LXXXIV. PYRUVIC AND OXALOACETIC CYANOHYDRINS

By DAVID EZRA GREEN[1] AND STANLEY WILLIAMSON

From the Biochemical Laboratory, Cambridge

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Traces of keto-acid have been found to inhibit the *in vitro* reaction of the lactic and malic dehydrogenases of animal tissues with molecular oxygen [Green & Brosteaux, 1936; Green, 1936]. The oxidation of lactate or malate was found to proceed more or less linearly only in presence of reagents which can combine with keto-acids. Cyanide was the most efficient of all the ketone fixatives in the sense that the speed of oxidation of lactate or malate was several times as great in presence of cyanide as in presence of other fixatives such as hydrazine, hydroxylamine and semicarbazide.

The chemical literature has no references to the formation of pyruvic and oxaloacetic cyanohydrins in aqueous solution. It was therefore only on the basis of analogy with other ketones that cyanide was assumed to combine with pyruvic and oxaloacetic acids. The present communication presents evidence that cyanide reacts rapidly at neutral *p*H with the above keto-acids to form the corresponding cyanohydrins.

Pyruvic cyanohydrin. When KCN and pyruvic acid were mixed in equimolecular proportions, the nitroprusside test became negative and no free keto-acid could be estimated by bisulphite-iodine titration. The potassium salt of the cyanohydrin was isolated by evaporating the aqueous solution *in vacuo* to a thick syrup and adding several volumes of ethyl alcohol. The salt gradually settled out as a sticky mass which became friable after several washings with absolute alcohol. Two recrystallizations from hot absolute alcohol yielded a pure product. The substance is extremely hygroscopic; M.P. 87°. (Found (Weiler). C, 30·80; N, 9·16; H, 2·80; K, 25·70 %. $C_4H_4O_3NK$ requires C, 31·37; N, 9·15; H, 2·61; K, 25·48 %.)

A solution of the cyanohydrin gave a negative nitroprusside test and failed to react with bisulphite (as shown by iodine titration). Addition of 2:4-dinitrophenylhydrazine produced no precipitate in 24 hours. Boiling the neutral or acid solution for 5 min. decomposed the cyanohydrin, and the presence of free pyruvic acid was demonstrated by the usual tests. The identification of pyruvic acid was accomplished with 2:4-dinitrophenylhydrazine. About 70 % of the theoretical yield of pyruvichydrazone was obtained; M.P. 213°; mixed M.P. 213°. (Found (Weiler). C, 40·53; N, 20·40; H, 3·22 %. $C_6H_8O_4N_4$ requires C, 40·28; N, 20·90; N, 3·01 %.)

Cyanohydrin formation took place rapidly in the *p*H range between 6 and 10. In all cases the cyanohydrin was decomposed by boiling in acid or neutral solution. Alkali in the cold was found to decompose the cyanohydrin within a few minutes; but unless the solution was acidified rapidly, recombination of pyruvate and cyanide occurred in the course of adding the acid.

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* Beit Memorial Research Fellow.

Oxaloacetic cyanohydrin. The oxaloacetic acid used in this study was prepared by the method of Wohl & Oesterlin [1901].

When KCN, oxaloacetic acid and KOH were mixed in equimolecular proportions, cyanohydrin formation was found to take place as judged by the negative tests for free keto-acid. The isolation followed the same procedure as above. The potassium salt of oxaloacetic cyanohydrin was even more hygroscopic than the pyruvic compound. The M.P. after drying over sulphuric acid *in vacuo* at room temperature was 95° (decomp.) but prolonged drying over P_2O_5 at 60° *in vacuo* raised the M.P. to 135° (decomp.). There appeared to be some decomposition of the cyanohydrin even at 60°. No method has yet been found for purifying the potassium salt of the cyanohydrin. It was found to be insoluble in all organic solvents.

A solution of the dipotassium salt of oxaloacetic cyanohydrin showed a negative nitroprusside test and failed to react with bisulphite. On boiling the aqueous or acid solution, decomposition took place, CO_2 being liberated and pyruvic acid formed. The latter was identified as the 2:4-dinitrophenylhydrazone.

Treatment of oxaloacetic cyanohydrin with dilute alkali (cold) regenerated the free keto-acid within a few minutes. When the alkaline solution was poured rapidly into a hydrochloric acid solution of 2:4-dinitrophenylhydrazine, the hydrazone of oxaloacetic acid was recovered in 75 % yield. The recrystallized hydrazone had a M.P. of 212° with a N content of 17·80 %, theory requiring 17·94 %.

Oxaloacetic acid can be estimated manometrically as CO_2 after decomposition by aniline in slightly acid solution [Ostern, 1933]. The cyanohydrin was not decomposed under the same conditions. The manometric method enabled therefore a determination of the speed of combination of oxaloacetic acid and cyanide to be made. Cyanide and oxaloacetic acid were incubated at 38° in the manometer for various lengths of time before the aniline-citrate solution was introduced. It appeared that reaction between oxaloacetic acid and cyanide was complete in less than 1 min.

REFERENCES

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