CCXII. THE 2:4-DINITROPHENYLHYDRAZONES OF SOME KETONIC ACIDS OF BIOLOGICAL IMPORTANCE.

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DURING the course of some experiments on the carbohydrate metabolism of the liver it was found necessary to prepare the derivatives of certain compounds which were thought to be of possible biological importance. Among numerous derivatives prepared were the 2 : 4-dinitrophenylhydrazones of mesoxalic, oxaloacetic and acetoacetic acids. As these compounds do not appear to have been described hitherto in the literature we record below our observations on their preparation, which requires some care, and their properties. We have also devised a quantitative method by which their identity may be verified.

Mesoxalic acid 2:4-dinitrophenylhydrazone.

Mesoxalic acid was prepared from diethylmalonate by the method of Curtiss [1906]. The acid was converted into the sodium salt and the latter purified by repeatedly precipitating it from its aqueous solution with alcohol.

Slightly more than the molecular equivalent of 2 : 4-dinitrophenylhydrazine dissolved in boiling $2N$ HCl (0·5 g. in 30 cc.) was added to an aqueous solution of sodium mesoxalate. The hydrazone was immediately precipitated in hair-like pale yellow needles. Sufficient strong hydrochloric acid was then added to render the solution $2N$ and the mixture allowed to stand overnight. The hydrazone was filtered off, washed with $2N$ HCl, and dried *in vacuo* over sulphuric acid. The yield was practically quantitative. The substance is appreciably soluble in water (1 part dissolves in approximately 400 of water at room temperature), but is almost insoluble in $2N$ HCl. It may be conveniently recrystallised by dissolving it in water and adding strong hydrochloric acid. The melting-point is 202° (uncorr.) unaltered by further recrystallisation. (Found N 18·73 %; Calculated N 18·79 %[3].)

The precipitation of the hydrazone occurs even in very dilute solution. Thus a precipitate is formed when 17 mg. of 2 : 4-dinitrophenylhydrazine

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dissolved in 1 cc. of $2N$ HCl are added to 4 cc. of a solution containing only 1·5 mg. of mesoxalic acid.

The hydrazone dissolves in saturated sodium carbonate to give a light brown solution from which it can be reprecipitated by acidification with hydrochloric acid. It is readily soluble in ethyl acetate and is completely thrown out from its solution in this solvent by the addition of a small amount of light petroleum. It is slightly soluble in alcohol, almost insoluble in ether. Although it is a malonic acid derivative its solution can be safely heated to 100°. An aqueous solution of the substance, saturated at the boiling-point, was boiled under reflux for 1 hour. On acidifying with hydrochloric acid the hydrazone was recovered unchanged in melting-point. The melting-point of glyoxylic acid 2 : 4-dinitrophenylhydrazone, the formation of which might be expected under these conditions is given by Brady [1931] as 190°.

The substance differs from other 2 : 4-dinitrophenylhydrazones in giving no colour with alcoholic potassium hydroxide.

Acetoacetic acid 2 : 4-dinitrophenylhydrazone.

Acetoacetic acid was prepared as the sodium salt in aqueous solution and freed from ethyl acetoacetate and acetone by the method of Shaffer [1921]. The hydrazone was prepared as described above, except that the hydrazine was added as a supersaturated solution in $2N$ HCl at room temperature. The yield of crude product was 85 % of the theoretical figure based on an estimation of the strength of the acetoacetic acid solution by the method of Van Slyke [1917]. The substance was purified by dissolving it in saturated sodium carbonate solution (20 cc. per g. of material), filtering from a trace of insoluble material, and precipitating it by the addition of hydrochloric acid. The precipitate was dried *in vacuo* over sulphuric acid. Crystallisation was effected by dissolving it in the minimum quantity of ethyl acetate at room temperature, and adding ten times the volume of ligroin. The substance crystallises in parallel growths of brownish-yellow pointed needles. It melts somewhat indefinitely at 125° (uncorr.) with evolution of carbon dioxide and the formation of a red liquid. The melting-point was unchanged by further recrystallisation. (Found, N 19·62 %; Calculated, N 19·86 %.)

The hydrazone gives a dark brown solution in saturated sodium carbonate and can be precipitated therefrom by acidification. It is slightly soluble in alcohol but almost insoluble in ether. It is practically insoluble in water. The substance gives a red colour with alcoholic potassium hydroxide.

At no stage in its purification should it be heated, either alone or in solution. In solution in ethyl acetate or glacial acetic acid it undergoes decomposition when warmed, considerably below the boiling-points of these solvents, yielding carbon dioxide and acetone 2 : 4-dinitrophenylhydrazone. The latter was obtained in a state of purity simply by two crystallisations of acetoacetic acid 2 : 4-dinitrophenylhydrazone from boiling ethyl acetate. The derivative melted sharply at 126° (uncorr.). The melting-point of the acetone hydrazone prepared from acetone and once recrystallised from ethyl acetate was 126°. A mixed melting-point of the two specimens showed no change from this figure. The crystals of both specimens were short rhombic prisms. (Found, N 23·71 and 23·81 %; Calculated, N 23·53 %.)

This hydrazone gives a red colour with alcoholic potassium hydroxide.

Oxaloacetic acid 2:4-dinitrophenylhydrazone.

The oxaloacetic acid used was the maleinoid form (melting-point 150° approx.) prepared by the method of Simon [1903]. The conditions described above for the preparation of the acetoacetic acid derivative were employed. The hydrazone separates slowly in glistening rhombic plates. The substance is thus obtained in a pure state, as judged by constancy of melting-point after recrystallisation, by the nitrogen content, and by the alkali titration figure (see below). (Found, N 17·91 %; Calculated, N 17·94 %.)

The hydrazone dissolves in saturated sodium carbonate to give a brown solution from which it can be reprecipitated by acidification. It is readily soluble in ethyl acetate and can be caused to crystallise from this solvent by the addition of ligroin or benzene. It is slightly soluble in alcohol but almost insoluble in ether. One part of the substance dissolves in about 140 parts of water at room temperature. It can be caused to crystallise from its saturated aqueous solution by adding enough strong hydrochloric acid to make the solution $2N$.

It softens at 205° and melts at 211° with evolution of gas. Like the analogous acetoacetic acid derivative it decomposes when heated, although rather less readily. Thus drying the substance at 110° for 2 hours caused the melting-point to fall to 208°. If a saturated solution of the substance is made in boiling ethyl acetate, crystallisation does not occur on cooling. The addition of light petroleum however causes the separation of a yellow semicrystalline precipitate. Titration with alkali indicates that partial decomposition into pyruvic acid 2 : 4-dinitrophenylhydrazone has occurred. 4·26 mg. of the product required 2·47 cc. of $N/100$ NaOH. The calculated titres for oxaloacetic acid hydrazone and pyruvic acid hydrazone are 2·64 and 1·63 cc. respectively.

Characterisation of small quantities of the 2:4-dinitrophenylhydrazones of ketonic acids by acidimetry.

In spite of the intense colour of these substances it is possible to dissolve them in excess of standard alkali and titrate the solution with standard acid, provided that an indicator is chosen the colour change of which is not masked by the colour change due to the derivatives themselves. Notwithstanding its low p_K, we have found that bromothymol blue gives satisfactory end-points. This titration may be performed with small quantities of material (2 to 5 mg.) weighed on a micro-balance. In the identification of an unknown substance it affords a useful check on the nitrogen determination. Determinations of C and H are not of great value with these derivatives owing to the fact that different compounds often have very nearly the same percentage composition with respect to these elements.

Examples of these titrations are shown in the table below. In each case the quantity of the substance indicated was weighed out, dissolved in 5 cc. of *N*/100 NaOH, and titrated with *N*/100 acid.

Microtitrations of 2 : 4 dinitrophenylhydrazones.

Parent acid	Weight taken (mg.)	cc. of *N*/100 NaOH Found	Calculated
Pyruvic	3·74	1·42	1·39
Acetoacetic	4·02	1·44	1·42
Oxaloacetic	3·06	1·97	1·96
Mesoxalic	3·07	1·96	1·96

SUMMARY.

1. The preparation and properties of the 2 : 4-dinitrophenylhydrazones of mesoxalic, acetoacetic and oxaloacetic acids are described.

2. A method is given by which these derivatives may be titrated as acids and which may be used in their identification.

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