PREPARATION AND PROPERTIES OF CRYSTALLIZED ALKALI SALTS OF L-CYSTINE

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A number of the normal salts of L-cystine with bases have been described by Neuberg and Mayer (1). Only the Cu salt was obtained in crystals while the salts with Ag, Hg, Pb, and Cd, as well as with Fe, Cr, Sn, and Zn, were amorphous. All of these salts are characterized by their insolubility in water. No data are available on the isolation of salts of the cystine anion with alkali metals. They form the object of the present report.

The principle used in the preparation of these salts is to dissolve the cystine in an alcoholic alkali solution to which just sufficient water to effect solution has been added, and, after filtering from excess cystine, to precipitate the salt by addition of a suitable indifferent solvent. While various solvents, such as acetone, ether, or large amounts of alcohol caused precipitations, these were either oily or amorphous. Only acetonitrile was found to possess the power of initiating regular crystallization in the salt solutions. While the solutions of the different salts require different amounts of the solvent for complete precipitation, a partial substitution of absolute ether for acetonitrile was found expedient in the case of the most soluble of the salts, the K salt.

Considerable experimentation led to the procedures described in the experimental part, and although the conditions given there are by no means unalterable, close adherence to them will avoid difficulties of crystallization. A method of reccrystallization was not found and reprecipitation under the conditions of preparation did not prove satisfactory with respect to improvement of purity or crystal form. However, analysis indicated a fair state of purity of the original preparations.
Comparison of the optical rotations of acid solutions of equal concentrations, of the original cystine on the one hand and the salt on the other, showed an almost unchanged recovery of the l-cystine from its alkali salt.

Of the three salts prepared, the Na, K, and Li salts, only the Na salt is obtained with 1 molecule of water of crystallization, which is given off at 78° in vacuo. The K salt, on the other hand, is distinguished by its deliquescent nature. On exposure to the atmosphere it soon gets sticky and loses its structure. The two other salts merely absorb a little moisture from the air which, however, can be removed by drying.

As regards the crystalline structure, the Na salt is the least satisfactory one, while the K and the Li salts were obtained in at least two different distinct crystal forms. It seems, however, improbable that these different crystal forms are related to the different optical modifications of cystine, since their qualitative distribution does not agree with the composition of the cystine used (with a maximum content of 6 per cent inactive cystine). Rather does it seem that in different concentrations different types of crystal growths prevail.

All three salts are very soluble in water and they are also soluble to some extent in the lower alcohols, the solubility in CH₃OH being considerably larger than that in C₂H₅OH, while the alcohol solubilities rapidly increase in the order Li→Na→K salt.

Determinations of optical rotation showed that the aqueous solutions are practically stable for a number of days, while the methyl alcoholic solutions undergo a rapid decomposition, evidenced also by discoloration of the solution and evolution of NH₃. These stability differences between the aqueous and alcoholic solutions undoubtedly are related to differences in the ionic state of the cystine molecule, differences that are accompanied by radical steric and energy changes (2). By the investigations of Levene and his associates (3), the existence of definite relations between ionization and optical rotation has been generally established. As regards l-cystine, earlier observations (4, 5) have indicated already that the rotation in alkaline solution is less than half as large as that in acid solution, and our data on the aqueous solutions of the alkali salts fully agree with those findings. The rotation of the alcoholic salt solutions, on the other hand, we find to be higher.
than that of the acid cystine solution. This large rotatory difference is paralleled, as mentioned above, by a significant difference of chemical stability. A shifting of the ionic equilibrium from the anionic form toward the non-ionized and hybrid ion forms, due to the weaker basicity of the alkali in the alcoholic solution, might account for both phenomena. A specific solvent influence of the alcohol on the rotation might also play a rôle, although observations of Abderhalden and Wybert (6) on the rotation of cystine derivatives (monochloroacetyl-, dichloroacetyl-, dibromoacetyl-, and diiodoacetyl-cystine) indicate that the solvent effect of alcohol on the rotation of cystine is of a minor order. For, by calculating and expressing the rotations given by the authors as specific rotations of the cystine molecule instead of the individual derivative, only comparatively small differences appear between the rotations of these compounds although they were determined partly in HCl and partly in C₃H₅OH.

Finally the stability of the aqueous salt solutions appears noteworthy in view of the known instability of cystine solutions when boiled with excess alkali (cf. (7)).

EXPERIMENTAL

The cystine used in most of this work had a specific rotation of \( [\alpha]_{29}^{n} = -192.1^\circ \), corresponding to a mixture of 94 per cent \( l \)-cystine and 6 per cent inactive cystine (8).

I. Sodium Salt—To 2.5 gm. of cystine, 43.5 cc. of a 0.46 n NaOH solution in 82 per cent C₃H₅OH are added. The solution of sodium cystinate is filtered through a dry filter from excess cystine; 2 volumes of acetonitrile (Eastman) are added and, after brief shaking, the solution is allowed to stand undisturbed for 1 to 2 hours. By this time, the milky solution should have changed to a crystalline mass with supernatant clear liquid. The crystals are filtered, washed with some acetonitrile, and freed from excess solvent in a vacuum desiccator (yield about 75 per cent).

In some cases a yellowish oily phase settles to the bottom soon after the solution has been filtered. In this case addition of a little 82 per cent C₃H₅OH will redissolve the oily layer. It is, however, always advisable to add the acetonitrile as quickly as possible after filtering in order to forestall any possible separation. If, soon after addition of the acetonitrile, crystal aggregations
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do not begin to appear on the glass walls, seeding with some crystals previously obtained in a test-tube experiment will initiate the crystallization.

The crystals consist of fine needles that have a silky appearance when dry; while under the microscope they appear mostly as irregular conglomerations of branched needles, they form occasionally

![Fig. 1. About × 80. Crystals of Na salt](image)

regular radiating groups, visible to the naked eye, on the walls of the glass vessel. Fig. 1 gives a photomicrograph of the Na salt.

A sample dried to constant weight in vacuo over $P_2O_5$ at 56° gave the following figures. (All alkali titrations of the salts were carried out with 0.02 N HCl, with methyl red as indicator.)

\[
\begin{align*}
\text{Calculated.} & & \text{Na} & & 16.58 \\
\text{CsH}_{10}O_4N_2S_2N_2 & & \text{Na} & & 15.23 \\
\text{CsH}_{10}O_4N_2S_2N_2 + H_2O & & \text{Na} & & \text{(volumetric) 15.45, 15.54;} \\
\text{Found.} & & \text{(as } Na_2SO_4) & & 15.12, 15.06
\end{align*}
\]
Samples previously dried at 56° to constant weight were now dried to constant weight at 78°, and the loss in weight was assumed to represent H₂O.

\[ \text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{S}_2\text{Na}_2 + \text{H}_2\text{O} \]

Calculated. H₂O 5.96

Found. " 5.90, 6.04

Drying at 100° did not cause any further loss of weight. Above 100° decomposition starts. Samples dried at 78° gave the following analysis.

\[ \text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{S}_2\text{Na}_2 \]

Calculated. Na 16.18, S 22.54

Found. " (volumetric) 16.48, 16.41

" S (as BaSO₄ according to Benedict-Denis (9)) 21.95, 22.03

These analyses indicate that the Na salt crystallizes with 1 molecule of H₂O.

The salt is very soluble in water. The approximate solubility of the anhydrous salt in anhydrous C₆H₅OH and CH₃OH at 25° was determined by saturating the alcohol with the salt at 30°, cooling to and keeping at 25° for at least 10 minutes with constant shaking, filtering, and titrating the alkali in measured volumes of the filtrate diluted with water.

C₆H₅OH solution. Found. 0.017 gm., 0.014 gm. per 100 cc.

CH₃OH " " 2.40 " " 2.28 " " 100 "

The specific rotation of the anhydrous salt was determined and calculated, for comparison, for cystine; 0.2506 gm. of salt dissolved to 25 cc. in H₂O gave [α]₀²⁵ = -89.6°, [α]₀²⁷ = -90.5° (after 24 hours); 0.2500 gm. of salt dissolved to 25 cc. in CH₃OH gave [α]₀²⁵ = -265°, [α]₀²⁷ = -250° (after 24 hours). While after 24 hours the aqueous solution shows no signs of decomposition, the methyl alcoholic solution has turned yellow and NH₃ is being given off.

II. Potassium Salt—To 2.5 gm. of cystine 20 cc. of a 1 N solution of K in absolute CH₃OH are added. The solution of potassium cystinate is filtered from excess cystine. A mixture of 75 cc. of absolute ether and 75 cc. of acetonitrile is added, and, after brief mixing, the whole is left in a refrigerator overnight. The crystalline mass is filtered and brought into a desiccator immedi-
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ately as the product is deliquescent in the air. With special care, the theoretical yield is obtained.

Under the conditions described the crystallization usually begins with the separation of droplets from the milky emulsion that first results on addition of the precipitating mixture. These droplets gradually assume a crystalline structure as shown in Fig. 2. Another crystal form also appears, consisting of irregular or plant-like conglomerations of spear-shaped growths. The latter form may be obtained especially well developed by adding at first about 3 volumes of the precipitating reagent to the alkaline cystine solution, resulting in the formation on the glass walls of crystallization nuclei of the first type, by pouring off the supernatant liquid after it has become clear, and adding to this at least twice its volume of precipitant; the crystals of the second type will now form in the more diluted solution. Fig. 3 shows this second type of the potassium salt crystals.

For analysis the salt was dried to constant weight over P_2O_5 in vacuo at 78° (at 56° the same result was obtained).

\[ \text{C}_3\text{H}_8\text{O}_4\text{N}_2\text{S}_5\text{K}_4 \]

Calculated. K 24.59, S 20.16
Found. " (volumetric) 24.63, 24.64; K(as K_2SO_4) 24.49; S(as BaSO_4 after oxidation in Parr Oxygen Bomb) 19.77.

The K salt also is very soluble in water. The solubilities in C_2H_5OH and CH_3OH were determined in the manner described for the Na salt.

\[ \text{C}_3\text{H}_8\text{OH} \]

Found. 1.75 gm., 1.91 gm. per 100 cc.
CH_3OH “ “ 31.74 “ 31.58 “ 31.81 gm. per 100 cc.

The following specific rotations, referred to free cystine, were obtained. 0.2500 gm. of salt dissolved to 25 cc. in H_2O gave \([\alpha]_D^{28} = -90.8°, [\alpha]_D^{29} = -89.5° \text{ (after 6 hours)}, [\alpha]_D^{29} = -88.9° \text{ (after 70 hours)}, [\alpha]_D^{29} = -88.9° \text{ (after 94 hours)}; 0.2698 gm. of salt dissolved to 25 cc. in CH_3OH gave \([\alpha]_D^{29} = -254°. \) No further readings could be taken as the solution became almost immediately turbid and colored and NH_3 was given off. On the other hand, even after 94 hours the aqueous solution was perfectly clear and colorless and no NH_3 could be detected.

III. Lithium Salt—To 7.4 gm. of cystine 150 cc. of a 0.40 m
Fig. 2. About X 53. First type of K salt crystals

Fig. 3. About X 53. Second type of K salt crystals
LiOH solution in 60 per cent C₆H₅OH are added. The resulting solution is filtered from excess cystine, 3 volumes of acetonitrile are added, and, after brief mixing, the whole is allowed to stand undisturbed for a few hours. In case of delayed crystallization seeding as mentioned with the Na salt is helpful. A yield of 95 per cent was obtained.

Also in the case of the Li salt the appearance of the crystals varies according to the conditions of crystallization. Figs. 4 and 5 give two typical views. For analysis the material was dried, as described, at 78°.

\[ \text{C}_6\text{H}_6\text{O}_4\text{N}_2\text{S}_2\text{Li}_2 \]

Calculated. Li 5.51

Found. “ (volumetric) 5.54, 5.52

The salt is very soluble in water, while the solubilities in C₆H₅OH and CH₃OH are lower than those of the Na and K salts.

\[ \text{C}_6\text{H}_5\text{OH} \text{ solution. Found. 0.0075 gm. per 100 cc.} \]
\[ \text{CH}_3\text{OH} “ “ 0.30 “ 0.31 gm. per 100 cc. \]

While all three salts form colorless crystals, the preparations of the Na and K salts usually could not be obtained without a very slight yellow tinge. The Li salt, however, had a pure white appearance. As the analysis also points to a high degree of purity, this salt was chosen for a careful optical study of the stability of the aqueous solution. The determinations were made in a water-jacketed tube with temperature control and the Hg line was used, on account of its greater accuracy. 0.2623 gm. of salt dissolved to 25 cc. in H₂O gave \([\alpha]_{\text{Hg}25.05} = -113.5^\circ, [\alpha]_{\text{Hg}28.64} = -114.6^\circ\), and \([\alpha]_{\text{Hg}28.91} = -114.7^\circ\) (after 20 hours); \([\alpha]_{\text{Hg}29.18} = -113.7^\circ\) and \([\alpha]_{\text{Hg}29.94} = -112.0^\circ\) (after 68 hours); \([\alpha]_{\text{Hg}29.90} = -94.5^\circ\) (after 68 hours). The two determinations at 29.18° and 24.94° after 68 hours indicate a temperature coefficient of about +0.4° [\(\alpha]_{\text{Hg}29.18} \) for +1°.

A new preparation of the Li salt, made from cystine of \([\alpha]_{\text{Hg}} = -238.7^\circ\) or \([\alpha]_{\text{D}} = -202.3^\circ\), corresponding to an \(\beta\)-cystine content of about 99 per cent (8), gave the following rotations. 0.3153 gm. of salt dissolved to 25 cc. in H₂O gave \([\alpha]_{\text{Hg}29.9} = -113.9^\circ\), or, corrected, \([\alpha]_{\text{Hg}} = -233.68^\circ\); \([\alpha]_{\text{Hg}29.7} = -236.35^\circ\), or, corrected, \([\alpha]_{\text{Hg}} = -233.68^\circ\); \([\alpha]_{\text{Hg}29.7} = -238.7^\circ\). This salt was dissolved in HCl to produce a solution corresponding to 1 per cent cystine in 1 N HCl. 0.2625 gm. of salt and 13.6 cc. of 2 N HCl diluted to 25 cc. with H₂O gave \([\alpha]_{\text{Hg}27.9} = -230.35^\circ\), or, corrected, \([\alpha]_{\text{Hg}} = -233.68^\circ\); \([\alpha]_{\text{Hg}27.7} = -238.7^\circ\).
<table>
<thead>
<tr>
<th></th>
<th>Li</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali solution used</td>
<td>0.4 N LiOH in 60 per cent C₂H₅OH</td>
<td>0.46 N NaOH in 82 per cent C₂H₅OH</td>
<td>1 N KOCH₂ in 100 per cent CH₂OH</td>
</tr>
<tr>
<td>Precipitant</td>
<td>3 volumes CH₃CN</td>
<td>2 volumes CH₃CN</td>
<td></td>
</tr>
<tr>
<td>Water of crystallization</td>
<td>0</td>
<td>1H₂O</td>
<td>7.5 volumes CH₃CN and ether</td>
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<tr>
<td>Alkali in anhydrous salt</td>
<td></td>
<td>15.23 per cent</td>
<td></td>
</tr>
<tr>
<td>Calculated</td>
<td>5.51 per cent</td>
<td>15.45, 15.54 per cent</td>
<td></td>
</tr>
<tr>
<td>Found (volumetric)</td>
<td>5.54, 5.52 per cent</td>
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<tr>
<td>Solubility</td>
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<tr>
<td>H₂O</td>
<td>Very soluble</td>
<td>Very soluble</td>
<td></td>
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<tr>
<td>CH₃OH</td>
<td>0.3 gm. per 100 cc.</td>
<td>2.3 gm. per 100 cc.</td>
<td></td>
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<tr>
<td>C₂H₅OH</td>
<td>&lt;0.01 “ “ 100 “</td>
<td>0.16 “ “ 100 “</td>
<td></td>
</tr>
<tr>
<td>Specific rotation ([\alpha]_D) (calculated for cystine at 29°)</td>
<td>-94.4°</td>
<td>-90.9°</td>
<td>-91.1°</td>
</tr>
<tr>
<td>In H₂O</td>
<td></td>
<td></td>
<td>-254°</td>
</tr>
<tr>
<td>“ CH₃OH</td>
<td></td>
<td></td>
<td>-257°</td>
</tr>
<tr>
<td>Stability of solution</td>
<td>Practically stable</td>
<td>Practically stable</td>
<td>Very slow decomposition</td>
</tr>
<tr>
<td>In H₂O</td>
<td></td>
<td></td>
<td>Slow decomposition with</td>
</tr>
<tr>
<td>“ CH₃OH</td>
<td></td>
<td></td>
<td>formation of NH₃</td>
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<td></td>
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<td></td>
<td>Rapid decomposition with</td>
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<td></td>
<td></td>
<td></td>
<td>formation of NH₂</td>
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* Correction factor of cystine in HCl used (8).
-236.80°, or, corrected, $[\alpha]^{29}_{429} = -233.64°$. For comparison, an identical solution was made of the cystine used for the preparation of the salt ($[\alpha]^{29}_{429} = -238.7°$), by adding the proper amount of LiOH to the acid solution. 0.2500 gm. of cystine, 0.66 cc. of 3.19 N LiOH, and 13.6 cc. of 2 N HCl diluted to 25 cc. with H2O gave $[\alpha]^{29}_{429} = -236.65°$, or, corrected, $[\alpha]^{29}_{429} = -235.68°$; $[\alpha]^{29}_{429} = -234.50°$, or, corrected, $[\alpha]^{29}_{429} = -235.96°$. An average difference of 2.16° $[\alpha]^{29}_{429}$ results between the original cystine and the cystine formed from its Li salt, indicating an almost unchanged recovery of the cystine.

A summarized comparison of the three salts is given in Table I.

**SUMMARY**

The Li, Na, and K salts of l-cystine were prepared by precipitation of alcoholic alkaline cystine solutions with acetonitrile. The properties of these salts, including their solubilities in H2O, CH3OH, and C2H5OH, the optical rotation, and the stability of the solutions, were determined.

Note—As this paper was being sent to press we read the publication of Voss and Guttmann (10) on the preparation of alkali salts of amino acids by the use of liquid ammonia. As the authors state that in the case of cystine partial reduction to cysteine interferes with their obtaining a pure disodium salt, we wish to mention that our salts do not give the nitroprusside reaction for sulfhydryl.

**BIBLIOGRAPHY**

Preparation and Properties of Crystallized Alkali Salts of l-Cystine

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