The Crystal Structure of Anhydrous Xanthosine Displays Intramolecular O(2′)H ... O(3′) Hydrogen Bond

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From an equimolar dipeptide/xanthosine mixture in water/methanol, only the nucleoside crystallized in anhydrous form, space group $P2_12_12_1$ with $a = 17.406\ (5)$ Å, $b = 12.378\ (4)$, $c = 5.350\ (2)$ Å, $Z = 4$, $C_{10}H_{12}O_6N_4$, $FW = 284.26$, $D_x = 1.639\ g \cdot cm^{-3}$. The structure was determined by direct methods on the basis of 984 X-ray data and refined to an agreement index of $R = 3.9\%$. Xanthosine occurs in the *syn* conformation with $\chi = -132.7°$ which is stabilized by an intramolecular hydrogen bond N(3)–H ... O(5′). The pseudorotation parameters for sugar puckering are $P = 156°$ and $\tau_m = 38.7°$ corresponding to C(2′)-*endo* form. The crystal packing of xanthosine in anhydrous and dihydrate modifications are very different, yet the molecular conformations are similar. In the anhydrous form, an unusual intramolecular hydrogen bond O(2′)–H ... O(3′) is observed.

Introduction

The structure and properties of xanthosine and of polyriboxanthilic acid have been the subject of experimental and theoretical studies [1–4]. These investigations explained in part the lack of success when trying to incorporate xanthosine into nucleic acids *in vivo*, the lethal effect of deamination of guanine in nucleic acids, and a model was proposed for the multistranded structure poly(X).

The structure of xanthosine dihydrate has previously been determined by means of X-ray diffraction analysis [5, 6]. The purpose of the present investigation was primarily to obtain a specific nucleoside/dipeptide complex to mimic a nucleic acid/protein interaction. The crystals which have been grown contained, however, only xanthosine (Fig. 1). Because the unit cell dimensions were different from those of xanthosine dihydrate [5, 6], we decided to determine the structure of this new crystal form which contains anhydrous xanthosine.

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Experimental

An equimolar mixture of xanthosine (SIGMA) and asparaginylarginyl-β-naphthylamine hydrochloride (SERVA) was dissolved in a water/ethanol solution. Crystals were grown after heating and slow cooling and contained only xanthosine. Systematic absence of X-ray reflections indicated space group $P2_12_12_1$, relevant crystallographic data are given in the abstract. 1033 diffraction data were measured on an automated four-circle STOE-diffractometer using Ni filtered CuK_α radiation with $2\theta/\theta$ scan mode. 49 reflections smaller than 2σ were rejected. Absorption corrections were not applied due to the small size of the crystal ($0.5 \times 0.2 \times 0.1\ mm^3$). The structure was solved by direct methods [7, 8] and refined by full matrix least squares using a weighting scheme according to counting statistics. All hydrogen atoms were included in the refinement when the R factor was 0.076. The final agreement index is $R = 0.039$. The atomic coordinates and thermal parameters are given in Table I.

Results and Discussion

In the papers of Koyama *et al.* [5] and Herceg *et al.* [6], two identical crystal structures of xanthosine dihydrate have been reported whereas we describe

Table Ia. Positional and thermal parameters of the non-hydrogen atoms with estimated standard deviations. The thermal parameters are of the form $\left(\exp - 2\pi^2 \sum_{ij}^{3} U_{ij} h_i h_j a_i a_j\right)$. All values are multiplied by 10^4.

Atom	*X/A*	*Y/B*	*Z/C*	U11	U22	U33	U23	U13	U12
N(1)	−1760(1)	4202(2)	1461(7)	271(14)	319(16)	336(18)	45(16)	−10(16)	11(13)
C(2)	−1085(2)	3820(3)	2498(7)	289(17)	304(18)	260(19)	−48(18)	4(17)	2(16)
O(2)	−461(1)	4143(2)	1737(5)	269(12)	464(14)	341(15)	44(15)	30(14)	−96(12)
N(3)	−1153(1)	3072(2)	4391(6)	206(13)	304(15)	283(16)	34(16)	−11(14)	−29(13)
C(4)	−1878(2)	2750(3)	5070(8)	257(16)	268(17)	255(18)	−17(17)	3(17)	−37(15)
C(5)	−2541(2)	3175(3)	4096(7)	245(16)	275(17)	316(19)	36(17)	−16(17)	−8(16)
C(6)	−2513(2)	4011(3)	2263(8)	287(16)	289(17)	302(19)	−1(17)	−19(18)	−27(16)
O(6)	−3048(1)	4530(2)	1432(6)	296(12)	436(15)	517(17)	164(15)	−35(15)	65(12)
N(7)	−3178(1)	2715(2)	5259(7)	248(14)	425(17)	391(18)	89(17)	−33(16)	−53(14)
C(8)	−2887(2)	2025(3)	6864(9)	203(16)	458(20)	384(21)	68(20)	−14(17)	−50(16)
N(9)	−2089(1)	2010(2)	6826(7)	207(13)	313(15)	292(17)	26(16)	−26(14)	−4(13)
O(1′)	−1032(1)	1976(2)	9392(5)	216(11)	349(13)	339(14)	−82(14)	−57(12)	21(11)
C(1′)	−1605(2)	1310(3)	8353(7)	231(15)	314(17)	274(18)	−12(18)	−16(17)	1(15)
C(2′)	−1181(2)	427(3)	6896(8)	319(16)	280(17)	298(19)	−32(18)	−14(18)	1(15)
O(2′)	−1639(1)	−489(2)	6502(6)	516(14)	298(13)	448(16)	−35(14)	−132(16)	−98(12)
C(3′)	−498(2)	226(3)	8641(8)	263(16)	277(17)	327(19)	43(17)	7(17)	0(15)
O(3′)	−784(1)	−483(2)	10 493(6)	331(12)	408(14)	451(17)	174(15)	−77(14)	−46(12)
C(4′)	−331(2)	1338(3)	9731(7)	204(15)	365(18)	268(18)	0(18)	−24(17)	35(15)
C(5′)	325(2)	1956(3)	8628(8)	251(17)	423(20)	405(21)	−2(20)	−72(18)	−68(17)
O(5′)	218(1)	2041(2)	5970(5)	240(12)	551(16)	352(15)	109(15)	26(13)	−38(12)

Table Ib. Positional parameters of the hydrogen bond atoms (X 10^4).

Atom	*x/a*	*y/b*	*z/c*
H(N1)	−1675	4789	134
H(N3)	−636	2870	5171
H(C8)	−3202	1540	8294
H(1′)	−2024	946	9664
H(2′)	−963	731	5185
H(O2′)	−1558	−1147	8099
H(3′)	−6	−37	7658
H(O3′)	−327	−874	11 580
H(4′)	−233	1231	11 425
H(5′1)	418	2808	9218
H(5′2)	795	1565	8973
H(O5′)	672	2346	5324

here the crystal structure of the anhydrous crystal form. In Fig. 1 are shown two views at right angles of the xanthosine molecule and Fig. 2 is a stereoscopic picture of the unit cell content looking down to *c*-axis. Tables II and III display bond lengths and angles, and the data of Koyama *et al.* for xanthosine dihydrate are included for comparison ([5]; *R*-value = 5.1%).

Concerning bond lengths, no significant differences are found between the two xanthosine structures in the purine system whereas the C(4′)−C(5′) and C(3′)−O(3′) bonds in the ribofuranose ring

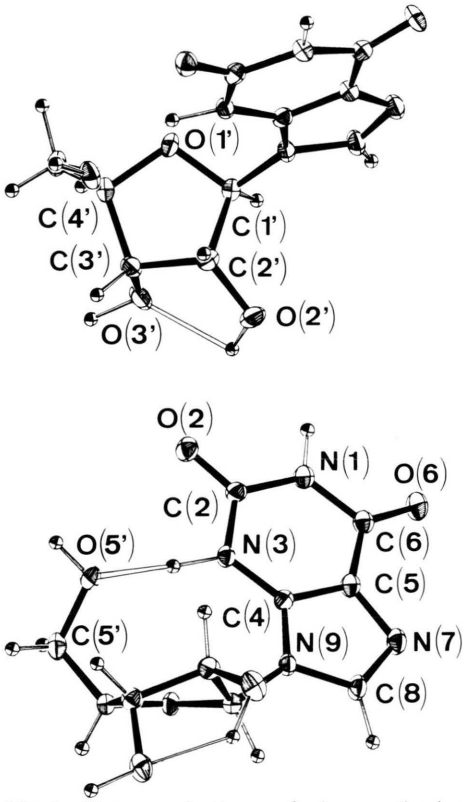

Fig. 1. Plane projections of the xanthosine structure. Bottom: projection perpendicular to the C(4′)−O(1′)−C(1′) plane; top: projection in the C(4′)−O(1′)−C(1′) plane; thermal ellipsoids are drawn at 30% probability level [12].

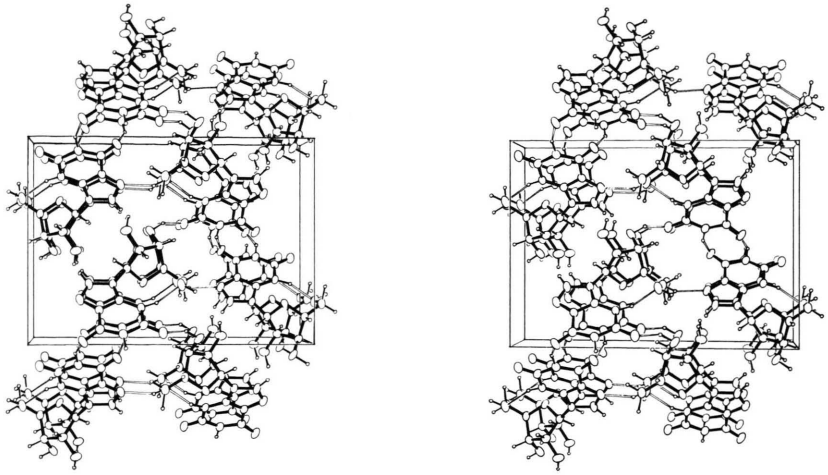

Fig. 2. Stereoscopic view of the xanthosine structure down the *c*-axis. Thermal ellipsoids are drawn at the 50% probability level [12]. O(2′)H...O(3′) bonds omitted.

Table II. Bond lengths [Å].

	xanthosine	xanthosine · $2H_2O$[5]
Pyrimidine group		
N(1)–C(2)	1.384(3)	1.380(5)
C(2)–N(3)	1.377(3)	1.380(5)
N(3)–C(4)	1.371(3)	1.364(5)
C(4)–C(5)	1.370(3)	1.367(6)
C(5)–C(6)	1.427(4)	1.429(6)
C(6)–N(1)	1.399(3)	1.394(6)
C(2)–O(2)	1.227(3)	1.229(6)
C(6)–O(6)	1.215(3)	1.223(6)
Imidazole group		
C(5)–N(7)	1.393(3)	1.386(6)
N(7)–C(8)	1.314(3)	1.304(6)
C(8)–N(9)	1.388(3)	1.389(5)
N(9)–C(4)	1.363(3)	1.376(5)
N(9)–(C1′)	1.459(3)	1.450(5)
Ribofuranose group		
O(1′)–C(1′)	1.409(3)	1.410(5)
C(1′)–C(2′)	1.531(5)	1.535(5)
C(2′)–C(3′)	1.532(5)	1.525(6)
C(3′)–C(4′)	1.523(5)	1.533(6)
C(4′)–O(1′)	1.464(4)	1.455(5)
C(2′)–O(2′)	1.402(4)	1.409(5)
C(3′)–O(3′)	1.414(4)	1.436(5)
C(4′)–C(5′)	1.495(4)	1.520(6)
C(5′)–O(5′)	1.438(4)	1.438(5)

differ by more than 3σ but are still in agreement with average data [9]. Bond angles are also comparable except those involving the secondary hydroxyl group. Thus, angles C(3′)–C(2′)–O(2′) and C(2′)–C(3′)–O(3′) differ by 3.5° and 6.7°, probably due to the variations in hydrogen bonding patterns discussed below.

Table III. Bond angles [°].

	xanthosine	xanthosine · $2H_2O$[5]
Pyrimidine group		
C(2)–N(1)–C(6)	127.9(4)	(4)
N(1)–C(2)–N(3)	116.9(3)	116.5(4)
N(1)–C(2)–O(2)	122.7(4)	121.6(4)
O(2)–C(2)–N(3)	122.7(4)	121.8(4)
C(2)–N(3)–C(4)	118.0(3)	118.3(4)
N(3)–C(4)–C(5)	124.2(4)	124.4(4)
C(4)–C(5)–C(6)	120.7(4)	120.3(4)
C(5)–C(6)–N(1)	111.4(3)	112.1(4)
C(5)–C(6)–O(6)	127.5(4)	126.7(4)
O(6)–C(6)–N(1)	121.1(4)	121.1(4)
Imidazole group		
C(5)–C(4)–N(9)	107.0(3)	106.3(3)
N(3)–C(4)–N(9)	128.8(4)	129.2(4)
C(4)–C(5)–N(7)	110.1(3)	110.6(4)
C(6)–C(5)–N(7)	129.0(4)	129.0(4)
C(5)–N(7)–C(8)	104.5(3)	104.7(3)
N(7)–C(8)–N(9)	112.6(3)	112.8(4)
C(8)–N(9)–C(4)	105.8(3)	105.5(3)
C(8)–N(9)–C(1′)	125.2(4)	126.2(3)
C(4)–N(9)–C(1′)	129.0(4)	128.4(3)
Ribofuranose group		
C(1′)–O(1′)–C(4′)	108.8(3)	109.2(3)
C(2′)–C(1′)–N(9)	114.7(3)	115.3(3)
C(2′)–C(1′)–O(1′)	106.1(3)	104.9(3)
N(9) –C(1′)–O(1′)	106.4(3)	106.9(3)
C(3′)–C(2′)–C(1′)	100.4(3)	100.8(3)
C(3′)–C(2′)–O(2′)	113.6(3)	117.1(3)
C(1′)–C(2′)–O(2′)	112.3(3)	114.5(3)
C(4′)–C(3′)–C(2′)	103.6(3)	102.4(3)
C(4′)–C(3′)–O(3′)	111.1(3)	108.3(3)
C(2′)–C(3′)–O(3′)	104.8(3)	111.5(3)
O(1′)–C(4′)–C(3′)	106.3(3)	106.4(3)
O(1′)–C(4′)–C(5′)	108.1(3)	108.1(3)
C(3′)–C(4′)–C(5′)	117.2(3)	116.0(3)
O(5′)–C(5′)–C(4′)	109.2(3)	109.5(3)

Some relevant torsion angles are given in Table IV. The conformation about the glycosyl bond, χ_{CN}, differs in the two crystal structures by only 7° and lies in the *syn* range, positioning the purine over the ribose and giving rise to an intramolecular N(3)−H ... O(5′) hydrogen bond. The sugar conformations are C(2′)-*endo* in both xanthosine molecules, with torsion angles (Table IV) leading to nearly identical pseudorotation parameters [10]: 156° and 156.4° for xanthosine and xanthosine dihydrate whereas pucker amplitudes τ_m differ slightly, 38.7° and 40.5° respectively, reflecting the larger deviation of C(2′) from the plane through the other four sugar ring atoms in the dihydrate crystal form (Table V).

Table IV. Relevant torsional angles.

	xanthosine	xanthosine · 2 H_2O[5]
Glycosyl bond C(1′)−N(9)		
C(2′)−C(1′)−N(9)−C(8)	110.3(3)	118.2
C(2′)−C(1′)−N(9)−C(4)	−68.1(3)	60.7
O(1′)−C(1′)−N(9)−C(8)	−132.7(3)	−125.7
O(1′)−C(1′)−N(9)−C(4)	48.9(3)	53.4
orientation of C(5′)−O(5′)		
O(5′)−C(5′)−C(4′)−O(1′)	−65.6(3)	−66.6
O(5′)−C(5′)−C(4′)−C(3′)	54.5(3)	52.7
Sugar, endocyclic		
C(1′)−C(2′)−C(3′)−C(4′)	−34.3(3)	−36.0
C(2′)−C(3′)−C(4′)−O(1′)	20.2(3)	21.3
C(3′)−C(4′)−O(1′)−C(1′)	3.8(3)	3.8
C(4′)−O(1′)−C(1′)−C(2′)	−26.7(3)	−27.4
O(1′)−C(1′)−C(2′)−C(3′)	38.0(3)	39.8
Sugar, exocyclic		
O(2′)−C(2′)−C(1′)−O(1′)	159.1(3)	166.4
O(2′)−C(2′)−C(3′)−C(4′)	−154.4(3)	−160.9
O(3′)−C(3′)−C(2′)−C(1′)	82.2(3)	79.6
O(3′)−C(3′)−C(4′)−O(1′)	−91.8(3)	−96.6

Table V. Displacement of ribose ring atoms (C(2′) is not included in the least squares plane calculations).

	xanthosine	xanthosine, 2 H_2O [5]
C(1′)	−0.014	−0.014
C(2′)	0.585	0.607
C(3′)	0.012	0.012
C(4′)	−0.020	−0.020
O(1′)	0.022	0.022

The crystal structure of anhydrous xanthosine features an unusual intramolecular O(2′)H ... O(3′) hydrogen bond between the vicinal ribose hydroxyl groups. Such interaction (O(3′)H ... O(2′)) has only been observed before in a modified nucleoside, 6-amino-10-(β-ribofuranosylamino)pyrimido[5,4-d]-pyrimidine [11] with exceptional O(1′)-*endo* sugar puckering giving rise to nearly eclipsed C(2′)−O(2′) and C(3′)−O(3′) bonds. We assume that the O(2′)H ... O(3′) bond observed in xanthosine with C(2′)-*endo* sugar pucker is due to the absence of any other hydrogen bond acceptor around the O(2′)H hydroxyl group. The torsion angle C(3′)−C(2′)−O(2′)−H of 21° nearly eclipses H with C(3′) and the angle O(2′)−H ... O(3′) of only 104.7° deviates largely from the usually adopted 160°−180°. This intramolecular hydrogen bond is therefore sterically unfavourable and other, intermolecular interactions are preferred if they can be formed.

The crystal packings of anhydrous xanthosine and its dihydrate are very different. In the latter, four hydrogen bonds between water molecules, purine N(1)H and sugar O(5′) groups and secondary hydroxyls O(2′)H, O(3′)H are probably responsible for the larger puckering amplitude of 40.5° whereas in the anhydrate crystal structure, there is only the intramolecular O(2′)H ... O(3′) interaction and a hydrogen bond O(3′)H ... O(2), see Table VI. Another difference in packing concerns stacking of purine heterocycles which is negligible in the dihydrate form but in anhydrous xanthosine, purines

Table VI. Hydrogen bonds in the anhydrous xanthosine crystal structure.

X H Y	Distances [Å]		Angle [°]	
	H−Y	X−Y	X−H−Y	H−X−Y
N(1) −H(N1) ... O(6) (−0.5 − *x*, 1.0 − *y*, −0.5 + *z*)	2.21	3.132	149.3	21.1
O(3′)−H(O3′) ... O(2) (−*x*, −0.5 + *y*, 1.5 − *z*)	1.64	2.665	153.0	16.3
O(5′)−H(O5′) ... N(7) (0.5 + *x*, 0.5 − *y*, 1.0 − *z*)	2.03	2.883	150.4	20.3
[a] N(3) −H(N3) ... O(5′) (*x*, *y*, *z*)	1.86	2.836	159.0	13.6
[a] O(2′)−H(O2′) ... O(3′) (*x*, *y*, *z*)	2.03	2.602	104.7	49.1

[a] Intramolecular hydrogen bond.

are stacked along the *c*-axis at about 3.4 Å interplanar separation and a tilt of 45° with respect to the *a*, *b* plane.

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