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피부 활성을 갖는 Phytosphingosine Ascorbate의 합성

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Preparation and Properties of Phytosphingosine Ascorbate with Retaining Skin Development Effects

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요 약: 피부 활성 물질로 넓이 알려져 있는 vitamin C (L-Ascorbic acid)는 콜라겐 생합성 촉진과 강한 항산화 작용으로 피부 항노화 및 주름 개선 효과 있을 뿐만 아니라 멜라닌 세포 활성 억제, 자외선 차단, 상처치유 등의 효능을 갖고 있다. 그러나 물성면에서 피부 자극과 수분 및 공기, 빛에 불안정하여 쉽게 산화된다는 단점을 갖고 있다. 이러한 문제점을 해결하기 위해 많은 연구가 진행되어 왔고, 그 결과 다양한 vitamin C 유도체가 개발되었다. 그러나 아직까지도 vitamin C의 불안정한 물성을 해결하면서 그 자체의 효능을 피부에 적용시키기에는 미흡한 점이 많다는 평을 받고 있다.

본 연구에서는 이러한 vitamin C의 불안정한 물성을 개선시키고 그 효능을 피부에 적용시킬 수 있는 vitamin C 유도체를 개발하기 위해, 피부 친화성이 우수한 스핑고지질류 중 하나인 phytosphingosine을 이용하여, 산염기 반응에 의한 vitamin C의 OH기와 phytosphingosine의 $-NH_2$기를 이온 결합시킨 새로운 vitamin C 유도체인 phytosphingosine ascorbate (SP-VC)를 합성하였다. 이렇게 합성된 phytosphingosine ascorbate (SP-VC)는 원소 분석(C58.3 : H9.3 : N2.8 : O29.5) 및 mass spectrosocopy (Maldi TOF-MS), UV/vis spectra (268.5 nm), ^{1}H NMR, FT-IR, 열분석 (m.p=154℃), HPLC 등을 통하여 구조 및 물성을 확인하였다. 또한 효능면에서는 우선적으로 phytosphingosine ascorbate(SP-VC)의 항균 및 항산화 효과를 확인하였다.

이러한 결과를 토대로 vitamin C와 phytosphingosine의 효능을 동시에 갖으며 불안정한 물성과 자극을 개선시킬 것이라 예상되는 새로운 소재를 합성하였다.

Abstract: In the human skin, vitamin C (L-ascorbic acid) that is well known as the activated materials has effects that is skin anti-aging and wrinkle repair by giving impetus to collagen biosynthesis and anti-oxidation, and that is the sun screen, a wound recovering, inhibition melanogenesis and so on. In spite of its effects, vitamin C has the defects of the skin stimulation and easily oxidized instability by water, air, heat and light. For solving their matters, many investigation is advanced and its results are synthesized the various vitamin C derivatives. And yet they have not solved the unstable property of vitamin C and were still insufficient for the comparing with the effect of the pure vitamin C itself.

In this study, in order to prepare vitamin C derivative of being improved the stability and to apply vitamin C effect in the skin, we prepared new vitamin C derivative, phytosphingosine ascorbate, by using phytosphingosine, one of sphingolipids, which have a distinguished skin affinity. Phytosphingosine ascorbate can be prepared as the ionic bond between amine group ($-NH_2$) of phytosphingosine and hydroxy group (-OH) of vitamin C by way of the relatively simple reaction. So the structure and properties of the synthesized phytosphingosine ascorbate was confirmed the use of elemental analysis (C 58.3 : H 9.3 : N 2.8 : O 29.5), MALDI TOF-MS (Mw=492.58), Ultraviolet spectra (268.5 nm), 1H NMR, FT-IR spectra, thermal analysis (m.p=154℃), HPLC and so on. And we could confirm the anti-bacterial and anti-oxidation effects.

Based on these results, we could confirm to prepare a new material that was expected of both effects of vitamin C and phytosphingosine and that is improved properties of vitamin C.

Keywords: *vitamin C derivative, phytosphingosine ascorbate, anti-oxidation, anti-bacterial activity*

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1. Introduction

L-Ascorbic acid (2), vitamin C, is well known to be susceptible toward thermal and oxidative degradation, and hence various stable derivatives of L-ascorbic acid have been searched for and prepared in a number of laboratories[1-13].

L-Ascorbic acid is a naturally occurring product found in many fruits and vegetables. In the human skin, vitamin C has the various effects that are anti-aging and wrinkle repair by giving impetus to collagen biosynthesis, and that is the sun screen, a wound recovering, inhibition melanogenesis and so on and is not expensive in comparison with the other vitamins. And vitamin C is not only reduced or eliminated a free radical and harmful oxygen as a high anti-oxidation effect but also strengthened an immunity of skin. However, as previously stated, vitamin C has the defects of the skin stimulation and easily oxidized instability by heat, light air, and water, especiallyan aqueous alkali solution[1-9]. Because of this defects, the applications of vitamin C have been limited in the cosmetics field.

For solving their matters, many investigations have advanced and its results are synthesized the various vitamin C derivatives. And yet they have not solved the unstable property of vitamin C and were still insufficient for the comparing with the effect of the pure vitamin C itself[1-13].

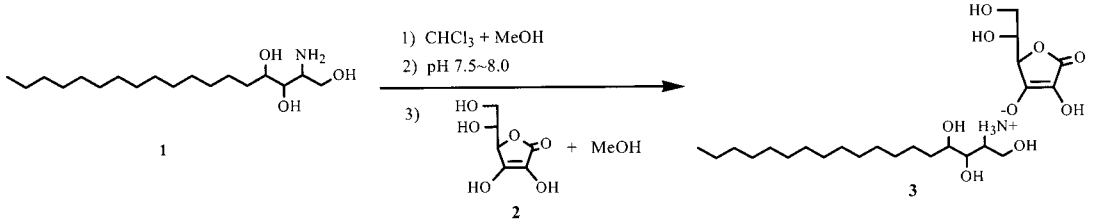
In this study, in order to prepare vitamin C derivative of being improved the stability and to apply vitamin C effect in the skin, we tried to synthesize the vitamin C derivative that was linked the specific-materials that were well known to be the effect in the skin with L-ascorbic acid and that was able to apply the effects of the linked material as well. In here, we investigated and prepared phytosphingosine ascorbate (SP-VC, 3) that was the new vitamin C derivative by using phytosphingosine (2S,3S,4R-2-amino-1,3,4-octadecanetriol; PS, 1), one of sphingolipids which have a distinguished skin affinity, that has the activation of skin-lipid strength and anti-bacterial effect for *Propionibacterium acnes* (*P. acnes*), on purpose to synthesize the new material that had the effect of vitamin C as well as phytosphingosine[16-18]. The prepared SP-VC was expected the synergism effects of vitamin C and phytosphingosine. Vitamin C has the instability and skin stimulation due to a self-oxidation and high acidity at 3-OH, respectively. Phytosphingosine ascorbate, which could be prepared the ionic bond between amine group ($-NH_2$) of phytosphingosine and 3-hydroxy group (3-OH) of vitamin C by way of the relatively simple reaction, acid-base reaction, is expected the improvement from the skin stimulation and the improvement of stability from oxidation of vitamin C as well as the ability that could apply the effect of the original vitamin C in the skin[6,7]. Thus the prepared SP-VC was expected the practicable to the cosmetics and pharmaceutical field. So that we have been investigating actively the skin effects, safety, and stability of SP-VC.

In this paper, we firstly were confirmed the synthesis and properties of phytosphingosine ascorbate and were carried out a free radical scavenging by using DPPH and anti-bacterial effect for *P. acnes* of the various effects. And we are supposed to establish that the effects of SP-VC that were expected of both effects of vitamin C and phytosphingosine.

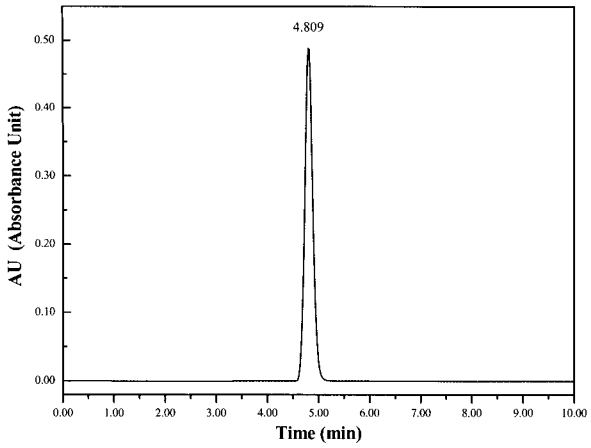
2. Results and Discussion

2.1. Synthesis of Phytosphingosine Ascorbate (3, SP-VC)

Scheme 1 is the synthetic process of phytosphingosine ascorbate (**3**) that can be obtained from acid-base reaction between L-ascorbic acid (**2**, vitC) and phy-



Scheme 1. Synthetic procedure of phytosphingosine ascorbate.



Eluent : Buffer Solution(Na_2HPO_4 pH=9) / MeOH = 1 / 9

Figure 1. HPLC chromatogram of SP-VC.

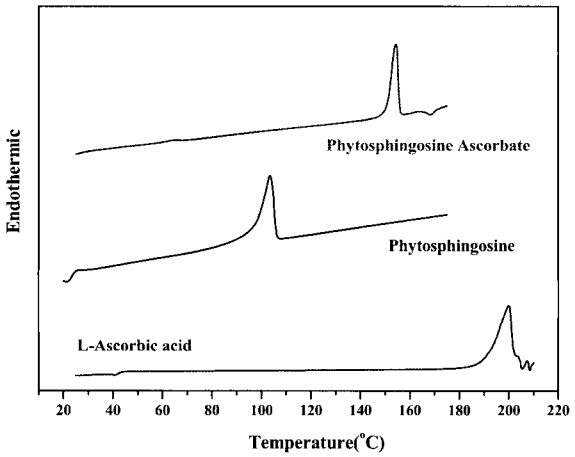


Figure 2. DSC curve of SP-VC, vitamin C & PS.

tosphingosine (**1**, PS). Exactly, **3**, which have the properties to differ from 1 and 2, could be obtained from the ionic bond between the negative (-) charge of 3-hydroxy (-OH) group of the dissolved **2** and the positive (+) charge of amine ($-NH_2$) group of **1**, in the controlled solution and could confirm the properties and structure through the various precision equipment.

TLC used silica gel 60 plate (F 254) of prepared

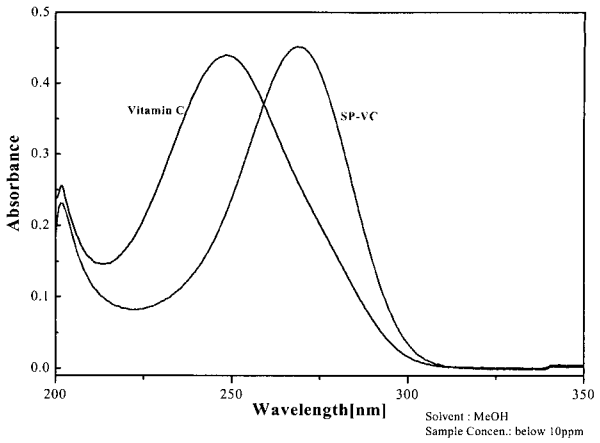


Figure 3. UV spectrum of SP-VC & vitamin C.

Table 1. Properties of SP-VC, Vitamin C & PS

	SP-VC	Vitamin C	PS
Tm (℃)	154~155	193~200	97~102
Td (℃)	215~225	195~200	280~290
Rf (TLC)	0.35~0.40	0.20~0.25	0.65~0.70
UV abs. (nm)	268.5~269.0	248.5~249.0	—

SP-VC became visible the one spot and confirmed the distinction of Rf value with 1 and 2 in an identical condition (eluent - $CHCl_3$: MeOH : water : acetic acid). Based on this result, we could obtain the characteristic peak of SP-VC through HPLC (Figure 1)[15]. And the characteristic thermal properties of SP-VC were measured through thermal analysis.

Figure 2 is the DSC curves of SP-VC, vitamin C, and phytosphingosine. The melting point (T_m) of SP-VC was measured in the range of 154~155℃ and was confirmed in higher ranges than one of PS (102℃) owing to vitamin C that had a high melting point. We measured the properties of SP-VC, PS, and vitamin C in the same condition (Table 1). The degradation temperature of vitamin C was known as equal with its T_m.

Figure 3 is the UV/vis spectra of SP-VC and vitamin C. SP-VC has the UV absorbance owing to vitamin C. However, the UV absorbance of SP-VC was measured on higher wavelength, 268.7nm, than vitamin C because of phytosphingosine back-bone (Table 1).

In order to confirm the structure of SP-VC, we confirmed and analogized by ^1H NMR, FT-IR spectrometer, elementary analysis, and MALDI TOF-MS

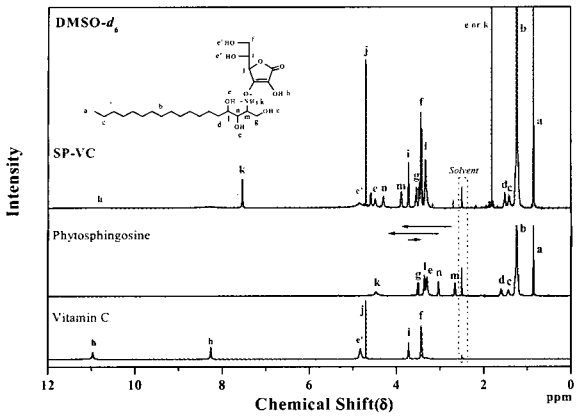


Figure 4. ^{1}H NMR spectrums of SP-VC, vitamin C & PS.

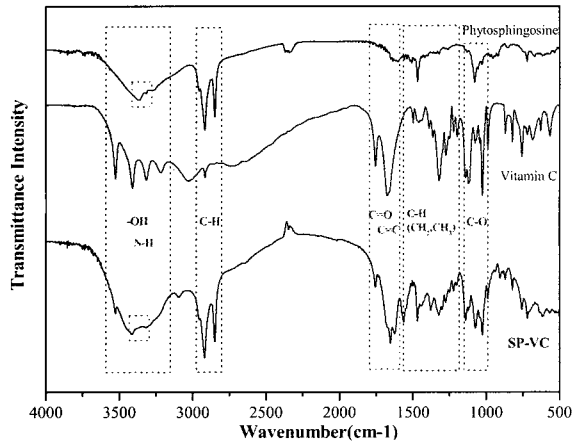


Figure 5. FT-IR spectrums of SP-VC, vitamin C & PS.

(time of flight-mass spectroscopy) to measure a molecular weight. Respectively, Figure 4 and 5 is ^{1}H NMR and FT-IR spectrums of SP-VC, vitamin C and PS. Through the assigned peaks, we could confirm the structure of them. In ^{1}H NMR spectrum, we could assign the shifted proton peaks on account of being combined two materials. And in FT-IR spectrum, we could analogize the structure of SP-VC from C=O, C=C and C-H absorbent band. Besides we measured C 58.3 : H 9.3: N 2.8: O 29.5 (Calc. C 58.4: H 9.7: N 2.8: O 29.2) and 492.58 (Calc. 493.63) by using elementary analysis and MALDI TOF-MS[13], respectively.

2.2. Free Radical Scavenging Effect by Using DPPH (1,1-diphenyl-2-picryl hydrazyl)

To confirm the anti-oxidation effect of SP-VC, which was considered vitamin C of typical anti-oxidant, we tested the anti-oxidation effect of SP-VC, vitamin C,

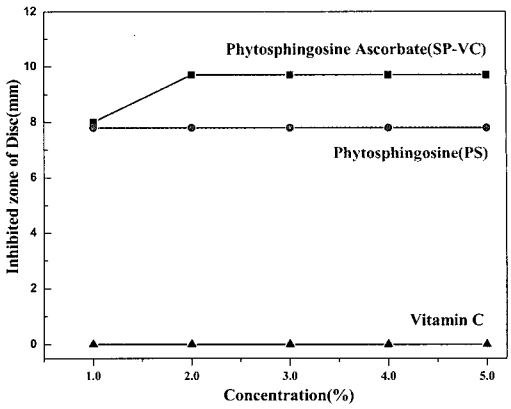


Figure 6. Anti-bacterial effect of SP-VC, vitamin C & PS.

Table 2. Comparison of Free Radical Scavenging Activity

Sample	SC50 (ppm)
BHT	356.26
Vitamin C	6.85
PS	—
SP-VC	21.48

PS, and BHT, one of the established anti-oxidants, as using the DPPH method. All of them were tested three times, respectively and measured SC50. As the results in Table 2, although the anti-oxidation effect of SP-VC was a little inferior to vitamin C, it was superior to BHT. As we know, phytosphingosine had no anti-oxidation effect.

2.3. Anti-bacterial Activity Tests for *P. acnes*

As the results of an anti-bacterial test for the *P. acnes* as concentration of them (SP-VC, PS, and vitamin C), we could confirm that the MIC of SP-VC and PS were 2% and 1%, respectively (Figure 6). All of them were tested three times, respectively. We were confirmed that the anti-bacterial effect of SP-VC was better than PS of being improved about this effect. And as the results that we tested SP-VC and the typical anti-bacterial materials, we also could confirm that SP-VC was better than them. And we couldn't find the anti-bacterial effect of vitamin C.

3. Conclusion

From the various analysis results, we could confirm that the prepared SP-VC was the single substance and

could improve the synthetic structure of SP-VC. And the prepared SP-VC had the effects of the anti-oxidation (Table 2) and anti-bacterial for *P. acnes* (Figure 6). Based on these results, we could estimated that SP-VC had the properties both of PS and vitamin C. Therefore, we are investigating the effects of the skin barrier strength, wound recovering, anti-aging, and skin lightening as considering vitamin C and PS, and are investigating the stability of SP-VC and its conditions.

4. Experimental Section

In the study, the used L-ascorbic acid (vitamin C; 99.5%) was purchased from Aldrich. Phytosphingosine (99%) was used as received from commercial sources. The other reagents and solvents were used chemicals grade. TLC was performed on plates coated with silica gel (silica gel 60 F254, Merck). The spots were located by UV light and indicator (1% phosphomolybdic acid in MeOH). Thermal analysis was measured of using a Pekin Elmer TGA/DSC 7 series in the nitrogen atmosphere at a heating rate of 10℃/min. ^{1}H NMR spectra were recorded on a Varian 500 MHz spectrometer (Unity Inora 500 NB) in DMSO-d6 and CDCl3 as a solvent. FT/IR spectra (KBr pellets) and UV/Vis spectra were measured on a Jasco FT/IR 410 and Jasco V-570 spectrophotometer, respectively. In order to measure the molecular weight, we used a MALDI TOF-mass spectroscopy (Perseptive Brosystem L-1004). Elementary analysis was performed with a CE instruments EA-1110 elemental analyzer. General procedure HPLC chromatogram was recorded on a Waters 2690 System with 996 Photo-Diode Array Detector (PDA, Waters).

4.1. General Procedure

In a dry, 500 mL, two-necked round-flask equipped with a mechanical stirrer and a dropping funnel, 18.0 g (57 mmol) phytosphingosine were added to 200 mL mixing solution of methanol and chloroform (7:3 v/v). The mixture were stirred until all the material dissolved. In the other 200 mL flask, 10.0 g L-ascorbic acid (0.057 mmol) were dissolved into 100 mL methanol. This solution was titrated by using 0.1 N aqueous KOH solution to pH 8.0. After stirring for 30 min, this solution was dripped slowly in the flask that was placed phytosphingosine solution. After stirring for 30 min, this solution was transferred in dropping funnel and then was dripped slowly at ambient temperature in the round-flask that was placed the phytosphingosine solution. The mixture were stirred vigorous for 2~3 h at ambient temperature and the white precipitate obtainedwas filtered, washed in mixing solution of acetone and hexane (3:7). The collected materials were dried in a vacuum oven. 25 g phytosphingosine ascorbate (SP-VC) were obtained (yield 86%).

m.p=154℃, Rf=0.35~0.40 (CHCl3: MeOH: Water: Acetic acid), Elemental analysis (C 58.3 : H 9.3 : N 2.8 : O 29.5), TOF-MS (Mw=492.58), ^{1}H NMR (DMSO-*d6*, 500 MHz) δ 0.87~0.90 (s, 3H), 1.27~1.4 (s, 2H), 1.4~1.5 (m, 3H), 1.50~1.53 (m, 3H), 3.3~3.4 (s, 1H), 3.44~3.48 (s, 2H), 3.5~3.6 (m, 2H), 3.72~3.73 (m, 1H), 3.8~3.9 (m, 1H), 4.19~4.21 (m, 1H), 4.7 (s, 1H), 7.53~7.55 (m, 3H), FT-IR (KBr) : 3412.4, 2918.7, 1754.9, 1653.6, 1468.5, 1379.8, 1322, 1140.7, 1073.2

4.2. Free Radical Scavenging Effect by using DPPH

As Blois methods[14], the free radical scavenging effect tests carried out using DPPH (Aldrich U.S.A) of SP-VC, vitamin C, phytosphingosine, and BHT. Equal volume samples were mixed 0.1mM DPPH solution (in methanol) and stayed 10 min, and then detection with UV spectrophotometer (570 nm).

4.3. Anti-bacterial Activity Tests for *P. acnes*

We tested anti-bacterial effect for *P. acnes* of SP-VC in the anaerobic condition. *P. acnes* were cultured and the activated for 72~96 h. The grew *P. acnes* were put with SP-VC, PS and the anti-acne effect materials as the concentration at the Gas-pak jur in the incubator (37℃). As the paper disc method, we tested SP-VC, PS, and the using anti-acne materials with anaerobic indicator envelope at gas-pak jur. This test performed at incubator for 4~5 days[16-18,23].

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