CCIII. THE PHOTOCHEMICAL DECOMPOSITION OF *L*-ASCORBIC ACID

BY ALEXANDER EDWARDS KELLIE
AND SYLVESTER SOLOMON ZILVA¹

From the Division of Nutrition, Lister Institute, London

(Received 28 June, 1938)

DURING the last few years evidence has been accumulating to show that the oxidation of *l*-ascorbic acid is catalysed by visible light in the presence but not in the absence of certain sensitizers. Martini & Bonsignore [1934] found that *l*-ascorbic acid reduced methylene blue to its leuco base when exposed to the light of an ordinary electric lamp. Kon & Watson [1936] observed that on exposure to visible light of short wave length (blue and violet) *l*-ascorbic acid in milk was oxidized to dehydroascorbic acid. A probable explanation for this phenomenon is found in the observation made by Hopkins [1937; 1938] that lactoflavin and to a lesser extent lumichrome could act as sensitizers in the oxidation of *l*-ascorbic acid when exposed to solar radiation.

In this communication experiments are described which show that light is capable of producing a chemical change in *l*-ascorbic acid even in the absence of sensitizers or catalysts. This photochemical action is, however, restricted to the ultraviolet region of the spectrum. It further appears that the photochemical change takes place not only with but also apparently without the intervention of oxygen. So far as cause and mechanism are concerned this reaction, therefore, differs from those observed by the above-mentioned workers.

TECHNIQUE

All the solutions were made up in phosphate buffer (*pH* 7) prepared with quartz-distilled water. This *pH* was employed in preference to *pH* 7.4, since it was more favourable to the stability of dehydroascorbic acid. The solutions (50 ml.) were exposed in glass or quartz round-bottomed flasks, according to the nature of the experiment, to a Hewittic Y 2 mercury vapour lamp at a distance of one foot. Control experiments carried out in the dark were kept at 37° since this was the temperature reached by the solutions, owing to heat emitted by the lamp, after one hour's exposure. In the anaerobic experiments the flasks were previously exhausted and filled with nitrogen three times and finally exposed in an atmosphere of this gas. Dehydroascorbic acid was determined by reducing the solutions with hydrogen sulphide at *pH* 3 after the addition of glacial acetic acid, one drop of 1 % NaCN and one drop of octyl alcohol. The hydrogen sulphide was removed in the usual way by a current of nitrogen. When sensitizers were present in solution the displacement of the hydrogen sulphide was carried out in the dark.

The behaviour of l-ascorbic acid on exposure to visible and ultraviolet light

The decomposition of *l*-ascorbic acid by the direct action of ultraviolet light can be conveniently demonstrated by following up the disappearance of *l*-ascorbic acid in solutions exposed to a mercury vapour lamp in quartz and in glass vessels

¹ Member of the Scientific Staff, Medical Research Council.

with and without sensitizers. In the experiment to be described *l*-ascorbic acid solutions in phosphate buffer at pH 7 were therefore irradiated in quartz and glass flasks in the absence of a sensitizer and in glass flasks in the presence of methylene blue and of lactoflavin. In addition, a solution of *l*-ascorbic acid in a glass flask was kept in the dark at 37° to serve as a control. At suitable intervals samples were removed and titrated at pH 3 with indophenol, whilst a duplicate of each sample was treated with hydrogen sulphide as described above. It was found possible to determine *l*-ascorbic acid in the presence of methylene blue, since the latter does not interfere with the end point; a very slight excess of indophenol produces a purple coloration in the blue solution. The results in Table I show that in the absence of a sensitizer the *l*-ascorbic acid in the glass

Table I. *Effect of exposure of l-ascorbic acid to mercury vapour lamp*

mg. of <i>l</i> -ascorbic acid per 100 ml.										
Exposure of <i>l</i> -ascorbic acid to mercury vapour lamp										
Time min.	In the dark		In quartz		In glass		In glass in the presence of methylene blue (0.5 mg./100 ml.)		In glass in the presence of lactoflavin (0.5 mg./100 ml.)	
	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S
0	4.00	4.00	4.00	4.00	4.00	4.00	4.00	3.95	4.00	3.88
15	4.00	4.00	2.50	3.00	4.00	4.00	2.00	3.57	0.80	2.70
30	3.98	4.00	0.68	1.97	4.00	4.00	0.60	2.70	0.00	1.92
60	3.97	4.00	0.00	0.78	3.80	3.75	0.00	1.20	0.00	0.90

flask, i.e. the solution which was exposed to visible light only, was not decomposed to any appreciable extent after 60 min. exposure, thus resembling the *l*-ascorbic acid solution kept in the dark. In contradistinction to this the solution in the quartz flask which was exposed both to visible and ultraviolet light simultaneously showed a progressive diminution in the *l*-ascorbic acid content. As was to be expected in the presence of methylene blue or of lactoflavin the *l*-ascorbic acid gradually disappeared when exposed even to visible light. It will also be noted that there was a formation of dehydroascorbic acid simultaneously with the disappearance of the *l*-ascorbic acid in each case.

The decomposition of l-ascorbic acid by ultraviolet light under anaerobic conditions

In the preceding experiment the exposure to the ultraviolet light took place in the presence of air and, as was seen, there was a simultaneous formation of dehydroascorbic acid in spite of the absence of a sensitizer. An experiment will

Table II. *Effect of exposure of l-ascorbic acid to mercury vapour lamp under anaerobic conditions*

mg. of <i>l</i> -ascorbic acid per 100 ml.					
Time min.	In the dark		Exposed to mercury vapour lamp		
	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	
0	4.00	4.00	4.00	4.00	
15	4.00	4.00	2.20	2.49	
30	3.90	4.00	0.90	1.13	
60	3.80	3.85	0.00	0.27	

now be described in which the irradiation of an *L*-ascorbic acid solution took place in the absence of atmospheric oxygen. The method employed to ensure this condition has already been described. The results obtained (Table II) leave no doubt that even in the presence of such traces of oxygen as may have been left there was a photochemical decomposition of *L*-ascorbic acid. Furthermore no formation of dehydroascorbic acid could be demonstrated in this case. The slight increase in the titration after treatment with hydrogen sulphide was of a similar order to that observed in the control solution kept in the dark.

The stability of dehydroascorbic acid to ultraviolet light under anaerobic conditions

Dehydroascorbic acid was prepared immediately before use by oxidizing *L*-ascorbic acid with the theoretical amount of potassium iodate in the presence of potassium iodide and hydrochloric acid. 1 ml. of a 0.2% solution was then added to 49 ml. of phosphate buffer (*pH* 7.0). The solution was exposed to the mercury vapour lamp in a quartz flask under anaerobic conditions. The determinations were carried out by titrating with indophenol the ascorbic acid formed after reduction with hydrogen sulphide. Dehydroascorbic acid is not very stable even under anaerobic conditions in the dark, most probably because of the gradual opening of the ring in the molecule [cf. Herbert *et al.* 1933], but, as will be seen from Table III, the deterioration in the exposed solution did not take place at a

Table III. *Effect of exposure of dehydroascorbic acid to mercury vapour lamp under anaerobic conditions*

mg. of *L*-ascorbic acid per 100 ml. after treatment with H₂S

Time (min.)	In the dark	Exposed to mercury vapour lamp
0	3.66	3.66
15	2.77	2.52
30	1.92	1.78
60	1.25	0.96

much higher rate than in the control solution kept in the dark. It may therefore be concluded that ultraviolet light has no very marked deleterious effect on dehydroascorbic acid in the absence of oxygen. This result is in consonance with the fact that a freshly made up solution of this compound shows no selective absorption in the ultraviolet region [Herbert *et al.* 1933].

The inability of lactoflavin to sensitize decomposition of L-ascorbic by ultraviolet light under anaerobic conditions

Although lactoflavin catalyses the oxidation of *L*-ascorbic acid in the light it was nevertheless desirable to ascertain whether it influenced the anaerobic decomposition of the compound by ultraviolet light. The results are given in Tables IV and V. They suggest that in the absence of oxygen lactoflavin has very little, if any, influence on the rate of the decomposition of *L*-ascorbic acid. It is true that a somewhat greater disappearance of *L*-ascorbic acid was observed when the solutions were exposed to the mercury vapour lamp in the presence than in the absence of the sensitizer. This, however, was undoubtedly due to the fact that in spite of all the precautions taken, a certain very short exposure to light and oxygen could not be avoided during the removal and titration of the samples. In the presence of oxygen lactoflavin, as was to be expected, accelerated markedly the oxidation of the *L*-ascorbic acid.

Table IV. *l*-Ascorbic acid exposed to mercury vapour lamp under anaerobic conditions in the presence and absence of lactoflavin

mg. of <i>l</i> -ascorbic acid per 100 ml.								
Time min.	In the dark				Exposed to mercury vapour lamp			
	Without lactoflavin		With lactoflavin (0.5 mg./100 ml.)		Without lactoflavin		With lactoflavin (0.5 mg./100 ml.)	
	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S
0	4.00	4.00	4.00	4.00	4.00	4.00	4.00	3.89
15	4.00	4.00	4.00	4.00	2.25	2.56	1.90	2.40
30	3.90	4.00	3.90	3.95	1.14	1.43	0.96	1.30
60	3.80	3.80	3.75	3.90	0.10	0.35	0.00	0.25

Table V. *l*-Ascorbic acid exposed to mercury vapour lamp under aerobic conditions in the presence and in the absence of lactoflavin

mg. of <i>l</i> -ascorbic acid per 100 ml.								
Time min.	In the dark				Exposed to mercury vapour lamp			
	Without lactoflavin		With lactoflavin (0.5 mg./100 ml.)		Without lactoflavin		With lactoflavin (0.5 mg./100 ml.)	
	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S
0	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
15	4.00	4.00	4.00	4.00	1.96	3.10	0.00	2.70
30	3.90	4.00	3.90	4.00	0.20	1.08	0.00	1.67
60	3.80	4.00	3.70	3.75	0.00	0.14	0.00	0.30

The influence of acidity on the decomposition of l-ascorbic acid by ultraviolet light

It is now well known that the atmospheric oxidation of *l*-ascorbic acid in the presence of metallic catalysts proceeds more slowly in acid solution than in neutral or alkaline solution and therefore a retardation in the disappearance of *l*-ascorbic acid when exposed to ultraviolet light in the presence of air in acid solution was to have been anticipated. It was, nevertheless, of interest to study the effect of pH on the anaerobic photochemical decomposition. *l*-Ascorbic acid solutions buffered at pH 3 and pH 7 were therefore exposed to ultraviolet light in the presence and in the absence of air. The results (Table VI) show that not

Table VI. *The influence of acidity upon the decomposition of l-ascorbic acid exposed to mercury vapour lamp*

mg. of <i>l</i> -ascorbic acid per 100 ml.								
Time min.	Exposed to mercury vapour lamp							
	Anaerobic conditions				Aerobic conditions			
	pH 3.0		pH 7.0		pH 3.0		pH 7.0	
	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S	Before treatment with H ₂ S	After treatment with H ₂ S
0	3.80	3.80	3.80	3.82	3.80	3.80	3.80	3.82
15	2.68	3.44	2.22	2.52	2.44	3.47	2.16	3.09
30	2.04	3.32	0.90	1.28	0.86	2.94	0.60	1.97
60	1.20	2.84	0.00	0.40	0.30	2.07	0.00	1.32

only under aerobic but also under anaerobic conditions the lower *pH* had a retarding influence on the disappearance of the *L*-ascorbic acid. Corresponding control solutions kept in the dark showed hardly any decomposition. It is of interest to note that after treatment with H_2S there was an appreciable rise in the indophenol-reducing capacity at *pH* 3 even under anaerobic conditions.

SUMMARY AND CONCLUSIONS

The results reported in this communication show that *L*-ascorbic acid, but not dehydroascorbic acid, undergoes photochemical decomposition when exposed to the light of the ultraviolet region of the spectrum without the intervention of oxygen. It may be argued that the disappearance of the *L*-ascorbic acid in the anaerobic experiments was due to the presence of the last traces of oxygen which were not removed after repeated exhaustion. The formation of dehydroascorbic acid owing to its comparative stability at *pH* 7 (Table III) should then have been detected, which as will be seen from Table II was not the case. Furthermore, we have observed that on exposing higher concentrations of *L*-ascorbic acid under anaerobic conditions the quantities which were decomposed were far in excess of those which could have been oxidized by any traces of oxygen which were left in solution. The following example will illustrate the point. A buffered solution (*pH* 7) of 50 ml. containing 50 mg. of *L*-ascorbic acid was exposed to the mercury vapour lamp anaerobically for 6 hr. under the above conditions. 22 mg. of the acid disappeared during this period. If the disappearance of the compound were due entirely to oxidation, 1.40 ml. of oxygen would have been necessary—a quantity which could not possibly have been present in solution even without previous exhaustion. In the presence of air, however, the oxidation of *L*-ascorbic acid is undoubtedly catalyzed by this light. Under both conditions the reactions proceed without the aid of a sensitizer. Lactoflavin which acts as a photocatalyst in the oxidation of *L*-ascorbic acid by visible light has no action on the photochemical change of this compound in the absence of oxygen. Acidity which retards the oxidation of *L*-ascorbic acid also retards, although to a lesser extent, its photochemical decomposition when oxygen is excluded. These observations taken in conjunction with the fact that the oxidation of *L*-ascorbic acid is catalyzed by visible light bring into prominence the complexity of the nature of the decomposition of *L*-ascorbic acid by light.

It may be observed in this connexion that the slightly increased decomposition of ascorbic acid recorded by Kon & Watson [1936] when milk was exposed in quartz rather than in glass vessels, was presumably due to the *direct* action of ultraviolet light. The greater part of the decomposition of the acid in the quartz vessel was, however, brought about probably in the same way as in the glass vessel, namely, as suggested by Hopkins [1938], by the action of visible light sensitized by the lactoflavin of the milk.

One of us (A. E. K.) is indebted to the Medical Research Council for a whole time grant. We should also like to express our thanks to Messrs Roche Products Ltd. for a gift of *L*-ascorbic acid and lactoflavin.

REFERENCES

- Herbert, Hirst, Perceval, Reynolds & Smith (1933). *J. chem. Soc.* 1270.
Hopkins (1937). *J. Soc. chem. Ind., Lond.*, **56**, 934.
— (1938). *C.R. Lab. Carlsberg*, **22**, 226.
Kon & Watson (1936). *Biochem. J.* **30**, 2273.
Martini & Bonsignore (1934). *Biochem. Z.* **273**, 170.