torily constant and showed no systematic trends with ionic strength, as is illustrated by the data in Fig. 1. Dielectric constants for 54.2% ethanol were computed from the equation, $\log D = 1.6806 - 0.00244\ [t(°C.) - 20]$, which is based on the data of Akerlöf.[9] Dielectric constants for water were taken from the work of Wyman.[10]

Acknowledgment.—This work was supported in part by the Office of Naval Research.

(9) G. Akerlöf, THIS JOURNAL, **54**, 4125 (1932).
(10) J. Wyman, *Phys. Rev.*, [2] **35**, 623 (1930).

DEPARTMENTS OF CHEMISTRY
FLORIDA STATE UNIVERSITY
TALLAHASSEE, FLORIDA, AND
UNIVERSITY OF COLORADO
BOULDER, COLORADO

Hydroxylysine in Proteins

BY PAUL B. HAMILTON AND R. A. ANDERSON

RECEIVED DECEMBER 3, 1955

Hydroxylysine was first recognized among the acid hydrolytic products of gelatin by Van Slyke and Hiller[1] and later isolated and identified by Van Slyke, Hiller, Dillon and MacFadyen.[2] Van Slyke, Hiller and MacFadyen[3] determined the hydroxylysine content of various proteins and found that collagen and gelatin were characterized by approximately 1% of their total nitrogen as hydroxylysine. Cotton seed globulin, casein, zein and aleuronate appeared to have approximately 0.2 to 0.5% of total nitrogen as hydroxylysine nitrogen. Other proteins appeared to have negligible amounts of hydroxylysine. MacPherson[4] concluded that hydroxylysine was present only in collagen and gelatin. Desnuelle and Antonin[5] were in essential agreement with Van Slyke, Hiller and MacFadyen.[3] They failed to find evidence for hydroxylysine in casein but claimed that small amounts (0.1%) were present in beef albumin, edestin, ovalbumin, rat muscle and Bence-Jones protein. Middlebrook[6a] reported 0.18% of total nitrogen in sheep's wool as hydroxylysine; Simmonds[6b] found 0.7% in sheep's wool. Inskip[7] could find no evidence for hydroxylysine in casein, lactalbumin, glycinin or zein. It was of doubtful occurrence in keratin (human hair) and the evidence for its presence in wool (sheep) was inconclusive.

Because of the ease of resolving mixtures of basic amino acids on short columns of ion exchange resins as described by Moore and Stein,[8] it seemed worthwhile to examine a number of protein hydrolysates. The analytical procedure employed is capable of detecting 0.05 micromole of amino acid (0.001 mg. approximately of hydroxylysine nitrogen) with certainty. Where zero values are reported (Table I), not even a trace of hydroxylysine was indicated in the position on the chromatogram normally occupied by hydroxylysine. The traces of hydroxylysine encountered by Van Slyke, Hiller and MacFadyen[3] in proteins other than the collagen group would seem likely attributable to the difficulties inherent in the quantitative specific precipitation of hydroxylysine at very low concentrations. From the evidence presented it appears that collagen is the only protein discovered so far that contains hydroxylysine.

TABLE I

	Lit. reference number (3)	(4)	(5)	(7)	Present paper
	Hydroxylysine N as % of toal N, %				
Collagen (ox cortical bone)	0.9				1.0–1.3
Collagen (human cortical bone)					0.8
Cartilage (beef)					1.5
Skin—Beef (raw)					1.0
Calf (raw)					1.3
Pork (raw)					0.7
Gelatin (from skin or bone)	0.9	1.1–1.2	1.0–1.1		1.0–1.2
Icthyocol (Sturgeon)					0.8
Elastin			0		0
Albumin (bovine plasma)			0.1		0
Fibrin (beef blood)					0
Plasma (human)					0
Hemoglobin (horse)	0	0			0
Serum (horse)					0
Casein (Hammarsten)	0.33	0	0	0	0
Lactalbumin	0.03	0		0	0
β-Lactoglobulin	0.02	0			...
Myosin		0			...
Muscle (rat)			0.1		...
Insulin		0			...
Bence-Jones protein			0.2		...
Keratin (hair, human)				?	0
Keratin (horn, cow)[a]					0
Keratin (hoof, cow)					0
Keratin (feathers, duck)					0
Keratin (wool, sheep)	0.11	0		+?	0[b]
Ovalbumin	0.09	0	0.1		0
Salmine		0			...
Protamine					0
Fibroin (silk)		0			0
Tobacco mosaic virus		0			...
Edestin		0			0
Gliadin	0.12	0	0.1		...
Zien (corn)	0.33	0		0	0
Gluten (wheat)					0
Globulin (pumpkin seed)	0.10				0
Aleuronate					0
Glycinine (soy bean)			0	0	
Peptone (Wittes)					0
Muscle (cod)					0[c]

[a] Some commercial preparations of keratin contain ornithine, but fresh untreated tissue, *i.e.*, hair, horn, hoof, feathers or wool, do not; its presence in the processed material is presumably an artifact.[9] [b] Middlebrook[6a] reported 0.18% of total nitrogen. [c] G. Agren (*Acta Physiol. Scand.*, **7**, 134 (1944)) reported 1.1% of total nitrogen in cod muscle (Swedish).

Experimental

Hydrolysates were prepared by refluxing approximately 1 g. of protein with 100 ml. of 6 *N* hydrochloric acid for 20 hours. The nitrogen content of each hydrolysate was determined by macro Kjeldahl. From a portion of hydrolysate, excess acid was evaporated and the residue dissolved in water so that approximately 1.5 mg. of nitrogen was contained in each ml. of solution. One ml. of solution was placed on an 0.9 × 15 cm. column of Dowex 50, 8% cross-linked, 200 to 400 mesh, operated in the sodium form. The columns were jacketed at 25°. The method was that of Moore and Stein,[8] but the buffer sequence employed to develop the column was that described by Hamilton and

(1) D. D. Van Slyke and A. Hiller, *Proc. Natl. Acad. Sci.*, **7**, 185 (1921).
(2) D. D. Van Slyke, A. Hiller, R. T. Dillon and D. A. MacFadyen, *Proc. Soc. Exper. Biol. Med.*, **38**, 548 (1938).
(3) D. D. Van Slyke, A. Hiller and D. A. MacFadyen, *J. Biol. Chem.*, **141**, 681 (1941).
(4) H. T. MacPherson, *Biochem. J.*, **40**, 470 (1946).
(5) P. Desnuelle and S. Antonin, *Biochim. Biophys. Acta*, **1**, 50 (1947).
(6) (a) W. R. Middlebrook, *Nature*, **164**, 321 (1949); (b) D. H. Simmonds, *Aust. J. Biol. Sci.*, **7**, 98 (1954).
(7) L. W. Inskip, THIS JOURNAL, **73**, 5463 (1951).
(8) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

Anderson,[9] to give more certain resolution of hydroxylysine from histidine.

(9) P. B. Hamilton and R. A. Anderson, *J. Biol. Chem.*, **211**, 95 (1954).

DEPARTMENT OF BIOCHEMISTRY
ALFRED I. DU PONT INSTITUTE
NEMOURS FOUNDATION
WILMINGTON, DELAWARE

The Stability of Metal Chelates of 5-Sulfo-anthranilic Acid

BY WILLIAM F. HARRIS AND THOMAS R. SWEET

RECEIVED DECEMBER 17, 1954

During an investigation of the analytical properties of derivatives of anthranilic acid, the stability constants of 5-sulfo-anthranilic acid with several transition metals were determined. The Bjerrum[1] titration method as modified by Calvin and Wilson[2] was adapted to the present work.

Experimental

Materials.—The 5-sulfo-anthranilic acid was prepared by a modification of the procedure suggested by Moser.[3] Thirty-four grams of anthranilic acid and 25 g. of sulfuric acid were thoroughly mixed in a beaker that was cooled in an ice-bath. The mixture was then ground in a mortar until it was a fine powder. The powder was heated in an oven at 150° for about 5 hours and was mixed. This was followed by heating at 180° for 9 hours. The product was crystallized 3 times from a 50% acetic acid solution, washed with acetone, and dried at 110°.

The metal solutions were prepared from the nitrate salts of the metals. The nickel, copper and zinc solutions were standardized by direct titration with a 0.015 *N* solution of disodium dihydrogen ethylenediaminetetraacetate. The cadmium and cobalt solutions were standardized by the addition of a known excess of a 0.015 *N* solution of disodium dihydrogen ethylenediaminetetraacetate and titration with a 0.015 *N* solution of zinc.

Procedure.—The titration vessel contained the metal ion in 100 ml. of aqueous solution. The 5-sulfo-anthranilic acid was added as a solid because it decomposed on standing in aqueous solution. The standard carbonate-free NaOH solution was added from a 10-ml. buret in which volume measurements could be estimated to within ±0.002 ml. After each addition of NaOH, the *p*H was measured with a Beckman model G *p*H meter that was standardized with Beckman buffer solutions at *p*H values of 4 and 7. During the titration the temperature was maintained at 35 ± 0.2°, carbon dioxide was excluded from the solution, and constant stirring was provided.

Calculation.—The calculations were made by adapting the method of Calvin–Bjerrum to the present situation.

H_2R represents the reagent, 5-sulfo-anthranilic acid

K represents the acid dissociation constant of the carboxyl group

T_M represents the total added metal concentration

T_{H_2R} represents the total added reagent concentration

From the equations for the conservation of species, charge balance and dissociation constant

$$T_M = [M^{++}] + [MR] + [MR_2^-]$$

$$T_{H_2R} = [HR^-] + [R^=] + 2[MR_2^-] + [MR]$$

$$[NO_3^-] = 2T_M$$

$$2T_{H_2R} + [OH^-] - [Na^+] = [H^+] + [HR^-]$$

$$K = [H^+][R^=]/[HR^-]$$

$$2[M^{++}] + [H^+] + [Na^+] = [NO_3^-] + 2[R^=] + [OH^-] + 2[MR_2^-] + [HR^-]$$

the following expressions for $\bar{n}$ and $R^=$ were obtained

$$\bar{n} = \left([H^+] + [Na^+] - T_{H_2R} - \frac{K}{[H^+]}(2T_{H_2R} - [Na^+] - [H^+])\right) \Big/ T_M$$

$$[R^=] = \frac{K}{[H^+]}(2T_{H_2R} - [Na^+] - [H^+])$$

The ionization of the sulfonic acid group was considered complete in all the calculations. Since a titration of the reagent with NaOH showed that the pK value of the protonated nitrogen is less than 2, a consideration of this ionization constant was omitted from the calculations. The treatment was further simplified by the fact that it was not necessary to add an excess of mineral acid to the solution prior to the titration.

Results and Discussion

The dissociation constant of 5-sulfo-anthranilic acid was found to be 2.00×10^{-5}. The chelate formation constants that were determined are given in Table I. The concentrations of metal salts and the concentrations of 5-sulfo-anthranilic acid in the table represent the total concentrations of each of these substances before the addition of NaOH.

TABLE I

CHELATE FORMATION CONSTANTS IN WATER AT 35°

Metal	Metal concn. $\times 10^3$ M	Reagent concn. $\times 10^3$ M	$k_1 \times 10^{-2}$	$k_2 \times 10^{-2}$	$K_{av} \times 10^{-2}$
Cu	1.01	6.84	2.45[a]	5.75	1.19[b]
	1.01	9.12	2.29[a]	5.75	1.15[b]
Zn	1.05	9.12	8.33	2.76	4.79
	1.05	13.68	7.58	2.28	4.16
Ni	1.02	6.84	7.58	2.24	4.12
	1.02	11.40	7.58	2.24	4.12
Cd	1.05	11.40	6.90	2.40	4.07
	1.05	13.68	7.09	2.51	4.22
Co	1.09	9.12	6.62	2.14	3.76
	1.09	11.40	6.62	2.14	3.76

[a] $k_1 \times 10^{-3}$. [b] $K_{av} \times 10^{-3}$.

The order of stability of the metals with 5-sulfo-anthranilic acid was found to be Cu, Zn, Ni, Cd and Co. This order agrees with that found for *o*-amino-phenol by Charles and Freiser,[4] with the exception that Cd was not included in their work and the positions of Zn and Ni were reversed. However, the values for the stability constants of these two metals are so nearly alike in the present work that their relative positions could have been reversed by experimental errors.

(4) R. C. Charles and H. Freiser, THIS JOURNAL, **74**, 1385 (1952).

DEPARTMENT OF CHEMISTRY
THE OHIO STATE UNIVERSITY
COLUMBUS, OHIO

Benzoylations of 2-Methoxylepidine and 4-Methoxyquinaldine by Means of Potassium Amide

BY CHARLES R. HAUSER AND JAMES G. MURRAY[1]

RECEIVED DECEMBER 23, 1954

Although 2-methoxyquinoline has been shown to react with potassium amide in liquid ammonia at room temperature to form 2-aminoquinoline,[2] it seemed possible to benzoylate the methyl group of 2-methoxylepidine (I) with methyl benzoate by

(1) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

(2) M. Calvin and K. W. Wilson, THIS JOURNAL, **67**, 2003 (1945).

(3) E. Moser, U. S. Patent 2,353,351.

(1) Eli Lilly Fellow, 1952–1954.

(2) F. W. Bergstrom, *J. Org. Chem.*, **3**, 233 (1938).