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The Apparent Ionization Constants and Ultraviolet Spectra of *o*-, *m*- and *p*-Chloro- and *p*-Sulfamyl-DL-phenylalanine

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At least four problems under investigation in these Laboratories, *i.e.*, the enzymatic synthesis of acylated α-amino acid amides,[3] the specificity and kinetics of chymotrypsin action,[4] the development of metabolic antagonists of the natural α-amino acids,[5] and the relation between chemical structure and thyroxine-like activity[6] have required the use of nuclear substituted phenylalanines or their analogs[7] and it thus has become imperative to have at hand more information regarding the physical and chemical properties of these compounds than is presently available. In this communication we wish to report observations relative to the apparent ionization constants and ultraviolet absorption spectra of the three isomeric nuclear substituted monochloro-DL-phenylalanines and of *p*-sulfamyl-DL-phenylalanine.

The apparent ionization constants of phenylalanine and the above four nuclear substituted phenylalanines, in 0.1 formal aqueous sodium chloride at approximately 25°, are given in Table I. It appears that the earlier values[8,9] for the pK'_{CO_2H} of phenylalanine are either too high (2.58) or too low (1.83) since the former is inconsistent with values reported for alanine and its derivatives[10] and the latter with values now available for additional derivatives of phenylalanine. A nuclear chlorine atom, irrespective of its position, was found to increase the acid strength of the ammonium group in phenylalanine by approximately 0.2 of a pK unit whereas a *p*-sulfamyl group caused an increase of approximately 0.5 of a pK unit. In contrast the above nuclear substituents had relatively little effect upon the acid strength of the carboxyl group.

An attempted independent determination of the acid ionization constant of the sulfamyl group in *p*-sulfamyl-DL-phenylalanine by a spectroscopic technique[11] was not successful and the assignment of the first, second, and third ionization constants found by titration, to the carboxyl, ammonium, and sulfamyl groups, respectively, was necessarily based upon comparison with representative values for other compounds containing these functional groups.[10]

The principal features of the ultraviolet absorption spectra of phenylalanine, the three isomeric nuclear substituted monochlorophenylalanines, and *p*-sulfamylphenylalanine are given in Table II. The spectrum of phenylalanine is in agreement with that reported by Smith[12] with the exception that the band in the 250-260 mμ region was found to have a fine structure not seen in Smith's curves. The replacement of any one of the nuclear hydrogen atoms by a chlorine atom, or of the *p*-hydrogen atom by a sulfamyl group, produced the expected[13] batho- and hyperchromic effects.

Experimental

***o*-Chloro-DL-phenylalanine** (I).[14]—Simultaneous reduction and hydrolysis[15] of 77 g. of 2-phenyl-4-(*o*-chlorobenzal)-5-oxazolone,[16,17] m. p. 159–161° (cor.) gave after successive recrystallizations from aqueous-ammonia and aqueous-methanol (90% methanol) 16 g. of I, long silky needles, m. p. 241–242°, dec. In contrast to phenylalanine and the other substituted phenylalanines described in this communication, I did not form well-defined crystals when recrystallized from water.

Anal. Calcd. for $C_9H_{10}O_2NCl$ (199): C, 54.3; H, 5.0; N, 7.0. Found: C, 54.6; H, 5.3; N, 6.8.

***m*-Chloro-DL-phenylalanine** (II).[18]—Reductive hydrolysis[15] of 109.5 g. of 2-phenyl-4-(*m*-chlorobenzal)-5-oxazolone,[18,19] m. p. 166–166.8° (cor.), gave after recrystallization from aqueous-ammonia 25.3 g. of II, colorless platelets, m. p. 239–241°, dec.

Anal. Calcd. for $C_9H_{10}O_2NCl$ (199): C, 54.3; H, 5.0; N, 7.0. Found: C, 54.3; H, 4.9; N, 7.1.

***p*-Chloro-DL-phenylalanine** (III).[20]—Proceeding as described above, 110 g. of 2-phenyl-4-(*p*-chlorobenzal)-5-oxazolone, m. p. 196–197° (cor.) gave 42.6 g. of III, colorless platelets, m. p. 258–259°, dec., after recrystallization from aqueous-ammonia.

Anal. Calcd. for $C_9H_{10}O_2NCl$ (199): C, 54.3; H, 5.0; N, 7.0. Found: C, 54.4; H, 5.4; N, 7.0.

***p*-Sulfamyl-DL-phenylalanine** (IV).—*p*-Formyl-benzenesulfonamide (V), m. p. 115–116° (cor.), was prepared by the oxidation of *p*-toluenesulfonamide with chloramine-

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(3) E. L. Bennett and C. Niemann, THIS JOURNAL, **70**, 2610 (1948).

(4) R. V. MacAllister, K. M. Harmon and C. Niemann, *J. Biol. Chem.*, **177**, 767 (1949).

(5) H. K. Mitchell and C. Niemann, THIS JOURNAL, **69**, 1232 (1947).

(6) C. Niemann and J. F. Mead, *ibid.*, **63**, 2685 (1941).

(7) C. Niemann, R. N. Lewis and J. T. Hays, *ibid.*, **64**, 1678 (1942).

(8) P. Hirsch, *Biochem. Z.*, **147**, 433 (1924).

(9) S. Miyamoto and C. L. A. Schmidt, *J. Biol. Chem.*, **90**, 165 (1931).

(10) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943.

(11) W. Stenstrom and N. Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).

(12) F. C. Smith, *Proc. Roy. Soc. (London)*, **B104**, 198 (1929).

(13) E. A. Braude, *Ann. Reports, Chem. Soc.*, **42**, 105 (1946).

(14) H. R. Henze, W. B. Whitney and M. A. Eppright, THIS JOURNAL, **62**, 565 (1940).

(15) H. B. Gillespie and H. R. Snyder, "Organic Syntheses," Coll. Vol. II, J. Wiley and Sons, Inc., New York, N. Y., 1943, p. 489.

(16) F. Mauthner, *J. prakt. Chem.*, **95**, 55 (1917).

(17) J. S. Buck and W. S. Ide, "Organic Syntheses," Coll. Vol. II, J. Wiley and Sons, Inc., New York, N. Y., 1943, p. 55.

(18) L. Flatow, *Z. physiol. Chem.*, **64**, 367 (1910).

(19) J. S. Buck and W. S. Ide, THIS JOURNAL, **54**, 3302 (1932).

(20) E. Friedmann and C. Maase, *Biochem. Z.*, **27**, 97 (1910).

T.[21-23] From 2 kg. of chloramine-T sufficient anilide was obtained to permit the isolation of 150 g. of twice recrystallized V. V gave a phenylhydrazone, yellow leaflets, m. p. 237–238° (cor.) after recrystallization from ethanol.

Anal. Calcd. for $C_{13}H_{13}O_3N_3S$ (275): C, 56.8; H, 4.8; N, 15.3; S, 11.6. Found: C, 56.9; H, 4.8; N, 15.2; S, 11.6.

A mixture of 18.5 g. of V, 17.9 g. of hippuric acid, 16.5 g. of sodium acetate, and 100 ml. of acetic anhydride was heated on a steam-bath for three hours, the reaction mixture cooled, the precipitate collected and washed with acetic acid and ethanol to give 40 g. of crude 2-phenyl-4-(*p*-sulfamylbenzal)-5-oxazolone (VI). Thirty grams of crude VI was dissolved in 50 ml. of 5 *N* methanolic sodium hydroxide and 100 ml. of water, the solution heated to boiling, chilled and acidified with 5 *N* hydrochloric acid. The crystalline precipitate was collected, recrystallized from aqueous ethanol and dried to give 19.5 g. of crude α-benzamido-*p*-sulfamyl-cinnamic acid (VII), m. p. 194–195° (cor.) with preliminary sintering at 150°. The crude VII was recrystallized twice from hot water to give VII, sheaves of colorless needles, m. p. 197.5–198.5° (cor.) with preliminary sintering at 150°. VII was dried at 100° prior to analysis.

Anal. Calcd. for $C_{16}H_{14}O_5N_2S$ (346). C, 55.5; H, 4.1; N, 8.1. Found: C, 55.0; H, 4.0; N, 8.1.

A suspension of 150 g. of VII, m. p. 197.5–198.5°, in 600 ml. of ethanol was reduced at 30–40 lb. of hydrogen pressure over 20 g. of 5% palladized charcoal, VII dissolving as the hydrogenation proceeded. After removal of the catalyst the ethanol solution was evaporated to dryness, the residue dissolved in 10 liters of hot water, the solution decolorized and allowed to stand for several days at 5°. The gummy precipitate was collected and dried *in vacuo* to give 103 g. of crude N-benzoyl-*p*-sulfamyl-DL-phenylalanine (VIII). One hundred grams of crude VIII was refluxed for two hours with 2 liters of 2.5 *N* hydrochloric acid, the hydrolysate allowed to stand at 5° for several days, the precipitate collected, the filtrate extracted with ether, and the aqueous phase adjusted to *p*H 2.6 with silver carbonate. The precipitate obtained from the chilled hydrolyzate (above) was washed with ether and dried to give 43 g. of crude VIII which was again refluxed with 800 ml. of 2.5 *N* hydrochloric acid for two hours. The hydrolysate was treated as described above and the aqueous phase, adjusted to *p*H 2.6, was combined with the aqueous phase obtained from the first hydrolysate. This solution was concentrated *in vacuo* to 100–150 ml., the concentrate adjusted to *p*H 6 with acetic acid and ammonium hydroxide, and placed in a desiccator over sulfuric acid. After standing for several months the crystalline residue was dissolved in the minimum quantity of hot water, the solution decolorized, an equal volume of ethanol added to the colorless filtrate and the solution allowed to stand several days at 5°. The crystalline precipitate was collected, washed with ethanol and dried to give 16.5 g. of IV, glistening colorless platelets, m. p. 246–251°, dec.

Anal. Calcd. for $C_9H_{12}O_4N_2S$ (244): C, 44.3; H, 4.9; N, 11.5; S, 13.1. Found: C, 44.4; H, 5.2; N, 11.3; S, 13.3.

The α-amino acid nitrogen content[24] of IV was found to be 5.67% which is in good agreement with the calculated value of 5.74%.

The residue remaining after the second hydrolysis described above was alternately recrystallized from aqueous ethanol and water to give VIII, blunt prisms, m. p. 204–205° (cor.).

(21) H. D. Dakin, *Biochem. J.*, **11**, 79 (1917).

(22) P. Koetschet and J. Koetschet, *Helv. Chim. Acta*, **12**, 669 (1929).

(23) P. Koetschet, J. Koetschet and P. Viaud, *ibid.*, **13**, 587 (1930).

(24) D. D. Van Slyke, R. T. Dillon, D. A. MacFayden and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941).

Anal. Calcd. for $C_{16}H_{16}O_5N_2S$ (348): C, 55.2; H, 4.6; N, 8.0. Found: C, 55.1; H, 4.6; N, 7.8.

Miscellaneous Preparations.—Synthetic DL-phenylalanine (Winthrop) was recrystallized three times from water. Toluenesulfonamide (IX), m. p. 135.7–137.0° (cor.) was obtained by dissolving crude IX in 5% sodium hydroxide followed by precipitation with dilute hydrochloric acid and recrystallization from aqueous ethanol. Eastman Kodak Co. White Label benzenesulfonamide was used without further purification.

TABLE I

APPARENT IONIZATION CONSTANTS OF NUCLEAR SUBSTITUTED PHENYLALANINES

In 0.1 formal aqueous sodium chloride at approximately 25°

Compound (1)	pK'_{CO_2H} (2)	$pK'_{NH_3^+}$ (3)	$pK'_{SO_2NH_2}$ (4)
Phenylalanine	2.16	9.15	
o-Chlorophenylalanine	2.23	8.94	
m-Chlorophenylalanine	2.17	8.91	
p-Chlorophenylalanine	2.08	8.96	
p-Sulfamylphenylalanine	1.99	8.64	10.26

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA OF SEVERAL NUCLEAR SUBSTITUTED DL-PHENYLALANINES

Compound	λ (mμ)	ε
Phenylalanine (in water)	232 (min.)	36
	252 (max.)	148
	254 (min.)	142
	258 (max.)	179
	262 (min.)	133
	263 (max.)	137
o-Chlorophenylalanine (in 0.1 formal aqueous sodium chloride)	213 (max.)	8300
	238 (min.)	43
	263	199
	264	200
	266 (max.)	203
	272 (min.)	142
	273 (max.)	144
m-Chlorophenylalanine (in 0.1 formal aqueous sodium chloride)	213 (max.)	8900
	237 (min.)	47
	260	215
	262	220
	267 (max.)	266
	272 (min.)	173
	274 (max.)	201
p-Chlorophenylalanine (in 0.2 formal aqueous sodium chloride or water)	221 (max.)	11200
	240 (min.)	68
	260 (max.)	226
	262 (min.)	214
	267 (max.)	261
	273 (min.)	169
	275 (max.)	182
p-Sulfamylphenylalanine (in 0.1 formal phosphoric acid or solution 0.1 formal in hydrochloric acid and 0.03 formal in sodium chloride)	224 (max.)	13000
	247 (min.)	269
	262	556
	263	558
	267 (max.)	631
	271 (min.)	431
	274 (max.)	527

Potentiometric Determination of Apparent Ionization Constants.—Twenty-ml. aliquots of 0.01–0.02 formal amino acid in 0.05, 0.10 and 0.20 formal aqueous sodium chloride were titrated at 24.4 ± 2.1° with standard 0.2 normal hydrochloric acid or sodium hydroxide using a Beckman Model G *p*H meter equipped with No. 1170 and No. 1190E electrodes. The constants were evaluated analytically[25,26] recognizing over-lapping ionizations in the case of *p*-sulfamyl-DL-phenylalanine and correcting in every instance for the amount of acid or base added which did not react with the amino acid. As a check, several of the constants were also evaluated by the method of Speakman.[27] The reduced data summarized in Table I were obtained from thirty-three independent titrations which revealed no significant dependence of the ionization constants upon ionic strength over the range studied. The values given are believed to be accurate to within 0.05 of a pK unit.

Absorption Spectra.—All spectra were determined with a Beckman Model DU Quartz Spectrophotometer at a temperature of 25 ± 3° and at intervals of 2 mμ or less from the lower limit of the instrument to 280 mμ and then at 10 mμ intervals to 320 mμ.

(25) H. T. S. Britton, "Hydrogen Ions," 3rd ed., D. Van Nostrand Co., New York, N. Y., 1943.

(26) R. G. Bates, THIS JOURNAL, **70**, 1579 (1948).

(27) J. C. Speakman, *J. Chem. Soc.*, 855 (1940).

Spectrophotometric Titrations.—The procedure of Stenstrom and Goldsmith[11] was employed using solutions approximately 0.001 formal in sulfonamide and adjusted to the desired *p*H maintaining the total ionic strength at approximately 0.12. The values found for $pK'_{SO_2NH_2}$ for benzenesulfonamide were 9.96 ± 0.05 and for *p*-toluenesulfonamide 10.21 ± 0.05.

Summary

The apparent ionization constants and ultraviolet absorption spectra of DL-phenylalanine, *o*-, *m*- and *p*-chloro- and *p*-sulfamyl-DL-phenylalanine have been determined. These nuclear substituents have been found to increase the acid strength of the ammonium group by approximately 0.2 of a pK unit for the chloro-compounds and by 0.5 of a pK unit for the *p*-sulfamido-derivative. Since these substituents effect the acid strength of the carboxyl group to a lesser degree all of the above substituted DL-phenylalanines have apparent isoelectric points which are more acidic than that of the parent amino acid.

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The Viscosities of Binary Liquid Mixtures: Monofluorodichloromethane and Acetone

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Viscosity determinations on a binary liquid mixture of a haloform and a solvent containing donor atoms have been carried out by various investigators[1] and it was recognized at once that it presented a case of marked non-ideality of liquid mixtures. However, all of the early investigators in this field failed to arrive at any satisfactory treatment of the experimental results. The activated complex theory proposed by Eyring[2] does present a method of drawing certain conclusions about the viscosity process which may be expressed as follows

$$\eta = \frac{Nh}{V} \exp. \left[\frac{N_1 \Delta F_1^{\pm} + N_2 \Delta F_2^{\pm} - \frac{\Delta F_m}{2.45}}{RT} \right] \quad (1)$$

This formula has been applied to the benzene–phenol binary mixtures.[3] Here V is the average molar volume and $\Delta F_1^{\pm}$ and $\Delta F_2^{\pm}$ are the free energy of activation for the pure components and ΔF_m is the excess free energy of mixing. There is not sufficient information available at this time to calculate ΔF_m for the mixture treated in this paper and hence the validity of this equation cannot be checked. However, the viscosity results on the monofluorodichloromethane–acetone mixture will be discussed qualitatively on the basis of this theory. The mixture chosen for investigation was of special interest, for the heat of mixing has been determined in this Laboratory[4] and the extreme electronegativity of the fluorine atom in monofluorodichloromethane should give results differing from the less electronegative bromoform and chloroform used by others.

Experimental Details.—A Fischer–Irany type viscometer was used in these determinations. It is a modification of the Ostwald type which allows the flow to take place in a closed system. It was housed in an insulated bath equipped with windows to allow one to view the flow of the liquid. The bath was ice water for the 0° determinations and dry ice–acetone for the −40 and −80° runs. It was stirred with an electric stirrer and the temperature was determined within 0.1° with a single junction copper–constantan thermocouple. The binary mixtures were weighed out and their densities determined all in one operation by employing a special pycnometer designed in this Laboratory. It is constructed of metal and capillary tubing and designed to withstand the high vapor pressures encountered in working with certain fluorine-containing compounds at room temperature. The results at −80° are given in Table I for rounded mole fractions. The acetone used was purified by the method of Shipsey and Werner[5] using sodium iodide. Monofluorodichloromethane

(1) O. Faust, *Z. physik. Chem.*, **79**, 97 (1912).

(2) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941.

(3) J. F. Kincaid, H. Eyring and A. E. Stearn, *Chem. Revs.*, **28**, 301 (1941).

(4) J. R. Lacher, J. J. McKinley and J. D. Park, THIS JOURNAL, **70**, 2598 (1948).

(5) K. Shipsey and E. A. Werner, *J. Chem. Soc.*, **103**, 1255 (1913).