study, as was the case in another report (Cascarano, Giacovazzo, Peerdeman & Kroon, 1982). However, the ability to distinguish atoms or ions with only a one-electron difference in macromolecules such as proteins must be seen as a great advance in X-ray protein crystallography. This has been achieved by the development of an intense, tuneable synchrotron radiation source.

This work was supported in part by a Grant-in-Aid for Scientific Research (60470153) from the Ministry of Education, Science and Culture of Japan.

References

CASCARANO, G., GIACOVAZZO, C., PEERDEMAN, A. F. & KROON, J. (1982). *Acta Cryst.* A**38**, 710-717.

EINSPAHR, H., SUGUNA, K., SUDDATH, F. L., ELLIS, G., HELLIWELL, J. R. & PARIZ, M. Z. (1985). *Acta Cryst.* B**41**, 336-341.

HUKE, K. & YAMAKAWA, T. (1980). *Nucl. Instrum. Methods*, **177**, 253-257.

International Tables for X-ray Crystallography (1974). Vol. IV, pp. 171. Birmingham: Kynoch Press. (Present distributor D. Reidel, Dordrecht.)

KITAGAWA, Y., TANAKA, N., KATSUBE, Y., KUSUNOKI, M., LEE, G. P., MORITA, Y., ASADA, K. & AIHARA, D. (1984). *Acta Cryst.* A**40**, C43.

KITAGAWA, Y., TSUNASAWA, S., TANAKA, N., KATSUBE, Y., SAKIYAMA, F. & ASADA, K. (1986). *J. Biochem.* **99**, 1289-1298.

KRAUT, J. (1968). *J. Mol. Biol.* **35**, 511-512.

NORTH, A. C. T., PHILLIPS, D. C. & MATHEWS, F. S. (1968). *Acta Cryst.* A**24**, 351-359.

SASAKI, S. (1984). *Anomalous Scattering Factors for Synchrotron Users, Calculated Using Cromer and Liberman's Method.* National Laboratory for High Energy Physics, Ibaraki, Japan.

SATOW, Y. (1984). In *Methods and Applications in Crystallographic Computing*, edited by S. R. HALL & T. ASHIDA, pp. 56-64. Oxford Univ. Press.

WILSON, A. J. C. (1979). *Acta Cryst.* **2**, 318-321.

Acta Cryst. (1987). B**43**, 275-280

A Statistical Stereochemical Model of the Flexible Furanose Ring

BY V. N. BARTENEV, N. G. KAMENEVA AND A. A. LIPANOV

Institute of Molecular Genetics, USSR Academy of Sciences, Kurchatov Sq., 46, *Moscow* 123182, *USSR*

(*Received* 27 *November* 1985; *accepted* 1 *December* 1986)

Abstract

The probabilities of alternative conformations of the furanose ring in nucleosides and nucleotides and the free-energy difference have been determined from statistical analysis of X-ray data. Approximate analytical expressions have been obtained defining the optimal one-parameter pathway of conformational changes for furanose.

Introduction

Furanose rings, which enter into the sugar-phosphate backbone of nucleic acids, possess a considerable conformational flexibility; the configurations assumed by the rings are important stereochemical characteristics of the three-dimensional structure of DNA and RNA molecules. Any description of the stereochemistry of furanose is rendered difficult by the fact that in order to simulate the conformational rearrangements of the ring one has to allow for the changing valence angles and bonds (Westhof & Sundaralingam, 1980; Pearlman & Kim, 1985). A complete description of the configuration of the five-membered flexible ring would mean defining nine $(5\times3-6)$ parameters, which is superfluous in many practical cases.

For a more economic description of the ring configuration, one can use the parameters of pseudorotation: phase angle P and pseudorotation amplitude τ_m (Altona & Sundaralingam, 1972). The furanose ring structures known from X-ray diffraction are distributed in the conformational range of the parameters P, τ_m close to the pseudorotation pathway with a roughly constant value $\tau_m \simeq 39°$ (Murray-Rust & Motherwell, 1978; Westhof & Sundaralingam, 1980), *i.e.* this is the most probable pathway for the conformational changes of furanose. The experimental data have been approximated to obtain analytical expressions (regression curves) for the ring's valence angles as functions of P along the pseudorotation pathway (Murray-Rust & Motherwell, 1978; Westhof & Sundaralingam, 1980). However, the use of these expressions for a one-parameter definition of the furanose conformation does not ensure the optimal stereochemistry of the ring as a whole, since the approximation coefficients were chosen for each individual angle regardless of the others and without stereochemical limitations. Even the simplest limitation, *viz* the condition of ring closure, alters the

0108-7681/87/030275-06$01.50

regression curves (De Leeuw, Haasnoot & Altona, 1980; Merritt & Sundaralingam, 1985).

The present paper proposes a simple statistical approach for the construction of a flexible furanose-ring model in which the steric properties (including the condition of ring closure) are described by a statistical function of the maximum likelihood. The function coefficients are average values and variances of the valence angles and bonds, so one can calculate the furanose-ring structures that are most likely in terms of the available experimental data on the geometric parameters of furanose.

The calculation of statistically optimal structures

How are the structures of flexible molecules (not only furanose rings) known from X-ray experiments distributed statistically in the conformational ranges of parameters?

On the one hand, if one assumes that intermolecular interactions have a random effect on the molecular structure in crystals, then the probability W_G of the structure being observed in a deformed state is determined by the free-energy difference ΔG between the deformed and energetically optimal conformations of an isolated molecule: $W_G = \exp(-\Delta G/RT_c)$, where RT_c characterizes the mean energy of deformation due to intermolecular interactions in crystals, by analogy with the probability $\exp(-\Delta G/RT)$ of the deformed conformation being realized in solution because of thermal motion with the mean energy RT.

On the other hand, the deformed conformation probability can be estimated from the available experimental statistical data for the structure's geometric parameters. To be specific, if the mean optimal values p_i^o and variances σ_i^2 are known for independent parameters, then the relative probabilities can be estimated from the value of the maximum-likelihood function

$$W_p = \exp[-\tfrac{1}{2}\sum_i (p_i - p_i^o)^2/\sigma_i^2].$$

The most likely three-dimensional structures correspond, according to the least-squares method, to the minimum of the function $-\ln(W_p)$. As a result the 'statistical' conformational energy maps can be constructed from the relation $W_p = W_G$.

The flexible furanose-ring model

If the correlation between the values of valence angles and bonds (which is due to the closure of the five-membered atomic chain into a ring) is disregarded, the values of the function W_p calculated from the statistical data for endocyclic valence angles and bonds (Table 1) determine the relative probabilities of the furanose conformation being deformed rather than relaxed.

Table 1. *Average values and standard deviations of furanose valence bonds and angles*

Valence bonds (standard deviation) (Å)

Phase angle P(°)	C1′–C2′	C2′–C3′	C3′–C4′	C4′–O4′	O4′–C1′
$0 < P < 360$	1·530 (0·020)	1·529 (0·020)	1·524 (0·018)	1·450 (0·015)	1·414 (0·019)
$-90 < P < 90$	1·533 (0·017)	1·529 (0·018)	1·527 (0·018)	1·448 (0·013)	1·414 (0·015)
$90 < P < 270$	1·527 (0·022)	1·528 (0·022)	1·522 (0·018)	1·453 (0·017)	1·414 (0·022)

Endocyclic valence angles (standard deviation) (°)

	C1′	C2′	C3′	C4′	O4′
$0 < P < 360$	106·56 (1·36)	101·66 (1·38)	102·45 (1·18)	105·28 (1·43)	109·77 (1·22)
$-90 < P < 90$	107·15 (1·04)	101·70 (1·37)	102·35 (1·09)	104·52 (0·95)	109·90 (1·23)
$-90 < P < 18$	(1·10)	(1·36)	(1·00)	(0·98)	(1·07)
$18 < P < 90$	(0·89)	(1·47)	(1·36)	(0·97)	(1·63)
$90 < P < 270$	105·99 (1·40)	101·63 (1·40)	102·54 (1·27)	106·01 (1·44)	109·65 (1·20)
$90 < P < 162$	(1·41)	(1·67)	(1·12)	(1·10)	(1·48)
$162 < P < 270$	(1·43)	(1·33)	(1·34)	(1·56)	(1·13)

Note: Atomic coordinates were taken from the Cambridge Structural Database (CSD), release 1984 (159 nucleosides and nucleotides). We disregarded about 100 chemically modified structures (some of them cyclized) whose furanose-ring conformations are strongly deformed. 65% of the crystal structures used in calculations are nucleosides. 85% contain riboses, the rest contain deoxyriboses.

A fairly accurate model represents the ring as a five-membered chain of atoms C2′–C3′–C4′–O4′–C1′ with four fixed bonds and one flexible bond (C1′–C2′), which closes the chain into a ring (Fig. 1). However, this fact decreases the ring's flexibility; to compensate for it, the effective variance $\sigma^2_{\text{eff}} = 5\sigma^2_{C1'-C2'}$ for the non-fixed closing bond length should be introduced into the calculation of W_p. The maximum-likelihood function for our model will be

$$W_p = \exp\{-\tfrac{1}{2}[\sum_i (\varphi_i - \varphi_i^o)^2/\sigma_i^2 + (l_{C1'-C2'} - l^o_{C1'-C2'})^2/\sigma^2_{\text{eff}}]\}.$$

Table 1 shows that l_i^o and φ_i^o remain unchanged upon transitions between the N and S conformation

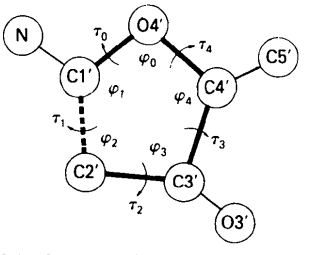

Fig. 1. Model of the furanose ring with fixed valence-bond lengths. The conformational parameters comprise valence angles $\varphi_0, \varphi_3, \varphi_4$ and torsion angles τ_3, τ_4. The torsion angles are roughly defined by the pseudorotation parameters P, τ_m: $\tau_i = \tau_m \cos[P + (i-2)144°]$. The length of the valence bond C1′–C2′, the valence angles φ_1, φ_2 and torsion angles τ_0, τ_1, τ_2 are functions of the chain's conformational parameters.

ranges, except for the mean values for the angles φ_1 and φ_4; *i.e.* the N and S ranges correspond to different energetically optimal values of φ_1^o and φ_4^o. We suppose that the $N \rightleftarrows S$ transitions are accompanied by continuous changes in the values of φ_1^o and φ_4^o, which can be approximated, for instance, by the relations (in °)

$$\varphi_1^o = 106{\cdot}6 + \cos(P); \; \varphi_4^o = 105{\cdot}3 - \cos(P).$$

Conformational maps

Fig. 2(*a*) shows the experimentally observed distribution of furanose structures in coordinates P, τ_m. In the N region the majority of structures are localized in the vicinity of the C3′-*endo* ($P = 18°$) conformation and in the S region - in the vicinity of the C2′-*endo* ($P = 162°$) conformation. The conformational map of furanose in the first approximation may be constructed using the statistical parameters calculated on the basis of the complete set of furanose-ring conformations in both the N region and the S region (see Fig. 2*b*). The positions of the energy minima correspond to the experimentally observed distribution of furanose structures. For the optimal pseudorotation pathway between C3′-*endo* and C2′-*endo*, for T_c = 300 K, the energy barriers of the transitions through the W and E regions are in the first approximation 15·1 and 14·7 kJ mol^{-1} respectively (Fig. 3*a*). Conformational transitions of the furanose ring through the flat state (with a barrier of about 41·9 kJ mol^{-1}) are far less probable.

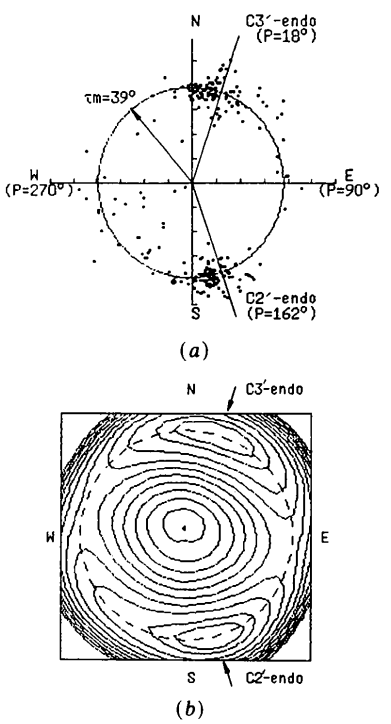

Fig. 2. (*a*) Experimental furanose conformations (dots) in nucleoside and nucleotide crystals for ~250 structures (CSD, release 1984). Similar distributions in coordinates P, τ_m were presented by other authors for a smaller number of structures [for example, Pattabiraman & Rao (1982)]. (*b*) 'Statistical' conformational map $\Delta G(P, \tau_m)$ (kJ mol^{-1}) for the furanose ring in crystal conditions. $\Delta G = 0$ if $(P, \tau_m) = (18°, 37{\cdot}4°)$ and $(P, \tau_m) = (162°, 38{\cdot}5°)$. In the vicinity of these minima, energy levels are drawn every 3·14 up to 12·6 kJ mol^{-1}, then every 4·2 kJ mol^{-1}; dashed line - the optimal pseudorotation pathway ($\tau_m = 37{\cdot}4°$). One can see that the distribution of the experimental structures and the conformational map are not symmetrical relative to the equator (W-E line): the optimal configuration ranges are somewhat drawn out from C3′-*endo* towards E ($P = 90°$) and from C2′-*endo* towards W ($P = 270°$).

However, such an approximation actually ignores the possible effects of the exocyclic components on the energy barriers. One may assume that the exocyclic components lead to extra steric obstacles which are asymmetric relative to the optimal furanose conformations on the pseudorotation pathway. In the N region, for example, where the relaxed structures are situated near the P value of 18°, the experimental distribution of all structures may reflect some asymmetry of the furanose deformation ability at $P < 18°$ and $P > 18°$. To take these possible effects into account, we used the valence-angle standard deviations calculated for several P-value intervals: $-90 < P < 18°$, $18 < P < 90°$, $90 < P < 162°$, $162 < P < 270°$ (Table 1). The average values of the valence angles were assumed to be the same as in the previous case.

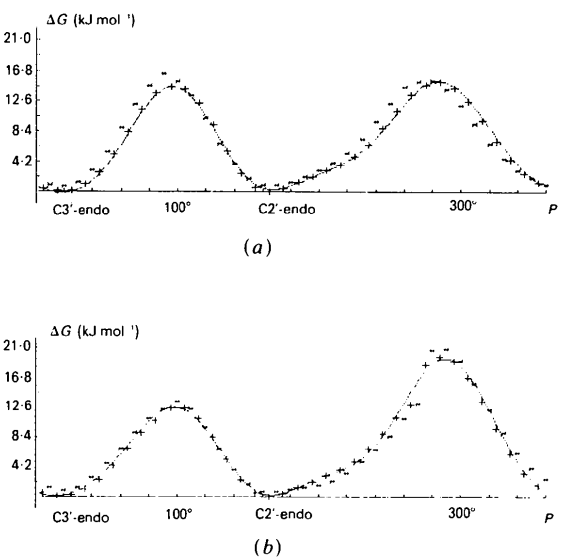

Fig. 3. (*a*) Curves depicting the free-energy change of furanose on the pseudorotation pathway in crystal conditions, calculated without allowance for the effects of the exocyclic components on the energy barriers (as in the case of Fig. 2*b*). (*b*) Analogous curves which take the above effects into account (see text for details). The optimal τ_m value in this case is 37·7°. +, Minimization curve; —, Fourier approximation curve: ΔG(kJ mol^{-1}) = $4{\cdot}2 \times (1{\cdot}7 + 0{\cdot}3\cos P - 0{\cdot}7\sin P - 1{\cdot}6\cos 2P - 0{\cdot}7\sin 2P - 0{\cdot}3 \times \cos 3P - 0{\cdot}2\sin 3P + 0{\cdot}1\cos 4P + 0{\cdot}3\sin 4P)$ [only for (*b*)]; *, curve for the pathway defined by analytical expressions for valence angles (see legend to Fig. 4).

As a result, a refined conformational map with different energy barriers in the W and E regions has been calculated. The energy barrier for the transition through the W region is in this case 19·7 kJ mol^{-1} and the one for the E region is 12·6 kJ mol^{-1} with an error of 0·8 kJ mol^{-1} (Fig. 3*b*). The difference between these values is statistically significant and the accuracy of the method (the maximum error does not exceed 2·5 kJ mol^{-1}) is quite sufficient to draw reliable conclusions.

To compare these values with the experimental NMR data, the effective barrier must be calculated from the conformation map, as some of the transitions do not follow the optimal pathway. Taking into account the population of the various transition pathways, the effective barrier value is estimated at 13·8 (8) kJ mol^{-1}. The NMR experiment gives 18·8 (21) kJ mol^{-1} for purine nucleosides in solution (Davies & Danyluk, 1975; Ludemann & Westhof, 1978). The statistical underestimation of the barrier value is probably due to the assumed $T_c = 300$ K. In reality, however, T_c must be higher than room temperature to allow the process of crystallization. For the correction coefficient $K_c = T_c/300$ K we get the value $K_c = [18{\cdot}8\,(21)]/[13{\cdot}8\,(8)] = 1{\cdot}4\,(2)$, and the mean energy of furanose ring deformation due to intermolecular interactions in nucleoside and nucleotide crystals is then $K_c R$ (300 K) = 3·3 (4) kJ mol^{-1}. To evaluate the free-energy change of the furanose ring upon its deformation in solution, the ΔG values in Figs. 2 and 3 must be multiplied by the coefficient K_c.

Furanose conformations on the pseudorotation pathway

The curves depicting the furanose free-energy change on the pseudorotation pathway are shown in Fig. 3. The energy values calculated for approximated analytical expressions of the valence angles (see below) are in good agreement with the minimization curve, showing the approximation to be accurate enough.

The changing endocyclic angles of furanose on the pseudorotation pathway are described by the minimization curves and the analytical curves chosen in the form $\varphi_i = A_i + B_i \cos(2P - 72i + C_i)$. Unlike the free-energy curves (Figs. 3*a*,*b*), they proved to be *rather insensitive to the statistical properties*. The approximation coefficients are given in the legend to Fig. 4. Note that furanose differs in the values of the C_i phases from the ideal five-membered ring for which $C_i = 0$ (Dunitz, 1972).

Correlation between exocyclic angles and the furanose conformation

To use the above flexible furanose-ring model for the calculation of nucleotide structures, one has to allow for the dependence of some exocyclic valence and

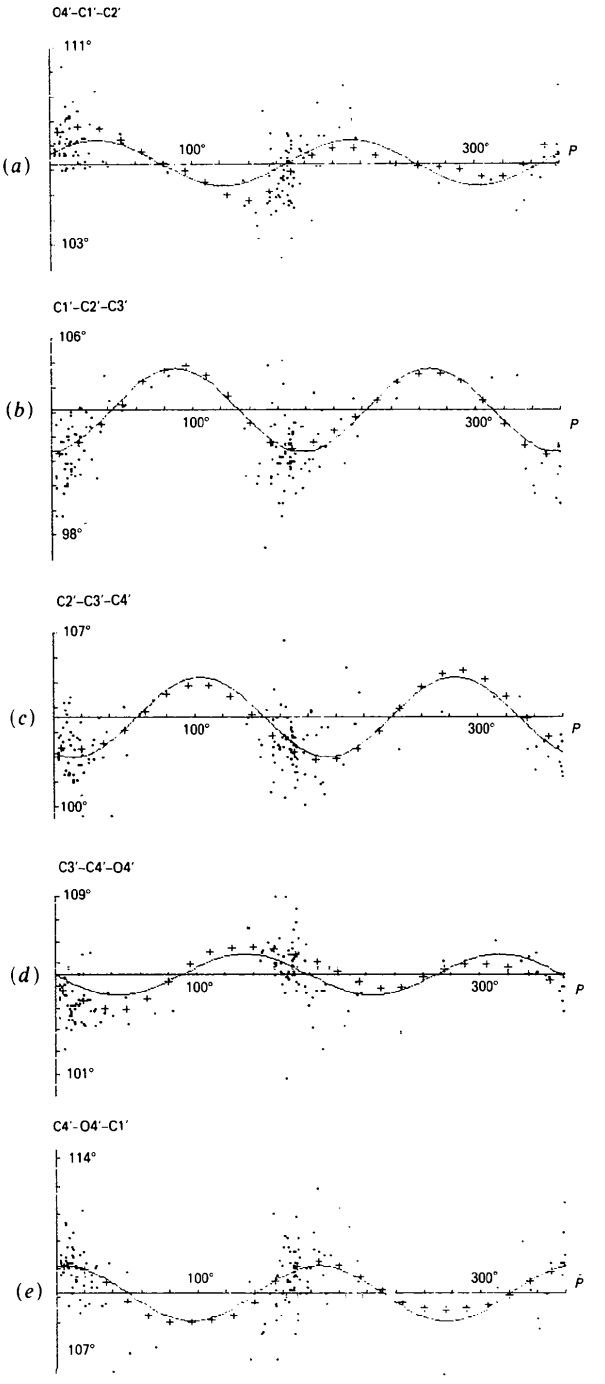

Fig. 4. Curves depicting the changing valence angles of furanose on the pseudorotation pathway: +, minimization curves; —, approximating curves; ·, experimental values. Analytical expressions (in °) for the approximating curves: (*a*) $\varphi_0 = 108{\cdot}8 + 1{\cdot}4\cos(2P - 0\times72 - 10)$; (*b*) $\varphi_1 = 106{\cdot}2 + 1{\cdot}1\cos(2P - 1\times72 + 16)$; (*c*) $\varphi_2 = 102{\cdot}9 + 1{\cdot}6\cos(2P - 2\times72 - 36)$; (*d*) $\varphi_3 = 103{\cdot}5 + 1{\cdot}5\cos(2P - 3\times72 + 9)$; (*e*) $\varphi_4 = 105{\cdot}2 + 0{\cdot}8\cos(2P - 4\times72 + 8)$. The minimization curves almost coincide with the approximating curves for the angles C2'–C3'–C4', C4'–O4'–C1', C1'–C2'–C3'. The curves for the angles C3'–C4'–O4' and O4'–C1'–C2' are not so accurately approximated by cosine dependences as could have been expected from the considerably different mean experimental values of these angles in the *S* and *N* regions.

torsion angles in furanose on the ring's folding geometry. Our algorithm uses the values of torsion angles C5′–C4′–C3′–O3′ (δ), O3′–C3′–C4′–O4′ (τ_3'), C4′–O4′–C1′–N (τ_0') and valence angles O3′–C3′–C4′, C3′–C4′–C5′, O4′–C1′–N (Fig. 1).

The statistical treatment of experimental data for nucleosides and nucleotides has revealed a certain dependence on the furanose conformation for only one of the valence angles, O3′–C3′–C4′: the mean values for the *N* and *S* regions are 111·9 and 109·6°, respectively.

For the exocyclic torsion angles the following approximate relations are valid: $\delta = \tau_3 + 120°$; $\tau_3' = \tau_3 - 120°$; $\tau_0' = \tau_0 - 120°$. While the third relation virtually does not depend on the conformation of furanose, for $\delta - \tau_3$ and $\tau_3' - \tau_3$ this dependence is statistically significant and can be analytically expressed in a linear approximation (Fig. 5).

The cosine dependence of the τ_3 angle on the phase angle *P* (see legend to Fig. 1) leads to an interesting effect. Equal changes in *P* (energetically close, see Fig. 3*b*) cause a considerably greater change in δ for the C2′-*endo* conformation than for the C3′-*endo* one. This fact can account for the distinct conservatism of *A*-type DNA, which is characterized by the C3′-*endo* furanose conformation as compared with *B*-type DNA characterized by the C2′-*endo* conformation (Leslie, Arnott, Chandrasekaran & Ratliff, 1980).

Discussion

The statistical conformational analysis proposed in this paper has obvious advantages over the traditional semi-empirical method of atom-atom potentials for those cases when sufficient experimental stereochemical statistical data are available for the conformational range under study. Naturally enough, the computed statistical conformational energy maps, optimal conformations and conformational transition pathways are in good agreement with the experimental distribution of alternative structures. The method's feasibility depends on the specific structure under study (the choice of model and geometric parameters, the modification of parameters on transitions between conformation families *etc.*).

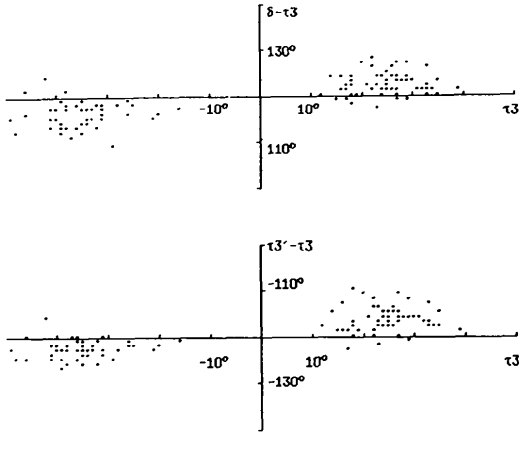

Fig. 5. Distribution of experimental values of the differences $\delta - \tau_3$ and $\tau_3' - \tau_3$ in nucleoside and nucleotide structures depending on the torsion angle τ_3. The corresponding linear approximation formulas are: $\delta - \tau_3 = 121° + 0{\cdot}09\tau_3$; $\tau_3' - \tau_3 = -118{\cdot}5° + 0{\cdot}09\tau_3$.

In the present study the method has been used to construct a stereochemical model of the flexible furanose ring. The model allows a fairly accurate analytical approximation of the experimental distributions for the geometric parameters of furanose, defined by the value of the pseudorotation phase angle only. We have successfully used this model for refining the structure of polynucleotides (Alexeev, Lipanov & Skuratovskii, 1987). This statistical approach can be applied to the construction of more flexible furanose-ring models (in preparation).

The results of the large number of calculations of the furanose geometry and the energy barriers on the pseudorotation pathway through minimizing the potential energy of intramolecular interactions within the ring are not equivalent (Lugovskoi & Dashevskii, 1972; Levitt & Warshel, 1978; Vorobjev, 1981; Zhurkin, Lysov, Florentiev & Ivanov, 1982; Olson, 1982). In such calculations an inadequate set of potential-function coefficients may lead to 'optimal' structures that are substantially different from those experimentally observed. Hence one needs a special selection of potential parameters that would ensure the agreement between the 'energetic' and experimental structures (Olson, 1982). The use of the maximum-likelihood function instead of the expressions for the intramolecular interaction energy makes the selection of numerous coefficients unnecessary; all parameters for the maximum likelihood function are determined directly from experimental data.

The statistical analysis of furanose-ring conformations in nucleoside and nucleotide crystals has shown the energy barrier of the optimal C3′-*endo* ⇆ C2′-*endo* transition (through the *E* region) to be no less than 12·6 (8) kJ mol^{-1}. The energy barrier for the transition through the *W* region is about 7 kJ mol^{-1} larger. However, since on average the rings are more strongly deformed in crystals than by thermal motion in solution at room temperature, the transition barriers in solution are K_c times larger than the above values. From the comparison of the results of our statistical analysis with NMR experimental results, the coefficient K_c is equal to 1·4 (2). This value of K_c can be used for finding conformational transition barriers in molecules chemically similar to nucleotides from the statistical analysis of their crystalline conformations.

We thank Professor M. A. Mokulskii for useful discussions, Dr Yu. T. Struchkov and Dr A. I. Yanovskii for making available CSD, Dr E. P. Smetanina for assistance in selecting bibliographic data on the structures not presented in CSD, and Mr D. A. Agrachev for his help in preparing the manuscript.

References

ALEXEEV, D. G., LIPANOV, A. A. & SKURATOVSKII, I. YA. (1987). *Nature (London)*. In the press.
ALTONA, C. & SUNDARALINGAM, M. (1972). *J. Am. Chem. Soc.* **94**, 8205-8212.
Cambridge Structural Database (CSD) (1984). Univ. Chemical Laboratory, Lensfield Road, Cambridge, England.
DAVIES, D. B. & DANYLUK, S. S. (1975). *Biochemistry*, **14**, 543-554.
DE LEEUW, H. P. M., HAASNOOT, C. A. G. & ALTONA, C. (1980). *Isr. J. Chem.* **20**, 108-126.
DUNITZ, J. D. (1972). *Tetrahedron*, **28**, 5459-5463.
LESLIE, A. G. W., ARNOTT, S., CHANDRASEKARAN, R. & RATLIFF, R. L. (1980). *J. Mol. Biol.* **143**, 49-72.
LEVITT, M. & WARSHEL, A. (1978). *J. Am. Chem. Soc.* **100**, 2607-2613.
LUDEMANN, H. D. & WESTHOF, E. (1978). In *Nuclear Magnetic Resonance Spectroscopy in Molecular Biology*, edited by B. Pullman, p. 41. Dordrecht: D. Reidel.
LUGOVSKOI, A. A. & DASHEVSKII, V. G. (1972). *Mol. Biol. (USSR)*, **6**, 440-448.
MERRITT, E. A. & SUNDARALINGAM, M. (1985). *J. Biomol. Struct. Dyn.* **3**, 559-578.
MURRAY-RUST, P. & MOTHERWELL, S. (1978). *Acta Cryst.* B**34**, 2534-2546.
OLSON, W. K. (1982). *J. Am. Chem. Soc.* **104**, 278-286.
PATTABIRAMAN, N. & RAO, M. J. (1982). *Int. J. Biol. Macromol.* **4**, 91-98.
PEARLMAN, D. A. & KIM, S.-H. (1985). *J. Biomol. Struct. Dyn.* **3**, 85-98.
VOROBJEV, YU. N. (1981). *Mol. Biol. (USSR)*, **15**, 517-525.
WESTHOF, E. & SUNDARALINGAM, M. (1980). *J. Am. Chem. Soc.* **102**, 1493-1500.
ZHURKIN, V. B., LYSOV, YU. P., FLORENTIEV, V. L. & IVANOV, V. I. (1982). *Nucleic Acids Res.* **10**, 1811-1830.

Acta Cryst. (1987). B**43**, 280-295

High-Resolution Neutron and X-ray Refinement of Vitamin B_{12} Coenzyme, $C_{72}H_{100}CoN_{18}O_{17}P.17H_2O$

BY H. F. J. SAVAGE,* P. F. LINDLEY AND J. L. FINNEY

Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, England

AND P. A. TIMMINS

Institut Laue-Langevin, 156X, 38042 Grenoble CEDEX, France

(*Received* 4 *June* 1986; *accepted* 1 *December* 1986)

Abstract

High-resolution neutron and X-ray diffraction data for vitamin B_{12} coenzyme have been obtained with the objective of elucidating the water organization in this crystalline system. Here, details of the data collection and refinement of the individual models are described. Neutron data: $M_r = 1939$ (assuming exchange of 19 coenzyme H atoms by D atoms and 17 D_2O solvent molecules) $P_12_12_12_1$, $a = 27{\cdot}849\ (6)$, $b = 21{\cdot}736\ (4)$, $c = 15{\cdot}368\ (3)$ Å, $U = 9303\ (2)$ Å^3, $Z = 4$, $D_m = 1{\cdot}381\ (15)$, $D_x = 1{\cdot}360$ Mg m^{-3}, pyrolytic-graphite monochromator, $\lambda = 1{\cdot}67$ Å, $\mu = 0{\cdot}024$ mm^{-1}, $F(000) = 34{\cdot}13$, $T = 279\ (1)$ K, final $R = 0{\cdot}085$ for 5601 significant reflections. X-ray1 data: $M_r = 1939$, $P2_12_12_1$, $a = 27{\cdot}701\ (7)$, $b = 21{\cdot}608\ (6)$, $c = 15{\cdot}351\ (4)$ Å, $U = 9189\ (2)$ Å^3, $Z = 4$, $D_m = 1{\cdot}381\ (15)$, $D_x = 1{\cdot}401$ Mg m^{-3}, Cu $K\alpha$ radiation, $\lambda = 1{\cdot}5418$ Å, $\mu = 25{\cdot}9$ mm^{-1}, $F(000) = 4024$, $T = 277{\cdot}0\ (5)$ K, final $R = 0{\cdot}088$ for 4390 significant reflections. X-ray2 data: $M_r = 1939$, $P2_12_12_1$, $a = 27{\cdot}809\ (7)$, $b = 21{\cdot}712\ (6)$, $c = 15{\cdot}333\ (4)$ Å, $U = 9258\ (2)$Å^3, $Z = 4$, $D_m = 1{\cdot}381\ (15)$, $D_x = 1{\cdot}401$ Mg m^{-3}, Cu $K\alpha$ radiation, $\lambda = 1{\cdot}5418$ Å, $\mu = 25{\cdot}9$ mm^{-1}, $F(000) = 4024$, $T = 277{\cdot}0\ (5)$ K, final $R = 0{\cdot}136$ for 5621 significant reflections. The orientation of the coenzyme molecule in these refined models is rotated in the unit cell by approximately 5° (about an axis close to the Co atom) with respect to the orientation observed in the original structure determination [Lenhert (1968), *Proc. R. Soc. London Ser. A*, **303**, 45-84]. One of the side chains of the corrin ring (*c* side chain) is disordered between two extreme positions. All the H- and D-atom positions for the coenzyme molecule and approximately 65% of the solvent D atoms were located from the neutron difference Fourier maps. Of the eleven methyl groups present, six are well ordered and five disordered. An acetone molecule (with partial occupancy) was

* Author to whom correspondence should be addressed. Present address: Medical Foundation of Buffalo, Inc., 73 High Street, Buffalo, NY 14203, USA.

0108-7681/87/030280-16$01.50