ISOLATION AND X-RAY CRYSTAL STRUCTURE OF A NOVEL 8-OXOPURINE COMPOUND FROM A MARINE SPONGE

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ABSTRACT.—A novel 8-oxopurine compound, $C_7H_9N_5O$, has been isolated from the sponge *Hymeniacidon sanguinea* Grant. The structure was determined as **1** on spectral grounds and by X-ray analysis of its acetyl derivative **2**.

Purines are widely distributed among marine organisms (1). However, unusual purines in the marine environment, namely, herbipoline (2) and spongopurine (3), have been isolated from only two varieties of the sponge *Geodia gigas*, and 2-amino-6,8-dioxypurine (4) was isolated from the ascidian *Microcosmus polymorphus*. We now wish to report the structure of a new marine purine **1**, 1,9-dimethyl-6-imino-8-oxopurine, characterized by the rare in nature 8-oxo function.

Compound **1** was found, together with 1-methyl-6-iminopurine (spongopurine) (3), during a reinvestigation of the butanolic extract of the English channel sponge *Hymeniacidon sanguinea* Grant, and its structure was verified by X-ray analysis of the acetyl derivative **2**. The sponge was extracted and worked up as previously described (5). After elution with H_2O of the 4-hydroxyphenyl-pyruvic acid oxime (5), a mixture of **1** and 1-methyl-6-iminopurine was obtained from the Dowex column by elution with 2 N NH_4OH. After acetylation, the mixture was resolved by preparative tlc. The acetylated spongopurine was identified by comparison with an authentic sample.

Compound **2** has the molecular formula $C_9H_{11}N_5O_2$ on the basis of the hrms and exhibits in the ir spectrum bands at $1730 \ cm^{-1}$, attributable to a carbonyl group in the purine skeleton, and at $1645 \ cm^{-1}$, due to the carbonyl of the acetyl substituent. The ^1H-nmr spectrum is in accordance with the depicted structure, showing singlets at δ 8.10 (H-2), 3.75 (N-CH₃), 3.35 (N-CH₃), and 2.22 (CH₃CO).
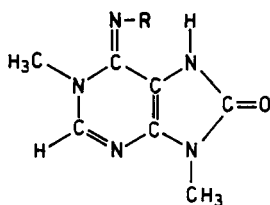
1 R=H
2 R=COCH₃

In order to define the structure unambiguously, a single crystal X-ray analysis of **2** was carried out, which definitively shows that the compound has a purine skeleton with an unusual oxo-function in position 8. The independent part of the monoclinic unit cell contains two molecules A and B (unprimed and primed labels, respectively) shown in the same orientation side by side in Figure 1. The two molecules have a strictly similar geometry as indicated by the values of the corresponding bond lengths and angles (Table 2), which compare well with those found for several purine derivatives (6). The trend of the internal ring angles can be consistently correlated to the number of bonds
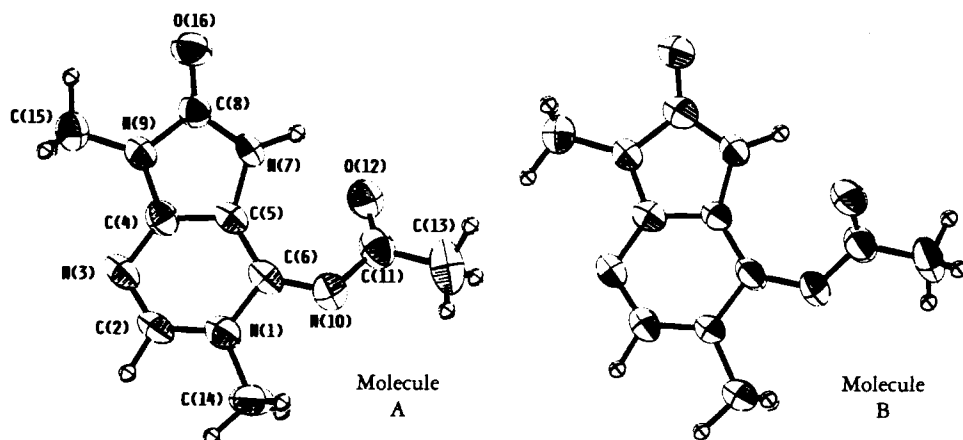
FIGURE 1. Molecular structure for the compound **2**, with thermal elipsoids at 50% probability and atomic numbering used (for the molecule B the primed labels were used).

TABLE 1. Positional Parameters ($\times 10^4$) and Equivalent Isotropic Temperature Factors ($\mathring{A}^2$) for Compound **2**[a]

$B_{eq}=4/3 \sum_i\sum_j b_{ij} a_i a_j$			
	x	y	z	B_{eq}
N(1)	3297(5)	495(1)	3881(6)	4.3(1)
C(2)	2902(6)	739(2)	5146(8)	4.2(1)
N(3)	2299(5)	569(1)	6313(6)	4.5(1)
C(4)	2043(5)	114(2)	6063(7)	3.7(1)
C(5)	2409(5)	− 165(2)	4818(7)	3.6(1)
C(6)	3104(5)	15(2)	3664(7)	3.7(1)
N(7)	1843(4)	− 601(1)	5009(6)	3.7(1)
C(8)	1161(5)	− 587(2)	6303(7)	3.7(1)
N(9)	1304(4)	− 143(1)	6978(6)	3.9(1)
N(10)	3509(4)	− 162(2)	2312(6)	4.4(1)
C(11)	3789(6)	− 621(2)	2248(8)	4.8(1)
O(12)	3919(4)	− 897(1)	3508(5)	5.2(1)
C(13)	4007(8)	− 778(2)	508(9)	7.3(2)
C(14)	3971(7)	752(2)	2695(8)	5.5(2)
C(15)	780(6)	9(2)	8441(7)	4.7(1)
O(16)	557(4)	− 901(1)	6869(5)	5.1(1)
N(1′)	1393(4)	−2172(1)	6893(6)	4.3(1)
C(2′)	2320(6)	−1827(2)	7467(9)	5.5(2)
N(3′)	3658(5)	−1873(1)	8284(7)	5.7(1)
C(4′)	4069(6)	−2318(2)	8620(8)	4.8(2)
C(5′)	3200(5)	−2695(2)	8066(8)	4.0(1)
C(6′)	1783(6)	−2638(2)	7143(8)	4.1(1)
N(7′)	4081(5)	−3076(1)	8591(7)	5.2(1)
C(8′)	5404(6)	−2937(2)	9439(9)	6.0(2)
N(9′)	5394(5)	−2457(2)	9439(8)	6.1(2)
N(10′)	749(5)	−2935(1)	6335(6)	4.6(1)
C(11′)	789(6)	−3376(2)	6952(8)	4.8(1)
O(12′)	1532(4)	−3510(1)	8408(6)	5.6(1)
C(13′)	− 244(7)	−3694(2)	5721(9)	6.6(2)
C(14′)	− 107(6)	−2056(2)	5989(9)	5.4(2)
C(15′)	6625(7)	−2160(2)	10171(12)	7.7(2)
O(16′)	6468(4)	−3172(1)	10135(8)	8.3(2)

[a]Unprimed and primed labels refer to molecules A and B, respectively.

shared by the nitrogen atoms. In the pyrimidine ring of the two molecules, the internal valency angles at N(1) are significantly larger than the values of 118.6° expected for purine systems without substituents in this position. Similarly in the five-membered ring, the geometrical details at N(7) and N(9) atoms fit well with the presence of extra-annular substituents at the nitrogen atoms. On the other hand, the valency angles at N(3) and N(10) indicate that these nitrogens are not protonated. The same conclusion on the protonation state of the nitrogen atoms was obtained from the difference electron density map, which clearly shows that among the three nonmethylated nitrogens, only N(7) has a hydrogen attached to it. Indeed, all the hydrogens attached to the ring atoms appear as the strongest maxima on the residual map and are close to the stereochemical positions. Moreover, in both molecules the values of C(2)-N(3) and C(6)-N(10) distances indicate that these two bonds have a stronger double bond character than the remaining C-N bonds.

TABLE 2.   Bond Lengths (Å) and Bond Angles (degrees) for Compound **2**

	Mol A	Mol B			Mol A	Mol B
N(1)-C(2)	1.354(7)	1.342(7)	C(2)-N(3)-C(4) . .	111.9(5)	113.4(5)
N(1)-C(6)	1.408(6)	1.405(6)	N(3)-C(4)-C(5) . .	126.8(5)	124.8(5)
N(1)-C(14)	1.478(7)	1.480(6)	N(3)-C(4)-N(9) . .	124.3(5)	125.1(5)
C(2)-N(3)	1.310(7)	1.294(7)	C(5)-C(4)-N(9) . .	108.8(4)	109.9(5)
N(3)-C(4)	1.347(6)	1.356(6)	C(4)-C(5)-C(6) . . .	120.5(5)	120.7(5)
C(4)-C(5)	1.384(7)	1.380(7)	C(4)-C(5)-N(7) . .	105.4(5)	105.1(4)
C(4)-N(9)	1.370(6)	1.339(7)	C(6)-C(5)-N(7) . .	133.9(5)	134.2(5)
C(5)-C(6)	1.372(7)	1.383(7)	N(1)-C(6)-C(5) . .	111.8(5)	112.4(4)
C(5)-N(7)	1.407(6)	1.392(6)	N(1)-C(6)-N(10) . .	114.7(5)	114.3(4)
C(6)-N(10)	1.328(6)	1.344(6)	C(5)-C(6)-N(10) . .	133.1(5)	133.1(5)
N(7)-C(8)	1.356(6)	1.342(7)	C(5)-N(7)-C(8) . .	110.0(4)	109.9(4)
C(8)-N(9)	1.383(6)	1.392(7)	N(7)-C(8)-N(9) . .	107.0(4)	107.2(5)
C(8)-O(16)	1.232(6)	1.236(7)	N(7)-C(8)-O(16) . .	128.9(5)	129.0(5)
N(9)-C(15)	1.442(6)	1.461(7)	N(9)-C(8)-O(16) . .	124.1(5)	123.8(6)
N(10)-C(11)	1.365(7)	1.364(7)	C(4)-N(9)-C(8) . .	108.8(4)	107.9(5)
C(11)-O(12)	1.244(7)	1.227(6)	C(4)-N(9)-C(15) . .	126.8(4)	126.3(5)
C(11)-C(13)	1.498(8)	1.501(8)	C(8)-N(9)-C(15) . .	124.4(5)	125.7(5)
C(2)-N(1)-C(6) . . .	123.3(5)	122.6(5)	C(6)-N(10)-C(11) .	121.0(5)	119.5(5)
C(2)-N(1)-C(14) . . .	117.2(5)	118.5(4)	N(10)-C(11)-O(12) .	125.2(6)	125.1(5)
C(6)-N(1)-C(14) . . .	119.5(5)	118.8(4)	N(10)-C(11)-C(13) .	114.1(6)	113.8(5)
N(1)-C(2)-N(3) . . .	125.5(5)	125.9(5)	O(12)-C(11)-C(13) .	120.6(5)	120.9(5)

For the two molecules, the five-membered ring is planar within the experimental errors, while the six-membered ring deviates from planarity at most 0.023(5) Å (molecule A) and 0.021(6) Å (molecule B), the two fused rings making an angle of 5.2° and 1.5°, respectively. Small deviations from planarity, of the same order of magnitude as those found in the present structure, have been reported for several purine derivatives and can be attributed to the packing forces (6). The *N*-acetyl group is not coplanar with the purine skeleton but is forced out of the mean molecular plane by the short intra-molecular contact between N(7) and O(12). The torsion angles C(5)-C(6)-N(10)-C(11) and C(6)-N(10)-C(11)-O(12) are 26.7(8)° and 12.0(9)°, respectively, for molecule A, and 28.9(8)° and 15.3(9)° for molecule B. Even so, the intramolecular N(7) . . . O(12) distance (2.751(5) Å and 2.766(5) Å in the two molecules respectively) is below the sum of the accepted van der Waals radii of the two atoms (7).

The molecular packing, shown in the stereoview of Figure 2, consists of layers normal to $\vec{b}$, built up alternatively by molecules A (layers A) and molecules B (layers B). The assemblage of the molecules in the layers is stabilized by van der Waals and stack-

ing interactions, although the overlap between the purine bases is different in the two layers and not very extensive. In layer A, the molecules pile up through a symmetry center with interplanar distances of 3.35 and 3.66 Å alternatively, forming rows in a direction parallel to $\vec{a}$, whereas in layer B, the molecules are related by the glide symmetry with an interplanar separation of 3.79 Å. In this case the rows are parallel to $\vec{c}$.
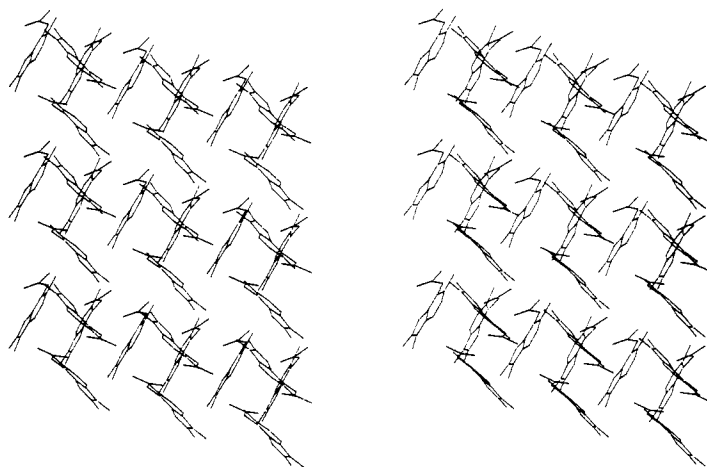

FIGURE 2.   Stereoview of the molecular packing.

Each pair of A and B molecules, belonging to adjacent layers, is held together by two hydrogen bonds, involving the N(7)-H group of one molecule and the acetylic oxygen of the other, and vice versa: [N(7) . . . O(12')=2.843(5), N(7') . . . O(12)= 2.985(5) Å]. The short intramolecular contacts (Table 3) N(7) . . . O(12') and N(7') . . . O(12') might be considered as the weaker components of an unusual system of two bifurcated hydrogen bonds (8,9), with N(7) and N(7') donors to the same pair O(12) and O(12'). However, it must be noted that the hydrogen atoms are well set along the lines N(7) . . . O(12') and N(7') . . . O(12), the angles N(7)-H . . . O(12) and N(7')-H . . . O(12') are less than 120°, and consequently the H . . . O distances are on the outer limits of the generally accepted values for this type of interaction.

The packing presents two additional short contacts between A and B molecules [C(2)-H . . . O(16')=3.241(6) and C(2')-H . . . O(16)=3.160(6) Å], which can be regarded as weak hydrogen bonds of the type already found in the crystal structures of other purine derivatives (10). The geometry of these interactions and their similarity, not required by the symmetry of the crystal, suggest that they contribute significantly to the stability of the crystal packing.

TABLE 3.   Interatomic Short Contacts for Compound 2[a]

D—H . . . . A		Symmetry	D . . . A (Å)	D-H (Å)	H . . . A (Å)	D-H . . . A (degrees)
N(7)-H(7)	O(12')	x,-½-y,-½+z;	2.843(5)	1.00	1.90	156.4
N(7')-H(7')	O(12)	x,-½-y,½+z;	2.985(6)	1.00	2.03	158.1
C(2)-H(2)	O(16')	1-x,½+y,½-z;	3.241(6)	1.08	2.17	170.7
C(2')-H(2')	O(16)	x,y,z;	3.160(6)	1.08	2.11	162.4
N(7)-H(7)	O(12)	x,y,z;	2.751(6)	1.00	2.20	113.1
N(7')-H(7')	O(12')	x,y,z;	2.766(5)	1.00	2.23	112.3

[a]Unprimed and primed labels refer to molecules A and B, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage microscope and are uncorrected. ^{1}H-nmr spectra were registered with a Varian XL 100 spectrometer (TMS as internal standard). Low resolution mass spectra were taken on an AEI MS-30, while high resolution mass spectra utilized the AEI MS-902 instrument. Ir spectra were measured for solutions in CHCl$_3$ on a Perkin-Elmer 257 Infrared Spectrophotometer. Uv spectra were recorded on Shimatzu-Baush and Lomb Spectronic 210 apparatus. Tlc analyses were carried out on silica gel plates (Merck F$_{254}$, solvent CHCl$_3$-MeOH, 92:8), and the compounds were visualized by exposure to mineral-light. The crystallographic work was carried out on the Enraf-Nonius CAD-4 diffractometer and PDP 11/34 computer of the "Centro di Metodologie Chimico-fisiche dell'Università di Napoli."

MATERIALS.—Fresh sponge (50 g dry weight after extraction) was collected in the sea of Roscoff (France).

EXTRACTION AND ISOLATION.—Fresh sponge was extracted (three times) with cold Me$_2$CO for 3 days; the solvent was removed, and the aqueous residue was extracted with Et$_2$O and n-BuOH. After evaporation, the material was dissolved in H$_2$O and applied to a column (2×20 cm) of Dowex 50W-X2 H$^+$ form. After elution with H$_2$O of the 4-hydroxyphenyl-pyruvic acid oxime, a mixture of **1** and 1-methyladenine was eluted with 2 N NH$_4$OH. The acetylation with Ac$_2$O and pyridine (1 h, reflux) allowed us to resolve the mixture on preparative SiO$_2$-tlc (Rf=0.26 and 0.4, respectively, for the acetyl derivative of 1-methyladenine and for **2**) with a yield of 0.5% dry weight for each compound.

Spongopurine, acetylated, was identified by comparison with an authentic sample; ^{1}H nmr (DMSO) δ 8.6 (s), 8.45 (s), 3.0 (s, N-CH$_3$), 2.1 (S, CH$_3$CO); ms *m/z* 191 (70%), 176 (100), 149 (55).

Compound **2**: Mp 245-246°(CHCl$_3$); ^{1}H nmr (CDCl$_3$) δ 8.10 (s, H-2), 3.75 (s, N-CH$_3$), 3.35 (s, N-CH$_3$), 2.22 (s, CH$_3$CO); uv λ max (MeOH) 326 (ε=15000), 237 nm (16500); ir ν max 1730, 1645, 1580, 1490, 1400, 1380 cm^{-1}; ms *m/z* 221 (M$^+$, 221, 0905, C$_9$H$_{11}$N$_5$O$_2$, 75%), 206 (100), 192 (51), 179 (57).

X-RAY STRUCTURE ANALYSIS.[1]—The acetyl derivative **2** was crystallized as small prisms by slow evaporation from CHCl$_3$. Accurate cell parameters were obtained by least-squares refinement of the setting angles of 20 reflections at medium ϑ accurately centered. Crystal data: C$_9$H$_{11}$N$_5$O$_2$, M$_w$=221.22, monoclinic, space groups P2$_1$/c, a=9.797(2), b=29.026(4), c=7.781(1) Å, β=106.22(2)°, V=2124(1) Å^3, Z=8, D$_c$=1.39 g cm^{-3}, μ (CuKα)=0.82 mm^{-1}. Intensities of 3140 independent reflections with ϑ⩽60° were collected at room temperature, using a single crystal (0.04×0.08×0.2 mm), ω−2ϑ scan mode and Ni-filtered CuKα radiation. The equipment and crystal stability were checked by three standard reflections monitored at intervals of 5 h (3% variation) during all the data collection. 1421 Intensities with I⩾3σ(I) were considered observed. Intensities were corrected for Lorentz and polarization factors but not for absorption effect. The structure was solved using MULTAN 79(11). The refinement of the positional and anisotropic temperature parameters for all nonhydrogen atoms was carried out by full-matrix (on F) least-squares method, providing a conventional R value of 0.088. At this point, the hydrogen atoms were generated at the expected positions (C-H= 1.08, N-H= 1.00 Å), taking into account the indications of the difference Fourier map. In the last refinement, all the H-atoms were included, as fixed atoms, with the isotropic thermal parameters set equal to the B$_{eq}$ of the parent atoms. At convergence, the discrepancy index R=Σ||F$_o$|−|F$_c$||/Σ|F$_s$| was 0.057 for the 1420 observations (0 2 0 excluded from final refinement for asymmetric background). R$_w$=0.072 with w= 1/σ^2 (F$_s$). The highest peak in the final difference Fourier synthesis showed a density of 0.2 e Å^{-3}. The atomic scattering factors used are from Cromer and Waber (12).

The final atomic coordinates for the nonhydrogen atoms of the two independent molecules are listed in Table 1. The numbering scheme used is shown in Figure 1; primed label refers to molecule B. Bond lengths and bond angles for the nonhydrogen atoms are reported in Table 2.

ACKNOWLEDGMENTS

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Center and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K.

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