The Position of Protonation of Anisole in Concentrated Aqueous Solutions of Strong Acids[1]

Sir:

It was recently reported[2] that the 270-mμ ultraviolet absorption band of phenol ethers dissolved in moderately concentrated aqueous H_2SO_4 decreases to about one-half its intensity as the acidity of the medium is increased, and that the relationship between absorbance and acidity takes the form of a normal sigmoid titration curve. These observations were interpreted in terms of protonation on ether oxygen, and the pK_a's of a number of phenol ether oxonium ions were calculated. This conclusion, however, has been disputed[3] on the basis that the nmr spectra of anisole in HSO_3F and in HF–BF_3 at low temperatures give evidence only of protonation on carbon[4] and that the ultraviolet spectrum of anisole in 100% H_2SO_4 shows no absorption at 270 mμ, but does contain bands attributable to C-protonated species at 240 and 285 mμ. It has also been reported[5] that the 270-mμ absorption of anisole dissolved in $HClO_4$ decreases only very gradually with no inflection point over the 8–9 H_0 units from pure H_2O to 70% acid, and that a new band then begins to appear at 285 mμ. We wish to report the following information with serves to reconcile these apparently contradictory observations.

The original experiments[2] give −6.8 (H_0 scale[6]) as the pK_a of anisole, making it half-protonated on oxygen in 77% H_2SO_4. From the carbon basicities of 1,3,5-trimethoxybenzene ("pK_a" = −3.7) and 1,3-dimethoxybenzene ("pK_a" = −6.5),[7] it can be estimated that anisole would be half-protonated on carbon in *ca.* 92% H_2SO_4. It is logical to except the conjugate acid of anisole to change from the O-protonated to the C-protonated species as the acidity of H_2SO_4 increases and the activity of free water goes down, for the former can be stabilized by hydrogen bonding to water whereas the latter cannot.[8] In support of this is the fact that the ultraviolet spectrum of anisole dissolved in 93% H_2SO_4 shows the band of the C-protonated species at 285 mμ[9] and a weak band of the O-protonated species at 270 mμ as well.

We and others[5,10] have observed that the "pK_a's" of non-Hammett bases are seldom the same in $HClO_4$ and H_2SO_4. It seems to be generally true that, whereas bases whose conjugate acids are stabilized by hydrogen bonding to the solvent are considerably less protonated in $HClO_4$ than in H_2SO_4 of the same H_0 value, the difference is much smaller or perhaps in the opposite sense for cases where such stabilization is not possible. This effect would tend to make the gap between O- and C-protonation of anisole smaller in $HClO_4$ than it is in H_2SO_4, and, in $HClO_4$, C-protonation might well begin to occur before O-protonation is half-complete. This, of course, would obscure the inflection point in the titration curve for O-protonation. There is in addition an appreciable medium effect on the intensity of the 270-mμ absorption of phenol ethers in $HClO_4$: in the case of 3,5-dimethylanisole and 3-hydroxyanisole, whose C-basicites are either greater than or roughly comparable to their O-basicities and for which O-protonation should therefore not be observable by ultraviolet methods, the intensity of the 270-mμ band nevertheless drops with increasing acidity to nearly 50% of its value in pure water before C-protonation becomes visible. These two effects can explain the absence of detectable O-protonation of anisole in $HClO_4$.

Acknowledgment. This research was supported by a grant (No. 1180-A1,4) from the Petroleum Research Fund of the American Chemical Society.

(1) Taken from a thesis submitted by L. E. Hakka to the Illinois Institute of Technology in partial fulfillment of the requirements for the Ph.D. degree.

(2) E. M. Arnett and C. Y. Wu, *J. Am. Chem. Soc.*, **82**, 5660 (1960).

(3) T. Birchall, A. N. Bourns, R. J. Gillespie, and P. J. Smith, *Can. J. Chem.*, **42**, 1433 (1964).

(4) There is now evidence that some O-protonation of anisole occurs along with C-protonation in HF–BF_3 solution at very low temperatures: D. M. Brouwer, E. L. Mackor, and C. Maclean, *Rec. Trav. Chim.*, **85**, 109 (1966).

(5) K. Yates and H. Wai, *Can. J. Chem.*, **43**, 2131 (1965).

(6) M. J. Jorgenson and D. R. Hartter, *J. Am. Chem. Soc.*, **85**, 878 (1963).

(7) The symbol "pK_a" is used to denote the value of H_0 at half-protonation; in the present case this is not equivalent to the negative logarithm of the true thermodynamic acidity constant for neither of these bases follows H_0 in its protonation.

(8) A. J. Kresge, G. W. Barry, K. R. Charles, and Y. Chiang, *J. Am. Chem. Soc.*, **84**, 4343 (1962); W. M. Schubert and R. H. Quacchia, *ibid.*, **85**, 1278 (1963); E. M. Arnett and G. W. Mach, *ibid.*, **86**, 2671 (1964).

(9) We have observed that the long-wavelength ultraviolet maxima of protonated polymethoxy- and methyl-substituted aromatic bases, whose nmr spectra indicate that these are C-protonated species, undergo a regular shift to shorter wavelengths as the number of methoxyl groups is decreased; a quantitative correlation of these spectral changes predicts that this absorption maximum occurs at 285 mμ in C-protonated anisole.

(10) E. M. Arnett and G. W. Mach, *J. Am. Chem. Soc.*, **88**, 1177 (1966).

A. J. Kresge, L. E. Hakka
Department of Chemistry, Illinois Institute of Technology
Chicago, Illinois 60616

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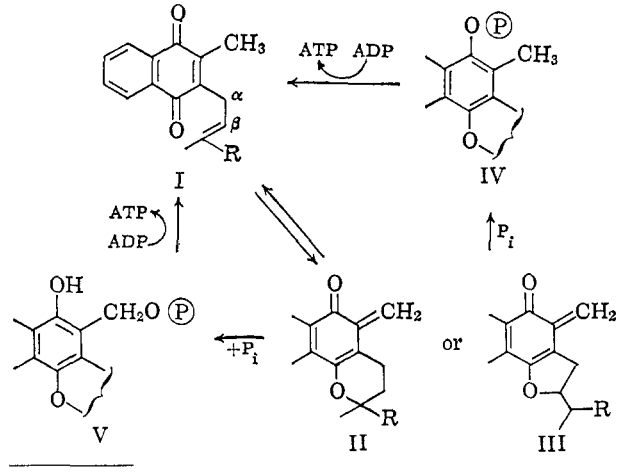
The Role of the Quinone in Oxidative Phosphorylation in *Mycobacterium phlei*. Evidence against Carbon–Hydrogen Bond Cleavage[1]

Sir:

Various mechanisms[2] proposed for the participation of the quinone in oxidative phosphorylation (sum-



(1) Sponsored in part by Grant AI-04888 from the National Institutes of Health, U. S. Public Health Service.

(2) (a) I. Chmielewska, *Biochim. Biophys. Acta*, **29**, 170 (1960); (b) E. Lederer and M. Vilkas, *Experientia*, **18**, 546 (1962); (c) K. Folkers, R. E. Erickson, and A. F. Wagner, *J. Am. Chem. Soc.*, **85**, 1534, 1535 (1963); (d) R. A. Morton, Ed., "Biochemistry of Quinones," Academic Press Inc., New York, N. Y., 1965.

Table I. Oxidative Phosphorylation with Intact and Light-Inactivated Reconstituted[a] Extracts[b] of *M. phlei*

Expt	System	Extract	Quinone added	Substrate	ΔP_i, μmoles	O_2, μatoms	P/O
1	Warburg[c] (duration 12 min)	Standard	None	Malate	14.5	12.6	1.2
		Inactivated	None	Malate	0.8	1.9	0.4
		Inactivated	Phylloquinone	Malate	10.1	10.3	1.0
	Macro[d] (duration 150 min)	Inactivated	Phylloquinone (49 mg/56 ml)	Malate	460	...	...
2	Warburg (duration 12 min)	Standard	None	Pyruvate	17.1	14.1	1.2
		Inactivated	None	Pyruvate	1.7	7.0	0.2
		Inactivated	Phylloquinone	Pyruvate	10.7	14.5	0.7
	Macro (duration 120 min)	Inactivated	Phylloquinone (104 mg/101 ml)	Pyruvate	900	...	...
3	Warburg (duration 12 min)	Standard	None	Malate	10.9	12.7	0.86
	Macro (duration 90 min)	Standard (51 ml)	None	Malate	350	...	...
4	Warburg (duration 12 min)	Standard	None	Pyruvate	13.7	14.1	0.96
	Macro (duration 90 min)	Standard (45 ml)	None	Pyruvate	510	...	...

[a] Extracts (20–28 mg of protein/ml) at 0° were inactivated by 25-min exposure to two 15-w GE black lights (long-wavelength ultraviolet). Phylloquinone was incorporated as an emulsion formed by sonication in a portion of light-inactivated extract at 0°. [b] All systems in each experiment were aliquots of the same bulk cell-free bacterial extract; inactivated systems were aliquots of a light-treated portion of this bulk extract. [c] Warburg systems contained components described previously[5a] (extract volume, 2.4 ml). [d] Same components as above in amounts proportional to indicated volume; reaction flask shaken at 30°. The reaction was followed by periodic phosphate analyses and terminated by centrifugation and addition of 95% ethanol.

Table II. Incorporation of Tritium into Quinone during Oxidative Phosphorylation with Extracts of *M. Phlei*

Expt	Quinone isolated	Phase	Yield of quinones (μmoles/ 50 ml)	Act. of quinone[a]	Spec act. of quinone[c,d]	Spec act. of T_2O[d] (dpm/μmole H)	Apparent incorp, %[e]
1	Phylloquinone	Particles	15	3.4/3.2	2.8	5.2×10^3	0.05 ± 0.03
	Phylloquinone	Supernatant	77	6.4/4.4	4.3	5.2×10^3	0.08 ± 0.03
2	Phylloquinone	Particles	27	4.9/5.9	4.7	5.8×10^3	0.08 ± 0.03
	Phylloquinone	Supernatant	69	4.3/6.1	4.1	5.8×10^3	0.07 ± 0.03
3	Native quinone	Particles	2.5	0/2.8[b]	0	2.5×10^3	0
4	Native quinone	Particles	1.8	4.2/4.7	30	6.6×10^3	0.50 ± 0.2

[a] Samples were counted by liquid scintillation; activity was measured in counts per minute/milligram; standard deviation is ±2 counts per minute/sample. [b] Less than standard deviation. [c] Calculated considering efficiency of counting and dilution factors. Efficiency was determined by internal standard. In expt 3 and 4 carrier native quinone was added during the purification procedure such that the dilution factors are 3 and 5, respectively. The carrier native quinone was isolated from *M. phlei* extracts in a yield of 25 μg (0.032 μmole)/ml. [d] In disintegrations/min μmole. [e] Calculated assuming one atom of hydrogen exchangeable per molecule of quinone.

marized schematically above) require hydrogen exchange between quinone and medium. For example, intermediacy of a quinone methide[2a-c] (II or III) implies exchange of the 2-methyl hydrogens; stabilization of the proposed intermediate *via* pyran ring formation (II)[2b] might also generate an exchangeable methine hydrogen (β position, I). Tautomerism to a vinylquinone, postulated[2a] in the route to the fused furan (III), would require exchange of the methylene hydrogens (α position, I). Also, mechanisms involving cyclization to the chromanol without accompanying methide formation[2d] might be detectable by isotopic exchange of the methine hydrogen.

Recently, tritium incorporation (from T_2O in the medium) specifically associated with oxidative phosphorylation has been reported[3] in the light-inactivated extract from *Mycobacterium phlei*, reconstituted with phylloquinone; however, an analogous study involving mammalian mitochondria and native ubiquinone failed to detect significant incorporation.[4] This difference might be reconciled by assuming the quinone acts by a different mechanism in the two systems (bacterial *vs.* mitochondrial; naphthoquinone *vs.* benzoquinone; light-inactivated system reconstituted with foreign quinone *vs.* intact system with native quinone). However, other observations indicate the two systems are similar[5] and phylloquinone parallels the action of native quinone in *M. phlei*.[6]

To resolve this possible contradiction, we have investigated tritium incorporation into the quinone during oxidative phosphorylation using cell-free extract[5a] from *M. phlei*. Both intact extracts (native quinone)[7] and phylloquinone-reconstituted, light-in-

(3) D. L. Gutnick and A. F. Brodie, *J. Biol. Chem.*, **240**, PC3698 (1965).

(4) (a) W. W. Parson and H. Rudney, *Biochemistry*, **5**, 1013 (1966), also observed no tritium incorporation into endogenous ubiquinone in *R. rubrum*. (b) D. L. Gutnick and A. F. Brodie, *J. Biol. Chem.*, **241**, 255 (1966).

(5) A. F. Brodie and C. T. Gray, *ibid.*, **219**, 853 (1956); (b) *Science*, **125**, 534 (1957); (c) A. F. Brodie, *J. Biol. Chem.*, **234**, 398 (1959); (d) A. F. Brodie and J. Ballantine, *ibid.*, **235**, 226 (1960).

(6) A. Asano and A. F. Brodie, *ibid.*, **239**, 4280 (1964).

(7) Native quinone of *M. phlei* is MK-9(H_2)[K_2(45)(H_2)]: P. H. Gale, B. H. Arison, N. R. Trenner, A. C. Page, Jr., and K. Folkers, *Biochemistry*, **2**, 200 (1963).

activated systems were used. Two substrates, malate and pyruvate, were employed to determine any effect of substrate upon the mechanism of quinone action. After the exposures, the systems were centrifuged and particulate and supernatant fractions were analyzed separately. If supernatant and particulate quinone pools do not equilibrate, isotopic exchange would appear only in the pool actively involved in oxidative phosphorylation, presumably the particulate fraction, and its detectability would be greatly enhanced by separate analysis.

The oxidative phosphorylation activity of the test systems is presented in Table I; the tritium incorporation into quinones recovered from the corresponding macro systems is shown in Table II.

All experiments displayed phosphate fixation coupled to oxidation both with malate and pyruvate. The tritium data indicate the lack of any significant isotope incorporation into the quinones. The extremely small residual activities are most likely due to traces of impurities since, particularly with native quinone, the quantities were small and there were accompanying highly radioactive lipoid fractions.[8] These could be a serious source of error if not completely removed and may explain the difference between our results and those reported.[3]

Lack of incorporation of tritium from T_2O into the quinone during oxidative phosphorylation indicates no carbon–hydrogen bond cleavage occurred in the quinone. Since this is a negative result, the possibility of undetected exchange must be considered. This might be possible if (a) there was a large isotope effect, and (b) only a small fraction of quinone was actively involved. However, the fact that the same results were obtained with particulate and supernatant fractions and exogenous and native quinones, and that the P_i fixed was large relative to the quinone present, argues against this assumption.

The best interpretation of our results is that the 2-methyl group is not directly involved, and therefore quinone methide mechanisms[2a-c] should be discounted. Complementary support for this conclusion is provided by similar experiments with 2-methyl-d_3-3-phytyl-1,4-naphthoquinone[9] in which we find that quinone recovered from reconstituted systems shows no loss of deuterium. The lack of incorporation of tritium also argues against involvement of the α-methylene hydrogens (as required in the fused furan mechanism[2a]) or the β-methine hydrogen. However, chromanol formation could occur without exchange because of the steric specificity of the β hydrogens in the pyran ring. The parallel experiments with phylloquinones-α-d_2[9] and -β-d are at hand.

(8) The quinone was extracted from the particulate and supernatant phases separately with 95% ethanol, and the ethanol extracts were diluted with an equal volume of saturated aqueous magnesium chloride solution and then extracted with pentane. The pentane extracts were repeatedly chromatographed on tlc-grade kiesel gel. Colorless bands both preceding and succeeding the quinone were highly radioactive.

(9) S. J. Di Mari, J. Supple, and H. Rapoport, *J. Am. Chem. Soc.*, 88, 1226 (1966).

(10) National Institutes of Health Predoctoral Fellow.

Clinton D. Snyder,[10] Samuel J. Di Mari,[10] Henry Rapoport

Department of Chemistry, University of California
Berkeley, California

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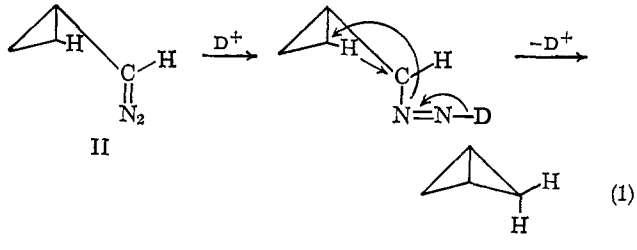
The Mechanisms of Base-Catalyzed Decomposition of Cyclopropanecarboxaldehyde *p*-Tosylhydrazone in Protic Solvents

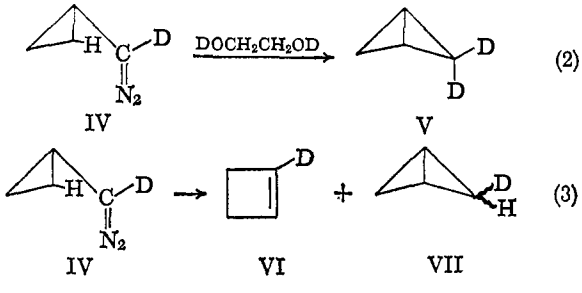
Sir:

Cyclopropanecarboxaldehyde *p*-tosylhydrazone (I) is decomposed by bases in aprotic solvents to cyclobutene, 1,3-butadiene, ethylene, and acetylene.[1] In protic environments, salts of I decompose primarily to bicyclo[1.1.0]butane and cyclobutene. It was suggested that salts of I thermolyze by carbenic processes involving cyclopropyldiazomethane, whereas in protic media the major path to bicyclo[1.1.0]butane involves cyclopropylmethyldiazonium or cyclopropylcarbonium intermediates. Recently[2] decomposition of I in ethylene glycol-d_2 by insufficient base was reported to yield bicyclo[1.1.0]butane which does *not* contain deuterium, and the following insertion mechanism was proposed (eq 1). Since these results and conclusions do not agree with present information, we shall summarize our studies of decomposition of salts of I in protic environments.



(1)

Decomposition of cyclopropanecarboxaldehyde-d_1 *p*-tosylhydrazone (III, Table I) in ethylene glycol-d_2 by insufficient, stoichiometric, or excess butyllithium gives bicyclo[1.1.0]butanes containing *two* (V, 77–87%) and *one* (VII, 12–19%) deuterium atoms, and cyclobutene essentially monodeuterated (VI, 93–98%). The *principal* process yielding bicyclo[1.1.0]butane results in incorporation of one deuterium from solvent to give V (eq 2); minor processes yield cyclobutene-d_1 (VI) and bicyclo[1.1.0]butane-d_1 (VII) without inclusion of external deuterium (eq 3).



(2)

(3)

Since reactions in excess base do not give appreciable quantities of dideuteriocyclobutene, neither III, nor its salt, nor IV undergoes significant deuterium exchange into the cyclopropane ring before decomposition. The near absence of trideuteriobicyclo[1.1.0]butane illustrates that deuterium exchange into bicyclo[1.1.0]-butanes is unimportant. Formation of cyclobutenes is enhanced upon effecting decompositions in excess base, in mixtures of protic and aprotic solvents, or in ethylene glycol-d_2 rather than protoethylene glycol.

(1) J. A. Smith, H. Shechter, J. Bayless, and L. Friedman, *J. Am. Chem. Soc.*, 87, 659 (1965).

(2) K. B. Wiberg and J. M. Lavanish, *ibid.*, 88, 365 (1966).