

# Electronic Spectroscopy of Isoelectronic Molecules. I. Nonlinear Triatomic Groupings Containing Eighteen Valence Electrons, with Comments on the $T_1$ State of the Peptide Linkage

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Electronic spectroscopy has had primary success in three molecular domains: diatomics, benzenoid hydrocarbons, and transition metal complexes. Many other molecular domains remain relatively uninvestigated. One of these areas—the classical inorganic domain which contains such molecular entities as  $\text{BO}_3^{3-}$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{ClO}_2^-$ ,  $\text{HN}_3$ , etc.—has been of interest to us for some time, and the present Account is an attempt to systematize the electronic spectroscopy of one series of such molecules, namely nonlinear triatomics which contain 18 valence electrons.

Previous attempts to correlate the spectra of the above set of molecules and to unify the description of their molecular orbitals (MO's) and electronic states were made by Walsh<sup>1</sup> and Mulliken.<sup>2</sup> Unfortunately, these two works were solely engrossed with stable, neutral molecules. We wish to consider a much broader series of molecular groupings which starts with ozone, which progresses through nitrite ion and formic acid, and which terminates in the peptide linkage. Some of the molecules (ions, groupings) in this series are shown in Figure 1. Others, not shown in Figure 1, are formamidinium ion, allyl anion, ONF, etc.

The common features of all these molecular groupings are: (i) each contains 18 valence electrons; (ii) each contains 4  $\pi$  electrons; and (iii) each consists, dominantly, of a nonlinear ( $C_{2v}$  or near- $C_{2v}$ ) triatomic group of atoms. At this point, we note the importance of some of these molecules: ozone from the point of view of upper atmosphere photochemistry; sulfur dioxide because of air pollution; the peptide linkage in view of its biological import; etc. We also note that the molecules listed in Figure 1 span the classical areas of inorganic, organic, and biological chemistry. Thus, apart from the importance associated with individual molecular groupings, the work also has ecumenical relevance!

Our aim is to provide a complete description of the lower energy electronic states of these entities. The approach we adopt is correlative. We have been impressed by correlative studies as applied to the unsaturated aromatic hydrocarbons (*e.g.*, polyacenes), and we believe that such studies constitute one of the more powerful tools available for the codification of electronic states and spectra. Purely computational techniques

are usually inadequate for handling complex molecules. Experimental investigations designed to answer a specific question may be difficult if not impossible and, in any case, they require a theoretical framework upon which to hang an interpretation. A combined theoretical-experimental approach which exploits the experimental regularities shown by similar molecules and which correlates these with the expected (*i.e.*, theoretical) trends benefits from the knowledge and insight provided by the interplay of both experiment and theory. We will now exemplify the correlative attitudes used in this work.

**Molecular Genealogy.** The interrelationships between the molecules in the series are displayed in Figure 1. This diagram may also be considered to represent a "proton push-pull synthesis" of the peptide linkage. In any event, extraction of a proton from the central oxygen of ozone produces nitrite ion. Extraction of a proton from the N atom of  $\text{NO}_2^-$  generates formate ion. Return of the proton discarded in the first step yields formic acid. Extraction of a proton from the O atom of the OH group of  $\text{HCOOH}$  yields  $\text{NH}_2$  and, hence, formamide. The further steps from formamide to *N*-methylacetamide to peptide are trivial in a spectroscopic sense.

If the "synthesis" of Figure 1 is to be of value, it must serve as a model capable of providing theoretical insights into the manner in which the electronic states vary from one molecule to another. We contend that such insights are provided in two ways.

(a) The continued deprotonations which occur in the series  $\text{O}_3 \rightarrow \text{NO}_2^- \rightarrow \text{HCO}_2^-$  bring about a reduction of the nuclear potential supplied by the central atom of the dominant triatomic grouping. These changes in nuclear potential generate readily determinable modifications of electronic energies and these, in turn, dominate the systematics of the observed molecular electronic spectra. We shall make extensive use of this attitude in the work to follow.

(b) Consider the deprotonation of fluorine to yield hydroxyl radical (*i.e.*,  $\text{F} \rightarrow \text{OH}$ ). Apart from the change of nuclear potential specified in item a, it is also evident that a pair of electrons which was nonbonding in the fluorine atom is now heavily involved in the O-H  $\sigma$  bond. If the bond is strong, the energy required to excite this bonding pair of electrons will have increased significantly relative to that needed for excitation of the corresponding nonbonding pair in fluorine.

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(1) A. D. Walsh, *J. Chem. Soc.*, 2266 (1953).

(2) R. S. Mulliken, *Can. J. Chem.*, **36**, 10 (1958).

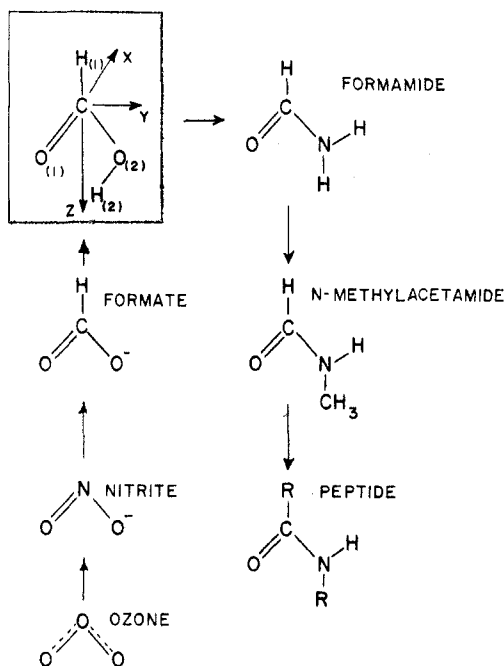


Figure 1. The molecular genealogy of the peptide grouping. Axes and atom-numbering conventions are provided for  $\text{HCOOH}$  in the top left corner.

The situation in molecules is not quite as simple as that outlined above. Nonetheless, this attitude can provide important information. In the work reported here, we make much use of the fact that in  $\text{NO}_2^-$  two electronic excitations of type  $\sigma \rightarrow \pi^*$  are expected to be of lower energy than the  $\pi \rightarrow \pi^*$  transition, whereas in the isoelectronic amidinium ion,  $\text{H}_2\text{NCH}=\text{NH}_2^+$ , all valence electrons of  $\sigma$  type are chemically bonded and the lowest energy excitation is expected to be of  $\pi \rightarrow \pi^*$  type.

This sort of attitude seems not to have been applied to the systematization of molecular electronic spectroscopy. However, its utility would appear to be both general and important: we need only mention the isoelectronic serialization of 1,1-difluoroethylene,  $\text{F}_2\text{CO}$ ,  $\text{H}_2\text{CO}_3$ , urea, acetone, and 1,1-dimethylethylene in order to provide illustration. We believe the power of this attitude will be amply demonstrated here.

**Plan of Article.** We will use the two basic ideas just outlined to develop attitudes about the variation of MO and state energies in a series of simple isoelectronic molecules for which considerable experimental information is available. The application of these ideas has been of a quantum chemical computational nature and the numbers we quote will be of similar origin. However, this presentation will stress the qualitative aspects in order to illumine better the basic physics. Energy considerations will be supplemented with simple transition probability conclusions, the theoretical and experimental data will be subsumed into a whole, and state identifications will be made.

With state identifications available for the simple members of Figure 1, we then feel free to extrapolate this knowledge to the peptide linkage. We will conclude,

contrary to accepted attitudes, that the lowest energy excited state of the peptide linkage should be of  ${}^3\Gamma_{\pi,\pi^*}$  type and that it should lie at  $\sim 4$  eV. The importance of this conclusion leads us into an evaluation of the literature which may have bearing on this point. The evaluation is mostly based on phosphorimetric data. The evaluation will be stringent. We will conclude that the  ${}^3\Gamma_{\pi,\pi^*}$  state has been identified in formamidium ion, in formate and acetate derivatives, and, possibly, in *N*-methyldiacetamide. However, no firm evidence for such a state has so far been provided by studies of proteins, peptides, or amino acids. Since a low-energy  ${}^3\Gamma_{\pi,\pi^*}$  state in the peptidic grouping would almost certainly have immense importance from the point of view of radiation damage, photoeffects on proteins (*e.g.*, denaturation, electrical conduction, etc.), energy transfer, etc., it is clear that our conclusions dictate a reopening of investigative effort in this area.

### Molecular Orbitals of the Isoelectronic Series

**Ozone to Formate.** The molecular orbitals of four representative molecules are shown in Table I. Only those MO's involved in the generation of the lower energy excited electronic states are shown. The MO's were obtained from Mulliken-Wolfsberg-Helmholz calculations;<sup>3</sup> these MO's will now be used to discuss the trends in MO and state energies as the central atom varies from carbon to nitrogen to oxygen (*i.e.*, from formate to nitrite to ozone).

The  $a_2$  MO is of  $\pi$  nature; it consists of a group orbital which is localized, *in its entirety*, on the two end atoms. Since the two end atoms are oxygens in all three molecules, the energy of the  $a_2$  MO should be more or less invariant in these molecules. The same conclusion is also applicable to the  $b_2$  MO: this MO consists *almost wholly* of end-atom atomic orbitals (Table I) and these pertain to oxygen in all three molecules. This conclusion is in apparent contradiction with the MO energies quoted in Table I. The reason for this contradiction is not obvious and must be sought in the details of the calculations. For our present purpose, we ignore these differences and use the results of the qualitative discussion.

The  $a_1$  MO, however, is not energy invariant. This MO, in nitrite ion, possesses dominant amplitude in the  $2p_z$  AO of nitrogen and the  $2p_z$  group orbital (GO) of the end oxygen atoms and is antibonding in this combination. Therefore, the topmost of the two  $a_1$  MO's which result from the AO-GO mixing (*i.e.*, the  $\sigma$  MO of Table I) will be destabilized relative to the  $2p_{zN}$  AO by an amount which will be inversely proportional to the energy separation between  $2p_{zN}$  and  $(2p_{zO_1} + 2p_{zO_2})$ . Furthermore, the  $(2p_{zN}, 2p_{zO})$ -overlap integral is large and, as a result, this destabilization will be severe. Indeed, we compute a bonding-antibonding  $a_1$  MO interval of 9.0 eV for  $\text{NO}_2^-$  and 7.5 eV for  $\text{HCOO}^-$ ; the same interval has been computed, by different means,

(3) D. G. Carroll, A. T. Armstrong, and S. P. McGlynn, *J. Chem. Phys.*, **44**, 1865 (1966).

Table I  
Molecular Orbitals of Some Representative Molecules<sup>a</sup>

$C_{2v}$	Nitrite ion	Formate ion	$C_s$	Formic acid	<i>N</i> -Methylacetamide
$a_1$	(0.61) $2p_{zN}$ - (0.56) $(2p_{zO_1} + 2p_{zO_2})$ [-6.63 eV; $\pi^*$ MO]	(0.60) $2p_{zC}$ - (0.57) $(2p_{zO_1} + 2p_{zO_2})$ [-5.63 eV; $\pi^*$ MO]	$a''$	(0.66) $2p_{zC}$ - (0.62) $2p_{zO_1}$ - (0.41) $2p_{zO_2}$ [-7.74 eV; $\pi^*$ MO]	(0.67) $2p_{zC}$ - (0.55) $2p_{zO_1}$ - (0.43) $2p_{zN}$ [-5.94 eV; $\pi^*$ MO]
$b_1$	(0.10) $2s_N$ + (0.52) $2p_{zN}$ - (0.60) $(2p_{zO_1} + 2p_{zO_2})$ - (0.07) $(2p_{yO_1} - 2p_{yO_2})$ [-8.07 eV; $\sigma$ MO]	(0.11) $2p_{zC}$ - (0.66) $(2p_{zO_1} + 2p_{zO_2})$ - (0.12) $(2p_{yO_1} - 2p_{yO_2})$ - (0.31) $1s_{H_1}$ [-8.34 eV; $\sigma$ MO]	$a'$	(0.28) $2p_{zC}$ - (0.40) $2p_{zO_1}$ - (0.17) $2p_{zO_2}$ - (0.31) $2p_{yC}$ + (0.64) $2p_{yO_1}$ + (0.32) $2p_{yO_2}$ - (0.27) $1s_{H_1}$ - (0.17) $1s_{H_2}$ [-12.99 eV; $\sigma$ MO]	Unavailable to us, but undoubtedly much the same as in formic acid
$b_2$	(0.11) $2p_{yN}$ - (0.44) $(2p_{zO_1} - 2p_{zO_2})$ - (0.54) $(2p_{yO_1} + 2p_{yO_2})$ [-8.73 eV; $\sigma_n$ MO]	(0.45) $(2p_{zO_1} - 2p_{zO_2})$ + (0.54) $(2p_{yO_1} + 2p_{yO_2})$ [-8.30 eV; $\sigma_n$ MO]	$a'$	(0.79) $2p_{zO_1}$ - (0.04) $2p_{zO_2}$ + (0.47) $2p_{yO_1}$ + (0.30) $2p_{yO_2}$ + (0.22) $1s_{H_1}$ - (0.09) $2p_{zC}$ [-11.25 eV; $n$ MO]	(0.73) $2p_{zO_1}$ + (0.58) $2p_{yO_1}$
$a_2$	(0.707) $(2p_{zO_1} - 2p_{zO_2})$ [-9.53 eV; $\pi$ MO]	(0.707) $(2p_{zO_1} - 2p_{zO_2})$ [-8.76 eV; $\pi$ MO]	$a''$	(0.70) $2p_{zO_1}$ - (0.67) $2p_{zO_2}$ + (0.25) $2p_{zC}$ [-12.66 eV; $\pi$ MO]	(0.72) $2p_{zO_1}$ - (0.60) $2p_{zN_2}$ [-10.78 eV; $\pi$ MO]

<sup>a</sup> The axes designations and atom-numbering conventions are given in Figure 1 (top, left). MO energy and type are given in brackets immediately below the analytic form of the orbital. *N*-Methylacetamide is assumed to be a "primitive  $C_s$ " molecule. The MO's of  $NO_2^-$  and HCOOH are quoted in more detail than those of HCOO<sup>-</sup> and CH<sub>3</sub>CONHCH<sub>3</sub>. The generation of the  $n$  MO, with dominant amplitude on one end atom, from the  $\sigma_n$  MO, with dominant amplitudes on two end atoms, should be noted.

as 10.73 eV for formate ion<sup>4</sup> and as 14.83 eV for ozone.<sup>5</sup> Both sets of numbers show a predictable trend. In  $O_3$ , the  $2p_z$  AO of the central oxygen and the  $2p_z$  GO of the end atoms are in close energy proximity and the interaction is stronger than in nitrite or formate where the energies of the interacting orbitals are more widely separated. Thus, the antibonding  $a_1$  MO is in an energetic order in  $O_3$ ,  $NO_2^-$ , and  $HCO_2^-$  which is precisely opposite to that expected from the valence orbital ionization energies (VOIE's) for the  $2p_z$  AO's of carbon, nitrogen, and oxygen. In other words, it is the off-diagonal terms of the energy matrix which are responsible for the energetic ordering of the  $a_1$  antibonding MO. These considerations are schematized in Figure 2A.

The same considerations applicable to the  $a_1$  MO also pertain to the  $b_1$  MO with one exception: since the overlap of the  $2p_x$  AO of the central atom with the  $(2p_{xO_1} + 2p_{xO_2})$  GO on the end atoms is relatively small—being of  $\pi$  nature—the energy of the resulting  $\pi^*$  MO (*i.e.*, antibonding  $\pi$ ) is dominantly determined by the diagonal terms of the interaction matrix. The results are shown in Figure 2B where it is seen that central atom VOIE's are the primary determinants of energy.

All of these conclusions are summarized in Figure 3. The lowest energy excitation is predicted to be  $b_1 \leftarrow a_1$  (*i.e.*,  ${}^1B_1 \leftarrow {}^1A_1$ ); it is of  $\pi^* \leftarrow \sigma$  type and its energy should increase in the order ozone < nitrite < formate. The transition  $b_1 \leftarrow a_2$  (*i.e.*,  ${}^1B_2 \leftarrow {}^1A_1$ ) is of  $\pi^* \leftarrow \pi$  type and is predicted to increase energetically in the same order as does the  ${}^1B_1 \leftarrow {}^1A_1$  transition; however, its increase should be less rapid and the  $\pi^* \leftarrow \sigma$  and  $\pi^* \leftarrow \pi$  energies should converge as we progress toward formate. The energy of the  $b_1 \leftarrow b_2$  (*i.e.*,  ${}^1A_2 \leftarrow {}^1A_1$ ;  $\pi^* \leftarrow \sigma_n$ ) transition should parallel that of the  $\pi^* \leftarrow \pi$  transition, but should be of lower energy.

**Formate to Formamide.** We will now discuss the effects on MO energies which ensue as the formate ion evolves to *N*-methylformamide. The results of calculations related to this point are diagrammed in Figure 4. Only MO energy differences, *not* the absolute values of the MO energies, are used in the construction of Figure 4. Qualitative arguments are used to fix the relative value of some one MO; thereafter, the positions of the other MO's are fixed using computed energy differences. For example, the  $\pi^*$  MO in formic acid is very similar analytically to that of formate ion (see Table I) and, therefore, the energies of these two MO's are equated. In formate ion, we note that the rate of descent of the  $a_1$  MO indicated in Figure 3 is such that this MO actually falls below the  $b_2$  MO in Figure 4. Nonetheless, formate ion still possesses two  $\sigma$  MO's intermediate between the  $\pi$  and  $\pi^*$  MO's. Thus, one would expect to observe two transitions of  $\pi^* \leftarrow \sigma$  type of lower energy than the  $\pi^* \leftarrow \pi$  transition.

The effects of the protonation which leads to formic acid are threefold. First, the  $a_1$  MO interacts strongly with the  $1s_{H_1}$  AO to form bonding and antibonding com-

(4) S. D. Peyerimhoff, *J. Chem. Phys.*, **47**, 349 (1967).

(5) S. D. Peyerimhoff and R. J. Buenker, *ibid.*, **47**, 1953 (1967).

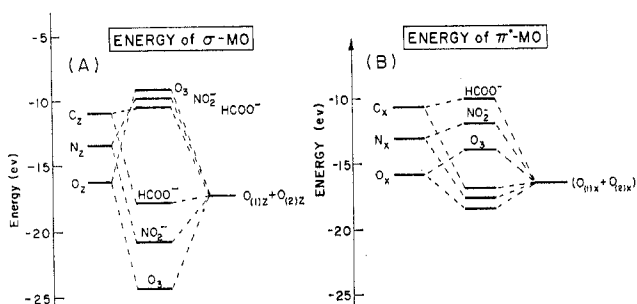


Figure 2. (A) Illustration of the formation of the  $a_1$  MO, large (*i.e.*,  $\sigma$ ) overlap.  $C_z$ ,  $N_z$ , and  $O_z$  denote VOIE's of  $2p_z$  AO's located on the specified centers. The symbol  $O_{1z}$  represents a  $2p_z$  AO on O-1 of Figure 1. A comment concerning the orbital  $C_z$  is in order: the extra hydrogen of formate ion dictates the replacement of  $2p_z$  by a hybrid involving  $1s_H$ ; it is this use of a hybrid carbon AO which accounts for the small  $2p_{zC}$  coefficient in the  $a_1$  MO of Table I. (B) Illustration of the formation of the  $b_1$  MO; small (*i.e.*,  $\pi$ ) overlap.

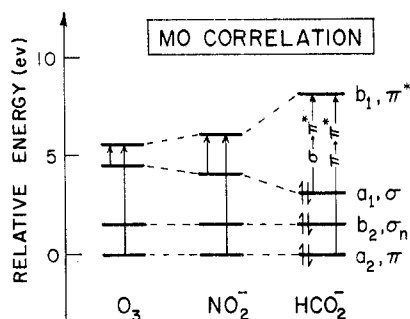


Figure 3. Trends in the orbital energies of ozone, nitrite, and formate.

binations. Thus, the bonding  $a'$  component drops energetically below the  $\pi$  MO, and we might expect to find in  $\text{HCOOH}$  a considerable increase in energy of the transition which is analog to the  ${}^1B_1 \leftarrow {}^1A_1$  ( $b_1 \leftarrow a_1$ ) excitation of  $\text{HCOO}^-$ . Secondly, the  $b_2$  ( $\sigma_n$ ) MO evolves toward an atomic-like orbital located on the carbonyl oxygen (*i.e.*, on  $O_1$ ); this evolution is evident in the eigenvectors of Table I. Thirdly, the  $a_2$  MO interacts with the  $2p_{xC}$  AO, and the MO relevant to our purposes (*i.e.*, the MO which consists of the bonding combination) is lowered in energy. The  $\pi$  MO's, therefore, attain a significantly greater energy spread. Thus, we expect a relative blue shift of the spectrum of  $\text{HCOOH}$ ; we expect to observe the  ${}^1B_1 \leftarrow {}^1A_1$  ( $b_1 \leftarrow a_1$ ) analog transition either at higher energy than, or close to, the  $\pi^* \leftarrow \pi$  transition; and we expect the  ${}^1A_2 \leftarrow {}^1A_1$  ( $b_1 \leftarrow b_2$ ) analog transition to be readily observable because it is no longer forbidden and because it should now have the lowest energy of all singlet  $\leftarrow$  singlet transitions.

The situation in *N*-methylacetamide is similar to that in  $\text{HCOOH}$ , with some exceptions. The  $-\text{C}-\text{H}$  bonding  $a_1$  component drops sufficiently low in energy that the  $b_1 \leftarrow a_1$  transition might no longer be observable spectroscopically. The  $\pi$  and  $\pi^*$  MO's are destabilized relative to those of formic acid because of the smaller electronegativity of the nitrogen atom which replaces

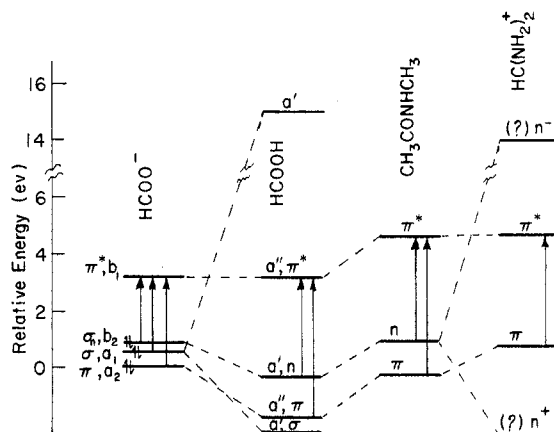


Figure 4. Molecular orbitals of some isoelectronic groupings.

oxygen. The  $\pi$  MO is further destabilized because bonding interactions with the carbon atom decrease. Similarly, the  $n$  orbital destabilizes relative to its energy in formic acid because of a decrease in the bonding interactions with the second oxygen atom of formic acid and because it localizes completely on one oxygen center.

**Amidinium Ion.** The situation which obtains in formamidinium ion is sketched on the extreme right of Figure 4. The evolution of  $=\text{O}_1$  to  $=\text{NH}_2^+$  causes  $\sigma$  bonding between the  $n$  AO and the  $1s_H$  AO's of the amide hydrogen atoms. As a result, the splitting shown on the extreme right of Figure 4 is obtained, and it is predicted that this molecule should possess only one low-energy transition which should be of  $\pi^* \leftarrow \pi$  nature.

### Experimental State Energies

The nitrite ion should exhibit three low-lying excited singlet states (see Figure 3). These and some of their characteristics are

$$\begin{aligned} \pi^* \leftarrow \sigma; {}^1B_1 \leftarrow {}^1A_1 & \text{ predicted: allowed; } f = 7.7 \times 10^{-5}; x \text{ polarized} \\ & \text{ observed: } f = 5 \times 10^{-4}; \\ & \quad x \text{ polarized} \end{aligned}$$

$$\begin{aligned} \pi^* \leftarrow \sigma_n; {}^1A_2 \leftarrow {}^1A_1 & \text{ predicted: forbidden} \\ & \text{ observed: } f < 3 \times 10^{-4} \end{aligned}$$

$$\begin{aligned} \pi^* \leftarrow \pi; {}^1B_2 \leftarrow {}^1A_1 & \text{ predicted: allowed; } f = 1.6 \times 10^{-1}; y \text{ polarized} \\ & \text{ observed: } f = 1.2 \times 10^{-1}; \\ & \quad y \text{ polarized} \end{aligned}$$

Experiment and theory are in very good agreement.<sup>6</sup> In addition, the  ${}^3B_1$  state has also been found and is well characterized. The lifetime of the  ${}^3B_1 \rightarrow {}^1A_1$  phosphorescence is  $10^{-2}$  sec.

These states and their attributes should persist in all molecules in the isoelectronic series. The states should be readily identifiable because of these attributes. Furthermore, their variation, energetically or otherwise, from molecule to molecule of the series should be

(6) L. E. Harris, H. J. Maria, and S. P. McGlynn, *Czech. J. Phys.*, **B20**, No. 9 (1970).

Table II  
Experimental Data and Assignments of Electronic States<sup>a</sup>

Molecule	Transition				
	<sup>3</sup> B <sub>1</sub> ← <sup>1</sup> A <sub>1</sub> ( <sup>3</sup> A'' ← <sup>1</sup> A')	<sup>1</sup> B <sub>1</sub> ← <sup>1</sup> A <sub>1</sub> ( <sup>2</sup> 1A'' ← <sup>1</sup> A')	<sup>1</sup> A <sub>2</sub> ← <sup>1</sup> A <sub>1</sub> ( <sup>1</sup> A'' ← <sup>1</sup> A')	<sup>3</sup> B <sub>2</sub> ← <sup>1</sup> A <sub>1</sub> ( <sup>3</sup> A' ← <sup>1</sup> A')	<sup>1</sup> B <sub>2</sub> ← <sup>1</sup> A <sub>1</sub> ( <sup>2</sup> 1A' ← <sup>1</sup> A')
Ozone	1.5 eV (7) $f \approx 2 \times 10^{-6}$ Gas	2.1 eV (8) $\epsilon_{\max} 1.3$ Gas	~3.5 eV Indistinct	~2.2 eV Estimated	5 eV (9) $\epsilon_{\max} 2800$ Gas
SO <sub>2</sub>	3.2 eV (10-13) $\epsilon_{\max} \approx 0.2$	3.7 eV (10, 11, 13) $\epsilon_{\max} \approx 400$	~4.4 eV (14) Indistinct	~3.7 eV Estimated	~5.3 eV (15, 16)
Amidinium ion				5.7 eV (17)	~3.0 eV (17)
Nitrite ion	2.8 eV (18-20) $\epsilon_{\max} \approx 0.003$	3.5 eV (21) $\epsilon_{\max} 25$	~4.1 eV (6) $\epsilon_{\max} \approx 10$ In water	~3.2 eV (22) Computational	5.9 eV (23) $\epsilon_{\max} \approx 5500$
Formate ion	~5.3 eV Estimated	~6.2 eV (24) $\epsilon_{\max} 100$ In water		~3.7 eV (25, 26) Phosphorescence	>7.7 eV (24) In water
Formic acid		7.3 eV (24, 27) $\epsilon_{\max} \approx 1200$ Assigned as Rydberg in ref 24	5.7 eV (27) $\epsilon_{\max} \sim 40$ Vapor	~4.3 eV Estimated	8.3 eV (24, 27) $\epsilon_{\max} \approx 7500$ Vapor

Molecule	Transition	
	$\pi^* \leftarrow n$ ( <sup>1</sup> A <sub>2</sub> ← <sup>1</sup> A <sub>1</sub> ; C <sub>2v</sub> )	$\pi^* \leftarrow \pi$ ( <sup>1</sup> B <sub>2</sub> ← <sup>1</sup> A <sub>1</sub> ; C <sub>2v</sub> )
Formamide	5.8 eV (24) Vapor	7.2-7.3 eV (24, 29, 30) Vapor
Acetamide	5.4 eV (28) In dioxane	6.8 eV (28) $\epsilon_{\max} 7600$ (water)
N-Methylacetamide	5.5 eV (28) In dioxane	6.67 eV (28) $\epsilon_{\max} 8800$ (water)
N,N-Dimethylacetamide	5.8 eV (24) Vapor	6.5 eV (24), Vapor 6.3 eV (28), $\epsilon_{\max} 9350$ (water)
Peptide	5.5 eV (28, 31) Circular dichroism; optical rotation	6.1 6.5 eV (31, 32) Two exciton components

<sup>a</sup> The numbers in parentheses following each transition energy specify references used in preparing this table. The state labels in the top row are for the point group C<sub>2v</sub>, and the labels between parentheses are for the corresponding transitions in C<sub>s</sub>.

readily predictable on the basis of our prior MO discussion. The resulting identifications are presented in Figure 5. The data used are given in Table II. We

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(21) J. W. Sidman, *J. Amer. Chem. Soc.*, **79**, 2669 (1957).

(22) L. E. Harris and S. P. McGlynn, unpublished work.

(23) W. G. Trawick and W. H. Eberhardt, *J. Chem. Phys.*, **22**, 1462 (1954).

(24) H. Basch, M. B. Robin, and N. A. Kuebler, *ibid.*, **49**, 5007 (1968).

(25) H. J. Maria and S. P. McGlynn, *ibid.*, **52**, 3399 (1970).

(26) L. W. Johnson, H. J. Maria, and S. P. McGlynn, to be published.

will not comment on the construction of this diagram; however, we will note those characteristics of the diagram which are readily related to the previous MO discussion.

(i) The lowest energy triplet state of O<sub>3</sub>, SO<sub>2</sub>, and NO<sub>2</sub><sup>-</sup> is <sup>3</sup>B<sub>1</sub>; it is of  $\pi^* \leftarrow \sigma$  MO excitation nature. The <sup>1</sup>B<sub>1</sub>-<sup>3</sup>B<sub>1</sub> split in each of these molecules is ~0.6 eV and is small. This number may be adopted for the magnitude of this split in all listed molecules.

(ii) The formamidinium ion, as expected, possesses a lowest energy excited singlet state of <sup>1</sup>B<sub>2</sub> type (*i.e.*,  $\pi^* \leftarrow \pi$ ). Since the lowest triplet state is <sup>3</sup>B<sub>2</sub>, this molecule provides unique information on the <sup>1</sup>B<sub>2</sub>-<sup>3</sup>B<sub>2</sub> split. The split is 2.8 eV and is large. This split will be larger in those molecules which contain the more electronegative atoms. Since the position of the <sup>1</sup>B<sub>2</sub> state is known in O<sub>3</sub>, SO<sub>2</sub>, and NO<sub>2</sub><sup>-</sup> and since the lowest triplet state in these molecules is <sup>3</sup>B<sub>1</sub>, it follows that the <sup>1</sup>B<sub>2</sub>-<sup>3</sup>B<sub>2</sub> split in these entities is <3.3, <2.3, and

(27) E. E. Barnes and W. T. Simpson, *J. Chem. Phys.*, **39**, 670 (1963).

(28) E. B. Nielsen and J. A. Schellman, *J. Phys. Chem.*, **71**, 2297 (1967).

(29) H. D. Hunt and W. T. Simpson, *J. Amer. Chem. Soc.*, **75**, 4540 (1953).

(30) D. L. Peterson and W. T. Simpson, *ibid.*, **79**, 2375 (1957).

(31) H. Okabayashi, T. Isemura, and S. Sakakibara, *Biopolymers*, **6**, 323 (1968).

(32) R. K. Momii and D. W. Urry, *Macromolecules*, **1**, 372 (1968).

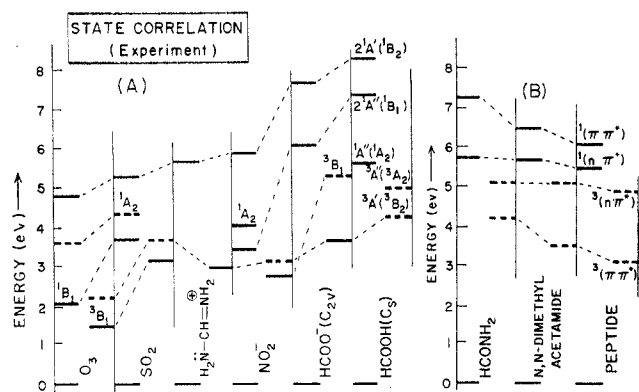


Figure 5. Electronic-state correlation diagram. Dashed levels are in doubt experimentally. The experimental data required are extensive and must be used judiciously. The sources of data are given in Table II. The symmetry notation used for HCOOH is:  $\Gamma$  in  $C_s$ , ( $\Gamma$  in  $C_{2v}$ ).

$<3$  eV, respectively. We may consider 3 eV as a representative estimate of this splitting. The low value of this S-T interval in  $SO_2$  is related to the more-diffuse nature of the sulfur AO's and the effects of this diffuseness in decreasing the exchange energies.

(iii) The states are expected to progress toward higher energies and to spread out over larger energy ranges as we proceed toward the right-hand side of Figure 5A (see also Figure 3). This is obviously the case (Figure 5A).

(iv) The  $^1B_2$ - $^1B_1$  split should decrease as we proceed to the right of Figure 5A (see also Figure 4). This, of course, is the case experimentally (see Figure 5A). In view of the representative magnitudes of the  $^1B_2$ - $^3B_2$  ( $\sim 3.0$  eV) and  $^1B_1$ - $^3B_1$  ( $\sim 0.6$  eV) splits, it follows, when the  $^1B_2$ - $^1B_1$  split is 2 eV or less, that the lower energy of the two triplet states should be  $^3B_2$ . This situation is assuredly achieved in all molecules to the right of nitrite ion in Figure 5.

(v) No experimental information appears to be available concerning the  $^1A_2$ - $^3A_2$  splitting. However, this split should also be of the order of 0.5 eV (*vide infra*). Thus, in view of the known location of the  $2^1A'$  ( $^1B_2$ ) and  $1^1A''$  ( $^1A_2$ ) states of HCOOH, and using reasonable singlet-triplet splits, there exists a distinct possibility that the lowest triplet state of HCOOH is of  $n, \pi^*$  type. However, given the energy specified for the  $^3B_2$  state of  $HCOO^-$  in Figure 5A, the lowest triplet state of HCOOH must be  $^3A'$  ( $^3B_2$ ). This last is the tentative identification made in Figure 5A.

(vi) The  $^1B_1$  state is of lower energy than the  $^1A_2$  state on the left of Figure 5A. As we progress toward the right of Figure 5A, the order of these two states tends to invert. However, the identification of the  $^1A_2$  state in HCOOH is not secure, and the fact that it has not yet been detected in  $HCOO^-$  is disconcerting.

### Computational State Energies

A series of computational results is collected in Figure 6.<sup>33,34</sup> A comparison of Figures 5 and 6 is in-

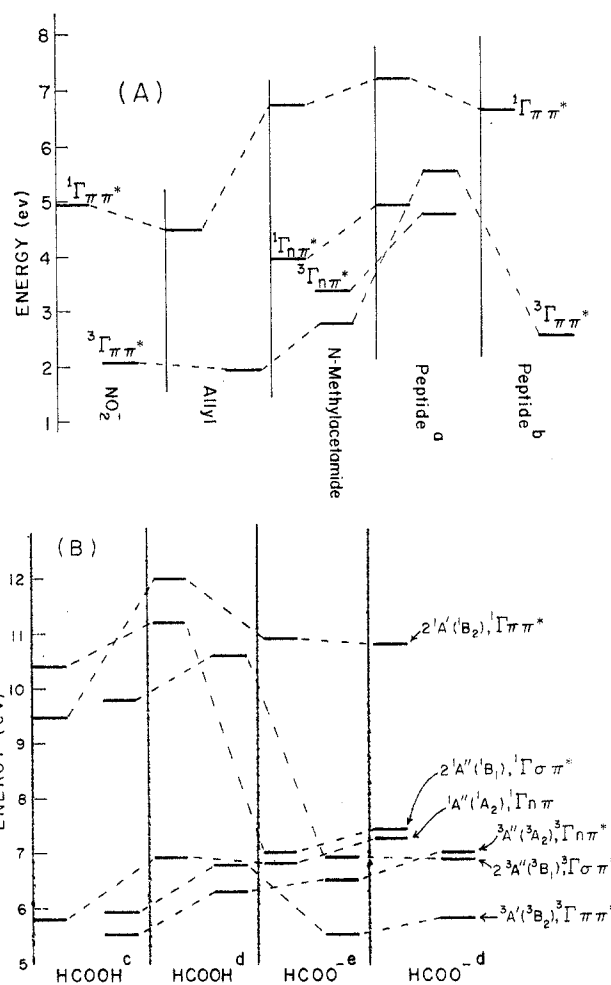


Figure 6. (A) Excited states of isoelectronic molecules. The calculation on  $NO_2^-$  was of a variable electronegativity self-consistent field type.<sup>22</sup> The allyl anion calculation was of Pariser-Parr SCF type.<sup>17</sup> The calculations on peptide a are from Pullmann,<sup>33</sup> those on peptide b are from Yomosa.<sup>34</sup> (B) Excited states of isoelectronic molecules. These are from (c) Peyerimhoff, *et al.*,<sup>35</sup> (d) Basch, *et al.*,<sup>24</sup> and (e) Peyerimhoff.<sup>4</sup>

formative. With regard to this comparison we note the following.

(i) The closest representative of formamidinium ion in Figure 6 is the allyl anion. The computed  $^1\Gamma_{\pi, \pi^*}$ - $^3\Gamma_{\pi, \pi^*}$  split is 2.5 eV, whereas the observed value in formamidinium ion is 2.8 eV.

(ii) The computed  $^1\Gamma_{\pi, \pi^*}$ - $^3\Gamma_{\pi, \pi^*}$  split in HCOOH is 3.6 eV according to Peyerimhoff, *et al.*,<sup>35</sup> and 5.7 eV according to Basch, *et al.*<sup>24</sup> (Figure 6B). In  $HCOO^-$  the same authors<sup>4,24,35</sup> find 5.4 and 4.9 eV, respectively. The lowest triplet state in  $HCOO^-$  is expected to be  $^3\Gamma_{\pi, \pi^*}$ . The experimental split in  $HCOO^-$  appears to be<sup>35</sup> 4 eV and the lowest state appears<sup>26</sup> to be  $^3\Gamma_{\pi, \pi^*}$ , in accord with theory. It may, perhaps, be of some significance that HCOOH does not seem to emit.<sup>26</sup>

(iii) Calculations performed on *N*-methylacetamide are compared with ones done on the peptide unit in

(34) S. Yomosa, *Biopolym. Symp.*, **1**, 1 (1964).

(33) A. Pullmann, "Modern Quantum Chemistry," Part III, O. Sinanoglu, Ed., Academic Press, New York, N. Y., 1965, p 283.

(35) S. D. Peyerimhoff and R. J. Buenker, *J. Chem. Phys.*, **50**, 1846 (1969).

Figure 6. It is clear that the LSU and Yomosa results are in very good agreement and in discord with the Pullmann results. Since the calculations of Yomosa have been criticized by Pullmann, it is perhaps well to detail the nature of the *N*-methylacetamide calculations.

Calculations on *N*-methylacetamide used a Clementi double- $\zeta$  AO representation. The AO basis set included all valence AO's. Computations were of a Mulliken-Wolfsberg-Helmholz (MWH) type and were processed to charge self-consistency. The Löwdin MO coefficients are shown in Table I. The exchange integrals  $K_{\pi,\pi^*}$  and  $K_{n,\pi^*}$  were then calculated using these MO's. All contributions to the exchange integrals were evaluated exactly.<sup>36</sup> The results are shown in Figure 7 where the assumption is made that the MWH energies correspond to singlet-triplet barycenters. Comparison with experiment indicates that this assumption is not very good for the  ${}^1\Gamma_{n,\pi^*}$  states. However, the results unambiguously indicate that the Pullmann  ${}^1\Gamma_{\pi,\pi^*}$ – ${}^3\Gamma_{\pi,\pi^*}$  splitting is too small by a factor of at least 2 and that the lowest triplet state of *N*-methylacetamide must be of  ${}^3\Gamma_{\pi,\pi^*}$  type.

(iv) In general, those calculations which are of greatest *ab initio* content tend to place all excited states at much too high energies. The *ab initio* content of the calculations of Peyerimhoff and Buenker<sup>4,35</sup> and Basch, *et al.*,<sup>24</sup> is greater than those of Figure 6A, and comparison of these two sets of results demonstrates our contention. On the other hand, those calculations which are of least *ab initio* content (*i.e.*, those of Figure 6A) tend to place the excited states at too low energies.

(v) It is noted that the  ${}^1\Gamma_{\sigma,\pi^*}$  and  ${}^1\Gamma_{\sigma_n,\pi^*}$  states of  $\text{HCOO}^-$  are predicted to be nearly degenerate. Is this the reason, one wonders, for the nondetection of the  ${}^1\Gamma_{\sigma_n,\pi^*}$  state in  $\text{HCOO}^-$  (see Figure 5)?

(vi) The lowest triplet state of  $\text{HCOO}^-$  is predicted to be  ${}^3\Gamma_{\pi,\pi^*}$ . This conclusion is unambiguous in Figure 5A. However, the triplet state of  $\text{HCOOH}$  is predicted by *ab initio* computations to be  ${}^3\Gamma_{n,\pi^*}$ ; however, we find it to be  ${}^3\Gamma_{\pi,\pi^*}$  in Figure 5A. We prefer, but do not insist on, the  ${}^3\Gamma_{\pi,\pi^*}$  designation. The lowest triplet state of *N*-methylacetamide (Figures 6 and 7) is predicted to be  ${}^3\Gamma_{\pi,\pi^*}$ ; we assume a similar designation of the  $T_1$  state for the peptide linkage.

(vii) The  $T_1$  state of the peptide linkage will not lie at energies less than 2.7 eV (*i.e.*, the experimental value for formamidinium); this energy may very well be of the order of 4 eV, a number which is based on comparison to  $\text{HCOO}^-$ , on considerations of electronegativity, and on actual computations. Thus, given a value for the  ${}^1\Gamma_{\pi,\pi^*}$  of *N*-methylacetamide at 54,000  $\text{cm}^{-1}$  we might expect the  ${}^3\Gamma_{\pi,\pi^*}$  of this same molecule to lie between the limits 30,000 (3300 Å, 3.75 eV) to 22,000  $\text{cm}^{-1}$  (3800 Å, 2.75 eV). Similar conclusions apply to the peptide linkage. It is clear that these conclusions are in significant discord with those of Pullmann<sup>33</sup> with regard to the nature of the  $T_1$  state, the magnitude of  $K_{\pi,\pi^*}$ , and the location of the  $T_1$  state.

(36) T. Janiszewski and A. C. Wahl, Quantum Chemistry Program Exchange No. 88.

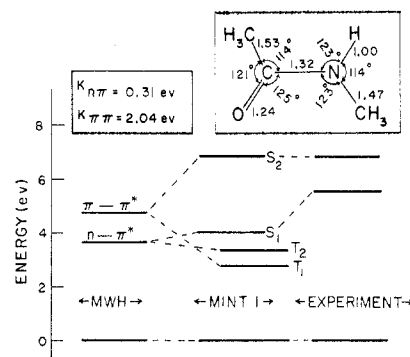


Figure 7. Energy levels of *N*-methylacetamide. Geometry is shown in upper right insert; planarity is assumed in all states; bond lengths are in ångström units.

(viii) Pullmann's calculations place the  ${}^1\Gamma_{\pi,\pi^*}$  state too high and the  ${}^1\Gamma_{n,\pi^*}$  state too low relative to experiment (compare Figures 5B and 6A). If we use the experimental energies for these two states and retain the singlet-triplet splittings computed by Pullmann for both configurations, we again predict that the lowest triplet state of the peptide group is  ${}^3\Gamma_{\pi,\pi^*}$ .

### $T_1$ State of the Peptide Bond

Our knowledge of the triplet manifold of electronic states of the peptide group is negligible. In particular, the dearth of recent literature on this topic indicates that it is either unimportant or not amenable to significant investigation using present techniques.

The simplest means of triplet detection is provided by phosphorimetry.<sup>37</sup> Such investigation is usually restricted to the lowest energy triplet state,  $T_1$ . It is widely believed that the localized  $T_1$  state of peptide does not luminesce; certainly, this is the gist of the assessment made by Konev:<sup>38</sup> "...Wels (1928)... visually observed blue fluorescence of serum albumin... This luminescence cannot be ascribed to protein itself... this author observed similar luminescence in amino acids known to be incapable of luminescence, such as glycine, glutamic acid, leucine, and alanine." The excellency of Konev's work makes it difficult to argue with his generalizations. At any rate, if he be correct, the peptide  $T_1$  state is not of importance as energy source, energy sink, or energy-transfer route in proteins which contain aromatic residues; furthermore, phosphorimetric study of this state in any protein, amino acid, amide, or enzyme will not be feasible. Since phosphorimetry is more sensitive for  $T_1$  studies than electron paramagnetic resonance (epr), it follows that epr is also not an adequate technique. Direct triplet  $\leftarrow$  singlet absorption spectroscopy does not appear feasible either: the difficulty associated with experimental studies of the  ${}^1\Gamma_{n,\pi^*} \leftarrow {}^1\Gamma_1$  transition in proteins<sup>39</sup>—a transition which

(37) S. P. McGlynn, T. Azumi, and M. Kinoshita, "Molecular Spectroscopy of the Triplet State," Prentice-Hall, Englewood Cliffs, N. J., 1969.

(38) S. V. Konev, "Fluorescence and Phosphorescence of Proteins and Nucleic Acids," S. Udenfriend, Trans. Ed., Plenum Press, New York, N. Y., 1967, pp 4-7 and 62-63.

(39) L. J. Sidel, *Arch. Biochem. Biophys.*, **54**, 184 (1955); **56**, 45 (1955).

is orders of magnitude more probable than any  ${}^3T_1 \leftarrow {}^1T_1$  transition—points up this nonfeasibility very clearly. Thus, Konev's conclusions indicate that the peptidic  $T_1$  state is not readily amenable to experimental investigation.

The computational works of the Pullmanns and their collaborators<sup>33</sup> indicate that the  $T_1$  state of the peptide grouping should be of  ${}^3\Gamma_{n,\pi^*}$  type and that it should lie at quite high energies ( $\sim 5$  eV). This result rationalizes the Konev maxim concerning the phosphorescence of peptides which contain phenylalanine, tyrosine, or tryptophan and the nonphosphorescence of ones not containing aromatic residues. Furthermore, since many other moieties present in proteins (*e.g.*, -S-S- linkage, phosphate group, carboxylate group, water, etc.) might very well possess triplets of lower energy than 5 eV, it is quite possible that the peptidic  $T_1$  state is just simply unimportant insofar as significant energy events in protein are concerned.

The attitudes of the last two paragraphs are rather well diffused throughout the literature of protein luminescence studies. These attitudes are: the triplet states of the peptide grouping are of high energy (Pullmann), and the triplet states of the peptide grouping are not luminophoric (Konev).

Our analysis of electronic states has led us to conclusions which are opposed to the first attitude and which, therefore, necessitate reexamination of the second. In particular, we seek answers to the following. (a) What is the energy of the peptidic  $T_1$  state? (b) What is the orbital excitation nature (*i.e.*,  $\Gamma_{n,\pi^*}$ ,  $\Gamma_{\pi,\pi^*}$ , etc.) of this state? (c) Is this state luminescent? (d) Is this state involved in any way in energy transfer events in proteins, in ultraviolet deactivation of enzymes, in protein semiconduction, etc.? We have provided the following answers to questions a and b: in the specific case of *N*-methylacetamide, for example, the  $T_1$  state should lie in the interval 3.8 to 2.8 eV; the  $T_1$  state is undoubtedly of  ${}^3\Gamma_{\pi,\pi^*}$  type. To answer questions c and d, we must turn to a survey of the relevant literature.

**Luminescence Data.** (i) *Formamidinium Ion.* The  ${}^3\Gamma_{\pi,\pi^*} \rightarrow {}^1T_1$  of formamidinium ion is well substantiated.

(ii) *Formates and Acetates.* Recent investigations<sup>26</sup> on the luminescence of formate and acetate salts indicate that the  $T_1$  state is  ${}^3\Gamma_{\pi,\pi^*}$ . Details of this work will be published elsewhere; here we simply enumerate those features which suggest that the emission is intrinsic to carboxyl. The phosphorescence spectra of acetate and formate derivatives contain a prominent vibrational interval of frequency  $\sim 1750$   $\text{cm}^{-1}$ . This interval correlates well with the known carbonyl stretching frequency of these same compounds. A heavy atom effect of a spin-orbital coupling nature is produced by both ionic substitution (*i.e.*,  $\text{Pb}^{2+}$  for  $\text{Na}^+$ ) and by intramolecular halogen substitution (*i.e.*, trichloroacetate as opposed to trifluoroacetate). A red shift occurs for the phosphorescence of the COSH derivative, which also has decreased lifetime. The lumines-

cence appears to be independent of the chemical synthetic route by which the compound in question was produced, of the mode of purification of the compound, etc. Finally, by study of the luminescence of the series  $\text{C}_6\text{H}_5(\text{CH}_2)_n\text{COOH}$  as a function of the number of methylene groups  $n$ , Maria, *et al.*,<sup>25</sup> have estimated the energy of the  $T_1$  state of the COOH group as 30 kK and its lifetime as 4 sec. These results are compatible with the observations of Johnson<sup>26</sup> for acetate and formate derivatives.

(iii) *N-Ethyldiacetamide.* Cheng and Nauman<sup>40</sup> report what appear to be *bona fide* phosphorescences for *N*-ethyldiacetamide. The origin of one phosphorescence appears to be at 26 kK, the other at lower energy. The different phosphorescences are attributed to *cis,cis* and *cis,trans* molecular conformations.

(iv) *Urea.* Akagi, Amako, and Azumi<sup>41</sup> report phosphorescence of an ethylene glycol-ether (1:9, v/v) solution at 90°K. The emission was attributed to a  ${}^3\Gamma_{n,\pi^*} \rightarrow {}^1T_1$  process because of the short lifetime.

(v) *Poly(*t*-butyl *N*-vinylcarbamate).* Monahan<sup>42</sup> studied the phosphorescence of poly(*t*-butyl *N*-vinylcarbamate) excited at 2537 Å at 77°K. The phosphorescence origin at 380 mμ is not sensitive to medium, but its lifetime changes from 4 sec in isopropyl alcohol-isopentane (3:7) and in thin film to 0.05–0.10 sec in 3-methylpentane. It was concluded that the emissive state in thin films and alcoholic medium was  ${}^3\Gamma_{\pi,\pi^*}$ , whereas in hydrocarbon solvent it was  ${}^3\Gamma_{n,\pi^*}$ .

(vi) *Proteins and Amino Acids.* Douzou, *et al.*,<sup>43</sup> report a serum albumin luminescence and describe it as a phosphorescence. In fact, they report one phosphorescence which is excited in the region 230–310 mμ and another excited at 350–430 mμ. This latter excitation region (usually indicated as "3650 Å excitation") has also been implicated in the phosphorescence of tryptophan and tryptophan-containing proteins by several workers (Szent-Györgyi, *et al.*,<sup>44</sup> Fujimori,<sup>45</sup> Kallman, *et al.*<sup>46</sup>). Douzou, *et al.*,<sup>43</sup> also report phosphorescence for several aliphatic amino acids as well as emissions excited by 2537 and 3650 Å incident light for tryptophan and glutathione.

Kallman, *et al.*,<sup>46</sup> present an interesting list of compounds and proteins which fluoresce and phosphoresce upon excitation at 310 and 365 mμ: acetone and cyclohexanone, acetamide and *N*-methylacetamide, polyglutamic acid, tyrosine and tryptophan, and several proteins—both fibrous and globular. The long-wavelength excitation produces a fluorescence (410 mμ) different from the one produced by short-wavelength

(40) L. T. Cheng and R. V. Nauman, Ph.D. Dissertation, Louisiana State University, Baton Rouge, La., 1968.

(41) M. Akagi, Y. Amako, and H. Azumi, *Nippon Kagaku Zasshi*, **87**, 689 (1966).

(42) A. R. Monahan, *Macromolecules*, **1**, 408 (1968).

(43) P. Douzou and J. Franço, *J. Chim. Phys. Physicochim. Biol.*, **59**, 578 (1962).

(44) R. H. Steele and A. Szent-Györgyi, *Proc. Nat. Acad. Sci. U. S.*, **43**, 477 (1957); **44**, 540 (1958); I. Isenberg and A. Szent-Györgyi, *ibid.*, **44**, 519 (1958).

(45) E. Fujimori, *Biochim. Biophys. Acta*, **40**, 251 (1960).

(46) H. Kallman, V. J. Krasnansky, and P. Person, *Photochem. Photobiol.*, **8**, 65 (1968); *Ber. Bunsenges. Phys. Chem.*, **72**, 340 (1968).



excitation and is always accompanied by a phosphorescence at slightly longer wavelength. Another phosphorescence with a lifetime of several seconds also appears in the model compounds and many globular proteins but not in fibrous proteins. They conclude that protein emission proceeds from the carbonyl bond of the peptide group.

#### Critique of Protein and Amino Acid Luminescence

**Data.** All experimental observations of phosphorescence from proteins and amino acids fall in the range 400–500  $m\mu$ ; the majority fall in the narrower range 400–450  $m\mu$ . However, no clear-cut conclusions may be drawn from these data since many other types of compound emit in this same interval and the presence of trace amounts of them—parts per billion or less—can produce the observed results.

It is probable that the Douzou–Francq results<sup>43</sup> are impurity conditioned. The observation, for glycine, that three different excitation wavelengths produce three different emissions is an obvious indication of this. There is a small possibility that such phenomenology might also be conditioned, as in *N*-methyldiacetamide, by different relative conformations of adjacent carbonyl groups, but this possibility must be considered very small. A similar consensus applies to the results of Kallman, *et al.*,<sup>46</sup> despite the concern exhibited by these workers for their sample purity.

Some comment must be made concerning the excitation of an emission by the 3650-Å mercury line. First, this constitutes excitation in a region where no known protein or protein constituent absorbs. Secondly, at least four different laboratories report this observation: Wels<sup>47</sup> (1928), Szent-Györgyi, *et al.*,<sup>44</sup> (1957–1958), Fujimori<sup>45</sup> (1960), Douzou and Francq<sup>43</sup> (1962), and Kallman, *et al.*,<sup>46</sup> (1968). This wavelength has excited emissions in proteins, amino acids, peptides such as glutathione, amides, and ketones. If this excitation is

(47) P. Wels, *Pflügers Arch.*, **219**, 738 (1928).

peptidic, it must represent a direct  $T_1 \leftarrow S_0$  absorption event. However, it must be emphasized that the literature does not document many electronic processes initiated by direct absorption to a  ${}^3T_{\pi,\pi}^*$  state except under strong perturbation such as is supplied by dissolved oxygen; on the other hand, phosphorescence induced by direct absorption to a  ${}^3T_{n,\pi}^*$  state is well known.

#### Conclusions

We have provided discussion of the electronic energy levels of the peptide linkage and its isoelectronic molecular groupings. The discussion has been theoretical and experimental and has included both singlet and triplet manifolds. We examined the changes in MO's, MO energies, and state energies which occur from one molecule to another in the series. We next compared predicted electronic state energies with experimental data and thereby identified a large number of transitions. We expect that this identification is correct in the majority of cases. We have concluded that the peptide linkage should possess a relatively low-energy  $T_1$  state and that this state should be of  ${}^3T_{\pi,\pi}^*$  type. This identification discords with the Pullmanns,<sup>33</sup> with Douzou and Francq,<sup>43</sup> and with Akagi, *et al.*<sup>41</sup> It is also concluded that this state has been observed in formamidinium ion, formate and acetate derivatives, and, possibly, in *N*-methyldiacetamide. It is concluded that no firm evidence for such a state has been provided by studies of proteins, peptides, or amino acids. Thus, no definitive answer to questions c and d can at this time be provided. However, we hope that our discussion has been provocative and that it will reopen a protein research area which has long lain dormant.

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