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# Proton Ionisation Constants of Some α-amino-acids Containing Sulphur Atoms in the Chain

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*Received March 15, 1971*

*The proton ionisation constants of DL-4,4'-dithio-bis-(2-aminobutyric acid), (abbrev. DIENK) and of S,S'-methylene-di-L-cisteine,(abbrev. HOMC) have been determined potentiometrically at different temperatures and ionic strengths, μ. The constants at 25.0°C and μ = 0.1 M KCl are for DIENK: –log* $K_{i}$ *=8.954(8), –log* $K_{i2}$ *= 8.158(11), –log* $K_{i3}$ *= 2.200(13), –log* $K_{i4}$ *~ 1.50; and for HOMC: –log* $K_{i}$ *=9.413(10), –log* $K_{i2}$ *= 8.676(13), –log* $K_{i3}$ *= 2.523(14), –log* $K_{i4}$ *~ 1.60. All the ionisation constants are larger than the corresponding ones of α–aminoacids without sulphur atoms in the chain. The capacity to increase the acidic character or to lower the basic character of any group seems to be a general property of sulphur atoms.*

*The thermodynamic functions ΔH and ΔS have been calculated from data of ionisation constants at temperatures between 5° and 30°C.*

*The ionisation constants at different ionic strengths up to 2 M (KCl) have been determined.*

## Introduction

As part of researches on equilibria in solutions concerning amino-acids[1], we have now undertaken the study of the properties of solutions of DL-4,4'-dithio-bis-(2-aminobutyric acid), $[S–(CH_2)_2–CH(NH_2)–COOH]_2$ or homocystine (abbrev. HOMC), and of S,S'-methylene-di-L-cysteine, $CH_2[–S–CH_2–CH(NH_2)–COOH]_2$ or djenkolic acid (abbrev. DIENK).

## Experimental Section

*Materials.* Reagent grade purity substances were employed without further purification. Analysis of the substances:

$C_7H_{14}O_4N_2S_2$ (DIENK):

calcd % = C, 33.06; H, 5.55; N, 11.02; S, 25.22;
found % = C, 33.24; H, 5.62; N, 11.18; S, 24.78;

$C_8H_{16}O_4N_2S_2$ (HOMC):

calcd % = C, 29.96; H, 4.99; N, 11.70; S, 23.90;
found % = C, 29.71; H, 4.83; N, 11.66; S, 23.34.

*Solutions.* Stock solutions of hydrochloric acid were prepared and their concentrations (0.10-0.12 *M*) determined potentiometrically against 2-amino-2-(hydroxymethyl)-1,3-propandiol. Stock solutions of potassium hydroxide (0.23-0.26 *M*) were prepared by diluting concentrated KOH (BDH production) by $CO_2$-free water and their titres checked potentiometrically against potassium hydrogenphtalate. Equivalent points were calculated by Gran's method;[2] the line of best fit was calculated by least squares. Solutions of aminoacid were prepared by dissolving the substance in KCl - solution at 90°C in closed vessel in water bath; then the exact volume of hydrochloric acid and the required amount of water were added. The initial volume of the total solution was 99.695 ± 0.013 ml in each titration. The total volumes of the titrant KOH-solution were 2-4 ml, subdivided by a Metrohm piston-burette into about 40 steps, measured with precision ± 0.005 ml.

*Potentiometric measurements.* Potentiometric determinations were performed in part by a potentiometer Metrohm E 388 equipped with EA 107 UX and EA 107 T glass electrodes and in part by a digital potentiometer Radiometer PHM52 equipped with G 2025B and G 202C glass electrodes. Reference electrode was a KCl sat. calomel electrode. The cell was calibrated twice a day by titrating solutions of hydrochloric acid with potassium hydroxide solutions; average $E_0$ was calculated from several points; the liquid junction potential was assumed to be constant. Values of $pK_W$ quoted by Harned & Owen,[3] for different temperatures and ionic strengths were used in alkaline solutions. Temperature was maintained at the chosen value within ± 0.1°C. The concentration ranges of each titration are reported in Tables I-II for DIENK and HOMC, respectively.

## Calculations

The proton dissociation constants have been calculated using the programs, LGST prepared by Vacca,[4] and GAUSS Z by Tobias.[5] For sake of comparison also the program GAUSS G by Tobias[4]

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(1) A. Braibanti, F. Dallavalle, E. Leporati, and G. Mori, *Inorg. Chim. Acta*, *5*, 449 (1971).

(2) G. Gran, *Analyst*, *77*, 661 (1952).

(3) H.S. Harned & B.B. Owen: The Physical Chemistry of Electrolytic Solutions, Reinhold Publishing Corporation, New York (1958).

(4) A. Vacca, private communication.

(5) R.S. Tobias and M. Yasuda, *Inorg. Chem.*, *2*, 1307 (1963).

**Table I.** Initial concentrations (*m*M), $-\log[H^+]$ and $\bar{n}$ ranges for the titrations of homocystine (HOMC)

| N. | Ionic strength | Temperature | HOMC | $T_H$ | $-\log[H^+]$ | $\bar{n}$ |
|---|---|---|---|---|---|---|
| 1 | 0.034 | 25° | 0.201033 | 0.803863 | 2.593-9.727 | 0.3-2.51 |
| 2 | | | 0.174278 | 0.697005 | 2.603-9.916 | 0.2-2.48 |
| 3 | | | 0.129637 | 0.518375 | 2.887-10.214 | 0.5-2.90 |
| 4 | 0.10 | 25° | 0.103785 | 0.415694 | 2.762-10.506 | 0.0-2.35 |
| 5 | | | 0.102485 | 0.410000 | 2.772-10.988 | 0.0-2.37 |
| 6 | | | 0.103290 | 0.457235 | 2.695-10.455 | 0.0-2.41 |
| 7 | | | 0.166080 | 0.683071 | 2.716-10.551 | 0.0-2.38 |
| 8 | | | 0.120433 | 0.541647 | 2.605-9.864 | 0.1-2.42 |
| 9 | 0.50 | 25° | 0.205814 | 0.821309 | 2.583-9.553 | 0.4-2.51 |
| 10 | | | 0.170291 | 0.681395 | 2.662-9.962 | 0.1-2.43 |
| 11 | | | 0.120135 | 0.650795 | 2.553-9.926 | 0.2-2.46 |
| 12 | 1.00 | 25° | 0.132097 | 0.528748 | 2.774-10.072 | 0.1-2.51 |
| 13 | | | 0.156496 | 0.625441 | 2.720-9.914 | 0.2-2.56 |
| 14 | | | 0.092635 | 0.370405 | 2.913-10.188 | 0.1-2.41 |
| 15 | 1.50 | 25° | 0.134407 | 0.537733 | 2.848-10.083 | 0.2-2.43 |
| 16 | | | 0.174427 | 0.697303 | 2.776-9.537 | 0.5-2.48 |
| 17 | | | 0.098299 | 0.393625 | 2.884-10.093 | 0.2-2.45 |
| 18 | 2.00 | 25° | 0.095244 | 0.381076 | 2.924-10.065 | 0.2-2.52 |
| 19 | | | 0.130015 | 0.518599 | 2.799-10.151 | 0.3-2.56 |
| 20 | | | 0.154715 | 0.618605 | 2.747-9.870 | 0.3-2.59 |
| 21 | 0.10 | 30° | 0.171521 | 0.686145 | 2.617-9.729 | 0.3-2.41 |
| 22 | | | 0.209268 | 0.837024 | 2.720-9.817 | 0.1-2.38 |
| 23 | | | 0.122781 | 0.491135 | 2.813-9.641 | 0.2-2.51 |
| 24 | 0.10 | 20° | 0.148716 | 0.615927 | 2.646-9.769 | 0.4-2.44 |
| 25 | | | 0.119427 | 0.477933 | 2.771-9.746 | 0.4-2.39 |
| 26 | | | 0.081382 | 0.325734 | 2.942-9.858 | 0.2-2.34 |
| 27 | 0.10 | 15° | 0.085481 | 0.541850 | 2.881-9.879 | 0.2-2.33 |
| 28 | | | 0.116036 | 0.464060 | 2.772-9.788 | 0.3-2.35 |
| 29 | | | 0.164590 | 0.658429 | 2.632-9.847 | 0.5-2.45 |
| 30 | 0.10 | 10° | 0.164660 | 0.658740 | 2.693-9.577 | 0.5-2.51 |
| 31 | | | 0.151920 | 0.607880 | 2.634-9.636 | 0.5-2.48 |
| 32 | | | 0.158700 | 0.635320 | 2.620-10.213 | 0.3-2.50 |
| 33 | | | 0.179790 | 0.718900 | 2.575-9.636 | 0.5-2.53 |
| 34 | 0.10 | 5° | 0.166664 | 0.660275 | 2.676-10.076 | 0.1-2.51 |
| 35 | | | 0.141157 | 0.559220 | 2.740-10.049 | 0.4-2.53 |
| 36 | | | 0.102448 | 0.405903 | 2.832-9.997 | 0.3-2.32 |
| 37 | | | 0.185889 | 0.736377 | 2.566-9.986 | 0.4-2.45 |

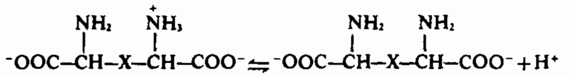
have been applied. All these programs have been assembled by us for the computer CDC 6600 of Consorzio Interuniversitario dell'Italia Nord-orientale, Bologna.

The program LGST minimises the function $\Sigma(\nu_c-\nu_o)^2$ where $\nu_c$ and $\nu_o$ are calculated and observed volume of the titrant KOH solution at each point; the program GAUSS Z minimises the function $\Sigma(\bar{n}_c-\bar{n}_o)^2$ which is the sum of the residuals in the formation function $\bar{n}$ and the program GAUSS G minimises the function $\Sigma(H_c-H_o)^2$, i.e. the residuals in analytical hydrogen ion concentration. Each program has been completed with an analysis of variance of the distribution of the residuals in the minimised function in order to assess if the distribution itself is, or is not, normal with mean $\bar{m} = 0$.

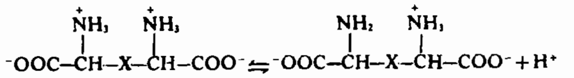
The results obtained for a typical titration from refinements with these three programs are reported in Table III. The results are practically equal. The constants $\beta_4$ have been estimated by trial and not introduced in the refinement because they gave rise sometimes to non-convergence of the refinement procedure. Were they introduced they would have changed $-\log \beta_3$ by $\sim -0.06$ units both for HOMC and DIENK.

## Results and Discussion

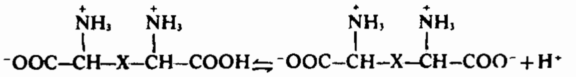
The dissociation constants refer to the equilibria:



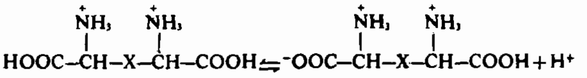
$$^{-}\mathrm{OOC}-\overset{\mathrm{NH_2}}{\mathrm{CH}}-\mathrm{X}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{COO^-} \rightleftharpoons {}^{-}\mathrm{OOC}-\overset{\mathrm{NH_2}}{\mathrm{CH}}-\mathrm{X}-\overset{\mathrm{NH_2}}{\mathrm{CH}}-\mathrm{COO^-}+\mathrm{H^+} \quad (1)$$



$$^{-}\mathrm{OOC}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{X}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{COO^-} \rightleftharpoons {}^{-}\mathrm{OOC}-\overset{\mathrm{NH_2}}{\mathrm{CH}}-\mathrm{X}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{COO^-}+\mathrm{H^+} \quad (2)$$



$$^{-}\mathrm{OOC}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{X}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{COOH} \rightleftharpoons {}^{-}\mathrm{OOC}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{X}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{COO^-}+\mathrm{H^+} \quad (3)$$



$$\mathrm{HOOC}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{X}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{COOH} \rightleftharpoons {}^{-}\mathrm{OOC}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{X}-\overset{\overset{+}{\mathrm{N}}\mathrm{H_3}}{\mathrm{CH}}-\mathrm{COOH}+\mathrm{H^+} \quad (4)$$

where X = $CH_2$-S-S-$CH_2$, $(CH_2)_2$-S-S-$(CH_2)_2$, $CH_2$-S-$CH_2$-S-$CH_2$ and are $K_1$, $K_{12}$, $K_{13}$ and $K_{14}$, respectively. A typical ditribution of the species is represented graphically in Fig. 1. The constants are compared at 25°C and ionic strenght $\mu$ = 0.1 *M* KCl with values for cystine[6, 7], [S-$CH_2$-CH($NH_2$)-COOH]$_2$, in Table IV. The constants obtained by us for HOMC agree fairly well with values obtained by Ryklan and Schmidt.[8] The constants for each equilibrium form

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(6) D.D. Perrin, *J. Chem. Soc.*, 1958, p. 3125.
(7) H. Borsook, E.L. Ellis and H.M. Huffman, *J. Biol. Chem.*, *117*, 281 (1937).
(8) L.R. Ryklan and C.L.A. Schmidt, *Arch. Biochem.*, *5*, 89 (1944).

**Table II.** Initial concentrations (*m*M), —log[H$^+$] and $\bar{n}$ ranges for the titrations of djenkolic acid (DIENK).

| N. | Ionic strength | Temperature | DIENK | $T_H$ | —log[H$^+$] | $\bar{n}$ |
|---|---|---|---|---|---|---|
| 1 | 0.034 | 25° | 0.179958 | 0.719820 | 2.563-10.261 | 0.1-2.31 |
| 2 | | | 0.228358 | 0.913389 | 2.470-9.771 | 0.2-2.36 |
| 3 | | | 0.130693 | 0.522668 | 2.738-10.039 | 0.1-2.24 |
| 4 | 0.10 | 25° | 0.220062 | 0.880165 | 2.446-9.968 | 0.1-2.37 |
| 5 | | | 0.201701 | 0.810658 | 2.591-10.212 | 0.0-2.28 |
| 6 | | | 0.218096 | 0.872424 | 2.809-10.075 | 0.0-2.15 |
| 7 | | | 0.237323 | 0.949377 | 2.379-9.912 | 0.1-2.35 |
| 8 | | | 0.198241 | 0.792910 | 2.561-10.027 | 0.1-2.33 |
| 9 | 0.50 | 25° | 0.225331 | 0.901333 | 2.483-9.962 | 0.1-2.41 |
| 10 | | | 0.152280 | 0.609150 | 2.706-10.038 | 0.1-2.25 |
| 11 | | | 0.117954 | 0.471880 | 2.745-10.089 | 0.0-2.31 |
| 12 | 1.00 | 25° | 0.234453 | 0.937905 | 2.476-9.869 | 0.1-2.43 |
| 13 | | | 0.185305 | 0.741207 | 2.568-9.711 | 0.2-2.38 |
| 14 | | | 0.139264 | 0.557047 | 2.664-9.977 | 0.1-2.29 |
| 15 | 1.50 | 25° | 0.197376 | 0.789567 | 2.563-9.791 | 0.2-2.47 |
| 16 | | | 0.092122 | 0.368505 | 2.897-9.997 | 0.0-2.38 |
| 17 | | | 0.136669 | 0.546621 | 2.713-9.816 | 0.1-2.40 |
| 18 | 2.00 | 25° | 0.135254 | 0.540954 | 2.711-9.957 | 0.1-2.42 |
| 19 | | | 0.183025 | 0.732063 | 2.600-9.728 | 0.2-2.45 |
| 20 | | | 0.148900 | 0.677200 | 2.522-10.893 | 0.1-2.54 |
| 21 | | | 0.150510 | 0.602370 | 2.640-10.314 | 0.1-2.49 |
| 22 | | | 0.210390 | 0.841440 | 2.513-9.817 | 0.2-2.55 |
| 23 | | | 0.199570 | 0.800910 | 2.533-9.823 | 0.2-2.55 |
| 24 | 0.10 | 30° | 0.174296 | 0.697270 | 2.560-9.380 | 0.1-2.24 |
| 25 | | | 0.188018 | 0.752087 | 2.588-9.799 | 0.1-2.46 |
| 26 | | | 0.077338 | 0.309373 | 2.953-9.493 | 0.1-2.29 |
| 27 | 0.10 | 20° | 0.186524 | 0.746044 | 2.608-9.870 | 0.1-2.44 |
| 29 | | | 0.223286 | 0.893099 | 2.464-9.791 | 0.1-2.29 |
| 29 | | | 0.122397 | 0.489602 | 2.723-9.752 | 0.1-2.25 |
| 30 | 0.10 | 15° | 0.113316 | 0.466065 | 2.736-10.017 | 0.1-2.31 |
| 31 | | | 0.080562 | 0.331303 | 2.886-9.977 | 0.1-2.24 |
| 32 | | | 0.151213 | 0.621984 | 2.601-9.808 | 0.2-2.33 |
| 33 | 0.10 | 10° | 0.173329 | 0.696125 | 2.588-9.938 | 0.3-2.34 |
| 34 | | | 0.186485 | 0.749007 | 2.542-9.722 | 0.4-2.36 |
| 35 | | | 0.170860 | 0.687017 | 2.567-9.826 | 0.2-2.32 |
| 36 | | | 0.148099 | 0.594787 | 2.591-9.825 | 0.1-2.41 |
| 37 | 0.10 | 5° | 0.176734 | 0.713539 | 2.694-10.079 | 0.2-2.30 |
| 38 | | | 0.125227 | 0.505674 | 2.691-10.103 | 0.1-2.40 |
| 39 | | | 0.159945 | 0.645884 | 2.586-10.239 | 0.1-2.45 |
| 40 | | | 0.096565 | 0.389965 | 2.763-10.156 | 0.2-2.48 |

**Table III.** Comparison of results from different refinement programs for a typical titration (N. 29 in Table I).

| Program | LGST | GAUSS Z | GAUSS G |
|---|---|---|---|
| log $\beta_1(\sigma)$ | 9.713(4) | 9.711(4) | 9.712(17) |
| log $\beta_2(\sigma)$ | 18.667(2) | 18.664(2) | 18.665(11) |
| log $\beta_3(\sigma)$ | 21.215(3) | 21.210(4) | 21.239(17) |
| $\Sigma\Delta_o^2$ | $4.34657\times10^{-4}$ | $86.63606\times10^{-5}$ | $49.51062\times10^{-9}$ |
| $A$ | $64.45\times10^{-5}$ | $56.16\times10^{-5}$ | $1.06749\times10^{-5}$ |
| $B$ | $0.3729\times10^{-3}$ | $0.1762\times10^{-3}$ | $-2.75809\times10^{-3}$ |
| $F$ | 0.2168 | 0.0201 | 0.5853 |

Line of best fit through $\Delta_o$, $\Delta_c = A+Bx_i$;

$x_i=\bar{n}_o$, $\nu_o$ or $[\mathrm{H}]_{tot}$ $\quad \Delta_o \equiv y_i=(\bar{n}_c-\bar{n}_o),(\nu_c-\nu_o)$ or $(\mathrm{H}_c-\mathrm{H}_o)$ $\quad F=\left\{\frac{[\Sigma(x_i-\bar{x})(y_i-\bar{y})]^2}{\Sigma(x_i-\bar{x})^2}\right\}\Big/\left\{\Sigma(y_i-y)^2-\frac{[\Sigma(x_i-\bar{x})\,(y_i-\bar{y})]^2}{\Sigma(x_i-\bar{x})^2}\right\}$

**Table IV.** Comparison of equilibrium constants of bis($\alpha$-amino) acids at 20°C.

| | Cystine | DIENK | HOMC |
|---|---|---|---|
| —log $K_1$ | 8.80[6] | 9.070 | 9.574 |
| —log $K_{12}$ | 8.03[6] | 8.310 | 8.819 |
| —log $K_{13}$ | 2.05[7] | 2.200 | 2.523 |
| —log $K_{14}$ | 1.04[7] | ~1.50 | ~1.60 |

a sequence obeying the rule cystine < DIENK < HOMC, in accordance with the increasing number of terms, either —CH$_2$— or —S—, separating the two ends of the molecule. This homogenous trend shows how there is no specific influence of the possible conformational isomers, whose existence is suggested by crystal structure determinations of L-cystine[9] and L-cystine hydrochloride.[10] Therefore, the conformational situation is probably common for the three compounds.

The values of the equilibrium constants are mainly influenced by —S— terms in the chain. The influence

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(9) B.M. Oughton and P.M. Harrison, *Acta Cryst.*, *12*, 396 (1959).
(10) A.F. Corsmit, A. Schuyff, and D. Feil, *Proc. K. Ned. Akad. Wet B*, *59*, 470 (1956).

**Table V.** Dependence of the equilibrium constants of DIENK upon temperature. Ionic strength, $\mu = 0.1$ *M* KCl.

| Temperature | | $-\log K_1(\sigma)$ | $-\log \beta_2(\sigma)$ | $-\log K_{12}(\sigma)$ | $-\log \beta_3(\sigma)$ | $-\log K_{13}(\sigma)$ | N.** | $F^{***}_{95\%}$ |
|---|---|---|---|---|---|---|---|---|
| 30° | obs | 8.752(34) | 16.773(24) | 8.021(42) | 19.015(45) | 2.242(51) | 102 | 1.29 |
| | calc* | 8.777 | | 8.038 | | 2.226 | | (3.94) |
| 25° | obs | 8.954(8) | 17.112(7) | 8.158(11) | 19.312(11) | 2.200(13) | 158 | 0.46 |
| | calc* | 8.931 | | 8.166 | | 2.239 | | (3.90) |
| 20° | obs | 9.070(18) | 17.380(14) | 8.310(23) | 19.619(25) | 2.239(29) | 106 | 1.39 |
| | calc* | 9.093 | | 8.300 | | 2.255 | | (3.93) |
| 15° | obs | 9.281(12) | 17.753(9) | 8.472(15) | 20.092(16) | 2.339(18) | 97 | 2.79 |
| | calc* | 9.263 | | 8.441 | | 2.275 | | (3.94) |
| 10° | obs | 9.510(7) | 18.130(5) | 8.620(9) | 20.424(8) | 2.294(9) | 81 | 1.06 |
| | calc* | 9.441 | | 8.590 | | 2.298 | | (3.96) |
| 5° | obs | 9.566(25) | 18.266(19) | 8.700(31) | 20.568(34) | 2.302(39) | 116 | 1.50 |
| | calc* | 9.629 | | 8.747 | | 2.324 | | (3.93) |

(*) Values calculated from the equations:

$$-\log K_1 = \frac{5395.5}{T} - 58.654 + 20 \log T \qquad -\log K_{12} = \frac{4910.5}{T} - 57.793 + 20 \log T \qquad -\log K_{13} = \frac{2852.8}{T} - 56.818 + 20 \log T$$

(**) N. = number of points measured; (***) $F_{95\%}$ = s. Table III.

**Table VI.** Dependence of the equilibrium constants of HOMC upon temperature. Ionic strength, $\mu = 0.1$ *M* KCl.

| Temperature | | $-\log K_1(\sigma)$ | $-\log \beta_2(\sigma)$ | $-\log K_{12}(\sigma)$ | $-\log \beta_3(\sigma)$ | $-\log K_{13}(\sigma)$ | N. | $F_{95\%}$ |
|---|---|---|---|---|---|---|---|---|
| 30° | obs | 9.296(10) | 17.830(7) | 8.534(12) | 20.281(11) | 2.451(13) | 126 | 1.29 |
| | calc* | 9.280 | | 8.540 | | 2.472 | | (3.92) |
| 25° | obs | 9.413(10) | 18.089(8) | 8.676(13) | 20.612(12) | 2.523(14) | 95 | 2.45 |
| | calc* | 9.423 | | 8.668 | | 2.513 | | (3.94) |
| 20° | obs | 9.574(8) | 18.392(6) | 8.819(10) | 20.961(9) | 2.569(11) | 88 | 0.74 |
| | calc* | 9.573 | | 8.803 | | 2.544 | | (3.95) |
| 15° | obs | 9.705(8) | 18.621(6) | 8.916(10) | 21.164(9) | 2.543(11) | 97 | 0.16 |
| | calc* | 9.731 | | 8.946 | | 2.585 | | (3.94) |
| 10° | obs | 9.901(11) | 19.009(7) | 9.108(13) | 21.688(9) | 2.679(11) | 221 | 0.92 |
| | calc* | 9.897 | | 9.096 | | 2.631 | | (3.89) |
| 5° | obs | 10.087(18) | 19.342(12) | 9.255(22) | 21.996(16) | 2.654(20) | 129 | 0.77 |
| | calc* | 10.072 | | 9.254 | | 2.681 | | (3.91) |

(*) Values calculated from the equations:

$$-\log K_1 = \frac{5194.6}{T} - 57.488 + 20 \log T \qquad -\log K_{12} = \frac{4931.2}{T} - 49.312 + 20 \log T \qquad -\log K_{13} = \frac{3227.1}{T} - 57.806 + 20 \log T$$

of sulphur atoms when substituted for $-CH_2-$ group is exemplified by S-methyl-cysteine, $CH_3-S-CH_2-CH(NH_2)-COOH$ ($-\log K_1 = 8.97$[11]) compared with 2-amino-pentanoic acid, $CH_3-(CH_2)_2-CH(NH_2)-COOH$ ($-\log K_1 = 9.808$[12]) and by cystine ($-\log K_1 = 8.80$[6], $-\log K_{12} = 8.03$[6], $-\log K_{13} = 2.05$[7], $-\log K_{14} = 1.04$[7]) compared with 2,7-diaminosuberic acid ($-\log K_1 = 9.89$[13], $-\log K_{12} = 9.23$[13], $-\log K_{13} = 2.62$[13], $-\log K_{14} = 1.84$[13]). In every case sulphur increases the acidic character of both amino and carboxylic groups. The same influence can be observed also in DIENK and in HOMC.

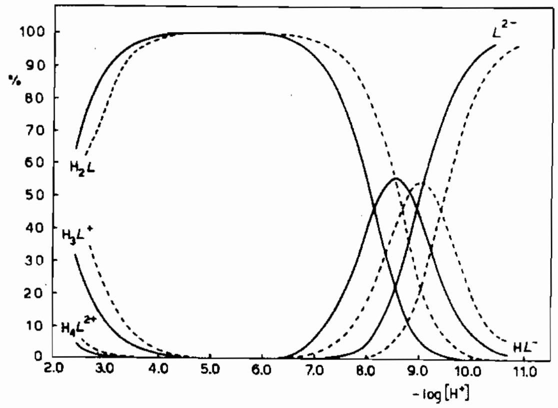

Figure 1. Representative distribution diagram for ionisation equilibria of DIENK(full lines) and HOMC(broken lines). The percentages relative to total ligand have been calculated for DIENK from the data of titration N. 4 in Table II, and for HOMC, from the data of titration N. 4 in Table I.

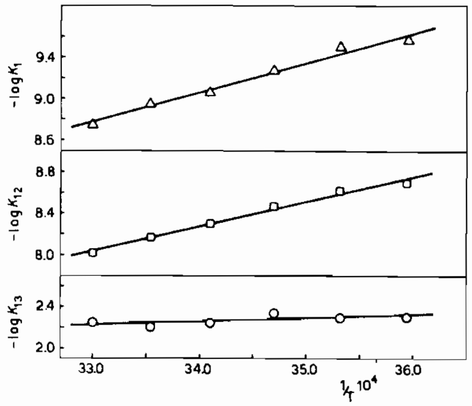

Figure 2. Dependence upon temperature of the proton ionisation constants of DIENK. Curves have been calculated from the equations in Table V.

**Table VII.** Thermodynamic functions for homocystine and djenkolic acid at 25°C.

| | HOMC<br>*A* | DIENK<br>*B* | $\vert\Delta\vert$<br>$\vert A\text{-}B\vert$ | *t** | *d.o.f.*** |
|---|---|---|---|---|---|
| $\Delta H_1(\sigma)$ (kcal mole$^{-1}$) | +11.916(.282) | +12.835(.818) | 0.919 | 1.06 | 5 |
| $\Delta S_1(\sigma)$ (cal mole$^{-1}$ degree$^{-1}$) | —3.09(.95) | +2.09(2.74) | 5.18 | 1.79 | 5 |
| $\Delta H_{12}(\sigma)$ (kcal mole$^{-1}$) | +10.711(.296) | +10.616(.531) | 0.095 | 0.16 | 5 |
| $\Delta S_{12}(\sigma)$ (cal mole$^{-1}$ degree$^{-1}$) | —3.76(.99) | —1.71(1.78) | 2.05 | 1.00 | 5 |
| $\Delta H_{13}(\sigma)$ (kcal mole$^{-1}$) | +2.917(.610) | +1.205(.643) | 1.712 | 1.93 | 5 |
| $\Delta S_{13}(\sigma)$ (cal mole$^{-1}$ degree$^{-1}$) | —1.76(2.05) | —6.02(2.15) | 4.26 | 1.43 | 5 |

(*) $t=\dfrac{|\Delta|}{(\sigma_A^2+\sigma_B^2)^{1/2}}$; (**) *d.o.f.* = degrees of freedom.

The influence of the chain length in the series cystine, DIENK, HOMC on the dissociation constants of the amino-groups is comparable in sign with increments in the constants of diamines ($-\log K_1 = 10.65$, $-\log K_{12} = 9.20$ for 1,4-diaminobutane[14] and $-\log K_1 = 10.93$, $-\log K_{12} = 9.83$ in 1,6-diaminohexane[15]) but the variations are slightly more pronounced in the sulphur-containing compounds. The comparison of the dissociation constants of carboxylic groups of compounds with –S– terms with those of dicarboxylic acids; as for example at 18° C those of pymelic acid[16], $CH_2[(CH_2)_2–COOH]_2$ ($-\log K_{12} = 5.427$), suberic acid[16], $[(CH_2)_3–COOH]_2$ ($-\log K_{12}=5.415$) and azelaic acid[16], $CH_2[(CH_2)_3–COOH]_2$ ($-\log K_{12} = 5.413$) show how the trend is of equal sign in the two series but the increments are much more pronounced in the sulphur-containing series. This behaviour is explained by the character of the sulphur atoms which attract electrons much more than $–CH_2–$ groups do.

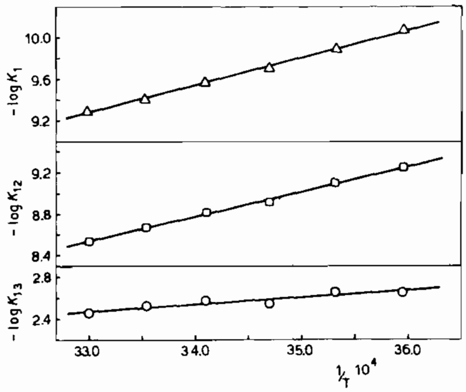

Figure 3. Dependence upon temperature of the proton ionisation constants of HOMC. Curves have been calculated from the equations in Table VI.

The dependence of the equilibrium constants upon temperature for DIENK (Table V, Fig. 2) and HOMC (Table VI, Figure 3) can be represented by the equation:[17]

$$-\log K=\frac{A}{T}-B+20 \log T \qquad (5)$$

Hence the thermodynamic functions $\Delta H$, $\Delta S$ can be calculated by differentiation of (5) (Table VII). The differences between the values of the corresponding thermodynamic functions for the two acids are not significant and no characteristic behaviour can therefore be put in evidence. The values are not very different from values of $\Delta H$, and $\Delta S$ obtained for diamines[18] and dicarboxylic acids[19].

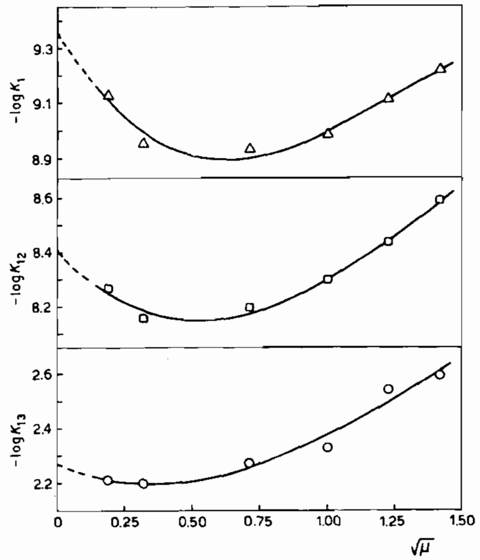

Figure 4. Dependence upon ionic strength at 25°C of the proton ionisation constants of DIENK. Curves have been calculated from the equations in Table VIII.

The dependence upon ionic strength $\mu$ (Table VIII) of $-\log K_1$, $-\log K_{12}$, $-\log K_{13}$ for DIENK is shown in Fig. 4. The behaviour of both constants of the amino-groups repeats the dependence upon ionic

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**Table VIII.** Equilibrium constants of DIENK at different ionic strengths at 25°C.

| Ionic strength | | $-\log K_1(\sigma)$ | $-\log \beta_2(\sigma)$ | $-\log K_{12}(\sigma)$ | $-\log \beta_3(\sigma)$ | $-\log K_{13}(\sigma)$ | N. | $F_{95\%}$ |
|---|---|---|---|---|---|---|---|---|
| 0.034 | obs | 9.127(5) | 17.395(4) | 8.268(6) | 19.605(7) | 2.210(8) | 115 | 0.89 |
| | calc* | 9.106 | | 8.252 | | 2.213 | | (3.93) |
| 0.10 | obs | 8.954(8) | 17.112(7) | 8.158(11) | 19.312(11) | 2.200(13) | 158 | 0.46 |
| | calc* | 8.992 | | 8.186 | | 2.197 | | (3.90) |
| 0.50 | obs | 8.940(6) | 17.140(4) | 8.200(7) | 19.410(8) | 2.270(9) | 77 | 1.04 |
| | calc* | 8.907 | | 8.178 | | 2.253 | | (3.97) |
| 1.00 | obs | 8.992(7) | 17.292(5) | 8.300(9) | 19.618(8) | 2.326(9) | 155 | 2.27 |
| | calc* | 9.008 | | 8.307 | | 2.373 | | (3.93) |
| 1.50 | obs | 9.122(10) | 17.555(8) | 8.443(13) | 20.098(13) | 2.543(15) | 97 | 1.20 |
| | calc* | 9.127 | | 8.452 | | 2.496 | | (3.94) |
| 2.00 | obs | 9.233(31) | 17.830(22) | 8.597(38) | 20.426(29) | 2.596(36) | 221 | 1.92 |
| | calc* | 9.228 | | 8.591 | | 2.612 | | (3.89) |

(*) Values calculated from the equations:

$$-\log K_1 = 9.3573 - 1.6887\mu^{1/2} + 1.8478\mu - 0.5081\mu^{3/2}$$
$$-\log K_{12} = 8.4086 - 1.0736\mu^{1/2} + 1.2638\mu - 0.2923\mu^{3/2}$$
$$-\log K_{13} = 2.2712 - 0.4332\mu^{1/2} + 0.6759\mu - 0.1409\mu^{3/2}$$

**Table IX.** Equilibrium constants of HOMC at different ionic strengths at 25°C.

| Ionic strength | | $-\log K_1(\sigma)$ | $-\log \beta_2(\sigma)$ | $-\log K_{12}(\sigma)$ | $-\log \beta_3(\sigma)$ | $-\log K_{13}(\sigma)$ | N. | $F_{95\%}$ |
|---|---|---|---|---|---|---|---|---|
| 0.034 | obs | 9.457(17) | 18.162(13) | 8.705(21) | 20.737(24) | 2.575(27) | 103 | 15.33 |
| | calc* | 9.463 | | 8.709 | | 2.573 | | (3.94) |
| 0.10 | obs | 9.413(10) | 18.089(8) | 8.676(13) | 20.612(12) | 2.523(14) | 95 | 2.45 |
| | calc* | 9.403 | | 8.668 | | 2.526 | | (3.94) |
| 0.50 | obs | 9.286(8) | 17.920(5) | 8.634(9) | 20.435(7) | 2.515(9) | 107 | 0.43 |
| | calc* | 9.297 | | 8.648 | | 2.515 | | (3.93) |
| 1.00 | obs | 9.322(10) | 18.077(6) | 8.755(12) | 20.702(9) | 2.625(11) | 86 | 2.04 |
| | calc* | 9.313 | | 8.733 | | 2.619 | | (3.95) |
| 1.50 | obs | 9.392(15) | 18.232(9) | 8.840(17) | 20.982(16) | 2.750(18) | 95 | 2.14 |
| | calc* | 9.395 | | 8.857 | | 2.757 | | (3.94) |
| 2.00 | obs | 9.521(15) | 18.526(10) | 9.005(18) | 21.437(15) | 2.911(18) | 67 | 2.32 |
| | calc* | 9.521 | | 9.000 | | 2.908 | | (3.99) |

(*) Values calculated from the equations:

$$-\log K_1 = 9.5613 - 0.5727\mu^{1/2} + 0.1773\mu + 0.1467\mu^{3/2}$$
$$-\log K_{12} = 8.7948 - 0.5575\mu^{1/2} + 0.4934\mu + 0.0025\mu^{3/2}$$
$$-\log K_{13} = 2.6776 - 0.6964\mu^{1/2} + 0.7117\mu + 0.0735\mu^{3/2}$$

strength of glycine. There is a sligth shift of the minimum of the curve of $-\log K_{12}$ at $\mu = 0.27$ *M* with respect to the curve of $-\log K_1$ with minimum at $\mu = 0.37$ *M*, but on the whole one can say that the interactions between the two α-amino-acid moieties of the molecule are very small and they behave as independent. The dependence of $-\log K_{13}$ shows a minimum at $\mu = 0.02$ *M*, and presents therefore a behaviour which resembles that of cation acids, like as glycinium ion, $H_3\overset{+}{N}-CH_2-COOH$, for which this constant increases smoothly with $\sqrt{\mu}$ [20]. On the other hand the dependence upon ionic strength μ (Table IX) of $-\log K_1$, $-\log K_{12}$, $-\log K_{13}$ for HOMC is shown Fig. 5. The constants relative to the two amino-groups are much more differentiated from one another than those of DIENK; the minimum of the curve of $-\log K_1$ occurs at $\mu = 0.65$ *M*, the minimum of the curve of $-\log K_{12}$ at $\mu = 0.32$ *M*. Moreover the curve of $-\log K_{13}$ presents a minimum at $\mu = 0.28$ *M* which means that the behaviour of this biprotonated molecule is very different from that of glycinium cation. Explanations of the different behaviour with ionic strength of the two acids could be only speculative at the present stage of our knowledge.

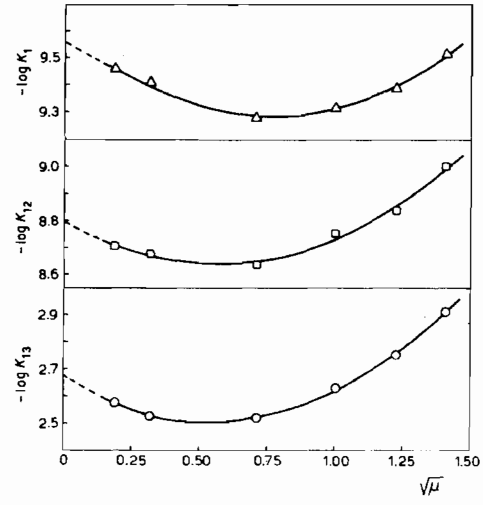

Figure 5. Dependence upon ionic strength at 25°C of the proton ionisation constants of HOMC. Curves have been calculated from the equations in Table IX.

*Acknowledgements.* Consiglio Nazionale delle Ricerche, Rome is kindly thanked for financial help.

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