Novel Pseudoceramides And Their Synthesis Using Alkyl Ketene Dimer

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Abstract

Nowadays, ceramides have been found to be an important component in the outermost layer of the skin — the stratum corneum (SC). It is understood that ceramides play an important role in structure and maintenance of the intercellular lipid lamella structure in the SC layer. Thus, many efforts have been made by the cosmetics and pharmaceutical industries to get human skin-identical ceramides or pseudoceramides which show similar performance with natural ceramides. The purpose of our study was to synthesize new pseudoceramides via an effective and economical synthetic pathway and to show their performance of skin restoration. Four kinds of the new pseudoceramides (PC-4, PC-4R, PC-5, PC-5R) were synthesized by the reaction of alcoholic amine and alkyl ketene dimer. First of all, PC-4 and PC-5 were synthesized by the reaction of 3-amino-1,2-propandiol and serinol with alkyl ketene dimer respectively. After that, PC-4R and PC-5R were produced by changing ketone group at β-position to amide bond of above synthesized PC-4 and PC-5 into hydroxyl group using $NaBH_4$ respectively. Their expected structures were conformed by the NMR, IR spectra, and elemental analysis. A study to show the restoration effectiveness was performed in which human skin was pretreated with high concentration of SDS surfactant solution. Using 0.5% solution of above synthesized pseudoceramides, there was the significantly faster restoration of the damaged than that of placebo itself treatment.

Introduction

The permeability barrier of the skin, which prevents transcutaneous water loss and penetration harmful compounds (including microorganisms) from the environment, is localized in the stratum corneum (SC) of the epidermis [1]. Also, as a protective layer, the SC must resist mechanical influence to a certain extent. And corneocytes, the components of SC, are filled with keratin and embedded in the lipids of the intercellular domains which form membraneous bilayers. The "Brick & Mortar" model in which the brick represented corneocytes and the mortar represented intercellular lipids was proposed to illustrate above configuration by Elias in 1981 [2, 3]. The corneocytes and the intercellular lipids make up the so-called permeability barrier. The intercellular space of the SC is mainly composed of neutral lipids and ceramides. Ceramides are the most prominent lipids pound in the SC and play an important role regulating barrier function and maintaining water holding capacity of the skin [4]. Skin damage caused by detergents which remove the lipids essential for the barrier function will result in an increased transepidermal water loss (TEWL), and deteriorated barrier function has negative consequences for the total condition of the skin. Moreover, a damaged skin barrier leads to increased skin sensitivity and potential irritation [5]. And it has been thought from recent investigations that ceramides play important roles in the functions as a reception, response and receptor of recognition and information, differentiation, proliferation, malignant change or behavior of cells [6, 7].

It has been found that topical applications of ceramide- or pseudoceramide- containing compositions are effective in relieving atopic eczema [8, 9]. They also have been found to exhibit therapeutic properties such as wound and ulcer healing through the promotion of cell restoration and growth [10]. With these reasons, many extensive efforts have been made by lots of the cosmetic and pharmaceutical companies to obtain access human skin-identical ceramide or pseudoceramide. In recent, our laboratories focused on the synthesis of pseudoce-

ramides using ketene dimer. These were designed to have certain properties similar to those of human skin-identical ceramides and to mimic the behavior of human skin-identical ceramides in relation to the skin so as to be usable in skin-care or hair-care compositions as cheaper substitutes in place of human skin-identical ceramides. Finally four kinds of the new pseudoceramides (PC-4, PC-4R, PC-5, PC-5R) were synthesized by the reaction of alkyl ketene dimer and alcoholic amine moiety, pretty much cost-effectively. The results of water retention capacity of the healthy human skin and restoration (TEWL test) of SDS-damaged human skin showed the possibility to use these compounds as the substitutes in place of human skin-identical ceramides.

Materials And Methods

1. Synthesis of the pseudoceramides

(1) PC-4: 2.79g of 3-amino-1,2-propandiol (Aldrich) and 15.02g of alkyl ketene dimer (Tae-Kwang Chemicals, Korea) were used to synthesize PC-4 under toluene (20ml) reflux system in a 250ml round-bottom flask for about 3 hours. After adding about 200ml of n-hexane and cooling this mixture, an off-white waxy solid precipitated. The solid was purified by recrystallization from n-hexane, followed by recrystallization from ethanol. The purified sample was obtained in a yield of 93%, and was a white waxy solid. This purified solid structure was confirmed by IR spectra, NMR-1H and elemental analysis.

(2) PC-5: Serinol, 2.2g (Aldrich) and alkyl ketene dimer, 12.00g (Tae-Kwang Chemicals, Korea) were used to synthesize PC-5 under toluene (20 ml) reflux system in a 250ml round-bottom flask for about 4 hours. After adding about 200ml of n-hexane and cooling this mixture, an off-white waxy solid precipitated. The solid was purified by recrystallization from petroleum ether, followed by recrystallization from ethanol. The purified sample was obtained in a yield of 95%, and was a white waxy solid. This purified solid structure was confirmed by IR spectra, NMR-1H and elemental analysis.

(3) PC-4R: In a 50 ml round-bottom flask, 5 g of PC-4 was placed under methanol (40 ml) reflux system. While stirring and heating the solution, 0.4 g of NaBH4 (Aldrich) was added and incubated for about 24 hours. Upon cooling, an off-white waxy solid precipitated. The solid was purified by recrystallization from methanol. The purified sample was obtained in a yield of 58%, and was a white waxy solid. This purified solid structure was confirmed by IR spectra, NMR-1H and elemental analysis.

(4) PC-5R: In a 50 ml round-bottom flask, 5 g of PC-5 was placed under methanol (40 ml) reflux system. While stirring and heating the solution, 0.4 g of NaBH4 (Aldrich) was added and incubated for about 24 hours. Upon cooling, an off-white waxy solid precipitated. The solid was purified by recrystallization from methanol. The purified sample was obtained in a yield of 68%, and was a white waxy solid. This purified solid structure was confirmed by IR spectra, NMR-1H and elemental analysis.

2. Efficacy of pseudoceramides

(1) Water retention capacity of the healthy human skin: 10 healthy young people (age 23 - 26) entered the study after obtaining informed consent. 6 sites were randomly selected on the volar forearm (left or right). Site 1, site 2, site 3, site 4 and site 5 were randomly pre-treated with a body lotion (placebo)[AK11] containing 0.5% PC-4 (site 1), 0.5% PC-4R (site 2), 0.5% PC-5 (site 3), 0.5% PC-5R (site 4) and the base alone (site 5) respectively. A sixth site served as control and therefore was not pre-treated. Treatment was performed twice a day for 7 days. After 2 hours later each treatment, water retention capacity was recorded by Corneometer CM 820 PC (Courage + Khazaka electronic GmbH, Germany). The value was measured in arbitrary value unit (temperature: 22±1°C, humidity: 70±5%).

(2) Restoration of SDS-damaged human skin (TEWL test): [AK12]A study was performed with 10 healthy young people (age 23 - 26) having informed consent. 6 sites were randomly selected on the volar forearm (left or right). On day 1 skin irritation on 6 sites was induced in the test site by applying Sodium Dodecyl Sulfate (SDS) 5% (Sigma) in distilled water under occlusive dressing for 2 hours. After SDS occlusion was removed, treatment was performed twice a day for 14 days. Site 1, site 2, site 3, site 4 and site 5 were randomly pre-treated with a body lotion containing 0.5% PC-4 (site 1), 0.5% PC-4R (site 2), 0.5% PC-5 (site 3), 0.5% PC-5R (site 4) and the base alone (site 5) respectively. A sixth site was not pre-treated. TEWL was recorded by Tewameter TM 210 (Courage + Khazaka electronic GmbH, Germany) after 2 hours later each treatment. Water loss was measured in g/m2hr (temperature: 22±1°C, humidity: 70±5%).

Results

1. Synthesis of the pseudoceramides

N-(2,3-dihydroxypropyl)-2-tetradecyl-3-oxo-stearamide (PC-4), N-(1,3-dihydroxyisopropyl)-2-tetradecyl-3-oxo-stearamide (PC-5), N-(2,3-dihydroxypropyl)-2-tetradecyl-3-hydroxy-stearamide (PC-4R), N-(1,3-dihydroxy-isopropyl)-2-tetradecyl-3-hydroxy-stearamide (PC-5R) were synthesized according to procedures described in **Materials And Methods**. FIGURE 1 shows the general synthetic scheme of the pseudoceramides from alkyl

ketene dimer and alcoholic amine. PC-4 and PC-5 were synthesized by the reaction of 3-amino-1,2-propandiol and serinol with alkyl ketene dimer respectively. And PC-4R and PC-5R were produced by changing ketone group at β-position to amide bond of above synthesized PC-4 and PC-5 into hydroxyl group using $NaBH_4$ respectively. FIGURE 2 shows the structures of PC-4, PC-5, PC-4R and PC-5R.

2. Efficacy of pseudoceramides

(1) Improvement of the healthy human skin: It was shown that the topical application of PC-4, PC-4R, PC-5 and PC-5R on healthy skin results in statistically increase in the water content of the human skin as compared to placebo and untreatment. However no significant differences were found among synthetic pseudoceramides. On day 3, it was shown that the best performance was recorded in the sites treated with PC-4, PC-5 and PC-5R. PC-4R recorded the best performance on day 1. Results (delta % value to placebo) are shown in FIGURE 3.

(2) Restoration of SDS-damaged human skin: A study performed in which human skin was pretreated (damaged) with a high concentration of SDS surfactant. FIGURE 4 showed the results of the recovery of SDS-treated human skin by pseudoceramides (PC-4, PC-4R, PC-5 and PC-5R) as measured by tewametry. A significantly faster restoration of the damaged skin was induced in the sites treated with 0.5% concentration of PC-4, PC-4R, PC-5 and PC-5R compared to placebo and control untreated site. This restoration was already reached within three days after SDS-treatment. No big differences were recorded after 7 and 14 days of treatment with pseudoceramides to placebo and untreated site except PC-4R. Unlike improvement test of healthy human skin, PC-4R showed on day 14, the most significant restoration effect of SDS-damaged human skin (about 97%) among PC series which had been synthesized.

Discussion

The ceramides detected in human SC so far were classified by Wertz et al. into six types [11]. As a characteristic feature, all ceramides contain two (Type 1, 2, 3, 4, 5 and 6II) or three (Type 6I) aliphatic unbranched chains, one (all types) amide bond and two (Type 1 and 2), three (Type 3, 4 and 5) or four (Type 6I and 6II) hydroxyl group. Therefore our lab focused on the synthesis of pseudoceramides which have the structure and functional group similar to those of human skin-identical ceramides. Four pseudoceramides, PC-4, PC-4R, PC-5 and PC-5R, which were synthesized by the reaction of alkyl ketene dimer and alcoholic amine moiety, have two aliphatic chain, one amide bond and two (PC-4 and -5) or three (PC-4R and -5R) hydroxyl group. It was expected that these structural similarity between newly synthesized pseudoceramides and human skin-identical ceramides would represent their behavior and performance similarity, and the results of corneometry test and TEWL test showed the possibility to be usable as economical substitutes in place of human skin-identical ceramides.

References

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FIGURE 1. Synthetic scheme

(1) Synthetic scheme of pseudoceramides from ketene dimer and alcoholic amine.
(2) Selective reduction scheme from pseudoceramides.
R_1 and R_2 mean each individually an aliphatic hydrocarbon group ($R_1 = R_0CH_2$).
R_3 = 2,3-dihydroxypropyl (3-amino-1,2-propandiol), 1,3-dihydroxyisopropyl (Serinol)

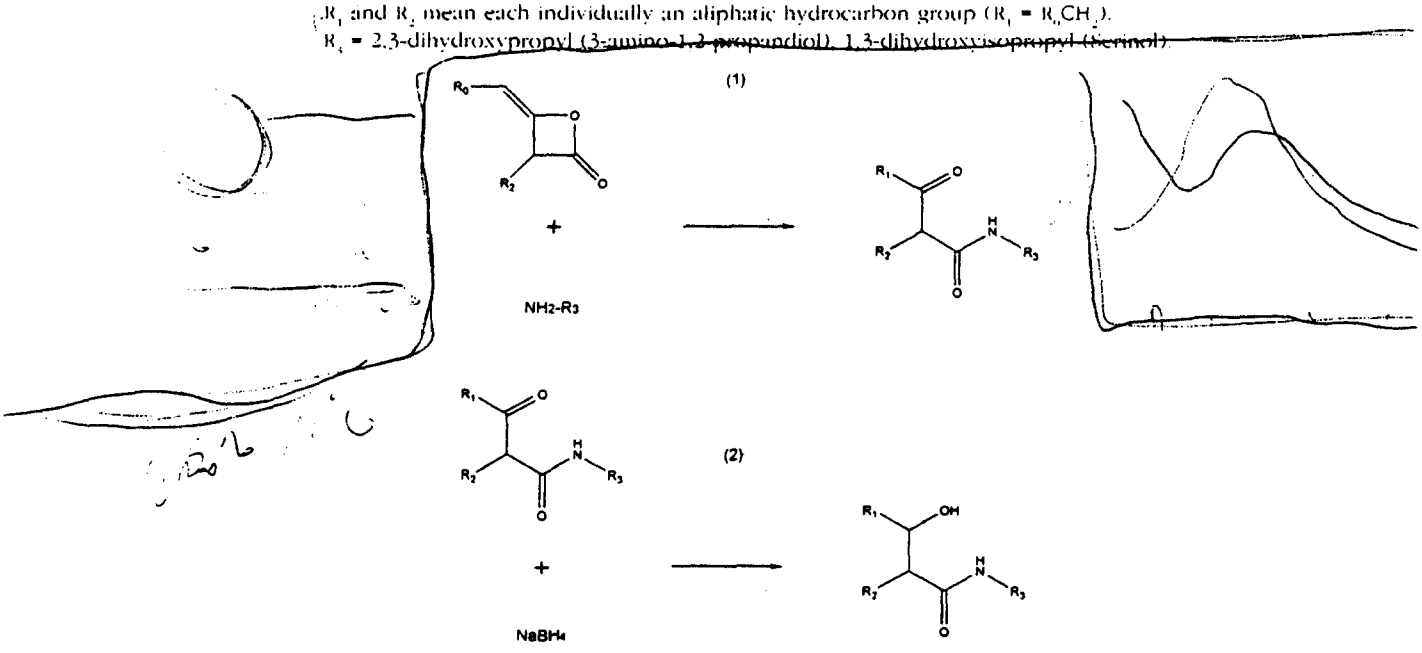
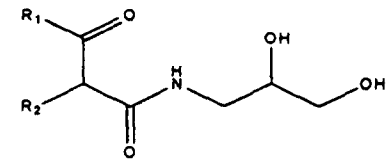


FIGURE 2. The structures of synthesized pseudoceramides

(1) PC-4: N-(2,3-dihydroxypropyl)-2-tetradecyl-3-oxo-stearamide
(2) PC-5: N-(1,3-dihydroxyisopropyl)-2-tetradecyl-3-oxo-stearamide
(3) PC-4R: N-(2,3-dihydroxypropyl)-2-tetradecyl-3-hydroxy-stearamide
(4) PC-5R: N-(1,3-dihydroxyisopropyl)-2-tetradecyl-3-hydroxy-stearamide

(1)



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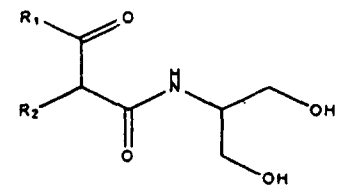

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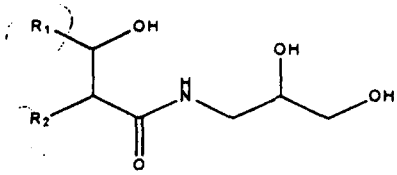

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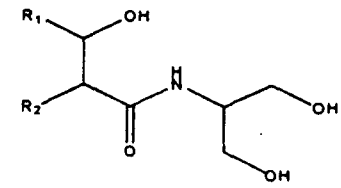

FIGURE 3. Effects of pseudoceramides (PC-4, PC-4R, PC-5 and PC-5R) on water retention capacity of healthy skin as measured by Corneometry.

Application phase: 7 days, twice daily.
Time of evaluation: before treatment (day 0) and 2 hours after last application on day 1, 3, and 7.

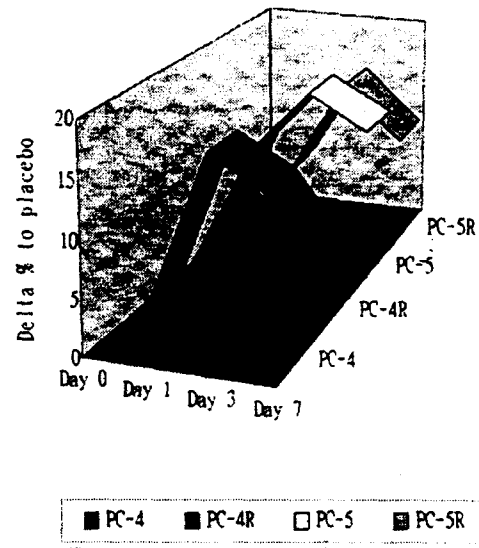

FIGURE 4. Recovery of SDS-treated human skin by pseudoceramides (PC-4, PC-4R, PC-5 and PC-5R) as measured by Tewametry.

Application phase: 14 days, twice a day.
Time of evaluation: right after treatment on the skin with SDS (5%, during 2 hours under occlusion) and 2 hours after last application on day 3, 7 and 14.