{}^*$	$2\sigma, 2\pi$	Linear[a]	(bent[a])	−111.247[b]
$(^1\Sigma^-) \rightarrow {}^1A''$	$\sigma^2\pi\sigma^*$	$3\sigma, 1\pi$	Bent	(bent)	−111.238[b]
$^3\Sigma^- \rightarrow (^3A'')$	$\pi_x{}^2\pi_y\pi_y{}^*, \pi_y{}^2\pi_x\pi_x{}^*$	$2\sigma, 2\pi$	Linear[a]	(bent[a])	−111.205[b]
$^1\Delta \nearrow {}^1A'$	$\pi^2\sigma\sigma^*$	$2\sigma, 2\pi$	Bent	(bent)	−111.202[b]
$\searrow (^1A'')$	$\pi_x{}^2\pi_y\pi_x{}^*, \pi_y{}^2\pi_x\pi_y{}^*$	$\begin{Bmatrix} 1\sigma, 3\pi \\ 3\pi, 1\sigma \end{Bmatrix}$	Linear[a]	(bent[a])	−111.177[b]
$(^1\Sigma^+) \rightarrow {}^1A'$	$\sigma^2\pi\pi^*$	$2\sigma, 2\pi$	Bent	(bent)	−110.717[b]

[a] For the three cases where the geometry disagrees with that calculated for HCN, the HCN bond angle is large (160° for $^3A'$, 157° for $^3A''$, 164° for $^1A''$).[13] Most probablyhere our limited CI gives only an approximation to the exact, slightly bent, geometry. [b] These high-energy states are not included in the correlation diagram. [c] Symmetries in parentheses correspond to unfavorable geometries. (Energies relative to the ground state are used in the Figure).

stabilized relative to their reactant counterparts. We will ultimately correct for this bias by properly positioning the ground states of reactants and products relative to each other. The energies of CH_3 and $CH_3{}^+$ are equal respectively to −39.077 and −38.779 au.[9] The bent $^2A'$ state of the ·HCO radical and its linear first excited $^2\Pi$ state[10] were calculated to have the following optimized geometries and energies: bent ·HCO r_{CO} = 1.30 Å, r_{CH} = 1.11 Å, ∠HCO = 124°, E = −111.737 au; linear ·HCO r_{CO} = 1.26 Å, r_{CH} = 1.07 Å, E = −111.696 au. The calculated energy difference of 1.12 eV can be compared with the experimental value[10] of 1.1 eV. We also include the lowest-excited π,π^* state ($^4A'$) of bent ·HCO. Its energy is estimated at 0.151 au above ground state (−111.586 au).[11]

The HCO^+ molecule-ion has received some attention recently.[12] It is isoelectronic with HCN, whose electronic states have been thoroughly investigated.[13] The HCO^+ system has a closed-shell ground state with a CO triple bond. Excitation of one π electron to the π^* manifold leads to *eight* states, which all correspond to the $\pi^3\pi^*$ configuration of the linear molecule. Some of these states remain linear while others bend. We calculated their energies via the restricted open-shell method,[6] accompanied by appropriate "zeroth-order" configuration interaction between degenerate configurations. Table II gives the energies of these states, together with their wave functions, electron count, and bent or linear geometry. Comparison is made with the geometries calculated for HCN.[13]

We now possess the relative energies of reactants, on the one hand, and the relative energies of products, on the other hand. In order to place reactant states and product states on a *common* sale, we recalculated the ground $^2A'$ state of CH_3CHO^+ by the unrestricted SCF method, to match the correlation energy obtained for the ground state ($^1\Sigma^+HCO^+$, $^2A'$ ·CH_3) of primary products. The energy of ground CH_3CHO^+ is found to be −150.703 au (instead of −150.606 au, Table I). This value is 76 kcal/mol below that (−111.505 − 39.077 = −150.582 au) of ground products. The difference appears to be a fairly reasonable estimate of the CC bond dissociation energy in the radical cation, where the hole in the n orbital must delocalize to some extent into, and thereby weaken, the CC bond.

The state correlation diagram is drawn in Figure 1.[14] Each state is described by a Pauling resonance structure, with the previously adopted conventions (π electrons as dots on the lhs of the CO bond; n electrons as dots in a single lobe of the n orbital). When there is a single π electron, we place it on the oxygen atom. A single π^* electron is placed on the carbon atom. Doublet-state energies are shown in full lines, quartet-state energies in dotted lines. Relative energies are shown on an electron-volt scale. The avoided crossing between the two lowest configurations of $^2A''$ symmetry is indicated by small circles. The lowest quartet state, $^4A'$ correlates with $^3A'HCO^+$ plus $^2A'$·CH_3 (coupled to a quartet). However, this correlation involves a barrier due to nonconservation of orbital symmetry (a $\sigma^2\pi n_\sigma\pi^*$ configuration is transformed into a $\pi^2\sigma p_\sigma\sigma^*$ configuration). The reaction is "feasible forbidden";[15] the corresponding barrier is indicated on the diagram.

The correlation diagram shows that ground-state acetaldehyde cation correlates with ground fragments HCO^+, ·CH_3 via a ~3 eV activation energy. *But, contrary to the case of photochemical α-cleavage* (where an excited triplet state also correlates with ground products) and to other photochemical reactions,[1] *no excited state leads adiabatically to ground primary product.* There thus appear to be two possible mechanisms of formation for the observed HCO^+ ions: (a) predissociation of the first excited state ($^2A''$) of CH_3CHO^+ by the ground state ($^2A''$). Although these two states do not intersect, at least along the reaction coordinate, such a predissociation is possible[16] because the excited surface has a set of vibrational levels, due to loss of a bonding (π) electron, which reaches above the dissociation limit of the ground surface. The photoelectron spectrum of acetaldehyde indeed shows[7] a vibrational progression for the second peak ($^2A''$ state) which runs from 2.7 to 3.5 eV above the first peak (ground ion). The observed threshold (2.5 eV)[17] for appearance of HCO^+ ions in the collision-induced fragmentation of CH_3CHO^+ also agrees with this mechanism. A second possibility is (b) direct adiabatic dissociation from the eighth excited state of the acetaldehyde radical-cation, at ~8-9 eV above ground state. However, probability of attaining this state, which requires a two-electron transition, is weak.

The correlation diagram also reveals that the second excited state ($2^2A'$) of CH_3CHO^+, obtained via ionization of the σ orbital, leads in a nearly equienergetic fashion to ground ·HCO plus $CH_3{}^+$. This state is therefore a likely precursor of the methyl cations which are observed in the mass spectrum. In this respect it is remarkable that (a) the experimental peak intensity for $CH_3{}^+$ increases with field intensity in the field-ionization mass spectrum of acetaldehyde;[18] (b) the observed threshold (3.8 eV) for the appearance of $CH_3{}^+$ ions[17] in the collision-induced dissociation of CH_3CHO^+ is identical with the difference in observed vertical energies[7] between ground CH_3CHO^+ and "σ-ionized" second excited state of CH_3CHO^+!

We hope to have demonstrated that consideration of the different electronic *states* of reactants and products is useful in interpreting mass spectrometric reactions.[19] The generation of products from distinct excited states of reactant confirms early discussions[20] of this possibility, as well as more recent experimental evidence[21] for excited-state pathways in the mass spectrometer.

Acknowledgment. We thank Florence Fiquet-Fayard for a stimulating discussion.

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On the Properties of Fused 4*n* π-Electron Systems. The 8,9-Benzobicyclo[5.2.0]nonatetraenyl Anion

Sir:

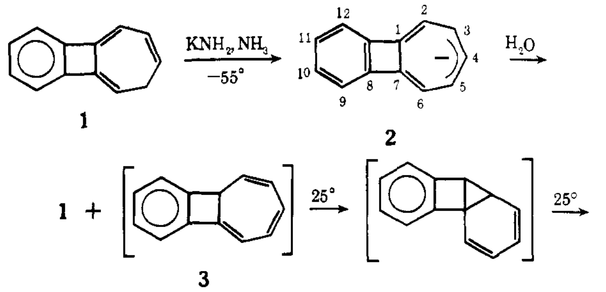
One of the more intriguing problems in connection with the concept of aromaticity concerns the electronic ground state character of 4*n* + 2 π-electron systems which formally result from the fusion of two 4*n* π-electron systems. A variety of systems have been investigated but relatively little evidence bearing on the aforementioned question has been obtained for planar delocalized compounds.[1-10] We now report the preparation of the title anion, the first 4*n* + 2 π-electron analogue of biphenylene, and present evidence which indicates that this compound displays properties characteristic of both 4*n* and 4*n* + 2 π-electron systems.

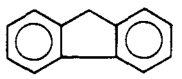
Table I. Proton NMR Data and Charge Densities (q) Calculated by the HMO ω-Technique (ω = 1.4) for Anion **2** and Cation **5**

Position	δ (**2**)[a] (ppm)	q (**2**)[b]	δ (**5**)[c] (ppm)	q (**5**)[d]
2	6.49	−0.008	7.56	+0.087
3	4.20	−0.138	8.20	+0.130
4	5.79	−0.008	7.67	+0.090
9	7.93	−0.045	7.2	+0.043
10	7.34	−0.073	7.2	+0.034

[a] Potassium salt in liquid ammonia at −55° with trimethylamine as internal standard; $J_{2,3} = J_{5,6} = 7.60 \pm 0.07$, $J_{3,4} = J_{4,5} = 9.87 \pm 0.07$, $J_{2,4} = J_{4,6} = 0.98 \pm 0.04$, $J_{9,10} = J_{11,12} = 8.00 \pm 0.02$, $J_{10,11} = 7.06 \pm 0.02$, $J_{9,11} = J_{10,12} = 0.73 \pm 0.02$, $J_{9,12} = 1.51 \pm 0.02$ Hz. [b] $q_1 = -0.123$, $q_8 = -0.110$. [c] Reference 11; $J_{2,3} = 9.0$, $J_{3,4} = 9.9$ Hz. [d] $q_1 = +0.139$, $q_8 = +0.021$.

Treatment of **1**[11] with potassium amide in liquid ammonia at −55° cleanly afforded a brown solution of anion **2**. A ca. 5:1 mixture of fluorene (**4**) and **1** was obtained in 50% yield when **2** was quenched with water and the product mixture never warmed above room temperature prior to analysis by NMR spectroscopy. This result suggests that **2** is protonated primarily at C_1, the position of highest calculated charge density. (Structure **2** emphasizes bond orders rather than charge densities.) Thus the previously suggested rearrangement of **3** to **4**[11] occurs in less than 30 min at room temperature.





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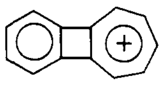

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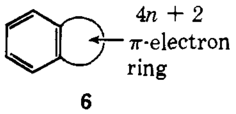
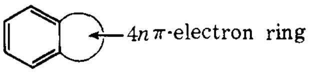
NMR data and self-consistent HMO charge densities are given in Table I. The calculated π-charge densities at C_3 and C_4 satisfactorily account for the difference in chemical shifts for H_3 and H_4[12] (which should experience similar ring current effects) in **2** and the corresponding cation **5**.[11]

Cyclic systems containing 4*n* or 4*n* + 2 π-electrons have been characterized in several ways by NMR spectroscopy: (a) by the presence of a paramagnetic or diamagnetic ring current,[13,14] respectively, and (b) by the degree and direction of π-bond fixation in the ring itself or in an adjacent benzo group.[15,16] In the absence of major perturbations resulting from bond angle strain and/or steric interactions between peri hydrogens, π-bond fixation has been found to occur in the benzo ring of benzannelated 4*n* + 2 and 4*n* π-electron annulenes as in **6**[16] and **7**,[16,17] respectively.



6



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