The Synthesis of Ascorbigen from Ascorbic Acid and 3-Hydroxymethylindole

EINO PIIRONEN and
ARTTURI I. VIRTANEN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

Some years after vitamin C was isolated and characterized as ascorbic acid, it was observed that the content of ascorbic acid in cabbage increased when cabbage was boiled for a short time [1]. McHenry and Graham [2], who detected a rise in the phenol-indophenol titration value in cooked cauliflower and in some other vegetables, believed the increase to be due to the liberation of »bound ascorbic acid», perhaps from an ester. Guha and Pal [3] found that the »bound ascorbic acid», which releases ascorbic acid on hydrolysis, can be extracted from cabbage with organic solvents. Sen-Gupta and Guha [4] gave the name ascorbigen to the substance although they did not isolate it. Procházka *et al.* [5] separated it from other substances. A comparison of their preparation (kindly sent by Dr. Ž. Procházka) with a synthetic ascorbigen prepared in this laboratory (Gmelin and Virtanen [6]) revealed that the former is identical with the synthetic preparation.

Gmelin and Virtanen found that ascorbigen can be synthesized either from 3-hydroxymethylindole and ascorbic acid or from indole, formaldehyde, and ascorbic acid. The synthesis from 3-hydroxymethylindole and ascorbic acid was accomplished in a phosphate buffer (pH 7) by heating for a few minutes on a water bath. Because the yield of ascorbigen varies greatly when this method is used, we have studied the factors that influence the reaction more closely.

In experiments in which ascorbigen was synthesized from 3-hydroxymethylindole and ascorbic acid, the optimum pH for the synthesis was found to be 4 to 5. In the first actual experiment performed after the preliminary experiments, the reaction temperature was 33°C, and the pH range 5 to 7. The same weight of both components was used in the reaction mixture, and hence 21 % of 3-hydroxymethylindole in excess of the theoretical value was added. The decrease in ascorbic acid during the experiment is shown in Table 1.

Table 1. The decrease in ascorbic acid in citrate buffer solutions (0.05 M) containing 9 mg of ascorbic acid and 9 mg of 3-hydroxymethylindole in 5 ml. pH values 5 to 7, temperature 33°C. The mixture was lightly shaken every two minutes during the whole reaction period.

Time	% of the original ascorbic acid consumed at		
min	pH 5	pH 6	pH 7
40	65	25	7
80	72	35	—
120	75	43	—
200	77	55	12

Thin-layer chromatograms of the reaction mixture after a reaction time of 200 min gave after staining with Ehrlich's reagent the following visually determined results.

1. Solvent: butanol saturated with water

	R_F	pH 5	pH 6	pH 7
3,3′-Di-indolyl-methane	0.94	30 %	50 %	35 %
3-Hydroxymethyl-indole	0.89	5 %	10 %	45 %
Ascorbigen	0.83	65 %	40 %	13 %

In addition Ehrlich-positive spots with R_F = 0.72 and 0.16 were obtained which became more intense as the pH rose.

2. Solvent: benzene:ethanol = 2:1

	R_F	pH 5	pH 6	pH 7
3,3′-Di-indolyl-methane	0.80	20 %	50 %	25 %
3-Hydroxymethyl-indole	0.67	0 %	0 %	50 %
Ascorbigen	0.59	80 %	50 %	25 %

In the second experiment the pH range was 2 to 7 and the temperature 37°C. 3-Hydroxymethylindole was used in an excess of 9 % over the theoretical value. The decrease in ascorbic acid at different pH values is shown in Table 2.

The thin-layer chromatogram (benzene: ethanol = 2:1) run after a reaction time of 10 min gave the following visually estimated ratios of the different components at different pH:

Table 2. The decrease in ascorbic acid in citrate-phosphate buffer solutions (McIlvaine) containing 6 mg of ascorbic acid and 5.4 mg of 3-hydroxymethylindole in 3 ml. pH values 2 to 7, temperature 37°C.

pH	mg and % of the orginal ascorbic acid consumed after					
	10 min		40 min		120 min	
	mg/ml	%	mg/ml	%	mg/ml	%
2	—	—	0.31	15	0.42	21
3	—	—	0.93	46	0.97	49
4	1.03	51	1.56	78	1.54	77
5	0.89	45	1.36	69	1.54	77
6	0.09	4	0.47	24	1.07	54
7	0.01	1	0.06	3	0.47	24

3,3′-Di-indolylmethane
pH 2 > pH 3 ≃ pH 4 < pH 5 < pH 6 ≃ pH 7;
3-Hydroxymethylindole
pH 2 < pH 3 > pH 4 > pH 5 > pH 6 < pH 7;
Ascorbigen
pH 2 < pH 3 < pH 4 > pH 5 > pH 6 > pH 7.

The formation of ascorbigen during 10 min at pH 7 was weak; after 40 and 120 min at this pH no ascorbigen was observed. Most of the 3-hydroxymethylindole had condensed to 3,3′-di-indolylmethane. After a reaction time of 120 min at pH 4, the yield of ascorbigen was nearly 80 % of the theoretical value. The photograph of the thin-layer chromatogram (Fig. 1) shows clearly the reaction products and the unchanged 3-hydroxymethylindole after a reaction time of 10 min. No 3-hydroxymethylindole could be detected at any pH value after 40 min.

Summary: Ascorbigen can easily be synthesized from 3-hydroxymethylindole and ascorbic acid at 20 to 37°C. The optimum pH is about 4 and the yield 70 to 80 %. At pH 5 the yield is still almost the same. The reaction time is about 1 h. At pH 7 small amounts of ascorbigen are formed during a short reaction time, but the ascorbigen disappears later on. At pH 6 and 7 large amounts of 3,3′-di-indolylmethane are formed from 3-hydroxymethylindole.

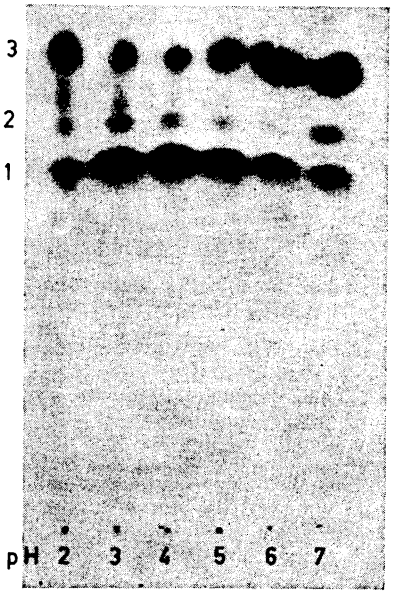

Fig. 1. Synthesis of ascorbigen from 3-hydroxymethylindole and ascorbic acid in water at 37°C and different pH. Reaction time 10 min. A paper chromatogram of the products formed; 1 = ascorbigen, 2 = 3-hydroxymethylindole, 3 = di indolylmethane.

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