Figure 1. Multiplexed TD spectra following acetylene adsorption on a Cu(110) surface at 90 K. The heating rate was 3 K s⁻¹.
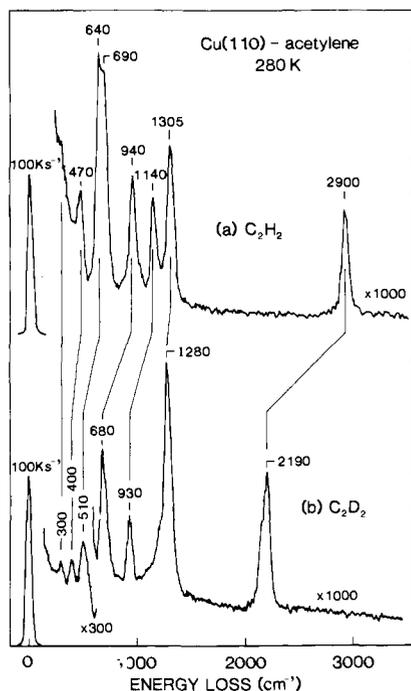
Figure 2. EEL spectra of (a) C_2H_2 and (b) C_2D_2 on Cu(110) obtained after saturating the surface at 100 K and annealing to 280 K.

Table I. Frequencies (cm⁻¹) and Assignments of the EEL Bands from Acetylene Adsorption on Cu(110) at 280 K

assignment	C_2H_2	C_2D_2	H/D
$\nu(CH)$	2900	2190	1.32
$\nu(CC)$	1305	1280	1.02
$\delta_{as}(CH)$	1140	930	1.23
$\delta_s(CH)$	940	680	1.38
$\gamma(CH)$	640	510ᵃ	1.25
Cu–acetylene modes	470	400	
		300	
$\gamma(CH)$ benzene	690	510ᵃ	

ᵃ Unresolved.

spectra of which are similar to those seen here on the Cu(110) surface except for the activity of the $\delta_{as}(CH)$ mode in the present spectra. Similarly, additional work in this laboratory has shown

that the Cu(100) surface produced acetylene spectra very similar to those from the Cu(111) surface. The insensitivity of the band near 1300 cm⁻¹ to deuterium substitution leaves little doubt as to its assignment to stretching of the CC bond which must be of ~1.5 order implying strong interaction with the surface. Furthermore, the similarity of the spectra on these low-index copper surfaces suggests a common adsorption site, most probably a two-fold bridge although it does not appear possible to distinguish between a CC bond vector parallel or perpendicular to CuCu axis on the basis of these EEL spectra.

After the sample was heated to 400 K the EEL spectrum reveal weak bands near 770, 870, and 3010 cm⁻¹ (550, 720, ~2250 cm⁻¹ with deuterated acetylene). The 870 (720) cm⁻¹ band was attenuated after heating to 600 K. The remaining two bands survived 700 K but were attenuated after 800 K. These spectra show that reaction-limited hydrogen evolution in this temperature region is associated with the destructive dehydrogenation of at least two new adsorbed hydrocarbon residues which must have been formed concomitantly with acetylene desorption and trimerization.

As a result of these observations the adsorption and reaction of acetylene on a Cu(110) surface may be written thus

$$C_2H_2 \xrightarrow{<200\ K} C_2H_2(ads) \qquad (1)$$
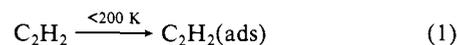
with three reactions occurring concurrently in the region 200–400 K

$$C_2H_2(ads) \xrightarrow{270,\ 330\ K} C_2H_2\uparrow \qquad (2)$$

$$C_2H_2(ads) \xrightarrow{325\ K} {}^1/_3C_6H_6\uparrow \qquad (3)$$

$$C_2H_2(ads) \xrightarrow{<400\ K} C_xH_y(ads) + C_{y-x} \qquad (4)$$

followed by

$$C_xH_y(ads) \xrightarrow{600–900\ K} C_x + (y/2)H_2\uparrow \qquad (5)$$
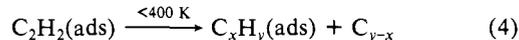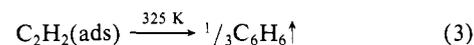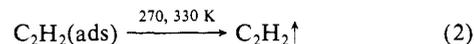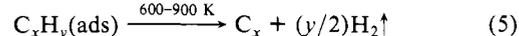
Equation 4 is written in a general form since speculation on the structure and bonding configuration of the species C_xH_y (at least two of them up to 600 K) is beyond the purpose of this paper.

Registry No. C_2H_2, 74-86-2; Cu, 7440-50-8; C_6H_6, 71-43-2.

Change in Rate-Limiting Step in Proton Removal from an Intramolecularly Hydrogen-Bonded Acid and the Rate of Opening and Closing of a Hydrogen Bond

N. E. Briffett, Frank Hibbert,* and R. J. Sellens

Department of Chemistry, King's College London Strand, London, England WC2R 2LS

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We wish to report that proton transfer from intramolecularly hydrogen-bonded phenylazoresorcinol monoanions to general bases (B⁻) in 70% (v/v) Me_2SO–H_2O, eq 1, shows a change in rate-

limiting step with base concentration. This has made it possible to obtain values for the rate coefficients and equilibrium constants
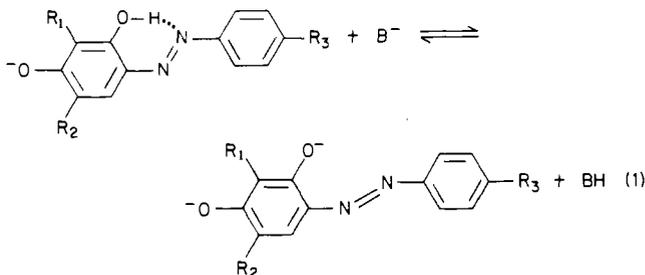
Table I

	BH	K^a	k_1, s^{-1} [b]	$10^3 k_{-1}/k_2$, mol dm^{-3} [b]
4-[(4-nitrophenyl)azo]resorcinol	benzimidazole	0.71 ± 0.08	77 ± 15	6.6 ± 2
4,6-bis(phenylazo)resorcinol	phenol	0.28 ± 0.03	(3.8 ± 0.8) × 10^4	5.3 ± 0.9
	2-methylphenol	0.74 ± 0.1	(3.7 ± 0.7) × 10^4	8.2 ± 1.5

[a] Experimental values (see text).   [b] Best-fit values (see text).
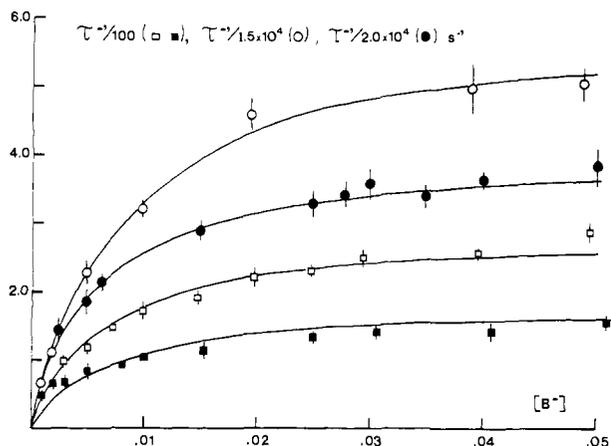
Figure 1. Reciprocal relaxation times for ionization of 4-[(4-nitrophenyl)azo]resorcinol monoanion in benzimidazole buffers at buffer ratios r = 1.0 (■) and 0.5 (□) and of 4,6-bis(phenylazo)recorcinol monoanion in phenol (r = 3.4, ●) and 2-methylphenol (r = 1.0, O) buffers.

for opening and closing of the intramolecular hydrogen bonds in these molecules.

Equilibration of the monoanions and dianions of various phenylazoresorcinols in the presence of aqueous hydroxide ion has been shown[1,2] to occur by the mechanism in Scheme I. Direct attack by hydroxide ion on the hydrogen-bonded proton (step 1) occurs in parallel with a two-step process (steps 2 and 3) involving an open non-hydrogen-bonded form of the monoanion. In the two-step process, to satisfy the observed kinetics, it is necessary that the rate of proton removal from the open form of the monoanion be greater than the rate at which the open form reverts to the hydrogen-bonded monoanion. In aqueous buffer solutions, the reaction is first order in general base and this is explained[3] in terms of direct attack of base as in step 1.

We have now studied the equilibration between the monoanions and dianions of 4-[(4-nitrophenyl)azo]resorcinol (eq 1 with R_1 = R_2 = H, R_3 = NO$_2$) and of 4,6-bis(phenylazo)resorcinol (R_1 = R_3 = H, R_2 = PhN$_2$) in 70% (v/v) Me$_2$SO–H$_2$O in the presence of buffers. The buffers were made up by partially neutralizing solutions of the buffer acids with tetramethylammonium hydroxide and the ionic strength was maintained at 0.1 mol dm^{-3} by addition of tetramethylammonium chloride. Kinetic measurements were made at 15 °C by using the temperature-jump technique and relaxation of the equilibrium between the monoanions and dianions following a temperature jump was observed spectrophotometrically. The values of the reciprocal relaxation times (τ^{-1}) as a function of buffer (B$^-$) concentration for 4-[(4-nitrophenyl)azo]resorcinol monoanion in buffers of benzimidazole and its anion and for 4,6-bis(phenylazo)resorcinol monoanion in phenol and 2-methylphenol buffers are shown in Figure 1. The vertical lines on the data points represent twice the standard deviation of 5–10 determinations of the reciprocal relaxation time at each buffer concentration. Values of the equilibrium constants for dissociation of 4-[(4-nitrophenyl)azo]resorcinol monoanion and 4,6-bis(phenylazo)resorcinol monoanion in each buffer (K = [dianion]·[BH]/[monoanion][B$^-$]) were determined spectrophotometrically and the results are given in Table I.

Scheme I
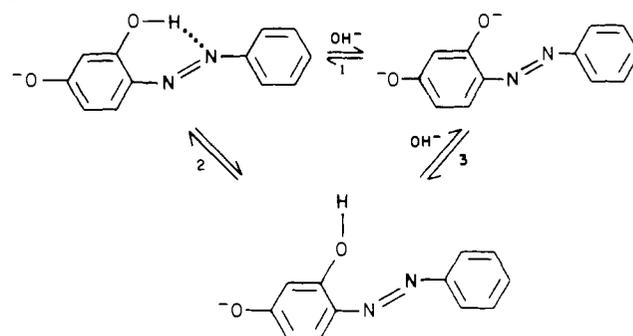
The buffer saturation plots[4] (Figure 1) are explained by the mechanism in eq 2, for which the dependence of reciprocal re-

laxation time on buffer concentration is given by eq 3, assuming
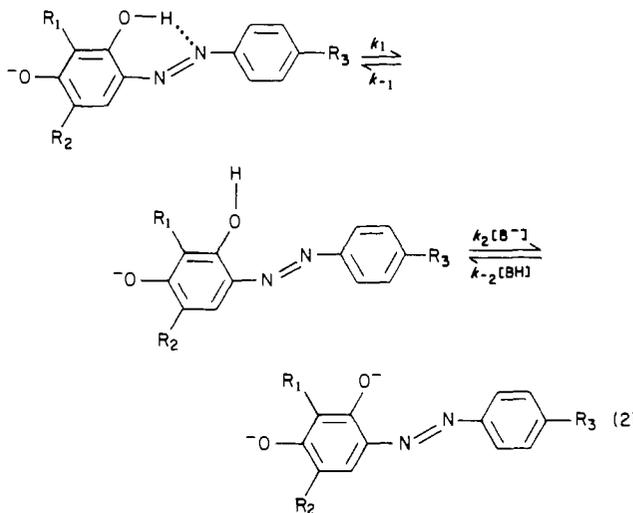
$$\tau^{-1} = (k_1 k_2 [B^-] + k_{-1} k_{-2} [BH]) / (k_2 [B^-] + k_{-1}) \quad (3)$$

$$\tau^{-1} = k_1 (1 + 1/Kr)[B^-] / ([B^-] + k_{-1}/k_2) \quad (4)$$

$$\tau = k_{-1}/k_1 k_2 (1 + 1/Kr)[B^-] + 1/k_1 (1 + 1/Kr) \quad (5)$$

that the intermediate open form of the (phenylazo)resorcinol monoanions is present in low concentration. Equations 4 and 5 are obtained from (3) by substituting the overall equilibrium constant K = $k_1 k_2 / k_{-1} k_{-2}$ and the buffer ratio r = [B$^-$]/[BH]. The mechanism in eq 2 resembles steps 2 and 3 in Scheme I except that for reaction with general bases under the present conditions, the balance between the rate of proton removal by base from the intermediate open form of the monoanion and the rate at which the open form returns to the hydrogen-bonded monoanion is dependent on buffer concentration. At low buffer concentration ($k_2 [B^-] < k_{-1}$) proton removal is rate-limiting with preequilibrium formation of a low concentration of the open form and at high

(1) Perlmutter-Hayman, B.; Shinar, R. *Int. J. Chem. Kinet.* **1975,** *7,* 453. Perlmutter-Hayman, B.; Sarfaty, R.; Shinar, R. *Int. J. Chem. Kinet.* **1976,** *8,* 741.
(2) Hibbert, F.; Simpson, G. R. *J. Am. Chem. Soc.* **1983,** *105,* 1063.
(3) Perlmutter-Hayman, B.; Shinar, R. *Int. J. Chem. Kinet.* **1977,** *9,* 1.

(4) Buffer association has been shown[5,6] to be insignificant at the concentrations and with the particular buffers used in this work. Thus buffer catalysis of proton transfer from a zwitterion[5] and from protonated 1,8-bis-(dialkylamino)-2,7-dimethoxynaphthalenes[6,7] gave practically linear rate against buffer concentration plots up to concentrations of 0.1 mol dm^{-3}, as did the buffer-catalyzed hydrolysis of 4-nitrophenyl 4-methoxybenzoate.[6]
(5) Bernasconi, C. F.; Terrier, F. *J. Am. Chem. Soc.* **1975,** *97,* 7458.
(6) Hibbert, F.; Robbins, H. J. *J. Am. Chem. Soc.* **1978,** *100,* 8239.
(7) Barnett, G. H.; Hibbert, F. *J. Am. Chem. Soc.* **1984,** *106,* 2080.

buffer concentration ($k_2[B^-] > k_{-1}$), opening of the intramolecular hydrogen bond is rate-limiting. Values for k_1 and k_{-1}/k_2 were obtained by least-squares analysis of the experimental data in the form of a plot of τ against $1/[B^-]$ as in eq 5, using experimentally determined values of K. The values of k_1 and k_{-1}/k_2 are given in Table I and the curves in Figure 1 were constructed by using these results. The contribution made by hydroxide ion to the observed relaxation time, which would appear as the intercept of a plot of τ^{-1} against buffer concentration at zero buffer concentration, is small and has been neglected in fitting the experimental results to eq 3–5.

The good fit of eq 3–5 to the experimental results supports the proposed mechanism. Further, if it is assumed that proton removal by buffers from the intermediate open forms is diffusion–controlled (k_2 ca. 1×10^{10} dm^3 mol^{-1} s^{-1}) the following values are obtained for opening and closing of the intramolecular hydrogen bonds: for 4-[(4-nitrophenyl)azo]resorcinol monoanion, k_1 77 ± 15 s^{-1} and k_{-1} ca. 7×10^7 s^{-1}, and for 4,6-bis(phenylazo)resorcinol monoanion, k_1 $(3.7 ± 0.8) \times 10^4$ s^{-1} and k_{-1} ca. 7×10^7 s^{-1}. The applicability of this approach in obtaining similar information about other intramolecularly hydrogen-bonded species is under investigation.

Functionalized Heteropolytungstate Anions Possessing a Modified Dawson Structure: Small, Individually Distinguishable Labels for Conventional Transmission Electron Microscopy

John F. W. Keana,* Marc D. Ogan, and Yixin Lü

*Department of Chemistry, University of Oregon
Eugene, Oregon 97403*

Michael Beer* and J. Varkey

*Department of Biophysics, The Johns Hopkins University
Baltimore, Maryland 21218*

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We describe herein the synthesis and properties of a versatile series of Dawson-type[1] heteropolytungstate (HPT) ions[2] that are monofunctionalized with protein-reactive organic groups. Organic transformations effected on the HPTs demonstrate that the HPT unit is stable toward a variety of reaction conditions. The new HPTs contain 17 tungsten atoms within an ellipsoid of about 11 × 18 Å and are shown for the first time to be visible individually using conventional transmission electron microscopy (CTEM),[3,4] constituting a new class of chemoselective labels for CTEM.

The functionalized HPTs were prepared as follows. Alkylation

of lithium cyclopentadienide with either 3,5-hexadienyl methanesulfonate[5] or 1-(3-bromopropoxy)-4-(dimethoxymethyl)benzene[6] gave **1a**[7] and **1b**,[8] respectively. Addition[9] of **1a** (309 mg)

to 1.1 equiv of Ti(NMe$_2$)$_4$ in dry benzene (4 mL) gave a red solution which, after 30 min at 60 °C, was cooled and added to a stirred suspension of 1 equiv of α_2-K$_{10}$P$_2$W$_{17}$O$_{61}$[10] in DMF (20 mL). After 10 min the pale yellow suspension was adjusted to pH 3–4 with 2 M HCl and the resulting orange supernatant was applied to a column of acidic Al$_2$O$_3$ packed in 7:3 DMF/water. The orange HPT was eluted with 1 M NaOAc buffer pH 5.6 and precipitated with Me$_3$NHCl in water. Crystallization from water gave **2a** (TMA salt)[7] (11% yield), which was ion exchanged to the K$^+$ salt.[11] Benzaldehyde–HPT **2c** (TMA[12] and K$^+$ salt) was obtained similarly. **2c** was shown to undergo smooth reductive amination with N^6-[[(aminohexyl)carbamoyl]methyl]adenosine 5'-triphosphate (Li salt)[13] to give the HPT-derivatized ATP **2e**, designed for the EM localization of ATP binding sites in certain proteins. HPTs **2a** and **2c** (K$^+$ salts) are highly water soluble and stable to storage in the solid state or in aqueous solutions (pH 2–8.5) under ambient conditions.

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(1) Dawson, B. *Acta Crystallogr.* **1953**, 6, 113. D'Amour, V. H. *Acta Crystallogr.*, *Sect. B* **1976**, B32, 729.

(2) Pope, M. T. "Heteropoly and Isopoly Oxometalates"; Springer-Verlag: New York, 1983.

(3) The *potential* of HPTs as EM labels has been recognized earlier: Wall, J. S. *Chem. Scr.* **1978**, 14, 271. Zonnevijlle, F.; Pope, M. T. *J. Am. Chem. Soc.* **1979**, 101, 273.

(4) Commercially available transmission electron microscopes can provide 2-Å point-to-point resolution with an electron optical magnification of about 800 000. Labels currently in use (for a review, see: Hicks, D.; Molday, R. S. In "Science of Biological Specimen Preparation"; Revel, J.-P., Barnard, T., Haggis, G. H., Eds.; Scanning Electron Microscopy, Inc.: AMF O'Hare, IL, 1984; pp 203–219) such as ferritin (~120-Å diameter) and colloidal gold (50–1600-Å diameter) are large relative to the resolving power of modern instruments and moreover do not lend themselves well to covalent attachment to biomolecules in a chemically well-defined manner. The recently described cationic undecagold clusters, visible by scanning transmission EM, constitute one approach toward improving labeling methodology through rational design: Reardon, J. E.; Frey, P. A. *Biochemistry* **1984**, 23, 3849. Yang, H.; Reardon, J. E.; Frey, P. A. *Biochemistry* **1984**, 23, 3857. Yang, H.; Frey, P. A. *Biochemistry* **1984**, 23, 3863.

(5) Nixon, J. R.; Cudd, M. A.; Porter, N. A. *J. Org. Chem.* **1978**, 43, 4048.

(6) Prepared by acetalization of the corresponding aldehyde: Schweizer, E. E.; Berninger, C. J.; Crouse, D. M.; Davis, R. A.; Logothetis, R. S. *J. Org. Chem.* **1969**, 34, 207.

(7) Satisfactory C, H, and N analytical values were obtained.

(8) MS *m/z* 274.157 (calcd for C$_{17}$H$_{22}$O$_3$, 274.157).

(9) A modification of: Lappert, M. F.; Chandra, G. *J. Chem. Soc. A* **1968**, 1940. In the Keggin series, (C$_5$H$_5$)TiPW$_{11}$O$_{39}$$^{4-}$ has been prepared independently by using C$_5$H$_5$TiCl$_3$: Knoth, W. *J. Am. Chem. Soc.* **1979**, 101, 759. Ho, R. K. C.; Klemperer, W. G. *J. Am. Chem. Soc.* **1978**, 100, 6772.

(10) Souchay, P. "Polycations et Polyanions"; Gauthier-Villars: Paris, 1963.

(11) **2a** K$^+$ salt: ^{1}H NMR (D$_2$O) 2.59 (t, 2, allylic), 3.10 (t, 2, CpCH$_2$), 4.90–6.50 (m, 5, diene), 6.52, 6.69 ppm (AA'BB', 4, Cp); ^{31}P NMR (D$_2$O) –9.93 (s), –13.19 (s) ppm (external H$_3$PO$_4$); ^{183}W NMR (D$_2$O) –92.29 (2 W), –130.19 (2 W), –169.70 (1 W), –174.68 (2 W), –180.88 (2 W), –192.64 (2 W), –196.05 (2 W), –197.01 (2 W), –219.07 ppm (2 W) (external Na$_2$WO$_4$).

(12) **2c** TMA salt:[7] calcd % W, 64.32; found, 63.5.

(13) The amine (Sigma Co.) (7 mg), HPT **2c** (20 mg), and NaBH$_3$CN (0.3 mg, added portionwise) were dissolved in 1 M phosphate buffer pH 6.5 (0.1 mL) and stirred at 25 °C for 10 days. The sample was diluted with water (0.2 mL) and then Me$_3$NHCl (50 mg) was added to precipitate out the product which was washed with water and ion exchanged to the K$^+$ salt (20 mg, 88%). 360-MHz NMR showed this to be about 20% of the HPT benzyl alcohol corresponding to **2c**. The entire product was purified by preparative TLC on silica gel, developed with CHCl$_3$/CH$_3$OH/H$_2$O, 3:3:1. The middle band was eluted off with water and precipitated as the TMA salt.[7] This was ion exchanged to the K$^+$ salt of **2e** (11 mg).