33. The Effect of Inorganic Salts on the Ketone Decomposition of Oxaloacetic Acid

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(*Received 24 February 1942*)

Oxaloacetic acid is known to decompose in aqueous solution into pyruvic acid and CO_2. At *p*H 4 and 20° the rate of the 'ketone decomposition' varies between 6 and 18 % per hr. The fact that this 'spontaneous' decomposition shows considerable variations suggests that trace impurities might affect the stability of oxaloacetic acid. Krampitz & Werkman [1941] recently reported that Mg ions accelerate the decomposition; this makes it probable that the impurities in question are inorganic salts. The effects of various salts on the rate of decomposition were therefore studied.

Oxaloacetic acid was prepared from tartaric acid according to the methods of Wohl & Oesterlin [1901] and Wohl & Claussner [1907], and recrystallized according to Fenton & Jones [1900]. The rate of decomposition was measured by the manometric determination of the CO_2 evolution.

Table 1 shows that many inorganic salts increase the rate of decomposition. Small quantities, amounting to 0·3 % of the oxaloacetic acid added, are in many cases sufficient to produce a pronounced effect. The effect is thus a catalytic one. The data in Table 1 suggest the following details:

Table 1. *Effect of inorganic salts on the rate of ketone decomposition of oxaloacetic acid*

0·02 *M* oxaloacetic acid in 3 ml. 0·3 *M* acetate buffer *p*H 5·0; 40°; the salts were added from a side-arm when the solution had been shaken at 40° for 15 min. The data on CO_2 evolution refer to the second 5 min. period after the additions.

Substrate added	Final molarity	μl. CO_2 evolved in 5 min.	Substrate added	Final molarity	μl. CO_2 evolved in 5 min.
None	—	48	$MnSO_4$	$6·6 \times 10^{-3}$	372
NaCl	$6·6 \times 10^{-3}$	49	$CdSO_4$	$6·6 \times 10^{-3}$	223
KCl	$6·6 \times 10^{-3}$	48	$FeSO_4$	$6·6 \times 10^{-3}$	622
KH_2PO_4	$6·6 \times 10^{-3}$	47	,,	$0·66 \times 10^{-3}$	389
Na_2SO_4	$6·6 \times 10^{-3}$	44	,,	$0·066 \times 10^{-3}$	166
$CaCl_2$	$6·6 \times 10^{-3}$	72·5	Pb-acetate	$6·6 \times 10^{-3}$	730
$MgCl_2$	$6·6 \times 10^{-3}$	132	,,	$0·66 \times 10^{-3}$	159
$BaCl_2$	$6·6 \times 10^{-3}$	49	$Fe_2(SO_4)_3$	$6·6 \times 10^{-3}$	600
$ZnSO_4$	$6·6 \times 10^{-3}$	460	$Al_2(SO_4)_3$	$6·6 \times 10^{-3}$	1300
$AgNO_3$	$6·6 \times 10^{-3}$	48	,,	$0·66 \times 10^{-3}$	364
$HgCl_2$	$6·6 \times 10^{-3}$	48	,,	$0·066 \times 10^{-3}$	178
$CuSO_4$	$6·6 \times 10^{-3}$	732	$K_3Fe(CN)_6$	$6·6 \times 10^{-3}$	45
,,	$0·66 \times 10^{-3}$	660	$K_4Fe(CN)_6$	$6·6 \times 10^{-3}$	54
,,	$0·066 \times 10^{-3}$	289			

(1) The catalytically active principle is associated with the cations. Anions are inactive.

(2) Univalent cations (Na, K, Ag) are inactive.

(3) All the multivalent cations tested were found active. The inactivity of $HgCl_2$ is no exception as the solution of this salt contains no significant amounts of Hg ions.

(4) No clear rule emerges as yet regarding the factor determining the degree of activity of the various cations. Among the tested substances Al, Cu, Fe II and Fe III are the most powerful catalysts at *p*H 5·0.

Effect of H^+ concentration. In strongly acid solution (*p*H 1 and below) oxaloacetic acid shows no measurable rate of decomposition at 20°. At 40° the decomposition is very slight. Addition of $Al_2(SO_4)_3$ (10^{-2} *M*) has no appreciable effect. In strongly alkaline solution (*p*H 13) oxaloacetic acid is equally stable. The optimum rate of decomposition is not sharply defined; it is near *p*H 4 (Table 2).

Table 2. *Effect of the H^+ concentration on the rate of decomposition of oxaloacetic acid in the presence of $Al_2(SO_4)_3$*

3 ml. medium; 20°; 0·02 *M* oxaloacetic acid; $0{\cdot}67 \times 10^{-4}$ *M* $Al_2(SO_4)$; phthalate buffer 0·2 *M*; acetate buffer 0·6 *M*.

*p*H	Buffer	µl. CO_2 evolved in 20 min.	*p*H	Buffer	µl. CO_2 evolved in 20 min.
0·0	*N* HCl	7·5	4·4	Acetate	63
1·2	Phthalate	29·5	4·7	,,	53
1·7	,,	48	5·1	,,	47·5
2·0	,,	60	5·7	,,	22
3·8	,,	58			

Reactivity of other ketonic acids. The stability of acetoacetic acid, mesoxalic acid, α-ketoglutaric acid and pyruvic acid is not influenced by the addition of Al salts, whilst acetonedicarboxylic acid is rapidly split, as shown in Tables 3 and 4. One molecule of CO_2 is rapidly liberated from acetonedicarboxylic acid; this is followed by the slow evolution of a second molecule. The rate of formation of the second molecule is the same as that of the 'spontaneous' decomposition of acetoacetic acid. Thus Al ions catalyse the conversion of acetonedicarboxylic acid into acetoacetic acid and CO_2, but have no effect on the ketone decomposition of acetoacetic acid.

Table 3. *Decomposition of acetonedicarboxylic acid by $Al_2(SO_4)_3$ and aniline*

Medium: 3 ml. 0·1 *M* acetate buffer *p*H 4·4; 20°; 0·0224 *M* acetonedicarboxylic acid. The catalysts were added after thermal equilibration.

Time (min.)	Catalysts added...	0·5 ml. 0·1 *M* $Al_2(SO_4)_3$	1·0 ml. aniline citrate
		µl. CO_2 evolved	
5	8·5	122	496
10	19	260	948
20	33	448	1300
35	54	608	1472
55	81	707	1490
95	121	745	1502
135	167	790	1508
160	180	802	1512

Table 4. *Relative rates of decomposition of oxaloacetic and acetonedicarboxylic acids*

20°. The data refer to 3 ml. solution. Medium: 0·1 *M* acetate buffer, *p*H 4·4.

Substances added... Time (min.)	0·02 *M* oxaloacetic acid	0·02 *M* oxaloacetic acid 3×10^{-3} *M* $Al_2(SO_4)_3$	0·02 *M* acetonedicarboxylic acid	0·02 *M* acetonedicarboxylic acid 3×10^{-3} *M* $Al_2(SO_4)_3$
		µl. CO_2 evolved		
10	19	213	22	41
20	39	422	40	76
30	59	626	56	119

It follows that the catalytic effect of the cations is limited to β-ketonic-dicarboxylic acids. α-Ketonic-dicarboxylic acids, or β-ketonic-monocarboxylic acids do not react. Amines, on the other hand, split all β-ketonic acids [Ljunggren, 1925] and aniline therefore liberates 2 mol. of CO_2 from acetonedicarboxylic acid (Table 3).

Qualitative test for oxaloacetic acid. In testing the effect of ions on oxaloacetic acid it was noted that $HgNO_3$ forms a precipitate with oxaloacetic acid which is still distinctly visible when 0·01 mg. oxaloacetic acid, dissolved in 3 ml. water, is mixed with 1 ml.

0·1 N $HgNO_3$. The precipitate is white in dilute solution and dark grey in more concentrated solutions of oxaloacetic acid. It is soluble in HNO_3 (unlike mercurous fumarate). It disappears on prolonged standing, or on heating, owing to the ketone decomposition of oxaloacetic acid. Acetonedicarboxylic acid forms a similar precipitate. Pyruvic and α-ketoglutaric acids do not react. The reaction can be used to detect small quantities of oxaloacetic acid in the presence of pyruvic acid, provided that other substances forming insoluble mercurous compounds (e.g. Cl ions) are absent.

Purification of oxaloacetic acid. Efforts to prepare stable solutions of oxaloacetic acid by repeated recrystallization or by treatment of the solutions with metal-binding reagents (dithizone; 8-hydroxyquinoline) were unsuccessful. Under the test conditions (0·2 M acetate buffer pH 4·0; 20°; 0·02 M oxaloacetic acid) the rate of decomposition did not fall below 6% per hour. It would therefore appear that the 'spontaneous' ketone decomposition of oxaloacetic acid is partly due to the inherent instability of the grouping $R.CO.CH_2.COOH$.

DISCUSSION

Two different types of catalysts—amines and multivalent cations—are now known to decompose ketonic acids; the former react with all β-ketonic acids and in certain cases with α-ketonic acids [Langenbeck, 1933]; the latter only with β-ketonic-dicarboxylic acids.

The catalysts concerned with these reactions in biological systems seem to involve cocarboxylase (an amine) and/or Mg (a divalent cation) [see Krampitz & Werkman, 1941]. Details of the biological mechanisms are still obscure; it may well be that forces similar to those acting in the 'model' systems are effective in the biological systems.

SUMMARY

A number of multivalent cations (Al, Cu, Fe II, Fe III, etc.) are shown to catalyse the ketone decompositions of oxaloacetic and of acetonedicarboxylic acids. The cations do not affect the stability of α- or β-ketonic-monocarboxylic acids, or of α-ketonic-dicarboxylic acids.

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