axial coordination by a strong π-bonding ligand (CN^-) lowers A_{zz} by approximately 20 G (Table I). Thus, the one *nonlabile* axial coordination site in GOase could be occupied and stabilized by a protein ligand capable of π bonding. This coordination would, therefore, account for the 20 G difference from GOase in A_{zz} in the model system and for the single coordination site available to exogeneous ligands in GOase.[2] Since the 20 G difference in the A_{zz} values for enzyme and model can be rationalized wholly on the basis of an extra axial π-bonding ligand in GOase, the model does appear to be an excellent one for the in-plane ligands to the copper in GOase.

The inference that the nonlabile endogeneous axial ligand to the copper in GOase has π-bonding character suggests that a sulfhydryl group may be the ligand involved. While one does not normally think of possible protein ligands, –S, –O, and –N, as capable of forming strong π bonds, a π-bond effect has been demonstrated for sulfur ligands.[10,11] Moreover, the presence of a cysteine sulfur in the axial position in GOase is a definite possibility. There is one free sulfhydryl group in GOase which is titrable only in the apoenzyme after it is denaturated.[3,4,8] Therefore, not only is a free sulfhydryl group present in GOase, it and its coordination position are also quite inaccessible in the native protein. The competition by galactose for CN^- binding, the availability of only one axial site, and the lack of effects of oxygen on the esr spectrum substantiate some inferences made from CD spectra about the binding of substrates to GOase. Namely, galactose must bind prior to oxygen in the enzymatic reaction scheme. Furthermore, the esr results are consistant with outer sphere coordination of the oxygen to the reducing equivalent on the sugar aldiol rather than direct coordination to the copper.

These considerations are in agreement with the observation that no copper oxidation change occurs during the enzymatic reaction. The binding of the sugar aldiol by copper produces a strongly polarized bond such that a weak base like oxygen may carry out the oxidation without an oxidation state change for copper. A mechanism for such a reaction in organic chemistry has been discussed by Corey.[12] A similar polarization–activation–oxidation by outer sphere oxygen seems reasonable here.

Acknowledgment. This work was supported by a grant from the donors of the Petroleum Research Fund, administered by the American Chemical Society, by GRS-USPHS Grant RR05400-11, and by Institutional Funds of the Faculty of Natural Science and Mathematics and Schools of Medicine and Dentistry, State University of New York at Buffalo. Recognition is also due the National Science Foundation for partial support of this work by Grant No. B040662.

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Biphenylene. Internuclear Distances and Their Root Mean Square Amplitudes of Vibration

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Abstract: The molecular structure of biphenylene in the gas phase was investigated by electron diffraction; the sample was maintained at 145°. Resolution of the radial distribution curve indicated that these molecules have a planar conformation. On the basis of the assumed D_{2h} symmetry and the relative bond lengths derived from the X-ray crystal structure, a least-squares analysis of the molecular scattering function gave the following r_g values for the interatomic distances: $(C–C)_{av} = 1.418 \pm 0.003$ Å, $C_1–C_{8b} = 1.372 \pm 0.012$ Å, $C_1–C_2 = 1.428 \pm 0.012$ Å, $C_2–C_3 = 1.370 \pm 0.015$ Å, $C_{4a}–C_{4b} = 1.524 \pm 0.006$ Å, $C_{4a}–C_{8b} = 1.432 \pm 0.018$ Å, $C–H = 1.096 \pm 0.009$ Å, $\angle C_{8b}–C_1–C_2 = 115.0 \pm 1.2°$, $\angle C_1–C_2–C_3 = 122.5 \pm 1.2°$, $\angle C_{4a}–C_{8b}–C_1 = 122.5 \pm 0.6°$. In the above list the angles were defined in the r_α representation and shrinkage corrections were inserted in the least-squares program. The listed uncertainties represent estimated limits of error. The structural parameters are in good agreement with corresponding values derived from a crystal structure analysis. The benzene rings are distorted, and "bond fixation" is clearly present. The $C_{4a}–C_{4b}$ distances are almost as long as in a normal single bond while the $C_{8b}–C_1$ separations are definitely shorter than the C–C separation in benzene. Another characteristic feature of the structure is the relatively small angle, $\angle C_{8b}–C_1–C_2$. Comparison between the observed and calculated mean square amplitudes suggests that this molecule may be "quasiplanar" due to the large amplitudes of the out-of-plane vibrational modes.

Biphenylene provided the first unambiguous example (Lothrop, 1941) of a molecule containing a formal cyclobutadiene ring and has been the subject of intensive chemical, physical, and theoretical study ever since.[1] According to almost all theoretical models the parent hydrocarbon, cyclobutadiene, should exhibit

(1) J. W. Barton, "Nonbenzenoid Aromatics," J. P. Snyder, Ed., Academic Press, New York, N. Y., 1969, Chapter 2.

pseudo-Jahn–Teller distortion and show extensive alternation of bond lengths.[2] Although biphenylene is not expected to have a triplet ground state there has been considerable discussion of the degree to which the presence of the formal cyclobutadiene ring moderates the chemical and physical properties of biphenylene. The question has been recast in terms of the "degree of bond fixation" (or equivalently, the "degree of bond alternation") displayed by the C_6 rings, and the associated reduction in benzenoid character introduced by the four-membered ring.

X-Ray crystallographic studies[3] show that in the solid state the molecules are planar (D_{2h}), and the bond lengths reveal the bond alternation predicted by LCAO calculations.[4] Whether this applies to the gas-phase structure has not been established. An early electron diffraction investigation of the vapor by the visual method could not assign individual bond lengths[5] with sufficient precision. A recent ir study[6] showed that the molecule has at least one low frequency associated with one of the out-of-plane bending modes: *ca.* 120 cm^{-1} (B_{3u}). This suggests that vibrational corrections to the structure analysis must be made. Hence, to shed light on the structure and intramolecular motions of the free biphenylene, the present study was undertaken.

Experimental Section

Biphenylene was prepared by the method of Friedman.[7] Recrystallization from methanol–water followed by sublimation yielded (21%) pale yellow needles, mp 110–110.5° (lit. mp 110°). The diffraction photographs were obtained with the Cornell University instrument in the convergent-beam mode.[8] The sample was introduced into the diffraction chamber (ambient pressure, 0.8–2 $\times$ 10^{-6} Torr) through a nozzle (diameter, 0.6 mm) maintained at 145°.[9] The nozzle-to-plate distances of 264.82 mm (LVL) at 40 kV and 95.84 mm (HVS) at 70 kV were calibrated by taking diffraction patterns of the standard (CS_2) concurrently with the biphenylene sample.[10] The exposure times for the LVL and HVS patterns were about 60 and 80 sec, respectively. The photographic plates (Kodak electron image) were scanned with a modified J-A microdensitometer, interfaced with an A-D Digitizing System. Transmittances were recorded at 200-μ intervals for the LVL plates and at 100-μ intervals for the HVS plates.[10] On conversion of the recorded transmittances to intensities, values were interpolated at unit q [$q = (40/\lambda) \sin (\theta/2)$]; the total range of q covered was from 11 to 125 Å^{-1}. These intensity data are listed in the microfilm edition (see paragraph at end of paper regarding supplementary material). Additional procedural details for data reduction have been reported elsewhere.[10]

Analysis

Experimental radial distribution curves, $f(r)$, and molecular intensity curves, $qM(q)$, were calculated from the diffraction intensity values with the Interactive Computer Programs for the augmented DEC PDP-9.[11] These are plotted in Figures 1 and 2, respectively, along with the corresponding theoretical curves. On the assumption that the molecule processes D_{2h} symmetry, ten independent parameters specify its geometrical structure, *i.e.*, a number equal to the number of totally symmetric vibrations (A_g species). In order to reduce the number of parameters, we made the additional assumption that all the bonded C–H distances were equal. The remaining nine parameters were chosen as follows: five bond lengths (C_{4a}–C_{4b}, C_{4a}–C_{8b}, C_{8b}–C_1, C_1–C_2, and C–H) and four bond angles ($\angle C_{4a}$–C_{8b}–C_1, $\angle C_{8b}$–C_1–C_2, $\angle C_{8b}$–C_1–H, and $\angle C_1$–C_2–H).

One should anticipate that for a molecule of this size the multiparameter surface would have one or more minima for the standard deviation computed *via* linearized least squares, adjacent to the deepest one which represents the structure compatible with all the available data. Indeed, when a starting model was inserted in which the generally accepted relative bond lengths were interchanged, a five-cycle iteration generated a structure with acceptable relative bond lengths but unacceptable magnitudes for the bond angles, and a large residual. This proved to be a false minimum. For the final radial distribution analysis, the X-ray crystal structure parameters were inserted as a starting model. The root mean square amplitudes of vibrations were deduced by a trial and error comparison between the contours of the experimental $f(r)$ and theoretical RD curves. The search and check operations are greatly facilitated by the PDP-9 computer system.[11] As shown in Figure 1, the first peak at 1.09 Å is the bonded C–H distance. The second high peak is composed of five nonequivalent bonded C–C pairs; their weighted average is 1.416 Å (r_a). The three peaks in the 2–3-Å range are comprised of a combination of several nonbonded distances; the main contributors are the nonbonded C–C pairs within each of the benzene rings and in the four-member ring. The peaks beyond 3 Å are due to the nonbonded C–C scattering from atom pairs situated in different benzene rings.

The peak positions in the $f(r)$ curve indicate that biphenylene has a planar structure in the gas phase. In spite of considerable overlapping the five bonded C–C distances could be resolved on the basis of the information derived from the nonbonded distances, since all five distances are not independent parameters. The structural parameters and the mean amplitudes derived from the above analysis were then used as a starting model for the more precise analysis of the $qM(q)$ data, taking into account the vibrational corrections (the effects of anharmonicity of the molecular vibrations and nonlinear shrinkage).

The molecular parameters derived in the radial distribution analysis were refined by least-squares fitting of the observed molecular intensity function to the following expression

$$qM(q) = K\sum_{i\neq j} A_{ij}c_{ij} \sin \{(\pi q/10)[r_{a,ij} - (\pi q/10)^2\varkappa_{ij}]\} \times \exp(-{}^1\!/_2(\pi q l_{ij}/10)^2] \quad (1)$$

where K is the index of resolution, A_{ij} is a constant

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(2) M. P. Cava and M. J. Mitchell, Ed., "Cyclobutadiene and Related Compounds," Academic Press, New York, N. Y., 1967. However, the infrared absorption spectrum of cyclobutadiene prepared in an argon matrix at 8°K is most simply interpreted on the basis of D_{4h} symmetry for this compound: O. L. Chapman, C. L. McIntosh, and J. Pacansky, *J. Amer. Chem. Soc.*, **95**, 614 (1973).

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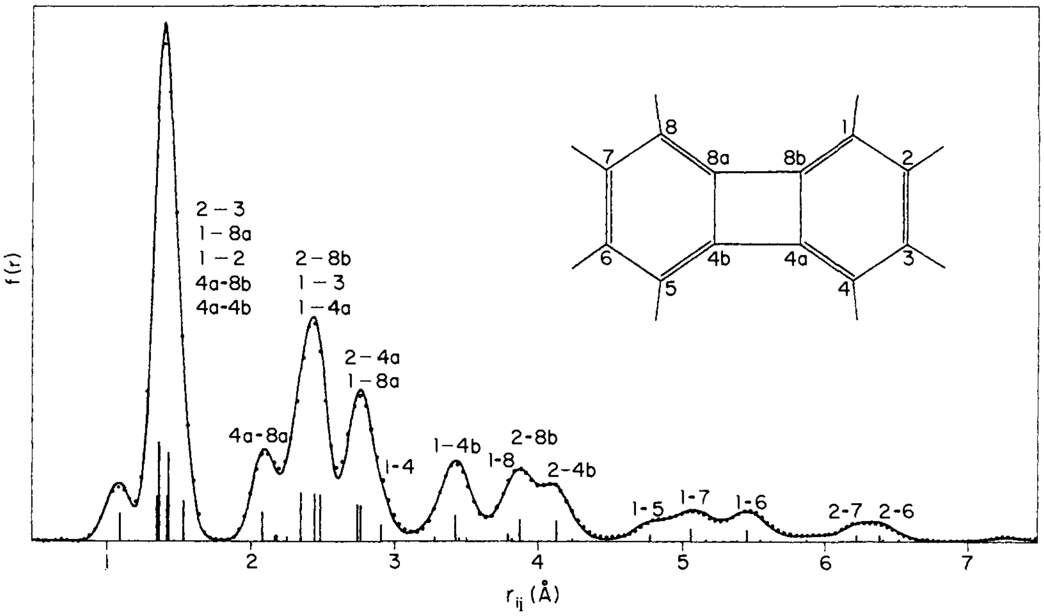

Figure 1. Radial distribution curves. The experimental values, $f(r)$, are given by the points, and the curve was calculated for the "best" model (Tables V and VI).

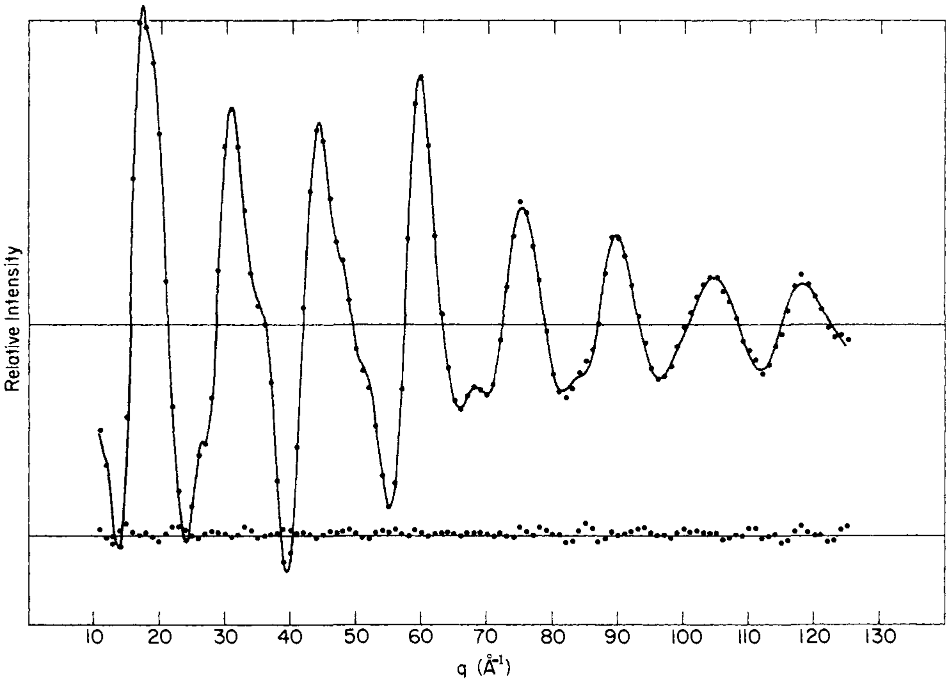

Figure 2. $qM(q)$ curves. The differences between that reduced experimental molecular intensity (dots) and the theoretical scattering are plotted along the lower q axis.

proportional to the product of atomic numbers of i and j, c_{ij} is a tabulated[12] function of q, determined by the elastic and inelastic electron scattering factors and phase shifts for i and j, and l_{ij} are the mean amplitudes of (i,j) pairs. For the bonded C–H pairs the vibrational asymmetry parameters, κ_{ij}, were set at 1.0×10^{-5} Å^3 [13] while for all other pairs they were assumed to be zero, since their contributions are quite small. The distance parameter r_a is related to the r_g, which is the directly measured thermal equilibrium averaged bond length: $r_g = r_a + l^2/r_a$.[13] When one takes into account the nonlinear shrinkage effect due to molecular vibrations, r_a may be expressed, in turn, by the following equation[14]

$$r_a = r_\alpha + \delta \qquad (2)$$

where r_α represents an *averaged projection* of the internuclear distance onto the line joining the equilibrium positions of the nuclei (ij).[15] In the present case the r_α values refer to the structure projected onto the molecular plane, and δ measures the shrinkage effect, which was calculated by means of a normal-coordinate

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analysis, and the mean amplitudes (see the next section).

In the first analysis the nine geometric parameters, and the mean amplitudes of the bonded C–H and all C–C's, were chosen as variables while the other mean amplitudes were fixed at calculated values. At this stage, some of the mean amplitudes were strongly correlated. Then, the following additional constraints had to be imposed on the mean amplitudes: (a) values for the bonded C–C pairs were set equal; (b) l(1–4a) and l(1–3) were set equal; (c) l(1–8), l(1–5), and l(2–6) were fixed at the values derived from the RD analysis (0.120, 0.105, and 0.114 Å, respectively). [The essential equality of l(C–C) for the bonded pairs was later checked by calculating the corresponding l_{ij}'s (Table IV), assuming that the stretching force constants were in inverse proportion to the C–C bond lengths (Table II).] The resulting least-squares reduction gave for the most probable values the magnitudes listed in Table I, together with their standard deviations. The corresponding error matrix is given in the microfilm edition. Systematic errors due to uncertainties in the nozzle-to-plate distances, accelerating voltages, sector imperfections, and densitometry, etc. have recently been examined by taking photographs of standard samples (CO_2, CS_2, and Ar).[10,11] On this basis the plausible limits of error for the structure parameters were estimated to be three times their standard deviations derived from the least-squares analysis, or 0.15% of the quantity listed, whichever has the larger magnitude. This was justified in ref 10a, and in the footnote to Table IV of ref 10c.

Table I. Least-Squares Results for Biphenylene

No.	Parameters[a]		No.	Parameters[a]	
1	K	0.098 (0.005)	13	l(1–6)	0.098 (0.007)
2	$l(\text{C–C})_{\text{av}}$	0.041 (0.001)	14	l(2–7)	0.118 (0.021)
3	l(4a–8a)	0.055 (0.003)	15	$l(\text{C–H})_{\text{av}}$	0.077 (0.003)
4	l(2–8b)	0.064 (0.004)	16	r(4a–4b)	1.522 (0.002)
5	l(1–4a)	0.059 (0.003)	17	r(4a–8b)	1.431 (0.006)
6	l(2–4a)	0.067 (0.003)	18	r(1–8b)	1.370 (0.004)
7	l(1–8a)	0.094 (0.008)	19	r(1–2)	1.427 (0.004)
8	l(1–4)	0.078 (0.009)	20	$r(\text{C–H})_{\text{av}}$	1.090 (0.003)
9	l(1–4b)	0.078 (0.004)	21	∠4a–8b–1	122.5 (0.2°)
10	l(2–8a)	0.094 (0.005)	22	∠8b–1–2	115.0 (0.4°)
11	l(2–4b)	0.075 (0.004)	23	∠8b–1–H	134.3 (1.6°)
12	l(1–7)	0.122 (0.009)	24	∠1–2–H	113.2 (2.3°)

[a] The most probable values derived from the least-squares fitting of the molecular intensity function, and their standard deviations; K (dimensionless) is an index of resolution. The mean amplitudes (l) and distances (r) are in Å units; the angles (degrees) represent an r_α structure, while the distances are in terms of the r_a structure; see eq 1 and 2.

Calculation of Mean Amplitudes

It has proved worthwhile to calculate the mean amplitudes for comparison with the observed values. The general theory for molecular vibrations gives for mean square amplitudes of the Cartesian displacement coordinates, $X(\Delta x, \Delta y, \Delta z)$[16]

$$\langle X\tilde{X}\rangle = \mathbf{L}_x \, \Delta \, \tilde{\mathbf{L}}_x \tag{3}$$

where the elements of the diagonal matrix Δ are given by [Cyvin[17]]

$$\Delta_s = \langle Q_s^2\rangle = (h/8\pi^2 c\omega_s) \coth (hc\omega_s/2kT) \tag{4}$$

and $\mathbf{L}_x$ is the normal coordinate transformation matrix ($X = \mathbf{L}_x Q$). The Δ_s and $\mathbf{L}_x$ matrices are obtained when the appropriate secular equation ($|\text{GF} - \text{E}\lambda| = 0$) for the molecular vibrations is solved [Wilson's GF-method[18]].

On the other hand, to a first approximation the root mean square amplitudes, l_{ij}, and the shrinkage corrections, δ_{ij}, are given by[17]

$$l_{ij}^2 \equiv \langle \Delta r_{ij}^2\rangle = \langle \Delta z_{ij}^2\rangle \tag{5}$$

$$\delta_{ij} = \langle \Delta\rho_{ij}^2\rangle/2r_{ij} - l_{ij}^2/r_{ij} \tag{6}$$

$$\Delta\rho_{ij}^2 \equiv \Delta x_{ij}^2 + \Delta y_{ij}^2$$

where the local z axis is taken along the line joining the equilibrium direction of the atom pair, and the local x and y axes are perpendicular to z. The relative displacements of the mean square amplitudes, $\langle \Delta x_{ij}^2\rangle$, $\langle \Delta y_{ij}^2\rangle$, $\langle \Delta z_{ij}^2\rangle$, are calculated from eq 3.

Biphenylene, with 20 atoms in a planar D_{2h} structure, has 54 fundamental frequencies: $10A_g + 4B_{1g} + 4B_{2g} + 9B_{3g} + 5A_u + 9B_{1u} + 9B_{2u} + 4B_{3u}$. To a first approximation the mean amplitudes, l_{ij}, are due principally to the in-plane vibrations only: $10A_g + 9B_{3g} + 9B_{1u} + 9B_{2u}$, while the shrinkage corrections, δ_{ij}, are related to the perpendicular amplitudes, $\langle \Delta\rho_{ij}^2\rangle$, of the out-of-plane vibrations only, and to the projected mean amplitudes of l_{ij}. In order to solve the secular equation, the potential energy for the in-plane modes was assumed to have a simple Urey–Bradley form[19]

$$V_{\text{in}} = \frac{1}{2}\sum_i K_i(\Delta r_i)^2 + \frac{1}{2}\sum_i H_i(r_{0i}\Delta\theta_i)^2 + \frac{1}{2}\sum_i F_i(\Delta q)^2 \tag{7}$$

where K_i, H_i, and F_i are the force constants for bond stretching, valence-angle deformation, and nonbonded interaction, respectively. For the out-of-plane modes, the potential energy was assumed to have the form

$$V_{\text{out}} = \frac{1}{2}\sum_i A_i\sigma_i^2 + \sum_i B_i\sigma_i\sigma_{i+1} + \frac{1}{2}\sum_i C_i\tau_i^2 \tag{8}$$

where A_i and B_i are the force constants for the out-of-plane bending coordinates (σ_i), and C_i represents the force constant for torsional motion around the bond. Precise definitions of these internal coordinates are given in ref 18 and 20.

Since no force constants for biphenylene have been reported, an initial set of values was assumed to be the same as in benzene.[21-23] These were then adjusted

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until they reproduced the observed frequencies [two in-plane species, B_{1u} and B_{2u}, and one out-of-plane species, B_{3u}] which have been assigned by Pecile and Lunelli.[6] The force constants are listed in Table II, while the corresponding calculated frequencies are given in Table III, together with the observed values; the agreement is fair, although the simple force field and the adjusted force constants merit further refinement. Using these frequencies and the listed force constants, the mean amplitudes and the shrinkage corrections were computed for biphenylene at 418°K; they are shown in Table IV.

Table II. Force Constants for Biphenylene[a]

K(4a–4b)	4.0	*H*(8a–8b–4a)	0.1	*H*(4a–8b–1)	0.05
K(4a–8b)	5.0	*F*(8a–4a)	1.0	*F*(4a–1)	0.58
K(1–8b)	5.6	*H*(8a–8b–1)	0.2	*H*(1–2–3)	0.36
K(1–2)	5.0	*F*(8a–1)	0.58	*F*(1–3)	0.58
K(2–3)	5.6	*H*(8b–1–2)	0.36	*H*(C–C–H)	0.17
K(C–H)	4.7	*F*(8b–2)	0.58	*F*(C–H)	0.38
A(C–H)	0.35	*B*(C′–H′,C–H)	0.04	*C*(4a–4b)	0.45
A(1–8b)	0.65	*B*(1–8b, 4–4a)	0.00	*C*(1–2)	0.35

[a] Estimated values: in-plane force constants (*K*, *H*, and *F*) in mdyn/Å unit; out-of-plane constants (*A*, *B*, and *C*) in mdyn/Å units; see text, eq 6 and 7.

Table III. Normal Vibrational Frequencies of Biphenylene[a]

(A_g)	(B_{1g})	(B_{3g})	(B_{1u})	(B_{2u})	(B_{3u})
3072	916	3074	3072 (3072)	3074 (3072)	915 (915)
3062	836	3065	3062 (3030)	3065 (3063)	748 (733)
1740	542	1587	1589 (1589)	1609 (1622)	368 (366)
1493	298	1417	1500 (1426)	1421 (1444)	100 (120)
1348		1232	1389 (1260)	1248 (1267)	
1232	(B_{2g})	1063	1115 (1151)	1103 (1128)	(A_u)
1112	922	977	1039 (1019)	1044	1009
963	769	643	965 (962)	745 (751)	887
825	575	531	610 (612)	229 (212)	768
411	182				348
					95

[a] Calculated normal frequencies (cm^{-1}) with the force constants listed in Table II (cm^{-1}); the values in parentheses are the observed (ir) frequencies reported in ref 6.

Table IV. Calculated Mean Amplitudes (l_{ij}) and Shrinkage Corrections (δ_{ij}) for Biphenylene (× 10^{-4} Å)

i	*j*	l_{ij}	δ_{ij}	*i*	*j*	l_{ij}	δ_{ij}	*i*	*j*	l_{ij}	δ_{ij}
1	8b	444	53	1	6	729	17	8a	H_3	967	108
2	3	469	90	2	7	762	14	2	H_8	1558	87
1	2	461	125	2	6	739	2	1	H_5	1203	38
4a	8b	454	39	1	H_1	768	202	2	H_5	1417	44
4a	4b	468	41	2	H_3	1015	201	1	H_7	1210	102
4a	8a	500	22	8b	H_1	996	157	1	H_6	1015	60
2	8b	578	94	1	H_2	1015	223	2	H_7	1138	53
1	4a	585	60	2	H_1	1002	218	2	H_6	1051	35
1	3	574	121	8a	H_1	1286	139	H_2	H_3	1656	249
3	4a	665	88	8b	H_3	966	192	H_1	H_2	1643	233
2	8a	586	63	1	H_3	979	215	H_1	H_8	2191	225
1	4	639	86	4a	H_1	970	143	H_1	H_3	1344	290
1	4b	567	26	2	H_4	968	220	H_1	H_4	1187	230
1	8	818	69	4a	H_2	971	180	H_1	H_7	1909	142
2	8a	637	61	1	H_8	1589	136	H_1	H_5	1474	51
3	8a	651	43	1	H_4	953	170	H_1	H_6	1593	73
1	5	622	10	8a	H_4	1124	78	H_2	H_7	1471	78
1	7	790	44	2	H_7	1027	136	H_2	H_6	1278	51

Results and Discussion

Geometric Structure. The "best" set of thermally averaged internuclear distances and bond angles for biphenylene derived from electron diffraction data is given in Table V along with the estimated limits of error. It is of interest to compare the structure of the benzene rings in this compound with the corresponding magnitudes in free benzene. The weighted average of the C–C bond lengths in the C_6 rings is 1.400 ± 0.006 Å, equal to that in C_6H_6: r_g(C–C) = 1.400_0 Å.[24] However, in biphenylene "bond fixation" is clearly present and the bond angles are distorted from 120°, especially $\angle C_{8b}–C_1–C_2$. The *r*(1–8b) and (2–3) distances are about 0.03 Å shorter while the *r*(4–8b) and *r*(1–2) distances are about 0.03 Å longer than in benzene. The average C–H length, 1.096 ± 0.009 Å, is slightly longer than that in benzene, 1.089_7 Å,[24] but this difference is not significant.

The bridge bonds connecting the two benzene rings, 1.524 ± 0.006 Å, are considerably longer than the sp^2–sp^2 bridge bond in biphenyl, 1.489 ± 0.007 Å;[25] indeed, they are rather close to a normal sp^3–sp^3 single bond, 1.533 ± 0.002 Å, in ethane.[26]

There is a close correspondence between the electron diffraction gas phase and X-ray diffraction crystal values in biphenylene (Table V). In making this comparison, one should note that the reported uncertainties in the positions of the atoms in the unit cell do not correspond to our estimated limits of error; rather, the former are standard errors, which were estimated to be 0.6–0.8 of the least-squares standard deviations.[3] On the other hand, the uncertainties in the present study are assigned error limits, taken to be at least three times the standard deviations derived from the least-squares analysis. Therefore, for comparison the corresponding limits of error in the crystal structure were simply taken to be three times their least-squares standard deviations and listed in Table V in parentheses.

The pattern of alternating bond lengths observed for biphenylene is well accounted for by MO calculations, as are the exceptionally long bonds joining the six-member rings. Quantitatively the agreement is less satisfactory. We propose that the residual discrepancy is associated with the abnormal in-plane hybridization of the atoms comprising the central four-membered ring. As with other cyclobutyl compounds, the hybrid orbitals forming the ring σ bonds must be assigned more than 75% p character for effective overlap so that the bonds external to the ring contain a corresponding large fraction of s character. This leads to long ring bonds and shorter than normal external bonds.[27] In addition, in biphenylene the constraints on the benzenoid bonds would be expected to result in exceptionally long bonds joining the six-membered rings *r*(4a–4b), modestly longer bonds for the remaining sides *r*(4a–8b), and shorter bonds radiating out from the central ring *r*(8b–1). The data in Table V are in accord with these expectations.

Theoretical calculations[28] using a variable β Hückel

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Table V. Comparison of the Geometric Parameters for Biphenylene

	ED[a]	X-ray[b]	MO[c]
r(C–C)$_{av}$	1.418 ± 0.003	1.416	1.410 (1.410)
r(1–8b)	1.372 ± 0.012	1.372 ± 0.002 (0.010)	1.389 (1.385)
r(1–2)	1.428 ± 0.012	1.423 ± 0.003 (0.011)	1.407 (1.410)
r(2–3)	1.370 ± 0.015	1.385 ± 0.004 (0.015)	1.389 (1.386)
r(4a–8b)	1.432 ± 0.018	1.426 ± 0.003 (0.014)	1.411 (1.413)
r(4a–4b)	1.524 ± 0.006	1.514 ± 0.003 (0.014)	1.478 (1.481)
r(C–H)$_{av}$	1.096 ± 0.009	1.06 ± 0.02	
∠4a–8b–1	122.5 ± 0.6°	122.6 ± 0.2°	
∠8b–1–2	115.0 ± 1.2°	115.2 ± 0.2°	
∠1–2–3	122.5 ± 1.2°	122.2 ± 0.2°	

[a] Gas phase structure according to the present study. The magnitudes for the distances (Å) are given according to the r_g definition and the angles in terms of the r_α representation. The uncertainties represent the estimated limits of error (see text). [b] X-Ray crystal structure analysis (ref 3); the uncertainties in parentheses are three times the least-squares standard deviations reported in ref 3. [c] Empirical SCF–MO calculations (ref 4); the values by PPP method and SPO method are in parentheses.

model, parameterized to give good fits for energies and bond lengths in normal hydrocarbons and hydrocarbon ions, lead to virtually identical bond lengths predicted according to the much more elaborate PPP and SPO models.[4] With this variable β Hückel model the equilibrium lengths of bonds around atoms 4a, 4b, 8a, and 8b were varied so as to best fit the observed lengths. These changes induced secondary changes in the lengths of the more remote bonds r(1–2) and r(2–3), in the observed direction. Without these hybridization increments the standard deviation between the observed and calculated lengths was 0.027 Å and with them it reduced to 0.012 Å, a value well within the combined uncertainties of experiment and the model.

Mean Amplitudes. The observed mean amplitudes in biphenylene are listed in Table VI, and compared with those in benzene.[29] The observed mean amplitudes for atom pairs within the benzene rings are in good agreement with the corresponding theoretical values, and quite close to the corresponding values observed in free benzene; indeed, on taking into account the different experimental temperatures (benzene at 291°K and biphenylene at 418°K), this correspondence is remarkable. In contrast, the observed values for the nonbonded atom pairs which belong to different benzene rings are appreciably larger than the calculated values (except for the 4a–8a or 4b–8b pairs). These differences seem significant, notwithstanding the magnitudes of the estimated uncertainties. In fact, the RD curve synthesized with the calculated mean amplitudes has strikingly sharp peaks in the region beyond 3 Å, and does not fit the experimental counterpart by a margin considerably greater than the estimated uncertainties in the experimental $f(r)$ curve. This indicates that the listed uncertainties for the mean amplitudes were overestimated, because of the strong correlations among them, and not because of experimental errors.

Table VI. The Comparison of Mean Amplitudes (Å)

Pairs	Obsd[a]	Calcd[b]	Benzene[c]
(C–H)$_{av}$	0.077 ± 0.009	0.077	0.075 ± 0.010
(C–C)$_{av}$	0.041 ± 0.004	0.046	0.046 ± 0.003
4a–8a	0.055 ± 0.009	0.050	
2–8b	0.064 ± 0.012	0.058	0.056 ± 0.005
2–4a	0.067 ± 0.009	0.066	0.061 ± 0.009
1–4	0.078 ± 0.027	0.064	0.061 ± 0.009
1–4a	0.059 ± 0.009	0.059	0.056 ± 0.005
1–3	0.059 ± 0.009	0.057	0.056 ± 0.005
1–8a	0.094 ± 0.024	0.059	
1–4b	0.078 ± 0.012	0.057	
2–8a	0.094 ± 0.015	0.064	
2–4b	0.075 ± 0.012	0.065	
1–5	0.103 ± 0.030	0.062	
1–6	0.098 ± 0.021	0.073	
1–7	0.122 ± 0.027	0.079	
1–8	0.120 ± 0.040	0.082	
2–6	0.114 ± 0.060	0.074	
2–7	0.118 ± 0.060	0.076	

[a] The mean amplitudes in biphenylene obtained by the present study; the uncertainties represent the estimated limits of error (see text). [b] Values calculated for 418°K in Table IV. [c] Reference 28 (at 291°K).

The discrepancy between the observed and calculated mean amplitudes is due to limitations of the theory. The mean amplitudes were calculated on the assumption of harmonic vibrations for small amplitude motions in which case only the in-plane motions contribute significantly to the mean amplitudes. For molecules with large amplitude (or slowly varying) motions due to out-of-plane displacements, an additional contribution to the mean amplitudes must be included. Thus, the experimental l_{ij}'s indicate that biphenylene executes large amplitude out-of-plane motions, a conclusion entirely consistent with the normal-coordinate analysis, which gave this molecule two low frequencies, 100 (B_{3u}) and 95 (A_u) cm^{-1}. The *relative* displacements of atoms in these motions can be estimated from the L_x-matrix elements. It is possible to estimate the potential functions and/or the contributions of such motions to the mean amplitudes by methods similar to those used for C_3O_2[30] and bicyclo-[2.2.2]octane,[31] etc. or treated classically, as in F_3CNO.[10c]

Acknowledgments. This work was supported by the National Science Foundation under Grant No. GP-34060. We thank Mr. Robert R. Karl for taking the excellent electron diffraction photographs used in this study.

Supplementary Material Available. Listings of the molecular intensity, background values and error matrices for biphenylene will appear following these pages in the microfilm edition of this

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volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-1026.

The Importance of Intramolecular Associations in the Chemical Ionization Mass Spectra of Monoenoic and Monoepoxy Fatty Acid Methyl Esters

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Abstract: The isobutane chemical ionization mass spectra are reported for some monoenoic fatty acids, methyl esters, and monoepoxy methyl esters. The enhanced fragmentation observed in the epoxy methyl esters is explained in terms of exothermic intramolecular hydrogen bond formation in the protonated molecular ions. The presence of intramolecular hydrogen bonding in bifunctional ions is supported by the observed efficient gas phase hydrolysis and transesterification reactions of the epoxy methyl esters when water and deuteriomethanol are used as reagent gases. The spectra of the epoxide derivative indicate the position, but not the stereochemistry, of the double bond in the olefinic side chain.

This study of the chemical ionization spectra of unsaturated fatty acids was initiated as an effort to determine the position and stereochemistry of the double bond in the alkyl chain. The data presented here demonstrate that the epoxide derivative can be useful in determining the position of the double bond. On protonation, extensive cleavage of the alkyl chain occurred at the epoxide ring by way of a long range interaction between the epoxide and carboxymethyl functions, but the subtle energy difference between the epoxide stereoisomers was not sufficient to influence the fragmentation pattern. This paper is directed toward understanding the nature of these long range interactions and how they play such a dominant role in the fragmentation of bifunctional molecules.

Chemical ionization induced fragmentation of monofunctional molecules appears to occur as a unimolecular dissociation of the protonated molecules, while intramolecular interactions in polyfunctional molecules have been reported to stimulate fragmentation or to stabilize the protonated molecular ion. Protonated alkyl esters have been shown to fragment by unimolecular mechanisms to give RCO^+, $RCO_2H_2^+$, and alkyl ions from the alcohol chain.[1] The relative abundance of the protonated molecular ion and its fragments are controlled in simple esters by the size and complexity of the alcohol group.[2-6] The dissociations leading to $RCO_2H_2^+$ and alkyl (CH_3^+) ions are endothermic for methyl esters and are not observed for these compounds.[1]

Chemical ionization mass spectra of dibasic acid esters fragment by the same mechanisms.[7] In addition, cyclic fragment ions are observed when the carboxyalkyl functions can become sterically proximate. For example, the protonated cyclic anhydride ion, *m/e* 149, was found only in the orthophthalate esters. The dominant feature of the ortho isomer spectrum, however, is the increased stability of the protonated molecular ion relative to the meta and para isomers.[7]

Carboxylic acids fragment on protonation to give the acylonium ion, RCO^+, with loss of water. Loss of CO_2H_2 occurs when it leads to the formation of a stable ion such as the immonium ion, $RCH{=}NH_2^+$, observed in α-amino acid spectra.[8,9] The amount of amino acid fragmentation is also increased through bifunctional interactions such as those observed in the aromatic amino acids.[8,9]

The isobutane chemical ionization induced fragmentation of monoenoic long chain carboxylic acids and methyl esters yields the acylonium ion with loss of water or alcohol as the only significant cleavage product. These observations are analogous to the simple carboxylic acids and methyl esters previously reported.[1] The epoxy derivatives, however, undergo more extensive fragmentation through pathways not available to the isolated functions. The data presented suggest a long range interaction between the epoxy and carboxymethyl functions which is explained by way of a proton complex postulate in which the epoxy and carboxymethyl oxygens act as ligands. The heat of formation

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