Structural Aspects of Nucleosides: Protonated and Complexed Adenosines

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To gain insight into the structural changes exerted by protonation or complexation of the adenine in nucleosides, the X-ray structures of adenosines were compared with their protonated or complexed congeners. Comparison of a variety of bond angles, bond lengths, and torsional angles in and around the ribose ring revealed only small differences. The specific case of the 5′-deoxy-5′-adenosyl moiety covalently bonded to the Co-atom in coenzyme B_{12} is discussed.

Introduction. – Adenosine, guanosine, cytidine, and thymidine are the four most important nucleosides found in nature. They consist of a sugar moiety bound to a heterocycle and are converted to nucleotides upon phosphorylation. Tri- and diphosphorylated adenosine, ATP and ADP, provide the energy for activating many enzymatic transformations. The 3′,5′-cyclic monophosphate of adenosine (cAMP) plays an important role in controlling and mediating the actions of peptide hormones. The propensity of these heterocylic bases to form H-bonds leads to specific interactions and associations. This is apparent in the unique base-pairing motifs found in DNA and RNA but is also observed in the specific interactions with proteins. For understanding the nature of the interactions, the three-dimensional structures of these nucleosides have been extensively studied [1a].

The typical structural features of nucleosides may be described as follows: The purine or pyrimidine ring is oriented either *anti* or *syn* relative to the ribose ring with no specific preference being apparent (*Fig. 1*) [1a][1]). This may be interpreted in terms of a low rotational barrier for a $C(sp^3)-N(sp^2)$ bond [3a]. The puckering of the ribose ring is best described by two conformations with a C(2′)-*endo* or a C(3′)-*endo* orientation (*Fig. 2*). In some structures, the intermediate conformation described as C(2′)-*endo*-C(3′)-*exo* and C(2′)-*exo*-C(3′)-*endo* is also populated. According to quantum-chemical results, these two conformations undergo rapid equilibrium *via* an O(4′)-*endo* orientation with a barrier of *ca.* 24 kJ/mol [1a]. The transannular distance between the N-atom of the heterocycle bound to C(1′) of the ribose and C(5′) of the exocyclic CH_2(5′)-OR group is in the range of 4.6 Å and depends on the puckering of the ribose

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[1]) For instance, the conformations around bond N(9)–C(1′) are given by the torsional angle τ(C(8)–N(9)–C(1′)–O(4′). For *anti* orientations, $180° \geq \tau \geq 0°$, whereas for *syn* arrangements $0° \geq \tau \geq -180°$ (*Fig. 1*). This definition includes the *anti*-periplanar (180°) and *syn*-periplanar (0°) orientations as defined by *Klyne* and *Prelog* [2]. It is different from the special definition used in nucleotide stereochemistry (*cf.* Fig. 2-5 and 2-10 in [1a]). For the numbering of atoms, see *Fig. 1*.

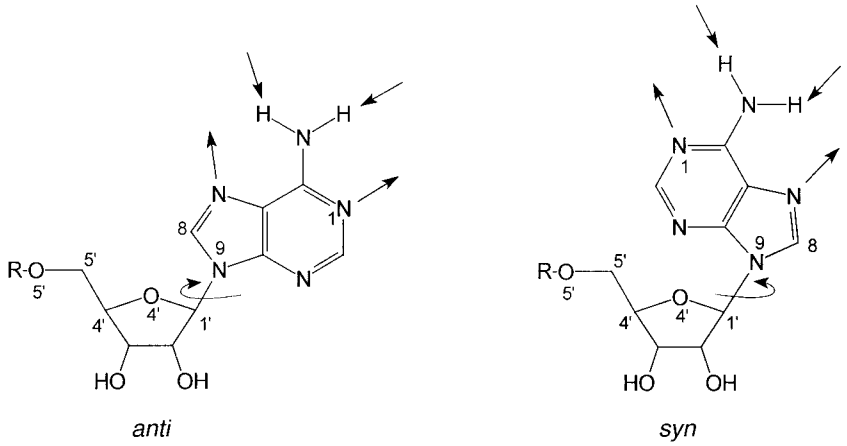

Fig. 1. *Conformation* (*anti* or *syn*) of the adenine moiety with respect to the ribose ring

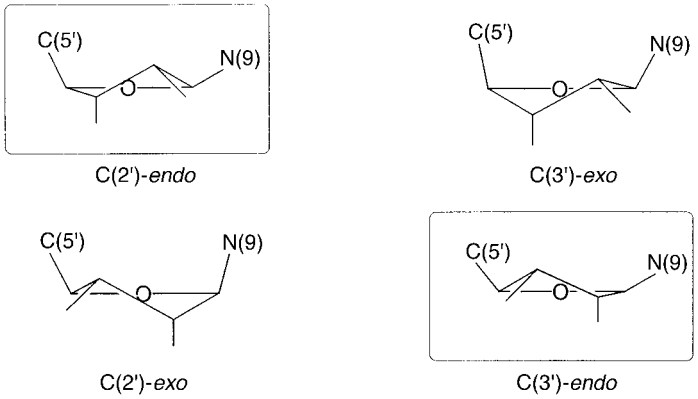

Fig. 2. *Ribose C(2′)*-endo, *C(2′)*-exo, *C(3′)*-exo, *and C(3′)*-endo *conformations of purine nucleosides*

ring. Three main conformations are associated with the torsional angle $\tau(O(5')-C(5')-C(4')-O(4'))$ (see *Fig. 3*)[2]).

Whereas the structural features of these nucleotides and, hence, of the nucleosides are well-established, the analoguous structures with the heterocyclic bases protonated or complexed have hitherto not been explored. In view of the importance of the complexation of nucleosides and nucleotides for activation of their reactions, the structural features of these two closely related classes of compounds are of interest.

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[2]) The notation used in *Fig. 3* is based on the *Klyne* and *Prelog* notation [2] and refers to the torsional angle $\tau(O(5')-C(5')-C(4')-O(4'))$. It differs from the definitions given by *Saenger* [1b], where the orientations around the C(5′)–C(4′) are described by $\phi_{OC}(O(5')-C(5')-C(4')-C(3'))$ with the torsional angle γ and the specifications $+sc$, *ap*, and $-sc$. In our notation, the $+sc$ conformation with $\tau(O(5')-C(5')-C(4')-O(4'))=+60°$ corresponds to ϕ with $\gamma=180°$ (*ap*, *gauche*, *trans*), while the $-sc$ arrangement ($\tau(O(5')-C(5')-C(4')-O(4'))=-60°$) corresponds to $\gamma=+60°$ ($+sc$, *gauche*, *gauche*) (*Fig. 3*).

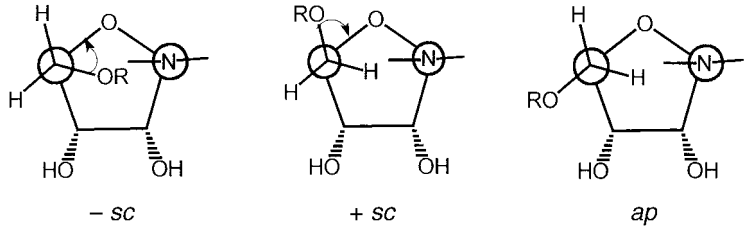

Fig. 3. *Torsional angle τ(O(5′)–C(5′)–C(4′)–O(4′)) in the ribose moiety*

Whereas H-bonding, apparent in the *Watson-Crick* and *Hoogsteen* base pairing of nucleotides in DNA, leads to rather small structural changes, the structural and, hence, the chemical consequences of a complete transfer of H^+ to one (or more) of the N-atoms of the hetereocylic moieties in nucleosides were not clear[3]). In particular, it was of interest to learn whether any structural change emanating from the base upon protonation would be felt across the ribose ring by the $CH_2(5')–O(5')–R$ group located *cis* to the heterocycle. To gain insight into the structural changes induced by protonation or complexation with transition-metal complexes, the structural features of these compounds were analyzed and compared with those of the 'free' nucleosides. For this analysis, the relevant structures were retrieved from the *Cambridge Structural Data Base* [5], and here we report the results of this study.

Results and Discussion. – syn *and* anti *Conformations.* From the *CSD* file, containing 215403 structures (July 2000), 399 structures were retrieved containing a nucleoside with a purine or pyrimidine base. A subset of 46 structures containing adenosine with a protonated (at N(1)) or complexed (mainly at N(7)) adenine moiety and 48 structures with a 'free' adenine moiety, both with $R \leq 10\%$, were obtained from this family of structures (*Fig. 1*). In the set of the 'free' adenosines (abbreviated as '*A*'), 32 structures show an *anti* conformation of the base relative to the ribose ring, with torsional angle $\tau = 3.7$ to $121.8°$, whereas 16 structures exhibit a *syn* conformation $\tau = -142.1$ to $-8.2°$) (*cf. Fig. 4,a*). In the set of protonated or complexed adenosines (abbreviated as '*AH*'), 36 structures with an *anti* conformation ($\tau = 7.8$ to $108.1°$) and 10 with a *syn* arrangement ($\tau = -144.3$ to $-2.6°$) were found (see *Fig. 4,b*).

Bond Lengths. Comparison of the average bond lengths of the *A* set with those of the *AH* series reveals very similar results. ANOVA tests on the hypothesis that the means of the bond distances $d(N(9)–C(1'))$ and $d(C(1')–O(4'))$ in the *AH* and the *A* set (*Table 1*) are equal cannot be rejected on a 1% significance level [6]. Thus, it appears that the protonation or complexation of the adenine moiety at one of the N-atoms is not translated into a change of these bond lengths.

The bond distances $d(O(4')–C(4')$ and $d(C(4')–C(5'))$ being very similar in the *A* and the *AH* set for both the *anti* as well as the *syn* conformers, it is apparent that

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[3]) Recently, *Lippert* and co-workers described a 10^9-fold acidification of the 6-amino group of adenine in an open, platinated nucleobase quartet consisting of two 9-ethyladenine, two 1-methyluracilate, two *trans*-$[Pt(MeNH_2)_2]$, and one $[Pt(NH_3)_2]$ moieties, with the Pt-atoms bonded to N(1) and N(7) in both 9-ethyladenines [4].

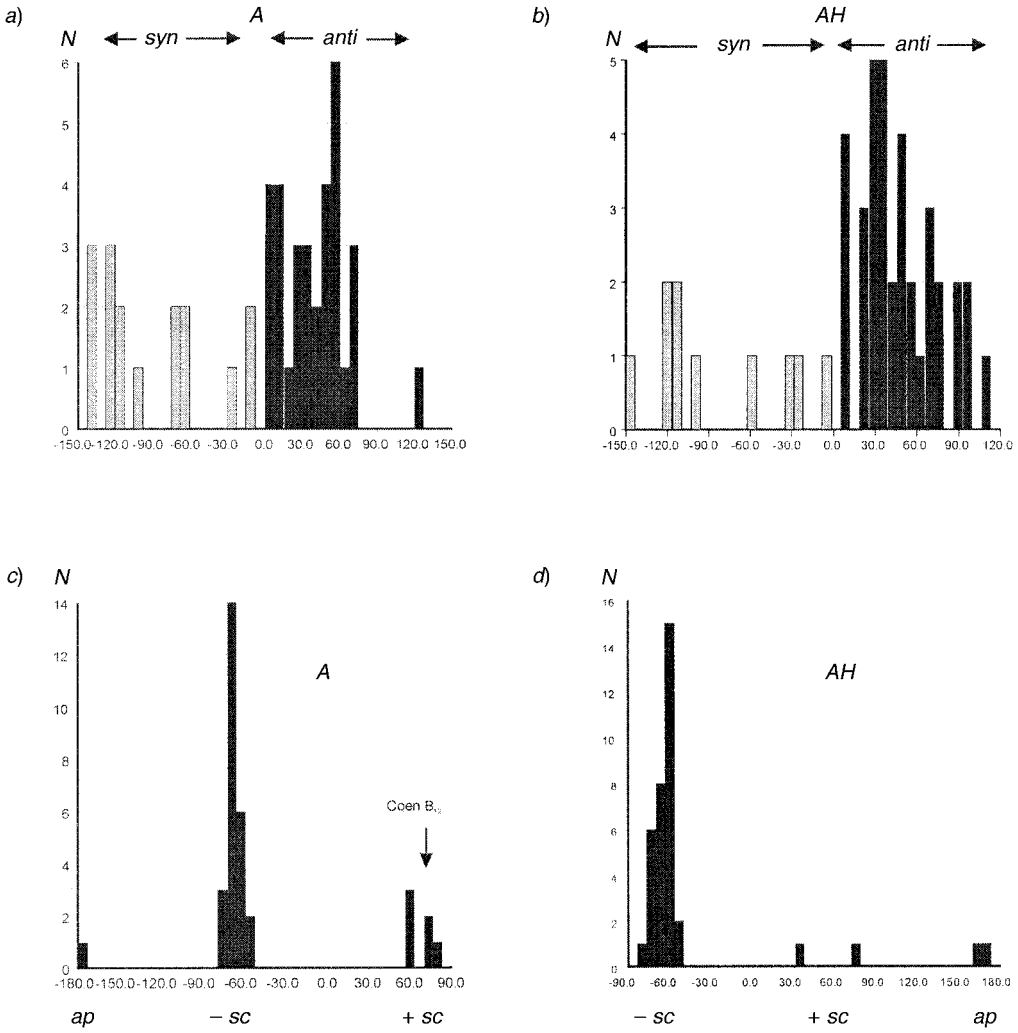


Fig. 4. a) b) *Population* N *of structures with* anti *and* syn *orientation of the adenine moiety relative to the ribose ring* (τ(C(8)–N(9)–C(1′)–O(4′), [°]) *for the* A *and* AH *set, respectively.* c) d) *Population* N *of conformations around C(5′)–C(4′)* [°] (*cf. Fig. 3*) *for the* anti *structures in the* A *and* AH *set, respectively.*

Table 1. *Average Bond and Nonbonding Distances* d [Å]. For numbering, see *Fig. 1.*

	d(N(9)–C(1′))	*d*(C(1′)–O(4′))	*d*(C(4′)–O(4′))	*d*(C(4′)–C(5′))	*d*(C(5′)–O(5′))	*d*(N(9)–C(5′))	*d*(C(8)–O(5′))
A: 32 *anti*	1.464(5)	1.406(5)	1.448(3)	1.506(5)	1.45(1)	4.22(3)	3.8(1)
16 *syn*	1.455(7)	1.421(6)	1.448(6)	1.516(7)	1.400(7)	4.34(7)	5.5(2)
AH: 36 *anti*	1.479(4)	1.410(3)	1.457(5)	1.516(4)	1.442(5)	4.19(1)	3.6(1)
10 *syn*	1.460(7)	1.428(7)	1.451(7)	1.517(7)	1.44(1)	4.29(5)	5.1(4)
Coenzyme B_{12}	1.477	1.448	1.451	1.450	–	4.363	–

protonation or complexation of the adenine moiety is not translated across the ribose ring.

Furthermore, the correlations between the bond distances d(N(9)−C(1′)) and d(C(1′)−O(4′)) in the *anti* and the *syn* sets of the *A* as well as of the *AH* structures are very small. This indicates that an anomeric effect [3b] in the N(9)−C(1′)−O(4′) substructure is apparent in neither the adenosine structures nor the protonated and complexed congeners.

The average of the transannular distances d(N(9)−C(5′)) in the *anti* set of the *AH* structures is $d = 4.19(1)$ Å, whereas $d = 4.29(5)$ Å for the ten structures with a *syn* conformation. For the set *A*, the corresponding values are $d = 4.22(3)$ Å (*anti:* 32 structures) and $d = 4.34(7)$ Å (*syn*; 16 structures) (*Table 1*). No correlations were detected between the conformations of the adenine moiety and the nonbonding distance d(N(9)−C(5′)) for the *anti* and *syn* subsets of the *AH* and *A* structures.

Bond Angles. Comparison of the bond angles ω(N(9)−C(1′)−O(4′)), ω(O(4′)−C(4′)−C(5′)), and ω(C(4′)−C(5′)−O(5′)) given in *Table 2* reveals that the corresponding values are very similar in the *A* and in the *AH* set, particularly for the *anti*-orientation of the adenine moiety.

Table 2. *Bond Angles* ω [°]. The terms *anti* and *syn* refer to the torsional angle τ(C(8)−N(9)−C(1′)−O(4′)).

	ω(N(9)−C(1′)−O(4′))	ω(O(4′)−C(4′)−C(5′))	ω(C(4′)−C(5′)−O(5′))
A: anti 32	108.3(3)	109.4(4)	109.4(4)
syn 16	107.9(7)	109.4(3)	113.4(4)
AH: anti 36	107.8(2)	109.1(5)	109.5(5)
syn 10	108.5(7)	108.3(7)	110(1.6)
Coenzyme B_{12}	105.807	109.270	(125.37)

Torsional Angles. In the *AH* as well as the *A* structures, the three staggered conformations given by τ(O(5′)−C(5′)−C(4′)−O(4′)) are each populated to a different extent (*cf. Fig. 3* and *Fig. 4, c* and *d*, resp.). The *-sc* conformations are highly populated in both the *AH* as well as in the *A* set. In the *AH* structures with the *anti* arrangement (with C(8) of the adenine moiety above the ribose ring; $180° \geq \tau \geq 0°$; 36 examples), an average torsional angle of $-64.3°$ is calculated for 32 structures, while each of two structures show a $+sc$ and an *ap* conformation. In the *anti* set of *A* structures (32 examples), an average $-sc$ torsional angle of $-66.4°$ is found for 25 structures. Only six structures are found with a $+sc$ and 1 structure with an *ap* conformation for O(5′)−C(5′)−C(4′)−O(4′) in the *A* set (*Fig. 3*). The strong preference of the conformers with $-sc$ orientations about the C(5′)−C(4′) bond found in the X-ray structures is reminiscent of the structure of 1,2-dimethoxyethane in the liquid phase, where the *gauche* conformation for C(1)−C(2) has the lowest energy, and in the crystalline phase, where only the *gauche* conformation is observed [7]. This preference is interpreted in terms of the anomeric effect, part of which may be associated with a hyperconjugative stabilization *via* a σ(C−H)/σ*(C−O) interaction. The number of examples with a $+sc$ conformation around the C(5′)−C(4′) bond is too small (six structures) for a more detailed discussion of a possible anomeric effect.

The strong preference for the $-sc$ conformations in the *anti* sets of the *AH* as well as of the *A* structures with rather short nonbonding distances between C(8) and O(5′)

corroborates the observations discussed by *Saenger* [1c]: In the highly populated *– sc* conformers (*Fig. 3*) with an *anti* orientation of the adenine moiety, the nonbonding distances *d*(C(8)–O(5′)) are rather short with average values of 3.6(1) Å for the 36 *AH* and 3.8(1) Å for the 32 *A* structures. If in the *anti* sets (C(8)–N(9)–C(1′)–O(4′): $120^\circ > \tau > 0^\circ$), the conformations around C(5′)–C(4′) are restricted to the *– sc* orientation, the nonbonding distances *d*(C(8)–O(5′)) are 3.39 Å in the *AH* set (32 of 36 structures) and 3.55 Å for the *A* set (25 of 32 structures). They are shorter than the sum of the *van-der-Waals* radii for C, H, and O, suggesting a bonding interaction between O(5′) and the H-atom at C(8) [1d]. In the *anti AH* as well as in the *anti A* set of structures, the values of the two parameters, τ(O(5′)–C(5′)–C(4′)–O(4′)) and *d*(C(8)–O(5′)) each cluster around the average values and do not show a distinct correlation.

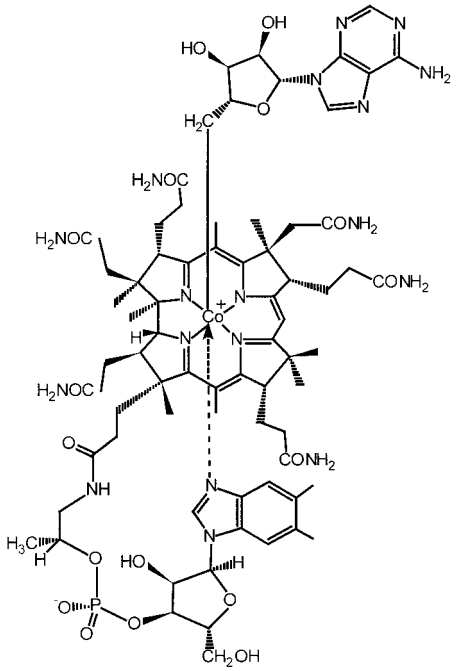
Puckering of the Ribose Ring. The analysis of the ribose puckering – given by the phase angle *P* for the pseudorotation (Fig. 4–3 in [1a]) – shows in both the *AH* and the *A* set a small preference for a C(3′)-*endo* over a C(2′)-*endo* mode (*Table 3*). The ratio C(3′)-*endo*/C(2′)-*endo* (*AH* 25:21, *A* 28:20) includes all structures regardless of whether the adenine moiety is *anti*- or *syn*-oriented relative to the ribose ring. A more detailed analysis, where only the structures with an *anti* conformation of the base ($180^\circ \geq \tau \geq 0^\circ$ for τ(C(8)–N(9)–C(1′)–O(4′)) and with the *– sc* conformations for the torsional angle τ(O(5′)–C(5′)–C(4′)–O(4′)) are considered, reveals for the 25 *A* samples a C(3′)-*endo*/C(2′)-*endo* ratio of 14:11 and for the 32 *AH* samples a ratio of 19:13. In the case of the C(3′)-*endo* conformation, the range of *P* is $360 - 50^\circ$ for the 28 *A* structures, and $347 - 52^\circ$ for the 25 *AH* structures. In the case of the C(2′)-*endo* conformation, *P* is in the range of $144 - 156^\circ$ for the 20 *A* structures, and in the range of $145 - 189^\circ$ for the 21 *AH* structures.

Table 3. *Puckering of the Ribose Ring* (phase angle *P* [°]) *and Nonbonding Distances* d *(N(9)–C(5′)) and* d*(C(8)–O(5′))* [Å]

	Σ of samples	Range of phase angle *P* [°]	*d*(N(9)–C(5′)	*d*(C(8)–C(5′))
A: C(2′)-*endo*	20	144.4–156.4	4.18(3)	4.3(2)
C(3′)-*endo*	28	359.9–49.7	4.33(4)	4.5(2)
AH: C(2′)-*endo*	21	145.5–189.4	4.21(2)	4.1(2)
C(3′)-*endo*	25	347.4–51.9	4.21(2)	3.7(2)
Coenzyme B_{12}	1	33.2 (C(3′)-*endo*)	4.363	–

This indicates that protonation or complexation of the adenine moiety does not affect the conformational preferences of the ribose ring in these adenosines.

The Unique Structure of Coenzyme B_{12}. Coenzyme B_{12} is the most unique adenosine structure, with a 5′-substituent different from OR (see *Formula*). It plays an important role as coenzyme in the methylmalonlyl CoA mutase and other mutases [8]. Triggered by the association with the substrate, coenzyme B_{12} is activated by homolysis of the Co–C bond, leading to Co^{II} and the 5-deoxy-5′-adenosyl radical. The latter activates the substrate by abstracting a H-atom from the appropriate position [9]. It is of particular interest to compare its structure with those of the neat adenosines described above. According to the torsional angle τ(C(8)–N(9)–C(1′)–O(4′)) $= +74.7^\circ$ the adenine moiety in coenzyme B_{12} adopts an *anti* conformation. The bond lengths *d*(N(9)–C(1′))



coenzyme B_{12}

and d(C(1′)–O(4′)) are both slightly longer than the average bond distances in the *anti A* set (*cf. Table 1*) and do not give a clue for an anomeric effect.

The phase angle P indicates a C(3′)-*endo* conformation of the ribose ring; the nonbonding distance d(N(9)–C(5′)) is very close to the average value calculated for the *A* set with a C(3′)-*endo* conformation (*Table 1*). From the torsional angle τ(Co–C(5′)–C(4′)–O(4′)) = +77.2°, it is concluded that a +*sc* (*gauche*) conformation (*Fig. 3*) around the C(5′)–C(4′) bond is apparent in coenzyme B_{12}. According to model considerations, an 'inside' (= –*sc*) conformation can be excluded for steric reasons. Since the 5′-deoxy-5′-adenosyl moiety in coenzyme B_{12} shows a +*sc* conformation (*cf. Fig. 3*, +*sc* conformation with RO(5′) replaced by {Co}) and not an *ap* conformation, a hyperconjugative interaction of the type σ(Co–C(5′))/σ*(C(4′)–O(4′)) cannot be very large. Comparison of the bond angles ω(C(4′)–C(5′)–O(5′)) in the *anti* set of the *A* (and the *AH*) structures with the bond angle ω(C(4′)–C(5′)–Co) in coenzyme B_{12} reveals a considerable opening of this bond angle (*Table 2*).

Conclusions. – A detailed structure analysis of adenosines and their congeners protonated or complexed at one of the N-atoms of the adenine moiety was undertaken to detect structural differences. Comparison of the 48 adenosine structures with those 46 structures containing a protonated or complexed heterocycle reveals only small changes in and around the ribose ring. Thus, a structural transfer of the base protonation into structural changes of the exocyclic CH_2(5′)–OR group is highly

unlikely. In view of these results, the activation of coenzyme B_{12} for enzymatic catalysis must require deep-seated structural changes rather than a simple H^+ transfer from the enzyme to the adenosine [10–12].

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