[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Studies in the Physical Chemistry of Amino Acids, Peptides, and Related Substances. IX. The Dissociation Constants of Some Amino Acid Derivatives

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The acidity of any dissociating group often is influenced profoundly by the presence of adjoining substituent groups in the molecule. The significance of such effects for the interpretation of the titration curves of amino acids and proteins has been clearly recognized in recent years.[1-3] As a further contribution to such studies, we have investigated the dissociation of a group of amino acid derivatives which reveal the influence of the amide group, the peptide linkage, and of other substituents upon the dissociation of carboxyl and amino groups. Our measurements of hydantoins permit an identification of the nature of the ionizing group in these ring structures involving CO and NH linkages. The basic dissociation of O-methyl isourea has been reinvestigated. Our measurements confirm earlier studies in revealing a profound difference between this molecule and urea. Also the results here reported give information regarding the superposed effects of two substituents in the same molecule upon a dissociating group.

Preparation of Materials.—In the preparation and handling of the materials here studied, we are profoundly indebted to Dr. Thomas L. McMeekin, who has previously prepared most of the substances which we have investigated and has investigated their physico-chemical properties.[4,5] Dr. McMeekin has supplied us with pure preparations of several of the materials which we have studied and has given us valuable advice in the preparation of the others. *Formylglycine* (m. p. 151–152°) and *hydantoic acid* (m. p. 169–170°) were prepared as has been described previously.[4] *Acetylglycine* was prepared by heating glycine (10 g.) with acetic anhydride (14 g.) in a reflux condenser until a pasty mass formed. The mixture was then collected quickly and dissolved in a little water. Chlorine was bubbled through the dark brown solution until the liquid became nearly colorless. The solution was cooled slowly to produce crystallization, then recrystallized from water (m. p. 205°). The *hydantoin of amino isobutyric acid* (5,5-dimethylhydantoin) was prepared from aminoisobutyric acid and potassium cyanate by the same methods employed for other hydantoins.[4,5] The melting point of the twice recrystallized material was 175–176°.

Pure samples of *hydantoin* (m. p. 218°), *glycylglycine-hydantoic acid* (m. p. 194°), *carbethoxyglycine* (m. p. 75°) were supplied by Dr. T. L. McMeekin. A pure sample of *chloroacetylglycine* was furnished by Mr. J. Sugarman, who had prepared it under the supervision of Dr. McMeekin. Dr. J. P. Greenstein supplied us with a sample of *O-methyl isourea hydrochloride*. After recrystallization from alcohol and ether, a chloride analysis gave Cl 31.97% (calcd. 32.09%).

Determination of Dissociation Constants

The dissociation constants were determined from e. m. f. measurements on the cell

H_2/Acid–Base Solution//Saturated KCl/HgCl/Hg

The hydrogen electrode was a water-jacketed bubbling electrode of the Simms[6] type; the saturated calomel electrode was also water jacketed, the same constant-temperature water supply flowing through both. The cell was calibrated with 0.1 *N* hydrochloric acid, whose *p*H was taken as 1.076, as in previous studies from this Laboratory.[2,3] The e. m. f. of the saturated calomel half cell was taken as 0.2473 volt at 20° and 0.2435 volt at 25°. No correction was made for liquid junction potential. Solutions of the substances to be studied at a known concentration (generally 0.05 molar) were measured out in definite proportions with standard sodium hydroxide (generally 0.10 normal). Glycine amide was treated similarly, with addition of standard hydrochloric acid. For each substance, the *p*H of five or six different solutions, with varying ratios of acid to base were studied, and the values of pK' were calculated from the equation

$$pK' = p\mathrm{H} + \log \frac{a - [\mathrm{H}]}{b + [\mathrm{H}]}$$

where a is the stoichiometric concentration of the acidic form of the molecule studied, and b the stoichiometric concentration of the conjugate basic form, these terms being used in the sense employed by Brönsted.[7] [H] is the estimated hydrogen ion concentration. $-\mathrm{Log}\ [\mathrm{H}] = p\mathrm{H} + \log \gamma\mathrm{H}$, and following Neuberger[8] we have estimated $\log \gamma\mathrm{H}$ by the equation

$$-\log \gamma\mathrm{H} = 0.5\sqrt{\mu}/(1 + \sqrt{\mu})$$

(1) Cohn, *Ergeb. Physiol.*, **33**, 781 (1931).
(2) Greenstein, *J. Biol. Chem.*, **98**, 479 (1931); **95**, 465 (1932); **101**, 603 (1933).
(3) Edsall and Blanchard, THIS JOURNAL, **55**, 2337 (1933).
(4) McMeekin, Cohn and Weare, *ibid.*, **57**, 626 (1935).
(5) McMeekin, Cohn and Weare, *ibid.*, **58**, 2173 (1936).
(6) Simms, *ibid.*, **45**, 2503 (1923).
(7) Brönsted, *Chem. Rev.*, **5**, 284 (1928).
(8) Neuberger, *Proc. Roy. Soc.* (London), **A158**, 68 (1937).

where μ, the ionic strength, is taken as the value of b (Table I) for an uncharged acid like hydantoic acid, or of a (Table I) for a cationic acid like the cation of glycine amide. The exact form of the equation used to calculate log γH affects the estimated pK' value very little; in the most unfavorable case, that of chloroacetylglycine, even the extreme assumption that log γH = 0 would change the calculated pK' value by only 0.004.

The pK' values obtained on all the substances studied are summarized in Table II.

TABLE I

ELECTROMETRIC TITRATIONS OF FORMYLGLYCINE, CHLOROACETYLGLYCINE, HYDANTOIC ACID AND GLYCINE AMIDE

"a" denotes the stoichiometric concentration of the acidic form of the molecule being titrated; "b" the stoichiometric concentration of the basic form. R is the ratio: $(a - [H])/(b + [H])$, where [H] is the hydrogen ion concentration, calculated as described in the text; and $pK' = p\text{H} - \log R$.

a	b	pH	log R	pK'
Formylglycine (Temp. 19.0°)				
0.0350	0.0100	2.948	0.477	3.425
.0227	.0182	3.344	.073	3.417
.0200	.0200	3.433	− .016	3.417
.0050	.0300	4.177	− .786	3.391
.00384	.0308	4.329	− .912	3.417
Chloroacetylglycine (Temp. 20.4°)				
.0350	.0100	2.877	.464	3.341
.0227	.0182	3.326	.072	3.398
.0200	.0200	3.396	− .020	3.376
.0050	.0300	4.145	− .786	3.359
.00384	.0308	4.281	− .912	3.369
Hydantoic Acid (Temp. 20.3°)				
.0350	.0100	3.289	.514	3.803
.0227	.0182	3.705	.086	3.791
.0200	.0200	3.810	− .008	3.802
.0050	.0300	4.587	− .782	3.805
.00384	.0308	4.702	− .908	3.794
Glycine Amide (Temp. 24.3°)				
.0250	.0073	7.395	.535	7.930
.0222	.0137	7.736	.209	7.945
.01875	.0216	8.008	− .061	7.943
.0143	.0319	8.284	− .348	7.936
.00833	.0456	8.644	− .738	7.906

TABLE II

ESTIMATED VALUES OF pK' FOR SUBSTANCES STUDIED IN THIS INVESTIGATION

Substance	Temp., °C	pK'	Earlier values (at 25°)
Hydantoic acid	20.3	3.80	
Glycylglycinehydantoic acid	20.2	3.54	
Acetylglycine	20.0	3.60	3.632[a,8] 3.65[9]
Chloroacetylglycine	20.4	3.37	
Formylglycine	19.0	3.42	
N-Carbethoxyglycine	21.8	3.65	
Hydantoin	24.0	9.12	9.12[10]
5,5-Dimethylhydantoin	23.7	9.19	
Glycine amide	24.3	7.93	
O-Methyl isourea hydrochloride	24.0	9.72	9.80[11]

[a] Neuberger's value at ionic strength 0.023.

The ionic strength in these investigations was varied between 0.01 and 0.03, by varying the relative amounts of the acid and basic forms in solution (see Table I).

(9) Ostwald, *Z. physik. Chem.*, **3**, 170 (1889).
(10) Wood, *J. Chem. Soc.*, **89**, 1831 (1906).
(11) Bruce, THIS JOURNAL, **26**, 457 (1904).

Discussion

The dissociation constants reported here are not, of course, the true dissociation constants valid at infinite dilution. It is doubtful, indeed, whether the latter can be determined accurately by cells with liquid junction of the type here employed.[12] For comparison, we may note that Cohn, Heyroth and Menkin,[13] using a cell of essentially the same type described here, determined pK' for acetic acid at 25° as 4.69 at an ionic strength of 0.03 and estimated pK at infinite dilution as 4.77, if the potential of the 0.1 N calomel half-cell be taken as 0.3357 volt. By very accurate measurements on cells without liquid junction, Harned and Ehlers[14] have determined pK for acetic acid at 25° as 4.756; and MacInnes and Shedlovsky[15] have determined an identical value by extremely careful conductivity measurements. These different figures for acetic acid give a fair indication of the probable discrepancy between the pK' values measured and the true pK values at infinite dilution. It should be noted that, for an acid of the charge type

$$RH \rightleftarrows H^+ + R^-$$

the equation for pK as a function of the ionic strength in very dilute solution at 20–25° should be, from the Debye–Hückel theory

$$pK' = pK - 0.5\sqrt{\mu}$$

Most of the substances studied in this investigation belong to this charge type. On the other hand, acids of the type

$$RH^+ \rightleftarrows H^+ + R$$

should, in very dilute solution, obey the equation

$$pK' = pK + 0.5\sqrt{\mu}$$

This latter relationship should hold for glycine amide and for O-methyl isourea. For acids of the former type, the true pK values may possibly be as much as 0.10 greater than the pK'

(12) See the discussion in a recent paper of Pedersen, *Det. Kgl. Danske Vidensk. Selskab*, **14**, No. 9 (1937).
(13) Cohn, Heyroth and Menkin, THIS JOURNAL, **50**, 696 (1928).
(14) Harned and Ehlers, *ibid.*, **55**, 652 (1933).
(15) MacInnes and Shedlovsky, *ibid.*, **54**, 1429 (1932).

TABLE III

EFFECT OF THE PEPTIDE LINKAGE AND OF OTHER SUBSTITUENTS ON THE DISSOCIATION OF THE CARBOXYL GROUP

The values for Nos. 6, 7, 9, 11, 13, and 14 are from the present investigation. For the indirectly estimated values, Nos. 4 and 12, see ref. 3, Table V (pK_D values). Other values from the review by Cohn[1] and from Landolt-Börnstein's "Tabellen," 5th edition (including the three supplementary volumes).

	Name	Formula	pK' (COOH)
1	*n*-Valeric acid	$CH_3CH_2CH_2CH_2COOH$	4.80
2	δ-Chlorovaleric acid	$ClCH_2CH_2CH_2CH_2COOH$	4.69
3	Levulinic acid	$H_3CCOCH_2CH_2COOH$	4.59
4	Glycine (uncharged)	H_2NCH_2COOH	(4.30)
5	δ-Aminovaleric acid	$^+H_3NCH_2CH_2CH_2CH_2COOH$	4.21
6	Hydantoic acid	$H_2NCONHCH_2COOH$	3.80
7	N-Carbethoxyglycine	$C_2H_5COONHCH_2COOH$	3.65
8	Malonic monoamide	H_2NCOCH_2COOH	3.64
9	Acetylglycine	$CH_3CONHCH_2COOH$	3.60
10	Acetoacetic acid	H_3CCOCH_2COOH	3.58
11	Glycylglycinehydantoic acid	$H_2NCONHCH_2CONHCH_2COOH$	3.54
12	Glycylglycine (uncharged)	$H_2NCH_2CONHCH_2COOH$	(3.46)
13	Formylglycine	$HCONHCH_2COOH$	3.42
14	Chloroacetylglycine	$ClCH_2CONHCH_2COOH$	3.37
15	Glycylglycine (charged)	$^+H_3NCH_2CONHCH_2COOH$	3.14
16	Pyruvic acid	$CH_3COCOOH$	2.49
17	Glycine (charged)	$^+H_3NCH_2COOH$	2.31

values here determined. For substances of the latter type, they may be as much as 0.10 less than our measured values. For a group of acids of the same charge type, however, a series of pK' values determined at approximately the same ionic strength and employing the same type of galvanic cell and e. m. f. measurement should give results comparable among themselves and form a satisfactory basis for estimating the effect of structure upon dissociation.

Influence of the Peptide Linkage.—The value of pK' for acetylglycine (3.60) compared to that typical of the fatty acids (4.80) reveals the strong effect of the peptide (CONH) linkage in increasing acid dissociation. The effect of the C=O group, the NH or NH_2 group, and the Cl atom on the dissociation of the carboxyl group is set forth in detail in Table III.

Hydantoin and 5,5-Dimethylhydantoin.—The fact that these two compounds have nearly identical dissociation constants indicates that the ionizing hydrogens arise from the NH groups, not from the CH_2 group. This behavior is in contrast to that of the closely related compound barbituric acid, for which the work of Wood[10] indicates that the opposite is the case.

The Effect of the Amide Group on the Acidity of the Ammonium Group.—The acid dissociation constant of the $-NH_3^+$ group is about five hundred times as great in glycine amide as in methylamine. Scarcely any other substituent except the —COOR linkage produces so powerful an effect on the $-NH_3^+$ group. (pK' of glycine methyl ester is 7.66.)[3]

O-Methyl Isourea, $HN{=}C(NH_2)OCH_3$.—Our measurements confirm those of Bruce[11] in showing this molecule to be more basic than ammonia, being half combined with acid at pH 9.72. In contrast urea shows no appreciable combination with acid until a pH approaching zero is reached.[16] The iso form of urea, $HN{=}C(NH_2)OH$, should presumably be a base of strength similar to that of O-methyl isourea. The difference, by a factor of more than 10^9, between the two dissociation constants suggests that urea cannot be present in any considerable amount in the iso form in aqueous solution.

An attempt was made to detect combination of O-methyl isourea hydrochloride with acid. The acidity of 0.01 *N* hydrochloric acid (pH 2.022), however, was found not to be appreciably affected by the presence of one equivalent of O-methyl isourea hydrochloride. If this substance combines with acid, then it must do so only at an acidity much greater than this.

Summary

Dissociation constants have been determined electrometrically for formylglycine, acetylglycine, hydantoic acid, glycine amide and several related compounds; also for hydantoin, 5,5-dimethylhydantoin, and O-methyl isourea. The

(16) Walker and Wood, *J. Chem. Soc.*, **83**, 484 (1903).

effect of the peptide linkage on dissociation, and other questions relating to these compounds, are briefly discussed.

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Dialkylaminoalkanol Esters of *p*-Aminobenzoic Acid

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From the time that Einhorn[2] described the preparation of diethylaminoethyl *p*-aminobenzoate and established its practical value as a local anesthetic, the study of analogous compounds with a modification of the alkyl groups on the tertiary nitrogen, of the character of the residue between the nitrogen and oxygen and of the position and kind of groups in the benzene nucleus has received much attention.

An investigation in this Laboratory on many of these compounds was started in 1917 and continued for five or six years thereafter, but the results were not published. In view of recent researches which have just been completed on anesthetic compounds of somewhat analogous structure in the oxazoline, thiazoline and related series, opportunity is taken here to record the accumulated data on the procaine homologs and to make a few general remarks on the deductions on pharmacological action and chemical constitution of these compounds.

Several series of compounds of the general formula, $H_2N-C_6H_4-C(=O)-O-X-NRR_1$ were prepared. They may be divided as follows:

(1) The grouping $NH_2C_6H_4COOCH_2CH_2-$ was kept constant and the $-NRR_1$ was varied. Compounds were synthesized in which the R and R_1 represented two methyls, ethyls, *n*-propyls, isopropyls, *n*-butyls, isobutyls, *s*-butyls, *n*-amyls, isoamyls, allyls, and in which R was allyl and R_1 was *n*-butyl.

(2) A series similar to (1) was prepared in which the grouping $NH_2C_6H_4COOCH_2CH_2CH_2-$ was kept constant. The compound with two cyclohexyl groups for the R and R_1 was included.

(3) The grouping $NH_2C_6H_4COOCH_2CH(CH_3)-$ was kept constant and the $-NRR_1$ was represented by diethylamine, di-*n*-butylamine and diallylamine.

(4) Three compounds of the general formula $NH_2C_6H_4COOCH_2CH(R)N(C_2H_5)_2$, where R was methyl, isobutyl and *n*-hexyl, were synthesized.

(5) The number of methylene groups between the oxygen and nitrogen was varied from two to five inclusive, maintaining in each case two ethyl groups on the nitrogen.

Pharmacological tests were made by Nielsen and Spruth of the Abbott Laboratories.[3] No attempt will be made here to give a detailed correlation, of anesthetic properties and chemical constitution; such would not be justified on the basis of the semiquantitative data available. General deductions, however, which are not without occasional exceptions, may be drawn.

With increase in size of the alkyl groups on the nitrogen, the toxicity increases. The anesthetic value also increases markedly, in fact more rapidly than the toxicity, especially in regard to duration of topical anesthesia. This statement applies in series (1), (2), and (3). A similar result is observed where alkyl groups are introduced on the carbons between the oxygen and the nitrogen as in series (4). Extending the distance between the oxygen and the nitrogen to four or five methylenes, results in increased toxicity, a gradual increase in anesthetic properties, more pronounced in case of topical anesthesia. It may be stated also that the compounds with iso or forked chain alkyls are generally less toxic and less anesthetic than the corresponding compounds with straight-chain alkyls. The majority of the compounds described had a more favorable ratio of M. L. D. to M. E. D. than procaine.

The di-*n*-butylaminopropyl *p*-aminobenzoate as the sulfate has found practical use as an anesthetic for topical anesthesia and is marketed under the name "Butyn."

The various anesthetics were prepared by two methods now well recognized as standard for such compounds: (1) condensation of *p*-nitrobenzoyl chloride with the proper aminoalkanol, and (2) condensation of a dialkylamine with an ω-halogen

(1) Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry.

(2) Einhorn, *Ann.*, **371**, 162 (1909).

(3) Unpublished results.