STRUCTURE OF β-THIOLPYRUVIC ACID*

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In a recent publication KUN[1] stated that further molecular properties of β-mercaptopyruvate will be discussed elsewhere, and this communication deals with evidence for the position of the keto–enol equilibrium in this compound. This is of considerable interest because of the manifold role of the compound in intermediary metabolism[1]. Although the ultraviolet spectrum[1] gave evidence of the presence of some enol, it did not indicate the amount. A comparsion of calculated and observed dissociation constants and a study of the infrared spectrum is here presented to clear up this point. Although the dissociation constants of the compound have been run previously[2] we felt it necessary to repeat them because the new modifications employed in the preparation of the compound[1] give rise to a purer product, the previous curve had only a few points and an irregular shape, and better instruments are now available for measuring pH.

Furthermore, the unexpected observations of SCHNEIDER AND REINEFELD[2] that acidification of a thiolpyruvate solution to pH 3.0 results in a crystalline precipitate could not be confirmed with our preparation which fact also indicates desirability of a reinvestigation of the chemistry of β-mercaptopyruvic acid.

EXPERIMENTAL

The ammonium thiolpyruvate was made as described previously[1]. The sodium thiolpyruvate was prepared from the ammonium salt by passing an aqueous solution of the ammonium thiolpyruvate through a column containing about a tenfold excess of Dowex-50 (Na^+ form). The effluent was collected directly in 15 volumes of 95 % ethanol. Sodium thiolpyruvate crystallized immediately and was filtered off after 12 h standing at 4°C. The conversion of the ammonium salt to the sodium salt is quantitative. The salts were dried over P_2O_5 in a vacuum in an Abderhalden pistol at 78° until they reached constant weight.

Calculated for $C_3H_7O_3NS$	*Found*	*Calculated for* $C_3H_3O_3Na$	*Found*
C = 26.27	26.17	C = 25.35	25.11
H = 5.15	5.23	H = 2.13	2.35
N = 10.21	10.12	S = 22.56	22.86
S = 23.38	23.20		

The titration curve in Fig. 1 was drawn directly by a Beckman Industrial pH Meter Model W fitted with a Brown Electronik recorder and a Synchron motor for introducing the acid or base by means of a hypodermic syringe. The instrument was standardized with buffers of known pH. The titration was carried out with approximately 20 mg of the sodium thiolpyruvate dissolved in 25 ml of water. The acid and base used in the titrations were approximately 1 *N*. The mid-points

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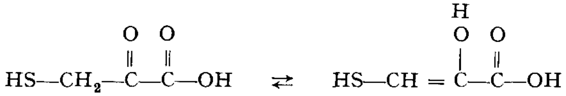
on the curve that was previously published[2] for this compound at pH 2.5 and 9.7, are to be compared with our values of 3.1 and 9.0. For the reasons stated previously we are inclined to believe our values are more reliable. Another titration which is not shown was run in approximately 50 % by volume alcohol–water solution, so that the second dissociation constant thus determined would be comparable to the constants for mercaptans in the literature. The mid-point of this curve is at a pH of 9.5 which is 0.5 higher than the mid-point in water as might be expected.

The infrared spectra were obtained on the solids in potassium bromide pellets made in vacuum under 25,000 lb. pressure. A Perkin Elmer Model 21 instrument with sodium chloride optics was used.

RESULTS AND DISCUSSION

β-Thiolpyruvic acid might be expected to exist as an equilibrium mixture of the keto (thiolketo) and the enol (enethiolol) forms.



$$HS{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}\overset{\overset{O}{\|}}{C}{-}OH \rightleftarrows HS{-}CH=\overset{\overset{\overset{H}{O}}{|}}{C}{-}\overset{\overset{O}{\|}}{C}{-}OH$$

keto (thiolketo) enol (enethiolol)

Evidence with regard to whether the compound is mainly in the keto or the enol form can be obtained from a comparison of the observed dissociation constants with those calculated for the keto structure. The first pK_a of β-thiolpyruvic acid can be estimated from the constant[3] of pyruvic $\log K_a = -2.49$ by taking into account the effect of the sulfhydryl group. Sulfur has an inductive constant of +3.4 on BRANCH AND CALVIN's scale[4]. Assuming that the equation they have developed for acetic acid derivatives holds also for pyruvic, the calculated value would be

$$\log K_{a_1} = -2.49 + \frac{0.3 \times \frac{+3.4}{2.8}}{(1 + 0.03) \times \frac{3.4}{2.8}} = -2.14$$

The titration curve for β-mercaptopyruvic acid, Fig. 1, shows the mid-point of the titration for the carboxyl group to be at pH of 3.1, indicating the actual compound

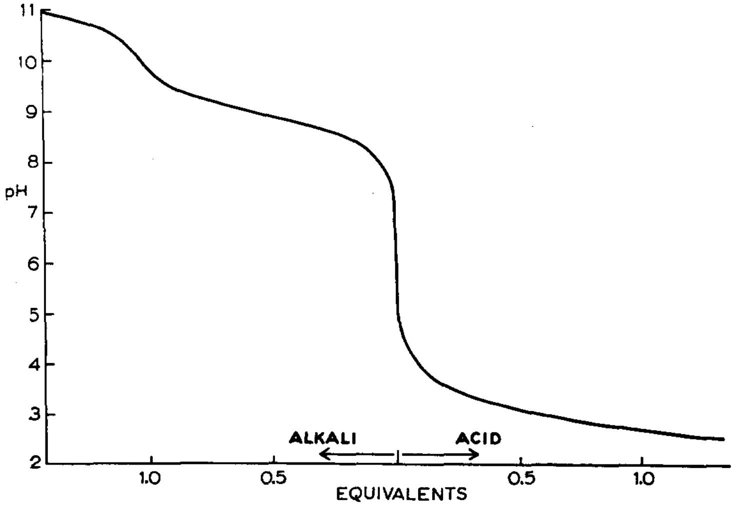
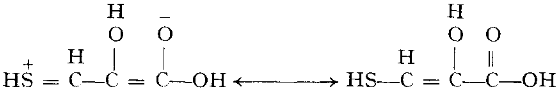


Fig. 1. Titration curve for β-thiolpyruvic acid in water.

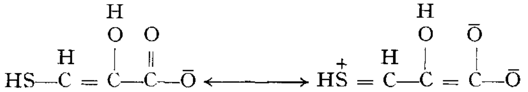
is considerably weaker than would be expected for the keto form. The enol would be expected to be slightly weaker because of the $> C = \overset{\overset{OH}{|}}{C}$-structure in place of the $-\overset{H}{\underset{|}{C}}-\overset{\overset{O}{\|}}{C}-$, but a more important factor, that is acid weakening for the carboxyl group in the enol is a contribution from the resonating form with a separation of charge



$$\overset{+}{HS} = \overset{H}{C}-\overset{\overset{\overset{H}{O}}{|}}{C} = \overset{\overset{\overset{-}{O}}{|}}{C}-OH \longleftrightarrow HS-\overset{H}{C} = \overset{\overset{\overset{H}{O}}{|}}{C}-\overset{\overset{O}{\|}}{C}-OH$$

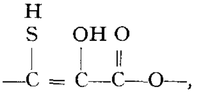
which puts a negative charge on the carboxyl oxygen. This would cause the positive proton to be held more tenaciously and consequently decrease the first dissociation constant. Since an analogous resonating form cannot be written for the keto structure, this difference between the observed and calculated pK_1 is evidence for the compound being in the enol form.

The second dissociation constant could arise from either the ionization of the SH or the OH group. If it arises from the SH group the contribution of the form with a separation of charge (B)

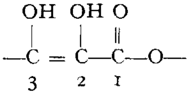


$$\underset{A}{HS-\overset{H}{C} = \overset{\overset{\overset{H}{O}}{|}}{C}-\overset{\overset{O}{\|}}{C}-\bar{O}} \longleftrightarrow \underset{B}{\overset{+}{HS} = \overset{H}{C}-\overset{\overset{\overset{H}{O}}{|}}{C} = \overset{\overset{\bar{O}}{|}}{C}-\bar{O}}$$

to the enol structure will have a noticeable effect on the value of the second dissociation constant. One would normally expect the SH group to be responsible for the second dissociation constant, since hydrogen sulfide, $K_a = 9.1 \cdot 10^{-8}$ is a stronger acid than is water $K_a = 1.8 \cdot 10^{-16}$ and mercaptans are stronger acids than are alcohols. β-Thiolpyruvic acid if mainly in the enol form is analogous to ascorbic acid



$$-\overset{\overset{\overset{H}{S}}{|}}{C} = \overset{\overset{OH}{|}}{C}-\overset{\overset{O}{\|}}{C}-O-,$$



$$-\underset{3}{\overset{\overset{OH}{|}}{C}} = \underset{2}{\overset{\overset{OH}{|}}{C}}-\underset{1}{\overset{\overset{O}{\|}}{C}}-O-$$

and the latter has a dissociation constant around 10^{-4} [5,6]. It has been shown[7] that the acid hydrogen in ascorbic acid is attached to the oxygen on the No. 3 carbon and not to the oxygen on the No. 2 carbon. Therefore, it is clear that the second dissociation constant of β-thiolpyruvic acid must come from the SH which is in the same relative position as the strongly acidic OH group in ascorbic acid.

The second dissociation constant for the keto structure can be estimated from the calculated inductive effect of the $-\overset{\overset{O}{\|}}{C}-\overset{\overset{O}{\|}}{C}-O^-$ group on the dissociation of methyl mercaptan. Ethyl mercaptan has a log K_a of -12.0 [8] and *n*-butyl mercaptan -12.4. Methyl mercaptan would then be a bit stronger, say -11.8. The $-\overset{\overset{O}{\|}}{C}-$ group will increase this by approximately $+5.0/2.8 = +1.78$ and the $-\overset{\overset{O}{\|}}{C}-\bar{O}$ group by $-1.9(2.8)^2 = -0.242$ so the calculated dissociation constant for the SH group in $HS-CH_2-\overset{\overset{O}{\|}}{C}-\overset{\overset{O}{\|}}{C}-\bar{O}$ is $-11.8-0.24 + 1.78 = -10.3$. The measured dissociation constant taken from the mid-point in the titration curve is -9.5. This was run in approximately 50% alcohol–water solution so it would be comparable to the calculated value which was based on the constant for the mercaptan which was also run in alcohol–water solution.

It is apparent that the observed dissociation constant for the SH ionization is 0.8 of a log K_a unit stronger than the calculated value. However, if the compound is in the enol form and has appreciable contribution from the form with a separation of charge which puts a plus charge on the sulfur, that is just what would be expected, for the plus charge on the sulfur would repel the positive proton and make the second dissociation constant larger.

The infrared spectra shown in Fig. 2 for sodium pyruvate, ammonium β-mercaptopyruvate and sodium β-mercaptopyruvate also gives evidence with regard to the amount of enol in β-thiolpyruvic acid. The spectrum of sodium pyruvate has a strong band at 5.85 μ which is attributed to the $C=O$ group and a band at 6.15 μ which is due to the carboxyl ion. One of the shoulders on the carboxyl ion band probably arises from the $C=C$ group. At 2.95 μ is a low band which is assigned to the enolic hydroxyl group. The height of the keto band as compared with the enol band suggests the compound is mainly in the keto form.

The curve for ammonium β-thiolpyruvate has a strong band at 6.23 which is attributed to the carboxylate ion but only a very low shoulder is present at 5.8,

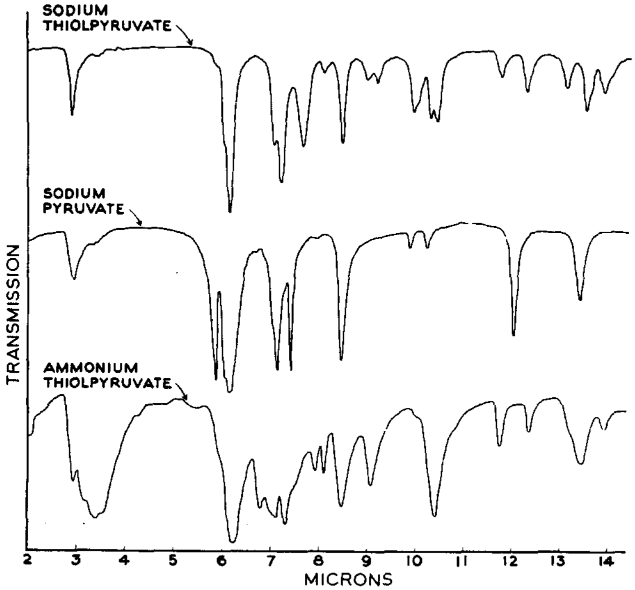


Fig. 2. Infrared spectra. Sodium thiolpyruvate 0.5 mg in 300 mg of potassium bromide. Sodium pyruvate 1.0 mg in 300 mg of potassium bromide. Ammonium thiolpyruvate 1.0 mg in 300 mg of potassium bromide.

indicating very little of the keto form is present. There is a strong band at 2.93 which is attributed to an enolic OH and one at 3.18 which may arise from a hydrogen bonded hydroxyl or from the ammonium ion. The bands at 3.40 and 3.53 also must come from the ammonium ion and the C–H. The band at 10.42 is attributed to the SH group.

The curve for sodium β-thiolpyruvate has a band at 6.14 due to the carboxylate ion, a low shoulder around 5.85 possibly due to the C = O and a sharp band at 2.94 due to the enolic hydroxyl group. The low extinction of the band giving rise to the shoulder at 5.85 plus the relatively high band at 2.94 suggests this compound is mostly in the enol form.

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SUMMARY

The discrepancy between the observed dissociation constants ($pK_1 = 3.1$ in H_2O and $pK_2 = 9.5$ in 50% ethanol) as compared to the calculated values ($pK_1 = 2.14$ in H_2O and $pK_2 = 10.3$ in 50% ethanol) for the keto form of β-thiopyruvic acid can be explained by the contribution of the enol form of this acid, which in solution tends to diminish the dissociation of the carboxyl group and simultaneously increase the acidity of the thiol group. This evidence indicates that β-thiolpyruvic acid when in solution in the range of pH 2 to 10 is more in the enol form than is pyruvic acid. Comparison of the infrared spectra of ammonium β-thiolpyruvate, sodium β-thiolpyruvate and sodium pyruvate shows that in the solid crystalline state β-thiolpyruvate ion exists almost completely in the enol form. Sodium pyruvate has a strong band at 5.85 μ attributed to the C = O group, while the crystalline β-thiolpyruvates have only low shoulders at 5.85 μ, indicating that only a small amount of the keto form is present. Both of the thiol salts have high bands at 2.93 and 2.94 μ arising from OH groups. They also have bands at 10.4 μ which are attributed to the SH group.

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