independent of the electron donor. The difference in the sign of the slopes between stirred and unstirred solutions is puzzling. The most reasonable interpretation seems to be that water and the rubrene cation react in the Nernst diffusion layer, yielding products which quench the chemiluminescent reaction between them. In stirred solutions, these products are swept away from the face of the electrode surface so that reaction always occurs at a clean surface whereas in unstirred solutions these products accumulate in the diffusion layer and interfere with the chemiluminescent reactions of the cation.

The effect of repetitively scanning into the positive potential region was studied for rubrene in dimethylformamide and a varying effect on light emission behavior was observed as shown in Figure 6. When scanning over the potential range 0 to +1.5 v, after four successive scans no light emission could be observed on subsequent scans. As the potential scan range was decreased, the light emission behavior depended less on the number of previous scans, until scanning over the potential range 0 to +0.95 v the light intensity-voltage curves of successive scans were virtually superimposable. This behavior can be explained by the fact that, in DMF, oxidation of rubrene to the cation occurs just on the edge of the solvent oxidation wave. At positive potentials of *ca.* +1.5 v, significant solvent oxidation accompanies oxidation of the hydrocarbon, and the products of the solvent oxidation are accumulated in the vicinity of the electrode. This was demonstrated by the observation of a large reduction peak when the electrode was made cathodic after several successive scans into the anodic range. This peak was found to correspond to one observed when solvent containing no rubrene was oxidized. The solvent oxidation products quench the luminescent reaction of the radical cation, and therefore as they accumulate on subsequent sweeps into the oxidation region, light emission vanishes. However, when one sweeps onto just the foot of the rubrene wave, solvent oxidation does not occur to a significant extent, and the effect of solvent oxidation products on the light emission reaction is not observed.

Acknowledgments. We wish to acknowledge the assistance of Professor K. Bowers in the esr work. Dr. Donald Maricle suggested the tetraphenylpyrene as a possible chemiluminescent hydrocarbon. We wish to thank Dr. Jack Chang for repeating some of our measurements. This work is supported in part through funds provided by the U. S. Atomic Energy Commission under Contract AT(30-1)-905.

The X-Ray Structure Determination of Thujic Acid (7,7-Dimethylcycloheptatriene-3-carboxylic Acid)

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Contribution from the Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut. *Received March 18, 1966*

Abstract: The structure of the *p*-bromophenacyl ester of thujic acid (7,7-dimethylcycloheptatriene-3-carboxylic acid) has been determined employing three-dimensional X-ray crystallographic techniques. The structure was solved by the heavy atom method and then refined through successive electron and difference density syntheses and by the method of least squares. All the hydrogen atoms except three (one methyl group) have been located. The bond distances in the cycloheptatriene alternate, the 1,6 distance is 2.42 A, and the ring assumes a boat conformation. These facts preclude a norcaradiene arrangement and the planar, pseudo-aromatic structure previously proposed for the ring. The C7 atom is undergoing a large thermal vibration which is compatible with the observation that, in solution, the molecule is inverting between conformers. The geometry of the molecule implies a small twist in the 1,2 and 5,6 double bonds. Some relevant $2p_z$–$2p_z$ overlap integrals of the ring have been calculated and they are discussed in terms of a novel π-bonding system. The X-ray results are very similar to those obtained for tropilidene by electron diffraction.

At the time this work was initiated there was still some controversy concerning the nature of the tropilidene ring. Although it seemed clear that the system was a monocyclic triene, the question of its planarity remained unsettled.[3] A preliminary communication of this structure determination indicated that in thujic acid (7,7-dimethylcycloheptatriene-3-carboxylic acid): (1) the cycloheptatriene ring is in a boat conformation, (2) the bond lengths alternate, and (3) the molecule is probably inverting between boat conformers in solution.[4] Subsequent studies have supported the boat feature of the molecule[5–8] and have

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(3) For a concise review of the problem and some pertinent references, see J. B. Lambert, L. J. Durham, P. Lepoutere, and J. D. Roberts, *J. Am. Chem. Soc.*, **87**, 3896 (1965).

(4) R. E. Davis and A. Tulinsky, *Tetrahedron Letters*, **No. 19**, 839 (1962).

(5) K. Conrow, M. E. H. Howden, and D. Davis, *J. Am. Chem. Soc.*, **85**, 1929 (1963).

(6) C. LaLau and H. DeTuyter, *Spectrochim. Acta*, **19**, 1559 (1963).

(7) A. P. ter Borg, H. Kloosterziel, and N. van Meurs, *Rec. Trav. Chim.*, **82**, 717 (1963).

(8) A. P. ter Borg and H. Kloosterziel, *ibid.*, **82**, 741 (1963); S. S. Butcher, *J. Chem. Phys.*, **42**, 1833 (1965).

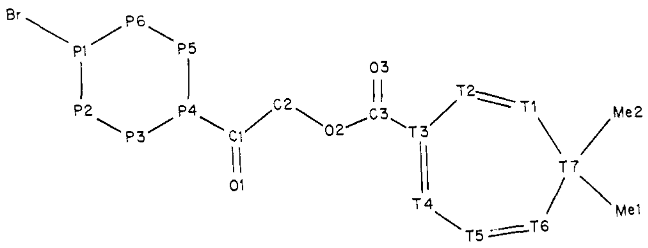

Figure 1. Numbering system; Me1 is equatorial.

established the inversion.[9,10] The final resolution of the problem came with the structure determination of tropilidene in the vapor by electron diffraction,[11] the results of which proved to be strikingly similar to those obtained for thujic acid. In what follows, the details of the X-ray determination of thujic acid will be given along with some inferences concerning the electron distribution in the molecule.

Experimental Section

One of the reasons for choosing thujic acid for an X-ray crystallographic structure determination was because of the equivalence of its methyl groups in the nmr spectrum at room temperature.[4,12] Such behavior is consistent with a planar structure or with a rapidly inverting pair of nonplanar conformers. In order to facilitate the structure determination, thujic acid was converted to its *p*-bromophenacyl ester. Good single crystals of this compound were grown by slow evaporation of an isopropyl alcohol solution. The crystals were well developed, displaying end faces, and had a prismatic morphology with a prominent (010) face; however, they were unusual in that their longest edges (along the *c* crystal direction) were perceptibly curved.

The X-ray work was carried out with Cu Kα radiation and a General Electric XRD-5 equipped with a single crystal orienter and a scintillation counter assembly. A preliminary survey of the diffraction pattern indicated that the crystal system was orthorhombic with $a = 6.29$, $b = 30.50$, and $c = 17.10$ A. From the systematic absence of reflections of the type ($hk0$), h odd, ($h0l$), l odd, ($0kl$), k odd, the space group was fixed to be Pbca. The observed crystal density measured by flotation in aqueous silver nitrate was 1.45 g cm^{-3} and the calculated density (based on eight molecules per unit cell) was 1.462 g cm^{-3}.

Before collecting three-dimensional intensity data, the mosaic spreads of several reflections at different ϕ and χ values were determined to aid in selecting the region of reciprocal space to be used for the intensity measurements. The spreads were found to be single peaked and adequately symmetrical and varied from 0.25 to 0.45° in width as a function of ω (background to background). The intensities were measured using the stationary crystal–stationary counter technique with balanced Ni–Co filters. Of the 2056 reflections possible to $d_{\min} = 0.94$ A ($2\theta_{\max} = 110°$), 1706 (83.0%) were taken to be observable.

Throughout data collection, three convenient reflections were monitored as a function of X-ray exposure to the crystal (the (200), (004), and (006) reflections). They showed a decrease in intensity of about 13% after a total of 35 hr of exposure (total of 31 measurements in approximately 2-hr intervals). Consequently, the intensity data, which was collected as a function of X-ray exposure, was corrected for this decrease. The intensities were also corrected approximately for absorption (as a function of azimuthal angle ϕ),[13] the maximum–minimum ratio of the correction being 1.66. Finally, Lorentz and polarization factors were applied, converting the modified intensities into relative structure amplitudes, and these were then treated according to Wilson's method to approximate an absolute scale.

Structure Analysis

The bromine positions were determined from the Harker sections at $u = {}^1/_2$, $v = {}^1/_2$, $w = {}^1/_2$ generated by the three mutually perpendicular glide planes of Pbca. The Harker sections were computed sharpened with $(z_{\mathrm{Br}}/f_{\mathrm{Br}})^2$, where z_{Br} and f_{Br} are, respectively, the atomic number of bromine and scattering form factor of bromine at rest. These planes gave bromine coordinates of $x = 0.077$, $y = 0.041$, $z = 0.121$.

Phases based on the bromine coordinates were then assigned to the observed structure amplitudes, but only to the 1258 reflections that had a bromine contribution of one-tenth that of its maximum (assuming an isotropic thermal parameter of $B = 3.5$ A^2), and a three-dimensional electron density, ρ_1, was computed. Twenty-two peaks greater than 2.4 e A^{-3} appeared in this density. The seven-membered tropilidene system, the phenyl ring, and atoms C1, C2 and O1 (see Figure 1 for numbering) were clearly distinguished. Actually, the entire molecule was recognized but the last five atoms not as well, so the latter were not included in the ensuing structure factor computation. In the next electron density (ρ_2), based on the foregoing 16 atoms and bromine, the only remaining uncertainty was Me2. An additional cycle fixed Me2 and this was followed by the introduction of individual isotopic thermal parameters and three more cycles of structure factor and electron and difference density computations.[14] At this stage, R was 0.29 and full-matrix least-squares refinement was undertaken.

The weighting scheme used in the least-squares refinement was as follows: if $I \leqslant 8I_{\min}$, then the standard error in $|F|_o$, $\sigma(|F|_o)$, was taken to be

$$\sigma(|F|_o) = (I + 2B_k)^{1/2}|F|_o/2I$$

and if $I > 8I_{\min}$, then

$$\sigma(|F|_o) = 0.075|F|_o$$

where I is the observed intensity, $I_{\min}$ the weakest intensity taken as observable, and B_k the background (with Co filter). In this way, the effect of the Lorentz polarization factor was accounted for since the weighting of reflections whose intensity is $I \leqslant 8I_{\min}$ is proportional to the estimated fractional error in intensity. Thus, of two reflections with the same $|F|_o$, the one observed at a lower scattering angle, and hence with the larger intensity, has the smaller standard error. For reflections with $I > 8I_{\min}$, counting statistics indicated an error of approximately 10% or less in intensity. Since this would imply some exceptionally accurate measurements for some of these reflections, the error for this group was taken to be 15% of the intensity measurement (about $2\langle\sigma/I\rangle$).

Several cycles of isotropic least-squares refinement, with two sets of electron and difference density computations interspersed (at $R = 0.26$ and $R = 0.233$), decreased R to 0.231. The difference density at this point (D_8) clearly indicated a need for general thermal parameters. These were introduced and one cycle of refinement on anisotropic thermal parameters varying only 15 atoms (Br, O1, O3, T1, T5, T6, T7, both methyls, and all six phenyl carbons) caused a remarkable de-

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[9] F. A. L. Anet, *J. Am. Chem. Soc.*, **86**, 458 (1964).

[10] F. R. Jensen and L. A. Smith, *ibid.*, **86**, 956 (1964).

[11] M. Traetteberg, *ibid.*, **86**, 4265 (1964).

[12] J. Gripenberg, *Acta Chem. Scand.*, **10**, 487 (1956).

[13] S. Silvers and A. Tulinsky, *Acta Cryst.*, **16**, 579 (1963).

[14] When reference is here made to difference density, it implies that line sections through peaks in ρ_o and ρ_c were examined in order to isolate peak-shape effects from positional shifts.

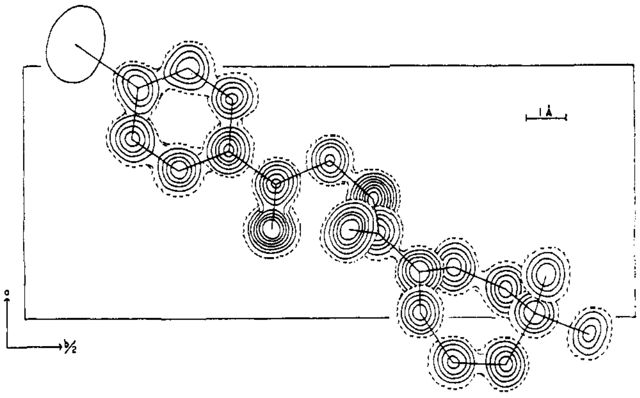

Figure 2. Composite electron density; contours at 1 e A^{-3}, 1 e A^{-3} broken; Br atom open and shown by single contour at 1 e A^{-3}

crease in R to 0.119; routine convergence to the final structure now seemed in view. However, before proceeding with the refinement, the reliability of the low-order data was reassessed.

Through the course of the structure analysis, it was becoming increasingly more evident that some of the large, low-order reflections were probably affected by extinction. Thus, of the 30 most intense reflections, 17 of the most intense 18, 5 of the next 6, and 24 of the 30 persistently calculated high in structure factor computations. If the first group of reflections is corrected for secondary extinction,[15] then R decreases from 0.119 to 0.113.[16] Attempts to grow smaller crystals so that such intensities could be remeasured failed to produce any crystals that were acceptable. Therefore, in order to reduce the effect of this class of reflections on the least-squares refinement, the standard errors of six were increased to $(||F|_o - |F|_c|)/2$, where $|F|_c$ is here the calculated structure amplitude at $R = 0.119$, and a corresponding increase was made in their observed structure amplitudes (see Table I). These changes, together with positional shifts and a new scale factor obtained from D_8, produced an increase in R to 0.128.

Table I. Reflections Adjusted for Extinction

h	k	l	2θ	Old $\lvert F\rvert_o$	Old $\sigma(\lvert F\rvert_o)$	New $\lvert F\rvert_o$	New $\sigma(\lvert F\rvert_o)$
0	0	4	20.60	705	52.8	846	141.4
1	3	2	19.38	529	39.7	587	58.2
2	0	0	28.18	208	15.5	227	18.7
1	4	2	20.86	185	13.8	206	20.7
1	4	1	18.82	159	12.0	173	13.8
0	2	1	7.56	133	10.0	146	13.0

Three more cycles of least-squares refinement, first varying the atoms for which D_8 had indicated large parameter shifts (47 mixed parameters, 17 positional) and then varying others, brought R to 0.115. Additional least-squares refinement on thermal parameters alone brought R to 0.095, and D_{10} was computed. Upon examination of this density, significant positive regions were found at or near the expected positions of all the hydrogen atoms except those of the equatorial methyl group (Me1). Elsewhere in D_{10}, the discrepancies between the observed and calculated density were essentially confined within the expected error. Small positional shifts were made according to D_{10} and a final difference density was computed from which individual isotropic temperature factors were assigned to the 14 observed hydrogen atoms. Final structure factors were then computed including these changes ($R = 0.094$ omitting hydrogens and $R = 0.088$ including hydrogens).

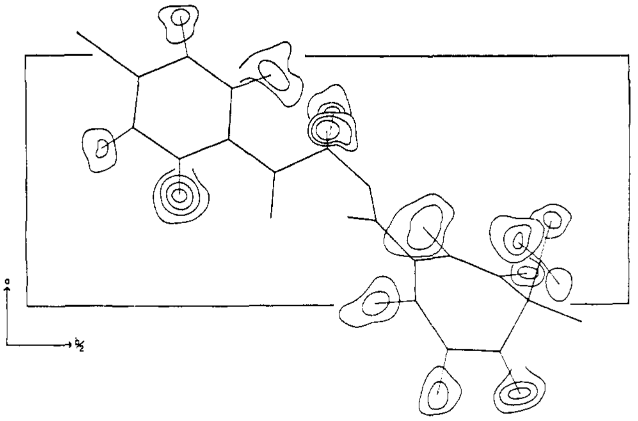

Figure 3. Composite of positive regions in difference density at or near expected hydrogen atom positions; contours at 0.25 e A^{-3}, beginning at 0.25 e A^{-3}.

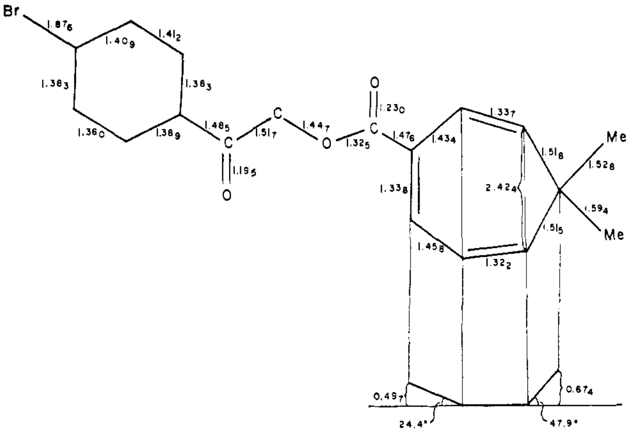

Figure 4. Interatomic distances, including some out-of-plane distances.

Results

The final electron density is shown in Figure 2; positive regions at or near expected hydrogen atom positions are shown in Figure 3. The final atomic coordinates are listed in Table II. Interatomic bond distances and several other relevant distances are shown in Figure 4, while Figure 5 gives interbond angles. The standard error in bond distances is estimated to be of the order of 0.015–0.020 A and that in the interbond angles about 1.0–2.0°

Discussion

The Tropilidene Ring. The T1–T6 distance of the seven-membered ring (2.42 A) precludes a norcaradiene structure for the ring system. A recent structure

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(15) R. W. James, "The Crystalline State," Vol. II, G. Bell and Sons, Ltd., London, 1962, Chapter VI, eq 6.23 and 6.24.

(16) Considering R for *only* these 18 reflections, R decreased from 0.148 to 0.051. Although such an improvement in agreement was satisfying, the calculated extinction correction was not applied and a more cautious approach was followed (see below).

Table II. Final Atomic Parameters[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	$\beta_{ij} \times 10^5$ β_{12}	β_{13}	β_{23}	Peak height, e A^{-3}
Br	0.0787	0.0414	0.1216	4732	105	1016	515	−329	−103	37.8
P1	0.9115	0.0917	0.1363	3634	61	433	208	−342	−30	5.2
P2	0.7052	0.0885	0.1637	3417	74	546	−88	−120	−47	5.6
P3	0.5894	0.1257	0.1735	3129	81	401	−34	−116	41	6.1
P4	0.6686	0.1667	0.1534	2433	68	408	166	−82	20	6.9
P5	0.8732	0.1705	0.1245	2535	115	435	−125	105	65	5.8
P6	0.9985	0.1325	0.1146	3074	102	472	254	−175	25	5.3
C1	0.5323	0.2059	0.1659	2745	87	362	−101	−70	−18	6.6
C2	0.6329	0.2504	0.1515	2740	75	643	207	26	−21	5.8
C3	0.3364	0.2875	0.1022	3713	82	329	34	−48	−14	6.3
O1	0.3505	0.2019	0.1849	2914	98	675	11	522	55	8.1
O2	0.4744	0.2844	0.1604	3156	66	490	204	−351	−31	8.9
O3	0.3532	0.2649	0.0429	5031	143	487	633	−324	−81	7.1
Me1	0.9321	0.4637	0.1498	5668	106	826	585	1026	−76	4.4
Me2	0.1651	0.4268	0.0497	3947	117	753	31	1099	52	5.0
T1	0.1119	0.3920	0.1836	3490	91	525	−119	−158	−123	5.8
T2	0.1959	0.3519	0.1767	3454	64	421	62	155	−37	5.8
T3	0.1774	0.3227	0.1113	2799	62	343	69	28	−36	6.3
T4	0.0160	0.3226	0.0603	2795	68	469	−0	−271	−42	5.6
T5	0.8298	0.3509	0.0635	2982	133	604	−45	−654	152	5.4
T6	0.8197	0.3914	0.0913	3697	85	610	165	−226	87	5.5
T7	0.0119	0.4176	0.1171	3274	60	634	102	347	2	5.3
							B			
H-P2	0.663	0.063	0.150				5.0			0.6
H-P3	0.417	0.125	0.200				4.0			1.2
H-P5	0.967	0.196	0.100				4.5			0.7
H-P6	0.167	0.125	0.075				4.5			0.7
H-C2	0.700	0.246	0.217				4.5			0.7
H′-C2	0.733	0.250	0.092				4.5			0.7
H-T1	0.133	0.417	0.200				5.0			0.6
H-T2	0.260	0.329	0.222				4.5			0.7
H-T4	0.033	0.292	0.000				5.0			0.6
H-T5	0.650	0.338	0.042				5.0			0.6
H-T6	0.667	0.411	0.070				4.5			0.8
H-Me2	0.240	0.407	0.042				4.5			0.7
H′-Me2	0.333	0.438	0.075				5.0			0.6
H″-Me2	0.040	0.442	0.008				5.5			0.4

[a] Anisotropic temperature factor = $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{33}kl)]$.

determination of a norcaradiene has shown that the corresponding distance in the norcaradiene is about 1.50 A.[17] The bond lengths in the tropilidene ring of thujic acid alternate, suggesting that the ring possesses alternate partial single and double bond character typical of a conjugated system. Furthermore, the ring is in a marked nonplanar boat conformation. Some of the out-of-plane distances are given in Table III.

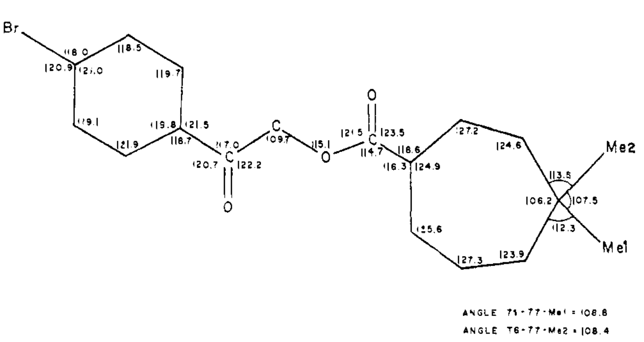

Figure 5. Interbond angles.

Table III. Some Out-of-Plane Distances

Plane	Atom	d, A	Inference
T1–T5–T6	T2	−0.03	T1, T2, T5, T6 coplanar within experimental error
T1–T5–T6	T7	+0.67	Angle between planes T1–T5–T6 and T1–T7–T6 is 47.9°
T1–T5–T6	T3	+0.50	Angle between planes T1–T5–T6 and T2–T4–T5 is 24.4°
T1–T5–T6	T4	+0.50	
T3–T4–T5	T2	+0.04	No twist about 3,4 double bond
T5–T6–T7	T4	−0.14	Average twist of approximately 7° about 5,6 double bond
T1–T2–T7	T3	−0.22	Average twist of approximately 11° about 1,2 double bond

From Table III, it can be seen that atoms T1, T2, T5, T6 are coplanar within the accuracy of the determination (±0.03 A) and that the T7 carbon atom and atoms T3 and T4 are, respectively, +0.67 and +0.50 A out of this plane. These results agree well with those of an electron diffraction investigation of tropilidene,[11] the results of which showed that: (1) the molecule has C_s symmetry, (2) the molecule is in a boat conformation, and (3) the ring possesses alternating bond lengths. Observations somewhat similar to the foregoing have also been made on cycloheptatriene-

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(17) C. J. Fritchie, Jr., *Acta Cryst.*, **20**, 27 (1966).

molydenum tricarbonyl, where six of the atoms of the ring were found to be nearly planar with the seventh atom (corresponding to T7) being 0.67 A out of the plane and the bond lengths in the ring alternated.[18]

From Table III, it will also be seen that atoms T3 and T4 are displaced from the planes of atoms T1, T2, T7 and T5, T6, T7, respectively, implying a twist about the 1,2 and 5,6 double bond. Since atoms T2, T3, T4, and T5 are essentially coplanar, no twist is present in the 3,4 double bond. The twists listed in Table III are regarded as limiting cases since they were obtained from considering only four atoms. The net effect of the twists is to decrease the distance of atoms T7 and/or T3 and T4 from the plane of atoms T1, T2, T5, and T6. A more exact description of the nature of the twists is possible if hydrogen atoms are included in the twist evaluation; however, the large uncertainty inherent in hydrogen positions precluded any such further considerations.

Overlap Calculations. The most interesting feature of the cycloheptatriene ring of thujic acid is probably the close proximity of the 1,6 positions in conjunction with the boat conformation of the ring system. The close proximity permits the $2p_z$ electrons of the 1,6 atoms to overlap to some extent, and the conformation is such that the 1,6 overlap is enhanced below but diminished above the boat. In this way, an unusual type of π bond can form. Thus, the π-bonding system of the cycloheptatriene ring resembles that of *cis,-s-cis,s-cis*-1,3,5-hexatriene on one side of a nodal plane and that of an irregular, nonplanar benzene on the other. Reproductions of a model attempting to illustrate this are shown in Figure 6, where the light and dark spheres are to be taken as the two different lobes of p_z orbitals.

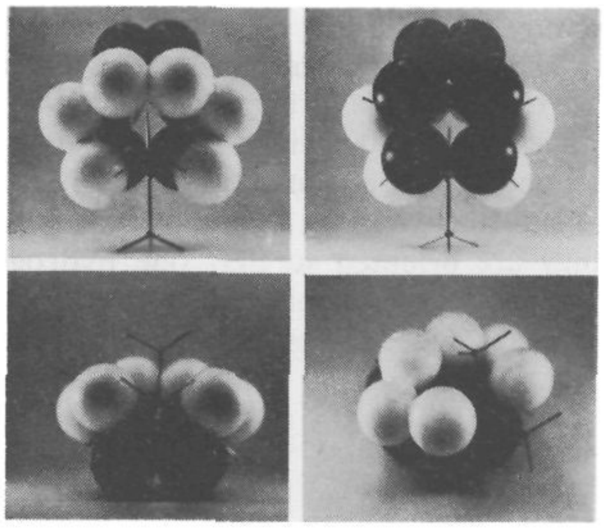

Figure 6. Representation of the p_z orbitals in thujic acid: (a) view from above the boat; (b) view from below the boat; (c) view along the Me1–T7 direction; (d) a general view.

Values of some pertinent overlap integrals for this system have been calculated according to Mulliken, *et al.*[19] The $2p_z$ orbitals of a pair of atoms in question were first decomposed into components along and perpendicular to their interatomic direction. The overlap integrals for these components were obtained by graphical interpolation of tables given by Mulliken, *et al.*,[19] multiplied by appropriate direction cosines, and then summed. The magnitudes of certain $2p_z$–$2p_z$ overlap integrals in the tropilidene ring of thujic acid are given in Table IV, while Table V gives for comparison the values calculated for the $2p_z$–$2p_z$ overlap integrals in *trans*-1,3-butadiene.[20]

The value of the 1,6 overlap integral for thujic acid is somewhat of a surprise: $S_{16} = 0.072$ is twice as large as the 1,3 overlap in butadiene and about one-third as large as the 2,3 overlap in butadiene. Thus, an appreciable interaction must exist between the 1,6 atoms of the ring and may be an important contributing factor in the formation of certain types of derivatives having a norcaradiene structure.[21]

The values of the S_{23} and S_{45} integrals of thujic acid are somewhat smaller than that obtained for S_{23} in butadiene. Although the environment of these bonds in the two different molecules is similar, less overlap is to be expected in the former because of the lack of planarity of the 3,4 double bond with either the 1,2 or 5,6 bonds. The value of S_{34} in thujic acid is as expected for a double bond of that length; however, S_{12} and S_{56} are slightly smaller because of the twist involved in these bonds.

Table IV. Some $2p_z$–$2p_z$ Overlap Integrals for Thujic Acid

Atoms T*i*, T*j*	Overlap integrals S_{ij}
T1, T6	0.072
T4, T5	0.191
T2, T3	0.210
T3, T4	0.272 (0.272)[a]
T1, T2	0.266 (0.272)[a]
T5, T6	0.275 (0.278)[a]

[a] Values in parentheses are for double bonds, with no twist, of the observed lengths.

Table V. $2p_z$–$2p_z$ Overlap Integrals for *trans*-1,3-Butadiene

Atoms *i*, *j*	Overlap integrals S_{ij}
1, 2	0.278
2, 3	0.233
1, 3	0.034
1, 4	0.002

Thermal Vibrations. All the atoms in the molecule appear to have relatively large over-all thermal motions which are markedly anisotropic, especially that of the bromine atom. Since tropilidene is known to be inverting between conformers in solution above about −120°,[9,10] and since the *gem*-dimethyl groups of thujic acid and its *p*-bromophenacyl ester are equivalent in nmr spectra at room temperature,[4,12] the large vibrations of thujic acid in the solid are consistent with the T7, T3, and T4 carbon atoms undergoing a cooperative inversion in solution, thereby converting to a mirror image conformer. Thus, the time-averaged structure in solution is probably planar. In the solid, however, such an inversion does not take place, apparently because of space limitations.

Hydrogen Atoms. Positive regions were observed in the final difference densities near expected hydrogen positions for all the atoms except Me1. The region

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(18) J. D. Dunitz and P. Pauling, *Helv. Chim. Acta*, **42**, 2188 (1960).

(19) R. S. Mulliken, C. A. Rieke, D. Orloff, and H. Orloff, *J. Chem. Phys.*, **17**, 1248 (1949).

(20) R. S. Berry, Ph.D. Thesis, Harvard University, 1956.

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in the vicinity of Me1 was obscure because Me1 possesses the largest thermal parameters in the molecule. The positive regions have been ascribed to hydrogen atoms and vary in peak height from 0.4 to 1.2 e A^{-3}, and their bond distances range from 0.9 to 1.1 A. The orientation of the hydrogen atoms of Me2 is suggestive in that they are oriented so that one hydrogen is directed toward the 3,4 double bond. In this way, Me2 can achieve a staggered configuration with respect to T7 (see Figure 6a).

Acknowledgment. We wish to thank Professor W. von E. Doering for bringing this problem to our attention and for providing the *p*-bromophenacyl derivative.

This research has been supported by the National Institutes of Health, Public Health Service, and by Lederle Laboratories Division, American Cyanamid Company. One of us (R. E. D.) is indebted to the Division of General Medical Sciences, Public Health Service, for Public Health Service Fellowship No. 5-Fl-GM-14, 844-03.

Structural Studies of Ribonuclease. XXV. Enthalpy Changes Accompanying Acid Denaturation[1]

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Abstract: An enthalpy titration curve (relative apparent heat content *vs.* pH) has been obtained for ribonuclease in 0.1 *M* KCl between pH 2.2 and 11.5 at 25°. A theoretical curve is able to fit the experimental data. From measurements of the heat change accompanying acidification from pH 9.3 to 2.2 at several temperatures ranging from 10 to 55°, the enthalpy of unfolding of ribonuclease at pH 2.2 and 45° was found to be 109 ± 5 kcal/mole. This result is discussed in relation to previous estimates of the heat of denaturation obtained from heat capacity measurements (70 ± 1 kcal/mole) and from calculations with the van't Hoff equation (114 ± 7 kcal/mole).

Much attention has recently been paid to the conformation of ribonuclease and its change under various conditions. In particular, the thermal transition has been studied by optical rotation and ultraviolet difference spectra measurements, and thermodynamic parameters for the denaturation process have been deduced by assuming models for the reaction.[3–7] The enthalpy of denaturation has also been obtained by calorimetric measurements[8] of the heat capacities of aqueous solutions of ribonuclease at a series of temperatures. Since there are discrepancies in the values of the enthalpy of denaturation obtained by these methods, it was decided to make a direct calorimetric measurement of the enthalpy of denaturation rather than obtain it from the heat capacities.

For this purpose, the heat change accompanying the acidification of ribonuclease solutions (*i.e.*, when the pH is lowered from 9.3 to 2.2) was measured at several temperatures ranging from 10 to 55°. The enthalpy change accompanying the denaturation of ribonuclease was obtained in a manner similar to that employed by Hermans and Rialdi[9] for myoglobin and by Bunville, *et al.*, for DNA.[10]

Experimental Section

Materials. Sigma Chemical Co. five-times crystallized ribonuclease (Lot 114B-1510) was chromatographed (to obtain ribonuclease A) and deionized, as described by Rupley and Scheraga.[11] A sample of the purified, lyophilized protein was found to be free of ribonuclease B by rechromatography on IRC-50 resin using an analytical column. The presence of a small amount of aggregate (less than 1%) was ignored. The lyophilized material was stored in an evacuated desiccator over P_2O_5. Concentrations were determined by weighing out the hydrated powder, which contained 7% moisture based on an extinction coefficient[6] of 0.738 cm²/mg at 278 mμ. Deionized distilled water was used, and all other chemicals were reagent grade.

pH Measurements. pH measurements were made at 25° with a Radiometer TTT 1a titrator, using Fisher Scientific Co. buffers for standardization.

Calorimetry. Calorimetric measurements were carried out with an adiabatic solution calorimeter modeled after the one described by Benjamin.[12]

In a typical experiment, 20–40 mg of ribonuclease was weighed into the sample cell with 3.0 ml (weighed to ±0.1 mg) of either 0.10 or 0.15 *M* KCl. The solution was brought to thermal equilibrium within about 45 min in the calorimeter vessel containing both the cell and about 40 ml (weighed to ±0.1 mg) of salt solution (containing the desired amount of either HCl or KOH) to be mixed with the protein solution. The cell was broken, and the heat of mixing was measured. The resulting solution of about 43 ml contained about 0.5–1.0 mg/ml of ribonuclease, the same con-

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[1] This work was supported by a research grant (AI-01473) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service, and by a research grant (GB-4766) from the National Science Foundation.

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