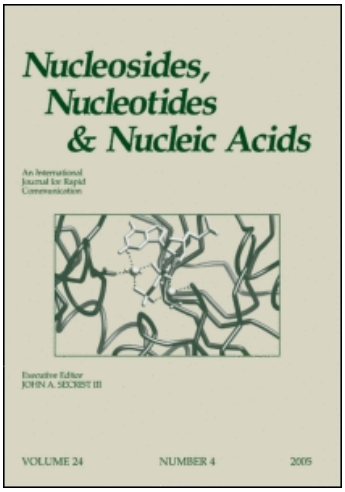

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Xanthine-7-Ribosides as Adenosine A_1 Receptor Antagonists: Further Evidence for Adenosine's Anti Mode of Binding

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XANTHINE-7-RIBOSIDES AS ADENOSINE A_1 RECEPTOR ANTAGONISTS: FURTHER EVIDENCE FOR ADENOSINE'S ANTI MODE OF BINDING.

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Abbreviations used: DPCPX: 1,3-dipropyl-8-cyclopentylxanthine; R-PIA: R-1-phenyl-2-isopropyladenosine; NECA: 5'-N-ethylcarboxamidoadenosine; HMDS: hexamethyldisilazane; TCS: trichlorosilazane; ABR: 1-O-acetyl-2,3,5-tribenzoylribofuranose; DMSO: dimethylsulfoxide; DCM: dichloromethane; GTP: guanosine-5'-triphosphate.

Abstract. The synthesis and A_1 adenosine receptor affinity of some xanthine-7-ribosides is described. It appears that these compounds are A_1 receptor antagonists. The orientation of the ribose moiety, as determined by ^{1}H-NMR spectroscopy and theoretical chemical calculations, is compared with the orientation of the ribose in the agonist adenosine. Implications for the *syn* vs *anti* modes of binding to the receptor are discussed.

INTRODUCTION

Adenosine receptors, which mediate the profound cardiovascular and central depressant effects of adenosine, have been divided into two major classes, A_1 and A_2.[1] All known adenosine agonists are closely related to the chemical structure of adenosine itself. Substitution at N^6 or C2 may enhance affinity and may impart A_1/A_2 selectivity.[2] Other modifications of the basic structure usually lead to inactive compounds. An intact ribose moiety seems to be especially important for full agonist activity.[3]

In contrast to adenosine agonists, adenosine antagonists may belong to quite dissimilar chemical classes, of which the xanthines are well known and best studied. The central stimulant caffeine (1,3,7-trimethylxanthine) and the anti-asthmatic theophylline (1,3-dimethylxanthine) belong to this class.

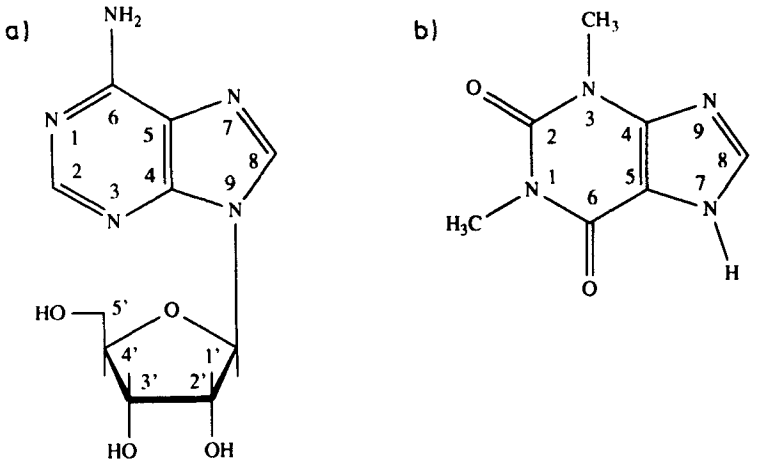


FIG. 1. Structures and ring numbering of adenosine (a) and theophylline in a flipped orientation (b).

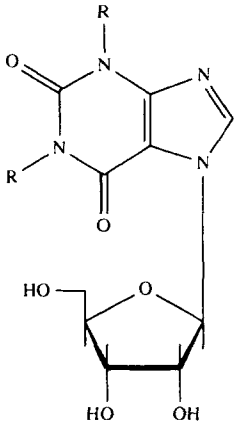
With the aid of molecular modeling methods, we recently developed a model for the antagonist binding site of the adenosine receptor, based on steric, electrostatic and hydrophobic properties of various adenosine antagonists.[4]

According to this model, theophylline binds to the adenosine receptor in a flipped orientation, i.e. the ring atoms N1, N3, N7 and N9 in adenosine coincide with C2, C6, N9 and N7 respectively in theophylline (FIG. 1). This implicates that the domain where the ribose moiety of adenosine binds must be adjacent to N7 in xanthines.

To further explore the role of the orientation of the ribose moiety in binding to the receptor, we have synthesized the 7-ribosides of theophylline, 1,3-dipropylxanthine and 1,3-dibutylxanthine (FIG. 2). Here, we report on their A_1 affinity and on the conformational properties about the glycosidic bond, as determined by ^{1}H-NMR spectroscopy and theoretical calculations, with special reference to the *syn* versus *anti* modes of binding to the receptor.

EXPERIMENTAL

Chemistry: The procedure for the synthesis of xanthine-7-ribosides was adapted from Vorbrüggen et al.[5-7] The appropriate xanthine was first silylated with



	R
theophylline-7-riboside	$-CH_3$
dipropylxanthine-7-riboside	$-nC_3H_7$
dibutylxanthine-7-riboside	$-nC_4H_9$

FIG. 2. Structures of xanthine-7-ribosides.

TCS and subsequently reacted with ABR in acetonitrile, in the presence of silylated nonaflate as a catalyst. After deprotection of the sugar with methanolic ammonia, purification over a silica column and subsequent crystallization, the product aimed for was obtained. The so-called one-step one-pot synthesis of Vorbrüggen was not succesful in our hands: the silylated xanthine had to be isolated before coupling with ABR would occur. Optimum yield was not aimed for.

Melting points (uncorrected) were determined on a Büchi-Tottoli capillary melting point apparatus. ^{1}H-NMR spectra were measured in D_6-DMSO on a JEOL FNM FX 200 at ambient temperature. Peaks of the ribose moiety could be assigned unambiguously by means of selective decoupling. UV-spectra were recorded on a Shimadzu UV-190. TLC was performed on Merck silica GF_{254} plates with DCM/methanol/ammonia 25% (85:15:1) as the eluent. Acetonitrile was purified as described.[5] Xylene was dried overnight over $CaCl_2$ and subsequently distilled from P_2O_5. Methanolic ammonia was prepared by leading NH_3 through ice-cold methanol until saturation (c.a. 3 hours). All evaporations were performed in vacuo in a rotary film evaporator.

HMDS, TCS and ABR were from Janssen Chimica, Beerse, Belgium. Potassium nonaflate was a gift of Bayer AG, Leverkussen, FRG. 1,3-

Dipropylxanthine and 1,3-dibutylxanthine were gifts from Duphar, Weesp, the Netherlands. An authentic sample of theophylline-7-riboside was provided by Dr. H. Vorbrüggen of Schering AG, Berlin, FRG. All gifts are gratefully acknowledged. All other chemicals were from standard commercial sources and of analytical grade.

Theophylline-7-β-D-ribofuranoside: Theophylline (300 mg, 1.7 mmol) was refluxed overnight in 10 ml HMDS, with 200 µl TCS added. The reaction mixture was coevaporated twice with 20 ml xylene and immediately dissolved in 20 ml acetonitrile. ABR (860 mg, 1.7 mmol), potassium nonaflate (1340 mg, 4.1 mmol) and TCS (650 µl, 5.1 mmol) were added and the mixture was heated until reflux. The reaction was monitored on TLC. After 30 min., a reaction product was detected with a hR_f of 0.78. After 2 hours, when no more theophylline (hR_f 0.32) and ABR (hR_f 0.89) was consumed, the reaction was stopped. 25 ml DCM was added and the solution was extracted twice with 30 ml of saturated $NaHCO_3$. The $NaHCO_3$-fraction was extracted twice with 20 ml DCM. The combined organic layers were extracted twice with 20 ml of saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4, dissolved in 50 ml methanolic ammonia and left at room temperature for three days. The reaction mixtured was evaporated to dryness and 20 ml water was added to the residue. The fraction that did not dissolve was filtered off and the solution was extracted twice with 10 ml ether. The precipitate which was formed was filtered off. The water layer was evaporated to dryness and the resulting yellow oil was applied to a silica 60 G column and eluted with DCM/methanol/ammonia 25 % (85:15:1). The fractions that contained the product aimed for were evaporated to dryness to yield a brown glass, which was crystallized from isopropanol/methanol to yield 30 mg of white needles.

The product has the same chromatographic properties as an authentic sample. Mp. 193 °C, undepressed by the authentic sample (lit.: 191 °C[5]). UV-spectrum (methanol): maximum at 276 nm, shoulder at 231 nm. The NMR spectrum was identical with the authentic sample: H^8: 8.47 (1H, s); H1': 6.10 (1H, d); 2'-OH: 5.50 (1H, d); 3'-OH: 5.19 (1H, d); 5'-OH: 5.08 (1H, t); H2': 4.30 (1H, q); H3': 4.06 (1H, q); H4': 3.90 (1H, q); H5': 3.62 (1H, m); $-CH_3$: 3.43 (3H, s) and $-CH_3$: 3.22 (3H, s) ppm.

1,3-Dipropylxanthine-7-β-D-ribofuranoside: 1,3-Dipropylxanthine (400 mg, 1.7 mmol) was refluxed with HMDS (10 ml) and TCS (250 µl) for 2.5 hours. The reaction mixture was coevaporated thrice with 20 ml xylene. The subsequent reaction with ABR, workup and deprotection with methanolic ammonia were exactly as described for theophylline-7-β-D-ribofuranoside. Following reaction with methanolic ammonia, the mixture was evaporated to dryness and the residue was dissolved in 20 ml water. This was extracted twice with 10 ml ether and the ether layer was back-extracted twice with 10 ml water. The water fraction was evaporated to dryness. The resulting brown oil was applied to a silica column and was eluted with chloroform/methanol (75:25). After combination of the appropriate fractions and evaporation of the solvent a glass was obtained which was crystallized from xylene to yield white needles (85 mg). Mp. 184 °C. UV-spectrum (methanol): maximum at 276 nm. The structure was confirmed by NMR spectroscopy: H^8: 8.46 (1H, s); H1': 6.10 (1H, d); 2'-OH: 5.50 (1H, d); 3'-OH: 5.18 (1H, d); 5'-OH: 5.05 (1H, t); H2': 4.32 (1H, q); H3': 4.06 (1H, q); H4' and $-CH_2-$: 4.00-3.75 (5H, m); H5': 3.60 (1H, m); $-CH_2-$: 1.58 (4H, m) and $-CH_3$: 0.84 (6H, m) ppm.

1,3-Dibutylxanthine-7-β-D-ribofuranoside: 1,3-Dibutylxanthine (425 mg, 1.7 mmol) was refluxed with HMDS (10 ml) and TCS (250 µl) for 1 hour. Reaction with ABR and workup were as described for theophylline-7-β-D-ribofuranoside. After reaction with methanolic ammonia for three days, the methanol was evaporated, the resulting brown oil was applied to a silica column which was eluted with DCM/methanol/ammonia 25 % (85:15:1). After combination of the appropriate fractions and evaporation of the solvent, a glass was obtained which was crystallized from xylene to yield off-white crystals (75 mg). Mp. 125-126 °C. UV: maximum at 276 nm (methanol). The structure was confirmed by NMR spectroscopy: H^8: 8.45 (1H, s); H1': 6.10 (1H, d); 2'-OH: 5.48 (1H, d); 3'-OH: 5.14 (1H, d); 5'-OH: 5.04 (1H, t); H2': 4.32 (1H, q); H3': 4.06 (1H, q); H4' and $-CH_2-$: 4.03-3.82 (5H, m); H5': 3.61 (1H, m); $-CH_2-$: 1.56 (4H, m); $-CH_2-$: 1.27 (4H, m) and $-CH_3$: 0.88 (6H, m) ppm.

Binding assays: Binding assays were performed with calf brain cortical membranes and [^{3}H]DPCPX as the radioligand. Membranes were prepared as described previously.[8] Assays were performed in 50 mM Tris-HCl buffer (pH 7.4)

in a final volume of 400 µl. The standard assay contained approximately 25 µg membrane protein and 0.1 nM [^{3}H]DPCPX. Where indicated, GTP was added to a final concentration of 500 µM. Incubations were carried out for 60 min. at 25 °C and were terminated by rapid filtration through Whatman GF/C glass fiber filters. Subsequently, tubes were washed twice with 1 ml buffer and filters were washed twice with 3 ml buffer. Filters were dried for 45 min. at 60 °C, 3.5 ml scintillation fluid (LKB OptiPhase MP) was added and radioactivity was determined in a LKB 1214 Rackbeta liquid scintillation counter. Solutions of tested compounds were made in DMSO and were diluted with buffer to a final DMSO-concentration of 1 % at the most, which had no influence on specific binding. Non-specific binding was determined in the presence of 10 µM R-PIA. K_i-values were computed with a non-linear regression program described previously.[9] K_i-values are means determined in three independent experiments. All concentrations were tested in duplicate.

Computations: Visualization and manipulation of structures was performed with the molecular modeling program Chem-X (april 1989 update)[10] running on a VAX 11/785 computer and employing a Pericom MX 7200 color display. Minimum energy conformations and conformational searches were computed on a Convex C-120 mini-super computer with the semi-empirical molecular orbital MOPAC program.[11] The AM1 Hamiltonian and Pulay's method of convergence were used.

The rotational flexibility about the glycosidic bond of adenosine was studied as follows. The crystal structure of adenosine[12] was fully minimized in MOPAC. The resulting structure was used as a starting point for a conformational search in which the glycosidic torsion angle was increased in steps of 10°, the calculation of each step starting from the conformation found in the previous step. Whereas the adenine skeleton was kept rigid, the ribose moiety was allowed to relax fully.

Similarly, a conformational search of theophylline-7-riboside was performed. To the crystal structure of theophylline[13], a ribose moiety was added on-screen. This structure was fully minimized in MOPAC, and the resulting structure in its turn was used as a starting point for the conformational search of theophylline-7-riboside. Again, the ribose moiety was allowed to relax fully, while the xanthine moiety was kept rigid.

TABLE 1. A_1 receptor affinities and Hill coefficients of tested compounds in the absence and presence of 500 μM GTP*

Compound	absence of GTP		500 μM GTP added	
	K_i	n_H	K_i	n_H
Theophylline	6.0 ± 0.3 μM	1.03 ± 0.03	7.2 ± 0.5 μM	0.99 ± 0.03
Dipropylxanthine	0.26 ± 0.02 μM	1.01 ± 0.07	0.27 ± 0.03 μM	1.00 ± 0.05
Dibutylxanthine	0.14 ± 0.01 μM	0.91 ± 0.08	0.14 ± 0.01 μM	0.95 ± 0.06
Theophylline-7-riboside	58 ± 3 μM	0.91 ± 0.08	95 ± 1 μM	1.04 ± 0.02
Dipropylxanthine-7-riboside	1.7 ± 0.1 μM	0.98 ± 0.05	1.8 ± 0.1 μM	0.94 ± 0.09
Dibutylxanthine-7-riboside	0.45 ± 0.05 μM	0.99 ± 0.11	0.51 ± 0.03 μM	0.93 ± 0.03
R-PIA	0.76 ± 0.03 nM	0.74 ± 0.03	7.3 ± 0.5 nM	0.92 ± 0.08

* Displacement of [^{3}H]DPCPX binding to calf brain membranes (means of triplicates ± S.E.M.).

RESULTS

K_i-values for the inhibition of [^{3}H]DPCPX binding to calf brain membranes with the respective pseudo-Hill coefficients (n_H) are listed in TABLE 1 for the various xanthines, their 7-ribosides and for the reference agonist R-PIA.

Full ^{1}H-NMR data for the xanthine-7-ribosides are listed in the experimental section. Details of the ribose signals in theophylline-7-riboside and adenosine are shown in FIG. 3.

The dependency of the intramolecular energy upon rotation about the glycosidic bond, as computed by MOPAC, is shown in FIG. 4 for (a) adenosine and (b) theophylline-7-riboside.

DISCUSSION

As predicted by our model, the xanthine-7-ribosides proved to inhibit binding of the A_1 receptor antagonist radioligand [^{3}H]DPCPX (TABLE 1). Affinity increases with increasing chain length of the substituents at positions 1 and 3 (butyl > propyl > methyl). The same effect is seen for the 7-unsubstituted xanthines, which is in agreement with known SAR for xanthines[2], and also in accordance with our model of the antagonist binding site, which states that increasing hydrophobicity at positions 1 and 3 should enhance affinity.[4]

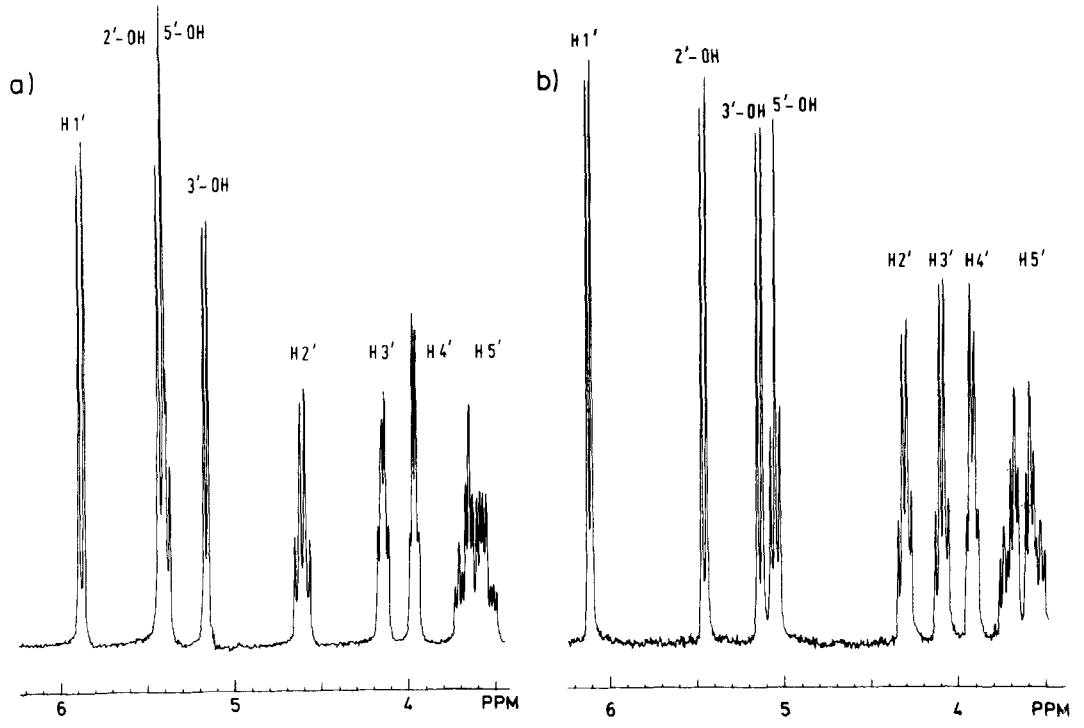


FIG. 3. Ribose signals in the ^{1}H-NMR spectrum of (a) adenosine and (b) theophylline-7-riboside.

Although the xanthine-7-ribosides are somewhat less potent than the corresponding xanthines, the ribose moiety can obviously be accommodated by the region adjacent to N7. Decreases in K_i-values vary from 10-fold for theophylline-7-riboside to 3-fold for 1,3-dibutylxanthine-7-riboside. The latter compound has an affinity of 0.45 μM, still 13-fold more potent than the prototypical adenosine antagonist theophylline. The substituents at N7 which have been reported so far are usually badly tolerated, both at A_1[14] and A_2 receptors.[14,15] Hence, the finding that the relatively large ribose moiety can indeed be accommodated by the N7-region, is in support of our model. Furthermore, in contrast to the xanthine-7-ribosides, theophylline-9-riboside is inactive at adenosine receptors.[16]

K_i-values are unaltered by the addition of a high concentration of GTP (500 μM). Also, the slopes of the displacement curves remain unchanged: they

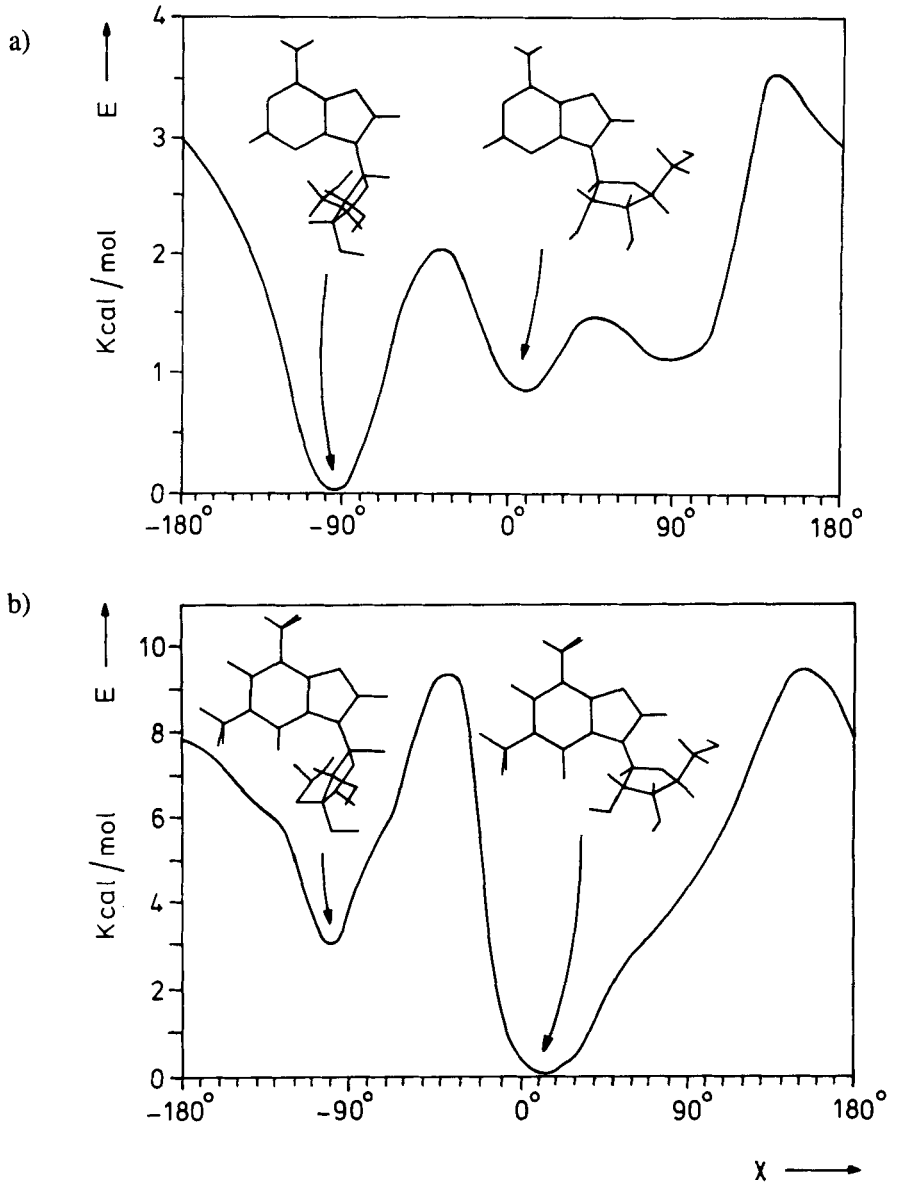


FIG. 4. Dependence of intramolecular energy, relative to the global minimum, upon variation of the glycosidic torsion angle (χ) for (a) adenosine (heat of formation at the global minimum: -89.9 kcal/mol) and (b) theophylline-7-riboside (global minimum at -126.3 kcal/mol). Note the different scales on the abscissa for (a) and (b).

approximate unity both with or without addition of GTP. Agonists, but not antagonists, differentiate between two states of the receptor: a high affinity and a low affinity state. GTP causes a transition towards the low affinity state, thereby causing a shift to the right and a steepening of the inhibition curve of agonists.[17] Such behaviour can be used to discriminate between agonists and antagonists, which is demonstrated for the reference agonist R-PIA (TABLE 1). Upon addition of GTP, the K_i-value increases 10-fold and the slope of the curve steepens. K_i-values and slopes of xanthines, as should be expected, are unaltered, and the same applies to the xanthine-7-ribosides. Therefore, the xanthine-7-ribosides must be considered to act as antagonists.

Thus, a major conclusion should be that an intact ribose moiety, coupled at the appropriate site to a purine moiety which is capable of binding to the receptor, in itself is not sufficient to activate the receptor. One explanation may be the possibility of an additional attribute of the purine moiety important for activation of the receptor. It has been suggested by Bruns[18] that (at least for A_2 receptors) the exocyclic amino group of adenosine is playing a role in the change in receptor conformation necessary to activate the receptor, presumably by acting as a hydrogen bond donor. Another possibility might be that in this compound the hydrogen atom attached to 2'-O, which is essential for agonist activity[3], cannot interact properly with the receptor, because it is involved in an intramolecular hydrogen bond with O^6 (see further). To test this second hypothesis, we have tried to synthesize the 6-thio derivative of theophylline-7-riboside, which is unlikely to form such an intramolecular hydrogen bond. These attempts however were unfruitful: 6-thiotheophylline could not be silylated, probably due to steric hindrance of the bulky sulfur atom.

An important feature which governs binding to the receptor is the orientation of the ribose moiety about the glycosidic bond. This orientation is characterized by the glycosidic torsion angle, χ (which is defined henceforth as the dihedral angle C8-N9-C1'-O1' in adenosine). In solution, there are two predominant rotamers, called *syn* and *anti* respectively. Typical values for χ found in crystal structures of nucleosides and nucleotides are 230 ± 30° for the *syn* and 45 ± 40° for the *anti* conformation.[19] Even relatively small variations in glycosidic torsion angle may result in a shift in the position of individual atoms of the ribose moiety of several angstroms, and hence this is an important factor.

On the basis of a detailed SAR analysis of nucleoside agonists and antagonists[18], Bruns has concluded that adenosine binds to the receptor in the *anti* conformation. It was found that in human fibroblasts (with receptors of the A_2-subtype) three 8,5'-cycloadenosines act as competitive inhibitors of adenosine-stimulated cyclic AMP production. These compounds are all restricted to the *anti* conformation by the cyclic bond, with χ-values ranging from 15 to 60°. On the other hand, compounds that have difficulty in attaining the *anti* conformation, due to steric hindrance by a large C8-substituent, proved to be inactive. Although this indeed suggests that adenosine binds in the *anti* conformation, some caution is warranted. The classic example of a nucleoside sterically constrained to the *syn* conformation is 8-bromoadenosine. For this compound, the *syn* conformation is estimated to be 8 kcal/mol more stable than the *anti* conformation. Still, two of its derivatives (8-bromoadenosine diphosphate and the 8-bromo derivative of NAD^+, both having the same preference for the *syn* conformation) cocrystallize with the appropriate dehydrogenase in the *anti* conformation. Apparently, in these cases the less favorable conformer can be stabilized by the enzyme it interacts with (for a review refer to[20]).

It has been argued by Prasad et al.[21] that the *syn* conformation might be the biologically active species. This is based on the parallels seen in a series of 5'-substituted adenosines between cardiovascular activity and the ability to adopt the *syn* conformation in solution, as assessed by NMR spectroscopy. For the potent A_1 and A_2 receptor agonist NECA, one of these 5'-substituted adenosine derivatives, it has recently been shown that also in the crystal structure the *syn* conformation is preferred.[22]

Miles et al[23] have suggested, on the basis of the rotational properties of a very small number of compounds, that adenosine binds to the receptor in a so-called *high anti* conformation, i.e. with χ c.a. 135°. This conclusion however seems highly improbable when Bruns' more extensive data are also taken into account.[18]

In order to gain more insight in this difficult matter, we have examined the orientation of the ribose moieties of adenosine and theophylline-7-riboside with the aid of NMR spectroscopy and quantum chemical calculations. Since agonists and antagonists are thought to bind in the same way to the receptor[18], and given

the apparently strict structural requirements for the N7-domain of xanthines and the ribose domain of adenosine derivatives, we must assume that the glycosidic torsion angles of adenosine and the xanthine-7-ribosides - when bound to the receptor - are comparable.

In FIG. 3, the ^{1}H-NMR spectrum of the ribose moiety of adenosine (a) and theophylline-7-riboside (b) is depicted. It is obvious that the chemical shifts of some protons, notably in 5'-OH and H2' are markedly different from the corresponding signals in adenosine. These differences reflect a different orientation about the glycosidic bond.

It has been shown by Stolarski, Dudycz and co-workers that in purine nucleosides and nucleotides, the chemical shift of H2' can be used as an indicator of the orientation of the sugar.[24,25] In DMSO, typical values for $\delta_{H2'}$ are 5.2 ppm for compounds that are virtually restricted to the *syn* conformation by a bulky 8-substituent and 4.2 ppm in compounds that exist exclusively in the anti conformation (e.g. 8,5'-cyclo-8-oxo adenosine derivatives). Since the *syn-anti* equilibrium is rapid on the NMR time scale, the chemical shift found for a certain compound will have an average value determined by the relative occurrence of the two rotamers. The intermediate value we obtained for adenosine (4.62 ppm) indicates that in DMSO solution, both the *syn* and *anti* conformers can exist and are rapidly interconvertible, with the *anti* conformation slightly preferred. This is in good agreement with the results obtained by other authors.[20]

If the same method is applied to the xanthine-7-ribosides, two situations must be distinguished. In the *anti* conformation, $\delta_{H2'}$ will only be influenced by the imidazole part of the xanthine ring, which is identical to the corresponding part in adenosine.[24] Hence, in the *anti* conformation, $\delta_{H2'}$ -values of purine-9-ribosides and xanthine-7-ribosides can be expected to be comparable. For adenosine in the *syn* conformation $\delta_{H2'}$ is determined by N3 and the ring current of the pyrimidine part of the molecule, causing a downfield shift. For xanthine-7-ribosides, $\delta_{H2'}$ is influenced by O^6 only, which can be expected to similarly cause a downfield shift. Thus, the low values found for the xanthine-7-ribosides (4.30 - 4.32 ppm) suggest that these compounds exist predominantly in the *anti* conformation.

This finding is corroborated in a Nuclear Overhauser Enhancement experiment. In adenosine, both H1' and H2' are enhanced upon irradiation of H8.

The same is found for theophylline-7-riboside, but relative to adenosine the enhancement of H2' is much larger. The spacial arrangement of these hydrogen atoms is such that in the *syn* conformation H8 is close to H1', whereas enhancement of H2' is only possible within the *anti* range. Thus, this provides additional evidence that in adenosine there exists an equilibrium between the *syn* and the *anti* conformations; for xanthine-7-ribosides this equilibrium is shifted towards the *anti* side.

The rotational flexibility about the glycosidic bond was further investigated with the aid of the semi-empirical molecular orbital program MOPAC, using the AM1 Hamiltonian. It has been shown that AM1 is quite satisfactory in the detection of rotational barriers and the estimation of hydrogen bonds. Rotational barrier energies however tend to be somewhat lower than experimentally determined values.[26]

Several calculations on the rotational flexibility of adenosine have been reported.[23,27-29] Although it is well established that the sugar pucker (i.e. the deviation from planarity in the pentose ring) varies considerably with the glycosidic torsion angle, in order to minimize unfavourable and to maximize favourable interactions between the two parts of the molecule, none of these calculations take this feature into account. This has led to considerable disagreement between the various calculations. However, in general each method detects at least two minima, one in the *anti* range (with χ varying from -10° to 60°) and one in the *syn* range (χ=210°-270°). Rotational barriers vary from 2-50 kcal/mol and will probably be overestimated in most cases, since the ribose is not allowed to adapt its pucker.[28]

In our own calculations, the ribose was allowed to relax fully. Upon rotation about the glycosidic bond of adenosine, MOPAC detects three minima (FIG. 4a). The global minimum has a *syn* conformation with χ=-92°. There are two local minima in the *anti* range with an intramolecular energy slightly higher than the global minimum, with χ=-6° (+ 0.8 kcal/mol) and with χ=67° (+ 1.1 kcal/mol). The energy barriers between the three minima never exceed 2 kcal/mol, indicating that all three conformations are readily accessible. This is in good agreement with the NMR data. In the solid state, two conformations have been reported, both in the *anti* range. In adenosine base, χ=10° is found[12], and for

adenosine hydrochloride, χ=43°.[29] Of course, it should be borne in mind that the surroundings of a molecule may have a decisive influence on its conformational preferences. This means that not necessarily the same minimum energy conformations will be found in the solid state (X-ray crystal structure), in solution (NMR measurements) and *in vacuo* (theoretical calculations).

The behaviour of theophylline-7-riboside (for which we have defined χ as the torsion angle C8-N7-C1'-O1') is altogether different (FIG. 4b). There is a global minimum with an *anti* conformation (χ=6°), close to the first *anti* conformation found for adenosine. There is also a local minimum with χ=-91°, corresponding with the global minimum found for adenosine. It is 3.2 kcal/mol less stable, and separated from the global minimum by a barrier of 9 kcal/mol. Thus, according to the AM1 calculations, the *anti* conformation is definitely preferred for this compound.

An explanation for this preference might be that in this conformation a hydrogen bond is possible between O2'-H and O^6 (distance 1.94 Å, angle 145°). In the NMR-spectrum of theophylline-7-riboside, such a hydrogen bond is not detected: the chemical shifts of O2'-H are identical in adenosine and theophylline-7-riboside. Since DMSO itself is capable of forming strong hydrogen bonds, it will compete with the intramolecular hydrogen bond. Unfortunately, adenosine and theophylline-7-riboside are not sufficiently soluble in NMR-solvents that do not impose this complication, e.g. chloroform or acetonitril. Therefore, it remains unclear if the proposed intramolecular hydrogen bond actually is formed and contributes to the preference of xanthine-7-ribosides for the *anti* conformation.

In conclusion, from both NMR experiments and theoretical calculations it follows that, whereas adenosine can readily adopt both the *syn* and the *anti* conformations, for theophylline-7-riboside the *anti* conformation is largely preferred. Since both compounds most probably bind to the receptor with the same orientation of the ribose moiety, these results provide further evidence in favour of the concept that adenosine binds to the receptor in the *anti* conformation.

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