

rapidly consumed approximately three moles of periodate, giving one mole of formaldehyde, two moles of ammonia, and between one and two moles of formic acid<sup>7</sup>; treatment of the periodate product with acidic 2,4-dinitrophenylhydrazine gave (in small yield) glyceraldehyde 2,4-dinitrophenylhydrazone.<sup>8</sup> The marked stability of I to acid is due to the fact that it is an N-glycoside of a 2-aminosugar.<sup>8</sup>

In respect to glycosidic attachment, a provisional choice among the guanidine nitrogens was made possible by quantitative Van Slyke nitrous acid deamination studies. The N,N'-diacetyl derivative of I (non-crystalline, purified by cellulose chromatography: Found: C, 43.87; H, 6.03; N-acetyl, 15.5) evolved nitrogen much more slowly than mono-N-(benzenesulfonyl)-streptolidine or any model 2-aminoimidazoline studied (Table I), suggesting the absence of a primary

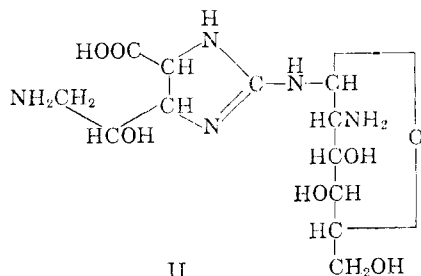
TABLE I  
VAN SLYKE DETERMINATIONS ON GUANIDINES<sup>a</sup>

Compound	—Moles of nitrogen evolved— in hr.			
	0.25	1.0	3	10
2-Aminoimidazoline	0.26	1.02	1.87	...
2-Aminoimidazoline-4,5-dicarboxylic acid	0.88	1.87	2.44	...
Streptolidine·HCl <sup>b</sup>	0.33	1.29	2.00	2.22
N-Benzenesulfonyl streptolidine	0.23	0.52	1.17	1.45
N,N'-Diacetyl-N(guan)-streptolidylgulosaminide	0.06	0.05	0.20	0.28

<sup>a</sup> Values obtained with a normal Van Slyke apparatus using sodium nitrite-glacial acetic acid at 25 ± 1°.

<sup>b</sup> Amount of nitrogen indicated in addition to that released by the non-guanidino primary amino group.

guanidino amino group in I, and therefore attachment of the aminosugar to the exocyclic nitrogen of the 2-aminoimidazoline moiety.<sup>9,10</sup>



(7) The release of the additional formic acid probably is the result of over-oxidation and to the acid-instability of the primary periodate product.

(8) In a simpler case, Dr. J. S. G. Cox (unpublished results, University of Wisconsin) demonstrated that N-(glucosyl)-guanidinoacetic acid is acid-hydrolyzed only slowly to the hexose and the guanidine components, but yet sensibly more rapidly than is N-guan-streptolidyl gulosaminide. The difference between these two systems may derive from the presence of a second, adjoining basic center in the latter case, which would be expected to inhibit acid cleavage of the glycosidic link.

(9) A like assignment was made by the Japanese workers (ref. 3), who depended upon the C=N stretching frequency of I and some model guanidines for their conclusions. Our own examination of this method, in which use was made of other representative guanidine models, demonstrated, however, that such exceptions to the working rules of the Japanese exist that deductions regarding alkyl substitution on guanidines, based on infrared spectral methods seem, at present, unjustified.

(10) Because of the positive rotations of streptolidine and α-D-gulosamine and the negative rotation of I, the β-configuration is tentatively assigned to the glycosidic linkage present in I.

Thus, structure II best accommodates the information available at the present time, and its incorporation into the complete formulas of streptothricin and streptolin is described in the accompanying Communication.<sup>11</sup>

(11) van Tameien, *et al.*, **83**, 4295 (1961).

NOYES LABORATORY OF CHEMISTRY  
UNIVERSITY OF ILLINOIS  
URBANA, ILLINOIS

H. E. CARTER  
J. V. PIERCE  
G. B. WHITFIELD, JR.  
J. E. McNARY

DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF WISCONSIN  
MADISON, WISCONSIN

E. E. VAN TAMELEN  
J. R. DYER  
H. A. WHALEY

RECEIVED JULY 24, 1961

### THE STRENGTHS OF CYANOCARBON ACIDS AND AN H-ACIDITY SCALE FOR CONCENTRATED ACID SOLUTIONS

Sir:

The syntheses of a number of cyanocarbon acids have been reported previously from this laboratory.<sup>1</sup> These were shown to be unusually strong organic acids, and pK<sub>a</sub>'s near 2 were reported. Further work now has proved them to be much stronger than originally thought and to possess an acid strength that is remarkable.

Reinterpretation of potentiometric titration curves shows the acids to be strong within the limitations of that method (pK<sub>a</sub> < ~0.5). Apparently the most feasible method of determining their strengths is to study their ultraviolet or visible spectra in solutions of strong mineral acids since the free anions usually have characteristic, strong electronic absorptions which should disappear on protonation. It has been found that a number of these acids do undergo reversible spectral changes characteristic of protonation in such solutions. The most promising approach to interpretation of the measured species concentration appears to be that of an "acidity function." Thus, this finding presents the possibility of determining the strengths of the cyanocarbon acids and simul-

TABLE I  
IONIZATION CONSTANTS OF CYANOCARBON ACIDS, 25°

Cyanocarbon acid	pK <sub>a</sub>		λ <sub>max</sub> (mμ) for longest wave length band	
	HClO <sub>4</sub>	H <sub>2</sub> SO <sub>4</sub>	Free anion	Protonated form
p-(Tricyanovinyl)-phenyl dicyanomethane	0.60	...	607	332
Methyl dicyanoacetate	-2.78	-2.93	235	<200
Hexacyanoheptatriene	-3.55	-3.90	645	347
Cyanoform	-5.13	-5.00	210	<200
Bis-(tricyanovinyl)-amine	-6.07	-5.98	467	366
Pentacyanopropene	<-8.5	...	395	?
Hexacyanoisobutylene	pK <sub>1</sub> <-8.5	...	370	?
	pK <sub>2</sub> (-2.9) <sup>a</sup>	...	336	370

<sup>a</sup> From a crude extrapolation of  $-H_0 + \log (C_A/C_{HA^-})$  to zero concentration in HClO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub>.

(1) W. J. Middleton, E. L. Little, D. D. Coffman and V. A. Engelhardt, *J. Am. Chem. Soc.*, **80**, 2795 (1958).

TABLE II  
 $H_-$  ACIDITY FUNCTION BASED ON CYANOCARBON ACIDS AS INDICATORS (COMPARED WITH  $H_0$ ), 25°

Acid concn. (molarity)	Acid						Acid concentration; wt. %	$H_2SO_4$		
	$H_-$	$H_0$	$H_-$	$H_0$	$H_-$	$H_0$		$H_-$	$H_0$	
0.1	...	+0.83	+1.10	...	10	-0.09	-0.31			
0.25	+0.73	+0.44	+0.75	...	15	-0.55	-0.66			
0.5	+0.44	+0.13	+0.33	...	20	-0.99	-1.01			
0.75	+0.19	-0.07	+0.04	-.04	25	-1.47	-1.37			
1.0	-0.03	-0.26	-0.19	-0.22	30	-1.96	-1.72			
1.5	-0.40	-0.56	-0.58	-0.53	35	-2.44	-2.06			
2.0	-0.75	-0.84	-0.93	-0.78	40	-2.93	-2.41			
2.5	-1.11	-1.12	-1.28	-1.01	45	-3.42	-2.85			
3.0	-1.47	-1.38	-1.60	-1.23	50	-3.91	-3.38			
3.5	-1.82	-1.62	-1.93	-1.47	55	-4.39	-3.91			
4.0	-2.15	-1.85	-2.25	-1.72	60	-4.90	-4.46			
4.5	-2.47	-2.06	-2.59	-1.97	65	-5.57	-5.04			
5.0	-2.76	-2.28	-2.93	-2.23	70	-6.21	-5.65			
5.5	-3.05	-2.51	-3.28	-2.52	75	-6.79	-6.30			
6.0	-3.34	-2.76	-3.62	-2.84	80	-7.28	-6.97			
6.5	-3.61	-3.03	-3.97	-3.22	..	...	...			
7.0	-3.86	-3.32	-4.38	-3.61	..	...	...			
7.5	-4.11	-3.60	-4.79	-3.98	..	...	...			
8.0	-4.36	-3.87	-5.21	-4.33	..	...	...			
8.5	-4.59	-4.14	-5.63	-4.69	..	...	...			
9.0	-4.85	-4.40	-6.05	-5.05	..	...	...			
9.5	-5.12	-4.65	-6.47	-5.42	..	...	...			
10.0	-5.40	-4.89	-6.90	-5.79	..	...	...			

taneously establishing an acidity scale based on their protonation. This scale would be an " $H_-$ " scale in analogy to the Hammett " $H_0$ " scale based on the protonation of neutral bases.<sup>2</sup> Previously it has not been possible to establish such a scale for concentrated acid solutions because suitable indicators have not been available.

Measurements were made on solutions of salts of 1,1,2,3,3-pentacyanopropene,<sup>1</sup> 2-dicyanomethylene-1,1,3,3-tetracyanopropene<sup>1</sup> (hexacyanoisobutylene), bis-(tricyanovinyl)-amine,<sup>1</sup> tricyanomethane (cyanoform)<sup>3</sup> 1,1,2,6,7,7-hexacyano-1,3,5-heptatriene,<sup>4</sup> methyl dicyanoacetate<sup>5</sup> and *p*-(tricyanovinyl)-phenyldicyanomethane<sup>6</sup> in sulfuric and perchloric acid solutions. The protonation of pentacyanopropene could not be detected in 11 *N* perchloric acid or 12 *M* sulfuric acid. In >90% sulfuric acid an irreversible change in the spectrum took place (probably hydrolysis of cyano groups). Hexacyanoisobutylene is a divalent acid which could be reversibly protonated once (second ionization); however, the first ionization could not be detected in 11 *N* perchloric acid.

The requirements for meaningful  $pK_a$  measurements and for establishment of an indicator acidity function<sup>2</sup> that plots of the log of the indicator ratio *vs.* concentration of acid should be parallel at the same acid concentration for different indicators and that the same  $pK_a$  should be obtained from measurements in different acids are adequately fulfilled for most of the cyanocarbon acids studied here. Figure 1 shows plots of  $\log C_{A^-}/C_{HA}$  against acid concentration for sulfuric acid solutions. Table I lists the  $pK_a$  values determined in both

(2) M. A. Paul and F. A. Long, *Chem. Revs.*, **57**, 1 (1957).

(3) E. Cox and A. Fontaine, *Bull. Soc. Chim. France*, 948 (1954).

(4) D. W. Wiley and J. K. Williams and B. C. McKusick, *J. Am. Chem. Soc.*, submitted for publication.

(5) F. Arndt, H. Scholz and E. Frobel, *Ann.*, **521**, 95 (1935).

(6) J. K. Williams, to be published.

sulfuric and perchloric acids. The stronger acids are determined relative to methyl dicyanoacetate which in turn is relative to *p*-(tricyanovinyl)-phenyldicyanomethane. The  $pK_a$  of the latter

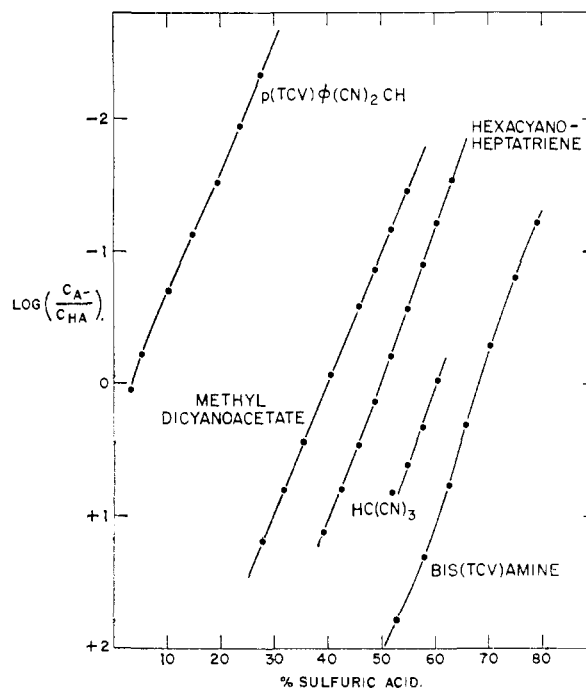


Fig. 1.—The log of the indicator ratio (ratio of concentration of anion to that of protonated form) plotted against sulfuric acid concentration (weight per cent.) for a series of cyanocarbon acid indicators.

was determined directly by measurements down to 0.1 molar where the activity coefficient of  $H^+A^-$  can be established from the Debye-Hückel theory and that of  $HA$  is close to unity.

The  $pK_a$  values thus determined show remarkable strengths for the cyanocarbon acids. Pentacyanopropene and the first ionization of hexacyanoisobutylene are comparable with the stronger mineral acids. Hydrochloric acid, for example, is thought to have a  $pK_a$  in the neighborhood of  $-7$ .<sup>7</sup> A high degree of resonance stabilization in the anions (which is not possible in protonated form) appears to be responsible for the high acidity.<sup>1</sup>

Smoothed values of the acidity function  $H_-$  calculated according to

$$H_- = pK_a + \log C_A/C_{HA}$$

are listed in Table II along with values of  $H_0$  for comparison.<sup>2</sup> The  $H_-$  function presented here is based entirely on *p*-(tricyanovinyl)-phenyldicyanomethane, methyl dicyanoacetate and bis-(tricyanovinyl)-amine.

$H_-$  behaves in a manner surprisingly similar to  $H_0$  although there are quantitative differences. At lower concentrations ( $<5 M$ ) these appear to be of the kind expected on the basis of the differences in charge types. At higher concentrations  $H_-$  roughly parallels  $H_0$ . The similarity of these two acidity functions is further evidence that these indicator acidity functions depend primarily on the properties of the hydrogen ion in solution relatively unencumbered by activity coefficient effects of the indicators themselves.<sup>7</sup>

(7) R. P. Bell, "The Proton in Chemistry," Chaps. VI, VII, Cornell University Press, Ithaca, N. Y., 1959.

CONTRIBUTION No. 714

CENTRAL RESEARCH DEPARTMENT

EXPERIMENTAL STATION

RICHARD H. BOYD

E. I. DU PONT DE NEMOURS AND COMPANY

WILMINGTON 98, DELAWARE

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#### DEMONSTRATION OF THE "CAGE" EFFECT

Sir:

In 1934, Franck and Rabinowitch<sup>1</sup> pointed out that collision times of reactive molecules should be much longer in solution than in the gas phase. Further, in solution, after the colliding molecules have diffused apart by a distance small compared to the mean distance between reactive molecules, there remains a finite probability of re-encounter before diffusion to the mean distance. Effectively the solvent holds the reactive molecules together, forming a "cage." A more sophisticated treatment using the theory of random walks has been given by Noyes.<sup>2</sup>

Numerous observations have been explained by invoking the cage effect with varying degrees of certainty. Probably the best is a recent study by Herk, Feld, and Szwarc,<sup>3</sup> in which they reaffirm Szwarc's previous conclusion that in the photolysis of azomethane in liquid isoöctane, ethane is formed chiefly by recombination of methyl radicals inside the solvent cage. This conclusion was inferred from three observations: that the  $CH_4/N_2$  ratio was independent of azomethane concentration,

(1) Franck and Rabinowitch, *Trans. Faraday Soc.*, **30**, 120, 9 (1934).

(2) R. M. Noyes, *J. Chem. Phys.*, **18**, 999 (1950), and the following papers.

(3) Herk, Feld, and Szwarc, *J. Am. Chem. Soc.*, **83**, 2998 (1961).

that ethane formation could be inhibited effectively by radical scavengers in the gas phase photolysis, but not in the liquid, and that the ratio of  $CH_4 + 2 C_2H_6/N_2$  was nearly 2.

We believe that since the cage effect is an important concept in solution kinetics, it is of value to confirm its existence directly and unequivocally. We wish in this paper to report such a confirmation.<sup>4</sup>

We have photolyzed mixtures of azomethane and  $d_6$ -azomethane in the gas phase and in isoöctane solution using a procedure essentially similar to that of Herk, Feld, and Szwarc.<sup>3</sup> Azomethane was prepared by oxidation of *sym*-dimethylhydrazine with mercuric oxide, while  $d_6$ -azomethane was purchased from Merck of Canada. Both materials were proven to be chemically pure by gas chromatography. The isotopic purity of the  $d_6$ -azomethane was checked by mass spectra. 3%  $CD_3N_2CD_2H$  and no other impurities were found. The isoöctane was spectroscopic grade, further purified by passing through silica gel. Its purity and the cleanliness of our handling procedure were checked by ultraviolet spectra.

The reaction products were analyzed by mass spectroscopy. The cracking patterns of  $N_2$ ,  $CD_3NNCD_3$ ,  $CH_3NNCH_3$ , and  $C_2H_6$  were determined for our mass spectrometer, while the cracking patterns of  $C_2D_6$ ,  $C_2D_5H$ , and  $CD_3CH_3$  were kindly provided by Mr. J. Bell of Harvard University.<sup>5</sup>  $C_2D_6$ ,  $C_2D_5H$ ,  $CD_3CH_3$ , and  $C_2H_6$  were determined from the mass 36, 35, 33, and 27 peaks, respectively. From these peak heights, the remaining peaks in the 36 to 24 range could be predicted within 4%.

In the gas phase photolysis of azomethane one would expect ethane to be formed by random recombination of methyl radical. For such a random process, the ethane is formed in proportions such that  $(CH_3CD_3)^2/(C_2H_6)(C_2D_6) = 4$ . The observed ratio of parent peak heights is  $4.1 \pm 0.8$ .

However in the isoöctane solution photolysis, the mass 33 peak is less than 2% of the 36 peak. This peak can be accounted for satisfactorily by the contributions of isotopic carbon and  $CD_3H$ . The authors estimate that if any  $CD_3CH_3$  is formed, it must be less than 0.3% of the total ethane.

(4) We have recently become aware of an unpublished study by Ausloos and co-workers at the National Bureau of Standards, whose results somewhat parallel our results.

(5) J. Bell, private communication.

ESSO RESEARCH & ENGINEERING CO.

LINDEN, NEW JERSEY

RICHARD K. LYON

DONALD H. LEVY

RECEIVED SEPTEMBER 11, 1961

#### ROTATORY-DISPERSION CHANGES DURING THE THERMAL DENATURATION OF CHYMOTRYPSINOGEN AND CHYMOTRYPSIN

Sir:

The effects of denaturing reagents such as urea in producing drastic unfolding of proteins have become so well known as to direct our attention away from the search for other interpretations of major denaturation reactions. In particular, the large entropy changes associated with reversible thermal denaturations may not be the result of