VIII.—*Oxalacetic Acid.*

By HENRY J. HORSTMAN FENTON, M.A., F.R.S., and H. O. JONES,
B.A., B.Sc.

IN the preceding communication, it is shown that malic acid is very energetically oxidised by hydrogen dioxide in presence of ferrous iron. The oxidation is attended with considerable evolution of heat, and unless special precautions are taken to keep the temperature low, carbon dioxide is evolved and pyruvic acid is found in the liquid.

On shaking up the original oxidised mixture with ether, scarcely anything could be extracted, and the addition of salt, sodium sulphate, &c., did not much improve the result. In the initial experiments, which were made in studying the oxidation product of tartaric acid (*Trans.*, 1894, **65**, 901), it was shown that the addition of strong sulphuric acid to the mixture greatly increased the yield on extraction with ether, and in the present instance the same is found to be the case. By adding slowly about one-tenth its volume of strong sulphuric acid to the oxidised mixture, with careful cooling, and repeatedly shaking with ether, a considerable quantity of substance can be extracted.

In the first experiments, no special care was taken to keep the mixture well cooled during oxidation and during the addition of sulphuric acid. In this case, the ether extract, on evaporation, gave a syrupy residue with a small quantity of a white, crystalline substance. The syrupy portion, when dissolved in water, gave immediately a white precipitate with phenylhydrazine acetate which, when recrystallised from alcohol, melted at 192° , and had all the characters of *pyruvic acid* hydrazone.

0.1494 gave 20.9 c.c. nitrogen at 25° and 749.4 mm. $N = 15.85$.

$C_9H_{10}O_2N_2$ requires $N = 15.73$ per cent.

It was afterwards found that if great precautions are taken to prevent rise of temperature during the operations referred to, the ether extract, when concentrated to a small bulk, deposits the white, crystalline substance in considerable quantity, and, on further evaporation, usually solidifies to a white mass.

The method of proceeding is as follows: Malic acid (1 mol.) is dissolved in the least possible quantity of water, and a ferrous salt is added in quantity corresponding to about $\frac{1}{8}$ at., or less, of iron. The nature of the ferrous salt is immaterial, and the sulphate, acetate, or malate may be employed. The mixture is cooled by ice and salt until

it begins to freeze, and hydrogen dioxide (20-volume strength*), cooled in a similar way, is added in very small quantities at a time with careful mixing, in proportion amounting to nearly 1 at. of oxygen. The liquid assumes an intense blood-red colour as soon as the first few drops of the dioxide have been added. The solution is now mixed with about one-tenth of its volume of strong sulphuric acid, great precautions being taken, as before, to prevent the temperature from rising more than a few degrees, and is repeatedly extracted with ether. The ethereal solution gives an intense blood-red colour with ferric chloride, so that the progress of the extraction, is easily tested ; even after 25 extractions, a further yield may be obtained.

The ethereal extract is concentrated to a small bulk and the residual solution, on cooling, begins to deposit the white, crystalline acid, the quantity being much increased on stirring. It is kept in a desiccator, partially exhausted, until the whole has solidified, and is then quickly washed three or four times with cold water, draining well each time with the aid of a pump. The yield of pure substance is about 22 per cent. of the acid oxidised.

Properties.—The substance separates from its solution in ether, acetone, or water, in white, crystalline aggregates which dissolve very slowly in cold water and easily, but with decomposition, in hot water. It is rather more soluble in ether, easily soluble in alcohol or acetone, and scarcely soluble in chloroform or benzene.

It crystallises from a mixture of acetone and benzene in blade-like crystals belonging to the prismatic system, and has a great tendency to form close aggregates. The aqueous solution gives an intense blood-red colour with ferric salts, which is scarcely affected by dilute mineral acids.

Heated in a capillary tube, it melts with sudden decomposition at 176—180°. Analysis of the substance dried in a vacuum desiccator gave the following result :

0.1766 gave 0.2361 CO_2 and 0.0488 H_2O . $\text{C} = 36.46$; $\text{H} = 3.07$.

$\text{C}_4\text{H}_4\text{O}_5$ requires $\text{C} = 36.36$; $\text{H} = 3.03$ per cent.

Owing to its sparingly solubility in appropriate solvents, and to its instability at high temperatures, the molecular weight could not well be determined by the freezing or boiling point methods. It will be shown, however, that the methyl ester dissolves easily in acetic acid, so that the value is easily determined in that case.

Titration with Alkalis.—The acid was dissolved in cold water and

* In later experiments, the dioxide has been allowed partially to freeze until the strength of the liquid portion is about doubled, and this procedure is found to be very advantageous, owing to the smaller quantity of water introduced.

titrated with pure caustic soda solution containing 0.01507 Na per c.c., prepared from metallic sodium. Phenolphthalein was used as indicator, and special precautions were taken to exclude carbon dioxide.

0.5188 required 11.85 c.c. soda solution for neutralisation, the calculated amount for a dibasic acid of the formula $C_4H_4O_5$ being 11.99 c.c.

After boiling the aqueous solution for a few minutes, carbon dioxide is evolved and the neutralising power is nearly halved, thus 0.6097 gram of the substance was heated for 5 minutes and then required 8.2 c.c. of the alkali, the calculated amount being 7.0 c.c. After heating for half an hour, a nearly similar result was obtained. On acidifying the resulting solution with acetic acid and adding phenylhydrazine acetate, a nearly white, crystalline precipitate was obtained which melted at 192° , and had all the other characters of *pyruvic acid hydrazone*.

Barium Salt.—A solution of the acid was exactly neutralised with pure soda and an excess of barium chloride added. The resulting white precipitate was well washed and dried in a vacuum desiccator.

0.4780 gave 0.3677 $BaSO_4$. Ba = 45.23.

$C_4H_2O_5Ba + 2H_2O$ requires Ba = 45.21 per cent.

This salt, when treated with ferric chloride, gives a deep, brick-red colour.

Calcium, strontium, lead, and silver salts, similarly, give white precipitates, the silver salt being quickly reduced on heating.

Methyl ester, $C_2H_2O(CO_2CH_3)_2$.—This was obtained by dissolving the acid in methyl alcohol, cooling the solution by ice, and partially saturating with dry hydrogen chloride. After the mixture had remained for about 24 hours, it was allowed to evaporate over solid potash and sulphuric acid, and the resulting solid crystalline mass well washed with cold water, in which it is scarcely soluble, then with a little cold methyl alcohol, and was finally recrystallised from hot methyl alcohol. It is thus obtained in beautiful, transparent, oblique prisms which melt at 77° .

The following results were obtained on analysing the substance dried in a vacuum desiccator :

- I. 0.1418 gave 0.2334 CO_2 and 0.0607 H_2O . C = 44.89 ; H = 4.75.
 II. 0.1571 „ 0.2598 CO_2 „ 0.0712 H_2O . C = 45.10 ; H = 5.03.

$C_6H_8O_5$ requires C = 45.00 ; H = 5.00 per cent.

Three molecular weight determinations by the freezing point method in the same quantity of acetic acid gave the following numbers, the calculated molecular weight being 160 :

	Solvent.	Substance.	Depression.	Mol. weight.
I.	13·90	0·1290	0·243	148·9
II.	—	0·2908	0·532	153·3
III.	—	0·4038	0·753	150·5

The alcoholic solution of this ester gives the deep red colour with ferric chloride.

The analysis, molecular weight, melting point, and other properties show that this substance is identical in every way with *methyl oxalacetate*, which was obtained by Wislicenus from the action of sodium on a mixture of oxalic and acetic esters, the melting point of which is given as 74—76°.

Action of Phenylhydrazine on the Acid.

When the acid (1 mol.) is dissolved in a little cold water and mixed with phenylhydrazine acetate (1 mol.), the solution at first remains clear, but after standing for some minutes it gives, on stirring, a pale, straw-coloured, crystalline precipitate of the *hydrazone of oxalacetic acid*, $\text{CO}_2\text{H} \cdot \text{CH}_2 \cdot \text{C}(\text{N}_2\text{H} \cdot \text{C}_6\text{H}_5) \cdot \text{CO}_2\text{H}$, which is seen under the microscope to consist of transparent, oblique prisms. This product is nearly pure, giving, on analysis, $\text{N} = 12\cdot14$ per cent. After recrystallisation from cold, dry ether, however, the crystals are lustrous and perfectly colourless. After drying in a vacuum desiccator, they yielded the following results on analysis :

0·1232 gave 0·2422 CO_2 and 0·0499 H_2O . $\text{C} = 53\cdot61$; $\text{H} = 4\cdot50$.

0·1841 „ 19·3 c.c. nitrogen at 17° and 767 mm. $\text{N} = 12\cdot49$.

$\text{C}_{10}\text{H}_{10}\text{O}_4\text{N}_2$ requires $\text{C} = 54\cdot05$; $\text{H} = 4\cdot50$; $\text{N} = 12\cdot61$ per cent.

The pure substance, when slowly heated, turns yellow, and without melting shows signs of decomposition at 95—100°; if suddenly heated to a little above 100°, the decomposition is violent. The yellow product of decomposition, however, melts at 182—183°.

This hydrazone dissolves in concentrated sulphuric acid with a deep red colour, and on addition of ferric chloride to the solution a fine purple colour is produced. It resembles in this respect the hydrazone of the methyl ester.

Wislicenus (*Ber.*, 1886, 19, 3225) and Buchner (*Ber.*, 1889, 22, 2929) have shown that the hydrazones of ethyl and methyl oxalacetates when acted on by dilute alkalis or acids, lose the respective alcohol and water, giving rise to a sparingly soluble acid having the formula $\text{C}_{10}\text{H}_8\text{O}_3\text{N}_2$, which begins to decompose without melting at 240—250°, and melts at 263°. This substance is shown to be 1-phenyl-5-pyrazolone-3-monocarboxylic acid. This acid gave a

red colour with nitric acid, a scarlet with nitrous acid, and a dark blue with ferric chloride in hot aqueous solution.

It was considered probable therefore that the hydrazone at present under consideration might behave in a similar way, and such is found to be the case. On heating the original pale yellow substance with dilute sulphuric acid, it is changed in a few minutes into a voluminous, white, crystalline magma, which dissolves sparingly in boiling water, and separates on cooling in beautiful, transparent needles. On heating, these turned yellow and showed signs of decomposition at about 243°, and completely melted at about 260°. For analysis, the substance was dried at 100°.

0.1804 gave 21.2 c.c. nitrogen at 18.5° and 764 mm. $N = 13.88$.

$C_{10}H_8O_3N_2$ requires $N = 13.72$ per cent.

The colour reactions of this product coincided exactly with those mentioned above, and in fact it is identical in every respect with the acid obtained by these authors.

Action of Phenylhydrazine on the Methyl Ester.

The ester (1 mol.) was dissolved in methyl alcohol and mixed with phenylhydrazine acetate (1 mol.); the clear mixture, on standing in a desiccator over potash and sulphuric acid, soon deposited brilliant, colourless, transparent plates, which, when recrystallised from methyl alcohol, melted at 117°.

Buchner (*loc. cit.*), by the action of phenylhydrazine on methyl acetylenedicarboxylate, obtained the hydrazone of methyl oxalacetate :

The product which he obtained melted at 118°, and the properties correspond exactly with those of the compound obtained in the present instance.

Oxidation of the Acid in presence of Iron.

It was pointed out in the preceding communication (p. 75) that the product obtained by oxidising malic acid in presence of iron gave, with phenylhydrazine acetate, a bright orange precipitate which crystallised from hot chloroform in prisms, and melted at 217—219°. The composition of this compound does not correspond with that of a direct derivative of oxalacetic acid, but of a more oxidised product.

It appeared possible that this was the result of oxidation of a neighbouring group by the phenylhydrazine, but such does not appear to be the case, since no such substance can be obtained by the action

of excess of the reagent on pure oxalacetic acid. Probably, therefore, the oxidation is due to the hydrogen dioxide, a portion of the oxalacetic acid being further oxidised. Although only the calculated quantity of oxygen, or less, was added, it is of course possible that the product may be attacked as readily as the malic acid. In order to test this hypothesis, pure oxalacetic acid (1 mol.) was mixed with a little ferrous sulphate, and hydrogen dioxide (1 mol.) added under the conditions previously mentioned. On now adding phenylhydrazine acetate (1 mol.), an *orange* precipitate was obtained exactly similar to that originally obtained from the malic acid oxidation, which, when recrystallised from chloroform, melted at 219° . Heated with sodium carbonate solution, it gave a crystalline sodium salt, and in fact showed exact similarity in every respect.

The orange compound obtained from malic acid on different occasions was remarkably constant in composition and melting point, whereas at first sight it might be supposed, on the above explanation, that it should be mixed with variable quantities of the hydrazone of oxalacetic acid. The latter compound, however, does not begin to separate for several minutes, or in dilute solutions for over half an hour, whereas the orange compound comes down immediately; the hydrazone, moreover, cannot be recrystallised from hot solvents, and would consequently be easily separated by the treatment described.

The nature of this further oxidation product has yet to be investigated. It gives a brownish-violet colour with ferric salts in alkaline solution, and the authors are inclined to conjecture that it may be the isomeric form of dihydroxymaleic acid, namely,

Oxidation of the Acid by Bromine.

When the acid (1 mol.) is mixed with glacial acetic acid and treated with dry bromine (1 mol.), the colour of the latter quickly disappears, and, after a short time, fumes of hydrogen bromide are evolved. A similar result is obtained by using water in place of acetic acid. The product in either case, when mixed with excess of caustic soda, gives an intense *violet* with ferric chloride, exactly resembling that produced by dihydroxymaleic acid with the same reagents. The authors have reasons, however, for thinking that this product is isomeric and not identical with the latter acid, and very probably it is identical with the product obtained in the previous experiment by oxidation with hydrogen dioxide. A further study of this reaction is in progress.

From the foregoing results, it is evident that the oxidation product of maleic acid described above is free *oxalacetic acid*. Whether this

acid is to be regarded as $\text{CO}_2\text{H} \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$, or as the tautomeric hydroxyfumaric acid, $\text{CO}_2\text{H} \cdot \text{C}(\text{OH}) : \text{CH} \cdot \text{CO}_2\text{H}$, is, of course, a problem similar to the much discussed question of the constitution of ethyl acetoacetate. Either formula would equally well explain its formation in the present case, and the reaction with phenylhydrazine, although lending support to the ketonic formula, is of course not conclusive.

Nef (*Annalen*, 1893, 276, 230) by the hydrolysis of ethyl ethoxyfumarate, obtained an acid corresponding approximately in composition to that required for the formula $\text{C}_4\text{H}_4\text{O}_6$; it melted at 172° , gave a dark red colour with ferric chloride, and when treated with phenylhydrazine hydrochloride, gave the phenylpyrazolonecarboxylic acid above mentioned. This acid appears to be hydroxyfumaric acid, but since it was not obtained in a pure state a strict comparison with the present acid cannot be made; the two acids may be identical or may represent the tautomeric (desmotropic) forms.

Michael and Bucher (*Ber.*, 1895, 28, 2511; 1896, 29, 1792) state that oxalacetic acid is obtained by the action of water on acetoxymaleic anhydride, and by acting with hydrochloric acid in the cold upon ethyl oxalacetate, unsymmetrical ethyl diethoxysuccinate, &c. There are only the bare statements, however; no details, analyses, or description of the acid are given.

Many interesting reactions of the acid suggest themselves, and these the authors hope shortly to investigate.