Crystal and Molecular Structure of 2-Thio-5-carboxymethyluridine and Its Methyl Ester: Helix Terminator Nucleosides in the First Position of Some Anticodons†

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ABSTRACT: The crystal and molecular structures of the odd wobble nucleoside 2-thio-5-methylcarboxymethyluridine (s^2mcm^5U) and its analogue 2-thio-5-carboxymethyluridine monohydrate ($s^2cm^5U \cdot H_2O$) are reported. Both nucleosides exhibit the ^3E conformation of the ribose which is connected with low dihedral angles about the glycosidic bonds of 16° for s^2mcm^5U and 3° for s^2cm^5U, respectively. Although the packing of the molecules is widely different, the location of the bases next to each other is nearly identical, showing a staggered conformation with little base overlap. The geometry of the two carboxymethyl substituents is very similar. Both show the carbonyl oxygen of the carboxylic group located next to C(5) with distances of 2.86 Å for s^2mcm^5U and 2.85 Å for s^2cm^5U. MINDO/3 calculations of the energy minimum of the carboxymethyl substituent reveal that this is the preferred conformation of the molecule and not an artifact resulting from packing forces. Modified uridine derivatives with substituents related to the carboxymethyl group in the wobble position may thus function as helix terminator nucleosides because steric hindrance prevents the continuation of the anticodon stack toward the 5′ end of the anticodon loop. The molecular structures of these compounds reveal no explanation regarding their restricted wobble recognition. We, therefore, substituted Gm(34) in the anticodon loop structure of $tRNA^{Phe}$ with s^2mcm^5U by computer methods in order to study the possible interactions of this nucleoside with its neighbors and the anticodon loop backbone. The location of the substituent at the 5 position in this plot differs from that reported by Berman et al. [Berman, H. M., Marcu, D., Narayanan, P., Fissekis, J. D., and Lipnick, R. L. (1978), *Nucleic Acids Res. 5*, 893–903]. The molecular aspects leading to this contradiction are discussed in detail. Our plot demonstrates that the slight distortion necessary for the formation of the G·U wobble pair might not be possible for s^2mcm^5U because of steric hindrance due to the bulky mcm substituent with the backbone of the so-called U turn.

With few exceptions, uridine in the 5′ position of the anticodons of all tRNAs sequenced so far is replaced by modified uridine derivatives. These include thio substitution of the 2-keto oxygen and/or the introduction of various substituents in the 5 position of the uracil moiety (Nishimura, 1972; McCloskey and Nishimura, 1977). Some of these nucleosides amplify the wobble recognition (Crick, 1966). For example, it has been shown that the nucleoside V (uridine-5-oxyacetic acid) in the first position of the *Escherichia coli* $tRNA^{Val}_1$ and $tRNA^{Ser}_1$ anticodons recognizes uracil in addition to adenine and guanine (Takemoto et al., 1973; Ishikura et al., 1971). In contrast, 2-thiouridine derivatives restrict the wobble recognition; they form base pairs with A but not with G (Sekiya et al., 1969). This behavior is presumably due to the reduced ability of sulfur to form a hydrogen bond with HN(1) of guanine (Yoshida et al., 1971; Agris et al., 1973). Among the odd uridine derivatives present in anticodons are the structures related to 5-carboxymethyluridine (cm^5U),[1] originally isolated from alkaline hydrolysates of bulk yeast and wheat embryo tRNAs (Gray and Lane, 1967, 1968). Further studies revealed that cm^5U derivatives also occur in different tRNA species. The methyl ester (mcm^5U) has been found in yeast tRNA (Tumaitis and Lane, 1970) and the amide in yeast and in wheat embryo tRNAs (Dunn and Trigg, 1975). The crystal structure of the latter has been recently reported (Berman et al., 1978). Its 2′-*O*-methyl derivative has been isolated from yeast tRNA hydrolysates (Gray, 1976).

It has been shown that mcm^5U occurs in the wobble position of $tRNA^{Arg}_3$ from brewer's yeast (Kuntzel et al., 1975). The analogue of mcm^5U, in which the 2-keto oxygen is replaced by sulfur (s^2mcm^5U), has also been isolated from yeast tRNA hydrolysates (Baczynskyi et al., 1968; Kwong and Lane, 1970) and was found in the wobble positions of $tRNA^{Lys}_2$ (Madison et al., 1972) and $tRNA^{Glu}_3$ (Kobayashi et al., 1974) from baker's yeast.

Sekiya et al. (1969) have shown by the ribosomal binding assay and by incorporation of Glu in in vitro synthesized hemoglobin, that $tRNA^{Glu}_3$ recognizes only GAA as a codon and not GAG. Recent studies of the coding properties of $tRNA^{Arg}_3$ from brewer's yeast also revealed that the sulfur lacking mcm^5U recognizes only AGA but not AGG (Weissenbach and Dirheimer, 1978). Therefore, it seems that in these wobble nucleosides the methyl ester of the carboxymethyl group in the 5 position of uridine rather than the 2-thio group causes the altered recognition pattern of the respective tRNAs. This behavior of mcm^5U was predicted by Sen and Gosh (1976) from desulfination of $tRNA^{Lys}_2$ from yeast.

Studies of the biosynthesis of mcm^5U support the conclusion that cm^5U is the precursor of its blocked derivatives because cm^5U residues in bulk tRNA can be methylated by a cell-free extract of wheat embryo (Bronskill et al., 1972). It is not clear as yet whether the methyl ester and the amide are alternative pathways or if they are metabolically interconvertible (Dunn

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[1] Abbreviations used: s^2cm^5U, 2-thio-5-carboxymethyluridine; cm^5U, 5-carboxymethyluridine; s^2mcm^5U, 2-thio-5-methylcarboxymethyluridine; mcm^5U, 5-methylcarboxymethyluridine; ncm^5U, 5-carbamoylmethyluridine; t^6A, N^6-(*N*-threonylcarbonyl)-adenosine; g^6A, N^6-(*N*-glycylcarbonyl)adenosine.

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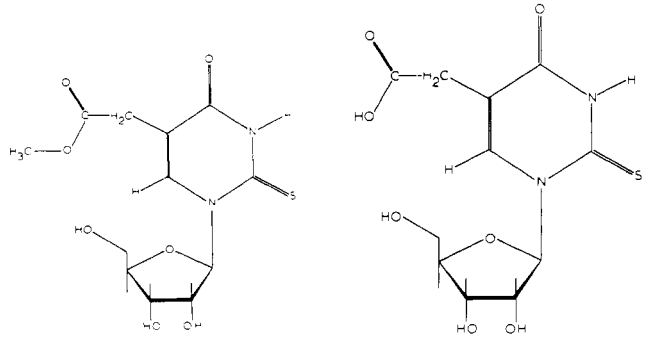
TABLE I: Crystal Data.

	$s^2cm^5U \cdot H_2O$	s^2mcm^5U
mol form.	$C_{11}H_{14}O_7N_2S \cdot H_2O$	$C_{12}H_{16}O_7N_2S$
mol wt	336	332
cryst dimens (mm)	0.2 × 0.1 × 0.8	0.1 × 0.02 × 0.6
space group	$P2_12_12_1$	$P2_12_12_1$
cell const (Å)	a = 23.15 (1)	a = 19.27 (1)
	b = 11.571 (6)	b = 14.006 (8)
	c = 5.316 (5)	c = 5.320 (7)
V (Å^3)	1424.2	1435.7
Z	4	4
ρ_{obsd} (g·cm^{-3})	1.57	1.53
ρ_{calcd} (g·cm^{-3})	1.567	1.536
μ (cm^{-1})	23.1	22.4

and Trigg, 1975). However, the carboxymethyl derivative itself has not been found in tRNAs.

In order to explain the unique coding properties of mcm^5U- and s^2mcm^5U-containing tRNAs and the absence of cm^5U in tRNA, we determined the crystal structures of both s^2mcm^5U and s^2cm^5U.



In addition, a calculation of the conformational properties of the carboxymethyl substituent in uridine using the MINDO/3 method to optimize the geometry was carried out to give further support to the assumption that the crystalline and solute conformations are identical. Furthermore, we computed a plot of the U turn from the crystal structure of $tRNA^{Phe}$ (Quigley and Rich, 1976) in which we replaced the 2′-*O*-methylguanosine(34) by s^2mcm^5U to demonstrate the possible interactions of substituents at the 5 position in modified uridine nucleosides with their neighboring nucleotides and the backbone of the anticodon loop, assuming that the so-called U turn is a general structural feature of all anticodon loops.

Materials and Methods

2-Thio-5-carboxymethyluridine methyl ester (s^2mcm^5U) was a generous gift of Dr. Vorbrüggen, Schering AG., Berlin. After several unsuccessful attempts with different solvents, one very small needle-shaped crystal was obtained from a mixture of acetone, methanol, and chloroform (1:1:2, v/v), which was allowed to evaporate slowly from an open vessel at ambient temperature. As soon as the first tiny crystals formed at the interface of the solvents, the vessel was tightly stoppered and slowly cooled to 10 °C. Treatment of s^2mcm^5U with formic acid/water (1:1, v/v) yielded clusters of thin needles of 2-thio-5-carboxymethyluridine monohydrate ($s^2cm^5U \cdot H_2O$).

The intensities were collected on a Stoe two-circle diffractometer (Cu Kα radiation) equipped with a graphite monochromator. Both crystals were oriented along c. In this way, 741 symmetry-independent reflections of $s^2cm^5U \cdot H_2O$ and 555 of s^2mcm^5U with $\theta \leq 60°$ were measured in the $\theta - 2\theta$ scan mode. The data were corrected for background and for Lorentz and polarization factors but not for absorption. 713 reflections of $s^2cm^5U \cdot H_2O$ had $|F| > 3\sigma_F$, and 460 reflections of s^2mcm^5U had $|F| > 2\sigma_F$.

The crystal structures were solved with SHELX-76 (Sheldrick, unpublished program). Isotropic refinement reduced R to 0.113 ($s^2cm^5U \cdot H_2O$) and 0.119 (s^2mcm^5U), which dropped to 0.084 for $s^2cm^5U \cdot H_2O$ with anisotropic temperature factors, whereas only the sulfur atom was made anisotropic for s^2mcm^5U because of the low number of measured reflections. Successive electron-density difference maps yielded the positions of 10 of the 16 hydrogen atoms in the respective structures. The six remaining hydrogen atoms were almost exclusively bonded to carbon atoms and could be positioned geometrically. The parameters of the hydrogen atoms were not varied. The refinement converged at R values of 0.078 and 0.097 for $s^2cm^5U \cdot H_2O$ and s^2mcm^5U, respectively. Parameters and structure factors are available as supplementary material.

The calculation of the preferred conformation of the carboxymethyl group was carried out with a revised version (Bischof, 1976) of the SCF-MO method MINDO/3, which considers all valence electrons and minimizes the total energy with respect to the geometrical variables (Bingham et al., 1975).

Results

Crystal data of the two nucleosides summarized in Table I show that both crystallize in the same orthorhombic space group $P2_12_12_1$ with needle axes c.

Conformation of s^2cm^5U and s^2mcm^5U. The bond lengths and angles (Figure 1) agree with those of similar nucleosides, but a detailed discussion is impossible due to the increased standard deviations which are the result of the very small crystals obtained from these compounds. The pyrimidine rings are, within experimental error, planar with a standard deviation of the ring atoms of σ = 0.01 Å. Only the sulfur atom shows a perceptible deviation (0.11 Å) from the base plane in s^2mcm^5U, whereas the bond to C(1′) is much less bent than in s^2cm^5U where C(1′) deviates by 0.22 Å.

The most striking molecular feature is the remarkable agreement between the conformations of the substituents in the 5 position (Figure 2). In both molecules the bond C(51)-C(52) is approximately perpendicular to the base plane and the carbonyl oxygen of the carboxylic group is situated next to the ring atom C(5), whereas the carboxyl oxygen points away from the base showing dihedral angles C(5)-C(51)-C(52)-O(52) of 163° and 166° for s^2cm^5U and s^2mcm^5U, respectively (Figure 1).

The ribosyl moieties have the C(3′)-endo conformation with C(1′), C(2′), C(4′), and O(1′) in the plane (σ = 0.03 and 0.01 Å), while C(3′) deviates by 0.55 and 0.44 Å for s^2cm^5U and s^2mcm^5U, respectively. As usual in nucleosides, the C(1′)-O(1′) bonds are shorter than C(4′)-O(1′). The orientation at the glycosidic bond N(1)-C(1′) is anti with dihedral angles C(6)-N(1)-C(1′)-O(1′) of χ = 3° (s^2cm^5U) and 16° (s^2mcm^5U), which are in the range between 0° and 20° typical for 2-thiopyrimidines (Lin and Sundaralingam, 1971; Lin et al., 1971; Kojić-Prodić et al., 1974, 1976; Hawkinson, 1977) and fit in the common relation between the puckering of the ribose and the orientation of the base (Sundaralingam, 1969; Egert et al., 1977). The two nucleosides differ, however, in the conformation around C(4′)-C(5′): s^2mcm^5U shows the usually preferred gauche-gauche arrangement with dihedral angles O(1′)-C(4′)-C(5′)-O(5′) and C(3′)-C(4′)-C(5′)-O(5′) of −71° and 47°, respectively, which allows the intramolecular interaction C(6)-H···O(5′) (Table II) often found in nucle-

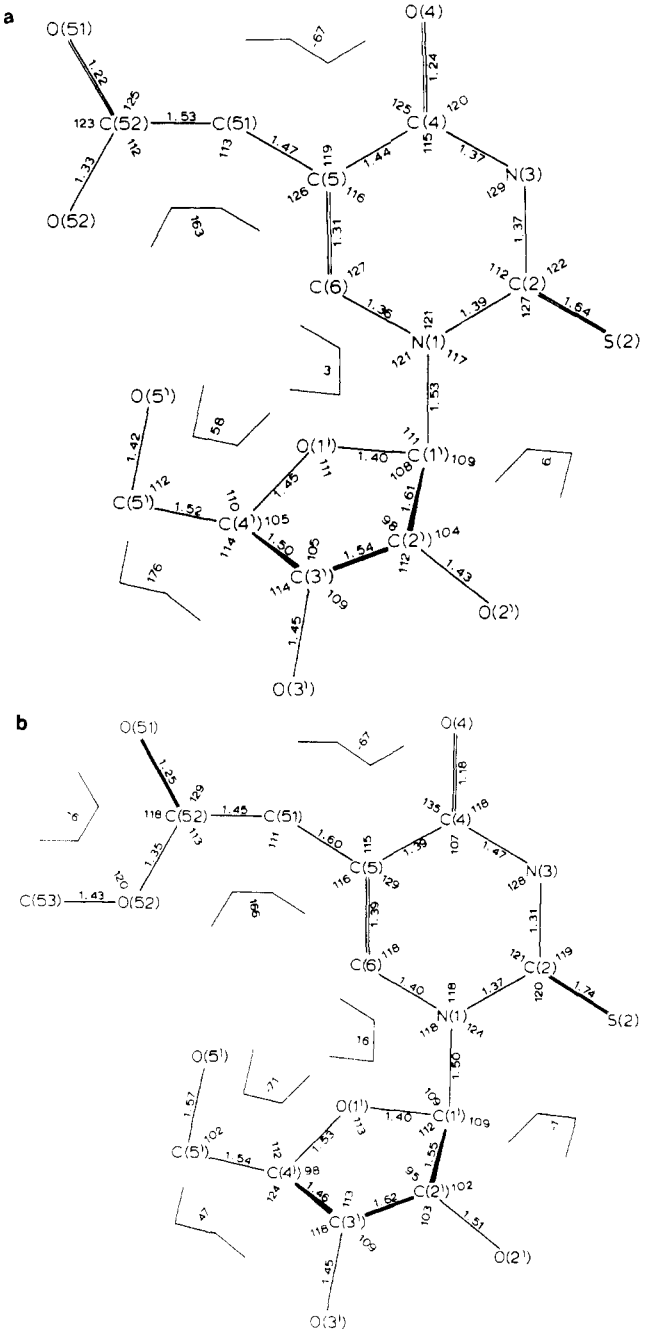


FIGURE 1: The bond lengths and the bond and dihedral angles of (a) s^2cm^5U (σ_{xx} = 0.02 Å, σ_{xxx} = 1°, x = C, O, N, S) and (b) s^2mcm^5U (σ_{xx} = 0.03 Å, σ_{xxx} = 2°).

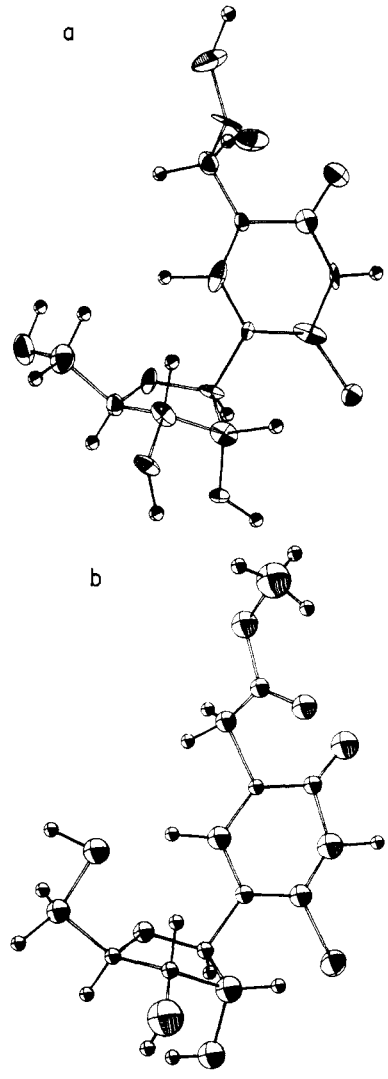


FIGURE 2: The structural representation of (a) s^2cm^5U and (b) s^2mcm^5U with the thermal ellipsoids of the heavy atoms (only in 2a).

TABLE II: List of Intermolecular Hydrogen Bonds of s^2mcm^5U, Including the Characteristic Intramolecular C(6)-H···O(5′) Interaction.

X-H···Y	X-H (Å)	H···Y (Å)	X···Y (Å)	∢ X-H-Y (deg)
N(3)-H···O(5′)	1.1	1.7	2.78	155
C(6)-H···O(51)	1.1	2.4	3.38	143
O(5′)-H···O(51)	1.2	2.1	2.76	112
C(6)-H···O(5′)	1.1	2.4	3.41	146

osides (Egert et al., 1978), whereas s^2cm^5U exhibits a gauche-trans conformation with dihedral angles of 58° and 176°, which was already found for some other 2-thiouridine derivatives (Kojić-Prodić et al., 1974, 1976; Hawkinson, 1977). The gauche-trans conformation seems to be mainly induced by packing forces.

Packing of the Molecules. A view down the *c* axis (Figure 3) illustrates the crystal structures which are determined by three screw axes parallel to the edges of the cell. The packing of s^2mcm^5U shows no definite separation of base and ribose regions. This arrangement is stabilized by one intermolecular hydrogen bond N(3)-H···O(5′), whereas interactions between riboses do not occur (Table II). The three-dimensional structure of $s^2cm^5U \cdot H_2O$ can be divided into layers parallel to the *bc* plane showing the well-known sequence: ribose-base···base-ribose-base···ribose-base···base-ribose, etc. Next to an interbase hydrogen bond N(3)-H···S(2) adjacent molecules are connected predominantly by a water molecule which participates in six hydrogen bonds (Table III). The water oxygen exhibits a tetrahedral alignment of two strong accepting and two donating hydrogen bonds, one of which is trifurcated. All together, the water molecule is linked with four bases and two ribosyl moieties and thus plays the dominant role in the intermolecular arrangement of the molecules (Figure 4).

In spite of the rather different crystal structures, the base stacking patterns of s^2cm^5U and s^2mcm^5U are nearly identical (Figure 5). The bases are packed along the *c* axis at a distance of 3.60 (s^2cm^5U) and 3.44 Å (s^2mcm^5U), showing a staggered

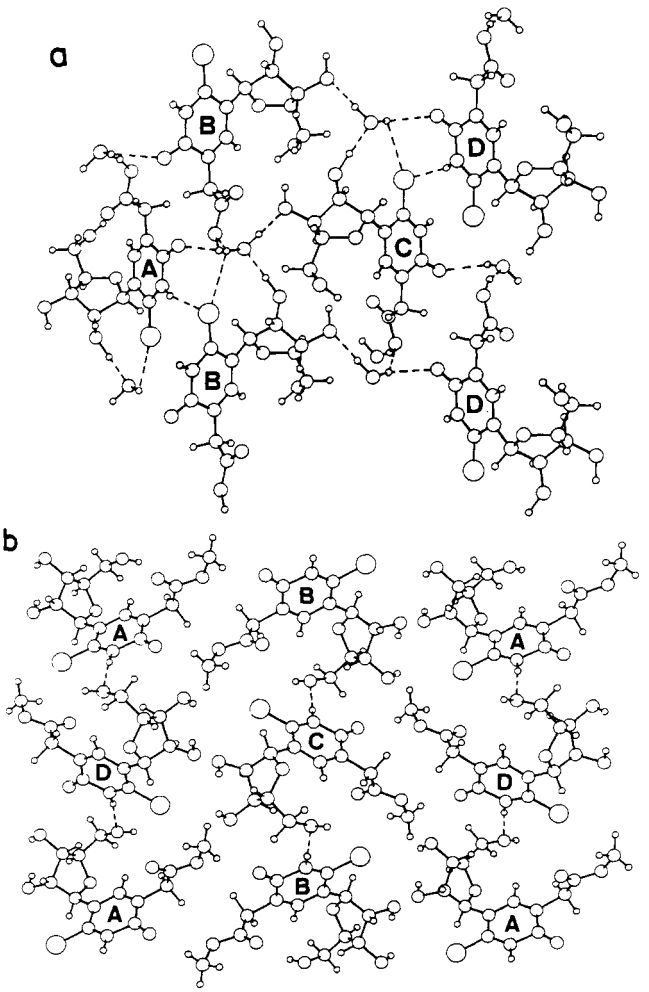


FIGURE 3: View down the *c* axes of (a) $s^2cm^5U \cdot H_2O$ and (b) s^2mcm^5U with *a* horizontal and *b* vertical. The individual base stacks are designated with letters. They are in relation with the following symmetry operations: (A ↔ B) screw axis parallel to *c*; (A ↔ C) screw axis parallel to *a*; (A ↔ D) screw axis parallel to *b*. The hydrogen bonds are indicated by dotted lines.

TABLE III: List of Intermolecular Hydrogen Bonds of $s^2cm^5U \cdot H_2O$.

X-H···Y	X-H (Å)	H···Y (Å)	X···Y (Å)	∢ X-H-Y (deg)
N(3)-H···S(2)	0.9	2.4	3.27	158
C(6)-H···O(51)	1.2	2.4	3.49	157
C(3′)-H···O(5′)	1.2	2.3	3.52	169
O(3′)-H···O(5′)	1.1	2.2	3.20	163
O(5′)-H···O(51)	1.0	1.8	2.82	180
O(W)-H···S(2)	1.3	3.0	3.66	112
O(W)-H···O(4)	1.3	2.3	2.96	110
O(W)-H···O(52)	1.3	2.2	3.31	146
O(W)-H′···O(3′)	1.0	1.9	2.90	180
O(52)-H···O(W)	1.3	1.4	2.70	164
O(2′)-H···O(W)	1.1	1.7	2.76	180

arrangement. This arrangement is stabilized by the hydrogen bond O(5′)-H···O(51) and the weak interaction C(6)-H···O(51) in *both* nucleosides. s^2cm^5U exhibits a further stabilization by the water molecule which connects the carboxyl groups of adjacent bases by means of two hydrogen bonds.

Calculation of the Carboxymethyl Group Conformation. The nearly identical conformation of the substituents at the 5 position of s^2cm^5U and s^2mcm^5U gave rise to theoretical

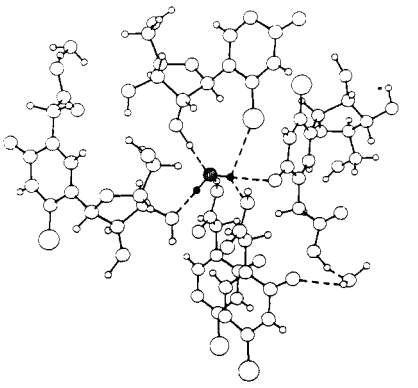


FIGURE 4: The dominant influence of the water molecule (hatched atoms) on the crystal structure of $s^2cm^5U \cdot H_2O$. The hydrogen bonds are indicated by dotted lines.

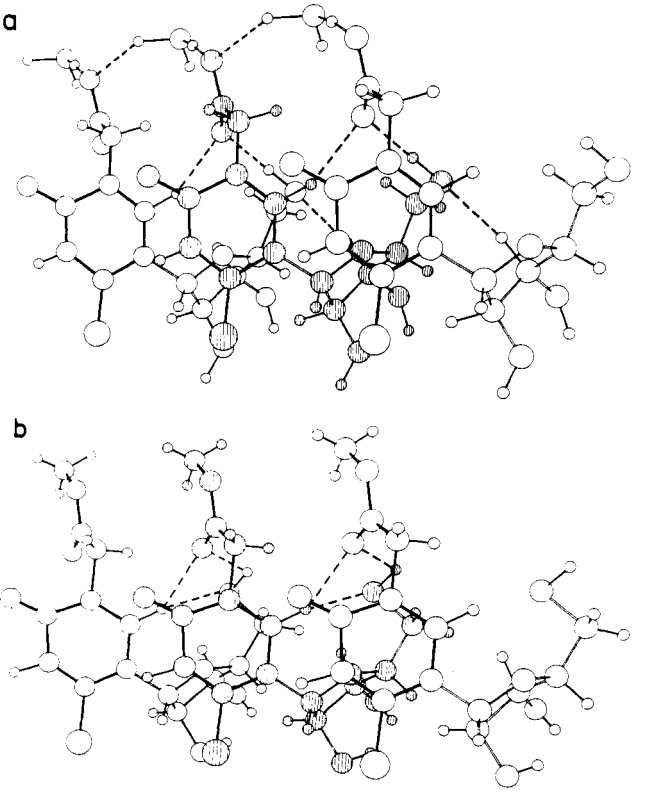


FIGURE 5: Views perpendicular to the base planes of (a) $s^2cm^5U \cdot H_2O$ and (b) s^2mcm^5U showing the staggered arrangement of the six-membered rings and the intermolecular interactions (dotted lines). The atoms of the middle one out of the three nucleosides are hatched.

calculations using the MINDO/3 method which should clarify whether this conformation represents an energy minimum because of electronic and steric reasons or if it is mainly induced by packing forces.

We chose cm^5U as our model compound and not the 2-thio derivative because it fits better into the series of uridines substituted at the 5 position, the conformational properties of which we have calculated. The ribose was replaced by the 1-hydroxyethyl group, $-CH(OH)CH_3$, which has a similar electronic influence on the base, which was verified by calculations of complete nucleosides and series of simplified models (manuscript in preparation). The optimization included all bond lengths except the bonds to hydrogen atoms (which were kept at 1.08 Å) and all bond angles except those of the 1-hydroxyethyl group (which were given the ideal tetrahedral angle of 109.5°). Furthermore, a free rotation of the hy-

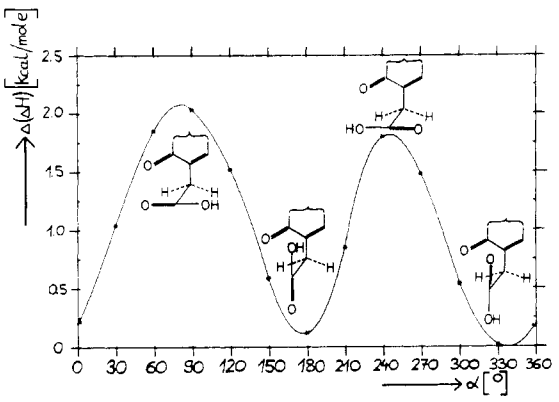


FIGURE 6: A plot of the energy relative to the global minimum at 340° vs. α [the dihedral angle C(5)-C(51)-C(52)-O(51)] with sawhorse projections of the conformations belonging to the extrema. The calculated values marked by asterisks are fitted by a polynomial approximation. The experimental values of α are 339° for s^2cm^5U and 351° for s^2mcm^5U.

droxyethyl group was permitted, leading to a dihedral angle C(6)-N(1)-C(1′)-O(1′) of about 50°.

The carboxymethyl group has three degrees of rotational freedom around the bonds C(5)-C(51), C(51)-C(52), and C(52)-O(52). The conformation of the latter, which defines the orientation of the carboxyl hydrogen H(52) with respect to O(51), stayed at the starting value of 0° for the dihedral angle O(51)-C(52)-O(52)-H(52). A free rotation around C(5)-C(51) is strongly hindered by repulsing interactions between the carboxymethyl group and the ring substituents O(4) and H(6), resulting in a high activation energy. Therefore, only two small ranges of this angle represent the favored conformations, which require the C(51)-C(52) bond approximately perpendicular to the six-membered ring with C(52) "above" or "below" the base plane. Following these arguments, the dihedral angle C(4)-C(5)-C(51)-C(52) was fixed at −90°. The conformational freedom of the carboxymethyl group is now reduced to the rotation of the carboxyl group around C(51)-C(52).

Therefore, we performed calculations varying the dihedral angle $\alpha \triangleq$ C(5)-C(51)-C(52)-O(51) from 0° to 360° in 30° steps and optimized all 34 variables (bond lengths, bond angles, and dihedral angles) mentioned above. The results are shown in Figure 6. The plot of the energy vs. α exhibits two minima at 180° and 340° separated by two maxima at 80° and 250°. The minima differ by only 0.1 kcal/mol and correspond to conformations with one oxygen of the carboxyl group "above" C(5), as indicated in Figure 6. The energy of the maxima is 2.1 and 1.8 kcal/mol higher than the energy of the global minimum at 340°.

Discussion

The crystal structures of $s^2cm^5U \cdot H_2O$ and s^2mcm^5U reveal two remarkable similarities: the conformation of the molecules and the base stacking. Although the packing of these compounds is different (Figure 3), the location of the base moieties next to each other is nearly identical (Figure 5), and no noticeable base overlap occurs in the crystals. In spite of its considerable polarizability, the 2-thio group is not at all involved in the base–base interaction as in 2-thiocytidine (Lin et al., 1971). However, it is not possible to deduce the base overlap of nucleosides within nucleic acids from their crystal structures because the energy minimum of the packing in the crystal is probably found by optimizing other interactions, for example, intermolecular hydrogen bonds.

Both nucleosides exhibit the C(3′)-endo conformation of the ribose combined with small dihedral angles over the glycosidic bonds. If this conformation is also the favored one in aqueous solution, stacking interactions in these nucleosides would be more stable because in stacked ribonucleic acids the C(3′)-endo conformation is exclusively found (for a recent review of this subject, see Kallenbach and Berman, 1977).

The results from the MINDO/3 calculations (Figure 6) show the preferred orientation of one oxygen of the carboxyl group either "above" or "below" C(5). The distances between O(51) and C(5) are only 2.85 and 2.86 Å for s^2cm^5U and s^2mcm^5U, respectively. The energy curve of the rotation around C(51)-C(52) in Figure 6 can be explained by a simple MO model which considers the interaction between the orbitals of the carboxymethyl group and those of the C(4)=O(4) double bond. Detailed studies reveal two possible interactions: (1) a bonding (i.e., stabilization) interaction between the lone pairs of one of the oxygens from the carboxyl group and the π^* orbital which has its greatest coefficient at C(4) and (2) an antibonding (i.e., destabilization) interaction between the same lone pairs of this oxygen and the π orbital localized mainly at O(4). These opposite effects determine the preferred conformation of the carboxymethyl group. The orbitals of the carbonyl oxygen, O(51), in the carboxylic group have the higher energy and are better adjusted for an overlap with the carbonyl group C(4)=O(4). This leads to the more pronounced stabilizing (α = 340°) and destabilizing (α = 80°) interactions when this oxygen is next to C(5) as compared to O(52) (α = 180° and 250°, Figure 6). Therefore, the carboxymethyl substituent has its energy minimum at the balanced distance of O(51) as close to C(4) and as far away from O(4) as possible. The experimental values for α obtained from the crystal structures are 339° for s^2cm^5U and 351° for s^2mcm^5U and agree with the calculations as well as the values for α of 16.1° for cm^5U and 329.2° for ncm^5U reported by Berman et al. (1978). The empirically found conformation, also derived only from electronic and steric properties by the MINDO/3 approximation, is also valid for the carboxyl-blocked derivatives like the methyl ester or the amide and for the respective 2-thio compounds. Thus, these results support the assumption that the molecular conformation found in these crystals is a common feature of odd uridines containing substituents at the 5 position related to the carboxymethyl group.

The molecular structure of s^2mcm^5U does not explain the influence of this substituent at the 5 position on the reduced wobble recognition of either s^2mcm^5U or mcm^5U in tRNAs (Sekiya et al., 1969; Weissenbach and Dirheimer, 1978). Therefore, this finding can most likely be explained by the sterical arrangement of these odd nucleosides within the anticodon loop. Assuming that the crystal structure of $tRNA^{Phe}$ represents the biologically active conformation, we substituted Gm(34) with s^2mcm^5U by computer methods using the Cartesian coordinates of the anticodon loop (Quigley at al., 1975). A similar procedure was used by Parthasarathy et al. (1977) to discuss the influence of t^6A and g^6A on the anticodon loop structure. Recently, Gm(34) was substituted with the molecular structure of 5-carbamoylmethyluridine (Berman et al., 1978). These authors postulated a hydrogen bond from the carbonyl oxygen O(51) to the 2′-hydroxyl group of the ribose from U(33) fixing the location of this wobble nucleoside. The analog plot for s^2mcm^5U with the bond lengths, bond angles, and dihedral angles taken from the crystal structure is shown in Figure 7. The interaction of O(51) with OH(2′) from U(33) is indicated by a line. The distance between the oxygens in this structure would be 2.05 Å, which is much less than the sum of the van der Waals radii. If one rotates the base around the glycosidic bond, lowering χ_{CN} to 5°—the value found in the

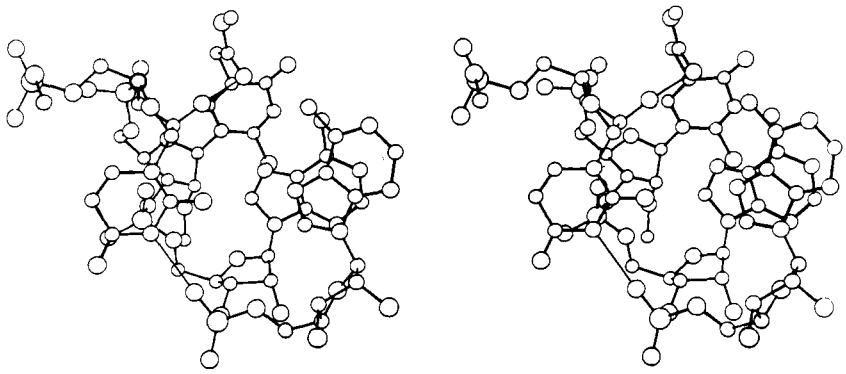


FIGURE 7: A stereo picture of the anticodon loop of $tRNA^{Phe}$ showing U(33) and the three anticodon nucleosides with Gm(34) replaced by s^2mcm^5U. The hydrogen bond from N(3) of U(33) to phosphate(36) stabilizing the U turn and the interaction of the carbonyl oxygen O(51) with the 2'-OH group from U(33) are indicated by thin lines.

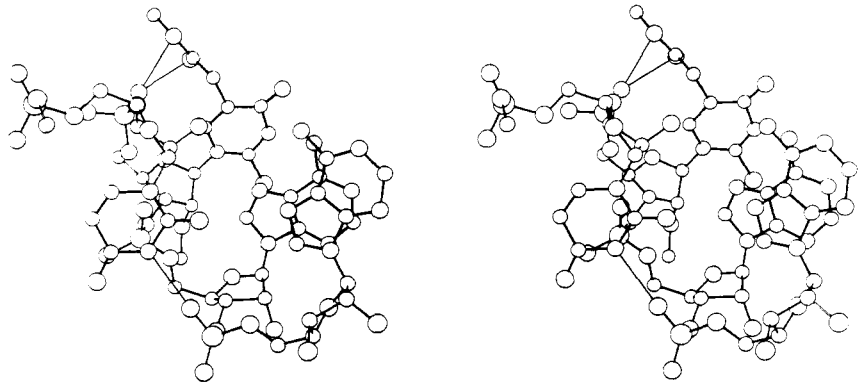


FIGURE 8: A stereo picture with the same atoms and the same orientation as in Figure 7 but with the substituent at the 5 position of U(34) rotated by 180°, resulting in an interaction of the mcm group with the phosphodiester(34) between U(33) and s^2mcm^5U (34), which is indicated by thin lines.

crystal structure of ncm^5U—the distance is 2.25 Å, which is still less than usually found for hydrogen bonds. Even if one decreases χ_{CN} to 0°, the distance (2.41 Å) is still shorter than a hydrogen bond. In addition, the angle O(2')-H···O(51) would be approximately 100°, which does not favor hydrogen bonding. These values indicate that the interaction of the mcm substituent with the ribose of U(33) must be repulsive.

In order to find a more stable arrangement of this nucleoside in the anticodon loop, we rotated the mcm substituent by 180° around C(5)-C(51) to reach an equivalent energy minimum on the other side of the six-membered uracil ring next to C(5). The resultant conformation of the carboxylic group was found in the crystal structure of cm^5U (Berman et al., 1978). The plot of the anticodon loop derived in this manner is shown in Figure 8. The interaction of the mcm group with the phosphodiester between U(33) and Gm(34) is indicated by lines between the carboxylic oxygens and one phosphate oxygen. These distances, derived from the replacement of Gm(34) by s^2mcm^5U without any distortion, are 2.75 and 2.97 Å, which are equal or only a little less than the sum of the van der Waals radii of these atoms. Therefore, this nucleoside might cause a slight deviation from this arrangement, which would bring the 2-thio group next to N(1) of a neighboring uridine, which is in position 35 of the $tRNA^{Glu}_3$ and $tRNA^{Lys}_2$ sequences from baker's yeast, allowing the preferred S(2)-N(1) stacking interaction (Mazumdar et al., 1974).

The U·G base pair is formed via hydrogen bonds between the 2-keto and 3-imino groups of the uridine moiety and the 1-imino and 6-keto groups of guanosine. This formation requires a slight rotation of the uridine residue within the anticodon stack toward the backbone of the U turn. Assuming that the anticodon loop conformation found in the crystal undergoes a codon-anticodon interaction, this rotation would be impossible for mcm^5U in the wobble position because of the steric hindrance of the mcm group, as discussed above. This bulky substituent in the 5 position may, for geometric reasons, prevent the G·U wobble pair. Also, the neighborhood of the negatively charged phosphodiester group could explain why cm^5U is not found in tRNAs. The small distance between these two negatively charged groups would result in an enhanced Coulomb repulsion and thereby disturb the helical structure of the anticodon stack.

Odd uridine derivatives containing one of these substituents at the 5 position in this conformation may be called "helix terminator nucleosides" because they always terminate a right-handed helix of stacked nucleic acids. The adjacent nucleotide in the polynucleotide chain cannot stack on these uridine derivatives due to the steric hindrance of the substituent at the 5 position. This behavior also agrees very well with their occurrence at the 5' end of the anticodons in some tRNAs next to the so-called U turn (Quigley and Rich, 1976) (Figures 7 and 8), where they may contribute to this possible common structural feature of the anticodon loops by preventing the stack of U(33) on the anticodon helix (Figure 8). Such a mechanism might be especially important for 2-thiouridine derivatives because 2-thiouridine is known to exhibit a highly thermostable stacking interaction in the homopolynucleotide (Bähr et al., 1973).

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Supplementary Material Available

Thermal parameters, the observed and calculated structure factors, and the positional parameters with their estimated standard deviations (11 pages). Ordering information is given on any current masthead page.

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