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Synthesis of N-ethanol-2-(myristyl/palmityl)-3-oxo(stearamide/arachidamide) and its physical properties for a cosmetic raw material

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Synopsis

As a cosmetic raw material, N-ethanol-2-(myristyl/palmityl)-3-oxo(stearamide/arachidamide) (PC-9^S) has been synthesized and its physical properties have been investigated. PC-9^S was synthesized by the reaction between alkylketene dimer (AKD) and 2-aminoethanol under the ethanol reflux system. The existence of intermolecular hydrogen bonding among the amide groups of PC-9^Ss was confirmed by an FT-IR study. PC-9^S showed a very wide range of lamellar liquid crystal formation, with a mixture of fatty acid and cholesterol, and the lamellar structure was formed with a high content of fatty acid composition. The cross-polarized microscopic result of PC-9^S confirmed its intrinsic characteristic of lamellar structure formation. PC-9^S was verified to be non-toxic, non-irritable, and non-sensitizing in skin irritation tests on animals and humans. This intrinsic power to form the lamellar structure made it possible to form a multilamellar emulsion, which could have applications in the cosmetic and drug industries. An effective and economical synthetic pathway with high purity and yield facilitates the industrial production of PC-9^S.

INTRODUCTION

Many physicochemical and biological actions of skin care products in the last decade were concerned with the horny layer of the stratum corneum (SC) (1). The structure of SC was proposed in an illustration as a "brick & mortar" model by Elias in 1981 (2,3). According to the Elias model, the brick represented corneocytes and the mortar represented intercellular lipids. The corneocytes are filled with keratin and embedded in the intercellular lipids that form lamellar structures, which are the origin of the skin barrier function. For this reason, many people have been investigating the components of the intercellular lipids. The main components of intercellular lipids are ceramide, fatty acids, and cholesterol. Ceramide is not only the most prominent lipid found in SC but also an important component for the formation and retention of lamellar structure in SC (4).

Topical applications of ceramide or pseudoceramide-containing compositions have been found to be effective in relieving atopic eczema (5,6), and also in exhibiting therapeutic properties such as wound and ulcer healing through the promotion of cell restoration and growth (7). For these reasons, many extensive efforts had been made by cosmetic and pharmaceutical companies to obtain access to natural ceramides (8–10) or pseudoceramides (11–14). In order to synthesize appropriate pseudoceramides, it is preferable that the structure of natural ceramides should be examined first, and that then pseudoceramides having similar structures should be designed and synthesized. Several previous reports had referred to the importance of multilamellar lipid vesicle structure in cosmetic and pharmaceutical formulation (15). A biphasic multilamellar lipid vesicle comprises a plurality of spaced lipid bilayers that include a liposome-forming component and optionally a biologically active agent (which means any agent that has a pharmacological, pharmaceutical, or cosmetic effect) entrapped within the lipid bilayers. The lipid vesicle also comprises peripheral aqueous solution compartments formed between the lipid bilayers and a central lipophilic core compartment located at the center of the multilamellar lipid vesicle. The liposome-forming component is usually selected from the group consisting of glycolipids, phospholipids, ceramides, and mixtures thereof, and optionally a fatty substance such as cholesterol is added to enhance the strength of the lipid bilayer (16).

Recently, we synthesized a pseudoceramide, N-ethanol-2-(myristyl/palmityl)-3-oxo(stearamide/arachidamide) (PC-9^S) by the reaction of 2-aminoethanol and AKD. It was designed to have properties similar to those of human skin (identical ceramides) and to mimic their behaviors. It showed easy lamellar liquid crystal formation in a mixture with fatty acid and cholesterol, and was demonstrated to be non-toxic, non-irritable and non-sensitizing in skin irritation tests on animals and humans. It was also demonstrated that the multilamellar emulsion using PC-9^S enhanced water barrier function into the skin.

EXPERIMENTAL

MATERIALS

Alkylketene dimer (Taekwang Co., Korea), 2-aminoethanol (Duksan Co., Korea), and ethanol (Dong Yang Co., Korea) were used for the synthesis of a pseudoceramide, PC-9^S. Stearic acid (Junsei Co., Japan), cholesterol (Junsei Co., Japan), POE (15) glyceryl monostearate (Nihon Emulsion Co., Japan), glyceryl monostearate (Nihon Emulsion Co., Japan), cetanol (Kao Co., Japan), liquid paraffin (Seojin Chemical, Korea), olive oil (Junsei Co., Japan), and carboxyl vinyl polymer (B.F. Goodrich, USA) were used as components to form a multilamellar lipid vesicle that was similar to the intercellular lipids of stratum corneum. All water was distilled from an all-glass apparatus.

SYNTHESIS OF PC-9^S

AKD (15 g, 31.5 mmol) and 2-aminoethanol (2.12 g, 34.7 mmol) were added to 50 ml of ethanol and refluxed for six hours. After adding about 200 ml of ethanol and cooling this mixture, white solids were precipitated. The solids were purified twice in ethanol to give pure PC-9^S as white crystalline solids (yield 90%). The physical properties of the obtained compound are as follows. TLC ($CHCl_3$: methanol : acetic acid = 95:5:1), R_f

= 0.36; melting point: 104°C ~ 107°C; IR (cm^{-1}, KBr): 3272, 1717, 1649, 1530; ^{1}H-NMR ($CDCl_3$): δ 0.8 ~ 1.9 (m, alkyl), 2.4 (2H, t, R-$\underline{CH_2}$-CO-), 3.4 (1H, t, CO-$\underline{CH}$(R)-CON-), 3.4 (2H, q, -CON-$\underline{CH_2}$-), 3.55 (2H, d, -CH_2-$\underline{CH_2}$OH), 6.8 (1H, t, -CO$\underline{NH}$-).

CHARACTERIZATION OF PC-9^S

The phase transition behavior and characterization of PC-9^S was determined by NMR (Varian, Jemini 200), FT-IR (Mattson, Mattson 7000), and melting point apparatus (Fisher).

OBSERVATION OF THE CROSS-MICROSCOPIC OPTICAL TEXTURE

A cross-polarized light microscopy (Nikkon) was used for the observation of the cross-microscopic optical texture after catching the meta-stable state of PC-9^S by controlling the temperature. The lamellar structure was shown from the "neat" pattern, and/or the "spherical" patterns with "Maltese crosses."

PREPARATION OF THE MULTILAMELLAR EMULSION

The process comprises forming a lipid phase melt by mixing multilamellar lipid vesicle-forming components (PC-9^S, stearic acid, and cholesterol) with oil droplets (liquid paraffin and olive oil) and emulsifying components (POE (15) glyceryl monostearate, glyceryl monostearate, and cetanol). The multilamellar emulsion was prepared by adding water into the lipid phase slowly, with vigorous stirring at appropriate temperature and cooling to room temperature.

MEASUREMENT OF THE TRANSEPIDERMAL WATER LOSS OF THE SKIN

The transepidermal water loss (TEWL) was measured by use of a tewameter before and after pretreatment with organic solvent (acetone : ethyl ether = 1:1) on the inner side of forearms in order to damage the skin. Then we applied 0.5 g of the multilamellar emulsion and controls to the damaged skin on each forearm and compared coutaneous barrier function before treatment and then one hour, three hours, and five hours after treatment.

RESULTS AND DISCUSSION

SYNTHESIS OF THE PSEUDOCERAMIDE, PC-9^S

Natural ceramides, which exist in nature, are widely divided into six types. Their features in terms of their structures are as follows: They have at least two alkyl groups and one hydroxyl group in their head position and also have more than one amide bond (17). The pseudoceramide, N-ethanol-2-myristyl/palmityl-3-oxostearamide/arachidamide (PC-9^S) was synthesized by a one-step reaction between AKD and 2-aminoethanol. Generally, the commercial AKDs were synthesized from acyl chlorides with a triethylamine catalyst. The used AKD was synthesized from a mixture of acyl chlorides, the main carbon chain of which comprised 16 and 18 atoms in the ratio of 3:7. Both protic solvent and aprotic solvent showed good results. As a protic solvent, ethanol was used. When the ethanol was added again as a solvent in the further purification step, the final product could be easily crystallized from the reaction medium. Two crystallizations resulted in pure PC-9^S product. In the case of toluene as an aprotic solvent, the final products showed a red-brown color when the reaction temperature was too high, and protic solvent such as ethanol needed to be added to purify the crude product. The characteristic features of PC-9^S are the following: two alkyl groups, one amide bond, one hydroxyl group, and one ketone group at β-position. The reaction scheme and the structure of the PC-9^S were represented in Figure 1.

The synthesis of new pseudoceramide was carried out via a one-step reaction. Thus, it is a very simple and effective process to prepare pseudoceramides. NMR and the FT-IR spectra confirmed the structure of the PC-9^S. These results are typically characteristic of ketene dimers in reaction with an amine and an alcohol. The FT-IR spectrum showed the typical amide peak, where an ester bond was not formed. This can be explained by the relative reactivity of amine and alcohol with AKD (18), since it is known that the amine group is much more reactive than the hydroxyl group with the AKD. In support of this, it has been reported that the reaction between alcohol and AKD was very sluggish without a catalyst.

INTERMOLECULAR HYDROGEN BONDING AMONG PC-9^Ss

The barrier function of ceramides is due to the lamellar structure formation with fatty acid and cholesterol. It was known that the intermolecular hydrogen bonding among head groups of the ceramides plays an important role in the formation of a stable lamellar structure (19). In the crystalline state, the carbonyl group within the amide bond takes

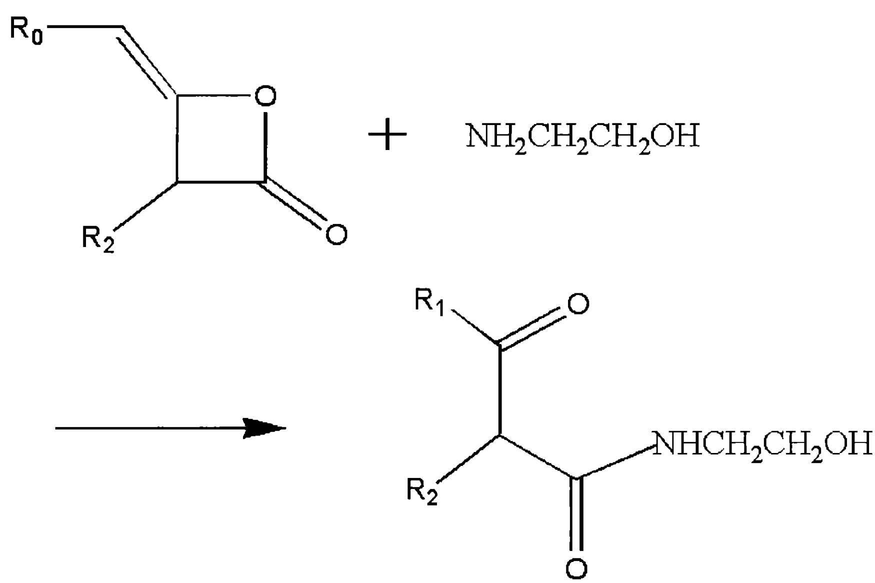


Figure 1. Reaction scheme of PC-9^S (R_1 = R_0CH_2).

part in hydrogen bonding with the hydroxyl group of another molecule. However, it was observed that when the pseudoceramide, PC-9^S was dissolved in a hydrophobic solvent such as chloroform (a liquid state), hydrogen bonding among the molecules weakened greatly or completely disappeared. The FT-IR spectra of PC-9^S in a solid state (KBr) and a liquid state (chloroform) was used to determine the existence of intermolecular hydrogen bonds. The reaction of AKD and 2-aminoethanol resulted in the ring-opening reaction of β-lactone and the new bond formation of a β-keto group (1717.13 cm^{-1}) and an amide bond (1649.15 cm^{-1}) shown in the KBr pellet, where the hydroxyl group of its head group shows a hydrogen bond with the amide bond. With PC-9^S dissolved in chloroform (a liquid state), it was observed that the wave number of the amide bond was shifted into the higher wave number (1662.72 cm^{-1}), which meant that the intermolecular hydrogen bonds disappeared in the chloroform, which was certified by the wave number shifting of the amide bond (Δ u = 13.57 cm^{-1}) (Figure 2). This demonstrates that there is an intermolecular hydrogen bond among molecules, which plays an important role in lamellar structure formation. Besides the intermolecular hydrogen bonding, there is the strong possibility to also form intramolecular hydrogen bonds. It was reported that an intramolecular hydrogen bond occurred in the six-member ring very easily (20). As a theoretical drawing, Figure 3 illustrates an intramolecular hydrogen bonding of PC-9^S.

PHASE DIAGRAM OF THE LIPID MIXTURE AND ITS OBSERVATION

Figure 4 shows the phase diagram of a PC-9^S/stearic acid/cholesterol complex system and a typically characteristic cross-polarized picture at a point within the phase diagram. The

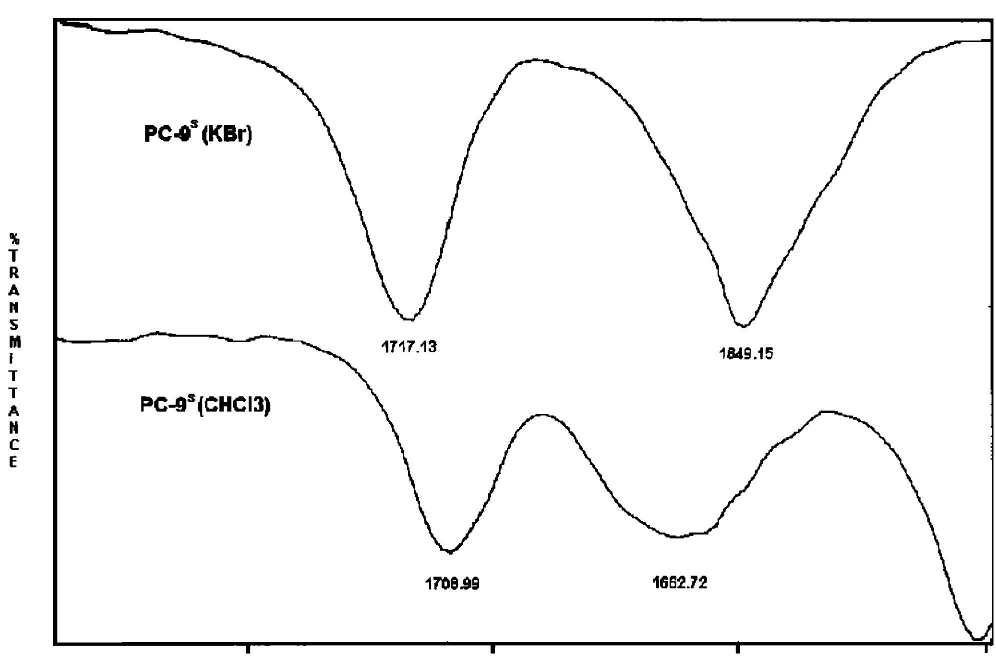


Figure 2. FT-IR spectrum of PC-9^S in the crystal state (KBr) and liquid state (chloroform).

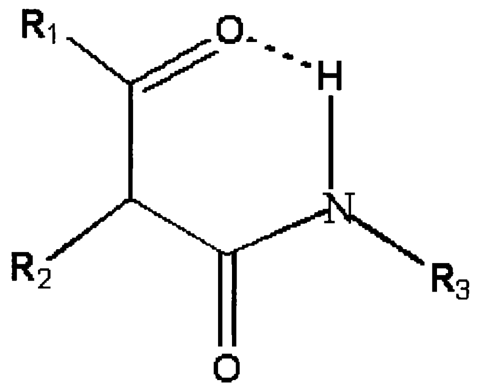


Figure 3. Schematic illustration of the intramolecular hydrogen bond of PC-9^S (R_1 = R_0CH_2).

results show the structure of the separated liquid crystalline phase to be a stable molecular associate of the lamellar structures as determined by use of the optical microscope. The lamellar liquid crystal was formed within the wide region surrounded by the solid line in Figure 4. The lamellar structure in an emulsion is known to be good for the emulsion stability, as shown in much published research on cosmetic and pharmaceutical formulations (21). As a newly synthesized ceramide, PC-9^S is effective in forming the multilamellar structure together with fatty acid and cholesterol. However, if considering

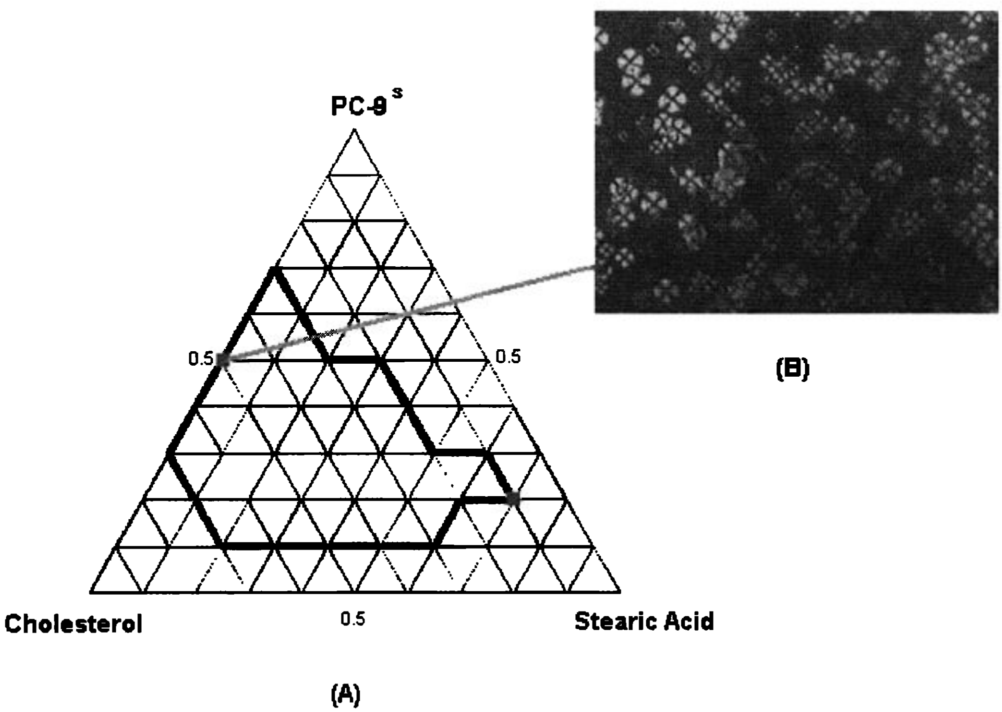


Figure 4. Phase diagram of lipid mixture and cross-polarized light microscopic photograph. A. Phase diagram having lamellar structure. B. Lamellar liquid crystal observed by use of a cross-polarized light microscope. Bold line in A indicates region having the lamellar structure.

the formula's cost in manufacturing emulsions on a large scale, it is recommended to have a low content of cholesteraol and a high content of fatty acid in the phase diagram, since the purified cholesterol is a high-cost material. The ratio of PC-9^S : cholesterol : stearic acid, 2:1:7, was used in the multilamellar emulsion formation, which is described in the following experiment.

FORMATION OF A MULTILAMELLAR EMULSION

The main objective is to use ceramides or pseudoceramides in a cosmetic formulation to improve the barrier function according to the maintenance of the lipid layer in stratum corneum. The improving barrier function of PC-9^S in the skin is mainly due to its ability to form a multilamellar structure with other components in the emulsion. In the phase study of the PC-9^S/fatty acid/cholesterol complex system, the ability to form a lamellar structure was determined by the use of the optical microscope. Table I shows an example of a multilamellar emulsion composition using PC-9^S and the stratum corneum lipid. Finally, it was demonstrated that PC-9^S was easily able to form the lamellar structure with emulsifier and oils.

A typical configuration of multilamellar mesophase texture, the optical anisotropy of "Maltese crosses" was shown by use of a cross-polarized microscope. It was also demonstrated that this aggregation structure in the emulsion was similar to that of human skin lipid lamellar bilayer (Figure 5).

SAFETY TEST OF PC-9^S

Table II represents the safety test results of PC-9^S, which was carried out with the dispersion state of PC-9^S in olive oil. In tests of acute oral toxicity and acute dermal toxicity with rat and rabbit, the LD_{50} (median lethal dose) values demonstrated that PC-9^S was very safe. That is, the LD_{50} of the acute oral toxicity was more than 5 g/kg and the LD_{50} of the acute dermal toxicity was more than 10 g/kg in rat. The LD_{50} of the acute oral toxicity was more than 5 g/kg and the LD_{50} of the acute dermal toxicity

Table I
Composition of a Pseudo-Stratum Corneum Lipid Emulsion

	Lipid components	Pseudo-stratum corneum lipid emulsion (wt %)	
Pseudo-stratum corneum lipid	PC-9^S	0.6	
	Cholesterol	0.2	2.3
	Stearic acid	1.5	
Emulsifier	POE (15) glyceryl monostearte	3.0	
	Glyceryl monostearte	1.5	7.5
	Cetanol	3.0	
Oil	Liquid paraffin	2.0	
	Olive oil	3.0	5
Thickener	Carboxyl vinyl polymer	0.2	0.2
Water phase	1,3-Butylene glycol	5	
	Water	80	85

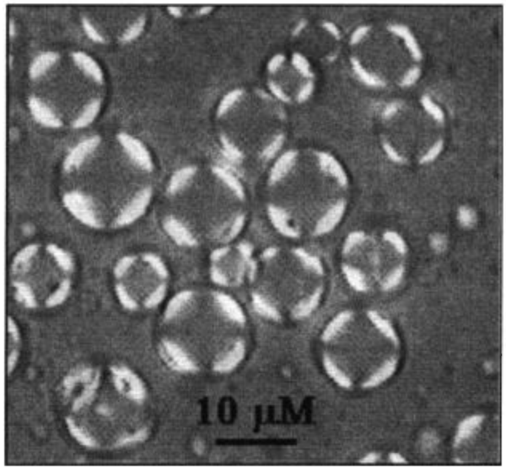


Figure 5. Cross-polarized light microscopic photograph of a pseudo-stratum corneum emulsion containing PC-9^S (×400).

Table II
Safety Test Results of PC-9^S

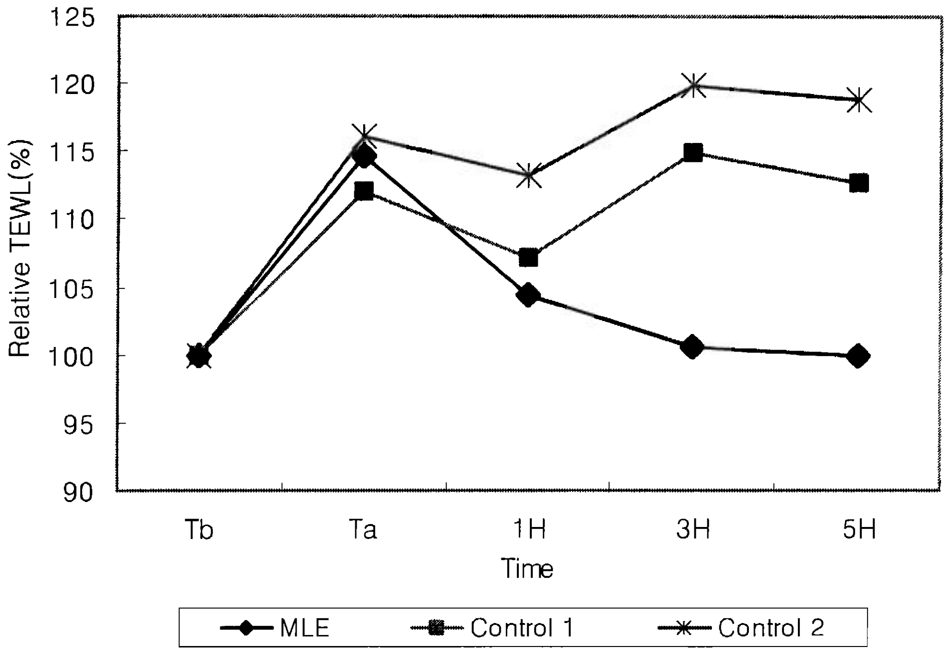
Test	Results	
Acute oral toxicity (rat)	LD_{50}* > 5 g/kg	Non-toxic
Acute dermal toxicity (rat)	LD_{50} > 10 g/kg	Non-toxic
Acute oral toxicity (rabbit)	LD_{50} > 5 g/kg	Non-toxic
Acute dermal toxicity (rabbit)	LD_{50} > 10 g/kg	Non-toxic
Primary skin irritation	P.I.I.** score = 0	Non-irritating
Eye irritation	Eye-washing score = 0.67 No eye-washing score = 4.0	Non-irritating
Skin sensitization	Score = 0 Rate = 0%	Non-sensitize
Human patch	Score = 0	Non-irritating

* LD_{50} = median lethal dose.
** P.I.I. = primary irritation index.

was more than 10 g/kg in rabbit. The primary skin irritation index (P.I.I.) score "0" means that there is not any problem with PC-9^S on the skin. For the eye irritation test, the solid particle of PC-9^S was applied to the eyes directly, and the eye washing group scored 0.67 and the non-washing group scored 4.0. According to the score analysis index, if its score is below 5, it can be classified as a non-irritating material. The human patch test was performed using PC-9^S dispersed in liquid paraffin. The irritation score resulted in "0." These safety test results demonstrate that PC-9^S is a very safe material as a cosmetic ingredient.

FAST RESTORATION FUNCTION OF THE MULTILAMELLAR EMULSION ON DAMAGED SKIN (FIGURE 6)

If the multilamellar emulsion application assists barrier function, application *in vivo* on human skin should decrease TEWL. To test this, we compared the barrier effect of the



T_b : Before treatment of organic solvent
T_a : One hour after organic solvent treatment
1H : After one hour from the emulsion treatment
3H : After 3 hours from the emulsion treatment
5H : After 5 hours from the emulsion treatment

Figure 6. Fast restoration function of the multilamellar emulsion on damaged skin.

multilamellar emulsion using PC-9^S with that of controls (regular emulsions) by measuring the decrease in TEWL. When the multilamellar emulsion using PC-9^S was applied to damaged skin, it was shown that restoration from water loss with the multilamellar emulsion treatment was significantly faster than with the controls. The fast restoration function of PC-9^S can be applied to the special dermatological preparation for sensitive skin or atopic skin.

CONCLUSION

The reaction of AKD and alcoholic amine provided an effective synthetic pathway for a new pseudoceramide, PC-9^S. Its high purity and yield enable an industrial application. The carbonyl group of amide bond can form the intermolecular and intramolecular hydrogen bond with the hydroxyl group, which was demonstrated by the wave number shifting in FT-IR spectra. According to cross-polarized microscopy measurement, it was determined that PC-9^S had high intrinsic characteristics to form a lamellar structure with fatty acid and cholesterol. It was also demonstrated, by observing the optical anisotropy of "Maltese crosses" by use of a cross-polarized microscope, that the pseudo-stratum corneum components had an intrinsic power with emulsifiers and oils to form a multilamellar emulsion. It was also demonstrated by TEWL measurement that the multilamellar emulsion has a water barrier function on the damaged skin. In the future, the effect of the multilamellar emulsion on an atopic, dry, scaly, and sensitive skin will be investigated in cosmetic formulations.

REFERENCES

(1) P. Wertz, D. Downing, R. Freinkel, and T. Trazyk, *J. Invest. Dermatol.,* **83,** 193–195 (1984).
(2) P. M. Elias, *Arch. Dermatol. Res.,* **270,** 95–117 (1981).
(3) H. J. Yardley, *Int. J. Cosmet. Sci.,* **9,** 13–19 (1987).
(4) D. Maes, K. Marenus, and W. P. Smith, *Cosmet. Toiletr.,* **105,** 43–50 (1990).
(5) M. Kerscher *et al., Eur. J. Dermatol.,* 1, 39–43 (1991).
(6) G. Imokawa, S. Akasaki, A. Kawamata, S. Yano, and T. Takaishi, *J. Soc. Cosmet. Chem.,* **40,** 273–285 (1989).
(7) E. A. Hannon and R. M. Bell, Duke University, *WO patent 92-03129* (1992).
(8) G. Rousset, Laboratories Inocosm, *WO patent 92-21321* (1992).
(9) J. Casey, P. Cheetham, P. Harries, D. Hyliands, J. T. Mitchell, and A. V. Rawlings, Unilever PLC, *WO patent 94-10131* (1994).
(10) H. Lambers, F. Farin, W. Keuning, and W. Vander Wilden, *2nd ASCS,* 106–125 (1995).
(11) S. Yano, A. Kawamata, Y. Minematsu, S. Akazaki, M. Zama, G. Imokawa, N. Takaishi, T. Ohtomo, and T. Higashi, Kao Co., *EP patent 0282816* (1988).
(12) P. Critchley, S. Kirsch, A. V. Rawlings, and I. Scott, Unilever PLC, *WO patent 92-06674* (1992).
(13) P. Critchley, S. Kirsch, A. V. Rawlings, and I. Scott, Unilever PLC, *WO patent 92-06982* (1992).
(14) Y. Ohashi, M. Suda, S. Yano, A. Kawamata, M. Okuda, and G. Imokawa, Kao Co., *US patent 5221757* (1993).
(15) T. Suzuki, J. Fukasawa, H. Iwai, I. Sugai, O. Yamashita, and A. Kawamata, *17th IFSCC, International Congress Yokohama,* Vol. 1, A101 (1992).
(16) M. Foldvari, University of Saskatchewan, *WO patent 95-03787.*
(17) P. W. Wertz and D. T. Downing, *J. Lipid Res.,* 24, 759–765 (1983).
(18) R. E. Kirk and D. F. Othmer, *Encyclopedia of Chemical Technology* (Interscience, New York, 1952), Vol. 8, pp. 110–111.
(19) H. Iwai, J. Fukasawa, M. Fukuda, I. Sugai, H. Uchida, and T. Suzuki, *J. Soc. Cosmet. Chem. Jpn.,* **30,** 310 (1996).
(20) J. March, *Advanced Organic Chemistry—Reactions, Mechanisms, and Structure,* 4th ed. (John Wiley & Sons, New York, 1992).
(21) G. Dahms, *Cosmet. Toiletr., 101,* 113–115 (1986).