The Synthesis and Safety of 3-Aminopropyl dihydrogen phosphate, a New Anti-aging Agent.

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Abstract

The novel synthesis of 3-aminopropyl dihydrogen phosphate(3-APPA; 3-Aminopropane phosphoric acid), and its applicability to the skin as a cosmetic raw material in terms of its efficacy and toxicology were presented.

The phosphorylation of 3-amino-1-propanol was carried out via cyclization into 6-membered 2,6-oxaza-phosphoryl ring in the presence of phosphorous oxychloride and an organic base. The subsequent ring-opening hydrolysis and crystallization afforded the highly purified product in 90% isoloated yield. The method is much superior to the previous literature phosphorylation methods, as the procedure is simple and high-yielding.

To confirm the efficacy of 3-APPA, several activities related to anti-aging capacity were measured. In-vitro human fibroblast, linear and 3-dimensional collagen matrix culture revealed that 3-APPA stimulated the proliferation of fibroblasts, and enhanced the synthesis of collagen, which showed 3-APPA's potency for skin wrinkle reduction.

The toxicological aspect of 3-APPA was also extensively examined. In vivo toxicity tests such as acute oral toxicity, eye irritation, human patch, and the repeat insult human patch test proved 3-APPA to be a safe material.

Thus 3-APPA can be used as an effective anti-aging agent for various cosmetic formulations.

I. Introduction

It has always been one of the most important issues in the cosmetic raw-material industry to develop effective anti-aging agents. So far, some raw materials of natural origin have been developed and utilized for this purpose. However, some compounds, like retinol and retinoic acid, are extremely expensive, and in some cases, the cell regenerating capacity of the materials is very low. Use of alpha-hydroxy acids as anti-aging agents have some limitations because their mechanism of action is believed to be based on the peeling of the outermost layer of skin membrane, and large dose, which is necessary for the efficacy, in the cosmetic formulations can cause severe damage to

the skin because of their acidity. Some compounds, like ceramides and placenta extracts, of animal origin suffer from so called "mad cow disease". So, they are recently being withdrawn from the market. These unfavorous situations over cosmetic anti-aging agents had led us to develop this effective and safe anti-aging agent, called 3-APPA.

Structurely, 3-APPA is similar to GABA(gamma-aminobutyric acid) which acts as a neurotransmitter and biochemical messenger in the living organism. The development of 3-APPA was based on the fact that phosphate group would be more readily compatible to the skin membrane than the carboxylic acid group. The resulting compound, 3-APPA, was proved to be safe and efficient for use as an anti-aging agent.

The phosphorylation of alcolols has been one of most important goals for organic-, and biochemists, especially for those who had interests in the synthesis of nucleosides. Thus, various phosphorylation agents and methods have been developed and utilized. The present synthesis of 3-APPA from 3-amino-1-propanol is much superior to the conventional phosphorylation methods of amino-alcohols, because the reaction proceeds almost completely, and the isolated yield is more than 90%, while highly purified product is obtained.

II. Methods

1. Synthesis

The synthesis of 3-APPA is based on the formation of 6-membered cyclic 2,6-oxaza phophoryl chloride by the reaction of 3-amino-1-propanol with phosphoryl chloride in the presence of an organic base such as pyridine or triethylamine. Thus, phosphoryl chloride was added into the solution of 3-amino-1-propanol in chloroform and triethylamine. After the triethylammonium salt formed at the reaction was removed by filtration, the remaining filtrate was hydrolyzed with water. The crystal formed from the dropwise addition of alcohol was filtered, and dried to afford the pure 3-APPA crystal.

2. Safety

3-APPA is formulated into a mixture which can be directly used as a ingredient for cosmetics without further alteration. The safety tests are based on the mixture containing 3-APPA, glyceryl polemethacrylate, glycerin, and water. The composition is as follows.

Composition of the test material

water	:	glyceryl polymethacrylate	:	glycerin	:	3-APPA
6.5	:	1.5	:	1	:	1

1) Acute Oral Toxicity

The acute oral toxicity was carried out according to CTFA guideline. The acute oral LD_{50} value was derived in mature ICR mice weighing from 20 - 28grams. A single dose regimen of 10ml/kg was employed for the test, and the test material was administered orally to 30 selected healthy mice. The LD_{50} value was determined by the Litchfield-Wilcoson method

2) Eye Irritation Test

The acute eye irritation test was done according to CTFA guideline. The potential toxicity of the test material was assessed in an acute eye irritation test on New Zealand White rabbits. In each animal, 0.1ml of the test material was introduced into the conjugational sac of the eye and an untreated eye was served as a control. Both eyes were examined at 1hr, day 1, 2, 3, after the treatment, according to the OECD's grading of occular lesions.

3) Human Patch Test

Human patch test was applied to 30 healthy female volunteers aged 20 - 28 according to CTFA guideline. The test was carried out using Finn chamber secured to the back site with scanpore tape. The test sites were cleaned with 75% ethanol prior to the test. 20ul of the prepared test material(10% in patch base) was dropped into each Finn chamber, and applied to the skin. The patches were removed after 24 hours. Each skin patch site was read on a 5-point scale according to the protocol of ICDRG at time 0.5, 24, and 48 hrs after the removal of the patch.

4) Repeat Insult Human Patch Test

Similar method to human patch test was employed for this test, but the patch was applied 9 times to the patch sites during 3 weeks of the induction period. After 2 weeks, challenge insult patch was attached to the original test sites and new sites for 48 hour. After the patch was removed, the sites were observed for 96 hours. The skin reaction was evaluated according to the regulatory of the International Contact Dermatitis Research Group (ICDRG).

3. Efficacy

For the evaluation of the activities of 3-APPA, human skin fibroblast proliferation, monolayer and three dimensional cell culture were measured.

1) Cell Proliferation

3-APPA was added into the culture system containing DMEM supplemented with 10% FBS, and the cell proliferation was measured by MTT assay.

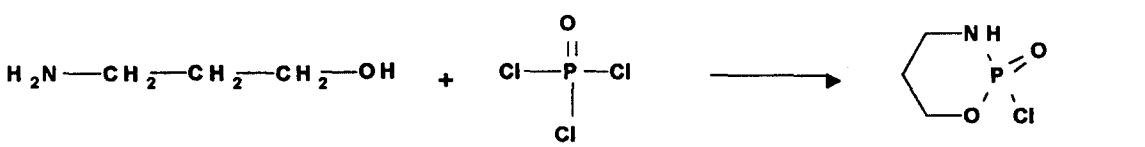
2) Collagen Synthesis

The activity of 3-APPA on the monolayer and three dimensional culture was measured by the 3[H]-proline incorporation method.

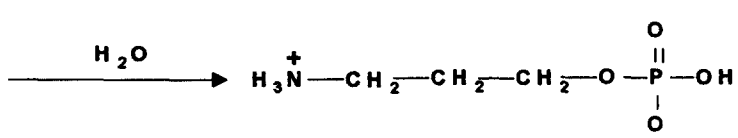
III. Results and Discussion

1. Synthesis

The cyclization reaction between 3-amino-1-propanol and phosphoryl chloride goes to completion in 15 minutes at 0 - 5oC, and the final isolated yield of 3-APPA is 90 - 95% based on the starting 3-amino-1-propanol <scheme>. This process has overcome the major problems of the conventional phosphorylation methods of amino alcohols, which were related to isolated yield, complexity of the reaction and work-up process. One of the most recent methods also utilizes 2,6-oxaza phosphate ring as the key intermediate, but the method uses phenoxy phosphoryl chloride, instead of phosphoryl chloride, and it requires expensive platinium oxide to release 3-APPA from the adduct. Furthermore, the isolated yield for the process is only 38%. The analytical data for the 3-APPA is shown in table 1.



3-Amino-1-propanol **Phosphoryl chloride** **2,6-Oxazaphosphoryl chloride**



3-APPA

< Scheme ; The preparation of 3-APPA >

Table 1. Analytical Data for the 3-APPA

	C 1*	C 2	C 3
1H-NMR (D_2O)	3.9ppm(q, 2H)	1.9ppm(quin, 2H)	3.1ppm(t, 2H)
13C-NMR (D_2O)	66ppm(d)	31ppm(d)	41ppm(s)

mp; 203 - 206°C, 31P-NMR; 1.12ppm(external standard; 85% H_3PO_4)
* The nearest carbon atom from the phosphate group is designated as C 1

2. Safety

1) Oral Toxicity

No noticeable irritational evidence appeared in all mice from the test, hence, the LD_{50} value exceeds 10ml/kg (LD_{50} > 10ml/kg).

2) Eye Irritation Test

At 48th hour observation, the M.O.I.(Mean Occular irritation Index) was zero for both 'washing' and non-washing' samples. Thus, the result of eye irritation test proved 3-APPA to be non-irritant.

3) Human Patch Test

According to the test, noticeable primary irritation did not appear, and minimul erythema reaction was detected in 4 out of 30 volunteers at 24hr observation, but, all disappeared at 48hr. For the patch base, 2 from 30 volunteers showed minimal erythema at 24hr, but the reaction disappeared at 48hr.

4) Repeat Insult Human Patch Test

No noticeable cumulative irritation and contact sensitization was caused by 3-APPA in all human volunteers.(Table 2)

Table 2 : Result of Repeat Insult Human Patch Test

	Induction Period			Mean Score	Challenge			Mean Score
Sample	1st Week	2nd Week	3rd Week	(%), n=20	24 hr	48hr	72hr	(%), n=20
3-PPA	1	1	1	0.42	-	-	-	0
Patch Base	-	1	1	0.028	-	-	-	0

1) Cell Proliferation

3-APPA increased cell proliferation by 120% at 10-4 % concentration, compared to control, whereas the increase by ascorbic acid was 50%.

Table 3. Effect of 3-APPA on Cell Proliferation* in Various Concentration

	Concentration				
	1 x 10-2%	1 x 10-3%	1 x 10-4%	1 x 10-5%	1 x 10-6%
3-APPA	110	185	220 + #	130	95
Ascorbic acid	105	130	150	140	105
Control	100	100	100	100	100

Data shown in the table are the cell viabilities(%). compared to control(100%)

+p<0.05 vs control, #p<0.05 vs ascorbic acid

2) Collagen Synthesis

3-APPA increased the monolayer collagen synthesis by 50%. In 3-dimensional culture, it increased the collagen synthesis by 20% compared to both ascorbic acid and control.

Table 4. Effect of 3-APPA on Collagen Synthesis (Monolayer)*

	Concentration		
	1 x 10 - 3%	1 x 10 - 4%	1 x 10 - 5 %
3-APPA	110	150 + #	128
Ascorbic acid	102	105	120
Control	100	100	100

*Data shown in the table are the synthesis ratio(%). compared to control(100%).
+p<0.05 vs control, #p<0.05 vs ascorbic acid.

Table 5. Effect of 3-APPA on Collagen Synthesis (3-D culture)*

	Concentration		
	1 x 10 - 1%	1 x 10 - 2%	1 x 10 - 3 %
3-APPA	120 + #	105	108
Ascorbic acid	89	98	88
Control	100	100	100

*Data shown in the table are the synthesis ratio(%). compared to control(100%).
+p<0.05 vs control, #p<0.05 vs ascorbic acid.

IV. Conclusion

1. The synthetic process via 2,6-oxaza-phophoryl chloride is an efficient method for the preparation of 3-APPA, in terms of time, yield and the purity of the product.

2. The extensive safety tests verified that 3-APPA could be safely applied to the human skin.

3. In the MTT assay, 3-APPA enhanced the proliferation of human fibroblasts.

4. Linear and 3-dimensional collagen culture using 3[H]-proline incorporation method showed that 3-APPA increased the collagen synthesis.

5. Thus, 3-APPA can be safely applied to the cosmetic formulations as an effective anti-aging agent.

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