J. Am. Chem. Soc., 92, 7587 (1970); 93, 7325 (1971).
(5) R. Grubbs, C. P. Lau, R. Cukier, and C. Brubaker, Jr., J. Am. Chem. Soc., 99, 4517 (1977).
(6) J. I. Crowley, T. B. Harvey, and H. Rapoport, J. Macromol. Sci. Chem., 7, 1117 (1973).
(7) P. Jayalekshmy and S. Mazur, J. Am. Chem. Soc., 98, 6710 (1976).
(8) C. D. Campbell and C. W. Rees, J. Chem. Soc. C, 742 (1969).
(9) Some precedent exists for this process; see R. O. C. Norman and C. B. Thomas, J. Chem. Soc. B, 421 (1970).
(10) M. E. Schafer and R. S. Berry, J. Am. Chem. Soc., 87, 4497 (1965).
(11) C. D. Campbell and C. W. Rees, J. Chem. Soc. C, 752 (1969).
(12) F. Schroeppel and J. Sauer, Tetrahedron Lett., 2945 (1974).
(13) L. F. Fieser and M. J. Haddadin, Can. J. Chem., 43, 1599 (1965).
(14) From catalytic hydrogenation of p-nitrophenethyl alcohol.
(15) F. W. McLafferty, "Interpretation of Mass Spectra", W. A. Benjamin, Reading, Mass., 1973.
(16) R. L. Letsinger, M. J. Kornet, V. Mahadevan, and D. M. Jerina, J. Am. Chem. Soc., 86, 5163 (1964).

Chemistry of Singlet Oxygen. 30. The Unstable Primary Product of Tocopherol Photooxidation[1]

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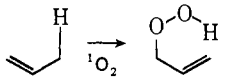
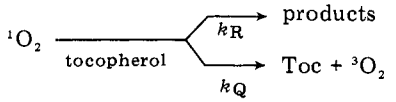
Abstract: α-Tocopherol (vitamin E) reacts with singlet molecular oxygen both by a quenching process and by irreversible reaction to give products, and this scavenging action may be one mode of its biological antioxidant function. Final reaction products such as α-tocopherol quinone and α-tocopherol quinone epoxide have been reported and a mechanism involving endoperoxide intermediates has been proposed by other workers. We have investigated the photooxidation of α-tocopherol at low temperature and identified the primary product as the modestly stable *hydroperoxydienone* (**6**). The structure of **6** has been characterized by IR, UV, ^1H and ^{13}C NMR, and mass spectroscopy. On decomposition of **6**, α-tocopherol quinone (**1**) and the quinone epoxide (**2**) are formed; reduction of the intermediate with triphenylphosphine gives **1** as sole product.

Lipid peroxidation is a significant pathological process which alters membrane activity and function. Singlet molecular oxygen ($^1O_2$) is believed to be involved in some cases in peroxidation of biological lipids;[3,4] it can be produced under the influence of light and a sensitizing pigment, or in a variety of nonphotochemical processes involving oxygen, peroxides, or superoxide.[3,5] Although many of the tests by which it has been detected in biological systems are not sufficiently specific to distinguish singlet oxygen unequivocally from other oxidizing species such as hydroxyl radical, halogens, or alkoxy radicals, the evidence is highly suggestive that $^1O_2$ does occur as a pathological byproduct of certain enzymatic processes. Once formed, $^1O_2$ is known to be capable of reaction with unsaturated lipids (such as fatty acids or cholesterol) to yield peroxides according to an "ene" mechanism as shown below.[3,5,6]

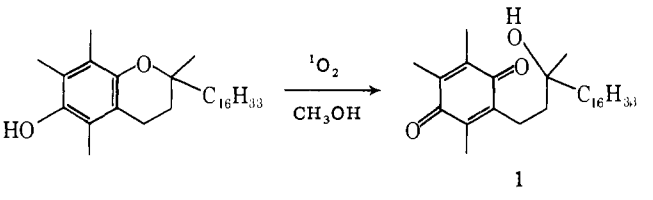


Tocopherols (vitamin E) act as biological antioxidants. Previous attention has centered on their importance as radical chain terminators,[7,8] and this is clearly an important mode of their antioxidant function.[3,4] However, it was recently demonstrated that tocopherols are capable of highly efficient singlet oxygen scavenging, both by a quenching process and by irreversible reaction to form products.[9-15]



Quenching tends to predominate, with the ratio of the two processes dependent on solvent polarity and ranging from 14:1 in CH_3OH to about 120:1 in less polar solvents. Rate constants for the reaction, determined by us and by other investigators, are summarized in Table I. The overall reaction efficiency is such that α-tocopherol at a concentration as low as 10^{-5} M can intercept 50% of the $^1O_2$ in nonpolar solvents. It has been proposed that singlet oxygen scavenging may be an additional protective biological role of tocopherol.[9-15]

The products of tocopherol photooxidation were reported by Grams et al.[10,11] to be a mixture of tocopherol quinone (**1**) and the quinone epoxide (**2**), along with methanol adducts.



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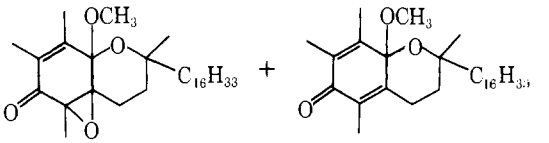

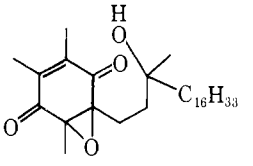

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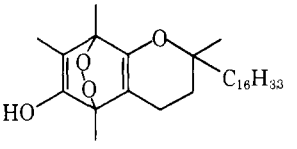
These authors suggested[10,11] that the products derive from several endoperoxide intermediates **3–5** which result from 1,4



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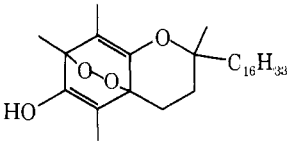

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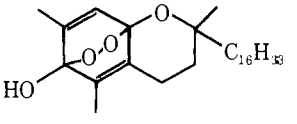

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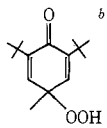
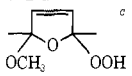
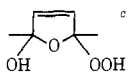
Table I. Rates of Reaction (k_R) and Quenching (k_Q) for α-Tocopherol and Singlet Oxygen, $\times 10^{-6}$ M^{-1} s^{-1}

solvent	$k_Q + k_R$	k_R
CH_3OH[a]	530	36
C_6H_6[b]	148	~1.5
pyridine[c]	250	2.1
cyclohexane[b]	90	
isooctane[c]	120	

[a] Reference 13. [b] Reference 14. [c] Reference 12.

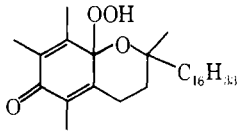
Table II. Spectral Data for Intermediate **6** and Various Model Compounds

	ν, cm^{-1} (CCl_4) OH	ν, cm^{-1} (CCl_4) C=O	δ OH or OOH (^1H NMR), ppm vs. Me_4Si[a]
6	3531	1735 1675 1637	8.9
α-tocopherol quinone (**1**)	3620	1640	
α-tocopherol	3632		4.2
		1710 1665 1645	7.8
t-BuOH[c]	3613		1.4
t-BuOOH[c]	3555		8.9
	3512		9.2
	3525		5.4[d]
	3638		

[a] Temperature and concentration dependent. [b] Reference 17. [c] Reference 22. [d] Time average of OH and OOH protons.

cycloaddition across the aromatic ring. Aromatic addition of singlet oxygen does have precedence, for example, in reactions with various substituted anthracene derivatives.[16]

However, photooxidation studies with substituted phenols both in our laboratories and elsewhere have revealed a similar combined quenching and chemical reaction with singlet oxygen.[3,17] In some cases of high steric encumbrance, the intermediate products were stable, and were shown to be hydroperoxydienones.[17-21] It seemed likely to us that the quinone products resulting from the tocopherol reaction were indeed secondary products, but were derived not from endoperoxide intermediates but rather from the hydroperoxydienone (**6**).



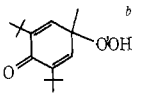
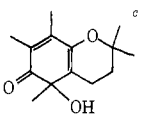
6

The work reported here shows that the hydroperoxydienone is indeed the product and is a modestly stable compound which can be isolated and characterized at reduced temperature.

Results and Discussion

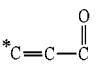
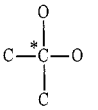
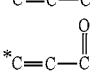
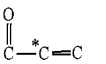
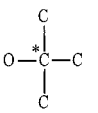
DL-α-Tocopherol was photooxidized below −30 °C in chloroform with tetraphenylporphine as sensitizer. Oxygen uptake was rapid, and the reaction was complete after 2.5 h, at which time the solvent was removed under vacuum at low temperature. The resulting yellow oil was highly peroxidic; iodimetric titration yielded a value of 86% peroxide.

Table III. Ultraviolet Spectrum of Intermediate I and Model Compounds

compd	λ_{max}, nm (ε, M^{-1} cm^{-1})
intermediate **6**	235 (1.6×10^4)[a] 275 (sh, 3.7×10^3)[a]
	236 (9.9×10^3) 270 (2×10^3)
 **7**	299 (5.5×10^3) 327 (2.8×10^3)

[a] ε values calculated assuming 100% purity of sample. [b] Reference 17. [c] Reference 24.

Table IV. ^{13}C Chemical NMR Spectrum for Intermediate **6**[a] and Assignments

shift	assignment	shift	assignment
186.5 (s)	*C=O	131.2 (s)	
150.0 (s)		97.7 (s) 97.4 (s)	
147.6 (s) 147.3 (s)			
131.8 (s) 131.6 (s)		77.3 (s)	
		11-41 (m)	alkyl

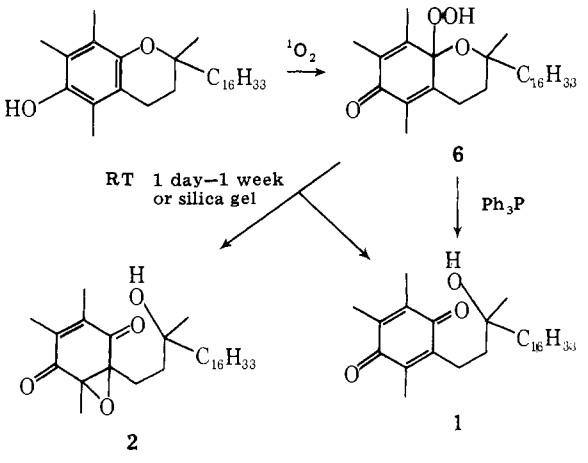
[a] $CDCl_3$, −20 °C; shifts in parts per million downfield relative to Me_4Si. Asterisk indicates a double peak due to epimeric isomers.

The mass spectrum was distinct from that of the quinone end products, and showed *m/e* 462 (tocopherol + O_2), with a major peak at *m/e* 430 (M − 32). Loss of oxygen is a characteristic fragmentation for *p*-hydroperoxydienones.[17] High-resolution mass measurement gave *m/e* 462.3713 (462.3702 calcd for $C_{29}H_{50}O_4$). The ^1H NMR ($CDCl_3$, −25 °C) was consistent with **6**. In particular, there was a one-proton resonance that was concentration and temperature dependent (δ 8-11.3 ppm) and which disappeared on shaking with D_2O, while the rest of the spectrum was unaffected. The peak position is characteristic for hydroperoxyl protons, and clearly distinguishes them from hydroxyl protons (see Table II).[22] The infrared spectrum, obtained at high dilution in nonpolar solvent, shows a band at 3531 cm^{-1}, clearly assignable to a hydroperoxyl group, which is expected to absorb at 3500-3600 cm^{-1}, and clearly different from a hydroxyl group, which would appear above 3600 cm^{-1} (Table II).[22,23] The carbonyl absorption at 1735/1675 cm^{-1} is in good agreement with that of hydroperoxydienones formed from phenols.[17] The UV spectrum is consistent with additivity calculations for a cross-conjugated but not a linear dienone. Table III compares the spectrum of the intermediate with that obtained from a model *p*-hydroperoxydienone[17] and with the longer wavelength spectrum reported for an *o*-hydroperoxydienone (**7**).[24]

The ^{13}C NMR spectrum of the intermediate (−20 °C, $CDCl_3$) showed no evidence of contamination by starting material, end products, or side products. The spectrum, summarized in Table IV, is readily assignable to structure **6**. Thus, the single carbonyl peak has a shift of 186.5 ppm (relative to Me_4Si), consistent with a cross-conjugated carbonyl.[26a] The alkene resonances, 150.0, 147.6 (147.3), 131.8 (131.6), and 131.2 ppm, may be compared to those of 2,3-dimethyl-2-cy-

clohexenone (153.7 and 130.0 ppm).[26b] The peak at 77.3 ppm is assignable to the tertiary ether; the 97.7 (97.4) ppm peak may be assigned to the carbon atom bearing the two oxy substituents. Several atoms resonate as closely spaced double peaks under complete proton decoupling, indicating that **6** is formed, as could be expected, as a mixture of the two possible epimeric hydroperoxides.

On standing at room temperature for periods of 1 day to 1 week, or on passage through a chromatographic column packed with silica gel, **6** was smoothly converted to the end products, tocopherol quinone (**1**) and the quinone epoxide (**2**) previously identified by Grams,[10] with the epoxide in predominance. On treatment with triphenylphosphine, intermediate **6** was converted to tocopherol quinone as the sole product. The quinone obtained by phosphine treatment derives from the hemiketal generated on reduction of the hydroperoxy group in **6**.[27]



Conclusions

The data show conclusively that the product of photooxidation of α-tocopherol at low temperature in chloroform is the *p*-hydroperoxydienone (**6**).[28] No other structure is consistent with all the spectroscopic data; in particular, various endoperoxide structures (**3–5**) suggested by Grams are inconsistent with the spectra of the unstable intermediate, although **5** could possibly open to **6**. The chemical behavior on warming is also consistent with the structure, since the hydroperoxy ketal function would be expected to be very labile. The phosphine reduction of the intermediate provides additional confirmatory evidence of the specific position of the hydroperoxyl group in **6**.[27]

After this work was completed, Matsushita et al. reported evidence that the photooxidation produces two epimeric products,[29] consistent with the ^{13}C evidence for two isomers reported here. Although the isomers may be separable by careful low-temperature chromatography, we have not attempted this.

It seems likely that the same *p*-hydroperoxydienone (**6**) is produced on reaction of $^1O_2$ and α-tocopherol under other conditions, including in biological systems, and it should be thermally stable for a period of hours to days, depending on the conditions, before undergoing decomposition to **1** and **2**, the final decomposition products.

Experimental Section

Reagent grade chloroform (Mallinckrodt) was distilled from calcium hydride immediately prior to use. DL-α-Tocopherol (ICN Pharmaceuticals) was used as received. *meso*-Tetraphenylporphine (TPP, Aldrich) was also used as received.

Photooxidation of α-Tocopherol. α-Tocopherol (0.75 g, 1.7 mmol) and 10 mg (16 μmol) of tetraphenylporphine were dissolved in 110 mL of chloroform. The solution was purged with oxygen and irradiated at −30 °C for 2.5 h with a water-cooled DWY tungsten–halogen lamp operated at 80 V with a 1% $K_2Cr_2O_7$ filter solution. The oxygen was kept bubbling through the solution during the irradiation period. The solvent was removed using a vacuum rotary evaporator at −30 °C, followed by subjection to high vacuum (10^{-5} mmHg) at below −30 °C for 6 h. A dark gold oil remained, which was colored by residual sensitizer. Shorter reaction times (1 h) were found to give only partial conversion.

Spectroscopy. IR spectroscopy was performed on a Perkin-Elmer 521 (0.4-mm cells in CCl_4): 3625, 3520, 3425, 1720, 1220 cm^{-1}, among others. UV was done on a Cary 14 (hexane) (see Table III). ^1H NMR spectroscopy was performed on a Varian A-60D at −25 °C (δ, $CDCl_3$): 10.55 (s, broad), 2.00 (s), 1.80 (s), 1.25 (s), 0.89 (d). After addition of D_2O to the NMR tube and shaking, the peak at δ 10.55 diminished to base line within 2 min. Mass spectra were done on an AEI MS-9 by Dr. K. Fang: *m/e* 462.3713 (calcd for $C_{29}H_{50}O_4$, 462.3709); there was also a large peak at *m/e* 430 (M − 32). ^{13}C NMR work was performed on a Varian CFT-20 spectrometer in $CDCl_3$ solution containing Me_4Si as an internal standard with proton decoupling (see Table IV).

Hydroperoxydienone Reduction. DL-α-Tocopherol was photolyzed as above, and the solvent was removed under vacuum at −30 °C. The product oil was redissolved in a solution of 25 mL of anhydrous diethyl ether and 10 mL of hexane, and 0.30 g (1.14 mmol) of triphenylphosphine (Matheson Coleman and Bell) in 10 mL of diethyl ether was added. After swirling for 10 min, triphenylphosphine oxide had precipitated. The solution was filtered and chromatographed on a 15-cm silica gel column (70–230 mesh, Merck), eluting with 1:1 $CHCl_3$–ether.

α-Tocopherol quinone (**1**), the sole product, was identified by spectral comparison with authentic material. α-Tocopherol quinone epoxide (**2**)[30] was treated with triphenylphosphine under the same conditions described above. ^1H NMR showed that no reduction of the quinone epoxide to the quinone took place.

Decomposition of Hydroperoxydienone. DL-α-Tocopherol (1.00 g, 2.23 mol) was photolyzed as above, and the solvent was removed under high vacuum at below −30 °C. Half of the product oil was set aside in a stoppered flask at room temperature for 1 day before spectroscopy. The other half was redissolved in a minimum of chloroform and chromatographed on a 35-cm silica gel column, eluting with $CHCl_3$. After sensitizer eluted, the yellow product band was eluted with 3:1 chloroform–ether. Solvent was removed using high vacuum at ambient temperature, leaving a clear yellow oil. Spectroscopic measurements were performed immediately. In either case, both tocopherol quinone and the quinone epoxide were formed (IR and NMR), the epoxide in predominance. Standing at ambient temperature resulted in complete breakdown within several days; chromatography gave immediate, complete conversion.

The spectral data for the quinone epoxide were essentially identical with the literature values:[10] IR 1680 cm^{-1}; ^1H NMR (δ) 1.60 (s, 3), 1.95 (s, 6); MS *m/e* 462, 419, 402, 237. The quinone spectra were superimposable with spectra from a commercial sample (ICN Pharmaceuticals). High-resolution MS showed 446.3753 (446.3760 calcd for $C_{29}H_{50}O_3$). The products were yellow oils with $R_f \approx 0.23$ (relative to starting material) for TLC on silica gel with $CHCl_3$ as eluent.

Iodimetric Peroxide Determination. The peroxide determination was performed according to the method given by Siggia.[31] Thus, 0.448 g of intermediate **6** was heated to reflux in 40 mL of 2-propanol containing 2 mL of glacial acetic acid, and 10 mL of 2-propanol saturated with NaI added. After refluxing for 5 min, 5 mL of water was added and the mixture was titrated immediately with a standard solution of sodium thiosulfate. Two such determinations gave values of 86 and 84 mol % peroxide. Blank solutions containing chloroform and the α-tocopherol quinone epoxide gave no peroxide assay using the same procedure.

References and Notes

(1) Part 29: S. Wolf, C. S. Foote, and J. Rebek, Jr., *J. Am. Chem. Soc.*, submitted. Supported by National Institutes of Health Grant GM-20080. CFT-20 spectrometer purchased from funds provided by National Science Foundation Grant GP-41978. A preliminary account of this work was given at the International Symposium on Tocopherol, Lake Yamanaka, Japan, Sept 1977, and appeared in "Tocopherol, Oxygen and Biomembranes", C. de Duve and O. Hayaishi, Ed., Elsevier/North Holland Biomedical Press, Amsterdam, 1978, pp 13–21.

(2) NSF Postdoctoral Fellow, University of California, Los Angeles, 1976–1977.

(3) C. S. Foote in "Free Radicals in Biology", Vol. II, W. A. Pryor, Ed., Academic

Press, New York, N.Y., 1976, pp 85–133.
(4) N. I. Krinsky, *Trends Biochem. Sci.*, **2**, 35 (1977).
(5) C. S. Foote, *Acc. Chem. Res.*, **1**, 104 (1968).
(6) C. S. Foote, *Pure Appl. Chem.*, **27**, 635 (1971).
(7) A. Tappel, *Ann. N.Y. Acad. Sci.*, **203**, 12 (1972).
(8) W. A. Pryor, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 1862 (1973).
(9) G. W. Grams, *Tetrahedron Lett.*, 4823 (1971).
(10) G. W. Grams, K. Eskins, and G. Inglett, *J. Am. Chem. Soc.*, **94**, 866 (1972).
(11) G. W. Grams and K. Eskins, *Biochemistry*, **11**, 606 (1972).
(12) S. R. Fahrenholtz, F. H. Doleiden, A. M. Trozzolo, and A. A. Lamola, *Photochem. Photobiol.*, **20**, 505 (1974).
(13) C. S. Foote, T.-Y. Ching, and G. G. Geller, *Photochem. Photobiol.*, **20**, 511 (1974).
(14) B. Stevens, R. Small, and S. Perez, *Photochem. Photobiol.*, **20**, 515 (1974).
(15) R. Yamauchi and S. Matsushita, *Agric. Biol. Chem.*, **41**, 1425 (1977).
(16) C. S. Foote and S. Wexler, *J. Am. Chem. Soc.*, **86**, 3880 (1964); E. J. Corey and W. C. Taylor, *ibid.*, **86**, 3881 (1964); K. Gollnick and G. Schenck in "1,4-Cycloaddition Reactions", J. Hamer, Ed., Academic Press, New York, N.Y., 1967, p 255; K. Gollnick, *Adv. Photochem.*, **6**, 1 (1968).
(17) M. J. Thomas and C. S. Foote, *Photochem. Photobiol.*, **27**, 683 (1978). The mass spectrum of compound II is incorrectly reported in this paper and should be *m/e* 252, 220, 57.
(18) T. Matsuura, N. Yoshimura, A. Nishinaga, and I. Saito, *Tetrahedron Lett.*, 1669 (1969).
(19) C. Taimr and J. Pospíšil, *Angew. Makromol. Chem.*, **39**, 189 (1974).
(20) K. Pfoertner and D. Böse, *Helv. Chim. Acta*, **53**, 1553 (1970).
(21) I. Saito, N. Yoshimura, T. Arai, K. Omura, and T. Matsuura, *Tetrahedron*, **28**, 5131 (1972).
(22) C. S. Foote, M. T. Wuesthoff, S. Wexler, I. G. Burstain, and R. Denny, *Tetrahedron*, **23**, 2583 (1967).
(23) R. F. Goddu, *Adv. Anal. Chem. Instrum.*, **1**, 347 (1960).
(24) S. Matsumoto and M. Matsuo, *Tetrahedron Lett.*, 1999 (1977).
(25) The ^{13}C spectrum of an *o*-hydroxydienone (7) has been reported,[24] and it provides a contrast to the para oriented (**6**).
(26) (a) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972, p 294; (b) *ibid.*, p 193.
(27) The known[24] *o*-hydroxydienone (**7**) is stable (mp 104–105 °C). If our intermediate hydroperoxide were the ortho isomer, reduction would have been expected to lead to a similarly stable ortho alcohol.
(28) It is interesting to note the difference between the singlet oxygen intermediate **6** and the product obtained on reaction of superoxide ($O_2^{-}\cdot$) with the tocopherol model compound 6-hydroxy-2,2,5,7,8-pentamethylchroman, which has been recently reported.[24] The resultant action of superoxide occurs at an ortho site to produce an *o*-hydroperoxydienone (**7**) by a mechanism presently not well understood.
(29) S. Matsushita, J. Terao, and R. Yamauchi in "Tocopherol, Oxygen, and Biomembranes", C. de Duve and O. Hayaishi, Ed., Elsevier North-Holland Biomedical Press, Amsterdam, 1978, p 23.
(30) Prepared by the method of Grams et al.[9–11] and purified by TLC.
(31) S. Siggia, "Quantitative Organic Analysis", 3rd ed., Wiley, New York, N.Y., 1963, pp 255–266; C. D. Wagner, R. H. Smith, and E. D. Peters, *Anal. Chem.*, **19**, 976 (1947).

Ion Thermochemistry of Low-Volatility Compounds in the Gas Phase. 1. Intrinsic Basicities of α-Amino Acids

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Abstract: Samples of biomolecules with low vapor pressures, specifically α-amino acids, were introduced by a direct insertion probe into the ion source of a pulsed ionization high-pressure mass spectrometer. The number density of an amino acid in the source was determined by a new technique based on the measurement of the rate of its protonation by t-$C_4H_9^+$, the chemical ionization reactant ion. The proton affinities (PA) of the amino acids were obtained from measurements of the thermodynamics of proton transfer equilibria between the amino acids and appropriate reference bases. The following PAs (referred to $PA(NH_3) = 202.3$ kcal mol^{-1}) were obtained: glycine (208.2), alanine (212.2), valine (213.9), leucine (214.5), phenylalanine (215.1), proline (218.4). Comparison with the proton affinities of alkylamines shows that substitution by a carboxyl group decreases the proton affinity of the amine function by 1.8–3.1 kcal mol^{-1}. Comparison with solution basicities shows that the effect of solvent (H_2O) on this substituent effect is minor, if any. On the basis of the measured PA values, the following gas-phase heats of formation $(\Delta H°_f)_g$ are determined: $GlyH^+$ (53), $AlaH^+$ (43), $LeuH^+$ (15), and $PheH^+$ (57). Comparison of $(\Delta H°_f)_g$ of the ion-molecule association products $CH_3NH_3^+\cdot CO_2$ and $C_2H_5NH_3^+\cdot CO_2$ with $(\Delta H°_f)_g$ of their isomers $GlyH^+$ and $AlaH^+$ shows that the hydrogen-bonded cluster ions are more stable by 11 and 8 kcal mol^{-1}, respectively, then their covalently bonded isomers. We also observed the formation of the hydrogen-bonded dimers $(Gly)_2H^+$ and $(Pro)_2H^+$. For the association reactions leading to these dimers we measured $\Delta H° = -31$ and -29 ± 2 kcal mol^{-1} and $\Delta S° = -33$ and -32 ± 5 cal mol^{-1} K^{-1}, respectively.

Measurements of proton transfer equilibria in the gas phase have yielded an extensive ladder of the relative intrinsic basicities and proton affinities (PA) of organic compounds.[1–3] These data make it possible to separate and identify the intrinsic structural effects and the solvent effects on the acid–base properties of molecules.[4,5] To date, such measurements have been performed generally on compounds which are gases or at least moderately volatile liquids under standard conditions. In the present work we extend such measurements to the gas-phase proton affinities of involatile biomolecules, specifically α-amino acids. The present measurements are made possible by the application of ion–molecule kinetics to determine the number density of the amino acid vapor in the ion source of our pulsed high-pressure mass spectrometer.

Experimental Section

The present work was performed using the Rockefeller University Chemical Physics Mass Spectrometer in the pulsed ionization mode which was described previously.[6]

To measure proton affinities of amino acids (A) we observe proton transfer equilibria (reaction 1) between A and reference bases B:

$$BH^+ + A \rightleftharpoons AH^+ + B \quad (1)$$

In order to determine the equilibrium constants K_1, the number density of A (N_A) in the ion source must be known. This presents a difficulty since N_A is lower by a factor of 10^3–10^4 than the number density of the carrier gas t-C_4H_{10} in the source and even then N_A is this high only at relatively elevated temperatures. Thus N_A cannot be measured by conventional methods. The experimental procedure that we use is to introduce the solid sample A into the source via a direct inlet probe which places the sample 6–10 mm from the zone in the source where ion–molecule reactions occur. The sample A volatilizes from the probe, and in the reaction zone it is protonated by the reaction

$$t\text{-}C_4H_9^+ + A \rightarrow AH^+ + C_4H_8 \quad (2)$$

The rate of disappearance of t-$C_4H_9^+$ and of the appearance of AH^+ is measured by pulsed high-pressure techniques, and the pseudo-

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