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THE EFFECT OF *p*H ON THE STABILITY OF *CIS*-ACONITIC ACID IN DILUTE SOLUTION

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In the first description of *cis*-aconitic acid, Malachowski and Maslowski (1) noted that in aqueous solution it was rapidly converted by heat into the *trans*-acid. In a study of the electrical conductivity of dilute solutions of the two isomeric acids, Malachowski (2) found that the proportion of the *trans*-acid in equilibrated mixtures is approximately 85% and decreases slightly with elevation of the temperature and with dilution of the solution. Krebs and Eggleston (3) showed that in neutral solution sodium *cis*-aconitate is much more stable and that the most rapid and extensive conversion to the *trans*-isomer takes place in strongly acidic and strongly alkaline solutions. They point out, however, that the values they obtained are of uncertain accuracy because of interferences in the enzymic and chemical reactions used to determine *cis*-aconitic acid.

The strontium salt of *trans*-aconitic acid (4) is soluble when formed by double decomposition at room temperature, and precipitates only when the solutions are heated, but the strontium salt of *cis*-aconitic acid precipitates as the hexahydrate almost immediately on the addition of soluble strontium salts to cold solutions of soluble *cis*-aconitates, even in the presence of large excesses of *trans*-aconitates, as shown in Table I. This characteristic difference in precipitability of the strontium salts of the two acids has made possible a study of the stability of *cis*-aconitic acid and its alkali salts at *p*H values from 1 to 14 and at different temperatures.

The experiments recorded in Table II were undertaken to determine the range of stability of *cis*-aconitic acid in solutions of different *p*H values and temperatures, rather than the rate of the isomeric change. By precipitation from dilute solutions, strontium *cis*-aconitate hexahydrate has an apparent solubility equivalent to approximately 0.15% aconitic acid. Since 0.87% solutions were used this placed an upper limit of 80% on the determinable extent of the change to the *trans*-form, and made impossible the study of the equilibria reported by Malachowski (2). The high values of 23 to 33% isomerization of the *trans*-acid and salts reported by Krebs and Eggleston (3) would, however, lie within the range of the procedure described below, but when a like series of experiments was carried out with *trans*-aconitic acid no precipitates of strontium *cis*-aconitate could be detected. Attempts to study the isomeric change by determining aconitic acid as the mixed calcium-magnesium salt (5) and as the cadmium salt (4) proved unsuccessful because *cis*-aconitates largely inhibited the precipitation of the former and interfered in the precipitation of the latter.

From Table II it is evident that *cis*-aconitates are stable in neutral and slightly alkaline solutions, but unstable at high alkalinities, especially if the solutions are heated. In acid solutions the stability decreases as the acidity increases. Only in highly acidic and highly alkaline solutions does temperature have a material effect on the amount of the *cis*-acid which disappears. In the *p*H range 2 to 6 there apparently is a progressive series of equilibria which are dependent on the *p*H of the solution. It appears that these solutions behave as mixtures of tri- or di-basic *cis*-aconitates with free *cis*-aconitic acid, and that the salts remain stable while the free acid undergoes the *cis* ⇌ *trans* isomerization. If this is so, the expected subsequent reaction *cis*-salt + *trans*-acid ⇌ *trans*-salt + *cis*-acid, whereby the same equilibrated mixture would result in all the acid

TABLE I

PRECIPITATION OF STRONTIUM *cis*-ACONITATE IN PRESENCE OF *trans*-ACONITATE

ACONITIC ACIDS TAKEN		FOUND	RECOVERY
trans	*cis*	$Sr_3(cis\text{-}C_6H_3O_6)_2.2H_2O$	$cis\text{-}H_3C_6H_3O_6$
g./100 ml.	*g./100 ml.*	*g./100 ml.*	%
3.5	0.0	0.0	0.0
3.0	0.5	0.608	66.0
2.5	1.0	1.536	83.4
2.0	1.5	2.435	88.1
1.5	2.0	3.310	89.9
1.0	2.5	4.139	89.9
0.5	3.0	5.034	91.1
0.0	3.5	5.835	91.0

TABLE II

EFFECT OF *p*H, TEMPERATURE AND TIME ON THE STABILITY OF *cis*-ACONITIC ACID IN 0.05*M* SOLUTION

*p*H	*cis*-ACONITIC ACID RECOVERED AS STRONTIUM SALT								
	25°C.					65°C.			90°C.
	0 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	4 Hrs.
	%	%	%	%	%	%	%	%	%
1	100.0	69.0	57.5	40.2	29.9	0.0	0.0	0.0	0.0
2	100.0	79.3	75.9	57.6	48.3	24.1	27.6	24.1	28.7
3	100.0	92.0	86.3	88.5	69.0	45.6	45.9	43.7	43.7
4	100.0	98.8	94.3	94.3	83.9	59.8	63.6	63.6	59.8
5	100.0	100.0	96.6	96.6	89.7	85.1	79.3	80.6	73.6
6	100.0	100.0	98.8	98.8	94.3	98.8	98.0	97.7	92.0
7	100.0	100.0	100.0	100.0	97.7	100.0	100.0	100.0	100.0
8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
10	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.8
11	100.0	100.0	100.0	100.0	100.0	98.8	100.0	97.7	96.6
12	100.0	100.0	98.9	98.8	96.6	94.3	92.0	86.4	89.7
13	100.0	98.8	86.3		73.6	57.5	54.2	48.5	58.6
14	100.0	92.0	45.0	0.0	0.0	0.0	0.0	0.0	0.0

solutions, must be greatly inhibited, possibly because of non-dissociation of the molecules of either the salt or the acid, or the formation of complex anions containing undissociated metallic atoms such as are indicated by the work of Greenwald (6). Informative studies of these apparent equilibria are impossible until more precise methods of determining each of the isomeric aconitic acids in the presence of the other have been found.

EXPERIMENTAL

Strontium was determined by incinerating the salts and converting the ash to the sulfate. Aconitic acid was determined by the decarboxylation method of Roberts and Ambler (7).

Tristrontium cis-aconitate hexahydrate, $Sr_3(cis\text{-}C_6H_3O_6)_2\cdot 6H_2O$. This salt precipitated almost immediately when an excess of a soluble strontium salt was added to a cold freshly prepared solution of *cis*-aconitic acid (8, 9) neutralized to *p*H 6.8 with potassium or sodium hydroxide. Precipitation was completed in a refrigerator. The salt was filtered from the cold solution, washed with ice-water and dried over calcium chloride. It forms small, spindle-shaped, colorless single crystals and aggregates of smaller crystals radiating from a common center. Yields: from 100 ml. of 3.5% *cis*-aconitic acid solution, 6.495 g. (91.1%); from 9 preparations, each from 100 ml. of 0.87% solution of *cis*-aconitic acid, average, 1.333 g. of strontium salt, the filtrates from which contained by analysis 0.153 ± 0.001 g. of aconitic acid.

Anal. Calc'd for $Sr_3(C_6H_3O_6)_2\cdot 6H_2O$: Sr, 36.88; $H_3C_6H_3O_6$, 48.80.
Found: Sr, 36.90; $H_3C_6H_3O_6$, 48.87.

The salt lost water of hydration easily and came to constant weight when heated several hours at 140°, with loss of 4 molecules of water.

Anal. Calc'd for $4H_2O$: 10.01.
Lost at 140°: 9.56.
Heated at 140°; Calc'd for $Sr_3(C_6H_3O_6)_2\cdot 2H_2O$: Sr, 40.90; $H_3C_6H_3O_6$, 54.30.
Found: Sr, 40.80; $H_3C_6H_3O_6$, 54. 25.

Tristrontium cis-aconitate trihydrate, $Sr_3(cis\text{-}C_6H_3O_6)_2\cdot 3H_2O$. When solutions of potassium or sodium *cis*-aconitate prepared as above were heated to 95–100° and a soluble strontium salt was added, no immediate precipitation occurred, but on continued heating small colorless plates of tristrontium *cis*-aconitate trihydrate slowly deposited. Two preparations, each from 100 ml. of 0.87% solution of *cis*-aconitic acid, heated 3 hours in the steam-bath, gave yields of 1.222 and 1.235 g. of the salt. The salt slowly lost water of crystallization at 140°.

Anal. Calc'd for $Sr_3(C_6H_3O_6)_2\cdot 3H_2O$: Sr, 39.90; $H_3C_6H_3O_6$, 53.82.
Found: Sr, 40.00; $H_3C_6H_3O_6$, 52.90.

Precipitation of strontium cis-aconitate in presence of trans-aconitate. Stock solutions of each of the isomeric potassium aconitates were prepared by dissolving 17.5 g. of pure *trans*-aconitic acid and 15.68 g. of *cis*-anhydroaconitic acid (8, 9, 10) in 400-ml. portions of water, neutralizing immediately with KOH solution to *p*H 6.8 and diluting to 500 ml. From the stock solutions, 100-ml. solutions containing a total of 3.5 g. of the isomeric acids in varying proportions were prepared. In each of these solutions 10.5 g. of strontium acetate hemihydrate was dissolved, and the solutions were allowed to stand in the refrigerator at about 0° overnight. The precipitates were collected in tared Gooch crucibles, washed with ice-water, dried at 140° overnight and weighed. The results are assembled in Table I.

When the weights of *cis*-aconitic acid in the 100-ml. solutions are plotted as abscissae against the corresponding weights of salt obtained as ordinates, a straight line results, the slope of which is slightly less than that of the line for complete precipitation, indicating a slight common ion effect of the excess of strontium ions at the lower concentrations. By extrapolation, the experimental line cuts the X-axis at approximately 0.15, indicating that solutions of *cis*-aconitic acid containing 0.15 g. or less per 100 ml. will produce no precipitate of the strontium salt in mixtures with *trans*-aconitate. By comparing this value with that of 0.153 g. per 100 ml. found by analyses of filtrates from strontium *cis*-aconitate hexahydrate preparations, it is evident that the presence of a large excess of *trans*-aconitate has but an insignificant effect on the precipitation of strontium *cis*-aconitate hexahydrate.

Determination of stability of cis-aconitic acid in solutions of pH 1 to 14. Series of freshly prepared 100-ml. solutions containing 0.87 g. of *cis*-aconitic acid adjusted with HCl or KOH to each unit of *p*H from 1 to 14 inclusive were prepared and allowed to stand at 25° (room temperature), at 65°, and at 90° for different lengths of time. The solutions were then cooled to room temperature, and HCl or KOH solution was added as necessary to bring each solution to *p*H 6.8. The volume of each was adjusted to 100 ml., and 2.6 g. of strontium acetate hemihydrate was dissolved in each. After the solutions had stood at about 0° overnight, the precipitates of strontium *cis*-aconitate were collected in tared Gooch crucibles, washed with ice-water and dried at 140° to constant weight. From the precipitation line previously described the weights of *cis*-aconitic acid corresponding to the weights of strontium salt found were determined and calculated to per cent of acid taken. The results are given in Table II, in which, because of the solubility of the strontium salt, 0.0% recovery of *cis*-aconitic acid does not indicate 100% conversion to *trans*-aconitic acid.

The only non-volatile organic acid which could be found in the filtrates was aconitic acid. Decarboxylation in solutions of like composition and *p*H of 2, 3, and 4 heated for 4 hours at 90° (11, 12) amounted to but 0.07%, 0.13%, and 0.66%, respectively, of the aconitic acid present, as determined by loss of acidity. Since solutions of aconitic acid are most actively decarboxylated at *p*H 2 to 4 (11), no determinations of extent of decarboxylation were made on solutions of higher or lower *p*H.

SUMMARY

Tristrontium *cis*-aconitate tri- and hexa-hydrates have been prepared. The hexahydrate precipitates almost immediately from cold solutions, and the presence of large excesses of soluble *trans*-aconitates has no significant effect on its precipitation. It has been used to demonstrate that, in dilute solutions at *p*H 7 to 10, *cis*-aconitates are stable at temperatures up to 90°. Isomerization to *trans*-aconitates takes place at lower and higher *p*H values. Only in highly alkaline solutions does increase in temperature cause extensive increase in the amount of *cis*-aconitic acid or aconitate transformed.

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