Tetrahedron Vol. 38, No. 24, pp. 3729 to 3736, 1982
Printed in Great Britain.

0040-4020/82/243729-08$03.00/0

SPECTROMETRIC AND POLAROGRAPHIC STUDY OF THE KETOL CONDENSATION OF 3-THIOHYDROXY-2-OXOPROPANOIC ACID IN ALKALINE SOLUTION

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(*Received in France* 21 *January* 1982)

Abstract—The product of two-electron reduction of 3-thiohydroxy 2-oxo propanoic 1 acid is either β-mercaptolactate or pyruvate when the C–S is cleaved. The first pathway predominates in acidic media, the second in slightly basic media. Comparison of the polarographic and UV and NMR spectrometric behaviour of 1 with that of thioether C_2H_5–S–CH_2–CO–CO_2H 2 indicates that, in the second dissociation step for 1 ($pk_2 = 9.6$), kinetically controlled formation of the thiolate anion occurs which is slowly converted into an ambident carbanion. A ketol dimeric product was isolated as sodium salt and its structure established by ^{13}C NMR study. The ability to form ambident carbanion in slightly basic medium is of importance in essential biological processes.

The formation of a carbanion-enolate and subsequent aldol condensation was discussed in a previous paper concerning 3-hydroxy 2-oxopropanoic acid.[1] This paper deals with the case of 3-thiohydroxy 2-oxopropanoic acid **1** and its 5-ethylderivative **2**. For **1**, there is polarographic, potentiometric and spectrometric evidence for the formation of a ambient carbanion in slightly basic medium.

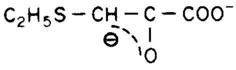
For organic compounds bearing an electronegative group Y on the carbon adjacent to a carbonyl group (Y-CH_2-CO-R), polarographic reduction in hydroxylic solvents involves transfers of electrons and protons which result, either in a cleavage of the C–Y bond, or formation of Y-CH_2-CHOH-R, in the first two electron step.

Which of the two-electron processes is preferred depends (a) on the nature of the group R, (b) on the kind and polarizability of the bond between C and Y, (c) on the substitutents in the group Y, (d) on the material and configuration of the electrode, (e) on the ionic form of the reduced species and hence on pH.[2] In the presence of a phenyl group in substituted acetophenones RS-CH_2-CO-C_6H_5, the cleavage of C-S bond is preferred.[3-5] When compared with a phenyl group, the presence of an electronegative COOH group adjacent to the carbonyl group facilitates reduction of the carbonyl group due to increased conjugation.

According to these concepts, **1** undergoes at the mercury electrodes two competitive pathways in which the product of a two electron reduction is either the α-ketoacid, when the C–S bond is cleaved, or the β-mercapto-α-hydroxy acid, when the keto group is reduced.

The first pathway predominates in acidic media. In neutral and slightly basic media, dissociation of both CO_2H ($pK_1 = 2$–3) and SH ($pK_2 = 9.6$) groups favors the C–S cleavage: the two reduction pathways occur simultaneously.

Dianion $CH_2(S^-)COCOO^-$ **1** shows an increased absorption at 290 nm with time consistent with formation of the carbanion enolate of thioether **2**:



$$C_2H_5S-\underset{\ominus}{CH}-\underset{O}{\underset{|}{C}}-COO^-$$

Also the polarographic wave in the pH range where the dianion **1** predominates decreases with time. From these results and additional points, essentially ^{13}C NMR data, it is reasonable to deduce that the ionisation of the thiol group of **1** initiates the formation of a ambident carbanion.

A ketol dimeric product was isolated as sodium salt and its structure established by a ^{13}C NMR study.

EXPERIMENTAL

Apparatus

The polarograms were recorded on a three electrodes Tacussel polarograph, Mark PRG 5. The characteristics of the capillary were as follows: $m = 6.4$ mg, $t_1 = 3.0$ s, mercury head = 60 cm; $m^{2/3}\ t_1^{1/6} = 4.15$. The cyclic voltammograms were recorded by means of a single sweep polarograph consisting of a rapid-response Tacussel potential sweep source, Mark PRT 30-01, a control module Mark TP-PRT a Tacussel GCMR module for drop-time control, a Tacussel differential amplifier, Mark ADTP, two Tektronic amplifiers Mark 2A60 and 3A75, and a Tektronic oscilloscope, Mark 564. The capillary had a natural drop-time of 16 s. Controlled potential electrolysis was carried out with a mercury pool electrode of area 68 cm^2 in a standard three electrodes system. The anode and cathode compartments were concentric and separated by sintered glass of porosity 4. The voltage source consisted of a Tacussel potentiostat, Mark PRT 20/2. A Tacussel IG 5 N integrator electronic milliammeter and voltmmeter were included into the electrolytic circuit. The electrolytic cell was sealed and thermostated.

pH Values were determined by means of a Tacussel TS 70 N 1 pH meter. The ultraviolet spectra were recorded on a Varian Electroscan Spectrophotometer.

Cell and electrodes

The cell for polarographic experiments was water-jacketed (Tacussel CPRA). An aqueous saturated calomel electrode (Tacussel C 10) to which all potentials cited are referred was used. The counter electrode was a platinum Tacussel Pt electrode.

Chemicals

The ammonium salts of 3-thiohydroxy-2-oxopropanoic acid (β-mercaptopyruvic acid **1**) was prepared according to Kun[6] starting with commercial (FLUKA) β-bromopyruvic acid. The free acid **1** was obtained by passing the ammonium salt over Dowex 50 WX resin in the H^+ form.

The 3-ethylthio-2-oxopropanoic acid (**2**) was prepared according to Parrod.[7] Sodium ethanethiolate, which crystallizes at 0°, was obtained by reaction of pieces of sodium (2.25 g) with ethanethiol (7.5 ml) in anhydrous ether solution (40 ml). It was used in absolute ethanol solution. Yield of 70% of $C_5H_8O_3S$ was obtained.

Product of ketol condensation

20 millimoles of 3-thiohydroxy-2-oxopropanoic acid (**1**) were dissolved in 200 ml 0.3 M carbonate buffer pH 10.6. After 20 h the alkaline solution was acidified by hydroiodic acid, carbon dioxide removed by a stream of nitrogen and volume reduced to 10 ml. Resulting solution was neutralized by addition of a dilute sodium carbonate solution, white precipitate filtered off, worked with ethanol and resulting ketol dried over P_2O_5 at reduced pressure for 24 h. Elemental analysis corresponded to empirical formula $NaC_3H_3O_3S$. The compound deecomposed before the m.p. was reached.

Stock solutions and buffers

0.01 M stock solutions of compounds **1** or **2** were prepared in 50% ethanol. For $pH < 2$ solutions of sulfuric acid for 2–8 McIlvain and for pH 2–10 Britton–Robinson buffers were used. For the pH-range between 9 and 11.5, 0.25 M carbonate buffers were used. Phosphate buffers were prepared from 0.5 M Na_2HPO_4: for the pH range between 6.5 and 8.5 solution of hydrochloric acid, for that above pH 10.5 solution of sodium hydroxide was added.

Procedures

For linear sweep polarography and cyclic voltammetry, the 0.01 M stock solutions of the β-mercaptopyruvic acid **1** in 50% ethanol or its derivative **2** was diluted tenfold with the buffer or supporting electrolyte used so that final solution contained 5% ethanol. The cyclic voltammograms were recorded at scanning rate of $0.5\ Vs^{-1}$.

For controlled potential electrolysis 200 ml of 10^{-2} M solution of the compound **1** and **2** was placed into the electrolytic cell and closed air-tight. After the chosen potential was applied the mercury pool was vigorously stirred using a magnetic stirrer and the electrolysis was carried out for about 4 h.

After the electrolysis in acidic solutions has been completed, hydrogen sulfide formed was transferred by a stream of nitrogen or argon into a trap containing 4 M NaOH solution. The concentration of the sulfide was determined either by anodic polarography or by spectrophotometry (using the absorption band at 230 nm with $\epsilon = 6 \times 10^3\ l\,mol^{-1}\,cm^{-1}$), after dilution with 0.1 M NaOH.

When electrolysis was carried out in alkaline media, polarographic determination of sulfide was possible by means of anodic waves after dilution of the resulting solution with 0.1 M NaOH in ratio 1 : 25. Direct spectrophotometric determination was complicated by the fact that sulfide anions absorb in the same wave-length region as dianion of acid **1**. Therefore the solution after completed electrolysis was first acidified, hydrogen sulfide transferred by a stream of nitrogen into a 4 M NaOH solution and determined as above.

In linear sweep polarography and cyclic voltammetry the beginning of the potential sweep was synchronized with recording. To avoid contamination with traces of sulfide formed in previous experiment, the sweep was triggered only after at least three drops elapsed from the previous experiment.

Electronic spectra of de-aerated solution of about 1×10^{-3} M solutions of compounds **1** and **2** were recorded in various buffers.

^{13}C NMR spectra were recorded on a Brüker W. P. 80 spectrometer operating at 20.18 MHz. The sample was dissolved in water or alkaline solution; a small amount of D_2O was added to provide a lock signal. Proton broadband decoupled, proton off resonance decoupled and undecoupled spectra were used for assignment of resonances. Dioxane was used as an internal standard. Chemical shifts were referenced to T.M.S.: $\delta_{TMS} = \delta_{dioxane} + 67.4$ ppm. ^1H NMR spectra were recorded on a Brüker W. P. 80 spectrometer operating at 80.13 LHz. The sample was dissolved in D_2O.

RESULTS AND DISCUSSION

Electronic spectra and acid base properties

Due to strong hydration of the carbonyl group of the β-mercaptopyruvic acid **1** (and possibly consecutive dimerization see ^{13}C NMR spectra), the free acid $CH_2SH{-}CO{-}CO_2H$, AH_3, and its anion AH_2^- show no characteristic absorption at pH lower than about 8, only bands corresponding to the carboxylic group and the carboxylate anion which were not studied in detail.

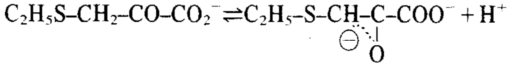
For β-mercaptopyruvic acid **1**, electronic spectra at $pH > 9$, recorded at 5° within few minutes after addition of the stock solution of the acid to the buffer exhibits two bands: at 230 nm ($\epsilon = 5 \times 10^3\ l\,mol^{-1}\,cm^{-1}$) and 280 nm ($\epsilon = 2 \times 10^3\ l\,mol^{-1}\,cm^{-1}$); the band at 280 nm is overlapped with increasing time by a strong absorption band at 292 nm ($\epsilon = 7 \times 10^3\ l\,mol^{-1}\,cm^{-1}$). This increase recorded at 292 nm followed first order kinetics and treatment of experimental data yielded rate constant ($k = 0.22\ min^{-1}$ at 5°) identical with that found from the decrease of polarographic limiting currents with time in this pH range. Resulting band resembles that of the carbanion enolate of the thioether **2**.

Product of the first order conversion underwent much slower ketol formation (see ^{13}C NMR spectra).

Potentiometric titration at 5°, where pH was measured within few seconds after addition of a given and gradually increasing volume of sodium hydroxide to an aliquot of β-mercaptopyruvic acid **1** yielded $pK_2 = 9.6$. When solution of the compound **1** was first made alkaline to pH 11.5 and then a solution of hydrochloric acid added, the titration curve corresponded to $pK_2 = 9.3$.

In solutions of sodium hydroxide of concentration greater than about 1.0 M, increase of an absorption band at 296 nm was observed instantaneously after addition of the stock solution of β-mercaptopyruvate to the alkaline medium. Absorbance at 296 nm increased with increasing sodium hydroxide concentration and reached in 4 M NaOH a value corresponding to $\epsilon = 8.8 \times 10^3\ l\,mol^{-1}\,cm^{-1}$. Change in spectra have shown an isosbestic point at 279 nm indicating that a simple acid-base equilibrium is involved. Using values of acidity function H_-[8] to characterize the activity of hydroxide ions, a pK_3 value of 13.7 was obtained

3-Ethylthio-2-oxopropanoic acid **2** shows no change in absorption spectra at $pH < 12$. In alkaline media absorption band at 297 nm is observed, reaching $\epsilon = 8.0 \times 10^3\ l\,mol^{-1}\,cm^{-1}$. From the change of absorbance at 297 nm with sodium hydroxide concentration, an approximate value of $pK \simeq 12.5$ was found for the equilibrium:



$$C_2H_5S{-}CH_2{-}CO{-}CO_2^- \rightleftharpoons C_2H_5{-}S{-}CH{-}\underset{\overset{|}{O}}{C}{-}COO^- + H^+$$

Acidification of solutions of dianion formed in alkaline solutions

When solutions of acid **1** in buffers of pH between 9 and 12 were acidified (few seconds after addition of the stock solution) to pH < 2, the band of the dianion at 280 nm ($\epsilon = 2.2 \times 10^3\ l\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$) decreased and a non-characteristic absorption band was formed. The resulting spectrum did not change with time.

For comparison the spectrum of the thioglycolate dianion ($\epsilon = 5.0 \times 10^3\ l\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$), at 230 nm, was recorded and its solution acidified. The resulting solution showed no absorption beyond 220 nm.

When a solution of β-mercaptopyruvate **1** was prepared in buffers pH 9 to 12 left to establish the equilibrium so that the absorption band at 292 nm reached its maximum value ($\epsilon = 7.0 \times 10^3\ l\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$) and then acidified, the immediate formation of a band at 270 nm ($\epsilon_0 = 5 \times 10^3\ l\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$ extrapolated for $t = 0$) was observed. This absorption band decreased with time, to ϵ_{eq} until equilibrium is reached between the enol form $CH(SH) = C(OH)COOH$ ($\lambda_{max} = 270$ nm, $\epsilon_{eq} = 3 \times 10^2\ l\ \mathrm{mol}^{-1}\mathrm{cm}^{-1}$ the keto form $CH_2(SH)COCOOH$ and the hydrated ketoform. Assuming the value of ϵ_0 as corresponding solely to the enol form, the equilibrium concentration of the enol form was calculated. For the acid AH_3 predominating at pH 2, 6% of the enol form $CH(SH) = CH(OH)COOH$ was found; for the carboxylate anion AH_2^- predominating at pH 4 practically 0% of the form $CH(SH) = C(OH)COO^-$ was found. When this solution was made alkaline, the band at 292 nm reappeared in original height.

The decrease of the band of the enol form at 270 nm produced by acidification followed reaction rate equation for a first order equilibrium reaction.

Plotting the value of $\log (At^{270} - A_{eq}^{270})/(A_0^{270} - A_{eq}^{270})$ as a function of the time in an aqueous sulfuric acid pH 0.95 at 25° gave the value $k = k_{keto} + k_{enol} = 1.1 \times 10^3.\mathrm{s}^{-1}$. Assuming that $K_{enol} = (\mathrm{keto})/(\mathrm{enol}) = 15.7$ approximate values of $k_{keto} \simeq 1.03 \times 10^{-3}\ \mathrm{s}^{-1}$ and $k_{enol} \simeq 6.6 \times 10^{-5}\ \mathrm{s}^{-1}$ were obtained.

When a solution of the ether **2** in 0.5 M NaOH containing 30% ethanol was rapidly acidified by sulphuric acid to pH 1.0 an absorption band at 289 nm is formed ($\epsilon_0 = 1.6 \times 10^4\ l\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$ extrapolated to $t = 0$). The height of this absorption band decreases with time until an equilibrium value ($\epsilon_{eq} = 6.5 \times 10^3\ l\ \mathrm{mol}^{-1}\ \mathrm{cm}^{-1}$) is reached, corresponding to about 40% of the enol form $C_2H_5SCH{=}C(OH)COOH$, or $K_{enol} \simeq 1.5$.

The decrease of the band at 289 nm with time followed reaction rate equation for a first order equilibrium process with $k_{keto} + k_{enol} = 1.5 \times 10^{-4}\ \mathrm{s}^{-1}$ at 25° in aqueous sulphuric acid pH 0.8 containing 30% ethanol. Using the above value of the equilibrium constant approximate values $k_{keto} \simeq 1.1 \times 10^{-4}.\ \mathrm{s}^{-1}$ and $k_{enol} = 4.0 \times 10^{-5}\ \mathrm{s}^{-1}$ were found.

From the above results, it may be deduced that the 3-thiohydroxy 2-oxopropanoic acid **1** undergoes four equilibria with increasing pH:

$$\underset{AH_3(a)}{CH_2SH\text{-}CO\text{-}CO_2H} \rightleftharpoons \underset{AH_2^-\ (a)}{CH_2SH\text{-}CO\text{-}CO_2^-} + H^+ \qquad (1)$$

$$\underset{AH_2^-(a)}{CH_2SH\text{-}CO\text{-}CO_2^-} \rightleftharpoons \underset{AH^{2-}}{CH_2(S^-) - CO - CO_2^-} + H^+ \qquad (2)$$

$$\underset{AH^{2-}}{CH_2(S^-)\text{-}CO\text{-}CO_2^-} \overset{\text{slow}}{\rightleftharpoons} \left\{ \begin{array}{l} CH(S^-) = C(OH)\text{-}CO_2^- \\ \qquad \updownarrow \\ \underset{AH'^{2-}}{S = CH\text{-}\overset{\ominus}{C}(OH)\text{-}CO_2^-} \end{array} \right. \qquad (2')$$

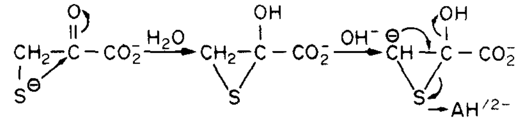
$$AH'^{2-} \rightleftharpoons \underset{A^{3-}}{CH(S^-) = C(O^-)\text{-}CO_2^-} + H^+ \qquad (3)$$

(a): or hydrated species on the CO group: see polarographic study.

The processes (1), (2) and (3) occur at widely separated pH values as pK_1 is about 2–3, pK_2 9.6 and pK_3 13.7. The value of $pK_3 = 13.7$ is in good agreement with values of second dissociation constants of some enediols like dihydroxyacrylate ($pK_2 = 13.7^1$) and triose reductone ($pK_2 = 13.0^9$).

That equilibrium (2′) is not rapidly established may account for some of the hysterisis phenomena observed in the potentiometric titration.

Concerning the mechanism of step (2′), it seems reasonable to assume that AH^{2-} undergoes a nucleophilic intramolecular attack generating a transient episulfide[10] which on further reaction yields AH'^{2-}.



The formation of this episulfide may be correlated with the appearance of the transient UV absorption band at 280 nm.

Polarographic reduction of 3-thiohydroxy 2-oxo propanoic acid

All species AH_3, AH_2^- and AH^{2-} in addition to the free keto form can also exit in the hydrated geminal diol form,[11,12] which may undergo dimerization (see ^{13}C NMR). The plot of the polarographic intensity i as a function of pH resembles those of other α ketoacids[11] (Fig 1).

In acidic solutions, polarographic reduction of **1** (forms AH_3 or AH_2), at the dropping mercury electrode (d.m.e.) occurs after preprotonation of the carbonyl group (as

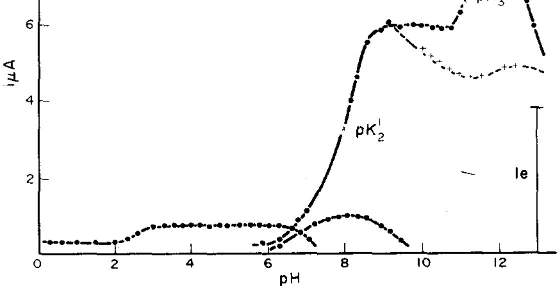


Fig. 1. Dependence of limiting currents of 1×10^{-3} M 3-thiohydroxy-2-oxopropanoic acid **1** on pH. Solutions of sulphuric acid, Britton-Robinson buffers and sodium hydroxide ($\mu = 1.0$) with 5% ethanol, at pH > 9 current i_o extrapolated to $t = 0$. Dotted line: wave i_e after establishment of equilibrium.

shown by the value $\Delta E_{1/2}/\Delta pH = -60\,mV/pH$), by electron and proton transfers to the CO group, according to eqns (4) and (5):

$$HS\text{-}CH_2\text{-}C(OH)_2\text{-}CO_2H + H^+ \rightleftharpoons HS\text{-}CH_2\text{-}\overset{+}{C}(OH)\text{-}CO_2H + H_2O \quad (4)$$

$$HS\text{-}CH_2\text{-}\overset{+}{C}(OH)\text{-}CO_2H + 2e + H^+ \rightarrow HS\text{-}CH_2CHOH\text{-}CO_2H. \quad (5)$$

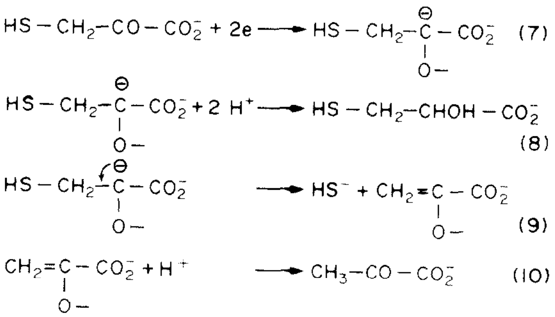
At pH 3–6, the monoanion AH_2^- is furthermore protonated on the carboxylate group ($\Delta E_{1/2}/\Delta pH = -170\,mV/pH$) and then reduced as AH_3.

In acidic media, the limiting current of the polarographic wave remains small, owing to the strong hydration, and the limiting current of the wave is kinetically controlled.

At pH 8–11, the dianion AH^{2-}, which predominate in this pH-range, is considerably less hydrated and is faster dehydrated than the monoanion AH_2^-: the height of the wave increases (Fig. 1):equilibrium (6) is shifted towards the right handside:

$$\underset{AH^{2-}}{{}^-S\text{-}CH_2\text{-}C(OH)_2\text{-}CO_2^-} \rightleftharpoons {}^-S\text{-}CH_2\text{-}CO\text{-}CO_2^- + H_2O. \quad (6)$$

The dianion AH^{2-} is protonated on the thiolate group ($\Delta E_{1/2}/\Delta pH = -60\,mV/pH$) and then reduced in two competitive processes, (8) and (9) (see controlled potential electrolysis):



$$HS-CH_2-CO-CO_2^- + 2e \longrightarrow HS-CH_2-\overset{\ominus}{\underset{\displaystyle O-}{C}}-CO_2^- \quad (7)$$

$$HS-CH_2-\overset{\ominus}{\underset{\displaystyle O-}{C}}-CO_2^- + 2\,H^+ \longrightarrow HS-CH_2-CHOH-CO_2^- \quad (8)$$

$$HS-CH_2-\overset{\ominus}{\underset{\displaystyle O-}{C}}-CO_2^- \longrightarrow HS^- + CH_2{=}\underset{\displaystyle O-}{C}-CO_2^- \quad (9)$$

$$CH_2{=}\underset{\displaystyle O-}{C}-CO_2^- + H^+ \longrightarrow CH_3-CO-CO_2^- \quad (10)$$

Comparison of the value of pK′ obtained from the pH-dependence of polarographic limiting currents (Fig 1) with values of pK_2 obtained from spectral and potentiometric data might indicate whether ionisation is a rapidly established equilibrium, and dehydration is rate determining, or whether the formation of the dianion AH^{2-} is rate determining.

When acid-base equilibrium (2) is rapidly established, $pK' = pK_2$; when rate of dianion formation is the governing process, $pK' < pK_2$.[13] In the case of **1**, $pK_2' = 8.0$ (Fig. 1) and $pK_2 \simeq 9.5$. Dianion AH'^{2-} formation is slow, according to the hypothesis of a rate determining step (2′).

At pH > 9.0, the height of wave i_0 decreases with time until it reaches an equilibrium value i_e. Te plot of this value i_e as a function of pH (Fig. 1, dotted line) does not show the increase at pH > 11.0 and decrease at pH > 12.5 observed for wave i_0. The decrease is first order in the ketocid and independent of hydroxide ion concentration, with rate constant ($k = 0.22\,min^{-1}$ at 25°), identical with that found from the increase of absorption band at 292 nm.

Formation of carbanion ambident AH'^{2-}, thermodynamically preferred, according to (2′), is probably responsible for these results.

$${}^-S\text{-}CH_2\text{-}C(OH)_2\text{-}CO_2^- + OH^- \rightleftharpoons {}^-S\text{-}CH_2\text{-}C(O^-,OH)\text{-}CO_2^- + H_2O \quad (11)$$

$$\underset{AH}{{}^-S\text{-}CH_2\text{-}CH_2\text{-}C(O^-,OH)\text{-}CO_2^-} \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} {}^-S\text{-}CH_2\text{-}CO\text{-}CO_2^- + OH^- \quad (12)$$

Resulting anion is then protonated and reduced in the sequence (7)–(10). Assuming that equilibrium (11) is rapidly established, inflexion point pK_3' (Fig. 1) corresponds to pK (11) and elimination of hydroxyl ion with rate k_{12} is the rate determining step.

At pH > 12.5, decrease of current for compound **1** is due to increase in rate of the reaction with constant k_{-12}.

Ketol formation involving β-mercaptopyruvate **1**

Absorbance at 292 nm and polarographic wave i_0 in pH 10.5 carbonate buffer decrease with time at 25°. The decrease corresponds to a second order reaction in **1** and consequently shows half-life time dependent on initial concentration of **1** (Table 1).

Simultaneously, it is possible to note the disappearance of roughly half of the SH acidity ($pK_a = 9.6$) and of the UV band at 230 nm: $\epsilon_0 = 5 \times 10^3\ l\ mol^{-1}\ cm^{-1}$; $\epsilon_e = 2.5 \times 10^{-3}\ l\ mol^{-1}\ cm^{-1}$ after equilibrium was reached.

The results are in agreement with the formation of a ketol product, as confirmed by potential electrolysis and by ^{13}C NMR. This ketol undergoes an intramolecular nucleophilic cyclisation concerning the $CH_2\text{-}S^-$ and CH=S groups; it was isolated as a sodium salt.

Controlled potential electrolysis

Comparison of the proposed mechanism at the d.m.e. with the results obtained in the controlled potential electrolysis using a mercury pool electrode indicates competition between C-S cleavage and C=O reduction.

Denoting as x the fraction of **1** in which the C-S bond is reductively cleaved in sulfide ion and pyruvate (reducible to lactate in a further step) and 1-x the fraction in which reduction of the keto group to a β-mercapto-α-hydroxy acid occurs, it is possible to write for the coulometric balance:

$$n(F) = 4x + 2(1 - x)$$

where F is the Faraday charge.

Coulometric balance calculated by means of this equation was in good agreement with experimental data (Table 2a). Thus, the acid form AH_3 is, at the mercury pool electrode reduced predominantly (95%) on the CO group according to reactions (4) and (5). In solutions

Table 1. Rate constant (k) of ketol formation in solution of β-mercaptopyruvic acid **1** in a carbonate buffer pH 10.5 (c = initial concentration of **1**)

$10^2c\ [mol\ l^{-1}]$	1.0	1.75	2.5	4.0
$10^3k\ [mol^{-1}\ l\ min^{-1}]$	8.0	8.1	8.0	8.0
$t_{1/2}\ [min]$	125	70	50	31

Table 2(a) Coulometric reduction of 3-thiohydroxy-2-oxopropanoic acid **1**

Buffer	pH	E[a] (V,SCE)	Yield H_2S %	Coulometric balance Coulombs Theor.[b]	Exp.
McIlvain	2.5	−1.05	13	436	420
McIlvain	7.3	−1.40	30	500	490
Carbonate	10.5	−1.60	60	617	500[c]
1M NaOH	14	−1.70	12	432	425

[a]Applied potential; [b]calculated from $n = 4x + 2(1 - x)$; [c]smaller yield may be due to the formation of a dimeric ketol.

Table 2(b). Yield of pyruvate or sulfide ions, expressed as fraction (x) of total conversion in controlled potential (−1.4 V, S.C.E.) electrolysis of β-mercaptopyruvic acid **1** as a function of pH

pH	1.0	3.5	4.7	6.5	7.5	12.0
yield (x) at 20°C	0.04	0.23	0.40	0.40	0.45	0.75

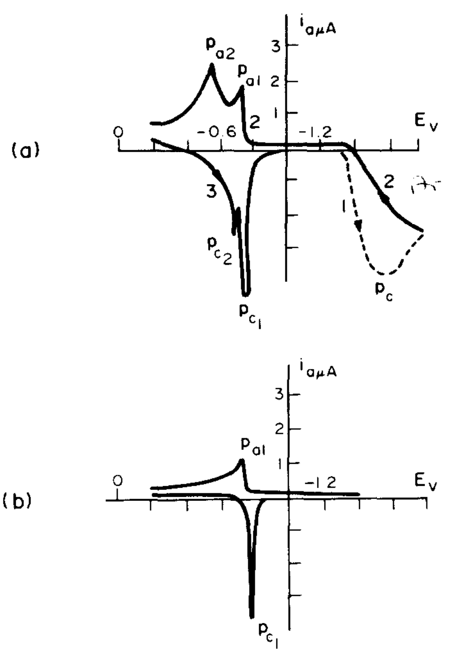


Fig. 2. Cyclic Voltammogram of 1×10^{-3} M 3-thiohydroxy-2-oxopropanoic acid in a carbonate buffer pH 10 containing 5% ethanol at a rate of scanning 0.5 V s^{-1} (cathodic currents increase below the current zero line). (a) **1** reduction from −1.2 V to −1.8 V; **2** oxidation from −1.7 V to −0.2 V; **3** reduction from −0.2 V to −1.2 V; (b) 2.6×10^{-4} M Na_2S, **1** oxidation from −1.0 V to −0.2 V; **2** reduction from −0.2 V to −1.4 V.

where AH_2^- predominates, cleavage of C=O (60%) and C–S (40%) are comparable as indicated by equations (8)–(9). The dianion AH^{2-} is, at the mercury pool electrode, reduced predominantly (75%) by the cleavage of the C–S bond (Table 2b).

Decrease of the yield of pyruvate at pH > 10 can be due to bulk chemical reaction: ketol formation.

Cleavage of the C–S bond is thus facilitated by a negative charge on sulphur and by presence of the carboxylate grouping in β position. This is supported by the observation that thioether **2**, unable to form thiolate, is reduced preferably on the CO bond.

Cyclic voltammetry

When the scan in started at -1,2 V and carried out to -1,8 V, only one single irreversible reduction peak (p_c) at -1,6 V is observed (Fig. 2 curve 1). Reversal at potential -1,8 V and scanning to 0,1 V yielded two anodic peaks (p_{a1} and p_{a2}) (Fig. 2 curve 2), which on further reversal give two cathodic peaks (pc_1 and p_{c2}). The reversible couple p_{a1} and p_{c1} corresponds to mercury salt formation involving sulphide ions and the reduction of the mercury compound, involving adsorption. The almost reversible couple p_{a2} and p_{c2} corresponds to a mercury salt formation of the thiol group β-mercaptopyruvate and the reduction of this salt, also affected by an adsorption phenomenon (Fig. 2b).

NMR study

In aqueous solution, 3-thiohydroxy- 2-oxopropanoic acid **1** could possibly exist in a bicondensed form exhibiting two asymmetric carbons.

The ^{13}C spectrum of **1** shows two groups of three signals of inequal intensity summarized in Table 3. In the undecoupled spectrum, the methylene carbon of each group shows four resonances, the typical pattern of a carbon coupled with two non equivalent protons (X part of an ABX spectrum). The ^1H spectrum exhibits two AB quartets of inequal intensity (Table 3). Similar results were obtained with ammonium 3-thiohydroxy- 2-oxopropanoate.

These data agree with a hemithioketal structure which could exist in two different forms, roughly in ratio 4 : 1, *dl*- and meso-

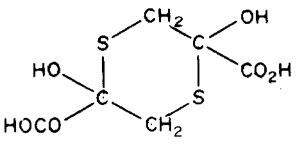


Table 3. NMR spectral data of 1 (D_2O solution, 25°)

Nucleus	δppm assignments	Major product ≈ 80%		Minor product ≈ 20%	
	C_1 (q)	175.1		174.9	
			[(a)]76.8		
^{13}C	C_2 (q)	76.6	76.6	79.2[(c)]	
			76.4		
			[(a)]42.7		44.7
	C_3 (b)	35.6	35.8	37.5	37.55
			35.4		37.45
			28.5		30.4
^1H	CH_2	3.0 3.9	J_{AB} = 1.5 Hz	3.2 3.6	J_{AB} = 15 Hz

[(a)]Undecoupled spectrum; [(b)]binary carbon; [(c)]splitting was not detected owing to low concentration of minor product; [(q)]quaternary carbon.

Stability of these species may account for the low polarographic intensity recorded in acidic media.

In NaOH 2M solution, the ^{13}C spectrum of thioether 2 shows five signals (Table 4). The signal at 96.0 ppm is splitted to a doublet in the off resonance spectrum. The occurrence of this strongly deshielded tertiary carbon and that of a quaternary carbon with a frequency intermediate between those of carbons linked to oxygen by double and single bond demonstrate the formation of an ambident carbanion:

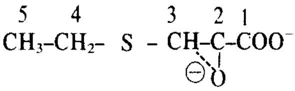
Table 4. ^{13}C NMR spectral data of 2 (NaOH 2M solution, 25°) δppm

C_1	C_2	C_3	C_4	C_5
176.5	157.7	96.0	28.2	15.6

Table 5. ^{13}C NMR spectral data of 1 ammonium salt, 25°

δppm assignments	Neutral D_2O[(a)]	NaOH 2M
C_1	177.4 (q)	176.5 (q)
C_2	77.9 (q)	153.1 (q)
C_3	36.8 (b)	108.6 (t)

(a) Major product; (b) binary carbon; (t) tertiary carbon; (q) quaternary carbon.



$$\overset{5}{CH_3}\text{–}\overset{4}{CH_2}\text{– S –}\overset{3}{CH}\text{–}\overset{2}{\underset{\ominus O}{C}}\text{–}\overset{1}{COO^-}$$

In NaOH 2M solution, the ^{13}C spectrum of 1 (as acid, sodium or ammonium salt) shows three signals (Table 5). Besides the quaternary carbon of the carboxylic anion, a tertiary and a quaternary carbon arising from the formation of an ambident carbanion give signals in the same chemical shift range as in the case of the ambident carbanion derived from 2.

Comparison with the chemical shifts of the same carbons in the oxo analogous carbanion[1] clearly shows that sulphur is less effective than oxygen as inductive attractor and mesomer donor.

The ^{13}C spectrum of the bicondensed ketol product 3 in D_2O shows six signals (Table 6). In the undecoupled spectrum, the tertiary carbon C-3 and one of the quaternary carbon C-2 exhibit small long range couplings. In saturated Na_2CO_3 solution, signals are only slightly shifted. The main feature is the disappearance of the long range couplings owing to the ionisation of the -SH group.

Table 6. NMR spectral data of bicondensed ketol 3, 25°

Nucleus	ppm, assignments	J Hz	Neutral D_2O		Saturated Na_2CO_3	
	C_1, C_5	(q)	178.4;	177.2	178.8;	179.8
	C_2	(q)	87.2	$^3J = 4$	88.1	
^{13}C	C_3	(t)	57.2	$^1J = 142$ $^2J = 6$	61.0	$^1J = 142$
	C_4	(q)	92.3		92.8	
	C_6	(b)	42.2	$^1J = 145$	42.0	$^1J = 145$
	CH		4.3			
^1H	CH_2		3.2 3.6	$J_{AB} = 11$		

(b) Binary carbon; (t) tertiary carbon; (q) quaternary carbon.

Table 7. ^{13}C NMR spectral data. pH dependent chemical shifts of bicondensed ketol 3 (3°) δppm

|NaOH| mol.l^{-1}	C_2(q)	C_3(t)	C_4(q)	C_6(b)	C_1 and	C_5(q)
0.3	88.2	61.8	93.7	41.6	179.5	180.4
0.6	88.2	61.7	93.7	41.8	179.5	180.4
0.9	88.2	61.3	96.2	41.7	179.4	180.4
1.2	88.0	61.1	97.4	41.6	179.2	180.4
1.5	88.0	60.6	≃101(a)	41.4	179.1	180.4
2.0	87.7	59.7	≃107(a)	41.0	178.7	180.4
3.0	87.1	57.6	(c)	39.7	177.7	180.5
(d)	88.0	60.8	92.6	41.8	179.1	180.0

(a) Very broad signals; (c) signal not detected; (d) 1, 5 mol.l^{-1} solution acidified by carbon oxide snow (for comparison see Table 6); (b) binary carbon; (t)-tertiary carbon; (q) quaternary carbon.

1, and its ammonium salt, gives identical signals after the ketol condensation had occurred in carbonate buffer solution.

The ^1H spectrum of 3 in D_2O exhibits a singlet for the tertiary proton and a AB quartet for the two non equivalent protons of the methylene group (Table 6).

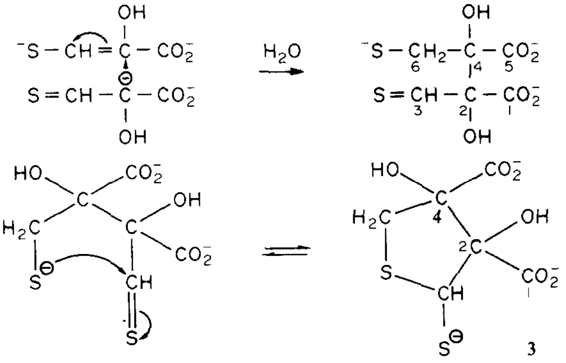
The results of the pH-dependent measurements in ^{13}C NMR are summarized in Table 7. An inspection of the chemical shift titration diagram shows that C-4 is deshielded towards higher pH values. Simultaneously, the corresponding signal becomes very broad.

These effects, which may be attributed to the deprotonation of the C-4 hydroxyl substitutent, are reversible: when a 2M NaOH solution is acidified by means of solid CO_2 a narrowing and high field shift of the signal are observed.

With time, the ^{13}C spectrum of the 2M NaOH solution of 3 changes: three new signals appear at frequencies previously attributed to the monomer ambident carbanion. After several hours, all other signals vanish.

It is reasonable to consider that, in 2M NaOH, with increasing time, the reverse reactions of ketolisation and cyclisation may occur, generating 1 (bi or triionised form according to the pH value).

These results agree with the occurrence of a ketolisation reaction according to:



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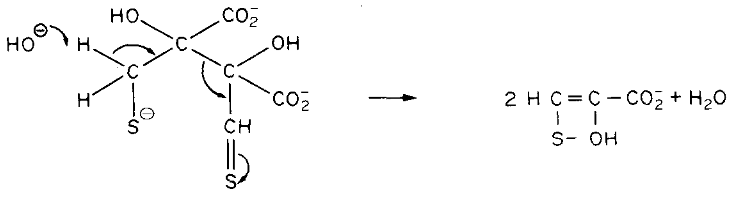
and retrodetolisation:



CONCLUSION

The polarographic behavior of 3-thiohydroxy- 2-oxo-propanoic acid (β-mercaptopyruvic acid) 1 was investigated according to two competitive pathways: the product of a two electron reduction is either the β-mercaptolactate CHSH-CHOH-COO⁻ or the pyruvate, when the C–S bond is cleaved. The first pathway predominates in acidic media; the second is slightly basic media.

By means of ^{13}C NMR it is established that 1 exists in acidic media as a bicondensed hemithioketal yielding a very small polarographic intensity.

In neutral and slightly basic media, dissociation of both CO_2H ($pK_1 = 2\text{-}3$) and SH ($pK_2 = 9.6$) groups favors the C–S cleavage: the two reduction pathways occur simultaneously.

With thioether $C_2H_5S\text{-}CH_2\text{-}CO\text{-}CO_2H$ 2, unable to form the thiolate, the behaviour resembles those of unsubstituted α ketoacids: 2 is reduced preferably on the oxo bond.

Comparison of the polarographic, potentiometric and spectrophotometric behaviour of the thioether 2 with that of 1 indicates that, in the second dissociation step of 1, kinetically controlled formation of the thiolate anion occurs, which is slowly converted into a ambident carbanion preferred thermodynamically:

$$\underset{AH_2^-}{CH_2SH\text{-}CO\text{-}CO_2^-} \overset{\text{fast}}{\rightleftharpoons} \underset{AH^{2-}}{CH_2(S^-)\text{-}CO\text{-}CO_2^-} + H^+ \quad (2)$$

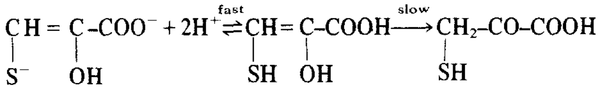
$$CH_2(S^-)\text{-}CO\text{-}CO_2^- \overset{\text{slow}}{\rightleftharpoons} \left\{ \begin{array}{l} CH(S^-)\text{=}C(OH)\text{-}CO_2^- \\ \quad\updownarrow\ominus \\ S = CH\text{-}C(OH)\text{-}CO_2^- \\ \qquad AH'^{2-} \end{array} \right. \quad (2')$$

Additional support for formation of this ambident carbanion are: (a) ionisation of 1 yielded trianion A^{3-};

value of $pK_3 = 13.7$ was in good agreement with the value of the dissociation constants of equilibria of the type:

$$HC(O^-)=C(OH)\text{-}CO\text{-}R \rightleftharpoons HC(O^-)=C(O^-)\text{-}CO\text{-}R + H^+$$

(b) acidification of alkaline solution of **1** after the equilibrium was reached, produced instantaneously 100% of the enol according to:



$$\underset{S^-}{CH}=\underset{OH}{C}\text{-}COO^- + 2H^+ \overset{fast}{\rightleftharpoons} \underset{SH}{CH}=\underset{OH}{C}\text{-}COOH \xrightarrow{slow} \underset{SH}{CH_2}\text{-}CO\text{-}COOH$$

(c) identification by ^{13}C NMR in alkaline solutions of **1**, (d) formation of the ketol **3** in the pH range where the dianion ambident AH'^{2-} predominates (concentration of trianion A^{3-} $CH(S^-)=C(O^-)\text{-}COO^-$ is very small); isolation of the dimeric ketol **3** as sodium salt and determination of its structure by ^{13}C NMR.

The ability to form a ambident carbanion in slightly basic medium distinguishes β-mercaptopyruvate **1** an attractive compound from biological point of view: its reactivity is of importance in biochemical point of view: its reactivity is of importance in biochemical essential processes in which carbanions add to carbonyl group, such in the acetylation of α ketoacids catalyzed by coenzyme A.

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