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THE SYNTHESIS, PHYSICAL PROPERTY, AND THE BIOLOGICAL ACTIVITY OF NOVEL NEO-CERAMIDES

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Abstract

Ceramides are currently emerging as the major skin care ingredients due to their barrier properties in the stratum corneum of the human skin. Thus, major cosmetic companies have developed synthetic ceramide analogs for their own use. In this study, several ceramide mimic compounds , new skin barrier lipids, were designed and synthesized, and their physical and biological properties were investigated to evaluate their skin care capability. Several structures were designed from the variation of hydrophobic alkyl chain and hydrophilic moiety by the use of molecular modeling software. The selected targets were synthesized, and their properties and activities were studied as the pure form, in the emulsion, or in the lamellar mixture containing cholesterol and fatty acid. Some compounds, such as 1,3-bis(N-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane, enhanced the restoration of skin barrier damaged by SDS(sodium dodecyl sulfate), and by acetone treatment. The rate of restoration was comparable to that of natural ceramides. The synthesized compounds alleviated SDS induced skin irritation and facilitated lamellar phase liquid crystal formation. The treatment of 1,3-bis(N-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane on the acetone damaged skin revealed that the compound promoted the recovery of intercellular lipid lamellar structure of stratum corneum layer. The replacement of palmitoyl groups of the compound with shorter alkyl chain gave lower emulsion viscosity and liquid crystal density, suggesting easier formulation and poorer barrier activity. Most of the synthesized compounds were non-irritable in various toxicological tests proving that they can be safely introduced to the skin care formulations.

1. Introduction

Stratum corneum of human epidermis plays an essential role in maintaining the consistency of the human skin[1]. The shape of the stratum corneum is such that the flat keratinized cells are embedded in the intercellular space filled with the bilayer lamellar lipid structure consisting of ceramides, cholesterol, and fatty acids[2)-4)]. This lamellar structure offers effective barrier against water loss through evaporation, and protects the internal body from various damages caused by the penetration of the external foreign materials[5)-7)]. Thus, relatively large number of cosmetic scientists showed great concern about the role of this intercellular lipid. And the importance of ceramides in maintaining healthy skin was revealed as a result of the extensive studies among the major cosmetic companies. As a first attempt, the ceramides, widespread in nature, were extracted from various animals and plants,

and utilized as cosmetic raw materials. However, ceramides of plant origin were too expensive to be used in sufficient amount into the cosmetic formulations[8)-10)]. The ceramides from animals[11)-13)]suffered from mad cow disease and animal right movements, and have been gradually discarded from the ingredient lists. Today, some semi-synthetic ceramide and pseudoceramides, most of them are still expensive, are commercialized in the market, and in some companies, ceramide analogs have been developed for their own use. Anyway, the importance of ceramides, as the ultimate humectant, cannot be too emphasized, and this fact drived us to develop our own synthetic ceramide analogs.

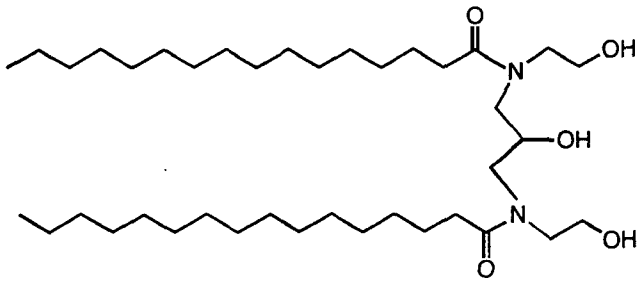
2. Methods

2-1. Molecular Design and Synthesis

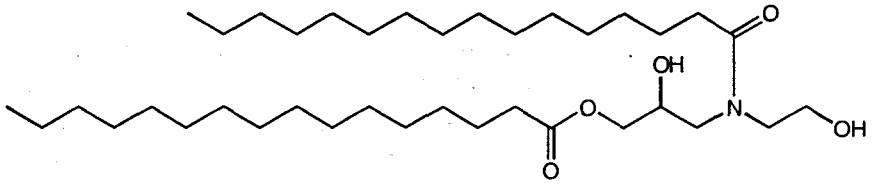
The structural characteristics of skin ceramides as the main constituent of the stratum corneum intercellular lipids are the presence of two long alkyl chains, more than two hydroxyl groups, amide bond, easy formation of intermolecular hydrogen bond, and the arragement of the two alkyl groups in the same direction within the lamellar mixture. Based on these structural characteristics and restraints of natural ceramides, several target molecules were designed and synthesized. Among the synthesized molecules, several groups of molecules were more active in TEWL measurement than the other. Therefore, four molecules(I, II, III, IV), each representing one of four different groups, diamides, ester amides, ether amides and amide acids, of the synthesized ceramide analogs were chosen for further efficacy experiment(Figure 1). In this report only the properties of diamide group are fully described. The evaluation of the other three groups are still going on and will be presented in the next report. As an illustration for synthesis, the route to diamides is shown at Scheme 1.

Figure 1. Structures of Synthesized Ceramide analogs

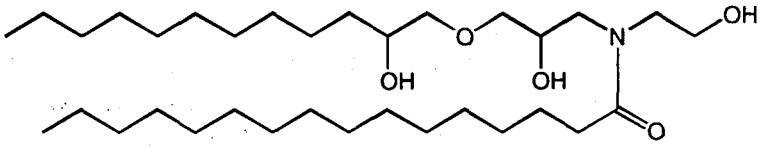
(I) 1,3-bis(N-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane
(Ceradiamide 16)



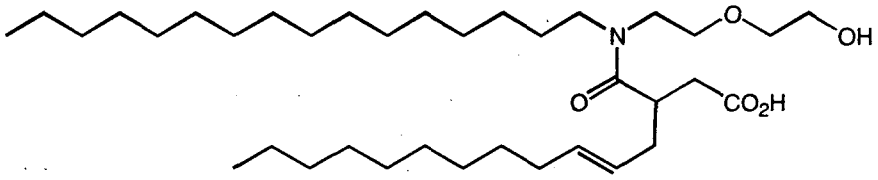
(II) 2-hydroxy-N-(2-hydroxyethyl)-N-palmitoyl-1-propanamine-3-palmitate



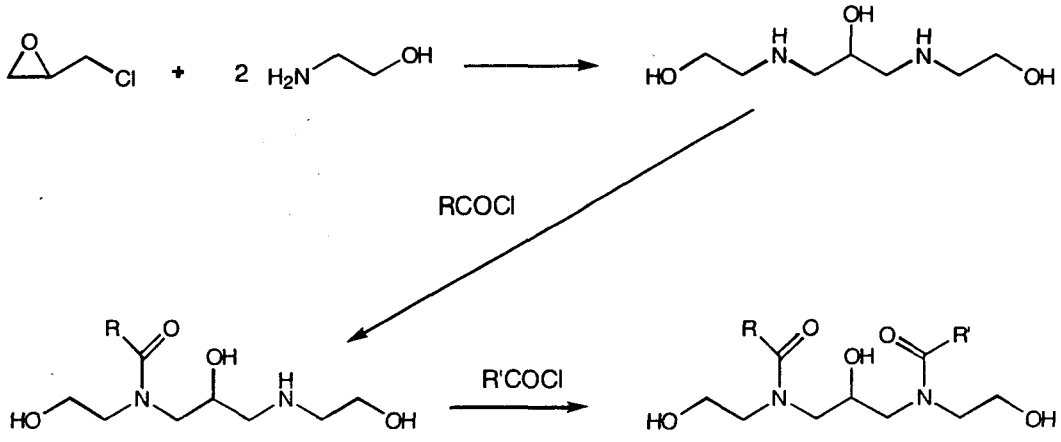
(III) 2-hydroxy-N-(2-hydroxyethyl)-N-palmitoyl-1-propanamine-3-((2-hydroxy) dodecyl) ether



(IV) N-((2-hydroxyethoxy)ethyl)-N-((2-carboxymethyl) tetradec-4-enoyl)-hexadecylamine



In this report only the properties of diamide group are fully described. The evaluation of the other three groups are still going on and will be presented in the next paper. As an illustration for synthesis, the route to diamides is shown at Scheme 1.



Scheme 1. Synthetic Route to Diamides

2-2. Safety

To insure the safety of the ceramide analogs, acute oral toxicity, acute dermal toxicity, primary skin irritation, acute eye irritation, skin sensitization, human patch and repeat insult human patch test were performed.

2-3. Evaluation of Efficacy

2-3-1. The TEWL Measurement

To compare the moisture retention capacity of the synthesized molecules to natural ceramides, 2.5% of SDS(sodium dodecyl sulfate) and acetone were used as the irritants, and mixtures (cholesterol: ceramide analog: stearic acid : linoleic acid) were used as the test materials for the recovery effect measurements. And, the skin recuperation was evaluated by measuring TEWL (transepidermal water loss) with the Evaporimeter EP1(ServoMed, Sweden). The test was carried out for 7 groups consisting of 5 hairless guinea pigs.

2-3-1-1. SDS Treatment

The animals were treated with 2.5% of SDS for 30 minutes using Finn chamber. After removing the SDS patch, the patch site was washed with water and was applied 200μl of each test material. Measurement of TEWL was carried out before, and 30 min, 1 hour, 2 hours, 4 hours, 7 hours and 24 hours after the test material treatment. The results are shown in Table 7. The scores was calculated by considering TEWL measured before SDS treatment as 10 ...Each score in Table 7 is average for five scores.

2-3-1-2. Acetone treatment

The back skin of the hairless mouse was washed with acetone for 5 minutes to impair the barrier recovery function of the stratum corneum layer, and the test material was applied to the site. Measurement of TEWL was carried out before, and 30 min, 1 hour, 2 hours, 4 hours, 6 hours and 8 hours after the test material treatment.

2-3-2. Observation of Liquid Crystal Formation

To evaluate the liquid crystal forming capacity of the synthesized ceramide analogs, Nikkon polarizing microscope was used to view the liquid crystals of the emulsion containing the lipid mixture(ceramide analog : cholesterol : stearic acid =5 : 2 : 3)

2-3-3. Observation of the Skin Surface

The skins of the hairless mouse and the guinea pig were treated with the test materials. The sections were cut and treated with 10% neutral formalin. After fixated with paraffin, theyt were stained with H&E (hematoxylin and eosin), type I pN procollagen(SP1. D8, Hybridoma Bank; ATCC), and ABC (avidin-biotin peroxidase complex) staining kit. The stained samples were subjected to scanning electron microscope measurements.

2-3-4. Thermal Behavior : DSC Measurement

5~15 Mg samples of ceramide analogs were applied to the DSC measurement(DSC 210, Seiko Instruments Inc.). The temperature was raised at the rate of 2°C/min from 20 °C to 130 °C and cooled again. Tm was calculated by the extrapolation at the abrupt heat capacity variation point.

3. Results and Discussion

3-1. Safety of the ceramide analogs

To estimate the safety of the synthesized compounds, various safety tests were carried out. All of four tested compounds(I, II, III, IV) were safe in these tests.,

3-1-1. Acute Oral Toxicity in mice

When 10g of each test material was administered orally per kg of healthy ICR mice, neither observable symptom nor death was observed.

3-1-2. Acute Dermal Toxicity in mice and rabbits

When 2g/kg of the test material was applied to the skin of selected healthy ICR mice and Newzealand White Rabbit, no observable change was observed.

3-1-3. Primary Skin Irritation Test in rabbits

The PII(Primary Irritation Index by Draize) value was 0.17 in rabbits. Thus, the test material was practically non-irritant.

3-1-4. The Acute Eye Irritation Test in rabbits

The M.O.I.(Mean Occular Irritation Index) of the test material was zero , hence the test material was non-irritant.

3-1-5. Skin Sensitization Test in Guinea Pigs

According to the Kligman grading for skin sensitization, the material scored grade I, proving the material to be highly safe.

3-1-6. Human Patch Test & Repeat Insult Human Patch Test

The results showed that the test material could be safely applied to the human skin

3-2. Effect on the Skin Barrier ; the TEWL Analysis

SDS treatment on the skin is different from that of acetone in that SDS penetrates into the lipid layer of the stratum corneum and disrupts the bilayer lamallar phase, while acetone removes the intercellular lipid from the skin. In both cases, the lipid barrier is destroyed, and as a result, the skin experiences the abnormal dryness and damages.

3-2-1. SDS Induced Dry Skin

Each of four compounds(I, II , III , IV of Figure 1) was mixed with other lipids(cholesterol, stearic acid, and linoleic acid) and subjected to TEWL measurement. The results are shown in Table 1.

Table 1: Restoration effect of ceramide analogs after SDS damage

Sample	Reduction ratio(%)*	
	7 hr	24 hr
Compound I	19.5	39.1
Compound II	18.9	38.2
Compound III	19.2	37.9
Compound IV	17.7	32.9
N-oleoyl phytosphingosine	19.0	39.2
Vehicle	17.5	25.3

* reduction ratio : (Tb-Ta)/Tb X 100
Tb : Initial TEWL, Ta : TEWL at specified time.

As shown in Table 1, compound I was most active in the SDS experiment.

3-2-2. Acetone Induced Dry Skin

The restoration effect of samples on the acetone induced dry skin is shown in Table 2.

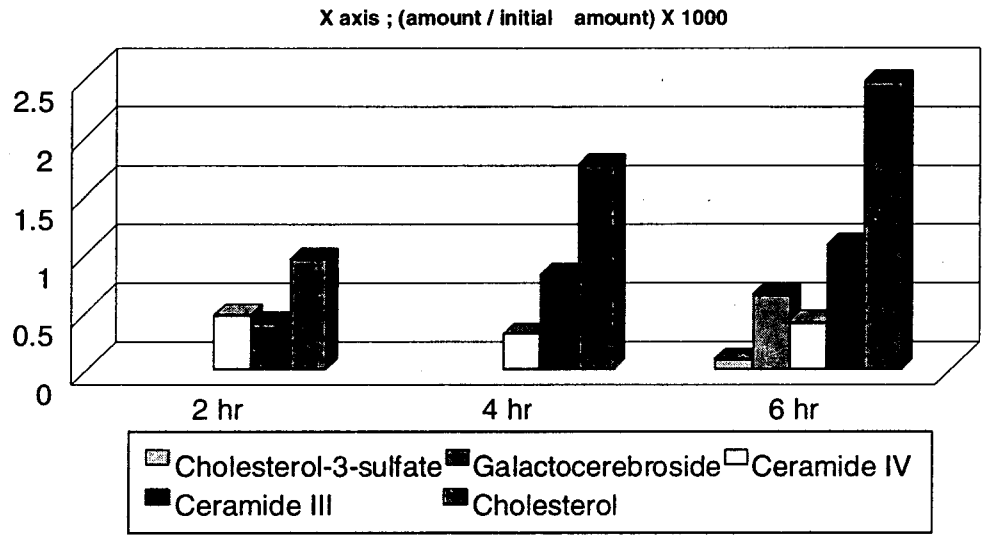
Table 2: Restoration effect of ceramide analogs after acetone damage

Sample	Reduction ratio(%)*	
	4 hr	8 hr
Compound I	40.2	57.3
Compound II	37.5	48.3
Compound III	38.2	55.2
Compound IV	27.3	45.2
Ceramide III	31.5	49.1
Vehicle	7.1	36.2

* reduction ratio : (Tb-Ta)/Tb X 1000, Tb : Initial TEWL, Ta : TEWL at specified time.

As shown in Table 2, the restoration effect for the acetone damaged skin was greater than for the SDS treated skin. As the acetone treatment washes out most of the intercellular lipids, the restoration is thought to be more visualized than the SDS damaged case. The lipid variation of acetone treated skin is shown in Figure 2.

Figure 2. Variation of Lipid composition of damaged hairless



.....Acetone damaged sample
.....Initial amount : the amount of given lipid before acetone wash

As can be seen in Table 1 and 2, compound I was most active in both cases. Therefore, compound I was selected for further experiments and its physical properties are described in comparision to natural ceramides.

3-3. Molecular Structure of Compound 1

The structure of compound 1 (named as Ceradiamide 16) is such that the two long alkyl chains are aligned to the same direction in the lamellar structure and the array of three hydroxyl groups is suitable to intermolecular hydrogen bonding or hydrogen bonding to water molecules, proving its most powerful recovery effect for SDS and acetone damaged skin as described above. The molecular strucure of Ceradiamide 16 is given at Figure 3.

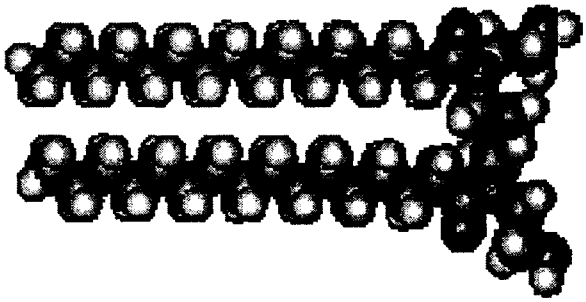


Figure 3. Molecular structure of Ceradiamide 16

Length 23.2415 , Width 7.4367 at the center, 10.5718 at the head

3-4. Liquid Crystal Formation of Ceradiamide 16

Figure 4 shows the polarizing microscopic picture of liquid crystals for the emulsion made from the lipid mixture. Ceradiamide 16 shows excellent lamellar phase liquid crystal forming capacity.

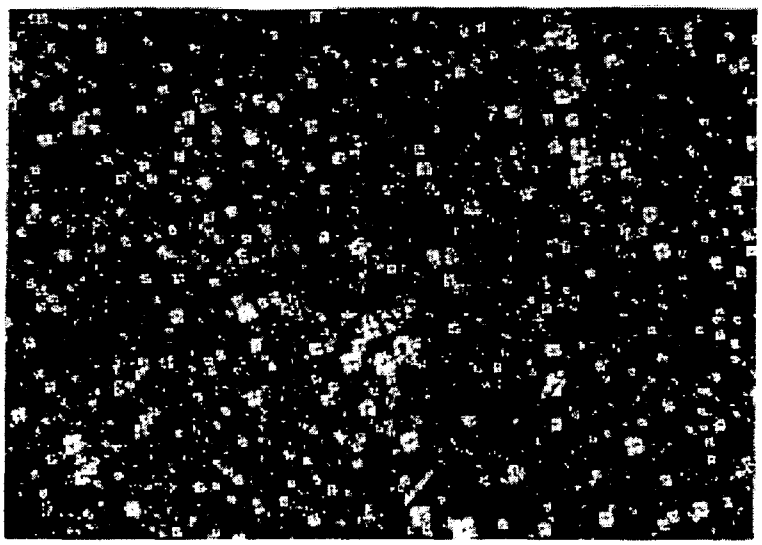
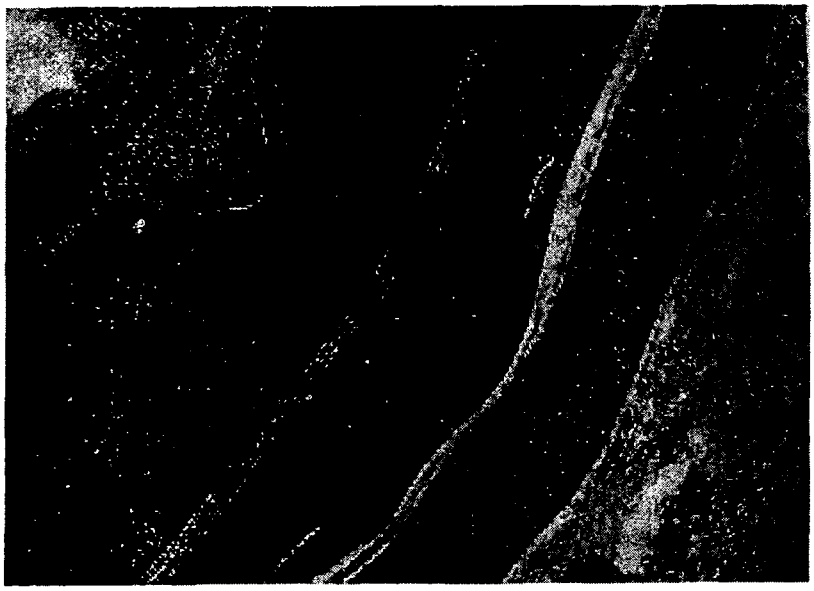


Figure 4. Liquid crystals of the emulsion from the lipid mixture containing Ceradiamide 16, cholesterol and: stearic acid (5: 2: 3)

3-5. Skin Surface Observation.

It is quite obvious, as shown in Figure 5, that intercellular lipid lamellar structure has almost been recontructed by the treatment of lipid mixture containig Ceradiamide 16.

(a)



(b)

Figure 5. Elecron microphotographs of the stratum corneum sheets before(a) and after(b) the treatment of lipid emulsion containg Ceradiamide 16

3-6. Thermal Behavior

Table 3 shows the data thermal behavior of Ceradiamide 16 and N-oleoyl phytosphingosine. As can be seen from the table, the temperature difference between the two cases for Ceradiamide 16 is almost 26 °C. This can be attributed to the fact that this material has a metastable state for relatively long temperature range. Big DS difference of Ceradiamide 16(not shown in the table) implies that this material has a quite stable a -gel state, suggesting that it can easily form the lamellar structure.

Table 3. Phase transition temperatures from DSC measurement

Sample	Melting (°C)	Crystallizing (°C)	Temp difference(°C)
N-oleoyl phytosphingosine	102.5	95.5	7.0
N-oleoyl phytosphingosine(wet)	103.9	93.4	10.5
Ceradiamide 16	64.2	38.3	25.9
Ceradiamide(wet)	68.8	38.3	30.5

4. Conclusion

Several ceramide analogs were designed and synthesized, and their safety and TEWL recovery effect were investigated. Among them, Ceradiamide 16 was most effective and, therefore, chosen to further efficacy study.

The molecular structure of the compound was so adequette for spacial arrangement that two long alkyl groups could be located in the same direction, and that the polar head could easily form intermolecular hydrogen bond or hydrogen bond to water molecules in the intercellular lamellar lipid mixture. The TEWL recovery of Ceradiamide 16 was comparable to N-oleoyl phytosphingosine and naturally occuring ceramide. The compound easily formed liquid crystals when mixed with other lipids present in the stratum corneum. Finally, electron microscopic observation on the Ceradiamide treated skin confirmed that this material is an active humectant for cosmetics, and can also be used as an effective substitute to natural ceramides for the dry skin cure.

Further study is still going on to investigate the effect of alkyl chain length variation or branched alkyl chain on the physical and biological properties. TEWL results, obtained so far, show that shorter alkyl chain length or the use of branched alkyl chain(the alkyl group of ceradiamide 16 is C16, palmitoyl) decreases the restoration effect. The study for alkyl chain vatiation and the efficacy investigation results for other groups of molecules specified earlier at section 2-1 will be discussed in the future.

5. References

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