bonds, as mentioned above) on the overall molecular conformation. Both of these studies as well as K_i and toxicity measurements[6] indicate that the hydroxyl group on the tryptophan 6′ position, which is present in all natural amatoxins except amanin and amaninamide, does not affect either conformation or biological activity.

Thus, the crystal structure of **10**, which is shown to be very similar to the structure of β-amanitin, supports these interpretations and demonstrates specifically that a derivative with isoleucine in position 3, tryptophan in position 4, and thioether group at the bridge still retains the general overall conformation of active amatoxins.

The reduction in binding affinity (about 30-fold) of **10** to RNA polymerase and lack of toxicity, relative to α-amanatin, are therefore *not* related to conformation. Since the presence of the thioether group and the lack of 6′-hydroxyl at the tryptophan have not affected the binding affinity or toxicity in other derivatives,[6,13] the observed decrease in activity of **10** could be associated with the nature of side chain 3 alone. Furthermore, the decrease in biological activity of the naturally occurring amatoxins amanullin (**4**) and amanullinic acid, and similar synthetic derviatives with altered side chain 3, is very likely due to the nature of side chain 3 and not due to different "inactive" overall conformations.

It seems unlikely that the local conformation of side chain 3 (which has been shown to vary among derviatives) influences the "fit" to the binding site of the enzyme. Only a relatively low rotational barrier (≈2.0 kcal/mol[29]) is expected around the α–β bond and the β–γ1 bond, consistent with the large temperature factors and partial disorder of the relevant atoms in the structures of β-amanitin and **10**.

A more likely possibility, mentioned before,[6,7] is the formation of a direct hydrogen bond between the γ-hydroxy group side chain 3 (present in active amatoxins) and a matching group in the binding site of the enzyme. The lack of this important interaction with the enzyme could account for the reduced affinity of **10** and similar derivatives and could also contribute to the decrease in toxicity.

As already discussed before,[6,13] there is no direct correlation between binding affinities of amatoxins and their toxicities. At least for some amatoxins, as noted above, the observed reduction in toxicity is significantly larger than expected from the reduction in binding affinity, as is the case with compound **10**. Thus, factors other than the binding to the enzyme may influence the toxicity of amatoxins;[13] these factors may include the ability to penetrate the cell or the membrane of the nucleous. In particular, compound **10** lacks three peripheral hydroxy groups, which might affect its ability to cross hydrophilic regions.

We have shown that molecule **10**, like most of the other amatoxin derivatives, is conformationally stable and relatively unstrained. It seems therefore unlikely that any signficant conformational changes occur inside the cell.

Acknowledgment. We thank R. Dumas for technical help in formatting the manuscript, the National Institutes of Health (Grant GM06920) for support, and the National Science Foundation (Grant PCM 77-1398) for initial support of the computational laboratory.

Registry No. 10, 59409-08-4.

Supplementary Material Available: A list of observed and calculated structure factors (Table S1), a list of coordinates and temperature factors for all hydrogen atoms (Table S2), a list of bond lengths involving hydrogen atoms (Table S3), a list of the torsion angles of the peptide (Table S4), and a stereodiagram of the final structure of **10** superimposed on the initial (molecular replacement) model (Figure S1) (28 pages). Ordering information is given on any current masthead page.

Communications to the Editor

Heat of Formation of Diphenylcyclopropenone by Photoacoustic Calorimetry

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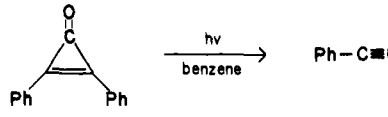
Since the first reports of the syntheses of cyclopropenone and its derivatives[1] there has been a great deal of interest in the chemistry and physical properties of this class of molecules.[2] One area of interest is the resonance stabilization energy provided by the delocalization of electron density.[3] One criterion used for establishing the magnitude of the resonance stabilization energy is the difference in energy between the experimentally obtained heat of formation of a molecule and a predicted heat of formation derived from an estimate of the strain in the molecule and a hypothetical heat of formation of the molecule ignoring any strain or resonance energy. On the basis of photoacoustic calorimetric measurements, we wish to report for diphenylcyclopropenone (DPCP) a resonance stabilization energy of 11 kcal mol^{-1}. The heat of formation of 86 ± 4 kcal mol^{-1} for DPCP is derived from the enthalpy of reaction for the photodissociation of DPCP to diphenylacetylene and carbon monoxide (eq 1).



(1)

Photoacoustic calorimetry is a method whereby one can determine the reaction enthalpy for ground-state reactants forming photogenerated products that are either stable molecules (as in this case) or have only transient existence.[4] The experiment involves integrating an early portion of an acoustic wave that is generated when heat is released by a molecule following absorption of a photon and consequential reaction. The acoustic wave thus generated is detected by a piezoelectric transducer,[5] amplified,

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0002-7863/84/1506-4615$01.50/0

Table I. Data Used to Calculate Φ_h for Compounds Used as Standards[a]

$$\Phi_h = (1/E_{exc})[E_{exc} - (\Phi_{isc})(E_T) - (\Phi_{fl})(\bar{E}_{fl})]$$

compd	Φ_{isc}	E_T, kcal mol^{-1}	τ_T	Φ_{fl}	$\bar{E}_{fl}$, kcal mol^{-1}	Φ_h
4-PhBZP	1.00[b]	60.8[c]	29 μs[d]	0.00[b]		0.283
ANT	0.75[e]	42.0[f]	77 μs[g]	0.25[h]	71.2[i]	0.419
XNT	0.97[j]		71 ns[k]	0.00[j]		1.00
TPE	0.00[l]			0.00[l]		1.00

[a] The excitation wavelength was 337 nm, which corresponds to an excitation energy (E_{exc}) of 84.8 kcal mol^{-1}. [b] The quantum yield for triplet formation in cyclohexane has been measured to be 1.02.[6] In addition no fluorescence has been detected in benzene solvent at room temperature.[7] [c] This value has been reported as 60.9[7] and 60.7 kcal mol^{-1}.[8] [d] In acetonitrile at room temperature.[7] [e] In benzene.[9] [f] References 9 and 10. [g] In *n*-hexane.[11] [h] Assuming $\Phi_{isc} + \Phi_{fl} = 1.0$. This assumption has been confirmed for ethanol as solvent[12a] as well as in liquid paraffin.[12b] [i] Essentially the same in cyclohexane, benzene, or ethanol.[13] [j] $\Phi_{isc} = 0.97 \pm 0.05$ in CCl_4 at 25 °C.[14] [k] Triplet lifetime in benzene solvent at 22 °C.[14] [l] At room temperature in benzene essentially all TPE internally converts much faster than the time scale of our detection apparatus.[15]

digitized, and transferred to a laboratory microcomputer. Photolysis is initiated by a radiation pulse from a nitrogen laser (Lumonics Series TE-260, 5-ns pulse, 337 nm, 4 mJ). Use of two fine apertures and colored-glass filters limited the pulse energy incident on the sample cuvette to ≤10 μJ. The pulse energy measured before and after the sample cuvette is used to determine the sample's optical density. An average of data from 100 laser shots is used to determine one data point. All experiments were carried out under oxygen-free conditions attained by a 5-min helium purge.

The signal amplitude, S_0, of the photoacoustic wave can be expressed as[4]

$$S_0/E_0 = K\Phi_h(1-10^{-A}) \quad (2)$$

In eq 2, K is the experimentally determined instrument response function that relates cell geometry and detection sensitivity to the observed signal for a given amount of heat released. Φ_h is the fraction of excitation energy that is released as heat in an amount of time that is shorter than the instrumental response time. E_0 is the energy of the excitation radiation, and A is the sample's absorbance at the excitation wavelength. $K\Phi_h$ is obtained for a compound as the slope of a plot of S_0/E_0 vs. $(1 - 10^{-A})$.

The calibration of the instrument to the solvent benzene is carried out similarly to a method previously described.[4] For this work, $K\Phi_h$ is measured for the four standards, xanthone (XNT), tetraphenylethylene (TPE), anthracene (ANT), and 4-phenylbenzophenone (4-PhBZP), whose Φ_h could be determined from data already in the literature (see Table I). For each determination, a plot of the calculated Φ_h values vs. the experimentally observed $K\Phi_h$ values yields a calibration curve, a sample of which is shown in Figure 1.

The quantum yield for photodissociation of DPCP is reported to be 1.00 ± 0.03 at 337 nm in benzene, and the dissociation is complete within 300 ns.[16] On the basis of the average of five sets of data, the experimentally determined heat of reaction is $\overline{\Delta H_{rxn}}$(eq 1) = −9.9 ± 2.9 kcal mol^{-1}. To obtain the $\Delta H_f°_{298}$(DPCP), we combine the experimentally obtained reaction enthalpy with the heat of formation of carbon monoxide ($\Delta H_f°_{298}$(CO) = −26.42 kcal mol^{-1})[17] and the heat of formation for diphenylacetylene obtained by Benson's group additivity method ($\Delta H_f°_{298}$(DPA) = 102.8 ± 1 kcal mol^{-1})[18] to produce $\Delta H_f°_{298}$(DPCP) = 86 ± 4 kcal mol^{-1}. This determination of the heat of formation of DPCP has not taken into account the differential heats of solvation of the reactants and products; however, it is anticipated that this difference in benzene will have at most a minimum effect on the derived heat of formation of DPCP.

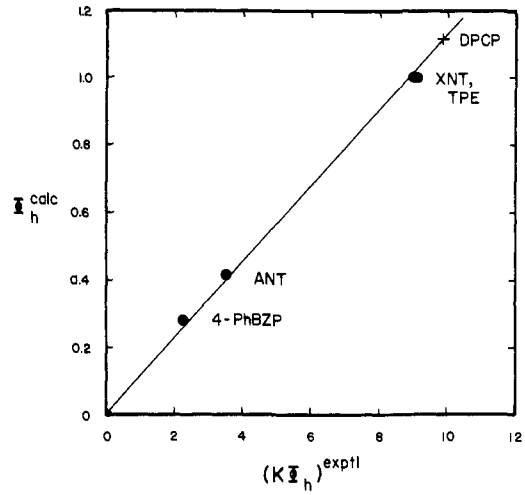

Figure 1. Calibration curve for the photodissociation of diphenylcyclopropenone in benzene at 337 nm. The four calibration points give a line whose $n = 0.9997$ and which predicts that Φ_h(DPCP) is 1.092. From this determination of Φ_h(DPCP), the reaction enthalpy of −9.9 kcal mol^{-1} is then calculated.

Greenberg and co-workers[3] recently reported a calculated value for the heat of formation of DPCP of 97.2 kcal mol^{-1} on the basis of a value of 30.2 kcal mol^{-1} (from application of Benson's group additivity methods[18]) for the heat of formation in the absence of any strain or resonance delocalization energy and a separately estimated strain energy of 67 kcal mol^{-1}. This predicted heat of formation (97.2 kcal mol^{-1}) does not take into account the energy associated with delocalization of the electron density. Thus the difference of 11 kcal mol^{-1} between our experimentally determined $\Delta H_f°_{298}$(DPCP) = 86 ± 4 kcal mol^{-1} and Greenberg and coworkers predicted value is attributed to the resonance stabilization energy of DPCP. This value is to be compared with the analogous value derived for benzene, which ranges from −21 to −36 kcal mol^{-1} depending upon the model employed.[19] It is further noted that the heat of formation of 86 ± 4 kcal mol^{-1} for DPCP obtained here is in remarkably close agreement to the Hartree–Fock value of 81 kcal mol^{-1} derived from isodemic analysis[3] and is in excellent agreement with the recently determined[20] bomb calorimetry value of 87 ± 5 kcal mol^{-1}.

Acknowledgment. This work was supported by the donors of Petroleum Research Fund, administered by the American Chemical Society. K.S.P. thanks the Camille and Henry Dreyfus Foundation and the Alfred P. Sloan Foundation for support.

Registry No. DPCP, 886-38-4; XNT, 90-47-1; TPE, 632-51-9; ANT, 120-12-7; 4-PhBZP, 2128-93-0.

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