$$\begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1/2 & 1/2 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 & 0 \\ 0 & 0 & 0 & 1/2 & -1/2 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

I

$$\begin{bmatrix} -1/2 & 1/3 & 1/6 & 0 & 0 & 0 \\ 1/6 & -1/2 & 0 & 1/6 & 1/6 & 0 \\ 1/6 & 0 & -1/2 & 0 & 1/3 & 0 \\ 0 & 1/3 & 0 & -1/2 & 0 & 1/6 \\ 0 & 1/6 & 1/6 & 0 & -1/2 & 1/6 \\ 0 & 0 & 0 & 1/6 & 1/3 & -1/2 \end{bmatrix}$$

II

either axial fluorine and the equatorial fluorine where τ is the mean time between fluorine exchanges. In the fluorine configurations, $\alpha\alpha\alpha$ and $\beta\beta\beta$, the intramolecular exchange of the equatorial fluorine with either of the axial fluorines leads to the same configurations; thus fluorine intramolecular exchanges have no effect on the first and last lines in the ^{31}P nmr spectrum. This can be seen as one of the main features in the experimental spectra in Figure 1A. Also, at high temperature where the fluorine lifetimes are very short, the three fluorines should become equivalent on the ^{31}P nmr time scale and therefore the ^{31}P nmr spectrum should show a 1:3:3:1 quartet as observed at 170° in Figure 1A and B.

Matrix II[6] is for an intermolecular exchange of fluorines where it is assumed that the three fluorines have the same mean lifetimes between exchanges. The principal feature of these spectra is the decoupling of the fluorines from the phosphorus which is not observed in the experimental spectra over the temperature range covered.

Since the spectra calculated using matrix I (B) are almost identical with the experimental spectra (A) and those calculated using matrix II are extremely different, the conclusion reached is that the fluorine exchange in Ph_2PF_3 is intramolecular. Also, a concentration study using nitrobenzene as a solvent showed that the spectra at all concentrations and temperatures were identical with those of the neat Ph_2PF_3 and indicates that the exchange process is first order in Ph_2PF_3.

On the other hand, ^{31}P nmr spectra obtained on Ph_2PF_3 in Pyrex tubes showed extreme line broadening and partial collapse of the quartet multiplet at ~140°. In fact, the temperature-dependent spectra closely resembled the calculated spectra given in Figure 1C and indicated an intermolecular fluorine exchange process. Concentration studies in nitrobenzene, however, showed no effect on the ^{31}P nmr line shapes and thus indicate a first-order process. We have tentatively concluded that Ph_2PF_3 in Pyrex tubes attacks the glass to produce phosphoryl compounds and F^- (or HF) which exchanges with the Ph_2PF_3. Cowley, *et al.*,[1] also observed collapse of the $^{31}P-^{19}F$ coupling at ~140° in the ^{19}F nmr spectrum of $(CH_3)_2PF_3$ and $(CH_3)_3PF_2$, in Pyrex tubes. Since they did not report on the concentration dependence of the multiplet collapse, it is possible they were not observing the same exchange process at high temperatures as they observed at low temperatures.

The caption for Figure 1 gives the τ values which were used to obtain the spectra shown in Figures 1B and C. Each τ value is reported with the temperature of the experimental spectrum in A that most nearly matches the calculated spectrum in B. An Arrhenius plot of these data excluding the limiting cases gives a $E_a \sim 20$ kcal. A more precise value of E_a is being obtained by following the disappearance of $^{19}F-^{19}F$ coupling by ^{19}F nmr.

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A Model Conformational Study of Nucleic Acid Phosphate Ester Bonds. The Torsional Potential of Dimethyl Phosphate Monoanion

Sir:

Knowledge of the conformational behavior of biopolymers has increased considerably in recent years, due to (1) the vast accumulation of experimental structure data from X-ray crystallography, nmr, and other physical methods,[1,2] and (2) the feasibility of carrying out large-scale potential-energy and statistical-mechanical calculations, using a variety of empirical and semiempirical techniques.[2a,3,4] Although the bulk of previous theoretical work has been directed toward polypeptide conformations,[3] several studies on polynucleotides have also been reported.[4] Associated with the three structural subunits of polynucleotides—the ribose sugar, the pyrimidine or purine base, and the phosphate group—are three major conformational problems: (1) the internal conformations of the ribose group; (2) the conformation between the ribose and the base (the glycosidic linkage); and (3) the conformations involving the phosphodiester linkage. The first two problems have been thoroughly examined in the

(1) For polypeptides, see, for example, R. E. Marsh and J. Donohue, *Advan. Protein Chem.*, **22**, 235 (1967); E. Meyer and W. C. Hamilton, Protein Data Bank, Brookhaven National Laboratory; F. A. Bovey, A. I. Brewster, D. J. Patel, A. E. Fanelli, and D. A. Torchia, *Accounts Chem. Res.*, **5**, 193 (1972), and references cited therein.

(2) For polynucleotides and related nucleosides, see (a) A. E. V. Haschemeyer and A. Rich, *J. Mol. Biol.*, **27**, 369 (1967); (b) M. Sundaralingam, *Biopolymers*, **7**, 821 (1969); (c) N. C. Seeman, J. L. Sussman, H. M. Berman, and S.-H. Kim, *Nature, New Biol.*, **233**, 90 (1971); J. L. Sussman, N. C. Seeman, S.-H. Kim, and H. M. Berman, *J. Mol. Biol.*, **66**, 403 (1972); (d) J. Rubin, T. Brennan, and M. Sundaralingam, *Science*, **174**, 1020 (1971); (e) R. E. Schirmer, J. P. Davis, J. H. Noggle, and P. A. Hart, *J. Amer. Chem. Soc.*, **94**, 2561 (1972); N. S. Kondo and S. S. Danyluk, *ibid.*, **94**, 5121 (1972); (f) H. Eisenberg and G. Felsenfeld, *J. Mol. Biol.*, **30**, 17 (1967); L. D. Inners and G. Felsenfeld, *ibid.*, **50**, 373 (1970).

(3) For polypeptides, see (a) H. A. Scheraga, *Advan. Phys. Org. Chem.*, **6**, 103 (1968), and G. N. Ramachandran and V. Sasiskharan, *Advan. Protein Chem.*, **23**, 283 (1968); (b) P. J. Flory, *Brookhaven Symp. Biol.*, **13**, 89 (1960); and D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).

(4) For polynucleotides and related constituents, see (a) W. K. Olson and P. J. Flory, *Biopolymers*, **11**, 1, 25, 57 (1972); (b) V. Sasisekharan and A. V. Lakshminarayanan, *ibid.*, **8**, 505 (1969), and preceding papers; (c) H. Berthod and B. Pullman, *Biochem. Biophys. Acta*, **232**, 595 (1971); **246**, 359 (1971); and B. Bullman, D. Perahia, and A. Saran, *ibid.*, **269**, 1 (1972).

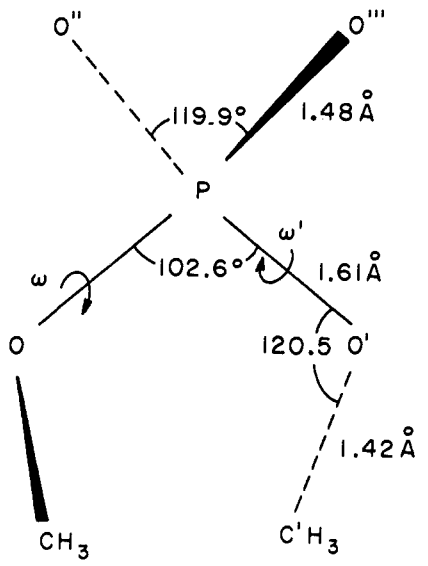

Figure 1. The dimethyl phosphate monoanion geometry used in the *ab initio* calculations, with averaged bond length and angles from ref 2c,d. Dihedral angles associated with the COPO′ and OPO′C′ fragments are denoted as ω and ω', respectively. ω = 0 and ω' = 0 represent eclipsed conformations.

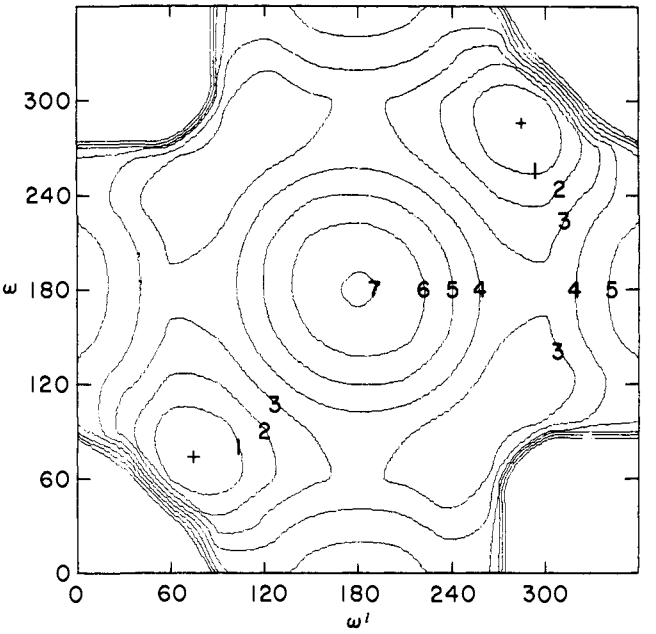

Figure 2. The *ab initio* torsional potential for dimethyl phosphate monoanion, as a function of the dihedral angles ω and ω', defined in Figure 1. Contours indicate kilocalories per mole above the minimum energy, indicated by crosses. Contours higher than 7 kcal/mol are not included.

literature,[2,4,5] and our understanding of the phosphodiester conformations, although hampered by a relative paucity of data, has also been extended recently.[2,4] For example, complete X-ray structural determinations for two distinct conformers of the dinucleoside phosphate, uridylyl-3′,5′-adenosine phosphate (UpA), have been reported.[2c,d,6] Furthermore, a survey of available data has suggested that the rotations about the PO ester bonds may often be the *major* conformational degrees of freedom in polynucleotides.[7]

In this communication we comment briefly on the previous calculations for the phosphodiester linkage and then present and discuss the results of model *ab initio* calculations on dimethyl phosphate monoanion (Figure 1), which effectively demonstrate the potential usefulness of sophisticated calculations on small fragments in refining and correcting larger scale empirical calculations. We wish to focus on the intrinsic[8] torsional potential energy associated with rotations about the PO ester bonds (*i.e.*, the dihedral angles ω and ω' in Figure 1). Empirical phosphate–ester conformational studies have traditionally employed a threefold ethane-type barrier, with a rather small amplitude (*e.g.*, 1.0 kcal/mol).[4a,b] Since these calculations have been primarily concerned with *overall* conformational energy effects, arising from the combined influence of rotational barriers and a variety of long-range electrostatic and dispersion effects, the importance of the nature and magnitude of the *intrinsic* torsional potential has not been fully appreciated, although the absence of trans-staggered phosphodiester conformations in available structural data has been noted.[2b,4] In actual fact, the absence of trans-staggered conformations might well be expected, since the conformations represented by ω and ω' in Figure 1 involve A–B̈–C–D moieties, in which a lone pair (B̈) is adjacent to a polar bond of the type $C^{\delta+}D^{\delta-}$. This is the same situation which gives rise to the "anomeric" effect[9] and other "gauche" effects.[9b,10,11] Partial donation of the lone pair to the polar bond is enhanced by gauche-type conformations.[11] To obtain more quantitative results, we have carried out *ab initio* molecular orbital (MO) calculations on the simplest phosphodiester—dimethyl phosphate monoanion—using an averaged set of molecular parameters (Figure 1) taken from the UpA structures,[2c,d] with C_{2v} symmetry imposed on the PO_4 moiety. The MO calculations employed a minimal set of Slater-type orbitals.[12] Minimal basis sets have previously been shown to give a good account of rotational barriers.[13] Calculationf were performed for about 40 unique combinations os ω and ω' (based on values of ω and ω' taken from 0 to 360° in 30° intervals). The results are displayed in Figure 2. Due to our simplified model, the ω,ω' map has four symmetry-equivalent regions, obtained by drawing the two diagonals from the corners of the square. We find that the trans–trans (ω = ω' = 180°)

(5) M. Sundaralingam, *J. Amer. Chem. Soc.*, 87, 821 (1969).

(6) A complete structure for adenosyl-2′,5′-uridine phosphate is also available: E. Shefter, M. Barlow, R. A. Sparks, and K. N. Trueblood, *Acta Crystallogr., Sect. B*, 25, 895 (1969).

(7) S.-H. Kim, H. M. Berman, N. C. Seeman, and M. D. Newton, *Acta Crystallogr.*, in press.

(8) By "intrinsic" we refer to the torsional energy of the COPO′C′ moiety (Figure 1) in the absence of contributions from interactions between the phosphate diester linkage and the ribose and base moieties. In previous empirical calculations, this "intrinsic" phosphodiester conformational energy has been decomposed into "torsional," "dispersion," and "electrostatic" terms.[4a,b]

(9) (a) R. U. Lemieux in "Molecular Rearrangements," Part 2, P. de Mayo, Ed., Interscience, New York, N. Y., 1964; (b) S. Wolfe, A. Rauk, L. M. Tel, and I. G. Csizmadia, *J. Chem. Soc. B*, 136 (1971).

(10) S. Wolfe, *Accounts Chem. Res.*, 5, 102 (1972).

(11) L. Radom, W. J. Hehre, and J. A. Pople, *J. Amer. Chem. Soc.*, 94, 2371 (1972), and references cited therein.

(12) W. J. Hehre, R. F. Stewart, and J. A. Pople, *J. Chem. Phys.*, 51, 2657 (1969); W. J. Hehre, R. Ditchfield, R. F. Stewart, and J. A. Pople, *ibid.*, 52, 2769 (1970).

(13) (a) W. H. Fink and L. C. Allen, *ibid.*, 46, 2261, 2276, 3270 (1967); (b) S. Wolfe, A. Rauk, and I. G. Csizmadia, *J. Amer. Chem. Soc.*, 91, 1567 (1969), and previous work cited therein; (c) L. Radom and J. A. Pople, *ibid.*, 92, 4786 (1970).

conformation, generally denoted as ap,ap (where ap stands for anti periplanar),[2b] corresponds to a local maximum, which lies well above (by ~7 kcal/mol) the calculated minimum ($\omega = \omega' = 75°$ or, by symmetry, $\omega = \omega' = 285°$). This minimum, as expected (see above), occurs near the gauche–gauche conformation and is denoted as −sc,−sc or +sc,+sc (where sc stands for syn clinal).[2b,14] Of the 18 phosphodiesters whose experimental ω,ω' values are listed in ref 2b–d, including many polynucleotides, and three dinucleoside phosphates, 11 are of the −sc,−sc or +sc,+sc type, with ω,ω' values corresponding to energies <1 kcal/mol above our calculated minimum energy. The remaining seven phosphodiesters are of the sc,ap type and have energies (based on the present calculations) within 3 kcal/mol of the minimum. It is reasonable that these sc,ap conformations should be accessible, if one recognizes that the intrinsic energy cost (~3 kcal/mol) could be moderated by longer range interactions involving the sugar and bases, effects beyond the scope of the present work. It is considerably less likely that the energetic demands of the ap,ap conformation would be satisfied, since the intrinsic barrier separating the sc,sc and ap,ap conformations is calculated to be ~7 kcal/mol. Hence, the absence of any experimentally observed ap,ap cases[15] is readily understood.[16] Our calculated torsional potential energy surface thus appears to be in good general accord with experimental ω,ω' data, in spite of the limitations inherent in using the symmetrical dimethyl phosphate as a model for much more complicated and less symmetrical phosphodiesters. We wish to emphasize the sharp difference between the present ω,ω' torsional potential, characterized by a large barrier and the absence of threefold periodicity, and the traditionally used threefold potential, with smaller barriers. Furthermore, it is suggested that the accuracy of future empirical or semiempirical calculations on phosphate esters would be improved by employing intrinsic ω,ω' torsional potentials for PO bonds, which are consistent with the results of the present model calculations.

As a final point, we consider the possible effect of phosphorus 3d orbitals on the calculated ω,ω' potential energy surface. Although charge densities might be sensitive to these functions, previous work[13b] indicates that addition of 3d orbitals does not strongly affect calculated rotational barriers. This point is currently being tested with further *ab initio* calculations. In the meantime, we have carried out semiempirical CNDO/2 calculations[17] on dimethyl phosphate, both with and without 3d functions on phosphorus. The 3d functions have a negligible effect on the rotational barriers and both calculated surfaces are in good agreement with the *ab initio* minimal basis results, with a sc,sc minimum at $\omega = \omega' = 66°$ and an ap,ap maximum 5 kcal/mol above the minimum.

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(14) Note that +sc,−sc conformation (*e.g.*, $\omega = +60$, $\omega' = -60$) is very unfavorable, due to combined dipolar and steric interactions. For regions where one of the angles is between ~120 and 240°, the coupling between the two rotors is rather small.

(15) Crystal structures for some triphosphate polyanions are available,[2b] which in some cases do indeed exhibit ap,ap conformations. There is, however, some indication that the preferred conformations may be dependent on the degree of negative charge in these polyanions.[2b] Simple electrostatic calculations for $P_3O_{10}^{5-}$, based on plausible point charges, suggest some preference for the ap,ap conformation over the sc,ap conformation. At any rate, conformation changes in polyphosphate anions include effects (*viz.* changes of interatomic distance for atom pairs in which *both* atoms have large formal charges) absent in the present model compound, dimethyl phosphate monoanion. Hence, one might approach the problem of polyphosphate conformations either by supplementing the intrinsic torsional contributions (Figure 2) with appropriate electrostatic terms or by carrying out molecular orbital calculations on larger model systems (*e.g.*, $P_3O_{10}^{5-}$). Such studies are presently being initiated.

(16) In some previous calculations,[4b] the ap,ap conformation was found to be less favorable than the sc,sc and sc,ap cases, for certain parameter choices; however, the energy differences were slight (−1.0 kcal/mol), and the ap,ap case always represented at least a local minimum.

(17) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966); D. P. Santry and G. A. Segal, *ibid.*, **47**, 158 (1967).

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Cyclic Peptides. V. ^1H and ^{13}C Nuclear Magnetic Resonance Determination of the Preferred β Conformation for Proline-Containing Cyclic Hexapeptides[1]

Sir:

Two C_2 symmetric intramolecularly hydrogen-bonded conformations are possible for the cyclic hexapeptide *cyclo*(Gly-L-Pro-Gly)$_2$. In a previous study,[2] 100-MHz ^1H nmr was used to analyze the solution structure of this peptide, but ambiguity in the nmr spectrum introduced by two pairs of magnetically nonequivalent glycine residues in the sequence did not allow a clear choice between structures A and B. With the aid of specifically deuterated and ^{13}C-enriched samples, the conformation of *cyclo*(Gly-Pro-Gly)$_2$ in DMSO and in aqueous solution has now been determined through the use of 250-MHz ^1H and 25.16-MHz ^{13}C nmr spectroscopy.

Previous nmr investigations of the solution conformations of *cyclo*(Pro-Ser-Gly)$_2$ and *cyclo*(Ser-Pro-Gly)$_2$[3,4] demonstrated that the favored conformation for each of these in aqueous solution contains type II β turns[5] (as in structure A) with the residue preceding the proline intramolecularly H bonded, and the proline in the trans′ ($\psi \approx 300°$)[6] conformation. In contrast, the proline-containing cyclic decapeptide gramicidin S assumes[7] type II′ β turns[5] (analogous to structure B), where the residue (Val) following the Pro is intramolecularly H bonded, and the Pro is cis′ ($\psi \approx 125°$).

(1) For the preceding paper, see C. M. Deber, D. A. Torchia, S. C. K. Wong, and E. R. Blout, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1825 (1972).
(2) R. Schwyzer and U. Ludescher, *Helv. Chim. Acta*, **52**, 2033 (1969).
(3) D. A. Torchia, A. diCorato, S. C. K. Wong, C. M. Deber, and E. R. Blout, *J. Amer. Chem. Soc.*, **94**, 609 (1972).
(4) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, *ibid.*, **94**, 616 (1972).
(5) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968).
(6) For an explanation of conventions used in dihedral angle nomenclature, see J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. Ramachandran, and H. A. Scheraga, *J. Biol. Chem.*, **241**, 1004 (1966).
(7) Yu. A. Ovchinnikov, V. T. Ivanov, V. F. Bystrov, A. I. Miroshnikov, E. N. Shepel, N. D. Abdullaev, E. S. Efremov, and L. B. Senyavina, *Biochem. Biophys. Res. Commun.*, **39**, 217 (1970).