CLXXIV. THE REGENERATION OF THE REDUCING PROPERTIES OF OXIDISED LEMON JUICE.

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(*Received July 3rd, 1933.*)

Tillmans *et al.* [1932] concluded, as a result of their work upon the relation between the indophenol-reducing capacity and antiscorbutic potency of fruit and vegetable juices, that these properties could be attributed to the same substance, which was most probably identical with Szent-Györgyi's hexuronic acid. These workers claimed that the capacity of lemon juice for reducing indophenol, after being destroyed by oxidation with this reagent, iodine or hydrogen peroxide, could be quantitatively regenerated by reduction with hydrogen sulphide, providing that the reduction was carried out soon after the oxidation and the oxidised juice was not exposed to air too long; that is to say, the oxidation of the reducing substance proceeded first to a stage from which it could be regenerated. Szent-Györgyi had previously claimed [1928] that hexuronic acid (ascorbic acid) was capable of being reversibly oxidised and reduced. It is the purpose of this note to record a few experiments which confirm these observations on the regeneration of the reducing capacity of lemon juice after oxidation with certain reagents.

In repeating the work of Tillmans *et al.*, various difficulties were encountered before their results could be substantiated. In the first place it was found necessary to use juices in which no traces of iron were present as this vitiated the results. The major difficulty, however, was in completely removing the hydrogen sulphide, which, of course, reduces both indophenol and iodine. The above workers claimed that they were able to remove the hydrogen sulphide in about 40 minutes by bubbling nitrogen through the juice. They tested their juices for its absence by dipping lead acetate papers into the solution. It was found in the experiments described below, however, that after passing nitrogen for about an hour, although the presence of hydrogen sulphide could not be detected by dipping lead acetate papers or by allowing the escaping gas to impinge upon wet lead acetate paper, the use of dry reaction paper revealed the presence of a considerable quantity of hydrogen sulphide in the solution. Furthermore, after bubbling nitrogen for 2 hours, the issuing gas also decolorised a solution of indophenol when bubbled through it. Using a dry paper, hydrogen sulphide could be detected even after passing nitrogen for 5–6 hours. Decitrated lemon juice gives a heavy precipitate with lead acetate; it is thus probable that the test for the presence of hydrogen sulphide would be vitiated by this precipitation when the paper was dipped in the liquid. Complete removal of hydrogen sulphide however was not necessary, for it was found that after passing nitrogen for about 3 hours, except in the case of oxidations with hydrogen peroxide, the persistent residuum of hydrogen sulphide was insufficient to affect

appreciably the titrations. The juices were considered to be free from hydrogen sulphide when the issuing gas failed to decolorise a solution containing 1 cc. $N/1000$ indophenol when bubbled through it for half an hour.

The ratio between the capacity of lemon juice for reducing iodine in acid solution and indophenol in neutral solution was almost invariably constant, and the amounts of these reagents reduced were roughly equivalent. Good agreement between indophenol and iodine titrations of the oxidised and untreated juices, and the absence of hydrogen sulphide by the above test, were therefore regarded as criteria that regeneration had been effected.

Experiments in which indophenols were used as oxidising agents gave rather low results after regeneration, but this was probably due to the relatively high concentration of the reduced form of the indophenol which very rapidly absorbs oxygen and may have catalysed the oxidation of part of the reducing substance before it could be titrated [see Zilva, 1927]. In these experiments also no iodine titrations could be undertaken as the indicator interfered.

The experiments with iodine as oxidising agent gave the most satisfactory results both with decitrated lemon juice and the raw juice.

Since hydrogen peroxide functions most satisfactorily in an acid medium, oxidations with this reagent were performed on the raw juice.

Some typical experiments and results are given below.

Experimental.

Decitrated lemon juice was prepared in the usual way. As oxidising agents were used 2:6-dibromophenolindophenol, iodine and hydrogen peroxide. The juices immediately after oxidation were placed in gas-washing-bottles, which, after hydrogen sulphide had been passed through for a suitable length of time, were sealed up for 24 hours. The hydrogen sulphide was subsequently removed by blowing oxygen-free nitrogen through the bottles.

Titrations with indophenol were made with a $N/1000$ solution of this reagent, which was standardised against titanous chloride. The titrations were carried out in neutral solution.

Titrations with iodine were carried out as follows. 5 cc. of the juice were mixed with 1 cc. glacial acetic acid and then 10 cc. $N/100$ iodine run in. After standing 5 minutes the excess iodine was determined with $N/100$ sodium thiosulphate. In the case of raw juice, no acetic acid was added.

Oxidations with iodine in acid solution.

(1) *Raw juice.* 5 cc. of this juice reduced 41 cc. $N/1000$ indophenol and 5·1 cc. $N/100$ iodine.

100 cc. of the juice were mixed with 10·2 cc. $N/10$ iodine and left to stand for 30 minutes. Through the mixture, which possessed no capacity for reducing indophenol, hydrogen sulphide was bubbled for 10 minutes. The bottle was then sealed up for 24 hours, after which a vigorous stream of oxygen-free nitrogen was blown through the juice for 3 hours. At the end of the experiment, the equivalent of 5 cc. of the original juice reduced 38 cc. $N/1000$ indophenol and 4·9 cc. of $N/100$ iodine.

(2) *Decitrated lemon juice.* The juice used was such that 5 cc. reduced 27 cc. $N/1000$ indophenol and 3·4 cc. $N/100$ iodine.

100 cc. of the juice were acidified with 10 cc. of glacial acetic acid; 6·8 cc. $N/10$ iodine and 3·2 cc. of water were then added. The remainder of the procedure was essentially the same as the above.

5 cc. equivalent of the original juice at the end of the experiment reduced 28 cc. $N/1000$ indophenol and 3·8 cc. $N/100$ iodine.

In both these experiments, good agreement was observed between the reducing capacities of the original and regenerated juices.

Oxidations with 2 : 6-dibromophenolindophenol.

Raw juice. 5 cc. of juice originally reduced 24 cc. $N/1000$ indophenol.

50 cc. of the raw juice were brought with N NaOH to p_H 6·8. The requisite amount of 2:6-dibromophenolindophenol in 10 cc. of water was added and the mixture diluted to 125 cc. 100 cc. of this mixture were next treated as in the previous experiment, except that the liquid was centrifuged before removal of the hydrogen sulphide. Bubbling nitrogen for 3 hours reduced the hydrogen sulphide to the required minimum. An amount of juice equivalent to 5 cc. of the original reduced 15 cc. of $N/1000$ indophenol.

Decitrated lemon juice. 5 cc. of juice reduced 24 cc. $N/1000$ indophenol.

The requisite amount of 2:6-dibromophenolindophenol in 10 cc. of water was added to 50 cc. of juice and the mixture diluted to 75 cc. It was then treated as described above. The hydrogen sulphide was removed by bubbling nitrogen for 3 hours. An amount of the treated juice equivalent to 5 cc. of the original juice reduced 12 cc. of $N/1000$ indophenol.

In both these experiments the low figures are probably to be attributed to the presence of the reduced form of the indophenol.

Oxidations with hydrogen peroxide.

Oxidations with this reagent are much slower than with either of those used in the previous experiments. Great difficulty was experienced in removing the hydrogen sulphide from the raw juices which were used. Even after passing nitrogen for 7 hours the indophenol and iodine titrations, as well as the qualitative tests, revealed the presence of hydrogen sulphide. The following experiment, however, shows that a quantitative regeneration of the reducing capacity of the juice can be obtained after oxidation with hydrogen peroxide.

5 cc. of the juice used reduced 24 cc. of $N/1000$ indophenol and 3·4 cc. $N/100$ iodine. 13·6 cc. of $N/10$ hydrogen peroxide were run into 200 cc. of the juice, and then 6·4 cc. of water were added. After standing for 3 hours 5 cc. equivalent of the original juice reduced 5·5 cc. of $N/1000$ indophenol. The juice was treated as in the previous experiments. Nitrogen was blown through the juice for $15\frac{1}{2}$ hours before the hydrogen sulphide was reduced to the required minimum. The equivalent of 5 cc. of the original juice then reduced 23 cc. of $N/1000$ indophenol and 3·4 cc. of $N/100$ iodine.

SUMMARY.

Tillmans's observation that lemon juice oxidised with indophenol, iodine or hydrogen peroxide can regain its reducing capacity when treated with hydrogen sulphide immediately after oxidation is confirmed.

My thanks are due to Dr S. S. Zilva for help and criticism and to the Medical Research Council for a whole time grant.

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