Electronic Structure of Thio-TEPA Antitumor Drug

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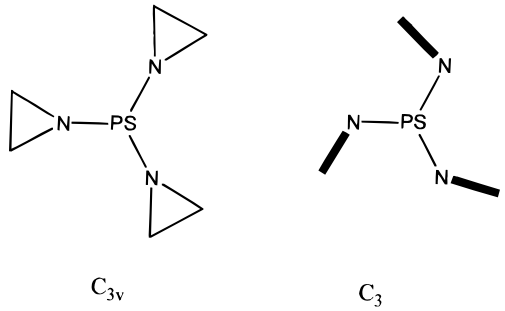
Introduction

The family of antitumor drugs based on TEPA (*N*,*N*:*N*′,*N*′:*N*″,*N*″-tris(1,2-ethanediyl)phosphoric triamide) has been the subject of several recent investigations. TEPA drugs of general formula $(C_2H_4N)_3PX$ are alkylating agents whose antitumor action consists mostly in alkylating the N7 position of guanine base in DNA.[1] Recent reports have included studies of TEPA biological activity in vivo[2] and a combined MNDO−mass spectrometric study of one member of the family, thio-TEPA[3] (ttp). The aim of the latter study[3] was to clarify the molecular structure of the compound and its relationship with biological activity. Kosevich et al.[3] have noted a discrepancy between molecular structure in the solid state (X-ray study) and the MNDO-optimized structure.[3] The authors suggested, on the basis of their field ionization MS studies, "...the high sensitivity of the molecule to changes in external conditions which may be a basis for its high biological activity...". They have also noted the absence of protonation or hydration of ttp in the gas phase under high electric field conditions. The structure−activity link that they proposed is not clear, partly because in biological systems high-field vacuum conditions do not apply. Since the results of X-ray study[4] are not precise (error ± 0.02 Å, due to decomposition of the crystal) and Kosevich et al.[3] did not specify molecular symmetry, we have decided to reexamine the problem using ab initio and semiempirical MO calculations and UV photoelectron spectroscopy (UPS).

Experimental and Theoretical Methods

The melting point of the sample from Acros Organics was measured (51 °C; lit.[12] 51.5 °C) and the sample used without further purification. The ab initio calculations with full geometry optimization inclusive of NBO analysis were performed at the MP2/6-31G(d,p)//HF/6-31G(d,p) level using the Gaussian 98 program[5] in order to determine the molecular structure of $(C_2H_4N)_3PX$ (X = O, S, Se). The calculations suggested that the molecular structures resemble a "windmill" shape, corresponding to C_{3v} symmetry with nitrogen lone pairs (Nlp) in the trans conformation vs PX bonds. This symmetry is higher than the C_3 symmetry observed for other phosphine chalcogenides[6] and is also higher than in the solid state (Table 1). The conformers with C_3 symmetry have a gauche arrangement of Nlp and are 18−20 kJ/mol higher in energy than C_{3v}. The crystal structure data[4] indicate the existence of a distorted C_3 symmetry with each ∠NlpPS angle slightly different. We have also performed semiempirical calculations at different levels (AM1, PM3, MNDO), and all of them suggest C_3 ("propeller-shaped") as the conformation with minimum energy. The small differences between conformer energies concur with the earlier suggestion[3] of the virtually free internal rotation of aziridine rings.



C_{3v} C_3

Photoelectron spectra were recorded using modified Perkin-Elmer PS16/18 spectrometers. HeI spectra (photon energy 21.22 eV) were recorded using a spectrometer modified to run at a fixed pass energy of 5 eV to give high sensitivity because of the low pressure of the sample. This resulted in a high resolution of 35 meV judged from Ar^+ ^2P calibrant lines. For recordings of HeII spectra (photon energy 40.81 eV), it was necessary to increase the sample pressure by using a heated ionization chamber at a temperature of 40 °C because of the lower signal usually associated with HeII spectra compared with HeI spectra. These spectra were recorded with a spectrometer in which the analyzer pass energy was scanned and electrons were not accelerated before analysis. These two modes of scanning should not affect the relative intensities of spectra in the electron energy range of interest in this paper. Spectra were calibrated with respect to the known positions of the Ar^+ ^2P (15.76 and 15.94 eV) and the He^+ (24.59 eV) lines. The He^+ line is generated when ground-state He atoms in the discharge are illuminated by 40.8 eV photons originating from excited states of He^+. Ionization energies corresponding to band maxima are judged accurate to ±0.05 eV at best because of the broad nature of the bands observed. The assignment of spectra was based on Koopmans theorem, which is valid for assigning bands whose ionization energies (IE) are below 20 eV. The applicability of the theorem was demonstrated in the work of Elbel et al.,[8] who studied UPS of similar compounds (Me_3PX). Furthermore, Green's function calculations (i.e., non-Koopmans approach) on aziridine also demonstrated that no shake-up bands are present for IE<20 eV.[7]

Results and Discussion

1. Electronic Structure. The electronic structure, revealed by UPS, was analyzed by comparison with the

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Table 1. Molecular Structure Parameters of TEPA and Phosphine Chalcogenides[4,6]

molecule/symmetry	geometry/Å, deg	method
Me_3PO/C_3	PO = 1.476, PC = 1.809, ∠OPC = 114.4	ED
Me_3PS/C_3	PS = 1.940, PC = 1.818, ∠SPC = 114.1	ED
Me_3PSe/C_3	PSe = 2.091, PC = 1.816, ∠SePC = 113.8	ED
$(C_2H_4N)_3PS/C_1$	PS = 1.91, PN = 1.64, ∠SPN = 118	X-ray
$(C_2H_4N)_3PS/C_{3v}$	PS = 1.968, PN = 1.67, ∠SPN = 116	HF/6-31G(d,p)
$(C_2H_4N)_3PO/C_{3v}$	PO = 1.472, PN = 1.669, ∠OPN = 115	HF/6-31G(d,p)
$(C_2H_4N)_3PSe/C_{3v}$	PSe = 2.114, PN = 1.670, ∠SePN = 115.1	HF/6-31G(d,p)

Table 2. NBO Analysis (Atomic Symbols Designate Atomic Charge, PX, PN Bond Populations)

molecule/symmetry	X	P	N	PX	PN
$(C_2H_4N)_3PO/C_{3v}$	−1.23	2.67	−0.92	22.1% O + 77.9% P	24.0% N + 76.0% P
$(C_2H_4N)_3PO/C_3$	−1.17	2.64	−0.95	23.2% O + 76.9% P	23.9% N + 76.1% P
$(C_2H_4N)_3PS/C_{3v}$	−0.77	2.13	−0.92	54.2% P + 45.8% S	25.2% N + 74.8% P
$(C_2H_4N)_3PS/C_3$	−0.62	2.03	−0.93	51.6% P + 48.4% S	25.4% N + 74.6% P
$(C_2H_4N)_3PSe/C_{3v}$	−0.70	2.06	−0.92	50.3% P + 49.7% Se	25.4% N + 74.6% P
$(C_2H_4N)_3PSe/C_3$	−0.60	1.94	−0.92	47.7% P + 52.3% Se	26.1% N + 73.9% P

assigned spectra of aziridine[7] and tertiary phosphine chalcogenides.[8] The comparison reveals important similarities in the number and types of bands present in different ionization energy (IE) regions. The band at 8.5 eV corresponds to ionization from the sulfur lone pair orbital of *e* symmetry, which has a pronounced S3p character. This assignment is based on HeI/HeII relative band intensity variation. The 8.5 eV band intensity falls strongly upon increasing photon energy as can be expected on the basis of the S3p HeII/HeI photoionization cross-section ratio,[9] which is 0.138. The corresponding ratios for C2p, N2p, and P3p are 0.307, 0.449, and 0.411, respectively. The cluster of bands at 9.6−10.1 eV can be attributed to orbitals of a+e symmetry which arise from linear combinations of nitrogen lone pairs on aziridine rings. This assignment is consistent with UPS data for aziridine.[7] Finally, band systems at 11.6 and 13.5 eV correspond to bonding orbitals localized on the aziridine moiety. The most interesting information provided by UPS concerns molecular structure and intramolecular interactions between aziridine and P═S moieties. The splitting of 9.6 and 10.0 eV bands in an approximately 1:2 intensity ratio suggests the presence of a C_3 symmetry axis as is the case in other phosphine chalcogenides (Table 1). The significance of the P═S−aziridine interaction can be estimated from the spectra without recourse to MO calculations that usually favor delocalized MO descriptions. A comparison of IE values and relative HeI/HeII intensities in the spectra of aziridine[7] and thio-TEPA demonstrates that such interactions are weak. The ttp bands at 9.6−10.0 eV and 11.6−13.5 eV correspond to aziridine bands at 9.85 and 11.9−13.6 eV, respectively. The comparable ttp and aziridine bands also show identical HeI/HeII intensity variations. Additional evidence of weak interactions stems from the comparison of sulfur lone pair (n_S) IE in ttp (8.5 eV) and Me_3PS, which differ by only 0.02 eV. This suggests that intramolecular interactions in all TEPA derivatives are likely to be weak. If this were not the case, one would observe IE shifts of n_S bands. The results presented in Tables 1 and 2 also give support to the notion of weak intramolecular interactions by indicating that bond length, overlap populations and net charges are fairly constant for PX and aziridine moieties. This general conclusion can be utilized to further our understanding of biological activity of the whole TEPA family of drugs.

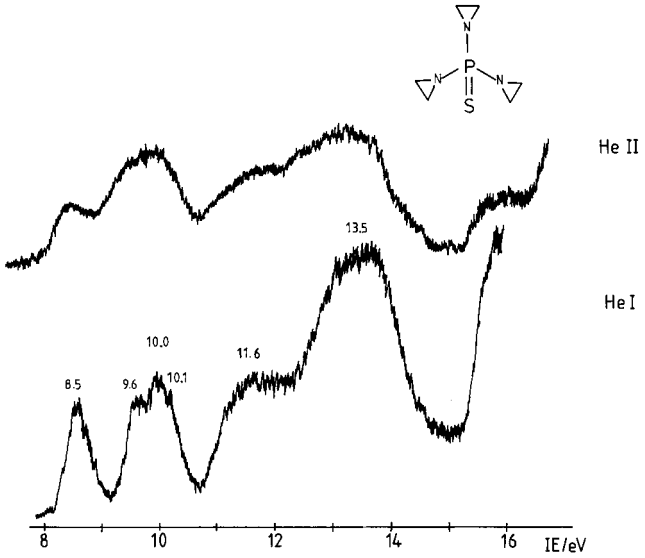

Figure 1. HeI and HeII UV photoelectron spectra of thio-TEPA.

A general discussion of structure and bonding in tertiary phosphine chalcogenides has been published recently.[10] In it the P═S bond was described as weak and of low polarity, especially when compared to the P═O bond. The P═Se bond is even weaker and less polar than P═S. These bond properties may help to rationalize the weak intramolecular interactions mentioned above and also to point out that weak intramolecular interactions are a generic property of the whole TEPA family of drugs.

UPS data indicate that there is virtually no (de)-stabilization of nitrogen lone pairs on going from aziridine to ttp. This conclusion follows from comparing n_N ionization energies (levels) in aziridine (9.85 eV) with ttp (9.6−10.0 eV). In ttp, the 10.0 eV level is doubly degenerate, with each level being stabilized by 0.15 eV vs aziridine. On the other hand, the 9.6 eV level is destabilized by 0.25 eV, i.e., by almost the same amount. Finally, some evidence of Jahn−Teller distortion exists in the shoulder (10.1 eV) of the 10.0 eV band, which corresponds to ionization from e symmetry orbital.

2. Biological Activity.[11] Most biological activity of drugs stems from the combined effect of several factors.

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The most important factors are transport properties (i.e., how successful is the delivery of drug compound to the cell interior) and receptor binding properties. The binding properties depend on how well the drug matches the receptor stereochemically and also how strong the various (non)bonding interactions between the drug and the receptor are. The bonding interactions between the drug and its receptor are related to the electronic structure of the drug and also to the receptor. However, since the receptors are often too large to be studied by UPS or even MO methods, we shall concentrate on the electronic structure of the drug itself. In case of TEPA drugs in general (although the one used in medical practice is ttp), the "receptor" is cellular DNA. The active form of the drug is the highly electrophilic ethyleneimonium ion, which has a positive charge located on nitrogen.[1] We propose that due to weak intramolecular interactions between PX and aziridine moieties the nature of heteroatom X does not influence overall biological activity. A recent comprehensive study[2] of antitumor activity of TEPA derivatives in vivo seems to bear this out. How can we rationalize this suggestion? The weak intramolecular interaction will not affect the stability or electrophilicity of the ethylenimonium ion, which is the established intermediate in the drug action[1] and thus presumably responsible for the drug's effectiveness. However, the complexity of drug actions is demonstrated by %ILS activity factors, which have values of 136, 141, and 64 for oxo-, thio-, and seleno-TEPA, respectively. Our MO calculations have shown that weak intramolecular interactions pertain to all three analogues. Why then is the activity of the Se analogue different? Sosnovsky et al.[2] suggested that lipophilicity may affect drug transport and hence overall activity. The nature of the heteroatom will influence lipophilicity and subsequently activity without changing the electronic structure.

(12) *The Merck Index*, 12th ed.; p 1648.

Conclusion

This work demonstrates that experimental study of the electronic structure can be linked directly to specific biological activity parameters. A better insight into the reasons for drug efficacy may be gained provided that one considers carefully other intervening factors (e.g., drug transport).

Finally, we wish to mention that our combined UPS/MO approach in studying drug activity is not unique, but it is nevertheless not common. The reason is that most biological molecules and drugs are either thermally unstable (UPS requires vaporization by heating) or too large to provide spectra where bands can be unambiguously resolved and assigned.

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