termination of the spectra of the compounds reported.

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Apparent Dissociation Constants of Cysteine Derivatives

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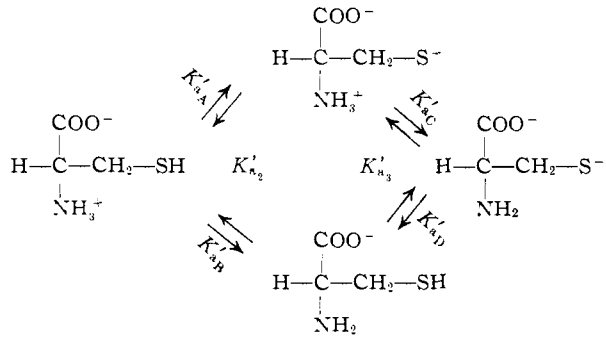
One of the characteristic reactions of the sulfhydryl (–SH) group is the ability to dissociate as a weak acid, $RSH \rightleftarrows RS^- + H^+$. The chemical and biological activity of this group, as it occurs in cysteine (I), coenzymes and proteins, would be expected to vary with the degree of ionization. For this reason, accurate knowledge of the ionization constant of the –SH group is a prerequisite to an understanding of its mechanism of action in both chemical and biological processes.

Inspection of the literature reveals lack of universal agreement concerning the ionization sequence of cysteine.[1] In 1927 Cannan and Knight[2] pointed out that whereas the primary apparent ionization constant (pK'_{a_1}) of cysteine is definitely that of the carboxyl group, a plausible argument could be made for assignment of the pK'_{a_2} and pK'_{a_3} to either the $-NH^+$ or –SH group. Textbooks[3] of protein chemistry list the pK'_{a_2} and pK'_{a_3} values of cysteine as those of the $-NH_3^+$ and –SH groups, respectively. Edsall,[4] on the other hand, regards the secondary and tertiary dissociation constants of cysteine as composite constants which are described by the equilibria



He showed that the individual constants and the two measured values, K'_{a_2} and K'_{a_3}, are related as

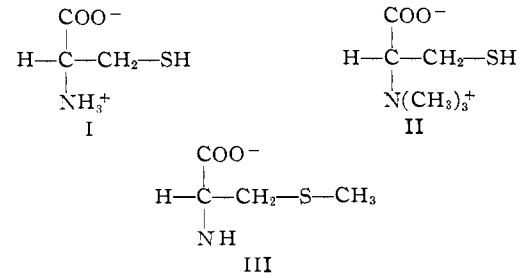
$$K'_{a_2} = K'_{a_A} + K'_{a_B}$$

$$\frac{1}{K'_{a_3}} = \frac{1}{K'_{a_C}} + \frac{1}{K'_{a_D}}$$

$$K'_{a_2}K'_{a_3} = K'_{a_A}K'_{a_C} = K'_{a_B}K'_{a_D}$$

From the experimentally determined values of K'_{a_2} and K'_{a_3} and a probable value of K'_{a_B}, the latter derived from the pK'_{a_2} of 8.60 for S-ethylcysteine,[5] Ryklan and Schmidt[6] have solved for the remaining constants. They found $pK'_{a_A} = 8.66$, $pK'_{a_C} = 10.45$ and $pK'_{a_D} = 10.51$.

In the present investigation we have examined the *alternate* model substance, cysteine betaine (II). This compound contains a permanent positive pole, $-N(CH_3)_3^+$, and the influence of this group on the ionization of the –SH group may be expected to be comparable to that of the $-NH_3$ group of cysteine. For an estimation of K'_{a_B} we have prepared and titrated S-methylcysteine (III), a somewhat closer analog than the S-ethyl derivative.



Experimental

Cysteine (I).—The Eastman Kodak Co. hydrochloride was used as received.

Cysteine Betaine (II).—The chloride was synthesized according to the method of Schubert.[7] The intermediate cystine betaine was crystallized. The crystalline cysteine betaine chloride contained 1.06 equivalent of –SH (as cysteine[8]). The neutral equivalent, with *p*H 7 as end-point, was 200.

Anal.[9] Calcd. for $C_6H_{14}O_2NSCl$: C, 36.08; H, 7.06; N, 7.01; S, 16.05. Found: C, 36.42; H, 7.25; N, 7.06; S, 16.30.

S-Methylcysteine (III).—The inner salt was synthesized according to Armstrong and Lewis,[10] m.p. 148° dec. (lit. 147–148° dec.[10]). Exactly one equivalent of alkali was consumed by 136 g. (one mole) over the *p*H range 6 to 11.

Titration Methods.—Titrations were carried out with standard 1 *N* NaOH in the automatic recording apparatus of Neilands and Cannon.[11] The gas phase was N_2 and the temperature 25°. The solvent was 0.15 *N* NaCl containing 10^{-3} *M* ethylenediaminetetraacetate. The substances were titrated at a concentration of 10 μM per ml. and the pK'_a values read directly from the corrected titration curves.[12]

Results

The measured apparent ionization constants for cysteine, cysteine betaine and S-methylcysteine are recorded in Table I.

TABLE I

APPARENT IONIZATION CONSTANTS OF CYSTEINE, CYSTEINE BETAINE AND S-METHYLCYSTEINE IN 0.15 *N* NaCl AT 25°

	pK'_{a_2}	pK'_{a_3}
Cysteine	8.30	10.40
Cysteine betaine	8.65	...
S-Methylcysteine	8.75	...

Substituting our pK'_{a_2} values of 8.65 and 8.75 for cysteine betaine and S-methylcysteine, re-

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spectively, in the relation $K'_{a_2} = K'_{a_A} + K'_{a_B}$ yields 8.39 as the pK'_{a_2} of cysteine. This is in fairly good agreement with the value 8.30 which we measure experimentally under the same conditions (Table I). From $K'_{a_C} = K'_{a_2}K'_{a_3}/K'_{a_A}$ we find pK'_{a_C} to be 10.05 and from $K'_{a_D} = K'_{a_2}K'_{a_3}/K'_{a_B}$ the figure for pK'_{a_D} is 9.95. The somewhat smaller values of K'_{a_C} and K'_{a_D} reported by Ryklan and Schmidt[6] are a consequence of the higher value which they selected for the pK'_{a_3} of cysteine, 10.78[13] at zero ionic strength. In any event, both K'_{a_C} and K'_{a_D} would be anticipated to be small because of the weakening effect of the net negatively charged intermediate ion species. The individual constants calculated as above give a ratio of *zwitterion form/neutral uncharged form* of 1.3:1, neglecting the full negative charge of the carboxyl group.

Our results support the concept that the acidic strength of the $-SH$ and $-NH_3^+$ groups of cysteine are almost identical. These data are therefore in agreement with the ionization scheme proposed by Edsall.[4]

Acknowledgment.—The authors are indebted to Dr. J. T. Edsall for advice and criticism. This research was supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

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Derivatives of Sulfenic Acids. XXI. Some Reactions of β-Chlorovinyl 2,4-Dinitrophenyl Sulfides[1]

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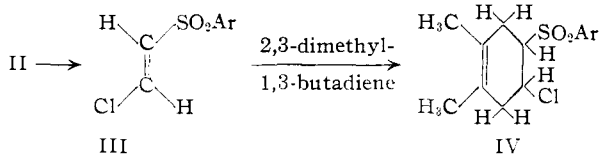
Incidental to other studies, it was of interest to examine the oxidation and catalytic desulfuration of certain β-chlorovinyl 2,4-dinitrophenyl sulfides (II, $R(Cl)C{=}C(R){-}SC_6H_3(NO_2)_2$) which result by adding 2,4-dinitrobenzenesulfenyl chloride (I) to symmetrical alkynes.[2] The results of these reactions and some related observations are reported now.

The adducts (II, R = H, C_6H_5, CH_3 and C_2H_5) did not respond to the usual unsaturation test with bromine in carbon tetrachloride, showing the combined effects which the chlorine and 2,4-dinitrophenylthio groups in these substances exert in lowering the reactivity of the olefin positions to electrophilic attack by bromine. This suggested the possibility that the adducts might be preferentially oxidizable to vinyl sulfones and this was shown to be so for II (R = H), which gave the corresponding 2-chlorovinyl 2′,4′-dinitrophenyl sulfone (III) in 75% yield using hydrogen peroxide in glacial acetic acid. The presence of the double bond in III was demonstrated by forming the Diels–Alder product IV, from III and 2,3-dimethyl-1,3-butadiene.[3] In analogy with evidence[4] which shows that I adds to olefins to give the *trans* adducts, the *trans* arrangement of Cl and $-SAr$ (Ar = 2,4-dinitrophenyl) in II, and of Cl and $-SO_2Ar$ in III and IV, is most likely.



Dienophiles similar to III have been recorded previously[5-7] but the inclusion of both the chloro and sulfone functions is unique to III. In contrast, attempts to add II (R = H or C_6H_5) to 2,3-dimethyl-1,3-butadiene, under conditions used to prepare IV, were not successful.

Treatment of the sulfenyl chloride–diphenylacetylene adduct (II, R = phenyl)[2] with Raney nickel, under typical desulfurating conditions, caused desulfuration, dehalogenation and hydrogenation, giving quantitative conversion to 1,2-diphenylethane.

By proving the presence of the olefin bond, in the case of II (R = H), and by showing that no rearrangements of the carbon chain are involved in the addition of I to diphenylacetylene, the above results—aside from their synthetic interest—also support the structures assigned[2] to the various examples of II.

Experimental[8]

2-Chloroethenyl 2′,4′-Dinitrophenyl Sulfone.—A solution of 0.50 g. (0.0019 mole) of the sulfide (II, R = H) and 5 ml. of 30% hydrogen peroxide in 25 ml. of glacial acetic acid was warmed on the steam-bath for 15 minutes, then left overnight at room temperature. The solution was warmed for two more hours, residual peroxides were decomposed catalytically with platinum wire, and water was added to the hot solution until it became turbid. Pale yellow, feathery plates (0.42 g., 75%), m.p. 114–120°, deposited on standing. The analytical sample was recrystallized from carbon tetrachloride and melted at 122–123°, with prior sintering from 116°.

Anal. Calcd. for $C_8H_5ClN_2O_6S$: C, 32.83; H, 1.72. Found: C, 32.91; H, 1.90.

The product decolorized permanganate in acetone instantaneously, but failed to react with bromine in carbon tetrachloride even at the boiling point of the solvent. In contrast (II, R = H) decolorized permanganate in acetone only slowly and reacted slowly with bromine in carbon tetrachloride, with evolution of hydrogen bromide.

6-Chloro-3,4-dimethyl-Δ³-cyclohexenyl 2′,4′-Dinitrophenyl Sulfone(IV).—Sulfone III (0.50 g., 0.0017 mole) and 5 ml. of 2,3-dimethyl-1,3-butadiene were refluxed in 30 ml. of benzene for 48 hours. Solvent and excess diene were as-

(1) This study was carried out under sponsorship of the Office of Ordnance Research, United States Army, Contract DA-04-495-Ord. 306.

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(3) The infrared spectra of each of the adducts, II, were examined, but a simple assignment to the double bond could not be made because of related absorptions in these complicated molecules. These spectra will be reported, together with a series of related compounds, in a separate manuscript.

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(8) The microanalyses were made by Mr. W. J. Schenck. Melting points are not corrected.