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ORIGINAL PAPER

Properties of dihydroasparagusic acid and its use as an antidote against mercury(II) poisoning

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Abstract Dihydroasparagusic acid is the first naturally occurring dimercaptanic compound that was isolated in 1948 from *Asparagus* concentrate. Although several synthetic procedures were proposed in the past decades for this natural substance, most of its chemical properties remain unstudied. In this work the capacity of the acid to act as an antidote against mercury(II) toxicity was evaluated in a simple biological model system, *Saccharomyces cerevisiae*, and is explained by the formation of a precipitate between mercury(II) and dihydroasparagusic acid. The precipitate was analyzed and studied. The solubility was determined by measuring in equilibrated solutions either the concentration of the total mercury(II) present in solution or the free concentration of hydrogen ions. The protonation constants were determined at 25 °C and in 1.00 M NaCl, as constant ionic medium, by means of potential difference measurements of a galvanic cell with a glass electrode. The experimental data are explained by proposing the chemical composition of the precipitate and the value of its solubility product. As the solubility of the precipitate increases by increasing the concentration of dihydroasparagusic acid, the further formation of a complex between mercury(II) and dihydroasparagusic acid is assumed.

Keywords Dihydroasparagusic acid · Antidote towards mercury(II) · Solubility product · Coordination chemistry · Protonation complexes · Stability constant

Introduction

Dihydroasparagusic acid (Fig. 1), the first dimercaptanic compound of natural origin, was first isolated from an *Asparagus* concentrate by Jansen [1]. Several investigations were performed on this product, but many of its properties remain unstudied. The presence of two mercapto groups allows one to hypothesize that H_3DA might display antidote properties. The search for such mercapto-based chemical species as suitable antidotes against mercury(II) toxicity has been underway since 1980 or earlier [2]. From a casual survey, Casas and Jones [2] found several anomalies in the behavior of compounds containing sulfhydryl groups as ligands of mercury(II). Many values of constants are cited, but it is not clear whether they refer to complexes with mercury(II) or to protonation constants of the studied compounds [2–7]. Values of constants can be compared when experimental conditions (i.e., temperature and ionic medium) are known. The comparison can take place if the protonation constants of the potential ligands are known.

The behavior of compounds containing sulfhydryl groups was studied in our laboratory to determine the protonation constants and to study their properties as ligands [8–11]. Cysteine [8], cystine [9, 10], and penicillamine disulfide [11] were studied at 25 °C and in constant ionic media. Their protonation constants were determined and in particular the complex formation between cysteine and cadmium(II) was studied at 25 °C and in 1.00 M NaCl

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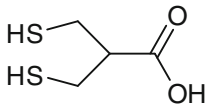
Fig. 1 Structure of dihydroasparagusic acid (H_3DA)



as constant ionic medium in a wide range of hydrogen ion concentrations.

Basinger et al. [12], by thinking about possible antidotes for mercury(II) poisoning, which was based upon earlier studies on arsenic, studied 2,3-dimercaptopropanol-1 (BAL) [13] as a ligand of mercury(II). However, the study was thus undertaken in order to develop a consistent understanding of the relationship between the coordination of mercury(II) and the structural requirements necessary if a molecule is to be an antidote for acute mercury(II) chloride intoxication. The study was carried out potentiometrically; however, only in few cases could the measurements be considered reversible. Furthermore, the ligand BAL was sparingly soluble in aqueous solution.

For the above reasons, dihydroasparagusic acid (H_3DA), the synthesis of which we recently reported [14], is presented and studied as an antidote against mercury(II) here. The presence of the carboxylic group increases the aqueous solubility of this compound compared with BAL. Its solubility depends on the hydrogen ion concentration of the solution. To study the behavior of H_3DA as a ligand towards mercury, it is necessary to investigate a wide range of concentration of the reagents. To minimize the variation of the activity coefficients of the reagents in spite of the variation of their concentration, the constant ionic medium method proposed by Biedermann and Sillèn [15] was adopted. All measurements were carried out at 25 °C and in 1.00 M NaCl as constant ionic medium.

The aim of this investigation was to verify the efficacy of H_3DA as an antidote against mercury(II), to evidence the complex formation between H_3DA and mercury(II), and to determine the protonation constants of H_3DA and the thermodynamic parameters of the compounds formed between H_3DA and mercury(II).

For the first purpose, an oxygen electrode was dipped in 10 cm^3 of 1.0 M glucose solution magnetically stirred at constant speed. A measured volume of *Saccharomyces cerevisiae* (0.25 cm^3) suspension was introduced into the glucose solution (1.0 M) very slowly to minimize the fluctuation of concentration of oxygen present in the solution. The presence of oxygen in solution decreases owing to the metabolic activity of *S. cerevisiae*. However, after about 1 h, the oxygen concentration reaches a quite constant value because the speed of oxygen consumption by the yeast and that of dissolution of oxygen coming from the atmosphere are the same.

The addition of the poisonous compound $HgCl_2$ (6 mg dm^{-3}) produces an immediate increase of the oxygen in solution so that its concentration approaches the initial oxygen saturation level. On the contrary, the simultaneous addition of $HgCl_2$ and H_3DA drastically limits the increase of the oxygen concentration in the culture medium. The resulting increase or decrease of oxygen concentration and its dependence on the addition of poisonous $HgCl_2$ and on H_3DA, respectively, will be explained in the "Results" section.

Figure 1 shows that dihydroasparagusic acid is a triprotic acid (H_3DA) with two hydrogen atoms bonded to two sulfur atoms and the third hydrogen atom bonded to the carboxyl group. The protonation constants of H_3DA can be defined by the following expression:

$$[H_nDA] = K_n[H_{n-1}DA]h \quad (1)$$

where n can be 1, 2, or 3 and h is the free concentration of the hydrogen ion. Charges are omitted for simplicity. The constants K_1, K_2, and K_3 are determined by measuring the potential difference (pd) of the following galvanic cell:

$$\text{RE/solution T/GE} \quad (\text{I})$$

where RE and GE are reference and glass electrode, respectively, and the solution T has the following general composition:

$$H\,\text{M in } H^+;\ (1-H)\,\text{M in } Na^+;\ 1.00\,\text{M in } Cl^-. \quad (2)$$

In Eq. (2), H is the analytical excess of hydrogen ions with respect to the ionic medium. At 25 °C, in a constant ionic medium and in millivolt units, the pd of cell (I) can be expressed as follows:

$$E_I = E_I^{\circ} + 59.16\,\log h + E_j \quad (3)$$

$E°_I$, a constant, and E_j, the liquid junction potential [15] which is dependent on h, are determined in the first part of each measurement when $H = h$, i.e., in the absence of H_nDA. To determine all three constants, an investigation in a wide range ($1 \le -\log h \le 12$) of hydrogen ion concentrations is necessary. In alkaline solution E_j' is dependent on OH^- and it is determined together with the ionic product of water, $-\log K_w$.

In the second part of the measurement, a known concentration of H_nDA is added to the solution T, and H is gradually varied by keeping $[H_nDA]_{tot}$ (total concentration of H_nDA) constant. From this approach, the analytical concentrations of H and H_nDA, and the h value, experimentally obtained from the measurements using cell (I) constitute the basis of the following elaboration.

The reaction between $HgCl_2$ and H_nDA is studied with different approaches. As by adding $HgCl_2$ to H_nDA the formation of a precipitate takes place, the determination of its solubility, S, is carried out. According to a first procedure, the solid is brought in contact with the ionic medium at known H, and according to a second one it is in contact

with a solution at known concentration of total H_nDA and known H. In both cases, suspensions are shaken until they reach equilibrium. After several tests, it could be deduced that in both cases 5 h is sufficient and necessary to reach equilibrium. The equilibrated suspensions are analyzed for $-\log h$ and for S. The former is determined by direct potentiometry and the latter by determining the Hg(II) concentration present in the filtered solutions by means of an atomic absorption spectrophotometer connected to a hydride kit. The values of H, analytically known, S and h, experimentally measured, are the basis of the elaboration carried out to obtain the results.

Results and discussion

H_3DA as an antidote

The capability of H_3DA as antidote versus mercury(II) was evaluated on a simple biologic system, *S. cerevisiae*, a yeast very sensitive to mercury, by measuring the oxygen concentration in a suspension with *S. cerevisiae*, mercury(II), and H_3DA. The oxygen concentration (as milligrams per cubic decimeter of O_2) in 1.0 M glucose solution at equilibrium with atmospheric oxygen was measured as a function of time. Three experiments were carried out by starting from the glucose solution. In the first one, a measured amount of *S. cerevisiae* was introduced and, after about 1 h, another solution of H_3DA was added. In the second experiment after the addition of *S. cerevisiae*, a solution of $HgCl_2$ was added. Finally, in the last experiment, after the initial *S. cerevisiae* addition, solutions of $HgCl_2$ together with H_3DA were added to the glucose solution.

In the first experiment, an initial plateau (ppm O_2 versus time) is observed before the introduction of the yeast. The presence of *S. cerevisiae* in the solution consumes oxygen thereby decreasing its concentration. After the test solution is in contact with air for about 1 h, the speed of oxygen consumption due to the yeast is equal to that of dissolution of oxygen from air and consequently a new plateau is again observed. At this point, to evaluate the eventual toxicity of H_3DA, H_3DA (with a concentration of 0.120 g dm^{-3}) was introduced and no remarkable modification was observed, showing a quite negligible toxicity. In fact, a little increase of oxygen concentration observed corresponding to the H_3DA addition was due mainly to the oxygen present in this last solution already in equilibrium with the atmospheric oxygen. The previous tendency to decrease the oxygen concentration in the medium culture was promptly restored by the yeast. This result, showing the negligible toxicity of H_3DA, allows one to use a relatively large amount of this compound as an antidote.

The comparison between the second and the third experiment is interesting. In all cases, the O_2 concentration is measured as a function of time. The first part of each experiment shows the same plateau. In the second experiment, the addition of yeast produces a decrease of oxygen, similar to that observed in the first one. By adding Hg(II) (with a concentration of 6×10^{-3} g dm^{-3}) an immediate and remarkable increase of O_2 concentration was observed. The O_2 concentration increase is observed because the yeast no longer consumes oxygen. The O_2 concentration increase is attributed to the mercury(II) which develops its poisonous effect on *S. cerevisiae*, with the inhibition of the yeast.

Vice versa, when, in the last experiment, mercury(II) was added together with dihydroasparagusic acid, with a mass ratio 1:600, the increase of O_2 concentration is relatively low. This observation shows that the yeast was little inhibited because the effect of the presence of free mercury(II) is very small. The simultaneously present dihydroasparagusic acid reacts with mercury(II) sequestrating it from the solution and therefore preventing its poisonous effect. As a consequence, the yeast's vitality was less affected by the presence of mercury.

The percentage of inhibition can be evaluated by using an internal normalization, because the yeast activity is related to atmospheric conditions, mainly the room temperature. It is assumed that the difference between the initial oxygen concentration and the oxygen concentration reached at the plateau corresponding to the addition of the yeast (second plateau in the first experiment) corresponds to 100 % vitality. The ratio of inhibition/toxicity may be calculated as follows.

From the above experiments, the oxygen consumption can be obtained from the decrease of oxygen level due to the presence of *S. cerevisiae* ($\Delta 1$), whereas the increase of oxygen can be calculated from the increase of oxygen corresponding to the introduction of mercury(II) ($\Delta 2$) or dihydroasparagusic acid ($\Delta 3$), respectively. The ratio $(\Delta 2/\Delta 1) \times 100 = 97$ % is evaluated for the inhibition of mercury(II), whereas the ratio $(\Delta 3/\Delta 1) \times 100 = 29$ % corresponds to the inhibition in the presence of mercury, provided that dihydroasparagusic acid is present as well. The effect of dihydroasparagusic acid as an antidote is evident.

Protonation constants

To obtain the K_n values, H and H_nDA, analytical concentrations and h, experimentally obtained from the pd measurements of cell (I) are elaborated. The material balance of analytical excess of hydrogen ion, H, by taking into account the mass action law and in a constant ionic medium, can be expressed as follows:

$$H = h + K_1 h\left[DA^{3-}\right] + 2K_1K_2h^2\left[DA^{3-}\right] + 3K_1K_2K_3h^3\left[DA^{3-}\right]. \quad (4)$$

From Eq. (4) and from the total concentration of H_nDA, the protonation function p of DA^{3-} (as the average number of protons bonded to DA^{3-}) can be calculated as follows:

$$p = (H - h)\left([H_nDA]_{tot}\right)^{-1} = \Sigma n K_n h^n (1 + \Sigma K_n h^n)^{-1}. \quad (5a)$$

In Fig. 2 the protonation function p is plotted versus $-\log h$. As experimental points obtained at different concentration of $[H_nDA]_{tot}$ fall on the same curve, the protonation function p is not a function of the dihydroasparagusic acid concentration, so that polynuclear species are not present and Eq. (4) is correct.

In a first approach the graphical method of normalized curves proposed by Sillèn [16] is applied. Equation (5a) can be normalized with the following:

$$p = \left(Ru + 2u^2 + 3R'u^3\right)\left(1 + Ru + u^2 + R'u^3\right)^{-1} \quad (5b)$$

where $\log u = 0.5 \log K_1K_2 + \log h$, $\log K_1 = \log R + 0.5 \log K_1K_2$, and $\log K_3 = \log R' + 0.5 \log K_1K_2$. The family of normalized curves of Eq. (5b) were superimposed onto the experimental points of Fig. 2 and the two plots were moved parallel to the abscissa until the best fit was reached. In this position the $-\log h$ value was read as a function of $\log u$ and, on the basis of the mathematical position proposed above, the values of the protonation constants K_1, K_2, and K_3 could be obtained.

By treating the experimental points independently using a graphical program [17] and a PC program BSTAC [18]

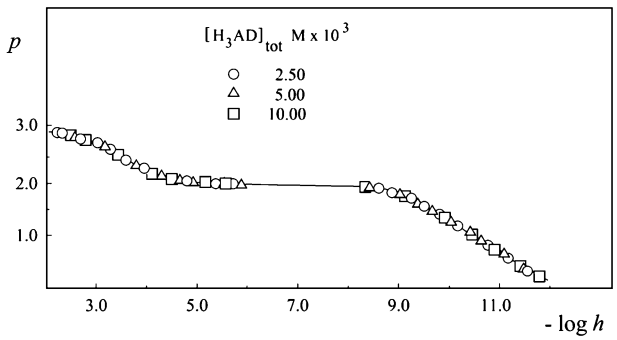


Fig. 2 Protonation function of dihydroasparagusate versus $-\log h$. The curve is the normalized one in the position of best fit

the same value for the protonation constants is obtained. In Table 1 the proposed values are collected.

Reaction between $HgCl_2$ and H_nDA and precipitation

To explain the trend of the reaction taking place between mercury(II) and dihydroasparagusic acid, the data of solubility, S, and of the free hydrogen ion concentration, $-\log h$, are elaborated. Experimental data of S at different H and $[H_nDA]_{tot}$ were obtained. The reaction between mercury(II) and DA^{3-} in aqueous solution can be written as follows:

$$Hg^{2+} + pH^+ + rDA^{3-} \leftrightarrow Hg\ H_p(DA)_r$$

defined by the constant

$$\beta_{1,p,r} = \left[Hg\ H_p(DA)_r\right]\left[Hg\right]^{-1}h^{-p}\left[DA\right]^{-r}. \quad (6)$$

In the reaction and in Eq. (6) most of the charges are omitted for simplicity and the square brackets indicate the free concentration (at equilibrium) of the species. Equation (6) formulates the hypothesis is that mononuclear species with mercury(II) are formed.

The solubility S can be expressed by the following relation:

$$S = \left[Hg^{2+}\right] + \Sigma\Sigma\beta_{1,p,r}\left[Hg^{2+}\right]h^p\left[DA^{3-}\right]^r = \left[Hg^{2+}\right]\left(1 + \Sigma\Sigma\beta_{1,p,r}h^p\left[DA^{3-}\right]^r\right). \quad (7)$$

Since the analysis of the precipitate provided the formula $Hg(H_2DA)_2$ and the solubility data are obtained at $-\log h \leq 7$, it seems reasonable to assume that in this range the dihydroasparagusic acid is present in the form H_2DA^- and then Eq. (7) can be written as follows:

$$S = \left[Hg^{2+}\right] + \Sigma\beta_{1,w}\left[Hg^{2+}\right]\left[H_2DA^-\right]^w = \left[Hg^{2+}\right]\left(1 + \Sigma\beta_{1,w}\left[H_2DA^-\right]^w\right)$$

which can be transformed into the following:

$$\log\left\{S\left[H_2DA^-\right]^2\right\} = \log\left\{\left[Hg^{2+}\right]\left[H_2DA^-\right]^2\right\} + \log\left\{1 + \Sigma\beta_{1,w}\left[H_2DA^-\right]^w\right\} \quad (8)$$

and also:

$$\log\left\{S\left[H_2DA^-\right]^2\right\} = \log K_s + \log\left\{1 + \Sigma\beta_{1,w}\left[H_2DA^-\right]^w\right\} \quad (9)$$

Table 1 Protonation constants of DA^{3-} at 25 °C and in 1.00 M NaCl as ionic medium

Species	log K_1	log K_2	log K_3	log K_1K_2	log $K_1K_2K_3$
HDA^{2-}	11.30 ± 0.05				
H_2DA^-		9.75 ± 0.05		21.05 ± 0.05	
H_3DA			3.50 ± 0.08		24.55 ± 0.10

The error limits correspond to the maximum possible shift between points and curves that still retains an acceptable agreement

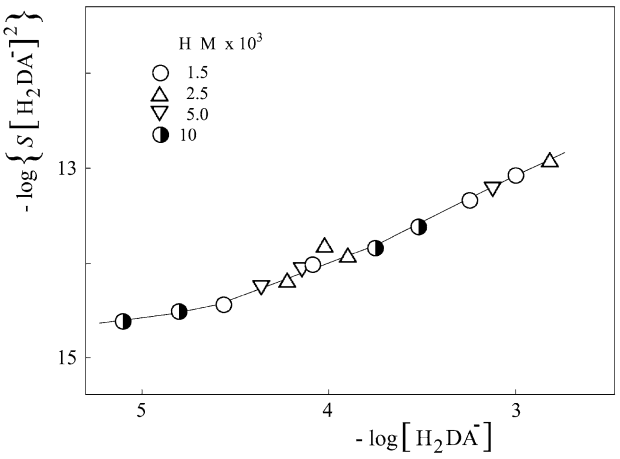


Fig. 3 Dependence of solubility on the concentration of $-\log [H_2DA^-]$

where K_s indicates the solubility product of the precipitate formed between mercury(II) and dihydroasparagusic acid and the constant $\beta_{1,w} = [Hg\,(H_2DA^-)_w]\,[Hg]^{-1}\,[H_2DA^-]^{-w}$ is the stability constant of final complexes formed between the reagents.

According to Eq. (9), the points are plotted in Fig. 3 in the form $\log \{S\,[H_2DA^-]^2\}$ versus $-\log [H_2DA^-]$. As points obtained at different H and at different concentration of H_3DA fall on the same curve, the validity of Eq. (9) can be deduced and K_s as well as the constant of complex formation can be obtained.

At first the method of normalized curve proposed by Sillèn [16] is applied and the formation of only one complex is assumed. A normalized curve can be obtained with the following equation:

$$y = \log(1 + v)$$

By comparing the normalized curve with Eq. (9), the following positions $\log\left\{S[H_2DA^-]^2\right\} - y = \log K_s$ and $\log v = \log \beta_{1,1} + \log[H_2DA^-]$ are assumed.

The normalized curve was superimposed onto the experimental points and the two plots were moved parallel to the two axes until the best fit was obtained. In this position and on the basis of the mathematical positions, the values of $\log K_s = 14.72 \pm 0.05$ and $\log \beta_{1,1} = 4.78 \pm 0.05$ are obtained. The error limits correspond to the maximum possible shift between points and curves that still retains an acceptable agreement.

Conclusions

This paper is a complete investigation of the properties of dihydroasparagusic acid. No literature data can be found about this subject because this natural product is present in very small quantities in its natural source and for this reason it was seldom investigated in the past. In light of its low abundance in the plant, it was recently synthesized by an improved procedure [14].

The validity of dihydroasparagusic acid as an antidote against mercury(II) poisoning is supported by the different oxygen consumption of the yeast (*S. cerevisiae*) in the absence and in the presence of H_3DA. It can be assumed that dihydroasparagusic acid, which also contains a carboxylic group, is more soluble than BAL and it is a triprotic acid. The three protonation constants are determined. As expected, the two hydrogen atoms are strongly bonded to sulfur and their dissociation as ions can take place at $-\log h \geq 9$. This means that, in acid or neutral ($-\log h \leq 7$) solution, the compound is present in the form H_2DA^-.

The analysis of the precipitate and the elaboration of the solubility data give consistent results. The value of the solubility product could appear relatively high, but it is calculated by considering mercury(II) and the compound in the form H_2DA^-. The concentration of this form is obtained from the product $K_1K_2h^2\,[DA^{3-}]$. On the basis of this calculation, the value of $\log K_s$ becomes $-\log K_s = \log \{[Hg^{2+}]\,(h^2\,[DA^{3-}])^2\} = 36.08$, which represents a low value. Furthermore, the value of $-\log K_s$ proposed here is determined in 1.00 M of NaCl as the ionic medium.

The high stability of the species $HgCl^-$ and $HgCl_2$ found by Ciavatta and Grimaldi [19] and the ionic medium adopted in this investigation (1.00 M NaCl) must be considered for a correct thermodynamic analysis of the solubility data and the proposed complex. The solubility product and the stability constant are valid under the selected experimental conditions and in the selected ionic medium. However, NaCl cannot be considered inert towards Hg(II). If the high stability of $HgCl^-$ and $HgCl_2$ is taken into account, the solubility product ($-\log K_s$) and the stability constant of the complex would be still higher than the proposed value. By taking into account the large excess of NaCl as the ionic medium, it seems reasonable to evaluate the presence of $HgCl_2$ with a constant $\log \beta_2 = 13.2$. This consideration gives more validity to the antidote property of H_2DA^-. Really, in the hypothesis of the absence of the ionic medium, the numerical values of $-\log K_s$ of $Hg[H_2DA]_2$ and of $\log \beta_{1,1}$ of $Hg(H_2DA)$ should be higher by a factor of approximately 13.2.

Although potentiometric measurements and analytical data are not able to provide information about the structure of the formed species, but taking into account the investigated concentration ranges and the literature data, some hypotheses regarding the mechanism of formation of the assumed compounds can be proposed.

The presence of the compound acting as H_2DA^- explains the composition of the precipitate. The formation

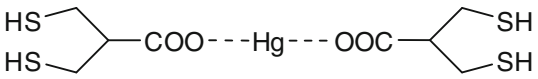


Fig. 4 Proposed structure of the $Hg[H_2DA]_2$ precipitate

of a chelate should be excluded because the form H_2DA^- can work as a monodentate compound and because the structure of mercury(II) is compatible only with an angle of 180°. The remaining possibility is the bond between mercury(II) and two H_2DA^- through the two carboxyl groups. Furthermore, the formation of the complex with H_2DA^- can be seen as a bond through the carboxylic group because the dihydroasparagusic acid, in the investigated $-\log h$ range, has the two protonated sulfur atoms and the carboxyl free to bind mercury.

This hypothesis is supported by the literature investigation on the formation and the stability of complexes of mercury(II) with acetate and chloride, respectively. Rossotti and Whewell [20] explained the experimental data obtained for the system mercury(II) acetate with the formation of two complexes with similar stability constants ($\log \beta_1 = 4.22$ and $\log \beta_2 = 4.23$). The values proposed in this paper for the complex $Hg(H_2DA)$ are similar to Rossotti's. Further support is provided by the investigation of the system Hg(II) Cl^-, studied by Ciavatta and Grimaldi [19]. The experimental data of this paper were explained by assuming the presence of two very stable complexes $HgCl^+$ and $HgCl_2$ ($\log K_1 = 6.72$ and $\log K_2 = 6.51$), the formation of $HgCl_3^-$ with low stability ($\log K_3 = 1.00$), and the formation of $HgCl_4^{2-}$ which is still less stable ($\log K_4 = 0.97$). The presence of the ionic medium does not interfere with the composition of the precipitate, because the analysis showed that its composition was $Hg[H_2DA]_2$ and no chloride was present in the precipitate.

By taking into account all these literature data and the mercury(II) properties, the structure shown in Fig. 4 is proposed for the precipitate. No chelate structure seems to be probable for the precipitate of $Hg[H_2DA]_2$.

The behavior of the dihydroasparagusic acid as an antidote against mercury(II) is confirmed by the very low value of the solubility product of the precipitate.

Experimental

Mercury(II) chloride (RPE product, C. Erba), was recrystallized twice from water and dried over H_2SO_4 in a vacuum desiccator for a week, as described by Ciavatta and Grimaldi [21]. Hydrochloric acid, sodium chloride, and sodium hydroxide were prepared and analyzed as described previously [8]. Dihydroasparagusic acid (H_3DA) is the first dimercapto compound isolated from a natural source [1]. It is contained in *Asparagus* species in the order of a few milligrams per kilogram of the fresh plant. In order to obtain a good yield of this acid, in a few steps and utilizing commercial reagents, we revised some existing syntheses [22–25]. The revised synthetic procedure for the preparation of dihydroasparagusic acid (H_3DA) was recently described [14] and is summarized as follows (Scheme 1): diethyl bis(hydroxymethyl)malonate was refluxed with concentrated hydroiodic acid to obtain a mixture of β,β′-diiodoisobutyric acid and iodomethylacrylic acid. After salification with 0.8 M KOH the solution was added with a solution containing thioacetic acid and potassium thioacetate. Under these conditions, in the presence of thioacetate anion and free thioacetic acid, two different reactions occur simultaneously: the substitution of iodide by thioacetate anion and the addition of free thioacetic acid to the double bond of the acrylic by-product, leading to the desired bis(thioacetyl) intermediate. The final alkaline hydrolysis under mild conditions allows one to obtain the final product.

A preliminary investigation of the reaction between H_3DA and $HgCl_2$ was performed by means of cyclic voltammetry [26]. A solution of 10^{-3} M of $HgCl_2$ was mixed with H_3DA in ratios of 1:4 or 1:8. The solution was buffered at pH 7.4 by adding phosphate. The shift of the potential to more positive values was explained by assuming the formation of a 2:1 complex between H_3DA and $HgCl_2$.

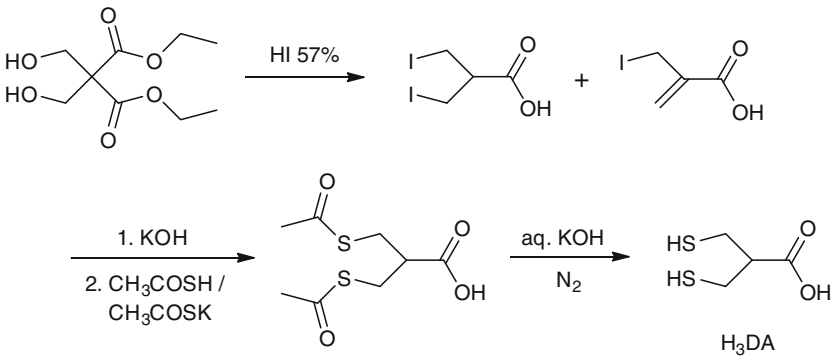
To evaluate the dihydroasparagusic acid capacity of the antidote against mercury(II), a suspension of *S. cerevisiae* was prepared by mixing 2.5 g of *S. cerevisiae* with 50 cm^3 of distilled water. The resulting suspension was kept at room temperature for at least 2 h before use. A volume of 0.25 cm^3 of this suspension, shaken before use, was introduced into the culture medium. A 1 M solution of glucose (10 cm^3) was used as the medium.

By adding a moderate excess of $HgCl_2$ to a solution of dihydroasparagusic sodium salt, a precipitate formed. The solid was filtered and washed several times to eliminate chloride ions. The solid was dried in an oven at 80 °C and analyzed according to Schwarzenbach and Flaschka's method [27] to obtain the percentage of mercury(II). The analysis provided a molecular mass of 501 with respect to the theoretical value of 500.61 corresponding to the formula $Hg(H_2DA)_2$.

Experimental apparatus

All the measurements were performed in a thermostat room at 25 ± 0.5 °C and in a thermostat at 25 ± 0.05 °C. A stream of nitrogen (99.999 %) further purified by passing through 10 % NaOH, 10 % H_2SO_4, and the ionic medium, was bubbled through all the solutions during the measurements.

Scheme 1



The capability of H_3DA to act as an antidote was evaluated by measuring the oxygen present in the *S. cerevisiae* mixtures both in the presence and absence of mercury(II) and H_3DA by means of an oxygen electrode (model 97-08, Orion Research). The O_2 electrode provides its measurements in milligrams per cubic decimeter of oxygen present. The solubility of the precipitate, *S*, i.e., the mercury(II) concentration, was determined by means of an atomic absorption spectrophotometer (model Solaar AA, Thermo Fisher), equipped with a hydride kit, able to determine mercury(II) up to 0.1 μg dm^{-3}.

The free hydrogen ion concentration, *h*, was obtained by potentiometric measurements of a galvanic cell containing a glass electrode (GE), by previous calibration in units of $-\log h$ with at least two or three solutions of known hydrogen ion concentration in the selected ionic medium. All the pd measurements were carried out by a Metrohm model 654 pH meter equipped with a glass electrode No. 6.0102.000 from the same firm. The reference electrode (RE) was prepared according to Brown's method [28] (RE = Ag, AgCl/1.0 mol dm^{-3} NaCl, saturated with AgCl/1.0 M NaCl). Constant values of galvanic cell measurements were obtained within a few minutes. The values were reproducible within ±0.2 mV. The GE response agreed with that of a hydrogen electrode until $-\log h \leq 10$. Beyond this limit, the values obtained from GE were adjusted assuming the response provided by the hydrogen electrode as correct.

As the measurements obtained from solutions prepared with different procedures agreed well, this proved that the measurements correspond to the real equilibrium.

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