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Cysteamine S-Phosphoric Acid

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Free cysteamine S-phosphoric acid has been prepared. The effect of pH on the hydrolysis of the synthesized compound has been studied and a mechanism for its reaction with iodine is suggested. Some solvent systems and spray reagents useful in paper chromatography of cysteamine S-phosphate are described.

A convenient, high yield method for the synthesis of trisodium phosphorothioate, used in the preparation of the title compound, has been worked out.

In two earlier papers [1,2] a method employing trisodium phosphorothioate for the preparation of some S-substituted phosphorothioic acids was reported. Among the compounds thus prepared was the sodiumhydrogen salt of cysteamine S-phosphoric acid (S-(2-aminoethyl) phosphorothioic acid). This compound was later found to be enzymically hydrolyzed to *ortho*-phosphate and cysteamine by human erythrocytes [3].

In the present paper the preparation of free cysteamine S-phosphoric acid is reported and some of its properties are described.

Cysteamine S-phosphoric acid has been prepared by dissolving the sodiumhydrogen salt in glacial acetic acid. Upon standing the free acid crystallizes out, whereas the simultaneously formed sodium acetate remains in solution.

The S-P bond in cysteamine S-phosphoric acid is extremely acid labile. Thus a 2.5 mM solution of the compound in 1 M HCl is completely hydrolyzed in less than one minute at 100°, giving a quantitative yield of cysteamine and *ortho*-phosphate. In neutral or slightly alkaline solutions the compound is relatively stable at room temperature.

The rate of hydrolysis of cysteamine S-phosphate as a function of pH at 37° is described in Fig. 1. The reaction was followed by the appearance of thiol groups [4] and (ethylenedinitrilo) tetraacetic acid was added to prevent heavy metal catalyzed oxidation of the cysteamine formed. Standardization of the colorimetric method was carried out as described previously [3]. A maximum rate of hydrolysis takes place around pH 3, where the compound is primarily in the form of $NH_3^+CH_2CH_2SPO_3H^-$ as concluded from acid-base titrations.

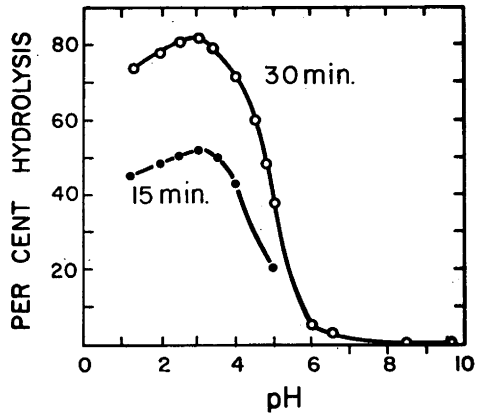

Fig. 1. Effect of pH on the hydrolysis of cysteamine S-phosphate (5 mM) at 37° in the presence of (ethylenedinitrilo)tetraacetic acid (13 mM). Ionic strength close to 0.15.

Such titrations were performed on both the free cysteamine S-phosphoric acid as well as on the sodiumhydrogen salt (10 mM solutions, 25°). Since the formation of some *ortho*-phosphate during the course of the titrations is inevitable in the acid region, other pK_a measurements were performed by adding just enough HCl to solutions of sodiumhydrogen cysteamine S-phosphate to reach the half titration points. The pH was then determined instantly. The apparent pK_a values obtained in this way agreed within ± 0.05 pK_a units with those obtained from rapidly performed acid-base titrations.

The results are reported in Table 1, from which it is evident that the second and third apparent pK_a values of cysteamine S-phosphate are relatively similar to those reported for 2-aminoethanol O-phosphate. The largest deviation in the results is to be found in the first pK_a, which for cysteamine S-phosphate is close to the lowest pK_a of *ortho*-phosphate ($pK_a = 2.1$), whereas the value for 2-aminoethanol O-phosphate is considerably lower.

In comparison with 2-aminoethanol O-phosphate cysteamine S-phosphate melts appreciably lower and is hydrolyzed by 1 M HCl at 100° some 1 000 times faster than the former (Table 1).

The observed rate of hydrolysis of cysteamine S-phosphate as a function of pH is qualitatively similar to that found for S-(*n*-butyl) phosphorothioate

Table 1. Comparison between some properties of cysteamine S-phosphoric acid and 2-aminoethanol O-phosphoric acid.

Property	$NH_2CH_2CH_2SPO_3H_2$	$NH_2CH_2CH_2OPO_3H_2$
Melting point	156°	244° [10]
Half life in 1 M HCl at 100°	<1 min	≈ 17 h [11]
pK_a	2.2; 5.0; 10.3	<1; 5.57; 10.10 [12]
		<1; 5.84; 10.89 [13]

by Herr and Koshland [5]. These authors proposed a mechanism for the observed hydrolysis. Several monoesters of *ortho*-phosphoric acid also give hydrolysis curves similar to those obtained for the S-substituted phosphorothioic acids mentioned here (see, *e.g.*, Ref.[6]).

Above pH≈2 cysteamine S-phosphate reacts momentarily with iodine, one atom of iodine being consumed per molecule of S-phosphate. During this process *ortho*-phosphate is formed together with a ninhydrin and cyanide-nitroprussiate positive substance with the same R_F-value in ethanol : pyridine : water, 2 : 1 : 1, and in *n*-butanol : acetic acid : water, 4 : 1 : 1, as cystamine (2,2′-dithiobisethylamine).

At pH≤1 the reaction between cysteamine S-phosphate and iodine is very slow *. At this pH at least part of the observed decolorization of iodine could be attributed to release of thiol groups by hydrolysis of the S-P bond.

The reaction between cysteamine S-phosphate and iodine in slightly acid and neutral solutions is similar to that described by Wieland and Lambert [7], who found that iodine is momentarily decolorized by a solution of S-(*n*-butyl) phosphorothioate around pH 7. These authors proposed a mechanism for the interaction between iodine and the S-substituted phosphorothioate used. However, this mechanism does not explain the failure of iodine to react rapidly with cysteamine S-phosphate at pH≤1.

A mechanism that explains the experimental results is given in Fig. 2. This is essentially a combination of the Herr and Koshland [5] mechanism with that of Wieland and Lambert [7].

In their suggested mechanism for acid hydrolysis of *S*-(*n*-butyl) phosphorothioate, Herr and Koshland [5] propose that one (and only one) of the OH-groups attached to the phosphate residue should be dissociated for maximum rate of hydrolysis. The dissociated OH-group should serve to activate a hydro-

* For instance, 3 mmole of cysteamine S-phosphate in 50 ml of 2 M HCl required more than 12 h to decolorize an equivalent amount of iodine.

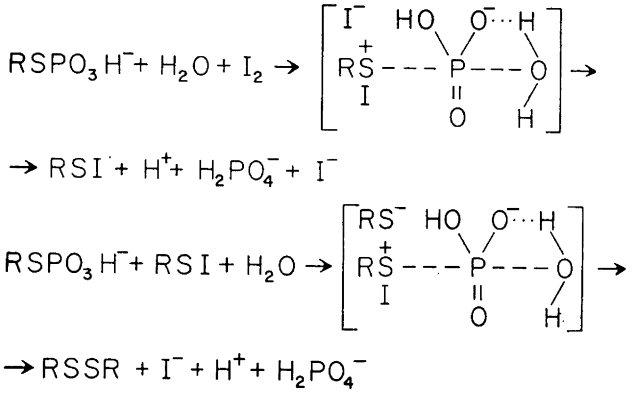

Fig. 2. Suggested mechanism for the reaction between iodine and cysteamine S-phosphate (R = $NH_3^+CH_2CH_2^-$).

gen bonded water molecule attached to the phosphate residue. The second (undissociated) OH-group on the phosphate residue should serve as a proton donor to the sulfur atom. When this OH-group is also dissociated, the molecule is thus stable.

In the scheme in Fig. 2 the dissociated OH-group serves the same purpose as in the Herr and Koshland mechanism as explained above. The presence of an undissociated OH-group, however, is not needed, since the intermediary step, like in the Wieland and Lambert scheme, is suggested to be RSI. This explains the fact that the cysteamine S-phosphate — iodine reaction (in contrast to the hydrolysis) proceeds rapidly also at pH 7.

Cysteamine S-phosphate can be separated from *ortho*-phosphate and from phosphorothioate by paper chromatography. Some solvent systems for this purpose are listed in Table 2. In addition to the commonly used phosphate sprays, the following spray reagents were useful in the identification of the above mentioned substances on the chromatograms:

1. 0.2 % ninhydrin in ethanol : pyridine, 8 : 2. Cysteamine S-phosphate gives a violet spot after heating for 5 min at 100°. (Ninhydrin in ethanol without added pyridine gives only weakly colored spots).
2. 0.1 % iodine in 5 % KI gives decolorized zones for cysteamine S-phosphate and phosphorothioate.
3. 1 % copper(II)chloride in 50 % ethanol containing 5 % NH_3 gives yellow brown spots with trisodium phosphorothioate and black spots with cysteamine S-phosphate.

Trisodium phosphorothioate, which was utilized for the synthesis of cysteamine S-phosphate [1], was previously prepared in about 40 % yield according to Yasuda and Lambert [8]. In this method $PSCl_3$ and a solution of sodium hydroxide in water are refluxed together. However, during this operation much phosphorothioate is hydrolyzed and large amounts of *ortho*-phosphate are formed. Improved experimental conditions for this reaction were therefore worked out. Lowering the reaction temperature to 75—85° gave 85—90 % final yield of anhydrous trisodium phosphorothioate of high purity. The decrease in reaction temperature did not unduly decrease the reaction rate *.

Table 2. Approximate R_F-values in three solvent systems. Solvents: 1, Ethanol:pyridine: water, 10:5:8. 2, 2-Methoxyethanol:NH_3(29 %):water, 3:2:2. 3, Ethanol:NH_3 (29 %): water, 6:1:3. The substances were chromatographed (ascending solvents) on Whatman No. 4 papers.

Substance	Solvent No. 1	2	3
$NH_2CH_2CH_2SPO_3HNa$	0.39	0.71	0.54
K_2HPO_4	0.18	0.54	0.29
Na_3SPO_3	0.30	0.62	0.31

* Yasuda and Lambert have later published a slightly modified procedure, which gives a yield of *ca.* 57 % Na_3SPO_3[14].

Binkley [9] performed the same reaction at 0°. In the present author's experience, however, the reaction rate at this temperature is inconveniently slow.

EXPERIMENTAL

Cysteamine S-phosphoric acid. 5.0 g of freshly prepared sodiumhydrogen cysteamine S-phosphate [1] were stirred at 25° in 100 ml of glacial acetic acid under exclusion of moisture for 30 min. During the process the S-phosphate dissolved completely and after a few minutes of stirring the crude free acid started to separate. The crystalline precipitate was filtered off and washed thoroughly with glacial acetic acid and with dry petroleum ether (b.p. 30–60°). The product was further purified by dissolving it in 50 ml of 10 % acetic acid and reprecipitating with 150 ml of ethanol. This purification process was repeated twice. 2.9 g (66 %) of crystalline substance were thus obtained (average of three prepns.) melting at 156° (corr.). Further reprecipitations did not increase the melting point. (Found: C 15.3; H 5.2; P 19.8. Calc. for $NH_2CH_2CH_2SPO_3H_2$ (157.14): C 15.3; H 5.1; P 19.7.)

Trisodium phosphorothioate. 80 g (2.0 mole) of sodium hydroxide were dissolved in 500 ml of water and 35 ml (0.34 mole) of $PSCl_3$ were added. The reaction flask was equipped with a reflux condenser and the synthesis was carried out on a combined magnetic stirrer-hot plate. After initial heating to about 80°, the reaction was allowed to proceed at 75–85° under constant stirring until all $PSCl_3$ had reacted (about 2 h). The slightly yellow solution was kept in the refrigerator (4°) over night. The precipitated white crystals were filtered off and washed with ethanol (100 ml). They were dissolved in 250 ml of distilled water at 45° and reprecipitated by the slow addition of 200 ml of ethanol under constant, rapid stirring. The solution was cooled under tap water, filtered and the crystals washed with ethanol (100 ml). The substance was dehydrated by stirring in 600 ml of dry methanol for 1.5 h, filtered and finally dried at 105° for 1 h. 52–55 g (85–90 %) of anhydrous trisodium phosphorothioate were thus obtained.

Phosphate determinations [9] and iodine titrations [8] showed the substance to be 99–100 % pure.

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