acid, with recrystallization from CH_3CN. At a mercury cathode, but not at Pt, it was stable to -1.9 V *vs.* sce. As before,[1] electrolytic reduction of tropylium fluoroborate in CH_3CN with any supporting electrolyte (0.4 *M*) at -1.40 V, before the second wave, resulted in the passage of 1 faraday/mol and the production of 7,7′-bis(cycloheptatrienyl) in 98–99% isolated yield, with <1% cycloheptatriene. The same result was obtained at -1.70 V, after the second wave, with tetrabutylammonium perchlorate (TBAP) supporting electrolyte, even in the presence of 50% acetic acid. However, with 0.4 *M* guanidinium perchlorate as the supporting electrolyte in CH_3CN, electrolysis at -1.6 V, after the second wave, led to the uptake of 1.30 faradays/mol and the production of 29% cycloheptatriene in addition to 65% of the dimer (which is stable under these conditions). Similarly, triphenylcyclopropenyl cation affords the 3,3′-bis(triphenylcyclopropenyl) with all electrolytes at the first wave and, under most conditions, at the second wave as well, but with guanidinium perchlorate as electrolyte 12% of 1,2,3-triphenylcyclopropene is also formed.

These trapping[3] experiments thus support our interpretation that the second electrochemical wave for these species does correspond to production of the anion. More interestingly, the fact that 0.4 *M* guanidinium ion can trap these anions, even though the more acidic and abundant acetic acid fails, confirms the idea that the medium in which the electrochemistry occurs is essentially a fused salt, from which solvent is excluded.

It is striking that even with guanidinium perchlorate some dimer persists, and the proportion is not changed by going to more negative potentials. Since the bulk guanidinium cation concentration is 200 times that of the tropylium cation, and carbon–carbon bond formation should be subject to more steric hindrance than is proton transfer, the ability of the tropylium ion or triphenylcyclopropenyl cation to compete in the trapping is remarkable. The most likely explanation is that it reacts with the anion by rapid prior electron transfer, followed by coupling of the resulting radicals.

Ordinary polarography of tropylium cation with TBAP supporting electrolyte shows almost no trace of the second wave (which was clearly visible by cyclic voltammetry, both in DMSO solvent[1] and in CH_3CN at a Pt electrode). This behavior is extremely unusual and reflects the fact that even at the second wave the overall chemical process is conversion of two tropylium cations to the dimer with two electrons, albeit by a different sequence from that observed at the first wave. Thus, the current does not increase. In polarography, an increase in diffusion current at the second potential can be observed only if some process other than coupling with the starting material takes place, and of course guanidinium perchlorate as the supporting electrolyte permits such a process. As Figure 1 shows, the ac polarogram[4] of tropylium fluoroborate has only the first wave with TBAP electrolyte, while with guanidinium perchlorate present a second wave can be seen at the potential we have observed previously using cyclic voltammetry. Similarly, in dc polarography the very small second wave is substantially increased using guanidinium perchlorate. Thus, this new supporting electrolyte is also of significance in permitting determinations of such potentials. The second wave could be seen in cyclic voltammetry even without a special electrolyte, since cyclic voltammetry is not a steady-state process. At the first potential essentially all the cation near the electrode is converted to radical, while at the second potential extra current passes as the surviving radicals are reduced to anions. Their subsequent precise chemical fate does not affect the current.

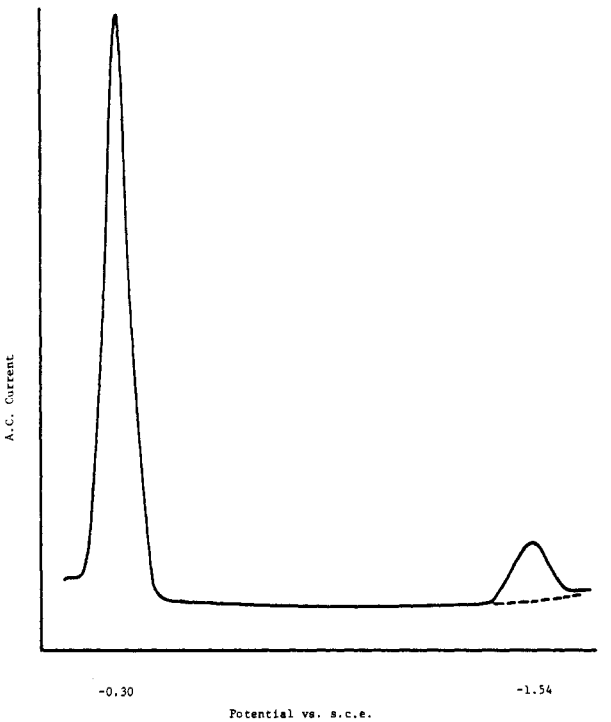

Figure 1. AC polarogram of tropylium fluoborate in CH_3CN: solid line, with guanidinium perchlorate as supporting electrolyte; dashed line, with tetrabutylammonium perchlorate as supporting electrolyte. The two polarograms are indentical except at the second wave, as shown.

Finally, these data indicate clearly that the most effective traps for electrochemically generated intermediates are those whose charge puts them into the double layer at the electrode. In other electroorganic syntheses this charge effect could also play an important role.[5]

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(3) Some unusual solvent acidity effects in trapping of electrochemically generated anions are reported by A. J. Fry and R. G. Reed, *J. Amer. Chem. Soc.*, **94**, 8475 (1972). It should also be noted that triphenylmethyl anions *can* be trapped[1] by acetic acid, in contrast with the tropylium and cyclopropenyl cases.

(4) D. E. Smith, *Electroanal. Chem.*, **1**, 1 1966.

Microenvironment of Histidine 12 in Ribonuclease-S as Detected by ^{13}C Nuclear Magnetic Resonance

Sir:

Histidine 12 is an essential amino acid residue for catalytic activity in bovine pancreatic ribonuclease-A

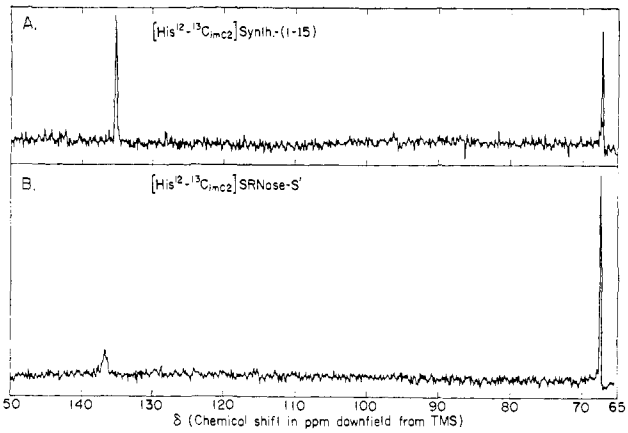

Figure 1. Downfield region of ^{13}C nmr spectra for enriched (1–15) peptide (2.5 m*M*) and semisynthetic complex (1 m*M*), in aqueous solution at pH 8.3, 32°, with 0.1 *M* NaCl. The sharp resonance on the upfield side of each spectrum is from the dioxane used as external standard. Spectral conditions were as described elsewhere,[7] except for operation with a 90° pulse, with samples in 8-mm o.d. tubes. Spectra were smoothed mathematically by use of a triangular apodization method. Pulse delay was 1 sec; the number of scans was 10,000 for (1–15) peptide and 47,525 for complex.

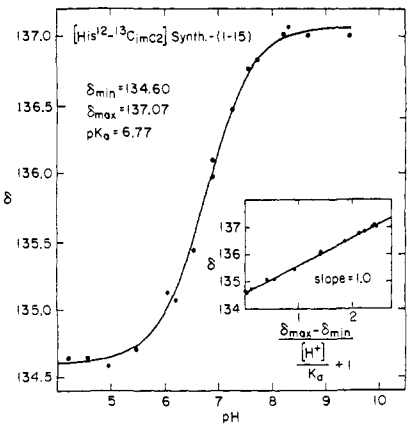

Figure 2. Variation of chemical shift with pH for [His^{12}-$^{13}C_{imC2}$]-synthetic-(1–15) at 32°. δ_{min} and δ_{max} are minimum and maximum values for chemical shift at pH extremes. The inset is a replot of data based on the formulation for a single-transition titration, $\delta = \delta_{min} - (\delta_{max} - \delta_{min})/([H^+]/K_a + 1)$. The solid lines in the major plot and replot are best fits of data based on this formulation.

and the structurally similar and equally active derivative ribonuclease-S, as established by chemical, kinetic, X-ray crystallographic, and ^1H nmr analyses.[1] With the purpose of introducing a nonperturbing probe for the further definition of the microenvironment of His 12 in ribonuclease alone and with interacting ligands, we have prepared [His^{12}-$^{13}C_{imC2}$]semisynthetic ribonuclease-S′.[2] This is the noncovalent complex containing [His^{12}-$^{13}C_{imC2}$]synthetic-(1–15) (the solid phase derived peptide corresponding to residues 1 through 15 of ribonuclease, with His 12 having the imidazole C-2 carbon enriched to 95% with ^{13}C)[3] and

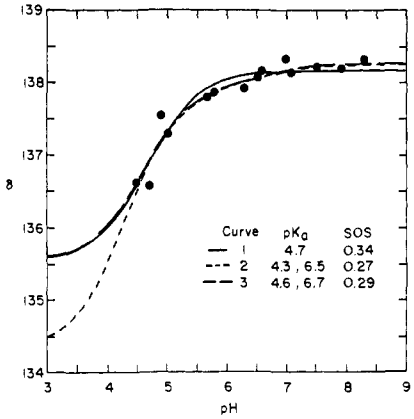

Figure 3. Variation of chemical shift with pH for [His^{12}-$^{13}C_{imC2}$]semisynthetic ribonuclease-S′ at 32°: curve 1, single transition; curve 2, double transition; curve 3, double transition with δ_{min} constrained at 135.53 ppm. SOS denotes the sum of squares values for the best fits. The maximum standard errors for calculated p*K* values are ±0.7 and ±1.2, respectively, for low- and high-pH transitions.

ribonuclease-S-(21–124) (the native subtilisin-derived fragment containing residues 21 through 124 of ribonuclease). Synthesis and purification of the semisynthetic complex on sulfoethyl-Sephadex were carried out by procedures described previously.[4,5] The complex is as active as native ribonuclease-A against cyclic cytidine 2′,3′-monophosphate, consistent with the nonperturbing nature of the ^{13}C introduced.

The environments of the ^{13}C-enriched histidine in both (1–15) peptide and semisynthetic complex were characterized by proton noise decoupled ^{13}C nmr,[6] using a Varian XL-100-15 spectrometer operated at 25.1 MHz for ^{13}C and equipped by Digilab for Fourier transformation.[7] Spectra, Figure 1, revealed a single observable resonance for each material which was assigned to the enriched C-2 carbon. Resonances due either to carbons at natural abundance or to trace amounts of variant species containing the enriched carbon atom are not detectable under the conditions used. As shown in Figure 1, the His C-2 resonance for the peptide is fairly sharp, whereas that for the complex is broadened. This most probably reflects the decreased rotational correlation time for the C-2 atom in the complex, suggesting the greater conformational rigidity of the His residue in this state. Additionally, the chemical shift in the complex is shifted downfield by about 1.3 ppm relative to that in the isolated, and presumably conformationally random, peptide. The broader resonance and downfield shift for complex *vs.* peptide is found at all pH values studied.

As shown in Figure 2, the variation of chemical shift with pH for the His C-2 resonance of the peptide describes a simple Henderson–Hasselbalch function, with a p*K* of 6.77. However, the downfield variation of chemical shift with increasing pH for [His^{12}-$^{13}C_{imC2}$]-semisynthetic ribonuclease-S′, as shown in Figure 3,

(1) Reviewed: (a) F. M. Richards and H. W. Wyckoff, *Enzymes*, **4**, 647 (1971); (b) R. Henderson and J. H. Wang, *Annu. Rev. Biophys. Bioeng.*, 1, 1 (1972).

(2) Ribonuclease-S′ refers to the ribonuclease-S-related complex reconstituted from isolated fragments, as distinguished from ribonuclease-S, the complex obtained directly from ribonuclease A by subtilisin treatment with no subsequent separation of noncovalently bound fragments.

(3) His^{12}-$^{13}C_{imC2}$ at 95% ^{13}C enrichment was a generous gift of C. Gregg, University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

(4) I. M. Chaiken, M. H. Freedman, J. R. Lyerla, Jr., and J. S. Cohen, *J. Biol. Chem.*, 248, 884 (1973).

(5) I. M. Chaiken, *Methods Enzymol.*, in press.

(6) ^{13}C nmr characteristics have been studied previously for His^{12}-$^{13}C_{imC2}$ introduced biosynthetically into α-lytic protease. M. W. Hunkapillar, S. H. Smallcombe, D. R. Whitaker, and J. H. Richards, *Biochemistry*, **12**, 4732 (1973).

(7) I. M. Chaiken, *J. Biol. Chem.*, **249**, 1247 (1974).

does not describe a simple titration. Linearizing analyses similar to that shown in Figure 2 indicate the presence of at least two transitions. Curve fits of the data according to titration processes involving either one or two independent pH-dependent transitions (with or without constraints[8]) are shown in Figure 3. Based on the sums of squares and pK_a values obtained (and further linearizing analyses), it appears that curve 2 gives the best fit.[9] Accordingly, the basic transition defined by $pK_a \simeq 6.5$[10,11] is assignable to the titration of the histidinyl 12 side chain itself. The low-pH transition, $pK_a \simeq 4.3$, may be due to at least one neighboring titrating group, which perturbs the His 12 imidazole chemical shift in ribonuclease-S′, although the presence of a low-pH conformational transition (local or more general) cannot be ruled out. Based on earlier considerations from enzymic modification,[12] kinetics,[13] and ^1H nmr,[11] the acid transition may represent, at least in part, the titration of the β-carboxyl group of Asp 121. The two pK values obtained here are fairly consistent with those suggested previously.[11,14]

(8) R. Shrager, J. S. Cohen, S. R. Heller, D. H. Sachs, and A. N. Schechter, *Biochemistry*, **11**, 541 (1972).
(9) Given the scatter of points and the inability to obtain data at very low pH values (below pH 4.4) due to the precipitation encountered with the present material, a choice between two-transition fits with varying δ_{min} must remain tentative.
(10) D. H. Meadows, O. Jardetzky, R. M. Epand, H. H. Ruterjans, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.*, **60**, 766 (1968).
(11) A. N. Schechter, D. H. Sachs, S. R. Heller, R. I. Shrager, and J. S. Cohen, *J. Mol. Biol.*, **71**, 39 (1972).
(12) C. B. Anfinsen, *J. Biol. Chem.*, **221**, 405 (1956).
(13) J. E. Erman and G. G. Hammes, *J. Amer. Chem. Soc.*, **88**, 5607 (1966).
(14) J. S. Cohen, J. H. Griffin, and A. N. Schechter, *J. Biol. Chem.*, **248**, 4305 (1973).
(15) (a) Laboratory of Chemical Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases; (b) Reproduction Research Branch, National Institute of Child Health and Human Development; (c) Laboratory of Chemistry, National Heart and Lung Institute.

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Enthalpies of Transfer of Aromatic Molecules from the Vapor State to Polar and Nonpolar Solvents[1]

Sir:

A significant development in the understanding of reactivity and equilibria in solution has resulted in recent years from studies of the gaseous state.[2] These data, combined with measurements of the interactions of gaseous species with solvents, can provide insights into solution behavior and into the modification of intrinsic reactivity by solvation.[3] Similar measurements in appropriate systems can also contribute to an understanding of the intermolecular forces which become operative as gaseous molecules interact with solvents. This communication reports such data for the interactions of aromatic molecules with the polar solvents *N*,*N*-dimethylformamide and methanol and with the nonpolar solvents benzene and cyclohexane.

We have recently been interested in the extent to which the solvation of aromatic substituents changes upon transfer from methanol (MeOH) to *N*,*N*-dimethylformamide (DMF).[4,5] The observation was made that values of the enthalpy of transfer ($\Delta\Delta H_s$) of aromatic compounds are, within experimental error, additive functions of the substituent groups present, regardless of the relative positions of the groups on the aromatic ring.[6] Individual group $\Delta\Delta H_s$ values can be rationalized in terms of the greater dipole moment, hydrogen bond acceptor ability, and solvent–solvent association forces of DMF (relative to MeOH), and the resulting more exothermic dipole–dipole, dipole–induced dipole, and hydrogen bonding interactions with polar, polarizable, and hydrogen bond donating groups, respectively.

Comparison of $\Delta\Delta H_s$ values (MeOH → DMF) for aromatic and aliphatic compounds with available $\Delta\Delta G$ values[7] indicates that the latter are largely determined by the enthalpy contribution. Thus the enthalpy of transfer is a meaningful indication of the change in solvation that a molecule or group undergoes upon transfer from one solvent to another.

Enthalpies of solution (ΔH_s) for a number of aromatic compounds in the nonpolar solvent cyclohexane and in the nonpolar but highly polarizable solvent benzene have now been measured and are presented in Table I.

Table I. Enthalpies of Solution of Benzene Derivatives

Compound	$-\Delta H_s$[a]			
	c-C_6H_{12}	Benzene	MeOH	DMF
Benzene	0.94	0.00	0.36[b]	0.04[b]
Toluene	0.77	0.12	0.45[b]	0.16[b]
p-Xylene	0.86	0.25	0.59	0.39
Mesitylene	1.22	0.62	1.04[b]	0.83[b]
Cyclohexane	0.00	0.90	1.21	2.87
Perfluoro-(methylcyclohexane)	3.79	5.46	3.98	5.95
Fluorobenzene	1.10	0.02	0.03[b]	−0.35[b]
Chlorobenzene	0.90	0.03	0.17[b]	−0.25[b]
Bromobenzene	1.07	0.05	0.30[b]	−0.19[b]
Iodobenzene	1.31	0.20	0.51[b]	−0.40[b]
α,α,α-Trifluorotoluene	1.66	0.56	−0.05[b]	−0.01[b]
Acetophenone	3.14	0.36	1.20[b]	−0.14[b]
Nitrobenzene	2.85	0.56	1.00[b]	−0.19[b]
Ethyl benzoate	1.54	0.05	1.21[b]	0.27[b]
Aniline	4.22	1.25	−0.62[b]	−2.68[b]
m-Cresol	6.02	2.58	−1.55	−3.35

[a] Calorimetric values ±0.05 kcal/mol. [b] Values from ref 5.

The enthalpies of solution were measured at concentrations of 10^{-4} to 10^{-3} *M*. No effect of concentration on ΔH_s was noted in this range. Values of ΔH_s and $\Delta\Delta H_s$ are thus essentially infinite dilution values.

Values of ΔH_s from Table I and previous values[5] have been combined with enthalpies of evaporation

(1) Presented in part at the Fourth Conference on Structure–Energy Relationships, San Juan, P. R., Jan 9–12, 1974.
(2) See, for example, J. I. Brauman, J. M. Riveros, and L. K. Blair, *J. Amer. Chem. Soc.*, **93**, 3914 (1971).
(3) E. M. Arnett, F. M. Jones, III, M. Taagepera, W. G. Henderson, J. L. Beauchamp, D. Holtz, and R. W. Taft, *J. Amer. Chem. Soc.*, **94**, 4724 (1972).
(4) R. Fuchs, L. L. Cole, and R. F. Rodewald, *J. Amer. Chem. Soc.*, **94**, 8645 (1972).
(5) R. Fuchs and R. F. Rodewald, *J. Amer. Chem. Soc.*, **95**, 5897 (1973).
(6) The additivity of group $\Delta\Delta H_s$ values of CH_2 and OH in alcohols and for CH_2 in hydrocarbons had previously been demonstrated by C. V. Krishnan and H. L. Friedman, *J. Phys. Chem.*, **73**, 1572 (1969); **75**, 3598 (1971).
(7) A. J. Parker, *Chem. Rev.*, **69**, 1 (1969).