THE CRYSTAL STRUCTURE OF N^6-(Δ^2-ISOPENTENYL)-2-METHYLTHIOADENINE, A MODIFIED BASE OF tRNA WITH CYTOKININ ACTIVITY.

R. K. McMullan and M. Sundaralingam*

Crystallography Laboratory
Department of Biochemistry
University of Wisconsin
Madison, Wisconsin 53706

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Summary

The crystal structure of the title compound, a modified base of tRNA has been determined from three-dimensional x-ray diffraction data. The plane of the isopentenyl side chain is rotated 91° from the plane through the adenine system and the methyl thio group. The substituents on the adenine ring prevent N(1) from hydrogen bonding; the molecule exhibits instead two types of pairing arrangements, one of which is compatible with the Hoogsteen or "reversed" Hoogsteen pairing scheme.

The modified base of transfer RNA(tRNA) N^6-(Δ^2-isopentenyl)-2-methylthioadenine(I)(1,2) is one in a family of four adenine derivatives (Figure 1)(3) that possesses cytokinin activity. The nucleosides of these bases are located adjacent to the 3' side of the anticodon in only those tRNA molecules that respond to codons starting with uridine(4). It has been demonstrated that the isopentenyl derivatives are essential in the codon-anticodon interactions(5,6), although their role at the molecular level is not yet understood. We report here the results of a crystal structure analysis which illustrates the hydrogen bonding and conformational properties of (I).

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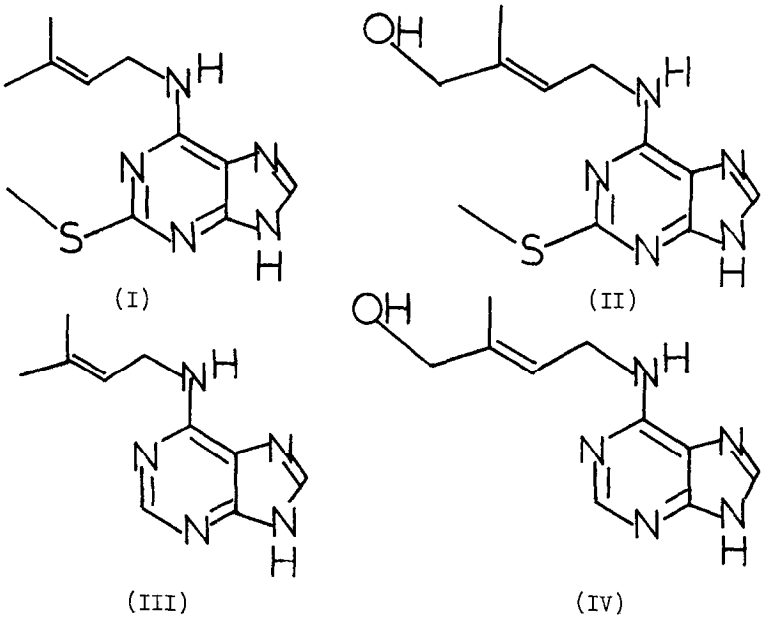

(I) N^6-(Δ^2-isopentenyl)-2-methylthioadenine
(II) N^6-(trans-4-hydroxy-3-methylbut-2-enyl)-2-methylthioadenine
(III) N^6-(Δ^2- isopentenyl) adenine
(IV) N^6-(trans-4-hydroxy-3-methylbut-2-enyl)adenine

FIGURE 1. The Four modified bases possessing cytokinin activity found in tRNA.

Materials and Methods

Colorless crystals of (I) suitable for x-ray diffraction analysis were generously supplied by Dr. Sidney Hecht of this University. The accurate unit-cell dimensions of the crystal measured on our diffractometer are given in Table 1.

Three-dimensional intensity data were collected on a Picker 4-circle automated diffractometer with nickel-filtered copper radiation. Of the 2096 independent reflections in the range $0^\circ \leq \theta \leq 128^\circ$, 1371 were observed with intensities greater than $1.5\sigma(I)$, where the standard deviations $\sigma(I)$ were determined by counting statistics. An interpretation of the (E^2-1) Patterson function calculated with these data provided positions of the sulfur atom and the ten carbon and nitrogen atoms of the adenine nucleus. The difference Fourier synthesis from which these atoms were removed then revealed positions of the six

TABLE 1

Crystal Data for (I)

Stoichiometry	$C_{11}H_{15}N_5S$
Molecular weight	249.34
Crystal system	Triclinic
Space group	$P\bar{1}$
Cell dimensions	a = 5.907 ± 0.002Å
	b = 11.487 ± 0.006
	c = 10.595 ± 0.004
	α = 95.73 ± 0.04°
	β = 100.00 ± 0.04
	γ = 114.27 ± 0.04
Cell volume	633.24Å^3
Z	2
Calculated density	1.308 g. cm^{-3}

carbon atoms of the side chains. Positions of the fifteen hydrogen atoms were found in the difference map following anisotropic refinement of the heavier atoms by the method of full-matrix least-squares. The present agreement index R is 0.048 for the 1371 reflections included in the analysis.

Results and Discussion

The observed values of the bond lengths and valence angles in the adenine nucleus are in good agreement with those found in the adenine nucleosides and nucleotides (7) and will be reported in detail elsewhere. Figure 2 illustrates the molecular conformation and mode of hydrogen bonding in the structure. Hydrogen bonding here occurs across inversion centers and involves N(9)-H...N(3) and N(6)-H...N(7) pairs. Both potentially active protons and the two most favorable acceptor sites in the molecule are thus utilized. This bonding gives rise to continuous ribbons along the b axis, which repeat with the third base in the sequence at the distance of 11.487Å. The conformation, apart from rotations of the methyl groups, can be described by two planes, which are defined respectively by the carbon atoms of the isopentenyl group and the ten atoms of the adenine nucleus. The dihedral angle between the planes is

approximately 91°. Atoms of the methylthio substituent, S(2) and C(10), lie in the plane of the adenine nucleus, apparently as a result of π interactions between S(2) and C(2) of the heterocyclic system. It is seen (Figure 2) that the molecular conformation is determined primarily by steric requirements for base pairing at N(6) and N(7).

The structure analysis demonstrates that the N(1) site of the base is prevented from participating in hydrogen bonding. It is of particular interest that the pairing(Figure 2) involves the Hoogsteen(8,9) sites N(6) and N(7), suggesting that these sites may pair with uridine(Figure 3) which with the isopentenyladenine nucleoside flanks the anticodon of many tRNA's. However, such pairing is contrary to existing chemical

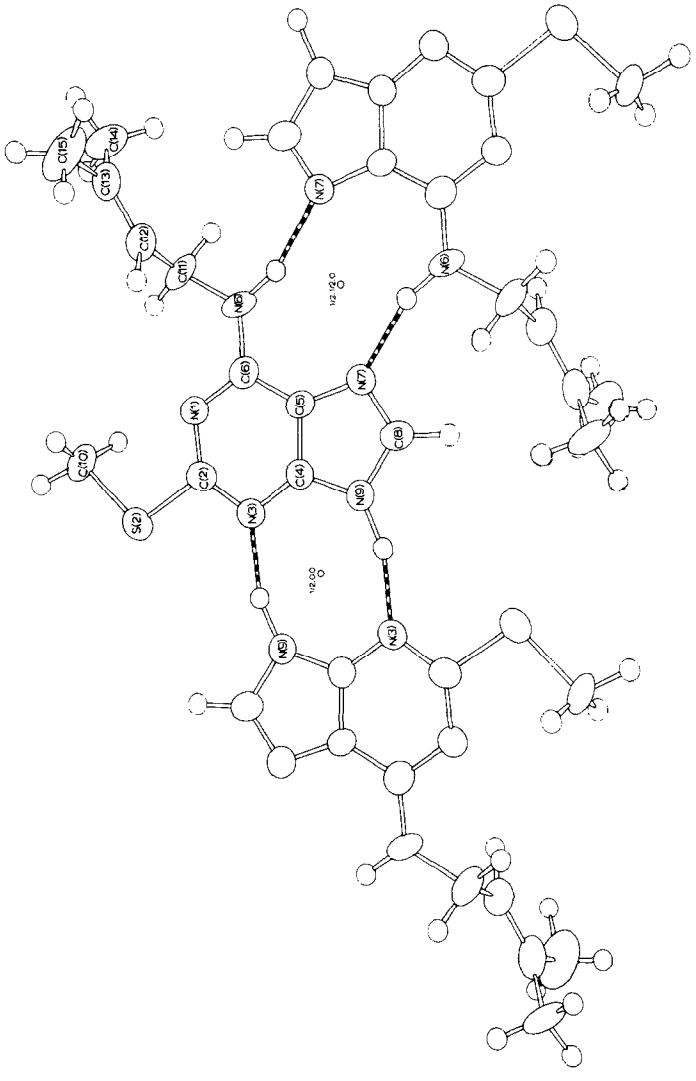

FIGURE 2. The hydrogen bonding ribbon in (I) viewed approximately normal to the base plane. Hydrogen bond distances are N(6)-H...N(7) = 2.99Å and N(9)-H...N(3) = 2.88Å.

evidence which indicate that the anticodon loop is not involved in secondary or tertiary structure(10).

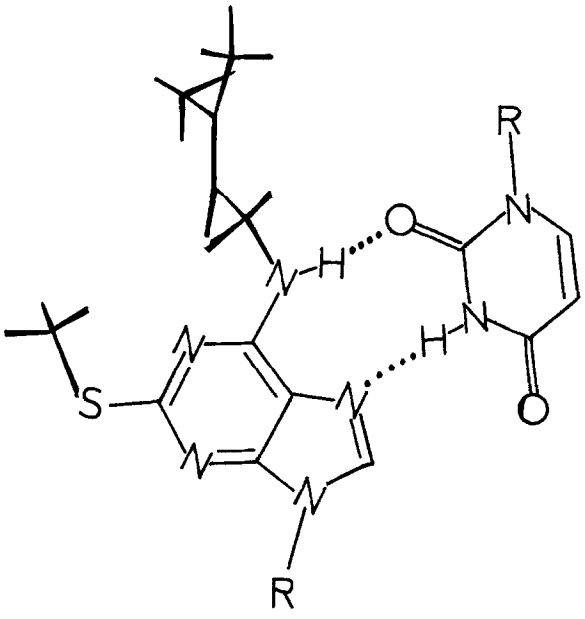

FIGURE 3. The "reversed" Hoogsteen(9) base pairing involving the isopentenyl nucleoside and uridine. From considerations of molecular models of tRNA it is seen that this pairing is possible.

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