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Studies on L-Ascorbic Acid Derivatives. VI.[1] Phosphorylation of L-Ascorbic Acid and Its Isopropylidene Derivative

Hiroaki Nomura, Masafumi Shimomura, and Shiro Morimoto

Research and Development Division, Takeda Chemical Industries, Ltd.[2]

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Phosphorylation of L-ascorbic acid and its 5,6-isopropylidene acetal with phosphoryl chloride under conditions using water as solvent and pyridine as base yielded almost quantitatively and selectively L-ascorbic acid 3-phosphate which has been a useful compound in cosmetic field. The reaction is suitable for manufacturing of the phosphate in a good yield. The effects of changes in solvent and base upon the phosphorylation reaction are also discussed by comparison of the yield and the composition of the products.

In the course of studies on the chemical modification of L-ascorbic acid much attention has been given to L-ascorbic acid 3-phosphate due to its vitamin C activity and the increased stability against treatment with alkali,[1,3] oxidation and prolonged storage.[3] Early attempts to prepare this compound by the phosphorylation of L-ascorbic acid (Ia) or its isopropylidene acetal (Ib) did not result in a useful preparative method.[4,5] By using acetone as solvent and pyridine as base the reaction of Ib with phosphoryl chloride yielded a mixture consisting of four phosphates, *i.e.*, L-ascorbic acid 2- (II) and 3-phosphates (III), L-ascorbic acid 3-pyrophosphate (IV) and bis (L-ascorbic acid-3,3′)phosphate (V).[5] In consequence the isolation of III from the reaction mixture has been necessarily achieved by chromatography in a poor yield.

As is commonly known, phosphoryl chloride is less satisfactory for the primary phosphate synthesis from an alcohol since the reaction proceeds often nonspecifically and leads to a mixture of primary, secondary and tertiary esters. Nevertheless, it is one of the most popular and industrially useful phosphorylating agents. The present study was undertaken to find a new facile method for preparing III by selective phosphorylation of I[6] with phosphoryl chloride.

Ia has two enolic functions with the ionization constants of $pK_1=4.25$ and $pK_2=11.79$ (at 25° in water)[7] respectively and in neutral or slightly alkaline solution predominant is the monoanionic species in which the enolic hydrogen at C_3 is ionized. Since the phosphorus atom in phosphoryl chloride is an electrophile and can interact strongly with nucleophilic centre of the ascorbate ion, the selective phosphorylation to III from the monoanionic species will be expected when the reaction is carried out in a solvent having a high dielectric constant. Actually, change of the solvent from acetone to water under the condition using weak base led to an exclusive product which was identified with the authentic sample[5] of III on paper chromatography, paper electrophoresis and by infrared spectrum.

1) Part V: H. Nomura, M. Kuwayama, T. Ishiguro, and S. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 341 (1971).
2) Location: *Juso, Higashiyodogawa-ku, Osaka.*
3) H. Mima, H. Nomura, Y. Imai, and H. Takashima, *Vitamins*, **41**, 387 (1970).
4) E. Cutolo and A. Larizza, *Gazz. Chim. Ital.*, **91**, 694 (1961).
5) H. Nomura, T. Ishiguro, and S. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 381, 387 (1969).
6) I is used to indicate inclusively both I_a and I_b.
7) R.P. Bell and R.R. Robinson, *Trans. Faraday Soc.*, **57**, 965 (1961); G. Nebbia and E.M. Pizzoli, *Acta Vitaminol.*, **13**, 269 (1959).

Paper electrophoresis and the following manipulation for the quantitative analysis of each phosphate by spectrophotometry[8] were carried out in order to estimate the effect of phosphorylation conditions upon the product compositions and to find the optimal condition for the selectivity. The results are summarized in Table I—III. Changing the solvent from a less aqueous to a more aqueous medium increased the selectivity (Table I, No. 1—4). This was preserved even in the reaction using unprotected ascorbic acid (Table I, No. 8). The solvent quantity appears to have effects on both the selectivity and the yield (Table II, No. 15—17).

The replacement of the phosphorylating agent by pyrophosphoryl chloride gave a similar result on the selectivity (Table I, No. 9), while by the reaction with phenylphosphoryl chloride I was recovered unchanged.

TABLE I. Phosphorylation of L-Ascorbic Acid and Its Isopropylidene Derivative by $POCl_3$ in Polar Solvent[a]

Run No.	I		Solvent[c]	Base		Phosphorylating agent		Conversion[e] %	Product composition %[f]			
	Ib g	Ia[b] g		Pyridine g	K_2CO_3 g	$POCl_3$ g	TCPP[d] g		II	III	IV	V
1	5		H_2O	11.9		4.25		78.5	0	97.9	0	2.1
2	5		H_2O–acetone (3:1)	11.9		4.25		80.8	1.5	81.9	8.7	7.9
3	5		H_2O–acetone (1:3)	11.9		4.25		85.5	17.2	70.6	0	12.2
4	5		acetone	11.9		4.25		91.4	26	42.1	7.9	24
5	5		acetone			21		0	0	0	0	0
6		4.09	acetone				20	0	0	0	0	0
7	5		H_2O (pH >12)		38.1	4.25		47.6	39.3	44.2	0	16.5
8		4.09	H_2O	11.9		4.25		83.4	3.8	89.8	0	6.4
9	5		H_2O	11.9			2.73	81.7	5.5	87.4	0	7.1

a) These reactions were performed at −10—0° for 30 min.
b) Ia=L-ascorbic acid, ib=5,6-isopropylidene-L-ascorbic acid
c) The solvent volume was adjusted to 30 ml in each run.
d) pyrophosphoryl chloride
e) 100–(% of I unchanged)
f) estimated by the spectrophotometric analysis after paper electrophoretic separation

For the present phosphorylation the use of base appeared to be essential. In fact, in the absence of base the phosphorylation did not occur (Table I, No. 5,6). As shown in Table II, the combined use of inorganic bases such as potassium carbonate with pyridine led to a more excellent yield as compared to the use of the individual base independently. The effect of the difference in base between pyridine and sodium or potassium carbonate on the product composition is striking. In a relatively strong alkaline solution, in which both of the enolic groups are ionized, the reaction proceeded nonselectively as expected due to the indiscriminate attack by phosphoryl chloride at the enolates at either C_2 or C_3 (Table I, No. 7, Chart 1, route c).

In the reaction using methanol, as shown in Table III, the influence of nature of the base upon the phosphorylation was more decisive, *e.g.*, the use of pyridine gave no phosphorylation product of Ib while by using sodim carbonate the good conversion to L-ascorbic acid 3-dimethylphosphate (VI) was observed. VI seems to be very acid-labile and on the subsequent working up of the reaction mixture yielded L-ascorbic acid 3-phosphate (III) and a more lipophilic compound (VII) which, after isolation by chromatography on cellulose powder, was tentatively assigned to be L-ascorbic acid 3-methylphosphate on the basis of its nuclear

8) M. Shimomura, I. Aoki, M. Miyazaki, M. Yasumatsu, M. Hori, and M. Hattori, *Ann. Rept. Takeda Res. Lab.*, **27**, 54 (1968).

magnetic resonance (NMR) spectra and the color reaction (λ_{max}=485 mμ) with ferric chloride.[1] In methanol–water, the phosphorylation even in the use of pyridine yielded III without any formation of the methylester, *e.g.*, VI and VII.

TABLE II. Phosphorylation of 5,6-Isopropylidene-L-ascorbic Acid (Ib) in Water[a]

Run No.	Ib g	H_2O ml	Base: Pyridine g	Base: Inorganic base g	$POCl_3$ g	TCPP g	I[b] remained %	Phosphorylated product %[c] (III %[d])
10	5	45		Na_2CO_3 10.6	4.25		47.7	
11	5	45		K_2CO_3 38.1	4.25		49.5	
12	5	45	11.9	—	5.2		13.6	76.8 (74.8)
13	5	45	11.9	K_2CO_3 2.6	5.2		5.9	95.1 (93.1)
14	5	45	8.3	K_2CO_3 3.2	4.25		9.9	92.3 (87.7)
15	5	45	8.3	Na_2CO_3 1.85		4.0	11.4	90.5 (88.2)
16	5	8.5	8.3	Na_2CO_3 1.85		4.0	2.9	62.0
17	5	8.5	8.3	Na_2CO_3 1.85	4.25		0.48	65.0 (51.2)

a) These reactions were performed at −10—0° for 30 min.
b) estimated by iodometry
c) estimated by the colorimetric analysis according to the previous method[1]
d) estimated by the spectrophotometry after the paper electrophoretic separation

TABLE III. Phosphorylation of 5,6-Isopropylidene-L-ascorbic Acid (Ib) in Methanol[a]

Run No.	Ib g	Solvent	ml	Base	g	$POCl_3$ g	I remained %	Product composition % II	III	IV	V	VII
18	5	MeOH–H_2O (1:1)	35	pyridine	11.9	4.25	36.7	0	86.8	13.2	0	0
19	5	MeOH	35	pyridine	11.9	4.25	100	0	0	0	0	0
20	5	MeOH	35	Na_2CO_3	4.9	4.25	20.7	0	52.9	0	0	47.1

a) These reactions were carried out at −10—0° for 30 min in each run.

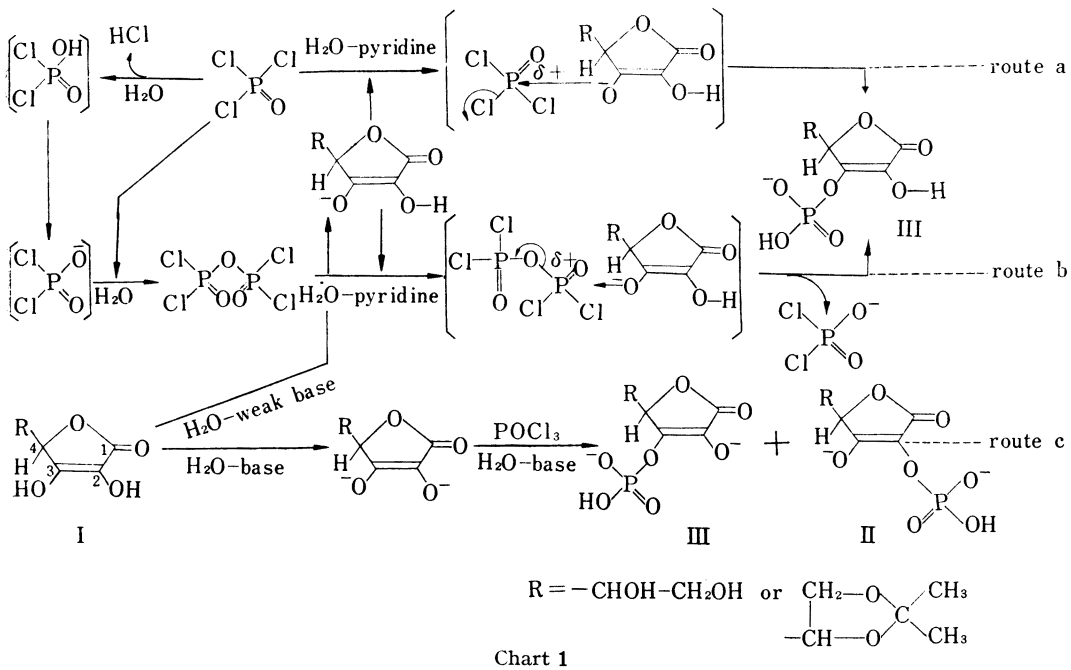

Chart 1

The mechanism of the hydrolysis of phosphoryl chloride has been studied by Hudson and Moss, who suggested that the formation of phosphodichloridic acid was very rapid (the half life time of the first step, $\tau_{1/2}=10^{-2}$ sec at 25°), while the following hydrolysis to phosphomonochloridic acid was relatively slow (the half life time of the second step, $\tau_{1/2}=250$ sec)[9] and therefore it is necessary to consider a possibility of reactions between phosphodichloridic acid and the ascorbate ion. However, this appears to be little since the reactivity of phosphodichloridic acid with hydroxyl group is fairly low as exemplified by lack of the reaction with sodium phenoxide.[9] In this connection the formation of pyrophosphoryl chloride has been suggested to be a result of the reaction of phosphoryl chloride with phosphodichloridate ion (route b in Chart 1).[9] From these consideration the following mechanism is suggested for the present phosphorylation.

Selective introduction of phosphoryl group into the enolic group at C_3 is primarily attributed to the complete and selective ionization of this group in a solvent having high polarity, namely, water ($\varepsilon=80$) is much better ionizing solvent than is low dielectric media such as acetone ($\varepsilon=21$). The experimental results indicate that the phosphorus atom in phosphoryl chloride or pyrophosphoryl chloride attacks the site of the highest electron density in the ascorbate ion. Secondly, the selectivity depends, partly at least, on the rapid hydrolysis of the intermediary produced dichlorophosphate of I into III, which prevents the formation of higher condensed phosphates such as V.

It is noteworthy that in an aqueous solution of a weak base the enolic group in I is phosphorylated selectively and almost quantitatively into III. The best procedure was to dissolve Ib (one equiv.), under mechanical stirring, in an aqueous solution containing both pyridine (6 equiv.) and a small amount of potassium carbonate (0.8 equiv.) and then to add dropwise a little excess of phosphoryl chloride (1.2—1.5 equiv.) at −10—0°. After a few minutes stirring the resulting mixture was worked up according to the procedure as described in the experimental section. The present procedure is suitable for the industrial preparation of III in an excellent yield.

Experimental

Separation and Determination for the Product Composition——The reaction mixture to be analyzed in each run was treated with a cation exchanger (IR-120, H-form) to remove pyridine. The eluent was neutralized with sodium hydroxide and lyophilized. The procedure for electrophoretic separation of the product was described in the previous paper.[8] The technique is summarized as follows: accurately 100 mg of the sample to be analyzed was weighed and placed in a 5 ml volumetric flask followed by dilution with water to the mark. Using a micrometer syringe 25 μl of the solution was applied in a narrow band about 4 cm long on a strip of Whatman No. 1 paper (6 × 50 cm), about 7 cm from one end. The phosphates were separated on electrophoresis in 0.06 M borate buffer at pH 10 using an electrophoretic apparatus (Sano-type electrophoretic apparatus, Shiraimatsuseisakusho, Osaka) for 100 min at 30 v/cm. During the electrophoresis the paper was immersed in *n*-hexane. After the paper being dried, the bands located in ultraviolet (UV) light were cut out. The phosphate existent in each band area was extracted with precisely 15 ml of 0.1 N NaOH in a Pyrex tube and the resulting solution, after filtration, was subjected to spectrophotometrical analysis (263 mμ). The recovery from runs of individual L-ascorbic acid phosphates was over 98%. The molar ratios of the phosphates produced under the selected conditions are listed in Table I—III.

L-Ascorbic Acid 3-Phosphate (III) from 5,6-Isopropylidene-L-Ascorbic Acid (Ib)——To a solution of 5, 6-isopropylidene L-ascorbic acid (10 g, 0.046 mole), pyridine (21.8 g, 0.276 mole), and potassium carbonate (5.2 g, 0.0525 mole) in water (100 ml) was added dropwise freshly distilled phosphoryl chloride (10.6 g, 0.069 mole) at the temperature −10—0° under mechanical stirring. After 30 min, aliquots of the solution were subjected to analysis for L-ascorbic acid (iodometry) and for L-ascorbic acid phosphates (colorimetry[1]), the results of which showed that the convertion was 94.7% based on 5,6-isopropylidene-L-ascorbicacid. The reaction mixture was passed through a column of Amberlite IR-120 (H-form, 400 ml). After washing of the column with water, the combined effluent and washing was neutralized with magnesium oxide, followed by the addition of 35 ml of ethanol and then allowed to stand for 18 hr at room temperature. After

9) R.F. Hudson and G. Moss, *J. Chem. Soc.*, **1962**, 3599.

the precipitate was removed by filtration and the solution was concentrated to 70 ml and adjusted to pH 4.6 by hydrochloric acid. Ethanol (250 ml) was added dropwise to the solution under stirring to afford a colorless precipitate. Recrystallization of the precipitate from water–methanol gave 7.5 g of colorless crystalline powder. $[\alpha]_D^{22}$ +54.7° (c=1.0, H_2O), NMR (60Mc, D_2O): 3.82, 3.83 (2H, $J_{5,6}$=7 cps, $J_{5,6'}$=5.5 cps, C_6–H_2), 4.18 (1H, multiplet, $J_{5,6}$=7 cps, $J_{5,6'}$=5.5 cps, $J_{4,5}$=1.6 cps, C_5–H), 4.64 (1H, quartet, $J_{4,5}$=1.6 cps, J^4_{PH}= 0.75 cps, C_4–H). Paper chromatographic analysis (propanol: water: trichloroacetic acid=75:24:1) indicated a single spot, the *Rf* of which was identical with the authentic sample. The electrophoresis and the following manipulation for the spectrometric determination[1] showed that the purity of III was 97.5%.

Preparation of L-Ascorbic Acid 3-Methyl Hydrogen Phosphate (VII)——To a solution of 5,6-isopropylidene-L-ascrobic acid (5 g, 2.31×10^{-2} mole) in methanol (35 ml) was slowly added anhydrous sodium carbonate (4.9 g, 4.62×10^{-2} mole) under mechanical stirring. The mixture was cooled to −10° in a dry ice bath and phosphoryl chloride (4.25 g, 2.74×10^{-2} mole) was added dropwise. The suspended particles of sodium carbonate gradually dissolved as the reaction progressed. The temperature was kept −10—0° during the process. After 30 min stirring and sodium chloride precipitated being removed by filtration, the volume of the filtrate was adjusted to 100 ml with methanol. One ml aliquot was subjected to iodometric analysis which revealed 21.7% of the initial amount of 5,6-idopropylidene-L-ascorbic acid being unchanged. Evaporation of the methanol solution gave pale yellow sirup which was dissolved in water and passed through a column of IR-120 (H-form, 150 ml) to remove both the isopropylidene group and sodium ions. The eluate was lyophilized to give 5.8 g of a pale yellow, hygroscopic solid. The electrophoretic analysis showed the presence of three ultraviolet absorbing components. These were identified as L-ascorbic acid (I), L-ascorbic acid 3-phosphate (III), and 3-methylhydrogenphosphate (VII). The relative mobility of VII to III in the electrophoresis was 0.62. The composition was shown in Table III (No. 20). A 200 mg sample of the lyophilized substance was dissolved in a small amount of methanol and added a small amount of cellulose powder. The mixture was placed on the top of a cellulose powder column (70 cm×2 cm) which was washed with *n*-propanol and then eluted with a solution composed of *n*-propanol–water–trichloroacetic acid (79:20:1). Each 5 ml fraction was collected and monitored by the ferric chloride test. The fractions containing L-ascorbic acid methylphosphate were combined and concentrated *in vacuo*. The addition of cyclohexane gave a white precipitate. After being collected by centrifugation the precipitate was recrystallized from cyclohexane–propanol. Yield 0.10 g, hygroscopic powder. Single spot on a polyamide thin-layer chromatography (polyamide 11 F_{254} Merck Co., mobile phase: *n*-propa nol–H_2O–trichloroacetic acid; 75:24:1). IR ν_{max}^{KBr} (cm^{-1}): 3400 (broad, OH), 2950 (CH), 1770 (lactone C=O), 1690 (C=O), 1610 (C=C), 1460 (CH_3), 1250 (P=O), 1050 (P-O-C). NMR (60 Mc, D_2O): 3.68 (3H, doublet, J_{HP}=11.0 cps, CH_3OP), 3.76, 3.78 (2H, defused doublet, $J_{5,6}$=7.0 cps, $J_{5,6'}$=5.5 cps, -CH_2OD), 4.06 (1H, multiplet, $J_{5,6}$=7.0 cps, $J_{5,6'}$= 5.5 cps, $J_{4,5}$=2.0 cps, -CHOD-), 5.02 (1H, triplet, $J_{4,5}$=2.0 cps, $^4J_{HP}$=1.3 cps, -CH-). The assignment fort he spectra was carried out according to the procedure described by Sawyer and Brannan.[10] The methoxyl protons gave rise to two peaks and the coupling constant, J_{H-P} for CH_3OP-O agrees with the previous studies.[11] This compound gave the characteristic coloration with ferric chloride, λ_{max}=485 mμ.

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10) D.T. Sawyer and J.R. Brannan, *Anal. Chem.*, **38**, 192 (1966).
11) J.B. Stothers and E.Y. Spencer, *Can. J. Chem.*, **39**, 1390 (1961); H. Finegold, *Ann. N.Y. Acad. Sci.*, **70**, 875 (1958).