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144. Yoshio Imai : The Antiscorbutic Activity of Some O-Benzoyl Derivatives of L-Ascorbic Acid in Guinea Pigs.*1

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In recent studies to obtain stable derivatives of L-ascorbic acid (L-AsA), some esters, *e.g.* of fatty acids, were shown to possess the antiscorbutic activity. Almost all of these potent substances had the ester linkage at 6- or 5,6-hydroxyl group of the parent structure,[1,2] while 2,3,5,6-tetraacetyl derivative showed only a weak activity in comparison with that of L-AsA.[1] The author of this paper studied some acyl derivatives of L-AsA, including a mono-ester of the endiol group, for their antiscorbutic activity in guinea pigs.

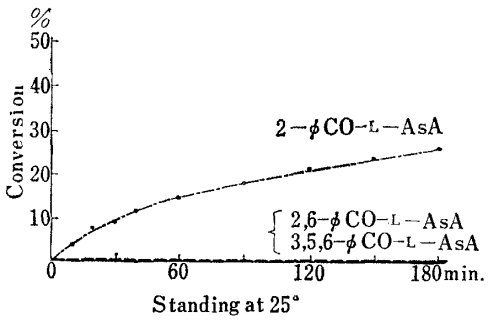

Fig. 1. Stability of O-Benzoyl Derivatives of L-Ascorbic Acid suspended with Arabic Gum in Distilled Water

10 μM. of sample/ml. of 0.1% arabic gum solution was titrated with 0.1N iodine solution at 25°(indicator : starch).

Materials

All the derivatives of L-AsA were supplied by Messrs. H. Nomura and K. Sugimoto of this company and their syntheses were described in the preceding paper,[3] except for ascorbigen prepared according to the method of Virtanen and Kiesvaara.[4] Fig. 1 shows the stability of ester linkage at the endiol group in an aqueous suspension at 25°. 2-O-Benzoyl-L-AsA seems to liberate free AsA, though the precise converted form, which is able to react with 2,6-dichlorophenolindophenol and 2,4-dinitrophenylhydrazine, still remains undetermined. The greater part of the dose of 2-O-benzoyl-L-AsA is, however, considered to be the original form at the time of administration to guinea pigs, as the suspension was prepared immediately before use. 2,6-Di-O-benzoyl-L-AsA and 3,5,6-tri-O-benzoyl-L-AsA did not consume any iodine at 25° within 3 hr.

Methods

Male guinea pigs weighing about 250 g. were divided into groups, each consisting of three to five animals, and housed individually in clean metal cages. The animals were fed a stock diet (Oriental RC-5*3) or a scorbutogenic diet[5] and water ad libitum. The scorbutogenic diet consisting of bran 45 g., crushed oats 25 g., skim milk 30 g. and vitamin A palmitate 2000 i.u. in 100 g. was daily given after mixing with one quarter volume of water. Daily dose of samples, suspended with arabic gum in 0.3 ml. of 50% sucrose solution per guinea pig immediately before use, was orally given from the 11th day. To the control group the sucrose solution was given. The animals were weighed three times a week and at the end of experiment the heparinized blood collected by decapitation was immediately centrifuged for the determination of plasma alkaline phosphatase activity according to a modification of the method of King and King[6] by means of "Technicon" autoanalyzer.*4 The subcutaneous haemorrhage was also noted at autopsy.

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Results

1) The Development of Scurvy and the Level of Plasma Alkaline Phosphatase

Thirty-five guinea pigs were divided into seven groups, of which three groups were fed the stock diet and the other three groups were fed the scorbutogenic diet. The last group was given 50 mg. of L-AsA/animal/day upon feeding of the scorbutogenic diet. The mean growth-curves of normal and scorbutic groups are shown in Fig. 2.

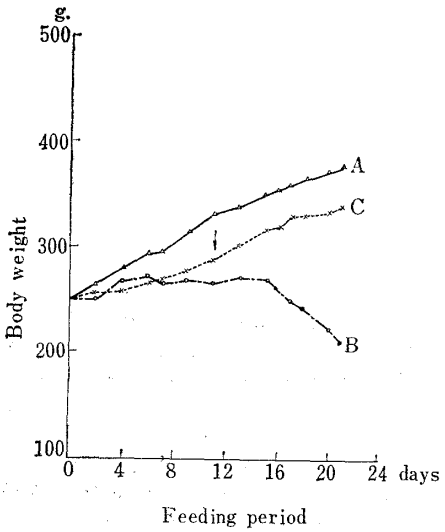

Fig. 2. Change in Body Weight

A: Stock diet
B: Scorbutogenic diet
C: Scorbutogenic diet+L-ascorbic acid 50 mg./day from the 11th day
Each curve represents the mean of five guinea pigs.

Guinea pigs fed the scorbutogenic diet showed a slightly less increase in body weight up to near the 10th day and lost rapidly their weight thereafter. No guinea pig, however, succumbed to death up to the end of three weeks. As shown in Table I, there was found no statistical difference in the activity of plasma alkaline phosphatase between control and scorbutic groups during the first week, but the fall of activity became apparent in the scurvy group within two weeks, *i.e.* 10.5± 1.8 and 5.0±1.0 after two and three weeks, respectively, as compared to initial 23.8±1.0 King-Armstrong unit. Subcutaneous haemorrhage was also noticed in some of the two week-fed group. Nevertheless, the restoration by giving L-AsA was easily recognized in all signs of deficiency.

2) The Activity of Derivatives of L-Ascorbic Acid

The antiscorbutic activity was compared by the degree of restoration in all signs of deficiency, *i.e.* plasma alkaline phosphatase level, body weight gain and incidence of haemorrhage. As shown in Table II, 1 mg. of L-AsA/animal/day might be minimum to prevent the development of scurvy in guinea pigs. Among the samples investigated, 2-O-benzoyl-L-AsA was shown to have almost the same order of activity as L-AsA, *i.e.* the former sufficiently prevented the incidence of subcutaneous haemorrhage at the equimolar dose to 1 mg. of the latter. Other compounds, including ascorbigen, showed a weak activity.

Table I. Development of Scurvy

Diet & treatment	No. of guinea pig	Experimental period (wk)	Plasma alkaline phosphatase[b]	Subcutaneous haemorrhage[c]
Start	5(253)[a]	0	23.8±1.0	0/5
A. Stock diet (Oriental RC-5)	5(257)	1	25.0±2.3	0/5
	5(256)	2	26.3±2.8	0/5
	5(253)	3	26.0±2.5	0/5
B. Scorbutogenic diet	5(256)	1	23.8±1.1	0/5
	5(254)	2	10.5±1.8	2/5
	5(255)	3	5.0±1.0	5/5
C. B+L-Ascorbic acid 50 mg./day p.o.	5(257)	3	24.5±3.0	0/5
			(M.V.±S.E.)	

a) Mean of initial body weight (g.) *b*) King-Armstrong unit *c*) Macroscopic finding

Table II. Antiscorbutic Activity of Derivatives of L-Ascorbic Acid (Oral)

Group	Dose[b] (mg./day)	No. of guinea pig	Body weight[d] gain (g.)	Plasma alkaline phosphatase[e]	Subcutaneous haemorrhage[f]
Control	—	25(255)[c]	53±5	23.8±1.0	0/25
Deficient	—	25(255)	−53±7	3.8±0.5	25/25
L-AsA[a]	5.0	25(267)	29±5	24.3±2.0	0/25
2-O-Benzoyl-L-AsA	8.0	8(253)	30±8	26.3±3.5	0/8
2,6-Di-O-benzoyl-L-AsA	10.9	5(265)	15±11	11.3±3.3	3/5
3,5,6-Tri-O-benzoyl-L-AsA	13.9	10(248)	−10±10	10.0±2.5	5/10
Ascorbigen	8.7	5(276)	−32±5	11.0±1.5	4/5
L-AsA	1.0	13(265)	40±7	20.0±3.0	1/13
2-O-Benzoyl-L-AsA	1.6	4(256)	29±14	24.5±4.0	0/4
L-AsA	0.5	9(248)	11±14	18.3±4.0	3/9
2-O-Benzoyl-L-AsA	0.8	4(249)	10±20	18.3±3.5	1/4
L-AsA	0.25	3(251)	−3±11	12.8±3.0	1/3
2-O-Benzoyl-L-AsA	0.4	3(269)	−26±21	12.0±4.8	3/3
			(M. V. ± S. E.)		

a) L-AsA: L-Ascorbic acid *b*) Equimole to L-Ascorbic acid *c*) Mean of initial body weight
d) During the 11~21st day *e*) King-Armstrong unit *f*) Macroscopic finding

Discussion

To evaluate the antiscorbutic activity the growth[5,7,8] or odontoblastic activity[9~12] has widely been used as the criterion in guinea pigs. There were, however, some problems to be considered in assessing the result, for instance, duration of test period or fluctuation of body weight gain and/or skill of the technique of histological examination. Schwachman and Gould[13,14] reported that there was a close relationship between the deficiency of vitamin C and the decrease in serum alkaline phosphatase activity. The author used the plasma alkaline phosphatase level as one of criteria in addition to the body weight gain. From the result in Table I, it was apparent that the deficient syndrome of vitamin C could be manifested after three week-feeding of the scorbutogenic diet. Both, in body weight gain and in recovery of plasma alkaline phosphatase, the response to L-AsA could not be always obtained as a linear line against the dose, but when the more quantity was given, the higher level of plasma alkaline phosphatase was observed. The weak vitamin C activity of 2,3,5,6-tetraacetyl derivative of L-AsA was already shown by Feldheim and Czerny,[1] while 6-stearoyl,[2] 6-lauroyl[2] and 5,6-diacetyl[1] derivatives have been reported to be about as active antiscorbutically as L-AsA. Though not acyl derivatives, 3-methyl-L-AsA[15,16] and 2,3-diphenacyl-L-AsA[17] were reported to show a very little or no antiscorbutic activity, while 1-methyl-heteroascorbic acid[16] exhibited the strong activity. Further, ascorbigen, described as a potent

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substance by Kiesvaara and Virtanen[18,19] was confirmed to possess only a weak activity in accordance with the result of Feldheim and Prochazcka.[20] It is of interest to note that 2-benzoyl derivative showed a strong antiscorbutic activity in spite of having an ester linkage at the endiol group, and that a great difference exists in the potency between 2-O-benzoyl-L-AsA and 2,6-di-O-benzoyl-L-AsA or 3,5,6-tri-O-benzoyl-L-AsA, since the ester linkage at 5- or 6-hydroxyl group may be considered to have only a slight effect on the biological activity. The strong antiscorbutic activity of 2-O-benzoyl-L-AsA may be partly due to its instability in aqueous state releasing non-esterified endiol group even at 25°, while 2,6-di-O-benzoyl-L-AsA and 3,5,6-tri-O-benzoyl-L-AsA are able to undergo the hydrolysis *in vitro* by tissue homogenates of guinea pig when the amount of ascorbic acid is estimated according to the method of Roe and Kuether.[21] This problem is still under study.

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Summary

Some derivatives of L-ascorbic acid (L-AsA), including a monoester of the endiol group, were studied for their antiscorbutic activity in guinea pigs. Among them 2-O-benzoyl-L-AsA was found to have almost the same activity as that of L-AsA, *i.e.* even the oral dose equimolar to 1 mg. of L-AsA/animal/day might be effective to prevent the development of scurvy. 2,6-Di-O-benzoyl-L-AsA and 3,5,6-tri-O-benzoyl-L-AsA were shown to be less active antiscorbutically, may be one-tenth or below, than L-AsA.

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