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Molecular Orbital Calculations on the Conformation of Nucleic Acids and Their Constituents

VII. Conformation of the Sugar Ring in β-Nucleosides: The Pseudorotational Representation*

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The conformational properties of the furanose ring of purine- and pyrimidine-β-nucleosides and -nucleotides are studied quantum-mechanically with the help of the PCILO method, using the pseudorotational concept. The computations point to the existence of two stable conformational zones centered around the C(3′)-*endo* and C(2′)-*endo* conformations which in the isolated furanose ring are separated by barriers of the order of 4 kcal/mole. In nucleosides one of the barriers (the one running through the O(1′)-*exo*-C(2′)-*exo* path) becomes very high. A detailed study is made of the relation between the phase angle of pseudorotation, P, and the torsion angle about the glycosyl bond, χ_{CN}. A very satisfactory agreement with the available experimental data is observed.

Die Konformationseigenschaften des Furanoserings in β-Nucleosiden und Nucleotiden von Purin und Pyrimidin werden nach der PCILO-Methode unter Berücksichtigung der pseudorotatorischen Betrachtungsweise studiert. Die Rechnung läßt auf die Existenz zweier stabiler Konformationszonen schließen, die in der Umgebung der C(2′)-*endo* und der C(3′)-*endo* Konformationen liegen, und die im isolierten Furanosering durch Energiebarrieren der Größenordnung von 4 kcal/mol voneinander getrennt sind. In Nucleosiden wird eine der Barrieren (die durch den Weg O(1′)-*exo*-C(2′)-*exo* gekennzeichnete) sehr hoch. Die Relation zwischen dem Phasenwinkel der Pseudorotation, P, und dem Drehwinkel um die Glycosylbindung, χ_{CN}, wird einer eingehenden Untersuchung unterworfen. Man beobachtet eine sehr zufriedenstellende Übereinstimmung mit den verfügbaren experimentellen Daten.

Les propriétés conformationnelles du noyau furanose des β-nucleosides et nucleotides des purines et pyrimidines sont étudiées par la méthode PCILO en faisant appel au concept de la pseudorotation. Les calculs indiquent l'existence de deux zones de conformations stables, centrées autour des conformations C(2′)-*endo* et C(3′)-*endo*, qui sont dans le sucre isolé séparées par des barrières de l'ordre de 4 kcal/mole. Dans les nucleosides, l'une de ces barrières (celle qui passe par le chemin O(1′)-*exo*-C(2′)-*exo*) devient très élevée. Une étude détaillée est effectuée sur la relation entre l'angle de phase de la pseudorotation P et l'angle de torsion autour de la liaison glycosylique, χ_{CN}. Un excellent accord avec les données expérimentales disponibles est observé.

Introduction

In continuation of our studies by the quantum mechanical PCILO (Perturbative Configuration Interaction using Localized Orbitals) method on the conformation of nucleic acids and their constituents [1–6], we present in this paper the results of computations carried out on the conformation of the sugar

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ring in β-nucleosides. In the previous publications of this series fixed conformations have been adopted for the furanose rings, corresponding generally to the most frequently encountered ones in the X-ray studies of nucleosides, nucleotides and polynucleotides, namely C(2′)–*endo* and C(3′)–*endo* [7, 8].

The present study has been carried out using the concept of pseudorotation which seems the most suitable one for a complete exploration of the conformational characteristics of the five membered sugar ring. It was first introduced in 1947 by Kilpatrick, Pitzer, and Spitzer [9] in the description of the conformation of cyclopentane. Their results showed an „indefiniteness" of the cyclopentane conformation, the angle of maximum puckering rotating around the ring without any substantial change in the potential energy. Later, in 1959, Pitzer and Donath [10] refining the earlier calculations, showed that the presence of substituents in cyclopentane induces potential energy barriers which restrict the free pseudorotation. Altona and co-workers [11–16] have explored the consequences of this limited pseudorotation for the conformation of ring *D* in steroids and of several other five-membered ring systems. Recently, Altona and Sundaralingam [17] have shown the usefulness of the pseudorotational concept in a slightly modified form for the description of the conformation of the sugar rings in β-nucleosides and β-nucleotides. In their study, each conformation of the furanose ring is unequivocally determined in terms of two parameters: the phase angle of pseudorotation, P and the degree of pucker, τ_M. The authors have evaluated these two parameters for a large number of β-nucleosides and nucleotides for which X-ray crystal data were available. Another useful utilization of the concept of pseudorotation in connection with NMR studies of the conformation of ribosides and arabinosides in solution is due to Hruska, Wood, and Dalton [18] and Hall, Steiner, and Pedersen [19].

From the theoretical point of view a few studies on the conformation of the sugar rings in β-nucleosides have also appeared recently [20–23], employing both classical and quantum mechanical approaches. Sasisekharan [20], working within the scheme of the empirical partitioned potential energy functions [24, 25], used four parameters to describe the pseudorotation in ribose and deoxyribose and then applied the pseudorotational concept to β-pyrimidine and β-purine nucleosides. Similarly, Lugovskoi and Dashevskii [21], also utilizing classical partitioned potential energy functions, have chosen two parameters to describe the pseudorotation in β-D-ribose and in a somewhat restricted fashion have extended their work to β-pyrimidine nucleosides [22], and very recently to β-purine nucleosides [22a].

In the field of the quantum mechanical methods, Govil and Saran [23] have utilized the EHT and CNDO procedures to describe, with the help of two parameters, the conformations of β-D-ribose. These authors did not call, however, upon the pseudorotational concept but used the more common representations of the conformations.

Finally in connection with the forthcoming discussion, we must indicate that a number of related theoretical studies, both by the classical [24–28] and quantum-mechanical methods [1, 2, 5, 29, 30] are available dealing with the relation between the furanose ring conformations and the glycosyl torsion angle χ_{CN}. In all these studies, however, fixed sugar ring conformations, whether

C(3′)–*endo*, C(2′)–*endo*, C(3′)–*exo* or C(2′)–*exo* have been selected and χ_{CN} was varied to establish the dependence between the sugar pucker and χ_{CN}.

The Procedure

A) The Method

The method employed for the calculation of the conformational energies is the PCILO method, as used in the previous papers of this series, the details of which may be found in original papers [31–33]. The computational program may be obtained from Q.C.P.E. (Quantum Chemistry Program Exchange) at the Chemistry Department of Indiana University, Bloomington, Ind., USA.

B) Definitions and Conventions

A schematic diagram of β-D-ribose is shown in Fig. 1. The conventions and notations of the torsion angles are essentially those of Sundaralingam [7]. The different torsion angles are defined as:

$$\tau_0 = C4' - O1' - C1' - C2'$$
$$\tau_1 = O1' - C1' - C2' - C3'$$
$$\tau_2 = C1' - C2' - C3' - C4'$$
$$\tau_3 = C2' - C3' - C4' - O1'$$
$$\tau_4 = C3' - C4' - O1' - C1'$$
$$\chi_{CN} = O1' - C1' - N1 - C2 \quad \text{(for pyrimidine nucleosides)}$$
$$\chi_{CN} = O1' - C1' - N9 - C8 \quad \text{(for purine nucleosides)}$$
$$\Phi_{C(2')-O(2')} = C1' - C2' - O2' - H(O2')$$
$$\Phi_{C(3')-O(3')} = C2' - C3' - O3' - H(O3')$$
$$\Phi_{C(4')-O(5')} = C3' - C4' - C5' - O5'$$
$$\Phi_{C(5')-O(5')} = C4' - C5' - O5' - H(O5').$$

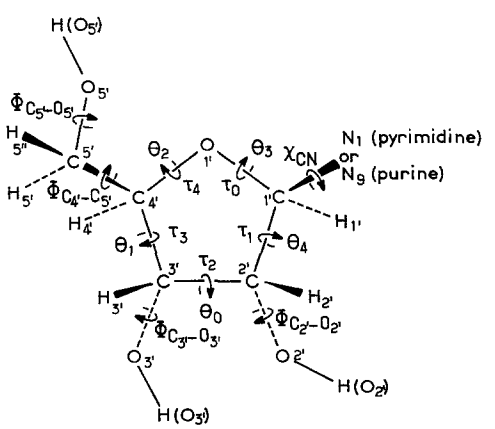

Fig. 1. Numbering of atoms and designation of torsion angles in the furanose ring

We remind that the torsion angle Φ_{B-C} of the bonded atoms A–B–C–D is the angle between the planes formed by atoms A, B, C and B, C, D. Φ_{B-C} is considered positive for a right-handed rotation: when looking along the bond B–C, the far bond C–D rotates in the clockwise direction with the respect to the near bond A–B. The zero value of the torsion angle corresponds to the *cis*-planar arrangement of the bonds A–B and C–D.

C) Pseudorotational Parameters, P and τ_M

In this paper we have adopted the description of pseudorotation of Altona and Sundaralingam [17] so as to have an easy comparison with the experimental data which they have collected. Following these authors, the five torsional angles $(\theta_0, \theta_1, \theta_2, \theta_3, \theta_4)$ of cyclopentane along the pseudorotational pathway are described by

$$\theta_j = \theta_m \cos(P + j\delta) \tag{1}$$

where $j = 0, 1, 2, 3, 4$ and $\delta = 144°$.

For $j = 0$, Eq. (1) becomes:

$$\theta_0 = \theta_m \cos P\,. \tag{2}$$

It is evident from Eq. (2), that θ_0 passes through the values of θ_m, 0, $-\theta_m$, 0 and θ_m, when P goes through a full pseudorotational cycle (0° – 360°), and so a pseudorotation over $P = 180°$ produces the mirror image of the original ring. From Eq. (1), one gets:

$$\tan P = \frac{(\theta_2 + \theta_4) - (\theta_1 + \theta_3)}{2\theta_0(\sin 36 + \sin 72)} \tag{3}$$

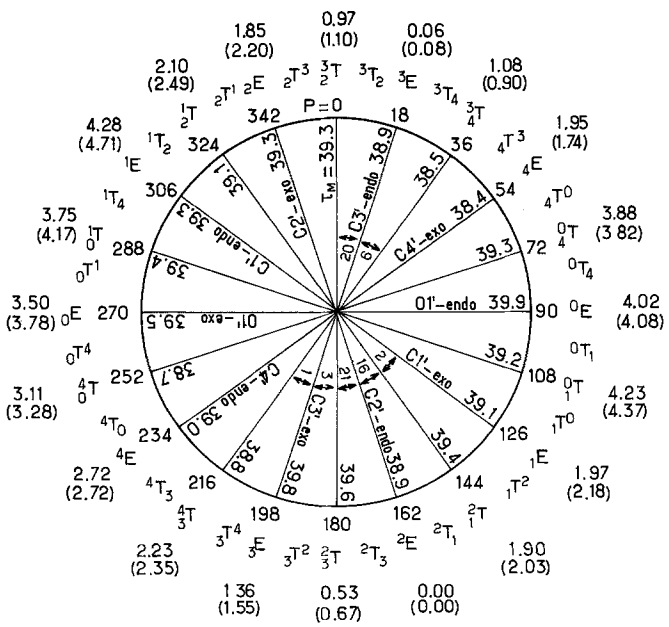

Fig. 2. The conformational wheel, in the pseudorotational representation, for *D*-ribose and deoxyribose (see text for details)

and knowing the five torsion angles about the sugar ring bonds, one can easily evaluate P from Eq. (3). For negative values of θ_0, 180° should be added to the calculated value of P from Eq. (3). A standard conformation is chosen for $P = 0°$ which corresponds to the maximum value of $C1'-C2'-C3'-C4'$ $(=\tau_2)$ torsion angle. It represents the C(3′)–*endo*–C(2′)–*exo* ($^3T_2$) conformation of the sugar. The simple relationships between θ's and τ's are:

$$\theta_0 = \tau_2\,, \quad \theta_1 = \tau_3\,, \quad \theta_2 = \tau_4\,, \quad \theta_3 = \tau_0\,, \quad \theta_4 = \tau_1 \quad \text{and} \quad \theta_m = \tau_M\,.$$

Figure 2 shows the continuously varying P values from 0–360°. The symmetrical twist (T) and envelope (E) conformations occur, respectively, at *even* and *odd* multiples of 18° in P, and are also indicated in the figure. The C(3′)–*endo* and C(2′)–*endo* conformations occur, respectively, at P equal to 18° and 162°.

D) Geometrical Input Data

Sundaralingam [8] has obtained average geometries for the C(3′)–*endo* and C(2′)–*endo* sugars from the known crystal structure studies on β-nucleosides and nucleotides. The five bond lengths of the sugar ring are exactly the same in these two fundamental conformations; however three out of five valence angles of the ring differ in the two conformations; the maximum difference beeing 1.7°. We adopted the bond lengths and valence angles of the C(3′)–*endo* geometry for all the different forms of the sugar with only a slight variation of $\pm 1.9°$ permitted for the valence angles. These geometrical parameters have been kept constant to get all the sugar forms corresponding to the different P and τ_M values.

We have not attempted to vary τ_M for each value of P but instead have adopted the mean value of 39° obtained for β-pyrimidine and β-purine nucleosides from the X-ray crystal structure studies of nucleosides and nucleotides [17]. P has been varied from 0–360° in 18° increments corresponding to the ten twist (T) and ten envelope (E) conformations of the sugar ring.

A computer program for the ring closure of the five membered cycle was developped, which for given bond lengths and valence angles and given P and τ_M values, provides the cartesian and internal coordinates of the ring with an accuracy of P within $\pm 0.5°$ and of $\tau_M = 39° \pm 1°$. The method employed for the minimization procedure used in this program is the Simplex method [34] which minimizes a function the value of which defines the magnitude of the closure on the stereochemical basis discussed above, by varying the valence and dihedral angles in conformity with the desired values of τ_M and P. In a practical way the dihedral angles are calculated from Eq. (1) for given P and τ_M values and used as initial values for the minimization procedure. The above mentioned function is designed in the following way: The torsion angles for the five atoms 1–5 of the sugar are kept initially at their respective values of P and τ_m. Three complementary fictitious atoms 6, 7 and 8 are considered such that the bond lengths 5–6 = 5–1, 6–7 = 1–2 and 7–8 = 2–3, that the valence angles 5–6–7 = 5–1–2 and 6–7–8 = 1–2–3 and that the torsion angles 3–4–5–6 = 3–4–5–1, 4–5–6–7 = 4–5–1–2 and 5–6–7–8 = 5–1–2–3. The function is defined as the sum of the distance between atoms 6–1, 7–2 and 8–3. These three

pairs of atoms are superimposed when the function is equal to zero and the ring is then fully closed.

For energy calculations, the ribose and deoxyribose have been constructed with the particular geometry of the sugar ring associated with the corresponding values of P and τ_M. All the constituents of ribose and deoxyribose have been taken into consideration. All hydrogens have been included. The bond lengths and the valence angles of other atoms have been taken following the proposal for a C(3′)–*endo* sugar given in Ref. [8].

For the energy calculations for the nucleosides, we have chosen adenosine and deoxyadenosine for the β-purine nucleosides and uridine and deoxyuridine for β-pyrimidine nucleosides. The geometry of adenine has been taken from the recent X-ray crystal structure study of adenosine by Lai and Marsh [35], and that of uracil from the recent experimental study of β-deoxyuridine by Rahman and Wilson [36].

E) Construction of the Conformational Energy Maps χ_{CN} vs. P

Conformational energy maps have been constructed as a function of the pseudorotational parameter P and the torsion angle χ_{CN} about the glycosyl bond in β-purine and β-pyrimidine nucleosides. The computations have been carried out in 20° increments of the torsion angle χ_{CN} for twenty values of P, from 0–360°, at an increment of 18°. The presentation of the results on the conformational energy maps has been confined to the 6 kcal/mole above the global minimum isoenergy curve.

Results and Discussion

A) Conformational Properties of Ribose and Deoxyribose

The conformational energies obtained by the PCILO computations for the ribose and deoxyribose rings as a function of the pseudorotation parameter P are presented in Fig. 2. The outermost numbers on the conformational wheel represent the values of the energy of the ribose (in kcal/mole) with respect to the global minimum taken as energy zero. The energies of the deoxyribose are given in parentheses. In the calculations of these energies for both ribose and deoxyribose, we have adopted the values of $\Phi_{C(4')-C(5')} = 60°$ (gg) and $\Phi_{C(5')-O(5')} = 180°$. The choice of these values results from our previous study [4] and also from experimental evidence [8]. The values of $\Phi_{C(3')-O(3')}$ for both ribose and deoxyribose and of $\Phi_{C(2')-O(2')}$ for ribose have been fixed at 60°. Figure 2 shows also the conformations of the sugar ring in terms of the envelope (E) and twist (T) forms as defined by Sundaralingam [37]. The values of τ_M associated in the computations with each P are also indicated in Fig. 2 along the corresponding radii of the conformational wheel. Finally the distribution of the observed experimental compounds from X-ray crystal structure studies is shown by numbers placed along the arrows in the populated P values. We have taken account in this distribution of a number of new compounds whose crystal structures have been determined [35, 36, 38–42] since the publication of Altona and Sundaralingam's compilation [17].

The results of Fig. 2, show the existence of two nearly equivalent global energy minima occuring at P equal to 18° and 162° and corresponding to the C(3′)–*endo* and C(2′)–*endo* conformations, respectively; the energy associated with the later being slightly lower than that of the former. The experimental data cluster around these selected conformations in very good agreement with the theoretical values. The areas of higher energies are unoccupied, with one exception, an O(1′)–*endo* conformation found in the crystal structure of dihydrothymidine (*vide infra*).

The two populated conformational zones may in fact be considered as separated by energy barriers on both sides, which in the isolated furanose ring are nearly equivalent and of the order of 4 kcal/mole. This order of magnitude seems to be confirmed by experiment [17, 18].

In a recent elegant analysis of the general aspects of the conformational wheel for ribose and deoxyribose Hruska, Wood and Dalton [18] have shown that the preference for the C(2′)– and C(3′)–*endo* conformations arises from the fact that these puckerings involve bond rotations which stagger all substituents on the furanose ring. They have also pointed out that interconversions *within* the two stable zones centered around the C(2′)–*endo* and C(3′)–*endo* conformations require a sign change for τ_0 and τ_4 but not for τ_1, τ_2 and τ_3. This situation accounts for the fact that the experimental values of τ_0 and τ_4 as found in crystals of nucleosides and nucleotides when presented in the form of conformational wheels [7] display a continuous range of values centered approximately at 0° (and ranging approximately from $-30°$ to 30°), while τ_1, τ_2 and τ_3 manifest a forbidden range around 0°. These observations substantiate the validity of the division of the conformational wheel of the sugar into two stable pseudorotational zones separated by two barrier zones. It may be remarked that although the two zones appear theoretically as energetically nearly equivalent, the zone centered around C(2′)–*endo* is both more populated and occupies a larger portion (in fact extends over C(3′)–*exo*) on the conformational wheel than the zone centered around C(3′)–*endo*.

Our results can now be compared with the results obtained for β-D-ribose by other theoretical studies. Sasisekharan [20], using classical partitioned potential energy functions, found also that there are two equally stable conformational zones around the C(2′)–*endo* and C(3′)–*endo* conformations, and that the barrier between the two minima along the pseudorotational path is about 2.5–3.0 kcal/mole for the ribose and about 2 kcal/mole for deoxyribose. Lugovskoi and Dashevskii, in a similar study using also partitioned potential functions, have calculated the energy of β-D-ribose as function of Φ_5 and Φ_4. (Their Φ's are related to our τ's or θ's (see Fig. 1) by the simple relations: $\Phi_1 = \tau_1 = \theta_4$, $\Phi_2 = \tau_2 = \theta_0$, $\Phi_3 = \tau_3 = \theta_1$, $\Phi_4 = \tau_4 = \theta_2$ and $\Phi_5 = \tau_0 = \theta_3$). Their computations have been carried out in two distinct approximations. When the electrostatic interactions are completely neglected, the results show two energy minima corresponding to the C(2′)–*endo* and C(3′)–*endo* conformations, the former being more restricted than the later. The transition from one stable conformation to the other along the pseudorotational pathway is through a barrier of the order of 2.5 kcal/mole. However, when the electrostatic interactions are included, a step which in principle represents a refinement, the minimum at the C(2′)–*endo*

conformation is strikingly destabilized, hardly representing even a local minimum, about 4 kcal/mole above the C(3′)–*endo* conformation which represents now the unique stable arrangement. The transition between C(3′)–*endo* and C(2′)–*endo* which could occur both ways around the pseudorotational pathway becomes now forbidden along the C(2′)–*exo*–C(3′)–*exo* road (barriers of about 13 kcal/mole) and remains possible only through the alternant C(1′)–*exo*–C(4′)–*exo* road with a barrier of 5 kcal/mole. This example shows the difficulties connected with the utilization of the empirical partitioned potential function procedures.

The only previous quantum-mechanical computation on the structure of D-ribose is due, as quoted above, to Govil and Saran [23] and was made through the use of the EHT and CNDO methods but did not involve the pseudorotational approach. These authors also found the C(2′)–*endo* and C(3′)–*endo* conformations to represent the stable arrangements of the ring system.

B) Relation Between P and χ_{CN}

1) β-Purine Ribosides

Adenosine and deoxyadenosine have been chosen for the study of the relation between the glycosyl torsion angle χ_{CN} and the pseudorotation parameter P in the series of β-purine ribosides. For each value of P, the geometry of the sugar was taken from the study of D-ribose described above. The torsion angles $\Phi_{C(4')-C(5')}$ and $\Phi_{C(5')-O(5')}$ have been kept the same as in the study of the ribose and deoxyribose moieties. The torsion angle $\Phi_{C(3')-O(3')}$ for both adenosine and deoxyadenosine has been fixed at 180° and the torsion angle $\Phi_{C(2')-O(2')}$ for adenosine was kept at 180°.

The results of the calculations on β-adenosine are shown in Fig. 3. The global minimum occurs at $P = 162°$ (i.e. C(2′)–*endo* conformation) for χ_{CN} varying from 70–100°. There is a large area included within the 1 kcal/mole isoenergy curve associated with this global minimum. There are also three local minima about 1 kcal/mole higher than the global one. Two of them are associated with the same value of P, and correspond to $\chi_{CN} = 240–260°$ and $\chi_{CN} = 300–320°$. The third local minimum also 1 kcal/mole above the global one occurs for $P = 18°$, corresponding to the C(3′)–*endo* conformation, with χ_{CN} varying from 70–125°.

Figure 3 contains also the representation of all the presently known experimental results from X-ray crystal studies on β-purine ribonucleosides and nucleotides. It is easily seen that all the representative points are concentrated around or in the vicinity of the above mentioned calculated global and the lowest local energy minima. Remembering that following Sundaralingam's convention [7] the *anti* conformations are defined by $\chi_{CN} = 0° \pm 90°$ and the *syn* conformations by $\chi_{CN} = 180° \pm 90°$, the results of Fig. 3 account satisfactorily for the fact that while both *anti* and *syn* conformation are found for the C(2′)-*endo* derivatives only *anti* conformations are observed in the case of the C(3′)-*endo* purine nucleosides.

Moreover, the conformational energy map of Fig. 3 also indicates that the possible transition between the C(3′)–*endo* and C(2′)–*endo* conformations of the sugar, if occurring, should do so through the C(4′)–*exo*–C(1′)–*exo* pathway. The energy barrier is about 5 kcal/mole. The transition through the

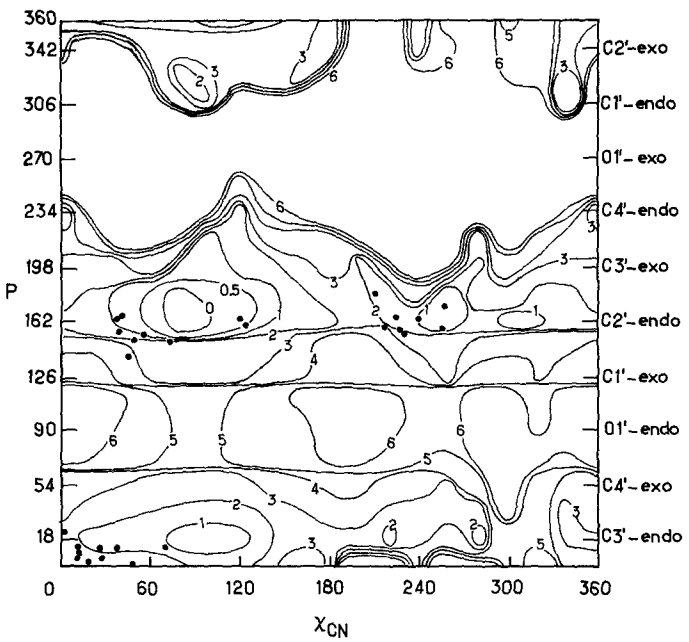

Fig. 3. Conformational energy map, P *vs.* χ_{CN} *in* β-purine ribosides. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero. ● Experimental X-ray results

C(3′)–*exo*–C(2′)–*exo* pathway is practically forbidden as it would involve a large energy barrier.

Figure 4 shows the conformational energy map obtained for β-deoxyadenosine. It is quite similar to that of Fig. 3, with some small variations: the local minimum at $P = 162$, $\chi_{CN} = 240°$ is only 0.5 kcal/mole above the global minimum, which remains at the same place as in Fig. 3 and the minimum at $P = 18°$, $\chi_{CN} = 90°$ although also 1 kcal/mole above the global minimum occupies a much smaller area.

Only two experimental results are known for β-deoxyribosides of purines. These are deoxyadenosine monohydrate [43] and 2′-deoxyguanosine in complex with 5-bromo-2′-deoxycytidine [44]. They are indicated by dots in Fig. 4. The first corresponds to a C(3′)–*exo* conformation of the sugar ($P = 198°$) and an *anti* value for χ_{CN} at about 2 kcal/mole above the global minimum, the second to a C(2′)–*endo* conformation of the sugar at $P = 162°$ and a *syn* value for χ_{CN}, also at about 2 kcal/mole above the global minimum.

The results of the maps of Figs. 3 and 4 may be compared to the results of Sasisekharan [20] obtained with the use of partitioned potential energy functions. Broadly speaking they are similar, showing comparable energy minima and energy barriers. The partitioned potential energy functions put, however, all the energy minima at the same level and appear thus unable to distinguish between them and in particular to pick up the global minimum. They remain therefore from this quantitative point of view behind the performance of the quantum-mechanical computations. This point has already been discussed in a related context in Ref. [3].

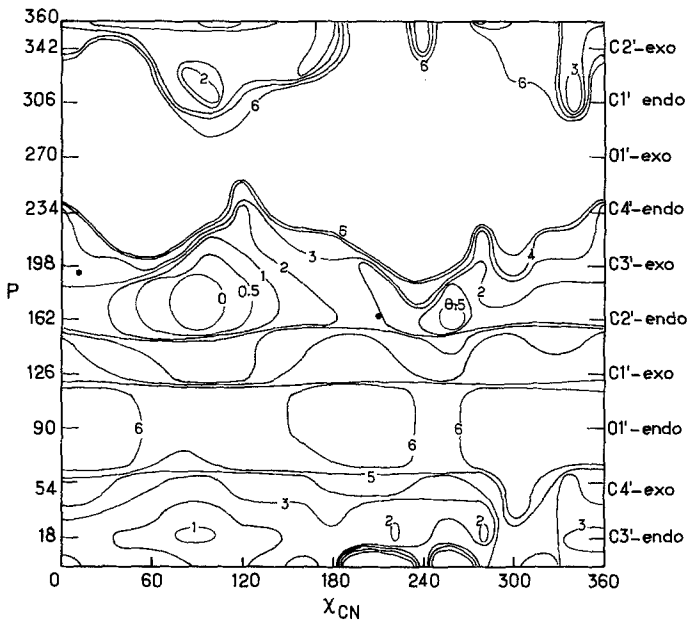

Fig. 4. Conformational energy map, P *vs.* χ_{CN} in β-purine deoxyribosides. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero. ● Experimental X-ray results

2) β-Pyrimidine Ribosides

The compounds utilized in this research were uridine and deoxyuridine. The values of the torsion angles $\Phi_{C(4')-C(5')}$, $\Phi_{C(5')-O(5')}$, $\Phi_{C(3')-O(3')}$ and $\Phi_{C(2')-O(2')}$ were kept the same as in the study of the purines.

Figure 5 shows the results of the computations carried out for β-uridine. This map shows a global minimum for $P = 18°$, corresponding thus to the C(3')–*endo* conformation with χ_{CN} in the *anti* region, varying from 0–45°. There is a large area within 1 kcal/mole associated with this global minimum. Then, there is a local minimum at $P = 162°$, corresponding to the C(2')–*endo* conformation only 1 kcal/mole above the global one, also in the *anti* region (χ_{CN} varying from 50–85°) and another local minimum, also 1 kcal/mole above the global one, associated with $P = 18°$ in the *syn* region (χ_{CN} around 260°). There are also other local minima in the *syn* region associated with $P = 18°$ and 162° but situated 2–3 kcal/mole above the global minimum.

All the experimental results obtained through X-ray crystallography for β-ribonucleosides of pyrimidines are indicated in Fig. 5. It is clearly seen that the great majority of the experimental conformations cluster around the two fundamental *anti* energy minima at $P = 18°$ and 162°. Three representative points are found in the *syn* region: 4-thiouridine [45] with a C(3')–*endo* conformation of the sugar and 6-methyluridine [39] with a C(2')–*endo* conformation which has two molecules in the asymmetric crystallographic unit. They occupy the secondary energy minima predicted for this region.

It may be worth stressing that while the theoretical global energy minimum of adenosine was found for the C(2')–*endo* conformation, the global energy

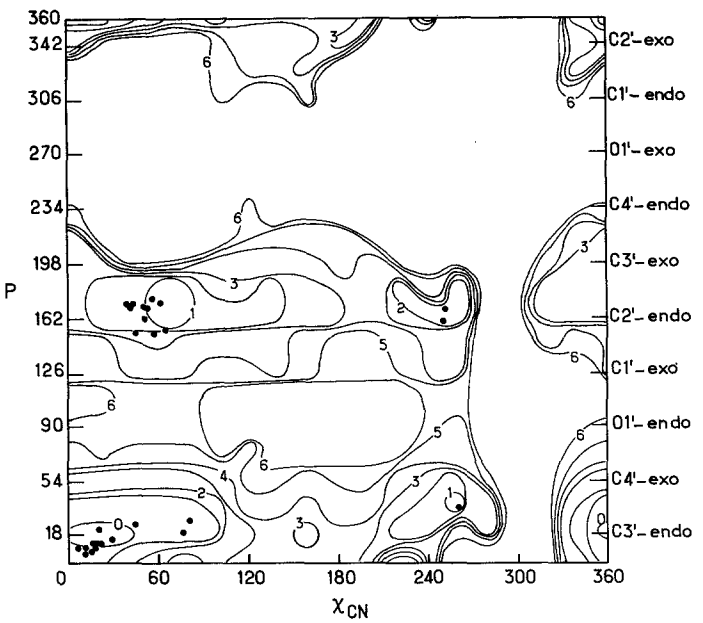

Fig. 5. Conformational energy map, P *vs.* χ_{CN} in β-pyrimidine ribosides. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero. ● Experimental X-ray results

minimum of uridine is associated with the C(3′)–*endo* conformation. The direction of the easiest transition between the two minima remains, however, the same for the pyrimidines as for the purines, namely *via* the C(4′)–*exo*–C(1′)–*exo* path. The barrier is about 6 kcal/mole as compared to 5 kcal/mole found for adenosine.

The results of the computations carried out for β-deoxyuridine are shown in Fig. 6. The map shows two global minima of almost similar depth associated with $P = 18°$ and $P = 162°$ and for χ_{CN} in the *anti* region. There is a particularly large area within the 0.5 kcal/mole isoenergy curve around the global minimum at $P = 162°$. The *syn* regions associated with both $P = 18°$ and $P = 160°$ present local energy minima but at 2 kcal/mole above the global ones.

The experimentally observed conformations in compounds of this category cluster practically exclusively around the large global minimum at $P = 162°$ (C(2′)–*endo* and *anti*). One compound dihydrothymidine [46], the only known compound with a O(1′)–*endo* conformation of the sugar ($P = 84.4°$) lies in a relatively high energy region, at 5 kcal/mole above the global minimum. Possibly the map of Fig. 6 is not well representative of this particular compound. Deoxycytidine 5′-phosphate monohydrate [47], which has a C(3′)–*exo* sugar pucker lies close to the 2 kcal/mole isoenergy curve, not far from the global minimum associated with $P = 162°$. There is only one compound, deoxycytidine hydrochloride [48], associated with the global minimum at $P = 18°$, corresponding to the C(3′)–*endo* conformation of the sugar ring.

The barrier for transition from C(3′)–*endo* to C(2′)–*endo* conformation is 5 kcal/mole, as compared to 6 kcal/mole in the case of uridine and this transition is possible only through the C(4′)–*exo*–C(1′)–*exo* pathway.

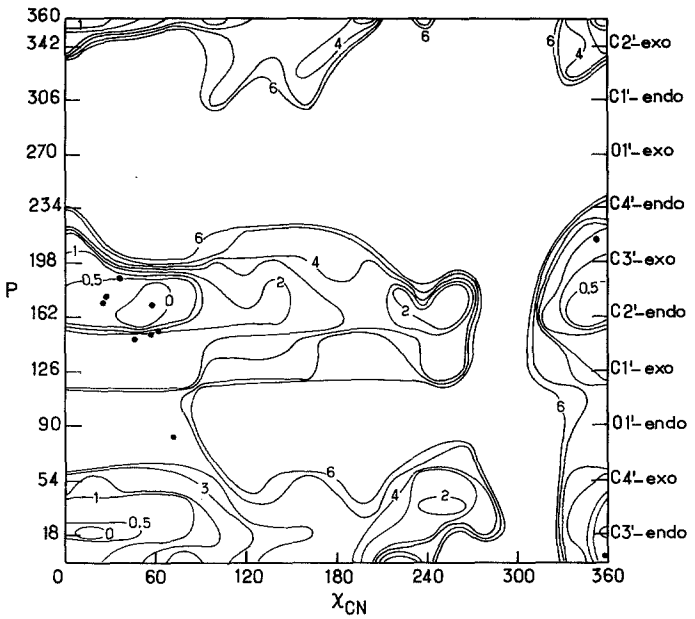

Fig. 6. Conformational energy map, P *vs.* χ_{CN} in β-pyrimidine deoxyribosides. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero. ● Experimental X-ray results

The results of the maps in Figs. 5 and 6 can also be compared with the results obtained from the computations done by using partitioned potential energy functions [20]. Again these last computations fail to distinguish the global minimum among the three minima obtained, one in the *anti* region for the C(3′)–*endo* conformation and two in the *anti* and *syn* regions for the C(2′)–*endo* conformation. 4-thiouridine lies in Sasisekharan's map [20] in high energy zone devoid of a local minimum. However, the transition from the C(2′)–*endo* to the C(3′)–*endo* conformation through the C(3′)–*exo*–C(2′)–*exo* direction is forbidden as in the PCILO calculations. The energy barrier for the transition through the C(4′)–*exo*–C(1′)–*exo* path is of the order of 3 kcal/mole as compared to 5–6 kcal/mole in PCILO computations.

Conclusions

The principal conclusions which can be drawn from this study seem to be twofold. In the first place we continue to observe the extremely satisfactory agreement between the results of the quantum-mechanical PCILO computations and the experimental data shown to exist for the other aspects of the conformations of nucleic acid and their constituents in the previous papers of this series. This satisfactory agreement, in particular with the data from X-ray crystallography, is worth stressing as the calculations are performed for isolated molecules. It indicates the general significance of the global and local energy minima put into evidence by the calculations. Secondly, the results point to the usefulness of the pseudorotational concept in its applications to the confor-

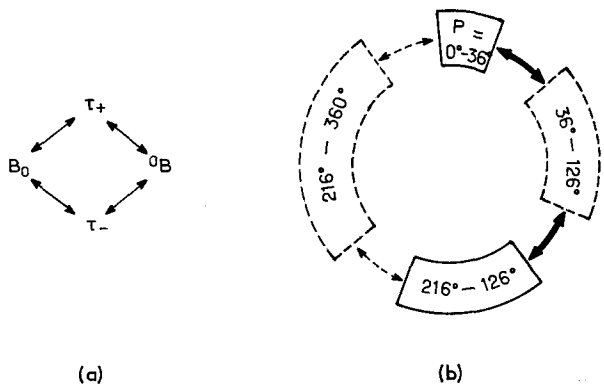

Fig. 7. Classes of states of the pseudorotational itinary of the sugar ring in nucleosides. a Following Hruska *et al.* [18], b Proposed here

mations of the furanose ring. It shows simultaneously the continuity of the deformability of the ring along the pseudorotational pathway and the existence of two zones of stable conformations separated by large zones of relatively unstable ones. This result is particularly important for the comprehension of the conformational equilibrium of nucleosides in solution. Hruska and coworkers [18], in particular, have deduced from their abundant studies of vicinal proton-proton couplings in the sugar rings of nucleosides that the furanose rings exist in dynamic equilibria involving well-defined states corresponding to the two stable conformational zones, centered around the C(3′)–*endo* and C(2′)–*endo* conformations, which they denote by the symbols τ_+ and τ_-, respectively, separated by two barrier zones, corresponding to the two pathways C(1′)–*exo*–C(4′)–*exo* (which they denote by the symbol ^0B) and C(3′)–*exo*–C(2′)–*exo* (which they denote by the symbol B_0). These authors give, however, a symmetrical representation of the overall situation (Fig. 7a). An unsymmetrical representation such as given in Fig. 7b seems to describe more adequately the real situation in nucleosides.

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