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ISOLATION AND X-RAY CRYSTAL STRUCTURE OF A DERIVATIVE OF 2,6-DIAMINOPURINE FROM A SEA ANEMONE

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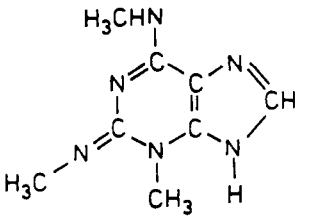
ABSTRACT.—A trimethyl derivative of 2,6-diaminopurine has been isolated from the sea anemone *Sagartia troglodytes*. The structure was determined on spectral grounds and by X-ray analysis.

Continuing our studies on polar compounds (1,2) from marine organisms, now we have isolated, from the sea-anemone, *Sagartia troglodytes* Price, a new purine **1**, 2-iminomethyl-3-methyl-6-amino-methyl-9H-purine, a derivative of the known synthetic 2,6-diaminopurine (3,4) that shows a wide variety of biological activities.

From the Me_2CO extracts of the animal, **1** was isolated (0.01% dry wt.) with a molecular formula of $C_8H_{12}N_6$ on the basis of hrms; the spectral data are in accordance with the depicted structure. The ^1H nmr showed a proton singlet at δ 7.72 (H-8) and three methyl singlets at δ 3.49, 2.95, and 2.89, all attributable to methyls on nitrogen atoms.



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The complete structure, finally established by a single crystal X-ray analysis, is characterized by a purine skeleton having three substituents, the iminomethyl, methyl, and aminomethyl groups, in positions 2, 3, and 6, respectively, of the six-membered ring. The non-hydrogen atoms of the molecule lie in a plane within 0.025 (8) Å. The base cocrystallizes with two H_2O molecules and one HOAc molecule which lies in the same plane of the purine base [maximum deviation from the purine least-squares plane is 0.23 (1) Å], and it is firmly linked to the base by two strong hydrogen bonds involving the N(7) and N(13) nitrogen atoms. The HOAc was not added to the crystallization batch but was used in the isolation procedure and explains the singlet at δ 1.75 observed in the ^1H-nmr spectra of **1**.

The two H_2O molecules O(W1) and O(W2) deviate from the plane of the purine moiety 0.607(6) and 0.065(6) Å, respectively, and, together with the carboxylic groups of the HOAc, give rise to an infinite chain of hydrogen bonds parallel to [101] direction (Figure 1). The crystal structure is assembled in layers (Figure 2); each layer extends parallel to the *b,a-c* plane and is formed by a pseudo-hexagonal packing of a basic unit comprising the purine derivative, the HOAc, and the two H_2O molecules. The packing of the layers is stabilized by hydrogen bonds between H_2O molecules O(W1) and O(W2) of different layers. Moreover, the centrosymmetrically related bases form stacked pairs at the interplanar distance of 3.35 Å and extensively overlap as indicated by the shaded area in Figure 1.

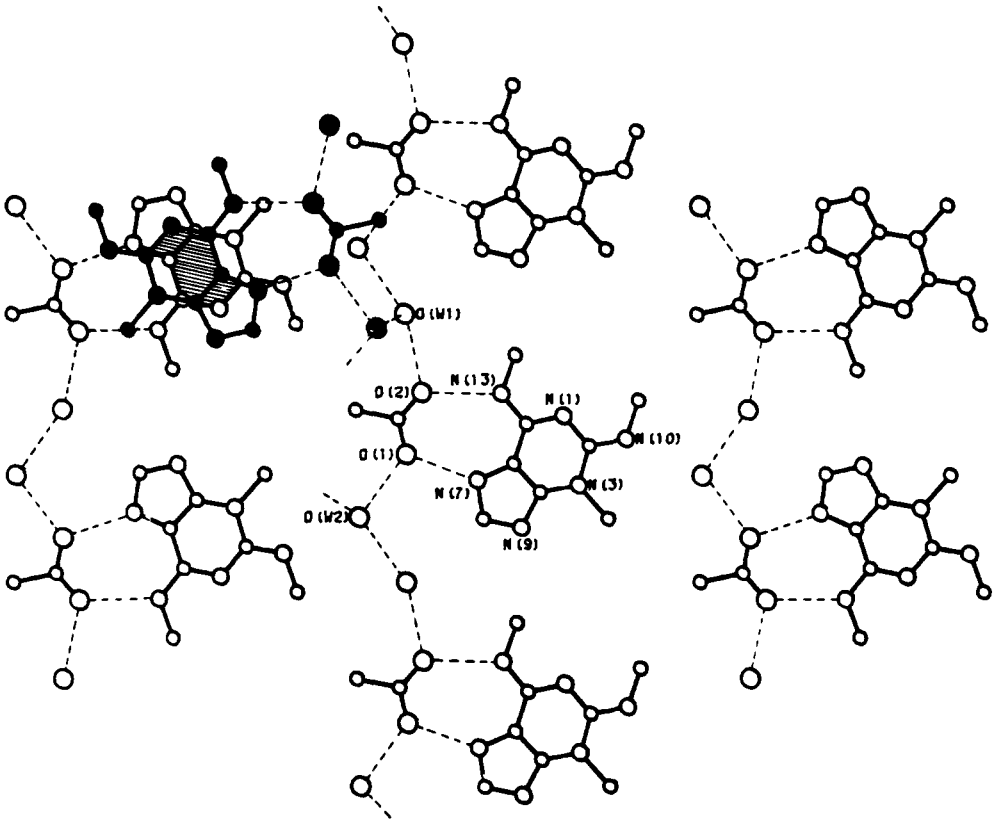

FIGURE 1. Assemblage of the structural units within a layer. Hydrogen bonds are indicated by dotted lines. On the upper left of the figure a structural unit (filled atoms) of the next layer is also shown. The shaded area indicates the overlapping between two bases related by a center of symmetry (see text).

TABLE 1. Bond Lengths (Å) and Bond Angles (Degrees)

N(1)-C(2)	1.358(7)	C(5)-N(7)	1.376(6)
N(1)-C(6)	1.318(6)	C(6)-N(13)	1.351(6)
C(2)-N(3)	1.395(7)	N(7)-C(8)	1.350(6)
C(2)-N(10)	1.305(7)	C(8)-N(9)	1.343(7)
N(3)-C(4)	1.358(6)	N(10)-C(11)	1.395(7)
N(3)-C(12)	1.548(7)	N(13)-C(14)	1.413(6)
C(4)-C(5)	1.332(7)	C(15)-C(16)	1.499(8)
C(4)-N(9)	1.384(7)	C(15)-O(1)	1.243(6)
C(5)-C(6)	1.435(7)	C(15)-O(2)	1.225(6)
C(2)-N(1)-C(6)	119.2(5)	C(6)-C(5)-N(7)	132.2(5)
N(1)-C(2)-N(3)	122.6(5)	N(1)-C(6)-C(5)	120.3(5)
N(1)-C(2)-N(10)	121.3(7)	N(1)-C(6)-N(13)	117.7(5)
N(3)-C(2)-N(10)	116.1(6)	C(5)-C(6)-N(13)	122.0(5)
C(2)-N(3)-C(4)	116.6(5)	C(5)-N(7)-C(8)	105.4(4)
C(2)-N(3)-C(12)	122.2(5)	N(7)-C(8)-N(9)	111.0(5)
C(4)-N(3)-C(12)	121.2(5)	C(4)-N(9)-C(8)	106.0(5)
N(3)-C(4)-C(5)	122.7(5)	C(2)-N(10)-C(11)	118.9(6)
N(3)-C(4)-N(9)	129.1(5)	C(6)-N(13)-C(14)	120.0(4)
C(5)-C(4)-N(9)	108.2(5)	C(16)-C(15)-O(1)	115.2(6)
C(4)-C(5)-C(6)	118.5(5)	C(16)-C(15)-O(2)	119.3(6)
C(4)-C(5)-N(7)	109.3(5)	O(1)-C(15)-O(2)	125.5(6)

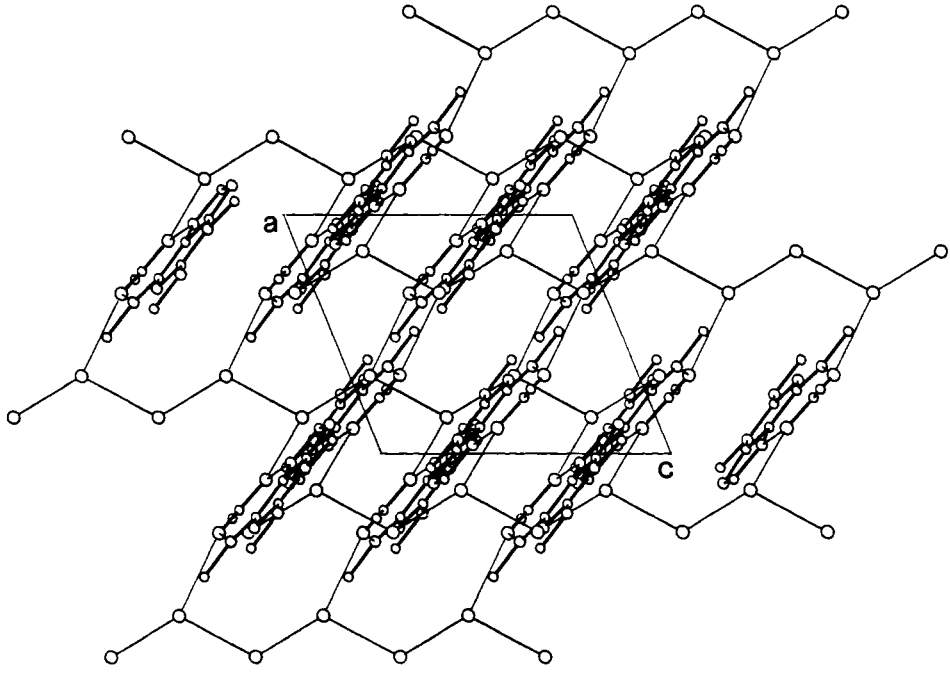

FIGURE 2. Crystal packing along the **b** direction

As mentioned in the Experimental section, the quality of the crystals is not sufficient to permit an authoritative determination of the positions for all the hydrogen atoms. Indeed, the errors associated with the geometrical parameters of the purine

TABLE 2. Positional Parameters ($\times 10^4$) and Equivalent Isotropic Temperature Factors ($Å^2$)

	$B_{eq}=4/3\Sigma_i\Sigma_j b_{ij}a_ia_j$			
	x	*y*	*z*	B_{eq}
N(1)	3082(4)	348(2)	450(7)	5.9(2)
C(2)	2077(9)	725(3)	−729(8)	5.7(2)
N(3)	579(8)	563(3)	−2163(7)	6.3(2)
C(4)	173(9)	3(3)	−2346(8)	5.5(2)
C(5)	1113(9)	−380(3)	−1213(8)	4.9(2)
C(6)	2641(9)	−192(3)	241(8)	5.0(2)
N(7)	369(8)	−908(2)	−1733(6)	5.5(2)
C(8)	−1067(9)	−823(3)	−3198(9)	6.3(2)
N(9)	−1230(9)	−273(3)	−3617(7)	8.1(2)
N(10)	2472(9)	1264(2)	−581(8)	8.2(2)
C(11)	3929(9)	1455(3)	842(9)	7.8(3)
C(12)	−575(9)	997(3)	−3488(9)	7.2(2)
N(13)	3648(8)	−554(2)	1453(7)	5.5(2)
C(14)	5125(9)	−349(3)	2875(9)	6.8(2)
Solvent molecules				
C(15)	2344(9)	−2061(3)	721(9)	6.5(2)
C(16)	2688(9)	−2680(3)	1074(9)	8.5(3)
O(1)	1068(8)	−1944(2)	−626(6)	8.2(2)
O(2)	3292(7)	−1716(2)	1748(6)	7.3(2)
O(W1)	6744(7)	−1956(2)	4292(6)	7.4(2)
O(W2)	−1520(7)	−2632(2)	−2770(6)	7.4(2)

skeleton, such as bond lengths and angles (see Table 1), are rather large with respect to the variations associated with the protonation state of the nitrogen atoms in different purine derivatives (5), so that their values cannot be used confidently to establish which nitrogen atom is protonated and which is not. However, in the difference Fourier map, peaks can be seen close to the expected position for the hydrogens attached to C(8), N(13), and N(9), although in the last case the distance is only 0.76 Å. A diffuse peak is found along the line joining N(7) to O(1), with the maximum close to O(1) so that in this remarkably short hydrogen bond, the hydrogen can be reasonably assigned to the HOAc. Indications for the hydrogen attached to the H_2O oxygens and to the methyl groups can also be found on the difference density map, although the maxima are of the same order of magnitude as the noise of the map. In conclusion, we feel confident that the assignment of the hydrogens, as shown in the scheme, is substantially correct. The scheme of hydrogen bonds is given in Table 3 and includes also the short contact O(W2)...N(10), which can be considered as the weak component of a highly asymmetric, three-center, hydrogen bond (6) involving O(W1), O(W2), and N(10).

TABLE 3. Short Contacts

D-H . . . . . A	Symmetry	D . . A
N(13)-H . . . . . O(2)	x,y,z	2.783(6)Å
O(1)-H . . . . . N(7)	x,y,z,	2.612(6)
O(W1)-H′ O2	x,y,z	2.803(5)
O(W1)-H″ . . . . . O(W2)	x+1,−y−1/2,z+1/2	2.797(6)
O(W2)-H′ . . . . . O(1)	x,y,z	2.702(5)
O(W2)-H″ . . . . . O(W1)	x−1,y,z−1	2.879(5)
O(W2)-H″ . . . . . N(10)	−x,y−1/2,−z−1/2	2.935(6)

The pharmacologic activity of diaminopurines is of widespread interest. The 2,6-diaminopurine (DAP) itself inhibits growth or multiplication in a wide variety of biological systems including tumors, viruses, plants, and bacteria (3,4). 2-Methylamino-6-aminopurine (MeDAP), a biosynthetic derivative of DAP, shows considerably less inhibitory capacity than DAP (7). Remy *et al.* have suggested that the methylation of DAP may be considered as a method of detoxification. Compound **1** is inactive in the *Artemia salina* test that detects some pharmacologic activities and shows a fair correlation with some cytotoxicity assays (8), but it is weakly active in the *Hordeum vulgaris* root growth bioassay (9). Our results may be a confirmation of the hypothesis of Remy *et al.* (7) on the role of the methylation of DAP in relation to the detoxification.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage microscope and are uncorrected. ^{1}H-nmr spectra were registered with Bruker WM 500 spectrometer (TMS as internal standard). Low and high resolution mass spectra were taken on AEI MS-50 instrument. Uv spectra were recorded on Shimatzu-Baush and Lomb Spectronic 210 apparatus. Tlc analyses were carried out on Si gel plates (Merck F_{254}, solvent $CHCl_3$-MeOH, 6:4), and the compounds were visualized by exposure to mineral-light, Sephadex LH-20 (Pharmacia), and cellulose powder CF11 (Whatman). 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 animals (45 g dry weight after extraction) were collected in the bay of Naples, Italy, and identified by Dr. Giovanni Russo of the Zoological Station of Naples. No voucher specimen is available.

EXTRACTION AND ISOLATION.—Fresh sea anemones were extracted with cold Me_2CO (3 times); the solvent was removed, and the aqueous residue was extracted with Et_2O and then with *n*-BuOH. The *n*-BuOH extract was taken to dryness in vacuo. The residue was dissolved in MeOH and applied to a column

of LH 20 (2×100 cm.), MeOH as eluent. The fraction containing the product (70 mg) was adsorbed on a column of cellulose (2×100 cm) equilibrated with *n*-BuOH-HOAc-H_2O (60:15:25). After examination by tlc, the fractions containing **1** were combined (10 mg) and crystallized from MeOH giving 5 mg of **1**. Compound **1**: mp 226-227° (MeOH); ^{1}H nmr (CD_3OD) δ 7.72 (5, H-8), 3.49, 2.95, 2.89 (ss, N-CH_3); uv λ max (MeOH) 288 (ε=14000), 248 (sh, ε=11200) nm; ms *m/z* 192 (M^+, 192.1114, $C_8H_{12}N_6$ 100%), 177 (10%), 163 (25%), 148 (15%), 135 (25%), 108 (60%).

X-RAY STRUCTURE DETERMINATION.[1]—A single crystal of approximate size 0.13×0.05×0.64 mm was used for X-ray study. Accurate cell parameters were obtained by least squares refinement of the setting angles of 25 reflections at medium θ (19°<θ≤27°) accurately centered. Crystal data: $C_8H_{12}N_6 \cdot C_2H_4O_2 \cdot 2H_2O$, M_w 288.3, monoclinic, space group $P2_1/c$, a=7.743 (3), b=23.645 (3), c=8.711 (2) Å, β=112.43 (3)°, V=1474 (1) Å^3, Z=4, Dc=1.299 g cm^{-3}, μ(CuKα) 8.20 cm^{-1}. Intensities of 2400 independent reflections with θ≤63° were collected at room temperature, using Ni-filtered radiation and ω scan mode with 2° of scansion required by the highly angular spread of reflections which denote disorder in the crystals. The equipment and crystal stability were checked by three standard reflections monitored at intervals of 5 h (4% variation) during all the data collection; 967 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 (10). The refinement of positional and anisotropic temperature parameters for all non-hydrogen atoms was carried out by full-matrix (on F) least-squares method. The H-atoms were placed in the calculated positions taking into account the indications of the difference Fourier map. The hydrogens were included in the last refinement as fixed atoms and with the isotropic thermal parameters set equal to the B_{eq} of the parent atoms. At convergence the final discrepancy index $R=\Sigma(|F_o|-|F_c|)/\Sigma|F_o|$ was 0.070: Rw=0.095 with $w=1/\sigma^2(F_o)$. The atomic scattering factors used are from Cromer and Waber (11). The final atomic parameters for non-hydrogen atoms are reported in Table 2.

BIOASSAYS.—The root growth bioassay technique was essentially that of Onckelen and Verbeeck (9). Compound **1** (2 mg) was dissolved in 100 ml of H_2O. The solution was poured into 10 dishes with Whatman grade 113 paper as support and 10 *H. vulgaris* seeds placed on each plate. After 4 days at 25° in the dark, the length of the main root was measured and compared with that of control plants, with the result being a decrease in root growth of 28%. The brine shrimp bioassay technique was that of Meyer *et al.* (8). At the concentration of 20 μg/ml in the brine medium; compound **1** was inactive.

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LITERATURE CITED

1. G. Cimino, S. De Rosa, and S. De Stefano, *Experientia*, **40**, 339 (1984).
2. G. Cimino, A. De Giulio, S. De Rosa, S. De Stefano, R. Puliti, C.A. Mattia, and L. Mazzarella, *J. Nat. Prod.*, **48**, 523 (1985).
3. G.H. Hitchings, G.B. Elion, H. Vander Werff, and E.A. Falco, *J. Biol. Chem.*, **174**, 765 (1948).
4. G.B. Elion and G.H. Hitchings, *J. Biol. Chem.*, **187**, 511 (1950).
5. D. Voet and A. Rich, *Prog. Nucleic Acid Res. Mol. Biol.*, **10**, 183 (1970).
6. G.A. Jeffrey and J. Mitra, *Acta Cryst.*, **B39**, 469 (1983).
7. C.N. Remy and M.S. Smith, *J. Biol. Chem.*, **228**, 325 (1957).
8. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
9. H.A. von Onckelen and R. Verbeeck, *Phytochemistry*, **11**, 1677 (1972).
10. P. Main, M.M. Woolfson, and G. Germain, "Multan 79: A Computer Program for the Automatic Solution of Crystal Structures," Department of Physics, University of York, England, 1979.
11. D.T. Cromer and J.T. Waber, in: "International Tables for X-Ray Crystallography," vol. 4. Kinock Press, Birmingham, England, 1974.

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