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Unusual conformational flexibility in N^1-substituted uncommon purine nucleosides

Crystal structure of 1-allyl-isoguanosine and 1-allyl-xanthosine

Yen-Chywan Liaw[a], Ji-Wang Chern[b], Gwo-Sen Lin[b] and Andrew H.-J. Wang[a]

[a]*Laboratory of Structural Biology, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC* and [b]*Institute of Pharmacy and Medical Laboratories National Defense Medical Center, Taipei, Taiwan, ROC*

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Several new N^1-substituted uncommon purine nucleosides, including doridosine (1-methyl-isoguanosine; m-iG), 1-allyl-isoguanosine (a-iG) and 1-allyl-xanthosine (a-X), have been synthesized and tested as agonists for the adenosine receptors. Some have smooth muscle relaxant or negative chronotropic activities. The X-ray crystal structure of these compounds has been determined at atomic resolution in order to understand the structure–activity relationship. The structures were solved by direct methods and refined by full-matrix least-squares refinement procedure. The crystallographic parameters are: a-iG, space group $P2_1$, a=10.573 (1) Å, b=21.955 (2) Å, c=14.360 (1) Å, β=110.65 (1)°, no. of 3σ Fo's=4585, R=0.047; a-X, space group $P2_12_12_1$, a=16.015 (2) Å, b=16.239 (1) Å, c=5.3723 (5) Å, no. of 3σ Fo's=1169, R=0.031. In the a-iG crystal, there are 4 independent molecules (with different conformation) per asymmetric unit. While all 4 molecules adopt *anti* χ_{CN} glycosyl torsion angle, their riboses have 3 distinct puckers ($C^{2'}$-exo, $C^{2'}$-endo and $C^{1'}$-exo). In contrast, the a-X structure adopts a *syn* χ_{CN} glycosyl torsion angle, which is stabilized by an intramolecular hydrogen bond between the N^3 of purine base and the $O^{5'}$ of the ribose (in $C^{2'}$-endo pucker). Both purine bases (a-iG and a-X) are mainly in the keto tautomer form. For the isoguanine base, the averaged N^1–C^2 bond distance (1.42 Å) is significantly longer than that (1.375 Å) of the guanine base. For the xanthine base, N^3 nitrogen has an imino proton attached which is unambiguously located in the electron density map. The surprising flexibility in the ribose ring of these N^1-substituted uncommon purine nucleosides suggests that the ribose moiety may not participate in the binding of nucleoside to the adenosine receptors.

Hypotensive drug; X-ray diffraction; Purine nucleoside; Tautomerism

1. INTRODUCTION

Many nucleosides and their derivates have been found to possess interesting biological activities. An important group of purine derivates has different effects on the biological activities of adenosine receptors which are sensitive to methylxanthine, such as caffeine and theophylline [1,2]. It appears that there are at least 2 subtypes of adenosine receptors, referred to as A_1 and A_2 types. The A_1 receptor from brain has been purified as a monomeric ~35-kDa glycoprotein [3]. A number of receptor-specific purine compounds have been developed as agonists or antagonists. For example, N^6-cyclopentyladenosine [4] (CPA) and 5′-chloro-N^6-(2-*endo*-norbornyl)adenosine [5] (CENBA) are extremely potent and specific agonists for A_1 receptors. Those compounds are N^6-substituted adenosine derivatives. Recently, N^1-substituted purine nucleosides such as doridosine (1-methyl-isoguanosine), a natural product isolated from marine animals, has been found to have the activity of lowering blood pressure [6–12], with the mechanism of its pharmacological action likely associated with the agonist action toward the adenosine receptor [9–12]. Attempts have been made to improve the therapeutic property of this class of compounds by synthesizing new N^1-substituted purine nucleosides, with varying degree of success [13–16]. 1-Cyclopropyl-isoguanosine showed strong negative inotropic effect without compromising the blood pressure and has a K_D of 6.8×10^{-8} M towards the A_1 adenosine receptor with an A_2/A_1 specificity ratio of 347 [17]. In order to better understand the structure– activity relationship of this class of N^1-substituted uncommon nucleosides, we have determined the three- dimensional structure of 1-allyl-isoguanosine (a-iG; K_D of 6.8×10^{-8} M) and 1-allyl-xanthosine (a-X) (Fig. 1), by X-ray crystallography. We are interested in discerning their structural functional motifs, in relation to the agonist/antagonist activity toward the adenosine receptor, which may aid the design of new compounds in the future.

In addition, there has been a recent resurgence of interest in the utility of uncommon bases in areas such

Correspondence address: J.-W. Chern, Department of Physiology and Biophysics, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

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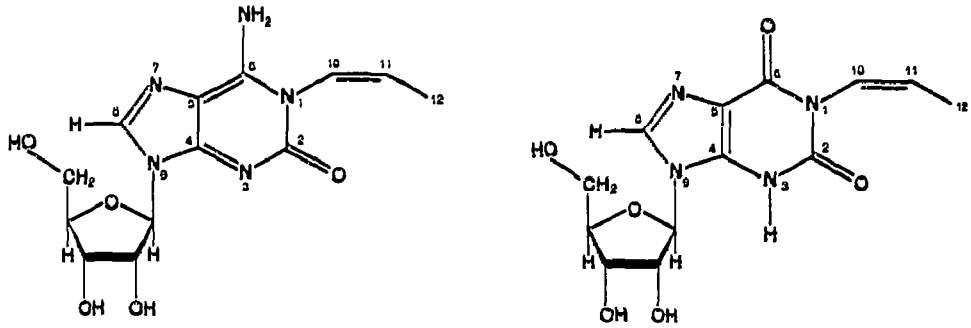


Fig. 1. Molecular formula of 1-allyl-isoguanosine and 1-allyl-xanthosine with their numbering system.

as extension of genetic codes [18] or the use in the third strand for the recognition of Watson-Crick base pair in a nucleic acid triplex [19]. The exact base pairing scheme involving those uncommon bases depends on the nature of their chemical structure. Our results in this paper provide new information in helping clarify the ambiguity regarding the tautomeric forms associated with isoguanosine and xanthosine.

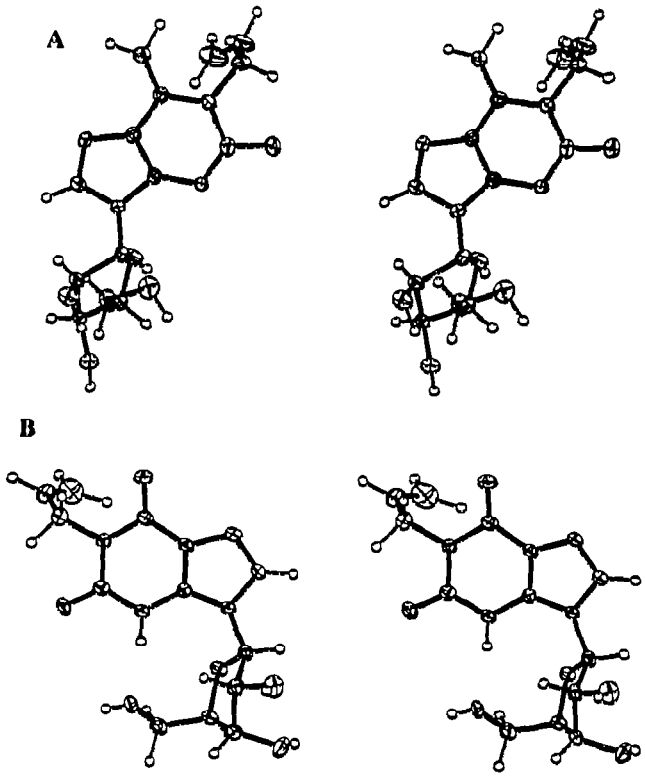


Fig. 2. Stereoscopic ORTEP drawing of the three-dimensional structure of (A) 1-allyl-isoguanosine (molecule A is shown), with χ_{CN} in the *anti* conformation and (B) 1-allyl-xanthosine, with χ_{CN} in the *syn* conformation.

2. EXPERIMENTAL

The syntheses of the N^1-substituted purine nucleosides have been described previously [16]. Crystals of a-iG and a-X were grown from a warm solution of the compound from DMF by slow cooling. The X-ray diffraction data were collected for each compound (to 2θ=120°) on a Rigaku AFC-5R (RU-300) rotating anode X-ray diffractometer at 22°C using the ω-2θ scan mode with graphite-monochromated CuK_α radiation (λ=1.5418Å). The power of the X-ray generator was set at 50 kV and 40 mA with 0.2 × 2 mm^2 fine-focus anode cup. Unit cell dimensions were determined using $CuK_{\alpha 1}$ radiation (λ=1.5406Å) and are listed in the Abstract. Both structures were solved by the direct methods using the program SHELXS-86 [20]. They were refined by the full-matrix least-squares refinement procedure using the NRCVAX package [21]. The crystal structure of a-iG was solved with considerable difficulty as there are 4 independent nucleosides (denotes molecules A, B, C and D) plus 5 water molecules (a total of 97 non-hydrogen atoms) per asymmetric unit. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were also included in the refinement with variable positions and isotropic temperature factors. The detailed structural analysis will be reported elsewhere. A search of the Cambridge Data Base revealed that no crystal structure of any N^1-substituted derivative of isoguanosine of xanthosine has been determined. The crystal structure of xanthosine [22–24] and an N^7-methylated xanthosine derivative has been determined [18].

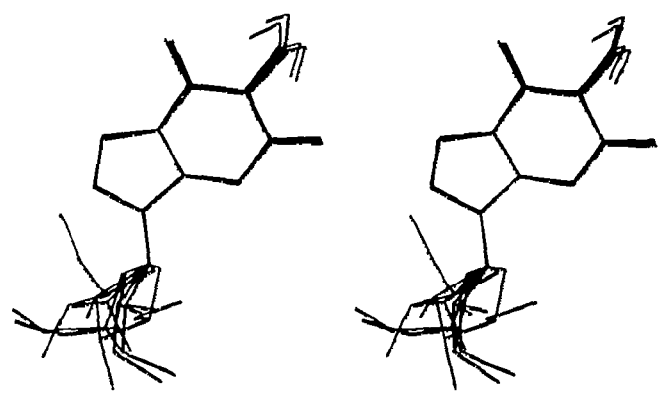


Fig. 3. Superposition of the 4 independent conformers of 1 allyl-isoguanosine.

3. RESULTS AND DISCUSSION

Fig. 2 depicts the three-dimensional structures of a-iG (only molecule A is shown) and a-X, which show that a-iG is in the *anti*, while a-X is in the *syn* conformation. When the 4 independent a-iG molecules are compared (Fig. 3), it is evident that a-iG has a high degree of conformational flexibility, especially in the sugar moiety. While the glycosyl torsion angle remains in the *anti* range, the ribose of a-iG adopts 3 distinct puckers: $C^{1'}$-exo (molecule A and B), $C^{2'}$-endo (molecule C) and $C^{2'}$-exo (molecule D). Interestingly, the *syn* glycosyl conformation of a-X is stabilized by an intramolecular hydrogen bond between the N^3 of purine base and the $O^{5'}$ of the ribose (in the $C^{2'}$-endo pucker). While it has been suggested by the so-called 'gauche effect' [25] that ribose sugar favors a $C^{3'}$-endo pucker conformation, the results here clearly indicate that other puckers are equally likely. The important conformational parameters are summarized in Table I.

Table I

Conformation of N^1-substituted isoguanosine and xanthosine

Molecule	Pucker	P	ν_{max}	χ_{CN}
a-iG				
A	$C^{1'}$-exo	110.5	40.0	−70.5 *(anti)*
B	$C^{1'}$-exo	137.6	42.0	−107.1 *(anti)*
C	$C^{2'}$-endo	159.7	42.8	−112.1 *(anti)*
D	$C^{2'}$-exo	343.2	29.7	−78.9 *(anti)*
a-X	$C^{2'}$-endo	161.8	37.2	39.6 *(syn)*

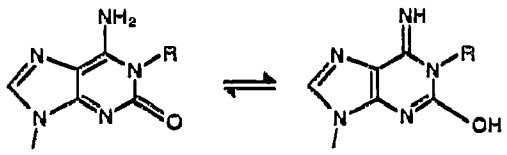
P, ν_{max} and χ_{CN} are pseudorotation angle, peudorotation amplitude and glycosyl torsion angle, respectively.

Table II

Bond Distances (Å) and bond angles (degree) in the bases of 1-allyl-isoguanosine (a-iG) and 1-allyl-xanthosine (a-X)

	a-X	a-iG				
		A	B	C	D	Ave
N1-C2	1.411(4)	1.428(7)	1.429(6)	1.423(6)	1.420(6)	1.425
N1-C6	1.401(4)	1.376(6)	1.385(6)	1.366(6)	1.376(6)	1.376
N1-C10	1.464(4)	1.472(6)	1.481(6)	1.471(7)	1.464(6)	1.472
N3-C2	1.361(4)	1.331(6)	1.342(6)	1.335(6)	1.349(6)	1.339
N3-C4	1.368(4)	1.359(6)	1.346(6)	1.346(6)	1.342(6)	1.348
N6-C6		1.317(6)	1.305(6)	1.323(6)	1.317(6)	1.316
O6-C6	1.239(4)					
N7-C5	1.390(4)	1.397(6)	1.394(6)	1.406(6)	1.386(6)	1.396
N7-C8	1.299(4)	1.306(6)	1.295(7)	1.281(6)	1.299(7)	1.295
N9-C4	1.362(4)	1.381(6)	1.373(6)	1.372(6)	1.372(6)	1.375
N9-C8	1.390(4)	1.376(6)	1.387(6)	1.387(6)	1.402(6)	1.388
N9-C1	1.456(4)	1.433(6)	1.429(6)	1.430(6)	1.454(6)	1.437
O2-C2	1.219(3)	1.255(6)	1.248(5)	1.248(6)	1.244(6)	1.249
C4-C5	1.362(4)	1.360(7)	1.369(7)	1.366(7)	1.354(7)	1.362
C5-C6	1.420(4)	1.380(7)	1.388(7)	1.397(7)	1.409(7)	1.394
C2-N1-C6	125.3(3)	122.7(4)	124.1(4)	123.6(4)	123.4(4)	123.5
C2-N1-C10	116.5(2)	117.2(4)	117.1(4)	117.1(4)	116.8(4)	117.1
C6-N1-C10	117.9(2)	119.7(4)	118.4(4)	119.3(4)	119.8(4)	119.3
C2-N3-C4	119.8(2)	112.9(4)	114.9(4)	113.8(4)	114.6(4)	114.1
C5-N7-C8	104.9(2)	104.6(4)	103.3(4)	103.5(4)	103.8(4)	103.8
C4-N9-C8	104.9(2)	105.4(4)	104.9(4)	105.3(4)	104.9(4)	105.1
C4-N9-C1'	128.8(2)	127.4(4)	128.5(4)	125.9(4)	125.5(4)	126.8
C8-N9-C1'	126.2(2)	127.2(4)	126.6(4)	128.7(4)	129.6(4)	128.1
N1-C2-N3	116.9(2)	122.7(4)	120.1(4)	121.1(4)	120.6(4)	121.1
N1-C2-O2	121.1(3)	113.7(4)	117.1(4)	115.6(4)	117.0(4)	115.9
N3-C2-O2	122.0(3)	123.6(4)	122.8(5)	123.3(4)	122.4(4)	123.0
N3-C4-N9	129.2(3)	126.1(4)	126.7(4)	125.7(4)	125.3(4)	126.0
N3-C4-C5	123.2(3)	127.1(4)	127.2(4)	128.4(4)	128.3(4)	127.8
N9-C4-C5	107.6(2)	106.8(4)	106.1(4)	105.9(4)	104.4(4)	106.3
N7-C5-C4	109.8(3)	110.1(4)	111.2(4)	110.9(4)	111.8(4)	111.0
N7-C5-C6	129.5(3)	129.0(4)	128.6(4)	130.8(4)	129.8(4)	129.6
C4-C5-C6	120.9(3)	120.8(4)	120.0(4)	118.3(4)	118.4(4)	119.4
N1-C6-N6		123.1(4)	121.9(4)	121.8(4)	121.2(4)	122.0
N1-C6-O6	120.3(3)					
N1-C6-C5	113.7(3)	113.7(4)	113.4(4)	114.6(4)	114.7(4)	114.1
O6-C6-C5	126.0(3)					
N6-C6-C5		123.2(4)	124.7(4)	123.6(4)	124.1(4)	123.9
N7-C8-N9	112.9(3)	113.1(4)	114.4(4)	114.4(4)	113.2(4)	113.8

The bond distances and bond angles of these N^1-substituted uncommon purine nucleosides (Table II) are of interest to the issue of tautomerism in isoguanosine (iG) and xanthosine (X). Recently, these 2 bases have been used to extend the letter of genetic code, with iG paired with isocytosine (iC), and X paired with 7-methyl-oxoformycin B (denoted as π base) [18]. An iC derivative has been used as a new base in the third strand of a triple helix [19]. However, iG seems to mispair with U frequently, which is attributed to the ability of iG to adopt an alternative tautomeric structure [26]. The bond distances (in Å; e.s.d. ~0.004 Å) of the a-iG (averaged) and a-X bases are listed in Table II. By the comparison of these values with those of normal bases [27], we conclude that they adopt mostly the *keto* form in resonance with another minor form shown in the Scheme. The conformation of allyl group in these 5 molecules adopts a planar 'hook' form with the torsion angle of N^1–C^{10}–C^{11}–C^{12} being nearly 0° (9.5°–7.5°). The plane of the allyl group is almost perpendicular to the base plane. The terminal hydrogen atom associated with the allyl C^{12} atom is situated underneath the base making a close contact ($H^{12'}$--N^1 distance is 2.52 (4) Å) and forcing the bond angles of N^1–C^{10}–C^{11} (112°) and C^{10}–C^{11}–C^{12} (125°) deviating from their normal values. The hydrophobic allyl groups in both structures are clustered together into hydrophobic pockets in the crystal lattices.



Scheme

Many adenine nucleoside analogs have been tested as agonist agents for adenosine receptors [1,2,4,5]. Inspection of their chemical structure suggests that the important regions for activity in the nucleosides are likely to reside in the base moiety [1,2,4,5]. A hydrophobic N^6-domain to which the substituent at the N^6-position of adenosine is bound has been identified in the receptor. For example, the cyclopentyl group in CPA or the norbornyl group in CENBA are hydrophobic moieties which can fit in the hydrophobic pocket (the so-called S3-A site) of the N^6-domain in an exquisitely matching manner, thus accounting for the high specificity. Those hydrophobic side chains at the N^6-position prefer to adopt a distal (to N^7-position) orientation as seen in several crystal structures of N^6-modified adenosine derivatives [27]. Interestingly, isoguanosine retains the N^6-amino group and the free N^7 (like those in the adenosine derivatives) to serve as the hydrogen bonding recognition site for the A_1-type adenosine receptor. It turns out that a hydrophobic side group (such as allyl, cyclopropyl or cyclohexyl) at the N^1-position of isoguanosine occupies nearly the same position of the side group of the N^6-derivatives of adenosine. Therefore it may not be highly surprising that N^1-substituted iG and N^6-substituted adenosine have similar A_1-specific agonist activity. However, it is surprising to see the high conformational flexibility (sugar puckers and *syn/anti* glycosyl angle) of the N^1-substituted uncommon purine nucleosides. This observation suggests that the ribose moiety may not participate significantly in the binding of nucleoside to the receptor. However, we cannot rule out the possibility that only a particular sugar pucker is selected when the compound is bound to the receptor. Nevertheless, the sugar moiety may be modified to test this hypothesis or to alter other properties (e.g. selectivity, solubility, membrane transport, stability) of the drug. More structural analysis like the present work, extending to other compounds, would enable us to more fully understand the detailed interactions of the binding of important agonist/antagonist compounds to adenosine receptors.

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REFERENCES

[1] Williams, M. (1987) Annu. Rev. Pharmacol. 27, 315.
[2] Linden, J. (1991) FASEB J. 5, 2668.
[3] Nakata, H. (1989) J. Biol. Chem. 264, 16545.
[4] Bruns, R.F., Lu, G.H. and Pugsley, T.A. (1986) Mol. Pharmacol. 29, 331.
[5] Trivedi, B.K., Bridges, A.J., Patt, W.C., Priebe, S.R. and Bruns, R.F. (1989) J. Med. Chem. 32, 8.
[6] Quinn, R.J., Gregson, R.P., Cook, A.F. and Bartlett, R.T. (1980) Tetrahedron Lett. 21, 567.
[7] Cook, A.F. Bartlett, R.T., Gregson, R.P. and Quinn, R.J. (1980) J. Org. Chem. 45, 4020.
[8] Grozinger, K., Freter, K.R., Farina, P. and Gladczuk, A. (1983) Eur. J. Med. Chem-Chim. Ther. 18, 221.
[9] Fuhrman, F.A., Fuhrman, G.J., Kim, Y.H., Pavelka, L.A. and Mosher, H.S. (1980) Science 207, 193.
[10] Baird-Lambert, J.A., Marwood, J.F., Davies, L.P. and Taylor, K.M. (1980) Life Sci. 26, 1069.
[11] Davies, L.P., Taylor, K.M., Gregson, R.P. and Quinn, R.J. (1980) Life Sci. 26, 1079.
[12] Bartlett, R.T., Cook, A.F., Poonian, M.S. and Taylor, K.M. (1980) Life Sci. 26, 1089.
[13] Bartlett, R.T., Cook, A.F., Holman, M.J., McComas, W.W., Nowoswalt, E.F., Poonian, M.S., Biard-Lambert, J.A., Baldo, B.A. and Marwood, J.F. (1981) J. Med. Chem. 24, 947.
[14] Kim, Y.H., Jee, S.J. and Mosher, H.S. (1985) Heterocycles 23, 21.
[15] Chern, J.-W. and Townsend, L.B. (1985) Tetrahedron Lett. 26, 6419.
[16] Chern, J.-W., Lin, G.-S., Chen, C.-S. and Townsend, L.B. (1992) J. Org. Chem. (in press).
[17] Data for biological activity are supplied by Dr. P.L. Tao, personal communication.
[18] Piccirilli, J.A., Krauch, T., Moroney, S.E. and Benner, S.A. (1990) Nature 343, 33.

[19] Ono, A., Ts'o, P.O.P. and Kan, L.-S. (1991) J. Am. Chem. Soc. 113, 4032.
[20] Sheldrick, G.M. (1985) in: Crystallographic Computing 3, (G.M. Sheldrick, C. Krüger and R. Goddard eds.) Clarendon Press, Oxford, pp. 175–189.
[21] Gabe, E.J., Lee, F.L. and Le Page, Y. (1985) in: Crystallographic Computing 3, (G.M. Sheldrick, C. Krüger and R. Goddard eds.) Clarendon Press, Oxford, pp. 167–174.
[22] Koyama, G., Nakamura, H., Umezawa, H. and Itaka, Y. (1976) Acta Crystallogr. B32, 969.
[23] Herceg, M., Fisher, J. and Weiss, R. (1976) Izv. Jug. Cen. Krist. 11, 130.
[24] Lesyng, B., Marek, C. and Saenger, W. (1984) Z. Naturforsch. C39, 720.
[25] Wolfe, S. (1972) Acc. Chem. Res. 5, 102.
[26] Switzer, C.Y., Moroney, S.E. and Benner, S.A. (1989) J. Am. Chem. Soc. 111, 8322.
[27] Saenger, W. (1987) Principles of Nucleic Acids Structure, Springer-Verlag, Berlin.