Theoret. chim. Acta (Berl.) 14, 402—410 (1969)

Molecular Orbitals for Components of Adenosine Triphosphate

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Received March 5, 1969

Energies and charge distribution properties are obtained for ortho-, pyro-, and tri-phosphoric acids, and for adenine, *D*-ribose, adenosine, and adenosine-5′-monophosphate (AMP). Results are based upon a molecular orbital method in which all electrons are included and in which the Hamiltonian matrix elements are approximated by reference to exact SCF matrix elements for the model compounds, PO, HCOOH, C_2H_4, C_2H_6, and hypothetical H_2CNH. Conclusions are in agreement with extended Hückel results on the charge distribution in the pyrophosphate bonds and on the participation of P 3*d* orbitals in the hybridization at P.

Die Energien und Ladungsverteilungen für Ortho-, Pyro- und Tri-Phosphorsäure sowie für Adenin, *D*-Ribose, Adenosin und Adenosin-5′-Monophosphat (AMP) werden angegeben. Den Rechnungen liegt eine MO-Methode zugrunde, die alle Elektronen berücksichtigt und in der die Elemente der exakten SCF-Matrix für die Modellverbindungen PO, HCOOH, C_2H_4, C_2H_6 und das hypothetische H_2CNH. Man erhält eine Übereinstimmung mit den Ergebnissen einer erweiterten Hückel-Rechnung bezüglich der Ladungsverteilung in den Pyrophosphatbindungen und der Beteiligung von 3*d*-Funktionen des Phosphors an der Hybridisierung.

Obtention des énergies et des distributions de charge pour les acides orthopyro- et triphosphoriques ainsi que pour l'adénine, le *D* ribose, l'adénosine et l'adénosine-5′-monophosphate (AMP). Les résultats sont obtenus par une méthode d'orbitales moléculaires pour tous les électrons, où les élements de la matrice hamiltonienne sont évalués d'une manière approchée par référence aux élements de la matrice SCF exacte pour les composés modèles PO, HCOOH, C_2H_4, C_2H_6 et l'hypothétique H_2CNH. Les conclusions sont en accord avec les calculs en méthode de Hückel étendue en ce qui concerne la distribution de charge dans les liaisons pyrophosphates et la participation des orbitales 3*d* dans l'hybridation du phosphore.

Introduction

Adenosine triphosphate (ATP) is of unique importance [1] for the transfer of free energy in oxidative reactions in plant and animal cells. Hydrolysis of ATP^{4-} to inorganic orthophosphate and ADP^{3-} in aqueous media near *pH* 7 and 25° C yields a free energy change ($-\Delta G^{0\prime} = 7$–8 kcal/mole when all substances, except H^+, are in their standard states) some 5 kcal/mole higher than for the hydrolysis of other phosphate esters such as AMP^{2-}. This has led to the terminology of "high-energy" and "energy-rich" compounds and bonds, although the reference is entirely to the free energy of hydrolysis near physiological *pH* and not to some localized property of a bond. Even though part of the free energy change may arise from the system of the solvated ions before and after reaction [2], the question remains whether some unique valence property exists in these molecules.

Earlier theoretical studies of ATP were limited to applying the simple Hückel molecular orbital methods to the π-electrons of hypothetical planar phosphate

chains [3, 4]. It appears from these studies that the differences in the "high-" and "low-energy" phosphates cannot be ascribed to π-electronic delocalization energies (opposing resonance) alone, and that electrostatic repulsions between the atoms of the phosphate chains must be added as an additional energy term. More recently, the extended Hückel (EH) method [5], including all valence electrons and using molecular geometries from X-ray diffraction studies, has been applied to ATP and related molecules [6]. The calculated electronic structures provided qualitative support for the roles of opposing resonance and electrostatic repulsions in determining the instability of the "high-energy" compounds relative to the products of hydrolysis.

The valence problem is investigated here in molecules less complex than ADP and ATP, but by a method [7] (NEMO II) which is free of the empirical parameters of the Hückel and EH methods and which includes all electrons. The molecules treated include AMP, H_3PO_4, $H_4P_2O_7$, $H_5P_3O_{10}$, adenosine, adenine, and *D*-ribose. The NEMO II method is based upon the use of self-consistent-field (SCF) results on small molecules to simulate the SCF Hamiltonian matrices, and hence eigenvalues, rough total energies, and charge distributions, in chemically related large molecules. The new results, as far as they can be carried, support the EH results [6] for these non-solvated, unionized molecules. Ions, although more closely related to the physiological situation, are not treated here because the number of ionic compounds on which SCF calculations have been done is so small that the dependence of the theoretical parameters (orbital exponents and Hamiltonian matrix elements) on ionic charge has not been clearly established [8].

Method of Calculation

The theory described in detail elsewhere [7] requires all overlap (S_{pq}) and kinetic energy (T_{pq}) integrals for the large molecule. These are combined with the potential energy elements ($\alpha_r - T_{rr}$) in a summation over basis functions, $U_{pq} = C_{pq} \sum_r S_{pr} \times (\alpha_r - T_{rr}) S_{rq}$, to form the approximate, large-molecule Hamiltonian matrix from which the LCAO-MO wave function and eigenvalues, ε_i, are obtained. The parameters, C_{pq} and α_r, are theoretically derived: the C_{pq} being obtained by substituting small molecule SCF matrix elements into the equation for U_{pq}, and the α_r being the diagonal Hamiltonian matrix elements of the small model compounds.

A minimum basis set of Slater-type orbitals, governed by the choice of model compounds, is employed for each molecule, except for those with P where the basis sets are augmented with the P 3*d* atomic orbitals. The orbital exponents are obtained by the usual Slater rules, expect for the preferred values of 1.2 for H 1*s* and 1.4 for P 3*d* [8, 9].

Atomic Cartesian coordinates, tabulated elsewhere [10], have been taken from X-ray crystallographic studies for H_3PO_4, [11], $H_4P_2O_7$ [12], $H_5P_3O_{10}$ [13], and AMP [14]. Coordinates for adenine, D-ribose, and adenosine are taken from the appropriate parts of AMP with atoms added at reasonable distances to complete these molecules (Figs. 1 and 2).

Rigorous SCF calculations on PO, HCOOH, C_2H_4, C_2H_6, and H_2CNH are the source of α's and C_{pq}'s which are used to compute the potential energy matrix

elements of the larger molecules. Appropriate parts of the model compounds for the α's and one- and two-center C_{pq}'s are indicated in Table 1, and the numerical values have been tabulated elsewhere [7]. In preliminary calculations on H_3PO_4, values of C_{pq} for P–O bonds were taken directly from diatomic PO, but a very large polarization $P^{\delta+}O^{\delta-}$ occurred, particularly for the double bond P=O. Although this difficulty could have been corrected by an ω-technique [15] there is as yet no satisfactory calibration of this technique with SCF calculations [8, 16]. A comparison of C_{pq} for HCOOH from SCF results indicated that the C_{pq}^{σ} and C_{pq}^{π} for the C=O bond are, respectively, 0.03 and 0.07 greater than for the C–O bond. Lacking more appropriate SCF results, we increased C_{pq}^{σ} and C_{pq}^{π} above the PO values

Table 1. *Source of parameters*

Part of large molecule	Part of model compound
P, P–O	PO
O, H, O–H, O of P–O–P	OH of HCOOH
O of P=O	=O of HCOOH
P=O (2-center C_{pq})	PO with $C_{pq}^{\sigma}+0.03$, $C_{pq}^{\pi}+0.07$
N, C, C⋯N, C–H, N–H	H_2CNH[a]
C⋯C of adenine[b]	C_2H_4
Non-bonded transannular C_{pq}	Average non-bonded C_{pq} in $HCCCH_3$, HCOOH, and HCOF[c]
C, O, H, C–O, O–H of *D*-ribose	C–O–H of HCOOH
C–C of D-ribose (2-center C_{pq})	C_2H_6
C–N of glycosidic bond	C=N of H_2CNH
C–O of C–O–P bond	C–O of HCOOH

[a] This hypothetical molecule is like ethylene, with N in place of one CH.

[b] Planarity is assumed for differentiating in-plane and out-of-plane C_{pq}^{π}.

[c] These averaged C_{pq}, 0.44 for $1s-1s$, 0.27 for $1s-2s$, 0.22 for $2s-2s$, 0.24 for $1s-2p$, 0.23 for $2s-2p$, 0.23 for $2p_{\sigma}-2p_{\sigma}$, 0.46 for $2p_{\pi}-2p_{\pi}$ in-plane, and -0.15 for $2p_{\pi}-2p_{\pi}$ out-of-plane, were used for all interactions between non-nearest neighbor atoms of the aromatic system within 3 Å of each other; for other non-nearest-neighbor interactions the approximation of non-bonded component C_{pq}'s was used.

by these amounts to use for the P=O bond in the phosphates, thus increasing the off-diagonal matrix elements and reducing the charge separation somewhat. Initial use of the non-bonded component C_{pq}'s [7] for all non-nearest neighbor interactions led to a slightly positive eigenvalue for the highest occupied molecular orbital (HOMO) of adenine because of poor approximation of the transannular matrix elements. Again lacking more appropiate SCF results, the C_{pq}'s for the matrix elements between non-bonded atoms of $HCCCH_3$, HCOOH, and HCOF were averaged and employed for adenine, adenosine, and AMP. Subsequently, the eigenvalue of the HOMO in adenine became negative, and other aspects of the wave function also seemed to improve. Whereas this investigation indicates that more appropriate model compounds are desirable and that previously suggested [7] improvements in the theory could advantageously be made, we present the following results as a test of the method in its present form and as a check of the charge distributions from the EH calculations.

Results and Discussion

Eigenvalues and Energies

The negative of the eigenvalue of the HOMO in Hartree-Fock theory is usually used to approximate the vertical (fixed nuclei) ionization potential (*IP*) [17]. For instance, in the case of P_2, the value (10.7 eV) calculated [8] from SCF theory is very close to the experimental [18] value (11.1 eV). The calculated values (Table 2) for the phosphates are reasonable compared to the value for PO obtained from mass spectral data [19] (8.0 ± 0.5 eV) and from SCF theory [8] (8.5 eV), but are several eV smaller than *IP*'s for alkyl phosphate esters [20] and phosphorous acid [18]. The HOMO of H_3PO_4 is found to have entirely $2p$ character on the three hydroxyl oxygens.

The unreasonably small *IP*'s for the organic molecules (Table 2) suggest that the model compounds, especially for the adenine moiety, are not entirely adequate.

Table 2. *Calculated energies*[a]

	H_3PO_4[b]	$H_4P_2O_7$	$H_5P_3O_{10}$	Adenine	D-Ribose	Adenosine	AMP
$1/2\ \Sigma N(i)\varepsilon_i$	−197.342	−371.109	−544.958	−151.049	−182.627	−310.107	−483.840
E(total)	−641.63	−1207.18	−1772.81	−464.85	−571.90	−960.68	−1526.20
KE	652.08	1227.40	1802.42	463.82	573.30	959.92	1534.75
A	−2.47	−4.40	−6.40	−2.93	−6.14	−8.54	−10.43
IP (eV)	8.63	8.73	8.63	2.69	5.82	2.58	2.58

[a] All quantities are in atomic units (1 u. a. = 27.2093 eV) unless otherwise noted. E(total) and A are computed using a hydrogenic eigenvalue and core integral corresponding to an exponent of 1.0 for H1*s*.

[b] A complete list of eigenvalues for a few of the low lying empty MO's and all the occupied MO's for this molecule of C_{3v} symmetry is: $12a_1$ (+1.263), $9e$ (+1.065), $8e$ (+0.420), $11a_1$ (+0.299, LEMO), $1a_2$ (−0.317, HOMO), $7e$ (−0.367), $10a_1$ (−0.435), $6e$ (−0.437), $9a_1$ (−0.580), $5e$ (−0.602), $4e$ (−0.796), $8a_1$ (−0.916), $7a_1$ (−1.239), $3e$ (−1.357), $6a_1$ (−1.433), $2e$ (−5.236), $5a_1$ (−5.237), $4a_1$ (−7.362), $3a_1$ (−20.556), $2a_1$ (−20.625), $1e$ (−20.626), $1a_1$ (−79.799).

The desirability of suitable SCF calculations on simple heterocyclic models is obvious. Experimental values [18, 21] for some nitrogen heterocycles are in the range 8–10 eV, and the value for glucose is 8.8 eV. Using the related NEMO I theory [16], we obtain 5.05 eV for adenine and 9.03 eV for D-ribose. Other MO methods [6, 22] give 8–12 eV for the *IP* of adenine. As suggested by the *IP*'s in Table 2 and in agreement with results [6] from EH theory, the HOMO's of adenosine and AMP are essentially localized on the aromatic base, but are fairly well delocalized over both the six- and five-membered rings.

The eigenvalues of the lowest empty MO's are computed to be positive as is common [8, 9] in SCF calculations on closed-shell, neutral molecules. Excitation energies, not discussed here, require two-electron Coulomb and exchange integrals.

Total electronic energies can be approximated from a sum of molecular eigenvalues and atomic core integrals [7], whereas binding or atomization energies are given by $A = 1/2 \sum_i N(i)\varepsilon_i - 1/2 \sum_i N(i)\varepsilon_i^a$, where the sums are over orbitals of

occupation number $N(i)$ and ε_i^a are eigenvalues for SCF atomic orbitals [23] using the same basis functions for the isolated atoms as for the atoms in the molecule. Total energies and electronic kinetic energies in Table 2 indicate that the virial theorem in the form $KE = -E$ (total) appears to be well satisfied, except in those compounds with P, where the satisfaction is typical of rigorous, but incomplete basis set calculations [8, 24]. Binding energies, clearly less reliable than those from rigorous SCF calculations, show internal consistencies, increasing with molecular size and having magnitudes near those found by summing bond energies [25].

Intramolecular Charge Transfer

Overlap populations, orbital occupancies, and net atomic charges are computed from a Mulliken population analysis [26, 10]. Overlap populations and net atomic charges for the phosphates are shown in Fig. 1, and the charges in the organic molecules are summarized in Fig. 2. Although the charges are probably somewhat exaggerated by lack of self-consistency conditions, their magnitudes are similar

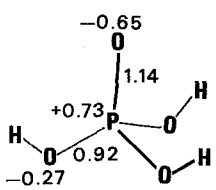

Fig.1a

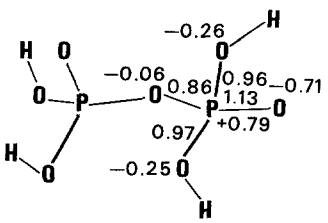

Fig. 1b

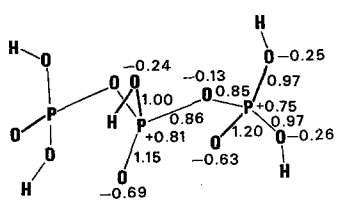

Fig. 1c

Fig. 1. Projections of the three-dimensional structures used for $H_3PO_4(C_{3v})$, $H_4P_2O_7(C_2)$, and $H_5P_3O_{10}$ (C_2 except for the H on the bridge phosphate group). Net atomic charges are shown as signed numbers and overlap populations as unsigned numbers

to those we obtain from EH theory [6], and are of even smaller magnitude than some published figures for adenine and adenosine [27].

The early π-electron Hückel calculations led to the generalization [3] that "high-energy" phosphates are characterized by a chain of atoms, each having a net positive charge. However, in the present method which requires no arbitrary separation of σ and π electrons, the charge on the bridge oxygen is -0.06 in $H_4P_2O_7$ and -0.13 in $H_5P_3O_{10}$, and both of these charges are very close to

those obtained from EH theory [6]. Quite probably the bridge oxygens are slightly more negative in the ions (e. g., $HP_2O_7^{3-}$) at *pH* 7 than in these neutral molecules. Judging from the EH results, esterification of the polyphosphate chains (i. e., in ADP and ATP) has only a small electron withdrawing effect on the backbone atoms. In any case, the bridge oxygens are not as negative as the terminal oxygens in the phosphate chains, and electron density maps currently being calculated may reveal significant aspects about the three-dimensional charge distribution at the bridge oxygens.

Description of σ and π bonding is facilitated by choosing the local coordinate system with the z axis along the bond. The contributions to the overlap populations between atoms from π orbitals correlate with increasing bond length; ~ 61 per cent of the P=O, ~ 34 per cent of the P–OH, and ~ 32 per cent of the P–OP overlap populations are from π interactions. Previous theoretical studies [3, 6] indicate opposing resonance may be a factor in the instability of the

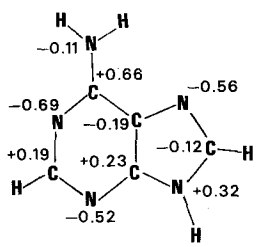

Fig. 2a

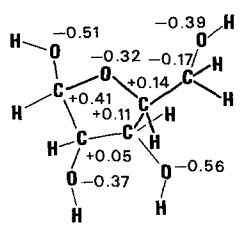

Fig. 2b

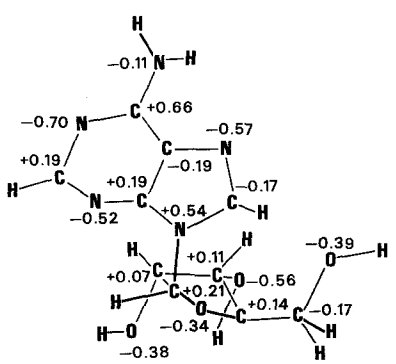

Fig. 2c

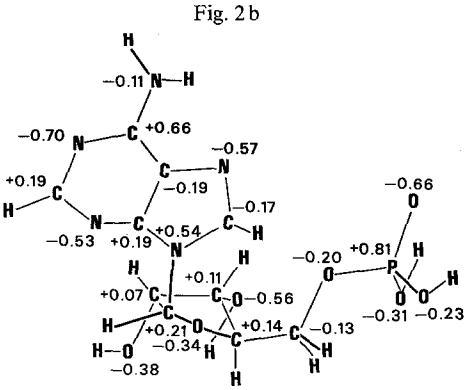

Fig. 2d

Fig. 2. Projections of the three-dimensional structures and net atomic charges for adenine, *D*-ribose (β-anomer), adenosine, and adenosine monophosphate (AMP)

polyphosphates, and hence in their high free energies of hydrolysis. Although this notion is difficult to express quantitatively in our all-electron method, the extent of delocalization as measured by bond lengths in the polyphosphates, which, in turn, affect the π overlap populations, suggests that more delocalization is possible after the hydrolysis of the pyrophosphate bond.

Opposing resonance may also play a role in the high free energy of hydrolysis [1, 2] of acetyl phosphate. Using a geometry based on AMP and methyl

acetate [28], the amount of π overlap population in the P–OC bond is calculated to be 35 per cent of the total overlap population. Delocalization in the acetate group is expected to increase after hydrolysis because in the ester the C–OP overlap population is 14 per cent π, whereas the C=O overlap population is 59 per cent from π interactions. The net atomic charges along the P—O—C=O chain are +0.80, −0.24, +0.56, and −0.45, clearly indicating no backbone of positively charged atoms in this "high-energy" compound when no arbitrary separation of σ and π electrons is made.

In the inorganic phosphates, as well as in AMP, the orbital populations of P indicate that the core orbitals (P 1*s*, 2*s*, 2*p*) are almost completely filled. The occupations of the valence orbitals of P (about 0.83 in 3*s*, 2.04 in 3*p*, 1.42 in 3*d*) indicate that the 3*d* orbitals are highly occupied even though the α_{3d} is over 0.6 a. u. higher in energy than α_{3p}. The 3*d* orbitals are found to contribute 45 per cent (21 per cent of σ and 61 per cent of π) of the P=O overlap population and 41 per cent (34 per cent of σ and 53 per cent of π) of the P–OH overlap population in H_3PO_4. The strong π contribution is expected from molecular SCF calculations [8] on phosphorus compounds, and the general question of *d* orbital bonding has been discussed extensively [24, 29].

Application of the point charge approximation to the molecules in Fig. 1 gives dipole moments of 1.9 *D* for H_3PO_4 (from P towards O of P=O), 5.8 *D* for $H_4P_2O_7$ (along C_2 rotation axis towards O bridge), and 7.5 *D* for $H_5P_3O_{10}$ (similar direction to that in $H_4P_2O_7$).

In the organic systems, adenine and *D*-ribose, the rigorous evaluation of the dipole moment integrals and point charge approximation yield values of 3.74 *D* and 3.43 *D*, respectively, for the dipole moment of adenine, and 3.43 *D* and 3.61 *D*, respectively, for the dipole moment of *D*-ribose. An experimental value [30] for a derivative of adenine is 3.0 *D*. The agreements by the exact and point charge methods are fortuitous, but they do suggest that our point charge approximation values of 4.48 *D* for adenosine and 7.44 *D* for AMP are reasonable predictions. As in the EH results [6], the five-membered ring of adenine is at the positive end of the dipole: if the origin is taken at N_3; the exact and approximate dipole moment vectors make angles of 67° and 72°, respectively, with the N_3–C_6 line. In *D*-ribose the dipole moment vector extends roughly perpendicular to the five-membered ring towards the same side as the CH_2OH group (Fig. 2). Both NEMO II and NEMO I [16] predict the hydroxyl oxygens of the sugar moiety to be more negative than the bridge oxygen. In adenosine and AMP, the dipole moment vectors happen to lie almost in the plane of the aromatic ring, and make angles of 43° and 29°, respectively, with the N_3–C_6 line.

Charge transfer among the base, sugar, and phosphate moieties of AMP is mainly inductive, since orientation [14] and valence saturation of the sugar minimize resonance effects. If AMP is severed at the glycosidic bond and at the P–OC bond, we find net charges of −0.018, −0.059, and +0.077 on the base, sugar and phosphoryl groups, respectively. If adenosine is severed, charges are −0.018 on the base and +0.018 on the sugar. Thus, a small charge transfer takes place towards the aromatic heterocycle in AMP and adenosine even though the electron population at N_9 decreases relative to adenine. In the formation of the N-glycosidic and ester bonds the effect on charge transfer carries through about two or three

covalent bonds from the reaction site. There is almost no covalent electronic interaction between the phosphate and base ends of the isolated nucleotide in spite of the 3*d* orbitals on P.

Atomic charges (Fig. 2) showing extreme values tend to correlate with chemistry. In adenine or in the adenine moiety of larger molecules, N_1 and N_7 are most negative, whereas the amino nitrogen has a relatively small charge; N_1 has been found to be protonated in crystallographic studies [14, 31], and N_7 is suggested by nuclear magnetic resonance studies [32] to chelate nucleotides to metal ions. Atom N_9 of adenine, which forms the glycoside link, becomes even more positive in the nucleoside, and has been correlated [3] with the high rate of hydrolysis of purine ribosides as compared with pyrimidine ribosides.

Acknowledgements. The author wishes to thank Professor William N. Lipscomb for invaluable discussions and assistance. Support from the National Institutes of Health while at Harvard is gratefully acknowledged.

References

1. Lehninger, A. L.: Bioenergetics. New York: W. A. Benjamin Inc. 1965; Racher, E.: Mechanisms of bioenergetics. New York: Academic Press 1965; Kaplan, N. O., and E. P. Kennedy: Current aspects of biochemical energetics. New York: Academic Press 1966.
2. George, P., and R. J. Rutman: Progr. Biophysics biophysic. Chem. **10**, 1 (1960); Phillips, R. C., P. George, and R. J. Rutman: Biochemistry **2**, 501 (1963).
3. Pullman, B., and A. Pullman: Quantum biochemistry, Chapter 7. New York: Interscience Publishers 1963.
4. Fukui, K., K. Morokuma, and C. Nagata: Bull. chem. Soc. Japan **33**, 1214 (1960).
5. Hoffmann, R., and W. N. Lipscomb: J. chem. Physics **36**, 2179, 3489 (1962); — J. chem. Physics **37**, 2872 (1962); Hoffmann, R.: J. chem. Physics **39**, 1397 (1963); — J. chem. Physics **40**, 2480 (1964).
6. Boyd, D. B., and W. N. Lipscomb: J. theoret. Biol., in press.
7. — — J. chem. Physics **48**, 4955 (1968).
8. — — J. chem. Physics **46**, 910 (1967).
9. Palke, W. E., and W. N. Lipscomb: J. Amer. chem. Soc. **88**, 2384 (1966).
10. Boyd, D. B.: Ph. D. Thesis, Harvard University, Cambridge, Massachusetts, 1967.
11. Furberg, S.: Acta chem. scand. **9**, 1557 (1955).
12. McDonald, W. S., and D. W. J. Cruickshank: Acta crystallogr. **22**, 43 (1967).
13. Cruickshank, D. W. J.: Acta crystallogr. **17**, 674 (1964).
14. Kraut, J., and L. H. Jensen: Acta crystallogr. **16**, 79 (1963).
15. Streitwieser, A., Jr.: Molecular orbital theory for organic chemists, p. 115. New York: John Wiley and Sons Inc. 1961.
16. Newton, M. D., F. P. Boer, and W. N. Lipscomb: J. Amer. chem. Soc. **88**, 2353 (1966).
17. Mulliken, R. S.: J. Chim. physique **46**, 497 (1949).
18. Kiser, R. W.: Introduction to mass spectroscopy and its applications. Englewood Cliffs, N. J.: Prentice Hall Inc. 1965.
19. Halmann, M., and Y. Klein: Adv. in mass spectrometry. W. L. Mead, Ed., Vol. 3, p. 267. London: Institute of Petroleum 1966.
20. Bafus, D. A., E. J. Gallegos, and R. W. Kiser: J. physic. Chem. **70**, 2614 (1966).
21. Fulton, H., and L. E. Lyons: Austral. J. Chem. **21**, 419 (1968); Lifshitz, C., E. D. Bergmann, and B. Pullman: Tetrahedron Letters 4583 (1967).
22. Pullman, A., and M. Rossi: Biochim. biophysica Acta **88**, 211 (1964); Pullman, A., and B. Pullman: Adv. in quantum chemistry, P.-O. Löwdin, Ed., Vol. 4, p. 267. New York: Academic Press 1968.
23. Roothaan, C. C. J., and P. S. Bagus: Methods in computational physics; Alder, B., S. Fernbach, and M. Rotenberg, Eds., p. 47. New York: Academic Press 1963.
24. Boyd, D. B., and W. N. Lipscomb: J. chem. Physics **48**, 4968 (1968).
25. Cottrell, T. L.: The strengths of chemical bonds, 2nd Ed. London: Butterworth and Co. Ltd. 1958.

26. Mulliken, R. S.: J. chem. Physics **23**, 1833, 1841, 2338, 2343 (1955).
27. Jordan, F., and B. Pullman: Theoret. chim. Acta (Berl.) **9**, 242 (1968).
28. O'Gorman, J. M., W. Shand, and V. Schomaker: J. Amer. chem. Soc. **72**, 4222 (1950).
29. Boyd, D. B.: J. Amer. chem. Soc. **91**, 1200 (1969); Boer, F. P., and W. N. Lipscomb: J. chem. Physics **50**, 989 (1969); Mitchell, K. A. R.: Chem. Reviews **69**, 157 (1969).
30. DeVoe, H., and I. Tinoco, Jr.: J. molecular Biol. **4**, 500 (1962).
31. Cochran, W.: Acta Crystallogr. **4**, 81 (1951); Sundaralingam, M.: Acta crystallogr. **21**, 495 (1966).
32. Cohn, M., and T. R. Hughes, Jr.: J. biol. Chemistry **237**, 176 (1962); Sternlicht, H., R. G. Shulman, and E. W. Anderson: J. chem. Physics **43**, 3133 (1965); Phillips, R.: Chem. Reviews **66**, 501 (1966); Hammes, G. G., and D. L. Miller: J. chem. Physics **46**, 1533 (1967); Sternlicht, H., D. E. Jones, and K. Kustin: J. Amer. chem. Soc. **90**, 7110 (1968).

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