The Structure of *dl* Photodimer C of 1,3-Dimethylthymine[1]

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Abstract: The crystal structure analysis of bis(dimethyl)thymine photodimer C, $C_{14}H_{20}N_4O_4$, confirms that it is a 5,6:5,6 *syn* stereoisomer. The cyclobutane ring is puckered, each atom lying 0.5 Å out of the plane of the other three. The thymine nuclei are planar if C(6) is omitted from the plane in each residue; the angle between these planes is 33°. The thymine residues are skewed with respect to each other, the angle of rotation being about 29°. The crystals are monoclinic, space group Cc, with cell dimensions $a = 7.849$, $b = 15.142$, and $c = 13.265$ Å, $\beta = 102.65°$; $Z =$ four dimers per cell. The structure was solved by obtaining a partial structure with phases determined by the symbolic addition procedure and the tangent formula, and the remainder with phases based on the partial structure.

Photodimers of thymine were first isolated and identified by Beukers, Ijlstra, and Berends,[3] as products of ultraviolet irradiation of frozen aqueous solutions of thymine and of aqueous solutions of DNA. These compounds are of considerable biological importance since there is good evidence that the formation of pyrimidine dimers is responsible for the majority of photobiological effects caused by ultraviolet irradiation of DNA; for example, it has been shown that this is the major factor in the inactivation of microorganisms by uv light.[4]

Beukers and Berends[5] suggested that the dimers were formed by linking of two thymines by a cyclobutane ring formed across their 5,6 double bonds, and Wulff and Fraenkel[6] pointed out that *cis* linkage of the thymines to the cyclobutane ring could give rise to four stereoisomeric dimers: 5,5:6,6 (or head-to-head) *syn* and *anti* (dimers A and B) and 5,6:5,6 (or head-to-tail) *syn* and *anti* (dimers C and D). Weinblum and Johns[7] obtained four different thymine dimers, and from chemical and spectroscopic evidence tentatively assigned to these the four isomeric structures. Recently the structures of dimer A of dimethylthymine[8] and of uracil[9] and dimer D of thymine[10] and of 1-methylthymine[11] have been determined by X-ray diffraction.

Bis(dimethyl)thymine dimer C was first described by Wulff and Fraenkel,[6] and a head-to-tail 5,6:5,6 *syn* structure was proposed for it.[7] A single-crystal X-ray diffraction analysis was undertaken to obtain unequivocal proof of its structure, and to compare the molecular geometry with the photodimers of known structure.

Experimental Section

Bis(dimethyl)thymine dimer C was prepared according to Wulff and Fraenkel,[6] and suitable crystals were obtained from aqueous solution. The crystals are monoclinic, and the systematic absences indicated space group C2/c or Cc. The unit-cell dimensions, determined from precession photographs and from diffractometer measurements using Cu Kα radiation (λ 1.5418 Å), are $a = 7.849 \pm 0.006$, $b = 15.142 \pm 0.010$, and $c = 13.265 \pm 0.009$ Å, $\beta = 102.65 \pm 0.1°$. The density measured by flotation is 1.294 g cm^{-3} while that calculated for four molecules per cell is 1.301 g cm^{-3}.

Intensity data were collected on an automated Picker four-circle diffractometer (Ni-filtered Cu radiation), and all independent reflections with 2θ (Cu Kα) < 133° (corresponding to a minimum interplanar spacing of 0.84 Å) were measured. The moving-crystal–moving-counter technique was employed (2θ scan) with stationary counts for background radiation on each side of the reflection. Absorption is low, and no corrections were considered necessary. A total of 1308 reflections were observed, out of a possible 1340 in the range recorded. Corrections were made for Lorentz and polarization factors, and structure amplitudes $|F|$ and normalized structure amplitudes $|E|$ were derived.

Structure Determination

The average of the normalized structure factor magnitudes (Table I) indicated a noncentric space group, so Cc was chosen. The origin of the cell was specified by assigning phases to two reflections; only two pairs of reflections having $|E| > 1.9$ satisfied the criteria for origin specification for space group Cc[12] and the pair with higher $|E|$ values was chosen. Phases of four additional reflections were specified by symbols (Table II) and phase determination was carried out with the sum-of-angles formula,[13] which holds for those reflections with large $|E|$ values

$$\phi(\vec{h}) \simeq \langle \phi(\vec{k}) + \phi(\vec{h}-\vec{k}) \rangle_{\vec{k}_r} \qquad (1)$$

where ϕ is the phase and $\vec{h}$ and $\vec{k}$ are the Miller indices.

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Reliable phases (variance[13] <0.5) composed of combinations of the assigned phases and symbols were obtained for 57 of the 173 reflections with $|E| > 1.4$. Relations between the symbols indicated very strongly that $a = d = b - \pi$, and that c was real (either 0 or π): the indications for $c = \pi$ slightly outweighed those for $c = 0$. Consequently as a first approximation the value of $\pi/2$ was assigned to a and to d, $-\pi/2$ to b, and π to c, and the 57 initial phases were refined and phases for 106 additional reflections with $|E| > 1.4$ were obtained (variance <0.5) with several cycles of the tangent formula[14]

$$\tan[\phi(\vec{h})] \simeq \frac{\sum_{k} |E(\vec{k})E(\vec{h}-\vec{k})| \sin[\phi(\vec{k}) + \phi(\vec{h}-\vec{k})]}{\sum_{k} |E(\vec{k})E(\vec{h}-\vec{k})| \cos[\phi(\vec{k}) + \phi(\vec{h}-\vec{k})]} \quad (2)$$

Table I. Statistical Averages of Normalized Structure Amplitudes

	Centric	Noncentric	Exptl
$\langle\|E^2\|\rangle$	1.000	1.000	1.010
$\langle\|E\|\rangle$	0.798	0.886	0.886
$\langle\|E^2 - 1\|\rangle$	0.968	0.736	0.762

Table II. Phases and Symbols Assigned to Implement Eq 1

$\vec{h}$			$E(\vec{h})$	$\phi(\vec{h})$
1	17	$\bar{3}$	2.73	0
1	15	$\bar{4}$	2.17	0
1	3	11	2.75	a
6	6	2	2.90	b
2	2	$\bar{2}$	2.02	c
6	6	0	2.50	d

A three-dimensional E map computed from these data revealed 15 of the 22 nonhydrogen atoms. A Fourier summation, carried out using all the reflections with phases based on these 15 atoms, clearly showed the remaining 7 atoms.

The atomic coordinates and thermal parameters were refined by full-matrix least squares using a modified version of the ORFLS program.[15] The function minimized was $\Sigma(F_0 - F_c)^2$, and the atomic scattering factors were taken from the International Tables for X-ray Crystallography.[16] Three cycles with isotropic thermal parameters lowered R to 13.0%, and two anisotropic cycles resulted in $R = 6.9\%$. A three-dimensional difference Fourier was computed, and all 20 hydrogen atoms were located. The hydrogens were included in structure factor calculations, with the H(6)'s given the anisotropic thermal parameters of the C(6)'s and the methyl hydrogens given isotropic temperature factors corresponding to the average of the anisotropic components of the carbons to which they were bonded ($R = 5.6\%$), and two cycles of least squares, varying everything but the hydrogen thermal parameters, brought convergence to $R = 3.5\%$ for the observed reflections. The atomic fractional coordinates and thermal parameters are given in Table III in which the β_{ij} are the coefficients in the expression

$$\exp -[\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{23}kl + 2\beta_{13}hl + 2\beta_{12}hk]$$

The table of final observed and calculated structure factors has been deposited as Document No. NAPS-00136 with the American Society for Information Science.[17]

Discussion

Figure 1 shows the configuration of the molecule, and confirms that photodimer C has a 5,6:5,6 thymine–thymine cyclobutane linkage with the thymines *syn* to each other (*i.e.*, it is a *syn* head-to-tail dimer).

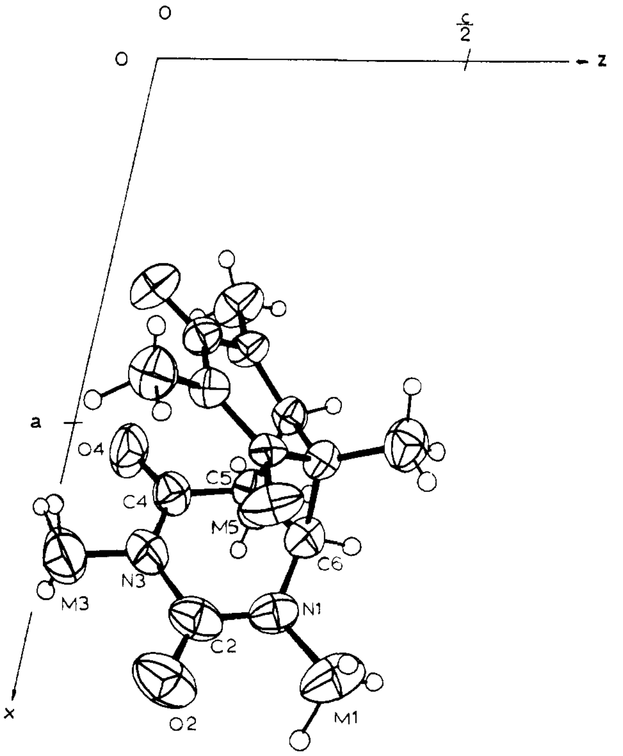

Figure 1. Perspective drawing of one molecule of dimethylthymine photodimer C viewed along [010]; O = oxygen, N = nitrogen, C = carbon, M = methyl-group carbon.

The cyclobutane ring is puckered, with dihedral angles of 153 and 154° (defined as the angle between planes having the transannular distances in common); each atom of the ring lies 0.5 Å out of the plane defined by the other three atoms.

The angle between the best planes through all six atoms of each of the two thymine nuclei is 40°. The residues themselves are not planar, but least-squares planes calculated through all combinations of five atoms

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(16) "International Tables for X-Ray Crystallography," Vol. III, The Kynoch Press, Birmingham, England, 1962.

(17) A copy may be secured by citing the document number and by remitting $1.00 for microfiche or $3.00 for photocopies to the ASIS National Auxiliary Publication Service, % CCM Information Sciences, Inc., 22 West 34th Street, New York, N. Y. 10001.

Table III. Fractional Coordinates and Anisotropic Thermal Parameters ($\times 10^5$)

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	0.6966	0.2475	0.2151	1825	330	647	−208	39	57
M(1)	0.6215	0.1592	0.1983	3132	435	992	−464	−109	99
C(2)	0.6440	0.3102	0.1433	1554	398	575	29	147	16
O(2)	0.5312	0.2980	0.0655	2342	516	787	−63	322	20
N(3)	0.7254	0.3932	0.1603	1556	310	567	46	176	72
M(3)	0.6630	0.4606	0.0817	2455	450	729	137	211	199
C(4)	0.8507	0.4178	0.2455	1503	310	669	91	226	−49
O(4)	0.9121	0.4916	0.2538	2163	272	1203	−41	−1	25
C(5)	0.9074	0.3504	0.3293	1765	325	544	27	149	−36
M(5)	0.8537	0.3814	0.4262	2491	560	662	234	233	−132
C(6)	0.8571	0.2562	0.2929	1800	322	472	2	121	54
N(1,2)	1.2308	0.3765	0.3101	1560	378	823	−63	92	−62
M(1,2)	1.3234	0.4383	0.3878	2388	571	1220	−161	−169	−211
C(2,2)	1.2319	0.3929	0.2108	1632	413	1058	25	530	−28
O(2,2)	1.3180	0.4510	0.1825	3024	565	1460	−381	1026	−48
N(3,2)	1.1260	0.3374	0.1352	1851	463	694	26	395	71
M(3,2)	1.1189	0.3597	0.0274	2532	886	819	122	549	207
C(4,2)	1.0381	0.2630	0.1555	2059	424	628	27	371	−55
O(4,2)	0.9619	0.2169	0.0863	3847	696	616	−435	430	−204
C(5,2)	1.0409	0.2418	0.2670	2002	327	571	56	218	−9
M(5,2)	1.1266	0.1523	0.2955	2742	380	991	295	326	14
C(6,2)	1.1027	0.3203	0.3386	1584	367	588	106	80	−43
H(6)	0.850	0.216	0.357	1714	345	459	59	98	38
Hm(1)	0.502	0.168	0.165	2528	647	885	0	328	0
Hm(2)	0.638	0.139	0.261	2528	647	885	0	328	0
Hm(3)	0.666	0.132	0.146	2528	647	885	0	328	0
Hm(4)	0.721	0.456	0.016	2067	529	724	0	268	0
Hm(5)	0.715	0.515	0.098	2067	529	724	0	268	0
Hm(6)	0.557	0.475	0.071	2067	529	724	0	268	0
Hm(7)	0.723	0.383	0.416	2169	555	759	0	281	0
Hm(8)	0.903	0.433	0.448	2169	555	759	0	281	0
Hm(9)	0.890	0.342	0.479	2169	555	759	0	281	0
H(6,2)	1.132	0.302	0.407	1445	375	659	90	130	−70
Hm(10)	1.246	0.489	0.389	2796	715	979	0	362	0
Hm(11)	1.349	0.414	0.446	2796	715	979	0	362	0
Hm(12)	1.423	0.473	0.358	2796	715	979	0	362	0
Hm(13)	1.207	0.345	0.015	2766	708	969	0	359	0
Hm(14)	0.991	0.380	−0.001	2766	708	969	0	359	0
Hm(15)	1.044	0.305	−0.018	2766	708	969	0	359	0
Hm(16)	1.059	0.106	0.259	2332	596	816	0	302	0
Hm(17)	1.110	0.141	0.366	2332	596	816	0	302	0
Hm(18)	1.248	0.149	0.282	2332	596	816	0	302	0
				Standard Deviations					
O	0.0004	0.0002	0.0002	62	13	20	24	29	14
N	0.0003	0.0002	0.0002	44	12	18	18	22	12
C	0.0004	0.0002	0.0002	54	13	20	21	27	13
M	0.0006	0.0003	0.0003	80	21	33	33	42	20
H	0.005	0.002	0.003						
Hm	0.007	0.003	0.004						

taken at a time showed that the rings are approximately planar if C(6) in each thymine residue is omitted from the calculation: maximum deviation from planes = 0.03 Å, and C(6) distances from the planes = 0.4 Å (inward toward the molecular center) in each case (planes 1 and 2, Table IV). This stereochemical feature is similar to that in the head-to-head *syn* dimer of dimethylthymine,[8] where C(6) of one thymine nucleus and C(5) of the other (which corresponds *in position* to C(6) in this molecule as the linkage is 5,5:6,6 in that dimer *vs.* 5,6:5,6 here) are out of the planes of the other atoms. The angle between five-membered planes is 33°. Table IV shows the deviations from the least-squares planes.

The two thymine residues are rotated with respect to each other (away from a position of thymine-nuclei atom overlap) by about 29° (calculated by averaging the

Table IV. Least-Squares Planes[a] and Deviations

Plane 1		Plane 2	
Atom	Deviation, Å	Atom	Deviation, Å
N(1)	0.014	N(1,2)	0.018
C(2)	−0.016	C(2,2)	−0.032
N(3)	0.004	N(3,2)	0.029
C(4)	0.010	C(4,2)	−0.011
C(5)	−0.011	C(5,2)	−0.004
M(1)	0.058	M(1,2)	0.100
O(2)	−0.057	O(2,2)	−0.138
M(3)	−0.056	M(3,2)	0.064
O(4)	0.012	O(4,2)	−0.047
M(5)	−1.312	M(5,2)	−1.274
C(6)	0.384	C(6,2)	0.412

[a] Plane 1: $0.8393x - 0.2977y - 0.4550z = 1.4118$; plane 2: $-0.8203x + 0.5706y - 0.0396z = -4.1101$, where x, y, z are coordinates in ångströms referred to orthogonal axes a^*, b, and c' ($= c \sin \beta$).

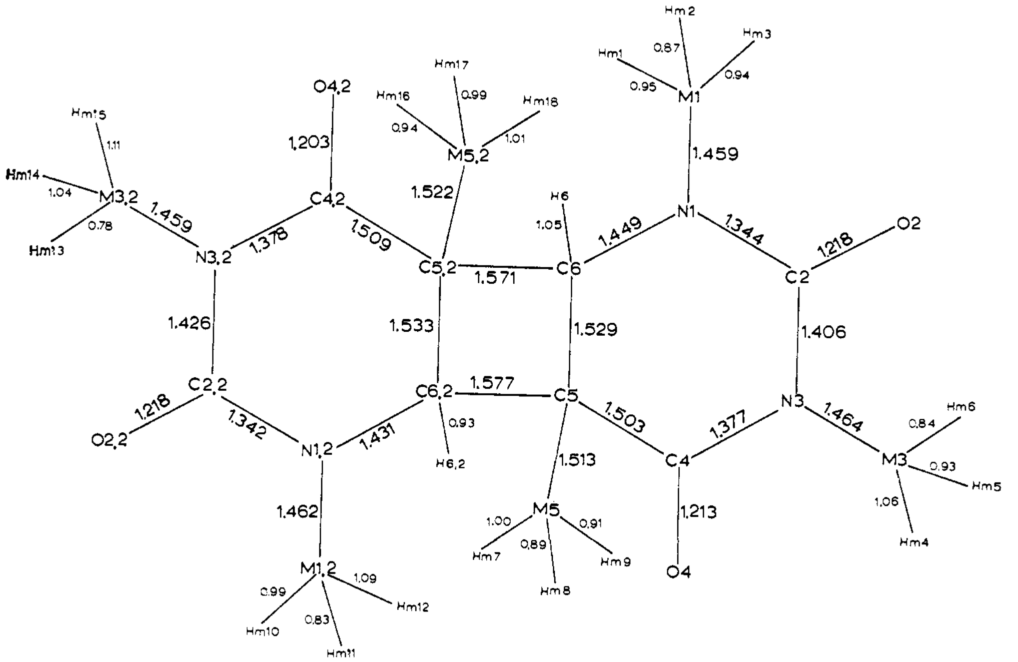

Figure 2. Bond distances (Å) in dimethylthymine photodimer C. Standard deviations of bond lengths are 0.003 Å for bonds involving "heavy atoms" and 0.05 Å for bonds involving hydrogens.

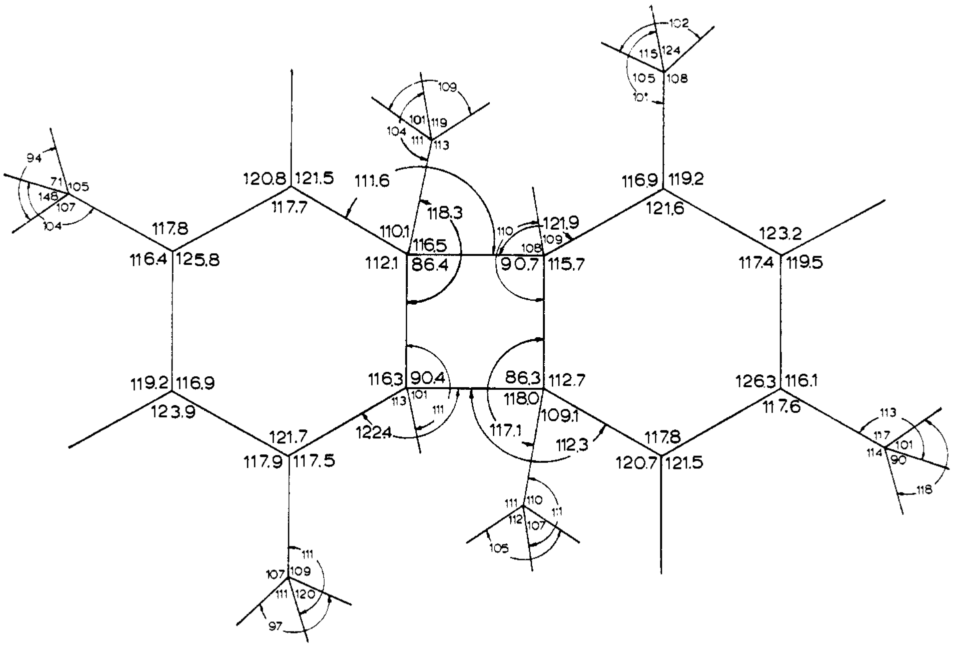

Figure 3. Valency angles (degrees) in dimethylthymine photodimer C. Standard deviations are 0.2° for "heavy atom" angles and 2-5° for hydrogens.

angles between normals to planes through C(5),C(6,2), C(4), and C(5),C(6,2),N(1,2); C(6),C(5,2),N(1), and C(6),C(5,2),C(4,2); and other equivalent sets); this is similar to the angular twist of 28° in the head-to-head *syn* dimer of dimethylthymine,[8] and 24° in the same dimer of uracil.[9] Thus the configuration of the thymine nuclei in the *syn* photodimers seems to be little affected by whether the linkage between them is head-to-head or head-to-tail (5,5:6,6 or 5,6:5,6).

The bond distances and angles are shown in Figures 2 and 3. The equivalent bond lengths in the two crystallographically independent halves of the dimer agree extremely closely with each other except for the C(2)-N(3) bonds, which differ by 0.02 Å (six times the standard deviation of each bond; the difference of each bond from the mean of their lengths is three times the standard deviation), and the N(1)-C(6) bonds, which differ by 0.018 Å. The inter-thymine cyclobutane bonds average 1.574 Å, significantly longer than the values of 1.531 Å for the intra-pyrimidine bonds, similar to what has been observed in the other photodimers cited. The averaged (between the two halves of the dimer) bond lengths in this molecule agree extremely closely with the corresponding distances in the head-to-head *syn* uracil photodimer[9] and the head-to-tail *anti* dimers of thymine[10] and 1-methylthymine:[11] the differences exceed 0.01 Å only in the case of the N(3)–C(2) and N(3)–C(4) bonds, where presumably the inductive effect of the methyl group in the present molecule causes a very slight lengthening of these bonds. The latter two dimers have planar cyclobutane rings; thus it appears that the conformation of the cyclobutane ring has little or no effect on the conformation of the heterocyclic rings.

The bond angles in the two halves of the dimer agree almost identically with each other. The values here also agree to within 1° with those in the head-to-tail *anti* dimers, excepting the angles at N(1) and C(5). These are smaller (~3°) than in the planar cyclobutane head-to-tail *anti* photodimers because of the puckering of the cyclobutane ring which causes C(6) to lie out of the plane of the other five thymine-nuclei atoms, and so reduces the angles at the neighboring atoms.

An outstanding feature of the structures of the thymine (and uracil) photodimers thus far elucidated is their remarkable consistency in molecular dimensions, which seems very little dependent on the nature of the pyrimidine–pyrimidine linkage (5,5:6,6 or 5,6:5,6), on the *syn* or *anti* relation of the two rings, or on the planarity of the cyclobutane ring.

The packing in the crystal is shown in Figure 4. The closest intermolecular contacts involve carbonyl oxygens and N-methyl groups and are 3.20, 3.28, and 3.30 Å. All other distances correspond to normal van der Waals contacts.

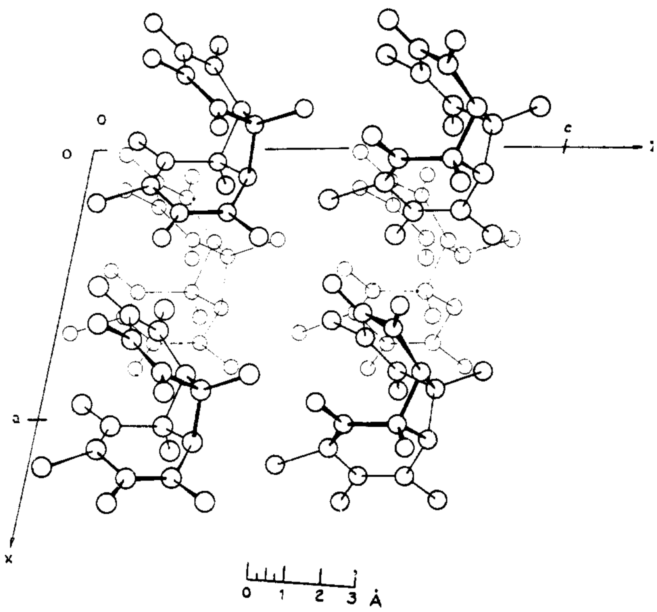

Figure 4. Intermolecular packing viewed along [010]. The bold-figured molecules at the right are related to the bold-figured ones at the left by the *c*-glide plane. The light-figured molecules are related to the bold-figured ones by translations of *a*/2 + *b*/2.

The ellipsoids in Figure 1 represent the relative thermal motions of the atoms, and their volumes are scaled to contain the region of 50% probability.

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The Rates of Recombination of Carbon Radicals in Solution

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Abstract: A series of azo compounds has been photolyzed in the cavity of an esr spectrometer. Use of a light-intermittency method allows estimation of the over-all rate constants (k_t) for bimolecular decay of the radicals. Comparison of the values of k_t for cumyl and a series of related radicals shows a pattern of behavior that is not easily rationalized on the basis of common structure–reactivity models. Serious discrepancies between known cage recombination parameters and the bimolecular rate constants are also noted. We tentatively suggest that relaxation of the solvent shell may play an important rate-determining role in reactions with rates close to the diffusion-controlled limit.

With the advent of electron spin resonance (esr) spectroscopy it has become possible to characterize free radicals and study the rates of their reactions by direct measurement. We have recently reported on the use of this method to measure the rate of bimolecular decay of radicals produced by photolysis of azo compounds.[1] We now wish to report the results of a more extensive study which reveals interesting reactivity relationships.

We assume that the following equations (1–6) describe the mechanism.

$$\mathrm{RN_2R} \longrightarrow \overline{\mathrm{2R\cdot + N_2}} \qquad (1)$$

$$\overline{\mathrm{2R\cdot + N_2}} \xrightarrow{k_c'} \mathrm{RR + N_2} \qquad (2)$$

$$\overline{\mathrm{2R\cdot + N_2}} \xrightarrow{k_{dis}'} \mathrm{R(-H) + RH + N_2} \qquad (3)$$

$$\overline{\mathrm{2R\cdot + N_2}} \xrightarrow{k_{dc}} \mathrm{2R\cdot + N_2} \qquad (4)$$

$$\mathrm{2R\cdot} \xrightarrow{k_c} \mathrm{RR} \qquad (5)$$

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[1] S. A. Weiner and G. S. Hammond, *J. Am. Chem. Soc.*, 90, 1659 (1968).