

study¹⁵, Tyr 76 and Tyr 92 are considered to be exposed on the surface of the molecule, Tyr 73 and Tyr 115 are halfway exposed, and Tyr 25 and Tyr 97 are buried. The last two are known not to be titrated even at high pH.¹⁶ In parallel with this, about two tyrosine residues are found to be deuterated at 24 °C (at 60 ms, for example), about four at 46 °C, and all six at 70 °C (see Figure 5). It is therefore suggested that the two tyrosine residues deuterated at 24 °C are Tyr 76 and Tyr 92, the other two next deuterated at 46 °C are Tyr 73 and Tyr 115, and the most slowly deuterated two are Tyr 25 and Tyr 97.

Let us next attempt to obtain some idea about the environment in which the tyrosine residues in the protein molecule are placed. Such an environment may be judged by the "attenuation factor" defined by

$$\gamma_j = k_j/k_e \quad (1)$$

for each of the exposed and buried tyrosine residues (j). By replotting the kinetic data illustrated in Figure 5, it is found that the rate constant (k_j) of the two tyrosine residues deuterated at 24 °C is about 120 s⁻¹. The rate constant of free tyrosine (k_e), on the other hand, is estimated to be 125 s⁻¹ (see Figure 4). Thus, the attenuation factor is nearly unity. In other words, these two tyrosine residues are considered to be fully exposed, while the attenuation factor γ_j for the most buried two tyrosine residues is as low as 10⁻² even at 70 °C, where this protein is denatured^{17,18} (k_j is estimated to be 80 s⁻¹ from data given in Figure 5 and k_e at 70 °C is estimated to be 7900 s⁻¹

by extrapolating the straight line obtained by the Arrhenius plot shown in Figure 4). Therefore, the "denatured" ribonuclease is suggested to have a considerable amount of a secondary structure.

References and Notes

- (1) Faculty of Pharmaceutical Sciences, University of Tokyo.
- (2) A. Hvidt and S. O. Nielsen, *Adv. Protein Chem.*, **21**, 287 (1966).
- (3) S. W. Englander, N. W. Downer, and H. Teitelbaum, *Annu. Rev. Biochem.*, **41**, 903 (1972).
- (4) M. Nakanishi, M. Tsuboi, and A. Ikegami, *J. Mol. Biol.*, **75**, 673 (1973).
- (5) M. Nakanishi and M. Tsuboi, *J. Mol. Biol.*, **83**, 379 (1974).
- (6) J. D. Glickson, W. D. Phillips, and J. A. Rupley, *J. Am. Chem. Soc.*, **93**, 4031 (1971).
- (7) H. Takesada, M. Nakanishi, A. Y. Hirakawa, and M. Tsuboi, *Biopolymers*, **15**, 1929 (1976).
- (8) J. H. Bradbury and B. E. Chapman, *Biochem. Biophys. Res. Commun.*, **49**, 891 (1972).
- (9) H. Takesada, M. Nakanishi, M. Tsuboi, and K. Ajsaka, *J. Biochem.*, **80**, 969 (1976).
- (10) D. G. Cross, *Biochemistry*, **14**, 357 (1975).
- (11) D. G. Cross, A. Brown, and H. F. Fisher, *J. Biol. Chem.*, **251**, 1785 (1976).
- (12) M. Nakanishi, H. Nakamura, A. Y. Hirakawa, M. Tsuboi, T. Nagamura, and Y. Saijo, *J. Am. Chem. Soc.*, **100**, 272 (1978).
- (13) T. T. Herskovits and Sr. M. Sorensen, *Biochemistry*, **7**, 2523 (1968).
- (14) D. G. Smyth, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **238**, 227 (1963).
- (15) F. M. Richards and H. W. Wyckoff, *Enzyme*, **4**, 647 (1971).
- (16) C. Tanford, J. D. Hauenstein, and D. G. Rands, *J. Am. Chem. Soc.*, **77**, 6409 (1955).
- (17) J. Hermans, Jr., and H. A. Scheraga, *J. Am. Chem. Soc.*, **83**, 3283 (1961).
- (18) T. Y. Tsong, R. P. Hearn, D. P. Wrathall, and J. M. Sturtvant, *Biochemistry*, **9**, 2666 (1970).

Photoelectron Spectra of Some Pyrazolthiones, 1,2,3-Triazolthiones, and 4-(1,2,3-Triazolio) Sulfides. Evidence of an Abnormal Effect of Methylation¹

C. Guimon,^{2a} G. Pfister-Guillouzo,^{*2a} and M. Begtrup^{2b}

Contribution from the Institut Universitaire de Recherche Scientifique, Laboratoire de Chimie Organique Physique, 64000 Pau, France, and the Institute of Organic Chemistry, The Technical University of Denmark, D.K.-2800 Lyngby, Denmark. Received September 24, 1976

Abstract: Photoelectron spectra of *N,N'*-dimethylpyrazol-3-ine-5-thione, 1,2,3-triazol-3-ine-5-thione, and 4-(1,2,3-triazolio) sulfide, together with their α -methyl derivatives, have been recorded and assigned. The *C*-methyl groups seem to stabilize the MOs in the compounds which possess a conjugated thiocarbonyl system. This unusual stabilization is discussed in terms of perturbation theory.

Previous photoelectron spectra of linear and five-membered cyclic thiocarbonyl compounds³ revealed that the first two bands are associated with ionization of electrons from the bonding π orbital of the thiocarbonyl group and from the nonbonding atomic orbital on the sulfur atom.

The two ionization potentials at best differ only slightly within experimental accuracy in saturated thiocarbonyl compounds. When distinguishable, the first band has been assigned to ionization of the *n* electrons. The order is reversed and the difference in energy is larger for β,γ -unsaturated cyclic thiocarbonyl compounds.

A feature common to saturated and β,γ -unsaturated thiocarbonyl compounds is a decrease in energy of the first band by introduction of a methyl group.

The present paper deals with the effect of α,β unsaturation and subsequent methyl substitution on the photoelectron

spectra of cyclic thiocarbonyl compounds using 1,2-dimethylpyrazol-3-ine-5-thione (a), 1,2-dimethyl-1,2,3-triazol-3-ine-5-thione (b), and their 4-methyl derivatives as models. In addition, the photoelectron spectra of the highly polarized thiocarbonyl group in 1,3-dimethyl-4-(1,2,3-triazolio) sulfide (c) and its 5-methyl derivative have been recorded.

Photoelectron Spectra of the Species a, b, and c. 1,2-Dimethylpyrazol-3-ine-5-thione (a). The photoelectron spectra of the pyrazolthione (a) (Figure 1) and its 4-methyl derivative exhibit an intense low-energy band corresponding to ionization of both a thiocarbonyl π electron and the sulfur *n* electron. This coincidence, also observed in other α,β -unsaturated thiocarbonyl compounds like the 1,2-dithiole-3-thiones^{4a} and the triazolthiones (b) below, is distinct from the marked separation found in the β,γ -unsaturated compounds.

The assignment of the photoelectron signals of the pyra-

Table I. Vertical Experimental and Calculated (CNDO/S) Ionization Potentials for Pyrazol-3-ine-5-thiones

Exptl values, eV		Calcd values, eV	Unsubstituted molecule	
			Contributions from atomic orbitals, %	
7.55	7.60	8.94	S = 72, N ₂ = 9, N ₁ = 7, C ₅ = 2, C ₄ = 8, C ₃ = 2	π
7.55	7.60	9.09	S = 96	n
9.78	9.30	12.18	S = 7, N ₂ = 18, N ₁ = 12, C ₅ = 11, C ₄ = 40, C ₃ = 13	π
10.22	10.20	13.06	S = 5, N ₂ = 25, N ₁ = 39, C ₅ = 14, C ₃ = 16	π
11.96	11.70	13.47	S = 55, C ₅ = 28, C ₄ = 7	σ
12.57	12.36	16.16	N ₂ = 9, N ₁ = 7, C ₅ = 12, C ₄ = 32, C ₃ = 13	σ
		17.25	S = 12, N ₂ = 5, N ₁ = 7, C ₅ = 16, C ₄ = 25, C ₃ = 22	σ
		18.59	N ₂ = 37, N ₁ = 30, C ₅ = 9, C ₄ = 9, C ₃ = 14	π

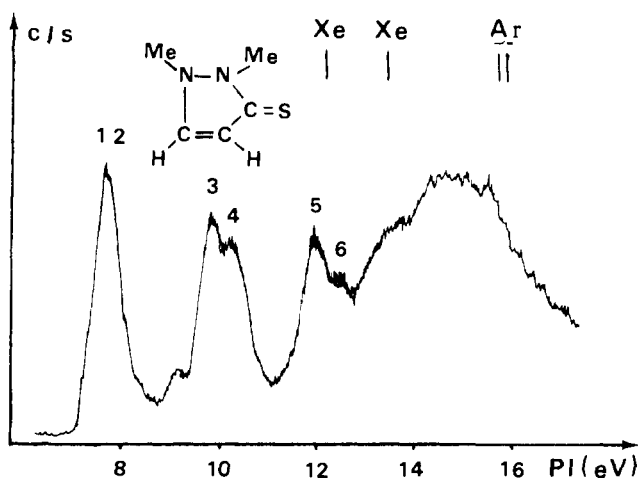


Figure 1. Photoelectron spectrum of 1,2-dimethylpyrazol-3-ine-5-thione.

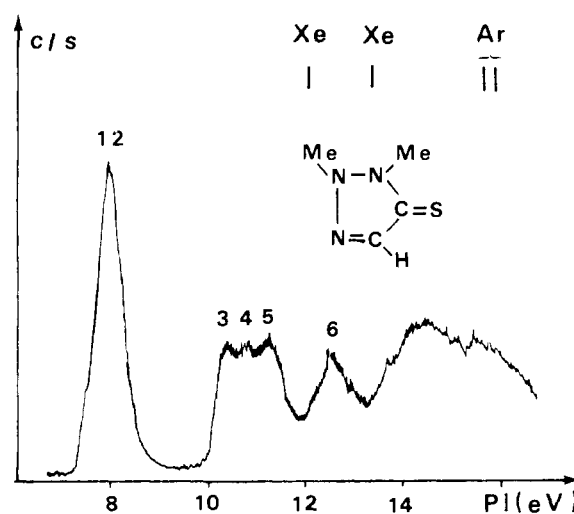
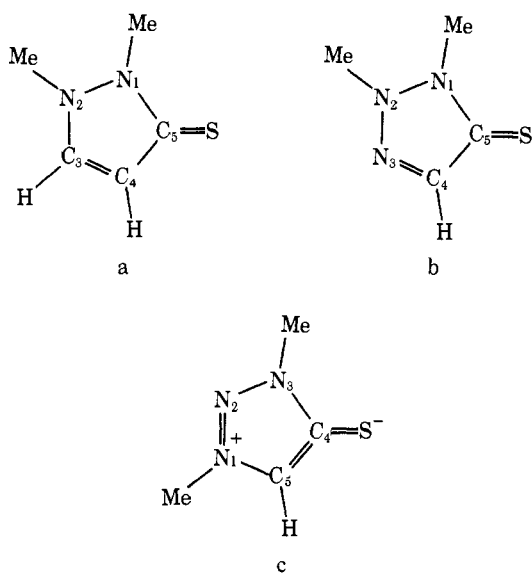


Figure 2. Photoelectron spectrum of 1,2-dimethyl-1,2,3-triazol-3-ine-5-thione.

zolphthiones is evident from Table I where the experimental values are compared with those calculated using the CNDO/S method,^{4b,c} Koopmans' theorem,⁵ and geometries based on x-ray data.⁶



As in all the previously studied cases the ionization potential most sensitive to methylation is that corresponding to the orbital most localized at the substituent site, C-4. In the present case this is the π orbital primarily localized at the C=C bond

giving rise to the band at 9.78 eV which is shifted to 9.30 eV on methyl substitution.

The first two orbitals highly localized on the sulfur atom of the α,β -unsaturated species (a) are slightly stabilized upon C-methylation. This contrasts with the methyl group destabilization observed in saturated and β,γ -unsaturated thiocarbonyl compounds as discussed below.

1,2-Dimethyl-1,2,3-triazol-3-ine-5-thione (b). The photoelectron spectra of b (Figure 2) and its 4-methyl derivative are similar to those of the corresponding pyrazolphthiones. The first two orbitals are almost degenerate and their ionization energies are insensitive to C-methylation. The next two bands are associated with the ionization of π electrons localized within the ring (Table II).

The first of these bands is more sensitive to C-methylation reflecting the somewhat higher localization at C-4 calculated for the corresponding orbital. The observed order of the closely situated fourth and fifth bands⁷ should be the opposite according to the calculations. The discrepancy may be inherent in the CNDO/S parametrization or in the inapplicability of Koopmans' theorem.

1,3-Dimethyl-4-(1,2,3-triazolio) Sulfide (c). The photoelectron spectra of c (Figure 3) and its 5-methyl derivative are quite different from those of the corresponding triazolphthiones since they exhibit the two low-energy n and π ionizations characteristic of thiocarbonyl compounds. Furthermore, between 10 and 13 eV there are five well-resolved bands relative to four for the triazolphthiones (Table III).

Table II. Vertical Experimental and Calculated (CNDO/S) Ionization Potentials for 1,2,3-Triazol-3-ine-5-thiones

Exptl values, eV		Calcd values, eV	Unsubstituted molecule	
			Contributions from atomic orbitals, %	
7.97	7.95	9.12	S = 69, N ₃ = 2, N ₂ = 9, N ₁ = 11, C ₄ = 7	π
7.97	7.95	9.35	S = 96	n
10.60	10.03	12.42	S = 10, N ₃ = 8, N ₂ = 30, N ₁ = 4, C ₅ = 16, C ₄ = 32	π
10.92	10.80	13.49	S = 3, N ₃ = 28, N ₂ = 12, N ₁ = 43, C ₅ = 11, C ₄ = 3	π
11.43	11.06	13.45	S = 40, N ₃ = 22, C ₅ = 24	σ
12.59	12.61	15.11	S = 22, N ₃ = 52, C ₅ = 8	σ
		16.89	N ₃ = 7, N ₂ = 3, N ₁ = 13, C ₅ = 19, C ₄ = 31	σ
		18.74	N ₃ = 16, N ₂ = 35, N ₁ = 30, C ₅ = 9, C ₄ = 9	π

Table III. Vertical Experimental and Calculated (CNDO/S) Ionization Potentials of 4-(1,2,3-Triazolio) Sulfides

Exptl values, eV		Calcd values, eV	Unsubstituted molecule	
			Contributions from atomic orbitals, %	
7.25 (1050 cm ⁻¹)	7.02 (1100 cm ⁻¹)	8.63	S = 74, N ₂ = 9, N ₃ = 6, C ₅ = 11	π
7.59 (1200 cm ⁻¹)	7.46 (1200 cm ⁻¹)	8.92	S = 97	n
10.35	9.88	12.15	S = 12, N ₂ = 32, N ₃ = 2, C ₄ = 24, C ₅ = 29	π
10.81	10.55	13.99	N ₁ = 39, N ₃ = 47, C ₄ = 8, C ₅ = 4	π
11.40	11.20	13.49	S = 56, N ₂ = 6, C ₄ = 25, C ₅ = 5	σ
11.74	11.49	15.72	S = 5, N ₁ = 12, N ₂ = 66, N ₃ = 6, C ₅ = 6	σ
12.60	12.19	17.30	S = 4, N ₃ = 18, C ₄ = 26, C ₅ = 27	σ
		19.07	N ₁ = 32, N ₂ = 24, N ₃ = 22, C ₄ = 9, C ₅ = 12	π

Destabilization of the two highest lying MOs is concordant with the presence of a highly negative sulfur atom in c. The π orbital is subject to a larger destabilization (0.7–0.9 eV) than the nonbonding orbital (0.4–0.5 eV) indicating in accord with a simple resonance formulation that the charge separation in c is primarily π in character.

The next two bands in c are associated with delocalized ring π electrons. The first of these bands corresponds to the most localized MO at C-4 and is therefore most sensitive to C-methylation. As in the triazolthione series, a discrepancy exists between the observed and calculated order of the closely spaced fourth and fifth bands.

Effect of C-Methylation on the Pyrazolthione a and the Triazolthione b. In agreement with previous workers⁸⁻¹⁴ we have observed that methylation causes destabilization of the highest occupied molecular orbitals. Destabilization is often associated with augmented negative charge in the orbital. Therefore, the stabilization of the highest occupied molecular orbital (HOMO) observed on C-methylation of the pyrazolthione (a) is inconsistent with the concept of a methyl group as an electron donor.

The possibility of stabilizing methyl groups is accommodated by theoretical considerations¹⁵⁻¹⁸ which indicate that interactions between orbitals of the methyl group¹⁹ and the residual molecule can lead to an MO energy lowering.²⁰⁻²² The two key molecular orbitals representing a methyl group are both mutually orthogonal and antisymmetric with respect to a plane. These orbitals have predominant π character. The π(CH₃) orbital respectively interacts with the π system and the π'(CH₃) orbital with the σ system of a planar moiety with delocalized electrons. The importance of these interactions depends on the energy difference between the concerned or-

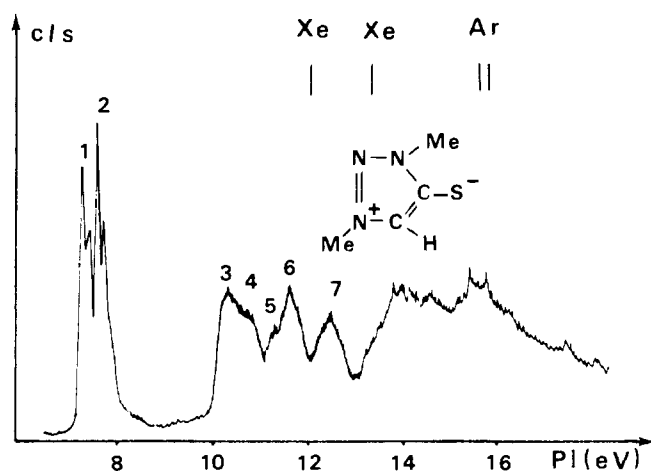


Figure 3. Photoelectron spectrum of 1,3-dimethyl-4-(1,2,3-triazolio) sulfide.

bitals and the magnitude of the orbital occupancy or orbital density at the site of interaction.

According to the perturbation theory^{19,23} the two effects of a methyl group on a molecular orbital ϕ_i may be written as¹¹

$$\delta E_i(\text{ind}) = \langle \phi_i | P | \phi_i \rangle$$

which represents the inductive effect and

$$\delta E_i(\text{hyp}) = \frac{\langle \phi_i | P | \pi\text{CH}_3 \rangle^2}{E_i^0 - E_{\text{CH}_3}^0}$$

representing hyperconjugation.

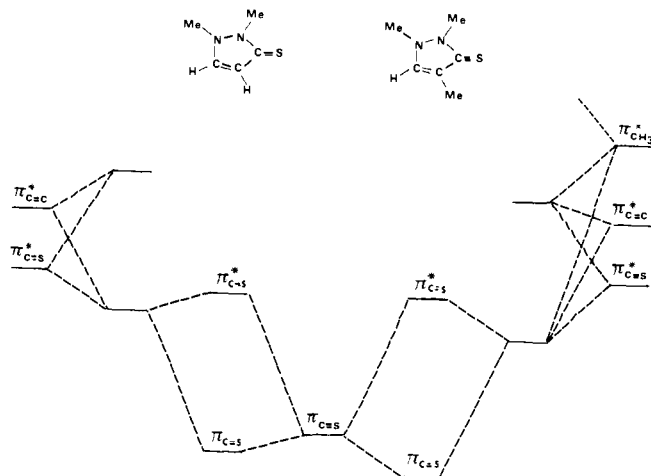


Figure 4. Stabilization of the last occupied molecular orbital of 1,2-dimethylpyrazol-3-thione by methylation at C₄.

If only interactions between occupied orbitals are considered methylation would tend to destabilize the assembly of highest molecular orbitals. The magnitude of the band shifts would then depend on the relative energies of the fragment MO and the degree of localization of the appropriate orbital of the residue molecule.

Now, methylation at C-4 of the pyrazolthione a induced a shift of the first two bands reflecting a stabilization of 0.05 eV. The corresponding bands were not significantly shifted by methylation of the triazolthione b. In contrast, the third band associated with the molecular orbital localized at the double bond is shifted toward lower energy by C-methylation of the pyrazolthione and the triazolthione (0.48 and 0.57 eV, respectively).

The stabilization of the first orbital in the pyrazolthione a and the absence of destabilization in the triazolthione b is only reasonable in terms of a second-order perturbation interaction²² between the $\pi_{C=S}$, the unoccupied $\pi_{C=C}$ in a or $\pi_{C=N}$ in b, and the $\pi_{C=S}^*$ orbital.

The vacant $\pi_{C=C}^*$ and $\pi_{C=S}^*$ orbitals are calculated to be of similar energy. In β,γ -unsaturated compounds with two heteroatoms α to the thiocarbonyl group, the C=C and C=S bonds are elongated and the interaction between the two virtual orbitals associated with these bonds may be considered as negligible.²⁴ This is not so for the α,β -unsaturated pyrazolthiones or triazolthiones where a significant interaction leads to the destabilization of the vacant $\pi_{C=S}^*$ orbital (Figure 4). The latter is energetically nearer to the occupied $\pi_{C=S}$ orbital and may stabilize it thus explaining the degeneracy of the $\pi_{C=S}$ and the n_s orbitals in the α,β -unsaturated compounds a, b, as well as in 1,2-dithiole-3-thione.³ The increased stabilization observed by C-methylation of the pyrazolthione is thus explained by a stabilizing interaction of the $\pi_{C=C}^*$ and the $\pi_{C=S}$ with the $\pi^*(CH_3)$ orbital (Figure 4). A reasoning similar to that presented in Figure 4 may be applied to the triazolthione case by noting that the $\pi_{C=N}$ and $\pi_{C=N}^*$ orbitals are respectively more stable than the $\pi_{C=C}$ and $\pi_{C=C}^*$ orbitals,¹⁹ and that the $\pi_{C=S}$ orbital is of lower energy in b than in a.

It follows that the triazolthione is less sensitive to stabilization by the $\pi^*(CH_3)$ but more sensitive to destabilization by the $\pi(CH_3)$ orbital. The experimental results may be interpreted by postulating that the two effects counterbalance each other.²⁵

Experimental Section

The spectra were recorded on a Perkin-Elmer PS-18 photoelectron spectrometer. The He(I) resonance line at 21.22 eV served as the ionizing source. The spectra were calibrated with the $2p_{1/2}$ and $2p_{3/2}$ lines of Ar and Xe and recorded with a resolution of 30 meV. The compounds studied were prepared by thiation of the corresponding azolium anions with sulfur.²⁶

Acknowledgments. We are grateful to the Société Nationale Elf-Aquitaine (Production) for financial support and to Professor P. Snyder for useful comments.

References and Notes

- (1) Part 23 of "Electronic Structure of Sulfur Compounds". Part 22: *J. Chem. Soc., Perkin Trans. 2*, **13**, 1652 (1977).
- (2) (a) Institut Universitaire de Recherche Scientifique de Pau; (b) The Technical University of Denmark.
- (3) (a) C. Guimon, D. Gonbeau, G. Pfister-Guillouzo, L. Asbrink, and J. Sandstrom, *J. Electron Spectrosc. Relat. Phenom.*, **4**, 49 (1974); (b) C. Guimon, G. Pfister-Guillouzo, M. Arbelot, and M. Chanon, *Tetrahedron*, **30**, 3831 (1974); (c) C. Guimon, M. Arbelot, and G. Pfister-Guillouzo, *Spectrochim. Acta, Part A*, **31**, 98 5 (1975); (d) *Tetrahedron*, **31**, 2769 (1975); (e) C. Guimon and G. Pfister-Guillouzo, *J. Electron Spectrosc. Relat. Phenom.*, **7**, 191 (1975); (f) C. Guimon, M. Arbelot, and G. Pfister-Guillouzo, *J. Mol. Struct.*, **30**, 339 (1975). These papers are extracts of the thesis of C. Guimon, Pau, 1976 (AO 11040).
- (4) (a) D. Gonbeau, C. Guimon, J. Deschamps, and G. Pfister-Guillouzo, *J. Electron Spectrosc. Relat. Phenom.*, **6**, 99 (1975); (b) J. Del Bene and H. H. Jaffe, *J. Chem. Phys.*, **48**, 1807 (1968); (c) C. Guimon, D. Gonbeau, and G. Pfister-Guillouzo, *Tetrahedron*, **29**, 3399 (1973).
- (5) T. Koopmans, *Physica*, **1**, 104 (1934).
- (6) K. Nielsen, *Acta Chem. Scand., Ser. A*, **29**, 647 (1975), and results to be published.
- (7) This order is deduced from the comparison with the spectra of other thiocarbonyl heterocycles.³
- (8) B. J. Coksey, J. H. D. Eland, and C. J. Danby, *J. Chem. Soc. B*, 790 (1971).
- (9) P. A. Clark, *Theor. Chim. Acta*, **28**, 75 (1972).
- (10) M. Klessinger, *Angew. Chem.*, **84**, 544 (1972); *Angew. Chem., Int. Ed. Engl.*, **11**, 525 (1972).
- (11) M. Beez, G. Bieri, H. Bock, and E. Heilbronner, *Helv. Chim. Acta*, **56**, 1028 (1973).
- (12) F. Brogli, P. A. Clark, E. Heilbronner, and M. Neuenschwander, *Angew. Chem., Int. Ed. Engl.*, **12**, 422 (1973).
- (13) P. Masclet, D. Grosjean, G. Mouvier, and J. Dubois, *J. Electron Spectrosc. Relat. Phenom.*, **2**, 225 (1973).
- (14) P. Carlier, J. E. Dubois, P. Masclet, and G. Mouvier, *J. Electron Spectrosc. Relat. Phenom.*, **7**, 55 (1975).
- (15) H. Kollmar and H. O. Smith, *Angew. Chem.*, **82**, 444 (1970); *Theor. Chim. Acta*, **20**, 64 (1971).
- (16) J. R. Grunwell and J. F. Sebastian, *Tetrahedron*, **27**, 4387 (1971).
- (17) G. R. Howe, *J. Chem. Soc. B*, 981, 984 (1971).
- (18) J. M. Andre, P. Degand, and G. Leroy, *Bull. Soc. Chim. Belg.*, **80**, 585 (1971).
- (19) W. L. Jorgensen and L. Salem, "The Organic Chemist's Book of Orbitals", Academic Press, New York, N.Y., 1973.
- (20) W. J. Hehre and L. Salem, *J. Chem. Soc., Chem. Commun.*, 754 (1973).
- (21) O. Eisenstein and Nguyen Trong Anh, *Bull. Soc. Chim. Fr.*, 2721, 2723 (1973).
- (22) L. Libit and R. Hoffmann, *J. Am. Chem. Soc.*, **96**, 1370 (1974).
- (23) M. J. S. Dewar, "The Molecular Orbital Theory of Organic Chemistry", McGraw-Hill, New York, N.Y., 1969.
- (24) In 1,3-dimethylimidazol-4-ine-2-thione this interaction is exactly zero for reasons of symmetry.
- (25) The energy of the $\pi_{C=S}$ orbital of 1,2-dithiole-3-thione is much lower (-8.42 eV). Hence, the destabilizing interaction with the $\pi(CH_3)$ orbital is more pronounced than the stabilizing interaction with the $\pi^*(CH_3)$ orbital.^{4a}
- (26) (a) M. Begtrup, *Acta Chem. Scand., Ser. B*, **29**, 141 (1975); (b) *J. Chem. Soc.*, **1**, 507 (1975); (c) to be published.