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Publication Date

1982-03-01

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March 1982

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Structure of a Psoralen-Thymine Monoadduct

Formed in Photoreaction with DNA

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Running Title: Psoralen-Thymine Monoadduct

ABSTRACT

A photoreaction product between calf thymus DNA and 8-methoxypsoralen, a naturally occurring psoralen, was acid-hydrolyzed to isolate a major species of the reaction, 8-methoxypsoralen-thymine monoadduct. The crystal structure of the monoadduct has been determined. The structure confirms the results of other spectroscopic studies (Kanne et al., 1982a) and allows one to conclude or suggest that (1) the photoreaction occurs preferentially at both T-A and A-T sequence in natural DNA; (2) the photocrosslink introduces a substantial kink in the DNA structure; (3) the configuration of the photoproduct is "cis-syn" at both ends of the psoralen; (4) the thymine and psoralen moieties remain planar even after the photoreaction; and (5) the angle between psoralen and thymine in the photoproducts is not fixed but has a limited range of flexibility.

1. INTRODUCTION

Psoralens are a class of heterocyclic aromatic compounds, which have been used as photosensitizing agents for the treatment of various pigmentation diseases and as chemical probes to study

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DNA structure (for a review see Hearst, 1981). On exposure to ultraviolet light psoralens form chemical crosslinks to the DNA bases in three steps (Dall'Acqua et al., 1971, 1970, 1968; Isaacs et al., 1977; Johnston et al., 1977). The first step is intercalation of psoralen between two adjacent base-pairs. The second step is formation of a monoadduct, that is, one psoralen photoreacts to one strand of DNA. The third step is the crosslinking of the same psoralen to the other strand of DNA, thus covalently linking the two strands of a double stranded DNA. Figure 1 shows the chemical structures and numbering system used for 8-methoxypsoralen and thymine. The photoaddition occurs at the double bonds between C12 and C13 of the furan ring (site I) and between C3 and C4 of the pyrone ring (site II). The preferred target is the double bond at TC5 and TC6 of thymine, forming cyclobutane rings by reacting at either site I or site II of the psoralen.

There are eight possible conformations for psoralen-thymine monoadducts (Figure 2) and 64 for diadducts. Spectroscopic and other studies have narrowed this down to a few possible conformations and recent NMR studies on psoralen monoadducts (Straub et al., 1981; Kanne et al., 1982a) have shown that the biologically relevant structures have the "cis-syn" conformation. Such drastic reduction in the number of conformers comes from the fact that the double helical DNA conformation imposes very stringent restrictions on modes of psoralen interaction. Thus, presumably, only a particular conformer can be formed.

To determine precise geometrical details of the psoralen photoadducts and to provide an independent identification of

the biologically relevant conformer, we have determined the crystal structure of a monoadduct between the thymine and 8-methoxypsoralen as isolated from the photoreaction product between calf thymus DNA and psoralen. Therefore, the resultant products we observe will be only those which can be formed in biological situations, that is, in natural DNA. During the preparation of this paper, Land et al. (1982) published a short communication reporting the structure of a photoadduct obtained by UV irradiation of a mixture of 8-methoxypsoralen and thymine in an ice-methanol matrix in the presence of benzophenone.

Although this adduct was made under non-biological conditions without the structural constraints of the double helical DNA, and was presumably one of many products formed in the reaction, the structure they obtained is the same as that reported here.

We describe here the structural details of a psoralen-thymine monoadduct obtained in a biological environment and the consequence of the photocrosslink between 8-methoxypsoralen and double helical DNA.

2. EXPERIMENTAL

(a) Synthesis

Figure 3 shows a brief summary of the way the compound was synthesized and purified (Kanne et al., 1982a). Briefly, 8-methoxypsoralen was added to DNA and then irradiated with ultraviolet light at 320 to 380 nm. The psoralen-DNA solution was extracted with chloroform to remove unreacted psoralen and its photodegradation products. This DNA-psoralen photoproduct

was precipitated, acid-hydrolyzed and purified by reverse phase and high performance liquid chromatography.

(b) Crystallization and X-Ray Diffraction Studies

The photomonoadduct between 8-methoxypsoralen and thymine (henceforth called 8MOP-T) was dissolved in 60% aqueous methanol solution in a partially airtight container and evaporated extremely slowly for a period of 4 weeks. Well formed crystals were obtained which had a triclinic space group with cell parameters $a = 11.297(2) \text{ \AA}$, $b = 14.046(3) \text{ \AA}$, $c = 15.77(1) \text{ \AA}$, $\alpha = 75.62(1)^\circ$, $\beta = 87.09(1)^\circ$, $\gamma = 84.77(1)^\circ$. (The estimated standard deviations in parentheses refer to the last digits.) This limits the possible choices of space group to $P1$ and $P\bar{1}$. Eventual structure solution and refinement confirms the latter space group to be the correct one. The measured density indicates that the unit cell contains 6 molecules of 8MOP-T and 2 molecules of methanol. Thus, the content of an asymmetric unit is one half of that amount. The X-ray diffraction data were collected on an automatic diffractometer using graphite-monochromated MoK-alpha-radiation. A total of 6079 reflections have been collected using the theta-2-theta scanning mode with variable scan rates.

Since there are 77 non-hydrogen atoms in an asymmetric unit of this space group, the structure was not immediately solvable by direct methods. However, examination of diffraction intensities showed that those with $l = \text{odd}$ were systematically weak. Taking advantage of this, we solved the partial structure of the half asymmetric unit by direct methods using the MULTAN 80 program (Main, Germain and Woolfson, 1980). Subsequently

the structure of the full asymmetric unit was determined by the difference Fourier method. After a few cycles of isotropic least squares refinement using 5496 reflections with structure factors greater than three times the estimated standard deviations, the difference Fourier map revealed the positions of all hydrogen atoms as well as one molecule of methanol. The entire structure was refined anisotropically by full matrix least squares method. The final R factor is 5.2%.

3. RESULTS AND CONCLUSIONS

(a) Psoralen Photoreacts to both A-T and T-A Sequences in DNA

Two of the three molecules (A and C) in an asymmetric unit have the same conformation and the third (B) is the mirror image of the two molecules. Thus in the entire unit cell there are three molecules of one-handedness and the other three have the other-handedness. This suggests that 8MOP can form photomonoadduct to an A-T sequence as well as T-A sequences in double helical DNA (see Figure 4). Although two isomers are present in a 1:1 ratio in crystals, isolation results suggest that the ratio may be 3:2 in the hydrolysate (Kanne et al., 1982a).

(b) Psoralen and Thymine Moieties Remain Planar even After Photoreaction

This crystal structure reveals that the reduction of double bonds in psoralen (C12-C13) and thymine (TC5-TC6) to form the 8MOP-T photoadduct does not distort the planarity of either moiety significantly. This is rather surprising because both the thymine and furan rings in 8MOP-T have two saturated

carbon atoms each. The atoms that deviate most from the psoralen planes are C12 (0.16 Å), C12 (0.14 Å) and O11 (0.10 Å) in molecules A, B and C respectively, and those from the thymine planes are TC5 (0.07 Å), TC6 (0.10 Å), and TC5 (0.04 Å) in molecules A, B and C respectively. The bond distances (Table 1 and Figure 5) near the cyclobutane ring reveal that bonds involving O11, C12, C13 and C6 of the furan ring and those involving TN1, TC6, TC5 and TC4 of thymine moiety of 8MOP-T still have some remaining double bond characteristics.

The cyclobutyl rings are also planar within 0.09 Å, 0.10 Å, and 0.06 Å respectively for the three molecules. The methyl group of thymine, of course, is out of the plane by 0.81 Å, 0.86 Å, and 0.92 Å respectively for the three molecules. This out-of-plane methyl group in 8MOP-T may in some cases interfere with normal stacking of the base-pairs in double helical DNA.

(c) Angle Between Planes of Psoralen and Base is Variable

The bond distances and angles are listed in Table 1 and 2 and the average values are also shown in Figure 5. There are no unusual bond distances and angles in the thymine or psoralen portion of the adduct other than those mentioned earlier. Corresponding bond distances or angles in three unique molecules do not differ by more than twice the estimated standard deviation. The difference among the three molecules is best manifested by the interplanar angle between the psoralen and thymine moieties as shown in Figure 6. The interplanar angle ranges between 44.11° and 53.45°. This suggests that the interplanar angle

is not fixed but has a limited range of flexibility, with its values depending on the environment.

(d) H-bonds and Stacking

All the psoralen moieties are stacked together with an interplanar separation of 3.2 Å and arranged in a herringbone pattern (Figure 7). Thymine molecules also fall into two sets of planes but there is no obvious overlap between thymine moieties. All hydrogen atoms that can form hydrogen bonds do so in the crystals. Probable hydrogen bonds are listed in Table 3.

(e) Cis-Syn Conformation on Both Sides of Psoralen Plane

The conformation found in this crystal structure (cis-syn) allows one to build molecular models for an 8MOP-T2 diadduct, where another thymine forms a photoadduct at the pyrone ring. Our model building shows that for each 8MOP-T monoadduct enantiomer, there is only one 8MOP-T2 diadduct that can be built and accommodated in double helical DNA. This unique diadduct has cis-syn conformation at the pyrone ring as shown in Figure 4. This is consistent with the observation by Kanne et al. (1982b).

(f) Psoralen Crosslinking Introduces a Sharp Kink in DNA Structure

As pointed out earlier, the angle between the thymine and psoralen moieties in the structure ranges from 44° to 53° when the cyclobutyl ring is formed at the furan ring. If one assumes similar geometry at the pyrone ring, then one can easily see that the extent of distortion to the DNA which would be induced by psoralen crosslinking is substantial. Molecular model building based on this structure suggests that the DNA may kink as much

as 70°. Psoralen crosslinking will not only unwind DNA as was previously shown by the superhelical density studies (Wiesehahn and Hearst, 1978), but it will also introduce a very sharp kink at the site where psoralen is introduced (Figure 8). This kind of distortion is important in interpreting the results of psoralen crosslinking experiments in that one has to be aware of the fact that the original structure near the site of psoralen crosslinking is drastically disturbed. This line of reasoning leads one to conclude that the psoralen-DNA photocrosslinking may occur only in those regions of duplex DNA that are flexible and are not constrained by proteins or a three-dimensional structure (Hanson et al., 1976). It also provides a possible explanation for the 1 microsecond relaxation time required between formation of the monoadduct and that of the diadduct (Johnston et al., 1981).

(g) Atomic Coordinates and Thermal Parameters

The atomic coordinates, thermal parameters and standard deviation derived from the full matrix least-square refinement are listed in Table 4. The thermal parameters of hydrogen atoms were refined isotropically and those of other atoms anisotropically. The parameters for the methanol hydrogen atoms were not refined.

We thank Drs. Steve Holbrook and Fred Hollander for their help in computations and data collection respectively. The work reported here has been supported by grants from NIH (CA27454 and GM25151), NSF (PCM8019468) and the Department of Energy (DE-AC03-76SF00098)

FIGURE LEGENDS

Figure 1. Numbering system for 8-methoxypsoralen and thymine used in this paper.

Figure 2. Nomenclature and schematic representation of the eight possible conformational isomers for 8-methoxypsoralen-thymine monoadduct. The large slabs represent 8-methoxypsoralen with the five membered furan ring at the left as indicated by the blackened markers. Handles projecting out from these slabs represent the methoxy group at C8 of the psoralen ring. The smaller slabs are for thymine bases with the handles representing TN1-TH1. The isomers on the top row are mirror images of the isomers on the bottom row. Two conformations found in the crystal structures are cis-syn.

Figure 3. Steps taken to synthesize and isolate 8MOP-T monoadduct from calf thymus DNA reacted with 8MOP.

Figure 4. (top) Schematic drawing to show the intercalation and 8MOP-T monoadduct formation at 5'...TA...3' and 5'...AT...3' sequences. (bottom) Schematic drawing of two diadduct enantiomers of cis-syn conformation. The representations are the same as those in Figure 2.

Figure 5. (a) Average bond distances among three independent 8MOP-T molecules (e.s.d.'s are listed in Table 1). (b) Average bond angles among three independent 8MOP-T molecules (e.s.d.'s are listed in Table 2). Only oxygen atoms are labeled.

Figure 6. Interplanar angles between thymine and 8MOP plane in three unique 8MOP-T molecules.

Figure 7. (a) Stereodrawing of a single 8MOP-T molecule.

(b) Stereodrawing of six 8MOP-T molecules and two methanol molecules in a unit cell.

Figure 8. Schematic drawing of double helical B-DNA (cylinders) linked by psoralen photocrosslinks. The T•A base pairs on both sides of psoralen moiety are shown as rectangular plates. This is based on a detailed molecular model built using skeletal atomic components.

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FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 6

reduce to 40% of the original

FIGURE 7-(a)

1000 1000 1000

FIGURE 7-(b)

FIGURE 8

BOND LENGTHS(A) AND STANDARD DEVIATIONS

BOND	MOL A	MOL B	MOL C	
C3 ---H3	1.07(4)	1.03(5)	1.02(5)	1.04(3)
C4 ---H4	0.97(5)	1.00(5)	1.00(5)	0.99(3)
C5 ---H5	1.01(5)	0.98(4)	0.91(4)	0.98(2)
C12---H12	0.93(5)	0.85(4)	0.90(4)	0.90(2)
C13---H13	0.91(4)	0.90(4)	0.97(5)	0.92(2)
C15---H15a	1.12(8)	1.05(11)	1.16(12)	1.11(6)
C15---H15b	1.11(10)	1.05(10)	0.94(5)	1.02(5)
C15---H15c	0.99(8)	0.93(11)	1.19(10)	0.99(5)
TC6---TH6	1.01(5)	1.03(5)	0.95(4)	0.99(3)
TC7---TH7a	1.03(7)	1.05(5)	1.02(5)	1.04(4)
TC7---TH7b	1.02(5)	1.10(7)	1.00(5)	1.03(3)
TC7---TH7c	1.09(6)	1.02(7)	0.93(7)	1.02(4)
TV3---TH3	0.95(4)	0.75(4)	0.93(4)	0.85(2)
TV1---TH1	0.75(5)	0.65(5)	0.68(5)	0.77(3)
* MeC---MeCH	0.92			
* MeC---MeCHa	0.93			
* MeC---MeCHb	0.93			
* MeC---MeCHc	0.95			

* INDICATES DISTANCES TO METHANOL HYDROGEN. THESE POSITIONAL AND THERMAL PARAMETERS WERE NOT VARIED DURING REFINEMENT

TABLE 1 (continued)

BOND LENGTHS(A) AND STANDARD DEVIATIONS

BOND	MOL A	MOL B	MOL C	AVL
O1 ---C2	1.373(6)	1.366(6)	1.377(6)	1.374(3)
O1 ---C9	1.377(6)	1.379(5)	1.373(6)	1.375(3)
C2 ---O2	1.211(6)	1.213(6)	1.219(7)	1.213(4)
C2 ---C3	1.453(7)	1.433(7)	1.422(7)	1.433(4)
C3 ---C4	1.335(7)	1.341(7)	1.341(7)	1.339(4)
C4 ---C14	1.434(7)	1.442(7)	1.433(7)	1.435(4)
C5 ---C6	1.374(7)	1.352(6)	1.353(7)	1.375(4)
C6 ---C7	1.384(6)	1.386(6)	1.382(6)	1.384(3)
C6 ---C13	1.513(7)	1.513(6)	1.525(7)	1.520(4)
C7 ---C8	1.395(7)	1.393(7)	1.386(7)	1.383(4)
C8 ---C9	1.397(6)	1.403(6)	1.399(7)	1.400(4)
C8 ---O14	1.357(6)	1.355(6)	1.362(6)	1.353(3)
C10 ---C5	1.402(7)	1.399(6)	1.410(7)	1.399(4)
C10 ---C9	1.381(6)	1.381(6)	1.373(6)	1.375(3)
O11 ---C7	1.353(6)	1.370(6)	1.353(6)	1.353(3)
C12 ---C11	1.423(6)	1.429(6)	1.436(6)	1.431(3)
C12 ---TC6	1.552(7)	1.554(7)	1.550(7)	1.552(4)
C13 ---C12	1.531(7)	1.523(7)	1.529(7)	1.523(4)
C13 ---TC6	1.555(7)	1.559(6)	1.561(7)	1.561(4)
C14 ---C15	1.427(9)	1.413(10)	1.421(9)	1.414(5)
TC6 ---TC5	1.541(7)	1.532(6)	1.540(7)	1.537(4)
TC6 ---TN1	1.445(6)	1.441(6)	1.426(7)	1.433(4)
TC6 ---TC7	1.530(9)	1.526(7)	1.526(9)	1.527(4)
TC6 ---TC4	1.503(6)	1.505(6)	1.503(7)	1.503(4)
TC4 ---TC4	1.205(6)	1.211(6)	1.213(6)	1.211(3)
TC4 ---TN3	1.365(6)	1.364(6)	1.364(6)	1.364(3)
TN3 ---TC2	1.384(6)	1.390(6)	1.384(6)	1.385(3)
TC2 ---TC2	1.223(6)	1.226(6)	1.235(6)	1.230(3)
TC2 ---TN1	1.337(6)	1.341(6)	1.326(7)	1.335(4)
MeO ---MeO	1.337(12)			

TABLE 1

BOND ANGLES(deg) AND STANDARD DEVIATIONS

ANGLES	MOL A	MOL B	MOL C	AVE
C2 -O1 -C9	122.4(4)	121.7(4)	121.5(4)	121.5(2)
O1 -C2 -O2	115.2(4)	116.4(4)	116.0(5)	115.5(2)
O1 -C2 -C3	115.3(4)	117.5(4)	117.0(4)	117.0(2)
O2 -C2 -C3	125.3(5)	125.1(5)	127.0(5)	126.5(3)
C2 -C3 -C4	120.5(5)	120.9(5)	121.7(5)	121.1(3)
C3 -C4 -C10	122.5(5)	121.2(5)	120.8(4)	121.4(3)
C10 -C5 -C6	119.3(4)	119.8(4)	115.4(4)	119.3(2)
C5 -C6 -C13	132.8(4)	133.0(4)	131.3(4)	132.4(2)
C5 -C6 -C7	119.1(4)	115.7(4)	120.2(4)	119.3(2)
C13 -C6 -C7	108.2(4)	105.3(4)	102.3(4)	105.3(2)
C6 -C7 -O11	114.1(4)	113.7(4)	113.9(4)	113.9(2)
C6 -C7 -C8	123.4(4)	124.0(4)	123.4(4)	123.5(2)
O11 -C7 -C8	122.5(4)	122.2(4)	122.7(4)	122.5(2)
C7 -C8 -O14	121.8(4)	121.5(4)	121.9(4)	121.7(2)
C7 -C8 -C9	115.7(4)	115.2(4)	115.3(4)	115.4(2)
O14 -C8 -C9	122.4(4)	123.0(4)	122.3(4)	122.7(2)
O1 -C9 -C10	122.0(4)	122.0(4)	121.9(4)	122.0(2)
O1 -C9 -C8	115.7(4)	115.5(4)	115.4(4)	115.5(2)
C10 -C9 -C8	122.3(4)	122.5(4)	122.7(4)	122.5(2)
C4 -C10 -C5	124.1(4)	123.6(4)	123.1(4)	123.6(2)
C4 -C10 -C9	115.2(4)	115.7(4)	115.9(4)	115.5(2)
C5 -C10 -C9	119.7(4)	119.7(4)	120.0(4)	119.8(2)
C12 -O11 -C7	105.9(4)	105.7(3)	107.1(4)	106.1(2)
C13 -C12 -O11	109.2(4)	110.2(4)	109.5(4)	109.8(2)
O11 -C12 -TC6	115.0(4)	117.4(4)	113.3(4)	115.7(2)
C13 -C12 -TC6	90.2(4)	89.8(4)	94.5(4)	90.2(2)
C5 -C13 -C12	100.0(4)	99.7(4)	100.3(4)	100.2(2)
C5 -C13 -TC6	117.7(4)	115.8(4)	119.1(4)	115.5(2)
C12 -C13 -TC6	83.7(4)	83.6(3)	89.2(4)	88.8(2)
C8 -O14 -C15	113.4(4)	115.0(5)	113.5(4)	115.0(2)
C12 -TC6 -TC5	82.5(4)	82.1(3)	89.3(4)	88.5(2)
C12 -TC6 -IN1	113.3(4)	113.5(4)	115.6(4)	114.3(2)
TC6 -TC6 -IN1	114.0(4)	113.9(4)	115.5(4)	114.5(2)
C13 -TC6 -TC6	89.6(3)	89.7(3)	89.7(3)	89.7(2)
C13 -TC6 -TC7	112.4(4)	112.3(4)	111.9(4)	112.2(2)
C13 -TC6 -TC4	112.1(4)	112.7(4)	113.5(4)	112.8(2)
TC6 -TC6 -TC7	112.6(4)	111.3(4)	113.6(4)	112.7(2)
TC6 -TC6 -TC4	116.9(4)	117.4(4)	115.3(4)	116.5(2)
TC7 -TC6 -TC4	111.5(4)	111.4(4)	111.1(4)	111.3(2)
TC5 -TC4 -TC4	122.3(4)	122.4(4)	121.4(4)	122.2(2)
TC5 -TC4 -TN3	116.8(4)	116.2(4)	117.5(4)	116.8(2)
TC4 -TC4 -TN3	120.3(4)	121.3(4)	121.2(4)	121.1(2)
TC4 -TN3 -TC2	127.5(4)	127.4(4)	127.7(4)	127.5(2)
IN3 -TC2 -TC2	119.0(4)	119.2(4)	119.4(5)	119.2(2)
IN3 -TC2 -TN1	115.3(4)	117.0(4)	115.1(4)	115.5(2)
TC2 -TC2 -TN1	124.2(4)	123.9(4)	124.6(5)	124.2(2)
TC6 -TN1 -TC2	126.9(4)	125.8(4)	127.6(5)	126.7(2)

TABLE 2

BOND ANGLES(deg) AND STANDARD DEVIATIONS

ANGLES		MOL A	MOL B	MOL C	AVE
C2	-C3 -H3	113.0(2)	115.0(3)	113.0(3)	113.7(1)
C4	-C3 -H3	125.0(2)	123.0(3)	125.0(3)	125.1(1)
C3	-C4 -H4	121.0(3)	122.0(3)	126.0(3)	123.0(2)
C10	-C4 -H4	117.0(3)	115.0(3)	113.0(3)	115.3(2)
C6	-C5 -H5	123.0(3)	121.0(3)	123.0(3)	122.3(2)
C10	-C5 -H5	117.0(3)	119.0(3)	119.0(3)	118.3(2)
C5	-C13-H13	114.0(3)	117.0(3)	119.0(3)	116.7(2)
C12	-C13-H13	122.0(3)	121.0(3)	117.0(3)	122.4(2)
TC5	-C13-H13	112.0(3)	109.0(3)	109.0(3)	109.7(2)
C11	-C12-H12	111.0(3)	111.0(3)	110.0(3)	110.7(2)
C13	-C12-H12	115.0(3)	114.0(3)	115.0(3)	114.7(2)
TC6	-C12-H12	113.0(3)	113.0(3)	117.0(3)	114.3(2)
C14	-C15-H15a	108.0(4)	94.0(6)	75.0(6)	94.5(3)
O14	-C15-H15b	114.0(5)	115.0(5)	111.0(5)	113.7(3)
O14	-C15-H15c	111.0(5)	95.0(7)	111.0(5)	107.7(3)
H15a-C15-H15b		114.0(7)	97.0(6)	112.0(6)	110.4(4)
H15a-C15-H15c		109.0(7)	135.0(9)	147.0(6)	125.7(5)
H15b-C15-H15c		101.0(7)	120.0(9)	92.0(7)	101.2(4)
C12	-TC6-TH6	118.0(3)	120.0(3)	111.0(3)	116.3(2)
TC5	-TC6-TH6	109.0(3)	111.0(3)	106.0(3)	109.3(2)
TN1	-TC6-TH6	111.0(3)	113.0(3)	115.0(3)	112.0(2)
TC5	-TC7-TH7a	100.0(4)	105.0(3)	106.0(3)	104.3(2)
TC5	-TC7-TH7b	106.0(3)	100.0(3)	104.0(4)	103.2(2)
TC5	-TC7-TH7c	105.0(3)	108.0(4)	107.0(4)	106.3(2)
TH7a-TC7-TH7b		121.0(5)	124.0(5)	110.0(5)	118.3(3)
TH7a-TC7-TH7c		124.0(5)	120.0(5)	118.0(5)	114.0(3)
TH7b-TC7-TH7c		100.0(4)	119.0(5)	109.0(6)	107.8(3)
TC4	-TN3-TH3	115.0(3)	118.0(3)	115.0(3)	116.7(2)
TC2	-TN3-TH3	115.0(3)	114.0(3)	115.0(3)	115.0(2)
TC6	-TN1-TH1	116.0(4)	124.0(3)	124.0(4)	121.9(2)
TC2	-TN1-TH1	115.0(4)	110.0(3)	109.0(4)	111.3(2)
* MeC -MeO -MeCH		108.0			
* MeO -MeC -MeCHa		111.0			
* MeO -MeC -MeCHb		103.0			
* MeC -MeC -MeCHc		122.0			
* MeCHa-MeC-MeCHb		91.0			
* MeCHa-MeC-MeCHc		90.0			
* MeCHb-MeC-MeCHc		132.0			

* INDICATES ANGLES TO METHANOL HYDROGENS, WHOSE POSITIONAL AND THERMAL PARAMETERS WERE NOT VARIED DURING REFINEMENT

TABLE 2 (continued)

HYDROGEN POSITIONAL AND THERMAL PARAMETERS

ATOM	X	Y	Z	B
H3A	0.712(4)	0.528(3)	0.426(3)	3.(1)
H4A	0.612(4)	0.383(3)	0.408(3)	3.(1)
H5A	0.452(4)	0.302(4)	0.358(3)	4.(1)
H12A	0.255(4)	0.384(4)	0.227(3)	4.(1)
H13A	0.240(4)	0.284(3)	0.254(3)	3.(1)
H15Aa	0.095(7)	0.533(5)	0.383(5)	12.(3)
H15Ab	0.127(9)	0.785(8)	0.340(6)	15.(3)
H15Ac	0.214(7)	0.685(6)	0.398(5)	10.(2)
H7A	-0.041(4)	0.312(3)	0.364(3)	4.(1)
H7Aa	0.263(5)	0.195(5)	0.288(5)	9.(2)
H7Ab	0.171(5)	0.113(4)	0.358(3)	5.(1)
H7Ac	0.044(5)	0.130(4)	0.408(4)	6.(2)
H3A	0.241(4)	0.285(3)	0.551(3)	2.(1)
H1A	-0.010(4)	0.439(3)	0.428(3)	3.(1)
H3B	0.277(4)	0.521(3)	0.252(3)	4.(1)
H4B	0.384(4)	0.659(3)	0.066(3)	3.(1)
H5B	0.564(4)	0.717(3)	0.115(3)	3.(1)
H12B	0.235(4)	0.517(3)	0.272(3)	2.(1)
H13B	0.767(4)	0.729(3)	0.235(3)	3.(1)
H15Ba	0.697(10)	0.292(8)	0.158(7)	17.(4)
H15Bb	0.215(8)	0.227(8)	0.195(6)	13.(3)
H15Bc	0.341(9)	0.348(8)	0.144(7)	17.(4)
H6B	1.052(4)	0.675(3)	0.136(3)	3.(1)
H7Ba	0.989(5)	0.862(4)	0.100(4)	7.(2)
H7Bb	0.958(5)	0.793(5)	0.221(4)	5.(2)
H7Bc	0.864(6)	0.889(5)	0.146(4)	7.(2)
H3B	0.807(4)	0.744(3)	-0.067(3)	1.(1)
H1B	0.997(4)	0.546(4)	0.072(3)	3.(1)
H3C	0.648(4)	1.229(3)	0.544(3)	4.(1)
H4C	0.431(4)	1.132(3)	0.483(3)	3.(1)
H5C	0.422(4)	1.064(3)	0.387(3)	2.(1)
H12C	0.553(4)	0.802(3)	0.238(3)	3.(1)
H13C	0.408(4)	0.585(3)	0.312(3)	3.(1)
H15Ca	0.565(11)	0.883(9)	0.358(8)	20.(4)
H15Cb	0.935(7)	0.957(6)	0.429(5)	9.(2)
H15Cc	1.035(8)	0.955(7)	0.341(6)	16.(3)
H5C	0.481(4)	0.895(3)	0.101(3)	2.(1)
H7Ca	0.302(5)	0.956(4)	0.195(4)	6.(1)
H7Cb	0.225(5)	0.855(4)	0.214(4)	6.(2)
H7Cc	0.253(6)	0.957(5)	0.115(5)	2.(2)
H3C	0.455(4)	1.208(3)	0.112(3)	2.(1)
H1C	0.635(4)	0.997(3)	0.067(3)	2.(1)
* MeOH	0.8347	0.1704	-0.0235	4.
* MeOHa	0.9702	0.1105	0.1146	4.
* MeOHb	0.8630	0.0899	0.1182	4.
* MeOHC	0.9854	0.2568	0.0526	4.

* INDICATES METHANOL HYDROGENS, WHOSE POSITIONAL AND THERMAL PARAMETERS WERE NOT VARIED DURING REFINEMENT

TABLE 4 (continued)

Table 3. Probable A-H---B Hydrogen Bonds.

A	H	B	$\text{H} \cdots \text{B}$ Å	$\text{A} \cdots \text{B}$ Å	A-H-B deg
MeO	MeOH	T02C a	2.389	2.874	109.71
TN1A	TH1A	T02A b	2.175	2.929	161.46
TN3A	TH3A	02A c	2.157	2.946	154.56
TN1B	TH1B	T02B d	2.115	2.966	175.78
TN3B	TH3B	02B e	2.551	3.037	121.72
TN3B	TH3B	T04C f	2.521	3.233	152.07
TN1C	TH1C	T04B g	2.455	3.016	140.77
TN3C	TN3C	02B h	1.937	2.827	158.90

a) -x, 1-y, 1-z

b) 1-x, 1-y, 1-z

c) 2-x, 1-y, -z

d) 1-x, 1-y, -z

e) 1-x, 2-y, -z

f) x, y, z

g) x, 1+y, z

g) x, y-1, z

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