Fatty Acid Monoesters of l-Ascorbic Acid and d-Isoascorbic Acid

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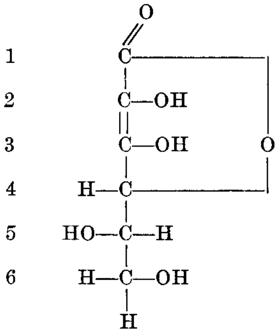
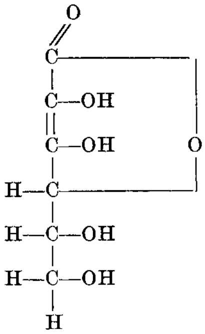
The oxidation-reduction properties of l-ascorbic acid (I) and d-isoascorbic acid (II) are well known (1) (2), and recently it has been shown that l-ascorbic acid and related substances may be valuable antioxidants for fats (3) and for oil-water emulsions (4) (5). d-Isoascorbic acid should be available at relatively low cost, since calcium 2-ketogluconate, which is readily convertible to d-isoascorbic acid in good yield (6) (7) (8) (9), is easily produced from glucose by fermentation (10).


I
l-Ascorbic acid
Vitamin C


II
d-Isoascorbic acid
Isovitamin C

The insolubility of l-ascorbic acid and d-isoascorbic acid in dry fats limits their effectiveness as antioxidants for fats and oils. However, esterification of these acids with higher fatty acids to render them fat-soluble should increase their effectiveness, provided that their oxidation-reduction properties are not adversely affected. It was the purpose of the present investigation to synthesize and study the properties of those fat-soluble monoesters of l-ascorbic acid and d-isoascorbic acid in which the primary hydroxyl group was esterified.

Several methods were tried for preparing pure fat-soluble fatty acid monoesters of these ascorbic acids. The methods employed were (a) treating the ascorbic acid with the appropriate fatty acid chloride in the absence of a solvent, or in pyridine or pyridine-chloroform solution, (b) heating the ascorbic acid and the fatty acid together in the presence or absence of catalysts, and (c) allowing the ascorbic acid and the fatty acid to react at room temperature in concentrated (95 per cent) sulfuric acid solution. Although the first method gave evidence of considerable reaction, no definite compounds could be isolated. By the second method no reaction was observed, and at least 90 per cent of the fatty acid was recovered unchanged. The last method was the only one that permitted ready separation and purification of the desired monoesters in good yield.

Concentrated sulfuric acid as a solvent as well as a catalyst in esterification reactions has been employed in a limited number of cases since first proposed by Meyer (11). Its most widespread application has been in the synthesis of glyceryl esters of fatty acids by Grün and coworkers (12) (13) (14), and it has also been employed by Bloor (15) to synthesize esters of mannitol and related carbohydrates with fatty acids. Grün has shown that none but primary hydroxyl groups take part in esterifications in sulfuric acid when the primary hydroxyl groups are free. Since l-ascorbic acid and d-isoascorbic acid contain only one primary hydroxyl group each, one would expect only monoesterification to take place. The products isolated in this investigation were monoesters, and although no conclusive evidence has been found that permits us to state unequivocally that esterification took place on the primary hydroxyl group only, it will be shown that this was most probably the case.

Pure monoesters of l-ascorbic and d-isoascorbic acids were prepared from lauric, myristic, palmitic, and stearic acids in 40-50 per cent yields. The esters were white, crystalline solids, soluble in most of the common organic solvents, and in fats and oils on gentle warming. They were insoluble in cold water. At room temperature dilute acetone solutions of potassium permanganate were readily decolorized by dilute acetone solutions of the esters. When titrated in alcohol solution with standard aqueous alkali, these esters gave theoretical neutralization equivalents for monoesters containing one titratable acidic enolic hydrogen.

A method was developed for determining the purity of the esters, based on the iodometric procedure for assaying l-ascorbic acid (16). Since both the esters and the ascorbic acids can be titrated with standard alkali and iodine solutions, it may be assumed that the enediol structure of l-ascorbic acid and d-isoascorbic acid has remained unaltered in the esters. Esterification, therefore, did not take place in the enediol group but on one of the other two available hydroxyl groups. The esters prepared in the present investigation gave a positive furfural test (17), indicating that carbon atoms one through five are unesterified unless it is assumed that under the conditions of the furfural test the ester is hydrolyzed. In the short time of contact between the 12-per cent alcoholic hydrochloric acid and the ester, little hydrolysis would be expected to take place. This leaves carbon atom six as the only one on which esterification had taken place. In view of Grün's (12) original work, it is highly probable that these monoesters of l-ascorbic acid and d-isoascorbic acid are esters of the primary hydroxyl group only.

Although we have usually found it convenient to operate with purified fatty acids, the less expensive, commercially available mixtures of higher fatty acids served just as well. The products, of course, were mixtures.

A study of the antioxidant properties of these esters is in progress, and a detailed report will be given separately.

In addition to their potential utility as antioxidants, monoesters of the type described in this publication may have utility as interfacial modifiers since they contain the necessary hydrophilic and lipophilic groups (18). Interfacial modifiers are used to improve the stability of emulsions such as aqueous dispersions of fat-soluble vitamins, mayonnaise, oleomargarine, chocolate coatings, and ice cream, and to improve the quality of cakes, to mention but a few of the uses related to the food industry. Qualitative experiments on the emulsification of peanut oil and water have shown that a small quantity of the l-ascorbyl or d-isoascorbyl ester prevents the emulsion from breaking for a considerable length of time.

The esters of l-ascorbic acid described above may have antiscorbutic activity because the structures to which this activity is attributed have presumably remained unaltered. Where l-ascorbic acid itself is unsuitable as a source of vitamin C these esters may find application. In addition, a fat-soluble form of vitamin C may be advantageous in certain vitamin preparations. d-Isoascorbic acid is reported to possess from one-fortieth to one-twentieth as much antiscorbutic activity as vitamin C (19). Tests on the antiscorbutic activity of these esters are in progress.

Experimental

Materials Used: The purest products that could be purchased were used. They had the following melting points: l-Ascorbic acid, 188-190° (decomposed); d-isoascorbic acid, 167.5-170° (decomposed); lauric acid, 42-43°; myristic acid, 52-53°; palmitic acid, 61-62°; stearic acid, 69-70°. The neutralization equivalents and carbon and hydrogen values for these compounds agreed with the theoretical values. l-Ascorbic acid and d-isoascorbic acid assayed 100 per cent (16).

Preparation of the Esters: To a solution of 0.05 mole of l-ascorbic acid or d-isoascorbic acid in 100 cc. of 95-per cent sulfuric acid at room temperature (23-25°), 0.04 mole of finely powdered fatty acid (lauric, myristic, palmitic, or stearic acid) was added. In the case of lauric, myristic, and palmitic acids the resulting mixture was shaken until complete solution was obtained. In order to dissolve the stearic acid, however, it was necessary to warm the mixture to 35°. The l-ascorbyl reaction mixture was allowed to stand overnight at room temperature (about 16 hours reaction time), whereas the d-isoascorbyl reaction mixture was allowed to stand for 6 hours. The solution was then poured slowly, with continuous stirring, on 500 grams of chopped ice, and the stirring was continued until all the oily liquid had solidified. When the mixture had reached room temperature, it was extracted with ether. The ether solution was carefully washed with water until the washings were free of sulfate ion. Stable emulsions that formed when the ether solution and the wash water were shaken together too vigorously were broken either by the addition of ammonium sulfate crystals or by allowing the emulsion to stand for several hours. The washed ether solution was dried over anhydrous calcium sulfate, and the ether was recovered. The dry, residual solid was ground to a fine powder and washed by decantation with three 200-cc. portions of petroleum ether (boiling range 35-60°). Each portion of petroleum ether was stirred with the solid for several minutes before the insoluble solid was allowed to settle. These washings removed the small amount of unreacted fatty acid (10 per cent or less of the original fatty acid), which was readily recovered and could be used over again. After the last washing, in order to obtain a product of analytical purity, the crude ester (about 70-80 per cent yield)[1] was crystallized to a constant melting point (one or two crystallizations) from a mixture of ether and petroleum ether (boiling range 35-60°). This was effected by dissolving the ester in the minimum quantity of boiling ether. Petroleum ether was then added in small portions until a slight turbidity was obtained at the boiling point of the solution. The esters usually crystallized as needles or plates. At this point, some evidence was available that the esters were monohydrates. In order to obtain concordant analytical results, however, the finely ground products were dried for at least one hour at 60-65° under reduced pressure (0.5 mm. or less) to remove the last traces of water. The esters were slightly hygroscopic, and in the isoascorbyl esters small quantities of water caused appreciable depressions of the melting points. The yields of pure products ranged from 40-50 per cent. Further quantities of esters could be obtained from the mother liquors. Table I gives a summary of the experimental data.

Neutralization Equivalent: About 1.0 gram of ester was dissolved in 100 cc. of neutralized ethyl alcohol.

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[1] These crude products were fairly pure and were satisfactory for most purposes. The d-isoascorbyl esters were pale yellow, whereas the l-ascorbyl esters were colorless.

TABLE I

Properties of l-Ascorbyl and d-Isoascorbyl Monoesters

Compound[1]	Neutralization Equivalent		Equivalent Wt. by Iodine Titration		Percent Fatty Acid Combined		Combustion Analyses Percent[2]				Melting Pt.
							Found		Calculated		
	Found	Calculated	Found	Calculated	Found	Calculated	C	H	C	H	
l-Ascorbyl[3] laurate	356.5	358.4	177.9	179.2	55.4	55.8	60.2	8.70	60.4	8.38	105.5-106.5°
d-Isoascorbyl laurate	360.0	358.4	178.8	179.2	56.2	55.8	60.1	8.23	60.4	8.38	78-79°
l-Ascorbyl myristate	383.5	386.5	193.1	193.3	59.7	59.1	62.3	8.92	62.2	8.87	110.5-111.5°
d-Isoascorbyl myristate	385.0	386.5	193.2	193.3	58.6	59.1	61.7	8.79	62.2	8.87	84-85°
l-Ascorbyl palmitate	413.2	414.5	206.4	207.3	62.4	61.9	63.3	9.24	63.7	9.24	116-117°
d-Isoascorbyl palmitate	413.0	414.5	208.1	207.3	61.8	61.9	63.5	9.21	63.7	9.24	88.5-89.5°
l-Ascorbyl stearate	441.7	442.6	222.1	221.3	64.0	64.2	64.9	9.56	65.1	9.57	117.5-118°
d-Isoascorbyl stearate	444.7	442.6	223.0	221.3	64.1	64.2	65.0	9.68	65.1	9.57	91.5-92.5°

[1] The data in this table were obtained on the anhydrous esters. The d-isoascorbyl esters were difficult to dry completely. They were heated at about 65° for 1 to 2 hours below 0.5 mm. to remove all traces of water.

[2] Semimicro analyses were made by the Analytical and Physical Chemistry Division of the Eastern Regional Research Laboratory.

[3] It has been suggested that these esters be named as derivatives of the ascorbic acids, for example, lauroyl l-ascorbic acid and lauroyl d-isoascorbic acid. This would be desirable from some standpoints since it would conform with the usual practice in sugar chemistry and would also indicate that the compounds still retain their acid function. Because of the probable application of these compounds in the chemical technology of fats, however, a name such as l-ascorbyl palmitate is more satisfactory, since it emphasizes the fat-soluble character of the compounds.

The solution was titrated with standard 0.1 N aqueous sodium hydroxide solution to a phenolphthalein end point. The end point was approached rapidly, the first pink color that permeated the entire solution being taken as the end point. The pink color faded quickly.

Iodine Titration: From 0.3 to 0.5 gram of ester was dissolved in 100 cc. of ethyl alcohol, and 25 cc. of 10-per cent sulfuric acid was added. The solution was titrated with standard 0.1 N iodine solution, a 0.2-per cent starch solution being used as an external indicator. A blank titration was also run. The theoretical equivalent weight is equal to one-half the molecular weight, since two hydrogen atoms are removed from the enediol group of the ester during the iodine titration.

Furfural Test: The furfural test was carried out in the usual manner (17), 12-per cent alcoholic hydrochloric acid being used as the solvent.

Summary

Fat-soluble fatty acid monoesters of l-ascorbic acid (vitamin C) and d-isoascorbic acid have been prepared from lauric, myristic, palmitic, and stearic acids in 40-50 per cent yields.

Evidence has been presented to show that only the primary hydroxyl group of each of the ascorbic acids has been esterified.

Antioxidant properties of these esters are being studied.

Preliminary tests on the esters have indicated that they may have useful properties as interfacial modifiers.

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FAC Color Standards for Commercial Fats

Governmental agencies have established trading grades for tallows and greases based upon FAC colors. Considerable confusion has been caused since the FAC color set as originally devised was not based on colors progressively darker as the number of tubes increased.

A.O.C.S. methods define the set as follows:

> "This set consists of 26 color standards, numbered with odd numbers from 1 to 45 and divided into 5 series. Numbers 1 to 9, inclusive, for light colored fats; numbers 11, 11A, 11B, 11C, for very yellow fats; numbers 13 to 19, inclusive, for comparatively dark fats of a reddish cast; numbers 21 to 29, inclusive, for fats with a greenish cast; numbers 31 to 45, inclusive, for very dark fats."

The tube numbering was done arbitrarily to identify the tubes on the basis of hue, so that in many cases a tube of higher number is *not darker* but distinctly *lighter* than tubes of lower number. Obviously, since all the FAC color numbers were not included when the governmental grades were established, there is much misunderstanding as to where fats of various FAC readings should be classified.

The Fat Analysis Committee, a joint committee of the American Oil Chemists' Society and of the American Chemical Society, and originators of the present FAC color standards, prescribe the following interpretation of the relationship of FAC color standards:

FAC Tube Number	Tubes Listed Below Are' Equal to or Lighter Than the Corresponding Tube in the Left-Hand Column
1	1
3	1, 3
5	1, 3, 5
7	1, 3, 5, 7
9	1, 3, 5, 7, 9
11	1, 3, 5, 7, 9, 11
11A	1, 3, 5, 7, 9, 11, 13, 11A
11B	1, 3, 5, 7, 9, 11, 13, 15, 11A, 11B
11C	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 11A, 11B, 11C
13	1, 3, 5, 7, 9, 11, 13, 11A
15	1, 3, 5, 7, 9, 11, 13, 15, 11A, 11B
17	1, 3, 5, 7, 9, 11, 13, 15, 17, 11A, 11B
19	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 11A, 11B, 11C
21	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 31, 33, 11A, 11B, 11C
23	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 31, 33, 35, 11A, 11B, 11C
25	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 31, 33, 35, 37, 11A, 11B, 11C
27	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 31, 33, 35, 37, 39, 11A, 11B, 11C
29	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 11A, 11B, 11C
31	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 31, 11A, 11B, 11C
33	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 31, 33, 11A, 11B, 11C
35	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 31, 33, 35, 11A, 11B, 11C
37	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 31, 33, 35, 37, 11A, 11B, 11C
39	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 31, 33, 35, 37, 39, 11A, 11B, 11C
41	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 31, 33, 35, 37, 39, 41, 11A, 11B, 11C
43	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 11A, 11B, 11C
45	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 11A, 11B, 11C

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