A Theoretical Study of H_3PO_4, nor-N-Mustard, and Cyclophosphamide

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Z. Naturforsch. **34 c,** 658 – 669 (1979); received December 18, 1978/March 13, 1979

Cyclophosphamide, Cytostatic Agents, *nor*-N-mustard, Alkylating Agents

Some physical and chemical properties of the cancerostat cyclophosphamide (generic name: ENDOXAN) and its basic constituents H_3PO_4 and nor-N-mustard have been calculated with the help of a modified CNDO/S-method. The spectroscopic data of the H_3PO_4, which is the starting-point for a corresponding calculation of cyclophosphamide, has been studied by taking account of the 3 d electron of the phosphorus. Nor-N-mustard is a very reactive compound, characterized by the ability to split off chloride ions and to act as an alkylating agent. The binding of the nor-N-mustard to the cyclic phosphate ester (cyclophosphamide) modifies the chemical reactivity of the mustard group in an essential way, and the 3 d electron of the phosphorus plays an important role with respect to the excitability of the C – Cl bonds. Cyclophosphamide must be metabolized in a suitable way to develop the same alkylating activity as the nor-N-mustard. The computation of the excited states of cyclophosphamide revealed a similar term scheme as it was found by Clar in the case of the carcinogenic polycyclic hydrocarbons.

1. Introduction

The cytostat cyclophosphamide (CP), synthesized 1958 by Arnold and Bourseaux [1], incorporates one of the most successful drugs against a wide class of tumors. According to a recommendation of the "International Conference on Screening Methodology for Anti-Tumor Drugs" [2] it serves also as a comparison standard for the development of new anticancer drugs. The contention of this study is to make apparent the physical and chemical properties of CP (2-oxo-2(bis-β-chloroethylamino)-1-hydro-1,3,2-azaoxaphosphoriane) and its basic constituents with the help of the methods of theoretical chemistry. In Fig. 1 it can be recognized that CP consists of an oxazaphosphorine ring (OPR) and of *nor*-N-mustard (NNM). For a theoretical treatment of CP it seems to be useful to start with the compounds H_3PO_4 and NNM, and we shall observe that the treatment of these basic compounds will provide some essential insights of the chemical behaviour of CP. NNM (see Fig. 2), being a precursor of CP, was used with some success as a drug against Hodgkin's desease. However, this drug turned out to be too toxic in normal tissue. The *in vitro* chemical reactivity of NNM is characterized by the ability to split off chloride ions Cl^- in aqueous solutions. If we consider chemical reactions of NNM with organic compounds and biomolecules in cells, then this ability of NNM involves the property to act as an alkylating agent (Brock and Hohorst [3]). CP was developed with the contention to improve the cytostatic activity of NNM by the means of a binding of NNM to a cyclic phosphate ester

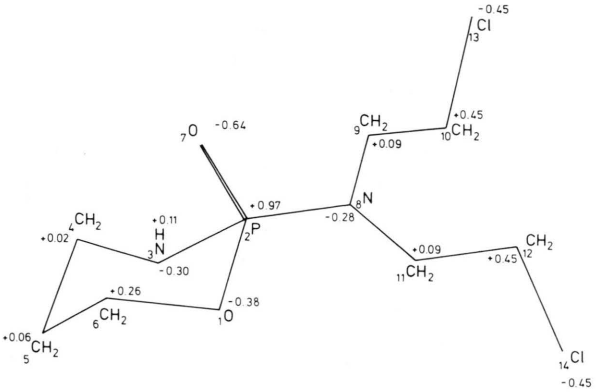

Fig. 1. Cyclophosphamide: conformation and charge densities.

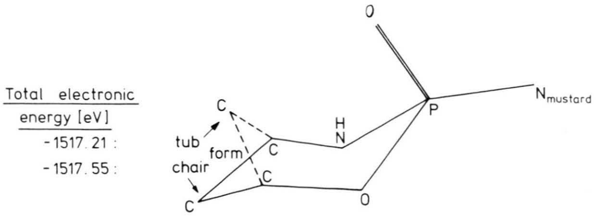

Fig. 1 a. Conformations of the oxazaphosphorine ring (OPR).

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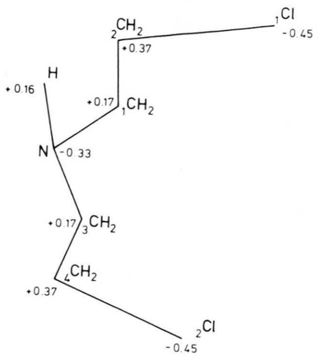


Fig. 2. Nor-N-Mustard (NNM): conformation and charge densities.

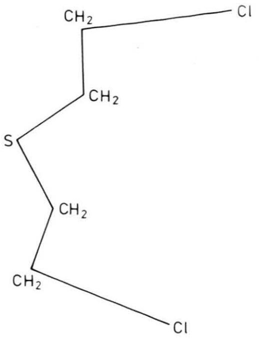


Fig. 3. Conformation of the mustard.

(OPR). CP is chemically almost inactive, and only by a proper metabolism it can be activated (Brock and Hohorst [4]). For a theoretical consideration it is also necessary to investigate the metabolites of CP, and we shall present them in a continuation [42]. A computation of CP and NNM exhibits a further theoretical background, overlapping with the chemical carcinogenesis of certain classes of molecules, as CP and NNM act as both cancerostatic and carcinogenic compounds. This fact is empirically known from other cytostatic agents – one may think of mustard (Fig. 3), derivatives of nucleic acids, X- and γ-rays – which may induce tumors after a certain latency period. However, the chemical carcinogenesis is most widely studied in connection with the polycyclic hydrocarbons (PHC). By experimental research one has found a significant correlation between the interaction of PHC to nucleic acids (DNA, RNA) and tumor induction. The binding of PHC to proteins has also been considered to be essential for tumor induction, but at present it is believed that the interaction of PHC with nucleic acids plays a more significant role (see Suess, Kinzel, and Scribner [5]).

The first theoretical study of biochemical problems in connection with chemical carcinogenesis was performed by the Pullmans [6]. These authors found a correlation between chemical reactivities of the K- and L-region of PHC and their carcinogenic activity. However, in spite of intensive incestigations, a link between covalent binding of cancerogenic molecules with nucleic and amino acids and cancer induction has not been established [7, 8]. Furthermore, the correlation of the authors [6] contains many exceptions [15, 16] and it exhibits also the disadvantage to be not applicable with respect to other classes of carcinogenic molecules. Other authors (*e. g.* Hoffmann and Ladik [9], Allison and Nash [10]) have postulated a characteristic charge transfer to be an essential effect for cancer induction. A further correlation was founded by Birks [11] via a consideration of the UV spectra of tryptophan and those of carcinogenic PHC. In similar fashion, Mason [12] could correctly classify the carcinogenic activity of many PHC by postulating a critical region 3.24 ± 0.11 eV, in which a carcinogenic hydrocarbon should possess an excited singlet state. However, it could be shown by A. and B. Pullman [13] that the correlations of the authors [9 – 12] also contain many exceptions. In the meantime, the PHC have thoroughly been studied and classified by Clar [14] and Sung [15]. Mason's observation can be ameliorated and a correlation of a very high significance was found, if two excited singlet states in the domain 3.1 eV – 3.5 eV *and* the corresponding transition moments are taken into account [15]. A classification of the cancerogenic activity of a certain class of molecules (*e. g.* the PHC) with respect to some characteristic excited states offers the possibility to consider other classes of molecules with respect to the same property, and calculations of CP and NNM can be viewed under the aspects of a correlation analysis. Other biochemical and pharmacological studies have been performed by many authors (see the review works of Pullman and Goldblum [17]). However, it should be pointed out that every theoretical or experimental contribution in the domain of biochemistry and pharmacology can only be considered as a conclusion from physical and chemical properties of a class of isolated molecules to certain biological actions. It may be very dangerous to postulate direct biological mechanisms on the basis of these correlations and conclusions even if these correlations hold with a high significance.

2. Properties of the Basic Compounds and Choice of Parameters

It was already mentioned that the treatment of CP with MO methods requires a detailed consideration of the physical and chemical properties of H_3PO_4 and NNM, as these compounds provide many useful informations, which are essential for the behaviour of the phosphate complex CP:

1. The geometrical structure of CP can be considered as a rather perturbed H_3PO_4, whereby the elucidation of the structure of NNM improves the knowledge about CP.

2. The chemical reactivity and physical properties of CP can be partially made apparent by a study of the constituents. Hereby we think preferrably of the charge densities and low excited states. In addition, the properties of the constituents may be used for testing of the parameters.

The UV spectrum of H_3PO_4 has been thoroughly investigated by Jander and Jahr [18]. They found a great dependence of the configuration on concentration, pH, and solvent:

I. In the very acid medium (*e. g.* presence of $HClO_4$) and at a very high selfconcentration of H_3PO_4 a configuration is realized, where the OH groups show only a weakly polar character and the dissociation of H^+ ions is negligible. However, under this condition we have rather $P(OH)_4^+$ than H_3PO_4. The UV absorption begins at ≈ 5.5 eV.

II. In the alkaline domain with $pH > 7$ another configuration is realized, where the OH groups show a considerably polar character, and H^+ ions can easily dissociate. The formation of phosphate complexes is derived from this structure. The UV absorption now occurs at 3 eV – 3.5 eV. An interesting special case is a solution of H_3PO_4 in a weakly acid medium with pH between 5 and 7, as now the absorption begins at 4.8 eV. This fact indicates that in this domain a double bond at the phosphoryl oxigen is much more present than in the domain with $pH < 5$. Although the structure II is realized under conditions, comparable with physiological systems, it seems to be reasonable to test MO calculations on H_3PO_4 for the structure I and the special case of structure II, respectively, and to get to the other configuration via the corresponding phosphate complexes.

The lowest excited singlet state can only be approximately described as a $\pi - \pi^*$-transition, as the π-bond of $P=O$ is formed by a 2p AO of the oxigen and a 3d AO of the phosphorus, and the overlap of the 3d AO with the AOs of the OH groups must not be neglected. So we have to be aware of contributions of n-π^*-transitions. This is in particular true for the transitions in the region 5.6 eV – 6.4 eV. We should note that the substitution of the OH groups by Cl atoms leading to $Cl_3P=O$ shifts the light absorp-tion to the region 4100 Å – 4900 Å (Naegeli [19]), and a similar behaviour was found by Buck [20] with respect to the molecule class $(RO)_2HO=O$. According to Robin [21] these molecules show also n-π^*-transitions in the region 5.6 eV – 6.4 eV. The basic compounds of NNM are ethylenimine and ethylchloride. Spectral data of interest can also be found in ref. [21], and these and some similar molecules (*e. g.* C_3H_6 and C_2H_4O) have been calculated by Clark [22] with the CNDO/2 method. However, one has to be aware of that cyclopropane and its derivatives have in the domain 60 000 cm^{-1} – 80 000 cm^{-1} only Rydberg transitions. The valence shell transitions lie beyond 80 000 cm^{-1} and thus many transitions are incorrectly assigned by ref. [22]. Ethylchloride [21] has its lowest excited singlet state at 58 000 cm^{-1}, where the C–Cl bond is preferrably involved. Calculations on phosphate complexes, performed by the authors [23 – 25], showed that one has to be very careful with respect to the treatment of the 3d phosphorus orbital, and it appears that the usual CNDO approximations, by taking account of resonance integrals $\beta_{\mu\nu}$ only between the nearest neighbours, do not work in the case of 3d orbitals. On the other hand, ab initio calculations of Perahia *et al.* [26] made clear that the neglect of the 3d orbital leads to unsatisfactory results with respect to ground state properties, and it is evident that this fact is in particular true for excited states, where the role of 3d orbitals is much more significant, because of their longer range compared with other orbitals. However, the results of the authors [23 – 26] serve as a starting-point for a more careful consideration, as the reliability of the results is an essential aspect with reference to the cited correlations, where excited states are of great relevance.

There arises the question which method of theoretical chemistry is an appropriate starting-point for the calculation of CP and its constituents with respect to some informations, desired for the already mentioned correlations between excited states of a certain class of molecules and their cancer induction. It appears that the CNDO/S method incorporates an adequate starting-point, but it is necessary to test and to prepare this method in a suitable way. As the CNDO/S method is prescribed by Del Bene and Jaffé [27], we only intend to discuss the problems of parameterizations in context of 3d orbitals. The following suppositions are made with respect to the hybridization of the atomic orbitals:

In the phosphorus center we positon besides the 3d orbital 4 sp^3 hybridized orbitals. For the phosphoryl oxigen we assume 1 sp^2 hybridized AO and 1 2p AO, whereas for the other atoms (C, Cl, O, N) we assume sp^3 hybridized AOs, which have to be weakly perturbed in order to test the reliability of the results. The parameters have to be varied within reasonable boundaries, *e. g.* ± 10%, as it is impossible to determine the best hybridization form for these atoms. If we assume for the C atom sp^3 hybridized AOs, then we would have to take for the VSIE (valence state ionization energy) value – 12.8 eV, whereas for sp^2 hybridized AOs we had to take – 13.8 eV. So the difference between the parameter for sp^3 hybridization and the corresponding one for sp^2 is small and lies within the "reasonable" boundaries. The VSIE values are listed in Table I. The Coulomb repulsion integrals $\gamma_{\mu\mu} = \langle \mu\mu \mid \mu\mu \rangle$ can be obtained by an empirical formula of Hinze and Jaffé [28], using the VSIE values and the electronegativity or by the evaluation of Slater functions with the help of appropriate table works [29]. It should be noted that the calculation of $< 3d\,3d \mid 3d\,3d >$ of the P yields 5.02 eV, whereas ref. [25] obtained 5.07 eV by empirical adaption. A thorough investigation is required for the determination of the integrals $\gamma_{\mu\nu} = < \mu\mu \mid \nu\nu >$ and the resonance integrals $\beta_{\mu\nu}$, as it is a main problem of semiempirical methods to treat systems, where more than one hybridized atomic orbitals have to be placed in the same atomic center, in a sufficient way. The integrals $\gamma_{\mu\nu}$ are usually calculated with a refined Mataga procedure (Ohno [30]) of the kind

$$\gamma_{\mu\nu} = \gamma_{\mu\nu}(\gamma_{\mu\mu}, \gamma_{\nu\nu}, R_{ab}) \tag{2.1}$$

where R_{ab} represents the distance between the atomic centers a and b. However, an empirical approximation formula of the type (2.1) for the integrals $\gamma_{\mu\nu}$ does not work without further sophistication in the following cases:

1. If several different AOs are positioned in the same atomic center ($R_{ab} = O$).
2. If $\gamma_{\mu\mu}$ and $\gamma_{\nu\nu}$ are related to AOs, formed by a different kind of hybridization.

Therefore we have taken into account the above cases by evaluation of the integrals $\gamma_{\mu\nu}$ with the help of Slater functions (ref. [29]) and, in particular, where the two center integrals $\gamma_{\mu\,3d}$ have to be determined (see Table II). By that, we have verified that there exists a discrepancy on the integrals $\gamma_{\mu\nu}$ obtained via (2.1) and computation via Slater functions. If we put

$$\gamma_{\mu\nu}^{\text{Slater}} = g_{\mu\nu} \cdot \gamma_{\mu\nu}^{\text{Mataga}}, \tag{2.2}$$

we are able to estimate rather precisely the difference between empirical adaption of $\gamma_{\mu\nu}$ and the corresponding exact computation (see Table II). Similar problems exist with the fixation of the resonance integrals $\beta_{\mu\,3d}$. The EHMO and CNDO methods have the common property that there is assumed $\beta_{\mu\nu}$ proportional to $S_{\mu\nu}$ (overlap integral). In the EHMO method (Mulliken [31], Wolfsberg and Helmholtz [32]) one uses the formula

$$\beta_{\mu\nu} = F(\text{VSIE}(\mu) + \text{VSIE}(\nu))\, S_{\mu\nu}. \tag{2.3}$$

For σ-orbitals, we have observed in agreement with Clark [22] that we have to put $F \approx 0.78$ in order to obtain the same values for $\beta_{\mu\nu}$ as by the parameterization of ref. [33]. Because of the longer range of the 3d AO of P we have calculated the coupling of the

Table I. Choice of parameters.

	VSIE/eV	γ_{aa}/eV (Hinze & Jaffé)	γ_{aa}/eV (Slater functions)
C (sp^2)	– 13.8	11.4	11.1
C (sp^3)	– 12.8	9.4	10.95
C*	– 13.0	–	11.1
N (sp^2)	– 18.0	16.2	13.95
N (sp^3)	– 16.5	13.6	13.74
N*	– 17.2	–	13.9
O (sp^3)	– 19.9	17.8	18.2 *
O (sp^2)	– 22.1	22.0	18.34 *
O (2 p)	– 16.0	9.8	15.2 *
P (sp^3)	– 12.5	11.2	9.2 *
P (3 d)	– 6.0	–	5.02 *
H (1 s)	– 13.5	13.2	12.8 *
Cl (sp^3)	– 17.2	13.6	10.76
Cl (sp^2)	– 18.3	14.8	11.23
Cl*	– 18.0	–	11.06

* Parameter used for the computational procedure.

3d electron with all other valence electrons via the mentioned tables [29] (see Table III c). It is obvious that with respect to the σ-bonds a good approximation is obtained when one only considers the resonance integrals, relating to the immediate neighbouring bonds. Arising by the use of many hybrid orbitals in one atomic center, we have also to take account of the fact that these orbitals can in general no longer be orthogonal to each other. This yields a dependence of the corresponding $\beta^{a}_{\mu\nu}$ on the hybridization according to (2.3). A neglect of this type of resonance integrals would lead to the consequence that the symmetry of the molecule (*e. g.* H_3PO_4) cannot be established in the symmetry of the corresponding MOs (see also the remarks of Pople *et a.* [33]). In the case of H_3PO_4 we have to be aware of a perturbed T_d group. In our semiempirical approach we assume the same coupling of the 3d AO with each other ligand orbital of the OH groups. By that, a C_{3V} symmetry is supposed to be an appropriate representation of the wavefunction of H_3PO_4 with reference to the symmetry adaption. In the case of CP, where the NNM is bound to the OPR, the weak coupling of the 3d AO to the AOs of the two Cl atoms is of great importance for the lowest excited states. This result was found by methods of perturbation theory and parameter variation in the domain of the calculated coupling (see Table III).

Table II. Calculation of some off-diagonalelements of the Coulomb repulsion integrals.

	$g_{\mu\nu}$	$\gamma_{\mu\nu}$/eV
	(see formula 2.2)	
P (μ, ν: sp^3 AOs)	0.47	4.32
P (μ: sp^3 AOs, ν: 3 d AO)	0.42	2.98
O(μ, ν: sp^3 AOs)	0.44	8.44
N(μ, ν: $sp^3 - sp^2$ AOs) *	0.43	5.98
C(μ, ν: $sp^3 - sp^2$ AOs) *	0.48	5.33

The table above lists those repulsion integrals where the refined Mataga procedure fails.

* Indicates that we have used for the calculation a mixture between sp^3- and sp^2-hybridized AOs.

Table III. List of the resonance integrals $\beta_{\mu\nu}$.

a. σ-bonds	$\beta_{\mu\nu}$/eV
C – C:	– 5.12
C – Cl:	– 2.9
N – H:	– 6.28
N – C:	– 5.4
C – O:	– 5.7
P – O (σ-bond of P = O):	– 7.1
P – O:	– 6.2
P – N:	– 5.8
b. π-bond	
P – O:	– 2.6
c. Coupling 3 d AO-ligands	
3 d – O:	– 1.1
3 d – N:	– 0.95
3 d – Cl:	– 0.4
d. Coupling of different AOs at the same atomic center	
C – C:	– 1.2
P – P (sp^3 AOs):	– 0.57
O – O:	– 1.1
N – N:	– 1.4

3. Results

In this section, we shall first discuss the two most important constituents of CP, H_3PO_4 and NNM. Some other calculations of essential constituents, such as chloroform, shall be presented in connection with a discussion of the metabolites of CP.

A. H_3PO_4

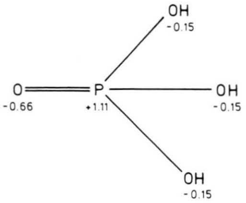
As already stated we assume the symmetry group C_{3V} for this molecule. The bond distances used in the calculations are: $R_{P=O} = 1.39$ Å and $R_{P-OH} = 1.6$ Å. With the help of some test calculations and a comparison with the results of the authors [23 – 26] it appeared that we need only 10 AOs, of which the enumeration is stated in Table IV. It can also be verified in this table that the diagonal elements of the density matrix P_{ik} and the 10 MOs satisfy the C_{3V} symmetry. One recognizes from the HOMO that it preferrably describes the π-bond of P = O and only to a little part a coupling with other ligands. When one regards the LUMO, which takes part in an essential way in the first transition, one observes that an excitation yields partially a charge transfer from the phosphoryl oxigen to the phosphorus, and a rather important contribution of the remaining AOs indicates a charge transfer to the other ligands. Therefore, the first excited singlet state (see Table V) is only in a rough approach a π*-state. According to Koopman's theorem the ionization energy is ≈ 9.2 eV; a calculation of Boyd [25] on a phosphate complex yields ≈ 8.2 eV. The classification of the excited singlet states in the domain 5.7 eV – 6.4 eV is also rather difficult, and we have to

Table IV. H_3PO_4. a. Eigenvectors of the MOs and the Orbital Energies.

	P_{sp^3}				P_{3d}	O_H	O_H	O_H	O_{sp^2}	O_π	
1.	0.00	0.00	0.00	0.66	0.02	0.00	0.00	0.00	0.74	0.01	
2.	0.37	0.37	0.37	0.00	0.12	0.44	0.44	0.44	0.00	0.02	
3.	–.46	0.46	0.00	0.00	0.00	–.54	0.54	0.00	0.00	0.00	
4.	–.27	–.27	0.54	0.00	0.00	–.31	–.31	0.62	0.00	0.00	
5.	–.06	–.06	–.06	0.02	0.45	0.01	0.01	0.01	0.08	0.88	HOMO
6.	0.33	0.33	0.33	0.06	–.57	–.24	–.24	–.24	0.05	0.39	LUMO
7.	–.53	0.54	–.01	0.00	0.00	0.46	–.47	0.01	0.00	0.00	
8.	–.31	–.31	0.62	0.00	0.00	0.27	0.27	–.54	0.00	0.00	
9.	0.28	0.28	0.28	0.01	0.67	–.29	–.29	–.29	–.03	–.26	
10.	0.00	0.00	0.00	0.74	–.02	0.00	0.00	0.00	–.66	–.01	

Orbital energies/eV
1. – 18.65; 2. – 17.16; 3. – 17.00; 4. – 17.00; *5. – 9.23 (HOMO)*;
6. – 0.92 (LUMO); 7. 0.03; 8. 0.03; 9. 1.21; 10. 1.99

b. Calculated charge densities



regard for every excited state (obtained via a mono-excitation) the corresponding MOs being involved and their dipole transition moments. The transition from an occupied MO i to a virtual MO k is denoted by | i, k⟩ and the contribution of | i,k⟩ for the configuration matrix by B_{ik}. With respect to the dipole transition moments we are here and in the other results only interested in the amount. One recognizes from Tables IV and V that the excited singlet states III and IV can be classified as *n*-π*-transitions within a good approximation. The other excited states in this domain exhibit also *n*-π*-character, but only to a less degree. We may conclude from Tables IV and V that the lack of unique classification of the transitions is a result of the long range coupling of the 3 d electron with the remaining valence elec-

Table V. Excited singlet states and the transition moments.

a. Energy of excited singlet states/eV	Contributions B_{ik} to the MO transitions | i, k⟩
1. 4.84	$B_{5,6}$: 0.82; $B_{5,9}$: 0.096; $B_{4,8}=B_{3,7}=B_{2,6}$: 0.028;
2. 5.74	$B_{5,7}$: 0.97; $B_{2,7}$: 0.03;
3. 5.74	$B_{5,8}$: 0.97; $B_{4,9}$: 0.03;
4. 5.98	$B_{5,10}$: 0.96; $B_{1,6}$: 0.04;
5. 6.35	$B_{5,9}$: 0.91; $B_{4,8}$: 0.045; $B_{3,7}$: 0.045

b. Absolute value $|X_{ik}|^2$ of the dipole transition moments X_{ik}/debye
$|X_{5,6}|^2$: 1.0; $|X_{5,7}|^2$: 0.1; $|X_{5,8}|^2$: 0.1; $|X_{5,9}|^2$: 1.8; $|X_{5,10}|^2$: 0.0;

$|X_{4,8}|^2$: 2.3; $|X_{4,9}|^2$: 4.6; $|X_{3,7}|^2$: 2.3; $|X_{2,6}|^2$: 0.2; $|X_{1,6}|^2$: 0.1

c. Modification of some resonance integrals $\beta_{\mu\nu}$
The description of $P(OH)_4^+$, classified by the symmetry group T_d, yields a change of the parameters $\beta_{\mu\nu}$, listed in Table 3 b and 3 c: $\beta_{5,10}=\beta_{5,6}=\beta_{5,7}=\beta_{5,8}=\beta_{5,9} \approx -1.4$ eV → – 1.8 eV. The lowest excited singlet state now varies from 5,4 eV to 5,7 eV. Such a model corresponds to the constitution I, found by Jander *et al.* [18].

trons. The pure $\sigma-\sigma^*$-transitions lie above 6.5 eV, and so we are not interested in a consideration of them.

With reference to $n-\pi^*$-transitions we should also mention that Bigelow *et al.* [34] have treated such transitions in organic compounds with the CNDO/S method. A realization of the T_d symmetry is obtained by adding a proton to the phosphoryl oxigen. Such a model preferrably describes the constitution I, observed by Jander *et al.* [18] with spectroscopic means. By that, we have to take account of a stronger coupling of the 3d electron with all other valence electrons of the OH groups; the coupling with the phosphoryl oxygen is lowered in an appropriate way. The lowest excited singlet state now lies at 5.5 eV, but the subsequent excited states in the domain 5.7 eV – 6.4 eV have not changed essentially. Therefore we may assume with some reason that we have obtained an acceptable starting-point for a calculation of CP (see Table V), as the approach presented here remains independent on parameter variation within "reasonable" boundaries.

B. Nor-N-Mustard (NNM)

The enumeration of the AOs is given in Fig. 2, where we may verify that the molecule exhibits C_{2V} symmetry. The elucidation of the geometrical structure is connected with some effort, as there are only σ-bonds. On the other hand, NNM is an essential compound of CP, and therefore a rather good knowledge on NNM facilitates the structure determination of CP. One may also regard Table VII a and Fig. 2, and ref. [35], respectively. Because of the C_{2V} symmetry of the molecule we have to analyze two HOMOs and LUMOs (see Table VI). These specific MOs indicate that the physical and chemical behaviour of NNM is preferrably determined by the C – Cl bonds. In the ground state the electric charge, induced by the valence electrons C 2 and C 4, is partially transferred to the Cl atoms (see also the diagonal elements of the density matrix P_{ik} in Fig. 2). These C – Cl bonds are responsible for the polar character of the molecule. The charge distribution at the remaining atoms yields a vanishing contribution for the static dipole moment. Thus the total molecule exhibits an ambiguous property: NNM is solvable in H_2O, and, on the other side, a lipophile character is also to verify. Therefore the molecule exhibits the ability for passive permeation through the cell membranes. The LUMOs indicate that the electric charge is transferred in first order from the Cl atoms to the neighbouring C2 and C4, and only in second order to the remaining atoms. Thus the

Table VI. Eigenvectors of the MOs of NNM.

	N 1	N 2	N 3	C 1		C 2		C 3		C 4		Cl 1	Cl2	H	
1.	0.44	0.43	0.43	0.34	0.17	0.13	0.06	0.34	0.17	0.13	0.06	0.03	0.03	0.33	
2.	–.54	–.32	0.32	–.36	–.33	–.34	–.16	0.36	0.33	0.34	0.16	–.14	0.14	–.47	
3.	0.30	–.31	–.31	–.25	0.25	0.32	0.20	–.25	0.25	0.32	0.20	0.22	0.22	0.29	
4.	0.0	–.38	0.38	–.34	0.22	0.30	0.21	0.34	–.22	–.30	–.21	0.24	–.24	0.00	
5.	–.01	0.08	0.08	0.04	–.26	–.23	0.28	0.04	–.26	–.23	0.28	0.54	0.54	–.02	
6.	0.00	–.09	0.09	–.06	0.26	0.24	–.27	0.06	–.26	–.24	0.27	–.53	0.53	0.00	
7.	0.00	–.09	0.09	–.06	0.26	0.24	–.27	0.06	–.26	–.24	0.27	–.53	0.53	0.00	HOMOs
8.	0.00	0.02	0.02	–.01	–.19	0.06	0.58	–.01	–.19	0.05	0.58	–.37	–.37	0.00	
9.	0.00	–.02	0.02	0.01	0.19	–.05	–.57	–.01	–.19	0.05	0.57	0.37	–.37	0.00	LUMOs
10.	–.08	–.29	–.29	0.30	0.36	–.40	0.14	0.30	0.36	–.40	0.16	0.06	–.06	0.14	
11.	–.01	0.21	–.21	–.16	–.44	0.46	–.16	0.16	0.44	–.46	0.16	0.06	–.06	–.01	
12.	–.20	–.29	–.29	0.40	–.32	0.27	–.09	0.40	–.32	0.27	–.09	0.03	0.03	0.31	
13.	0.00	–.45	0.45	0.47	–.21	0.15	–.05	–.47	0.21	–.15	0.05	0.01	–.01	0.00	
14.	–.62	0.19	0.19	–.19	0.07	–.05	0.01	–.19	0.07	–.05	0.01	–.01	–.01	0.68	

Orbital energies/eV

1. –21.93; 2. – 18.00; 3. – 17.49; 4. – 16.62; 5. – 16.38; *6. – 14.74; 7. – 14.71;*
8. – 4.57; 9. – 4.60; 10. – 1.49; 11. – 1.18; 12. –.29; 13. 0.70; 14. 1.00

The underlined energy values belong to the HOMOs and LUMOs.

Table VII. Excited singlet and triplet states of NNM and the transition moments.

	$B_{7,8}$	$B_{6,8}$	$B_{7,9}$	$B_{5,8}$	$B_{6,9}$	$B_{7,10}$	$B_{4,8}$	$B_{5,9}$	Excitation energy/eV	
$\mid X_{ik} \mid^2$:	0.0	0.0	0.0	20.25	226.0	0.0	170.0	2.3 *		
B_{ik}:	0.45	0.0	0.0	0.09	0.37	0.0	0.07	0.02	6.95	Singlet states
B_{ik}:	0.0	0.36	0.40	0.0	0.0	0.0	0.09	0.15	7.04	
B_{ik}:	0.01	0.52	0.28	0.0	0.0	0.0	0.19	0.0	8.67	
B_{ik}:	0.17	0.0	0.0	0.36	0.1	0.25	0.0	0.12	8.68	
B_{ik}:	0.46	0.0	0.0	0.03	0.46	0.05	0.0	0.0	3.40	Triplet states
B_{ik}:	0.0	0.42	0.42	0.01	0.0	0.0	0.05	0.1	3.37	
B_{ik}:	0.0	0.49	0.48	0.0	0.0	0.0	0.03	0.0	7.27	
B_{ik}:	0.05	0.0	0.0	0.48	0.01	0.04	0.0	0.42	7.50	

* Square of the dipole transition moments/debye ^{2}.

Table VII a.

Geometry of the NNM [Å]			
N:	0.	0.	0.
H:	0.95	–.25	0.
C 1:	0.	0.95	1.03
C2:	1.31	1.73	0.98
C3:	0.	0.95	– 1.03
C4:	1.31	1.73	– 0.98
Cl 1:	1.22	1.91	2.57
Cl2:	1.22	1.91	– 2.57

chemical reactivity of NNM is preferrably determined by the C – Cl bonds, and it can be measured by the ability to split off Cl^- ions in aqueous solutions. The C_{2V} symmetry of NNM can also be seen from the eigenvectors of the MOs states Table VI. Some interesting features can be found by considering the lowest excited states, calculated by taking into account monoexcitations, and the corresponding dipole transition moments. The lowest excited singlet states do not provide any new information (Table VII). Their energy levels are rather similar to those of ethylchloride [21], and the transitions are allowed within the frame of the usual dipole approximation. More attribution deserve the lowest triplet states: By the means of parameter variation within sufficient boundaries and methods of perturbation theory we found a rather considerable singlet-triplet-splitting. The two lowest triplet states lie in the domain 3.1 eV – 3.3 eV. One may easily conclude from Table VII that the transition from the singlet ground state to the lowest triplet states would be allowed, if they were also singlet states. However, such transitions are only possible by taking account of the spin-orbit coupling, which shall be discussed together with the metabolites of CP. We should point out that the lowest excited states (singlet and triplet) yield a charge transfer from the Cl atoms to the neighbouring C2 and C4, and the chemical reactivity of NNM, measured by splitting off the Cl^- ions in H_2O, is related to triplet excitations in the domain of 3.2 eV. This fact seems to be interesting from the aspect of the already mentioned correlation between the lowest excited states of a class of molecules and their carcinogenic activity, as it is well-known that NNM as well as the usual mustard (Fig. 3) exhibit a carcinogenic activity. From the aspect of chemical reactivity we may conclude that these two triplet states stand in a close relationship to a characteristic chemical reaction, known as alkylation of certain biomolecules. We should further note that a similar calculation of the mustard (Fig. 3) leads to the same results. The lowest excited triplets play also an important role in the physical and chemical behaviour of CP. However, we shall see that their excitability will be changed and, in addition, depends on the metabolism.

C. Cyclophosphamide (CP)

With the help of the preceding considerations we are able to discuss the chemical and spectroscopical properties of CP. There are two main questions of interest: 1. The geometrical structure and conformation of CP. 2. The chemical reactivity (characterized by the charge density and free valence) and the role of the low excited (singlet and triplet) states.

It was already pointed out that the treatment of H_3PO_4 and NNM provides a rather rough knowledge of the conformation of CP. The nitrogen N 8 of the mustard and the cyclic N 3 of the OPR (Fig. 1) may be considered as modified ligands of H_3PO_4. So the additional questions arise in which way the two chloroethyl groups are oriented with respect to the P=O bond and which conformation of the OPR is energetically favoured. The lowest total electronic energy is obtained, if the geometrical structure of CP is in rather good agreement with the structure, represented in Fig. 1. The NNM is orientated such that the distance of the Cl 13 to the phosphorus becomes small (3.3 Å), whereas the other Cl atom (Cl 14) has a distance of 6 Å to the phosphorus. It should also be noted that the Cl 13 exhibits a smaller distance to the cyclic nitrogen N 3; the Cl 14 is "much more neighbouring" to the cyclic oxigen O 1. The OPR is energetically favoured in the chair-form conformation. These results are in accordance with X-ray studies of CP and its derivatives in cristal form [35 – 39]. The calculated energy differences between the energetically favoured and other conformations are of the order 0.2 eV – 0.3 eV (see Fig. 1 a).

With respect to the chemical behaviour of CP there are two questions of interest, which should be answered by MO calculations. We have found that the NNM is a very reactive compound, and its chemical reactivity is preferrably determined by the CH_2Cl bonds. It turned out to be too toxic for the use as anti-tumor drug. In which way does the binding of NNM to the OPR modify the chemical reactivity of NNM? A further point to be discussed is the chemical behaviour of the OPR. We have to look at the reactivity of the corresponding atomic centers of OPR. In Fig. 1 the charge densities of the ground state can be verified. A comparison of the mustard group of CP with NNM shows that we have obtained in the first case analogous results. The two Cl atoms carry in both cases the same negative electric charge. A further interesting information is provided by the 3 cyclic carbons of the OPR. The C 4 carbon, neighbouring to the cyclic N 3, carries more electric charge

Table VIII. Eigenvectors of the relevant MOs of cyclophosphamide.

	P_{sp^3}				P_{3d}	O_7		O_1		C_6		C_5		C_4		
11.	–.02	–.01	0.39	–.25	–.02	–.03	–.01	–.04	–.11	–.06	0.24	0.26	0.12	0.07	–.32	
	N_3		N_8			C_9		C_{10}		C_{11}		C_{12}		Cl_{14}	Cl_{13}	
	0.43	–.54	–.34	–.31	0.06	0.04	0.08	0.09	0.08	–.17	0.07	0.09	–.09	–.09	–.19	
12.	0.00	0.02	0.09	–.18	0.05	0.01	0.02	0.03	–.03	–.02	–.01	–.07	0.12	–.07	–.19	
	0.14	0.18	0.10	–.28	–.28	0.16	0.35	0.14	0.03	–.01	–.35	–.35	0.20	0.03	0.45	
13.	0.00	0.01	0.00	–.02	0.01	0.00	0.00	0.01	–.01	–.01	0.00	0.00	–.01	–.01	–.01	
	0.00	0.00	–.02	0.20	–.17	0.16	–.36	–.36	0.22	–.14	0.29	0.29	–.18	0.48	–.38	
14.	0.00	0.03	–.06	0.01	0.00	0.00	0.00	0.03	–.13	–.06	0.48	0.34	–.44	–.48	0.36	
	–.05	0.20	0.01	–.01	–.01	–.01	0.00	0.00	–.01	–.01	–.01	–.01	0.00	–.01	0.00	
15.	–.03	–.04	–.04	–.03	0.48	–.01	0.86	–.02	–.03	–.03	–.03	–.05	–.03	–.03	–.04	
	–.02	–.03	–.02	–.03	–.03	–.03	–.01	–.02	–.06	–.03	–.01	–.02	–.06	–.03	–.03	HOMO
16.	0.00	0.00	0.00	0.00	–.13	–.01	0.16	–.01	0.00	0.00	0.00	0.00	0.02	0.07	–.03	
	–.01	–.01	–.01	–.01	–.01	–.01	–.03	0.01	0.51	–.01	–.07	0.02	0.65	–.31	–.41	LUMO
17.	0.00	0.00	0.00	0.00	–.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	0.00	0.00	0.00	0.01	–.01	–.01	–.16	0.02	0.65	0.00	0.12	–.02	–.52	–.41	0.32	LUMO
18.	0.13	0.29	0.32	0.41	–.21	–.20	0.15	–.33	–.02	0.07	0.01	–.01	–.01	0.01	0.19	
	–.36	–.04	–.40	–.11	–.12	0.16	0.04	–.05	0.01	0.16	0.03	–.05	0.01	0.01	0.01	
19.	–.05	–.18	–.22	0.12	0.10	0.07	–.07	0.16	0.03	–.06	–.02	0.01	0.03	–.03	–.11	
	0.20	0.07	–.01	–.32	–.34	0.35	0.24	–.28	0.06	0.37	0.26	–.31	0.07	–.03	–.03	
20.	0.00	0.00	–.01	–.01	–.01	0.00	0.03	–.02	0.03	0.00	0.00	–.01	0.01	–.03	–.08	
	0.03	0.03	–.01	0.28	–.25	–.24	0.42	0.45	–.10	0.21	0.40	–.43	0.10	0.04	–.03	

Orbitalenergies/eV
11. – 14.80; 12. – 14.55; 13. – 14.46; 14. – 13.60; 15. – 9.67 (HOMO); 16. – 4.65 (LUMO);
17. – 4.44 (LUMO); 18. – 1.70; 19. – 0.92; 20. – 0.55

Table IX. Cyclophosphamide:
The MO transitions of the excited singlet states $B_{ik} \cdot \mid i \rightarrow k>$

	Excitation energy/eV
$0.99 \mid 15 \rightarrow 16 > +0.01 \mid 15 \rightarrow 20 >$	3.22
$0.97 \mid 14 \rightarrow 16 > +0.03 \mid 14 \rightarrow 19 >$	7.12
$0.99 \mid 15 \rightarrow 17 > +0.01 \mid 15 \rightarrow 20 >$	3.28
$0.4 \mid 13 \rightarrow 16 > +0.11 \mid 14 \rightarrow 19 > +0.4 \mid 12 \rightarrow 17 > +0.03 \mid 15 \rightarrow 20 >$	6.93
$0.98 \mid 14 \rightarrow 20 > +0.02 \mid 15 \rightarrow 20 >$	7.25
$0.96 \mid 15 \rightarrow 18 > +0.03 \mid 12 \rightarrow 20 >$	4.42
The MO transitions of the excited triplet states $B_{ik} \cdot \mid i \rightarrow k >$	
$0.97 \mid 15 \rightarrow 16 > +0.01 \mid 12 \rightarrow 16 > +0.01 \mid 15 \rightarrow 20 >$	3.09
$0.97 \mid 14 \rightarrow 16 > +0.01 \mid 14 \rightarrow 17 > +0.02 \mid 12 \rightarrow 16 >$	7.12
$0.01 \mid 15 \rightarrow 16 > +0.97 \mid 15 \rightarrow 17 > +0.02 \mid 12 \rightarrow 17 >$	3.24
$0.02 \mid 15 \rightarrow 16 > +0.4 \mid 12 \rightarrow 16 > +0.4 \mid 13 \rightarrow 17 > +0.1 \mid 12 \rightarrow 18 >$	4.73
$0.06 \mid 14 \rightarrow 16 > +0.94 \mid 14 \rightarrow 18 >$	7.26
$0.9 \mid 15 \rightarrow 18 > +0.1 \mid 14 \rightarrow 20 >$	4.04

Cyclophosphamide:
Square of the dipole transition moments X^2_{ik} of the MO transitions $\mid i \rightarrow k > \mid$ debye²

$X^2_{15,20}$	$X^2_{14,19}$	$X^2_{14,20}$	$X^2_{13,18}$	$X^2_{13,19}$	$X^2_{12,17}$	$X^2_{12,18}$	$X^2_{12,19}$	$X^2_{12,20}$
0.07	0.03	12.85	26.75	12.84	13.75	0.09	0.02	0.04

The dipole transition moments $X_{ik}{}^2$, which have not been listed above, are 0 (*e. g.* $X^2_{15,16}$, $X^2_{15,17}$, $X^2_{15,19}$ etc.).

than the other cyclic carbons. A consideration of the free valence

$$F_i = \text{const.} - \sum_k P_{ik} \qquad (3.1)$$

(P_{ik} is the density matrix) leads also to the result that the C4 carbon plays an essential role in the OPR, whereas the reactive centers of the mustard group are the two C – Cl bonds. It should be noted that the Pullmans [6, 13] also used the free valence to obtain the reactive centers of some PHC. However, in the case of CP there arises the question, whether these informations are sufficient to characterize the chemical behaviour of CP. The usual NNM possesses the ability to split off Cl^- ions in aqueous solutions, and it may appear that CP has also this ability. One the other side, the C4 carbon of the OPR has become of interest as a reactive position. Thus the above informations do not permit to answer this question in a decisive way, and we have to consider the excited states and the transition moments, and the MOs involved, respectively. There is no essential distinction between the HOMO of H_3PO_4 (Table IV) and the HOMO of CP (Table VIII). The two π-electrons of the P=O bond are preferrably localized at the phosphoryl oxygen (total engative charge of O7: –0.64) and in second order at the P and some ligands, respectively. However, the LUMO of H_3PO_4 and the LUMOs of CP (MO 16 and 17) differ in a decisive way: In the case of H_3PO_4 the electric charge wanders in first order from O to the central atom P and only in a second order to the remaining ligands. In the latter case (CP) this fact has changed. As a consequence of the weak coupling of the 3d electron with the valence shell electrons of the Cl atoms we have obtained two LUMOs, and the excitation of the P=O bond yields a charge transfer to the C – Cl bonds. If we consider the lowest excited states (Table IX) we can verify that the MO transitions $\mid 15 \rightarrow 16\rangle$ and $\mid 15 \rightarrow 17\rangle$ play the main part. The contribution of $\mid 15 \rightarrow 20\rangle$ is negligible. However, only the weak contributions $\mid 15 \rightarrow 20\rangle$ and $\mid 12 \rightarrow 20\rangle$ exhibit a small dipole transition moment (Table IX a). So we can classify the lowest two excited singlet states (and triplets, if we treat them as singlets) as excited states with so weak transition probabilities that the two C–Cl bonds cannot be excited via P=O bond. Therefore we consider the higher excited states, which are correlated to the chemical behaviour of the OPR (MO transitions $\mid 14 \rightarrow 19\rangle$, $\mid 14 \rightarrow 20\rangle$). The excited states in the domain 7.25 eV and 6.93 eV are connected with allowed dipole transitions, and the MO transitions $\mid 14 \rightarrow 19\rangle$ and $\mid 14 \rightarrow 20\rangle$ are preferrably related to a change of the charge density at the C4 carbon. However, it should be pointed out that the excitability of the lowest singlet states (3.22 eV and 3.28 eV) may depend on additional influences, *e. g.*

pH, solvent etc. Such influences may be taken into account to a certain degree by external electric fields with the strength ≈ 0.1 V/Å. If we consider a homogeneous electric field with this strength, we do not obtain any change of the transition moments, and only some energy levels will be lowered (about 0.1 eV – 0.2 eV). However, an inhomogeneous field of the same strength at the phosphoryl oxigen and at the Cl atoms yields an excited state in the domain 3 eV, which can be classified as weakly allowed. Such an excited singlet state has also been found experimentally [40].

4. Conclusions

The consideration of the ground state charge densities, free valence, excited states and transition probabilities shows that CP and its chemical behaviour can be characterized by the following statements:

1. CP must be metabolized in a suitable way to be able to act as an alkylating agent (NNM behaves conversely). Therefore, CP has only a potential alkylating ability, as the excitability of the C – Cl bonds via the π-electrons of the P = O bond can be neglected. The only reactive center to be considered is the C 4 carbon of the OPR. However, we are only able to make *plausible* that metabolism occurs at this atom, but we *cannot* explain via MO calculations that the first metabolite of CP is 4-hydroxy-CP (see ref. [41]).

2. The main difference between CP and NNM is the fact that we have in the case of CP two triplet states being quasi-degenerate with two nonexcitable singlet states, whereas NNM has only excited singlet states in the domain 7 eV. Therefore a suitable metabolization of CP may lead to a chemical behaviour, where the excited states in the domain 3.2 eV – 3.4 eV, related to an excitation of the C – Cl bonds, may play a much more specific role than in the case of NNM. The triplet states of CP could be occupied via transitions from excited singlet states (radiationless transitions), if the lowest excited singlet states at 3.3 eV would possess allowed dipole transitions. These questions can only be answered in a sufficient way by a juxtaposition of the metabolites of CP, which we shall present in a forthcoming consideration [42]. In this connection we shall also discuss the spin-orbit coupling.

3. With respect to the already mentioned fact that CP, NNM and the usual mustard (Fig. 3) show a carcinogenic activity we should note that the results of our MO calculations can also be considered in relation to the investigations of Clar, Mason, Birks, and Sung with reference to the carcinogenic activity of some PHC. The cancerogenic PHC exhibit a similar term scheme, which, in particular, Clar [14] has studied and classified. In the case of CP and NNM the excited states in the domain of 3.3 eV stand in a close relationship with a chemical reaction, known as alkylation. This fact seems also to be supported by a consideration of the metabolites of CP [42].

Acknowledgements

The author is indebted for many valuable discussions to Dr. Peter, Prof. Hartmann, Prof. Hohorst, Universität Frankfurt, and Prof. Petzold, Universität Marburg.

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