Extraction and Analysis of Ceramides from Internal Wool Lipids

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ABSTRACT: This study sought to obtain internal wool lipid extracts rich in ceramides from different wool types. Extraction methods, i.e., Soxhlet extraction with different organic solvents and supercritical fluid extraction with CO_2 using several polarity modifiers such as MeOH or EtOH, were optimized. The internal wool lipid content varied from 0.2 to 1.9% (based on wool weight) with a ceramide content ranging from 15 to 30% (based on extract weight). The Spanish and Russian Merino wool extracts were the richest in ceramide compounds. TLC–FID was used to quantify the different internal wool lipid extracts. A new experimental protocol that enabled us to identify most of the different ceramide types is presented. These internal wool lipid extracts, especially the ones with a high ceramide content, may be regarded as an alternative source of animal ceramides, which could be of value in the cosmetic and dermopharmaceutical industries.

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Wool is a natural fiber composed mainly of protein with an external lipid content (lanolin) and with a minor internal lipid content (1.5%), which could arouse considerable interest due to its high proportion of ceramides. Internal wool lipids (IWL) are rich in cholesterol, FFA, cholesteryl sulfate, and ceramides and resemble those from membranes of other keratinic tissues such as human hair or the stratum corneum (SC) of skin (1,2).

This particular composition allows a highly ordered arrangement of lipids known as lamellar lipid bilayers. The intercellular lipids of the SC, especially the ceramides, play an important role in the barrier function of the skin, preventing penetration of external agents and controlling the transepidermal water loss to maintain the physiological skin content of water (3). Recent studies have demonstrated that topical application of optimized molar ratio mixtures of SC lipids (SCL) on insulted skin accelerates the barrier recovery process (4,5).

In fact, the composition of IWL, similar to the ones present in the SC (1), allows for the formation of liposomes with a stable bilayer structure (6–8). Furthermore, topical application of IWL liposomes on intact and disturbed skin has been demonstrated to improve barrier skin properties (9). Accordingly, IWL could be regarded as a new natural extract, beneficial for topical application and suitable for incorporation into pharmaceutical or cosmetic formulations in the treatment and care of skin.

Most ceramides used in cosmetic or dermatological formulations are biotechnologically synthesized, but they do not have the identical composition or the variety of those present in keratinized tissues such as skin, hair, or wool. Recent physicochemical studies based on intermolecular and intramolecular lipid organization provide a basis for speculation on the particular role of each ceramide species in SC organization and function (10,11). This demands the full mixture of ceramides with the composition and proportions similar to the ones present in the skin.

The aim of this work was to obtain IWL extracts rich in ceramides from different types of wool. The extraction methods, i.e., Soxhlet extraction with different organic solvent mixtures or supercritical fluid extraction (SFE) with CO_2 and several polarity modifiers, were optimized to achieve wool lipid extracts similar in composition to SCL. Moreover, TLC–FID was used to quantify the different IWL extracts. A new experimental protocol that enabled us to identify the majority of different ceramides is presented.

EXPERIMENTAL PROCEDURES

Raw Merino wool samples from Australia (19.0 μm fiber diameter, 56.4 mm fiber length), Spain (22.9 μm, 51.6 mm), South Africa (19.8 μm), and Russia (22.3 μm) and Romney wool samples from New Zealand (38.0 μm) were supplied by SAIPEL (Terrassa, Spain).

In an earlier study, surface lipids and contaminants were removed from the raw, unprocessed Australian Merino wool following a sequential extraction procedure with *t*-butanol (5) and heptane (2.5 h) and rinsing with water (12). In the following studies, all wool samples were industrially cleaned with sodium carbonate and a nonionic polyoxyethylene surfactant to remove lanolin before being mechanically shaken and rinsed with water to eliminate vegetable matter and dust. Prior to internal lipid extraction, the wool was equilibrated in a conditioned room (20°C, 60% RH).

Soxhlet extraction was performed for 5 h with a solvent ratio of 1:30 in pure organic solvents such as acetone (b.p. 56.2°C), methanol (b.p. 65.2°C), and the azeotropes acetone/methanol (88:12, vol/vol; b.p. 56.1°C), chloroform/methanol (79:21, vol/vol; b.p. 53.4°C), and chloroform/methanol/water (85:13.7:1.4, by vol; b.p. 52.3°C). The lipid extracts were concentrated and stored in chloroform/methanol (2:1, by vol) at 6°C. Aliquots were dried and weighed, and lipid extraction percentages were determined (1).

SFE was performed using a dual-syringe pump (SFC 3000; Fisons, Milan, Italy) for the delivery of CO_2 (SFE 99.998%; Praxair España, Barcelona, Spain) and a modifier (MeOH or EtOH; Merck, Darmstadt, Germany) as described elsewhere

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(13). The extraction cell (6.94 mL; Keystone Scientific Inc., Bellefonte, PA) was filled with a conditioned wool sample (4.8 g). The flow rate as liquid was adjusted by a metering valve (Hoke Inc., Cresskill, NJ) at *ca.* 1.5–2.0 mL min^{-1}, measured from the LCD of the pump, holding the temperature at 100°C. The total volume of fluid used per extraction was 4–5 cell volumes. Temperature (60, 100, or 160°C), pressure (100 or 360 bar), and modifier percentage (10 or 20% vol/vol MeOH or EtOH) were selected. The extract was collected in an empty screw-capped vial (15 mL) with a septum (Supelco, Bellefonte, PA) weighed previously, with an outlet to vent the decompressed CO_2. The extracts were concentrated to dryness under a gentle stream of nitrogen or maintained at 60°C to eliminate the excess solvent. The extracts were quantified gravimetrically and stored in chloroform/methanol (2:1, vol/vol) at 6°C.

The quantitative analysis of the samples was performed by a thin-layer chromatograph coupled to an automated ionization detector (Iatroscan MK-5 analyzer; Iatron, Tokyo, Japan) (1). Samples (15 μg) were spotted on silica gel-coated Chromarods SIII using an SES (Nieder-Olm, Germany) 3202/15-01 sample spotter.

A general lipid analysis was performed, developing the rods four consecutive times using the following mobile phases: (i) and (ii) chloroform/methanol/water (57:12:0.6, by vol) twice to a distance of 2.5 cm; (iii) *n*-hexane/diethyl ether/formic acid (50:20:0.3, by vol) up to 8 cm; and (iv) *n*-hexane/benzene (35:35, vol/vol) up to 10 cm. A more precise analysis of ceramide compounds was performed by developing the rods initially to a distance of 10 cm with *n*-hexane/diethyl ether/formic acid (53:17:0.3, by vol) to separate polar and nonpolar lipids. After a partial scan of 85% to quantify and eliminate the nonpolar lipids, a second development, again to a distance of 10 cm, was performed with chloroform/*n*-hexane/methanol/acetone (55:5:3:7, by vol). Thus, a suitable separation and quantification of the ceramides and other polar lipids was achieved.

These procedures were applied to the following standard compounds to determine their calibration curves for quantification of each compound: palmitic acid and cholesterol from Merck; type II and type IV ceramides, 7 hydroxycholesterol, galactoceramides, and cholesterol sulfate from Sigma (St. Louis, MO); and type III and type VI(II) ceramides from Cosmoferm (Delft, The Netherlands).

Tenacity of the extracted wool fibers (previously conditioned at 20°C, 60% RH) was evaluated following International Wool Textile Organisation (IWTO) guidelines (14). Each wool sample was evaluated 10 times at a clamp separation of 5 mm and a 60%/min (3 mm/min) strain gradient using an Instron 5500 dynamometer coupled to a PC. Tenacity results were expressed in cN/tex.

RESULTS AND DISCUSSION

Soxhlet extraction was first performed with different solvent systems to determine the most appropriate procedure for obtaining the maximum amount of IWL, especially the ceramide compounds. Even though the chloroform/methanol azeotrope (az.) (79:21) is the most widely used procedure for extracting IWL (1,12,15,16), other solvents or solvent az. such as acetone, methanol, acetone/methanol az. (88:12), and chloroform/methanol/water az. (85:13.7:1.4) were also assayed to determine the amount of lipids and the lipid composition of different extracts as well as the possibility of excluding the use of chlorinated solvents.

Australian Merino wool fibers, first cleaned in the laboratory with *t*-butanol and heptane and then conditioned (13.9% water content), were Soxhlet-extracted using different solvents. The lipid extracts were concentrated and lipid extraction percentages were determined (Table 1). Application of the TLC–FID technique to these fractions allowed us to determine their composition. Multiple developments of Chromarods with solvent systems were performed, as described in the Experimental Procedures section, and the main components were quantified as FFA, cholesterol, and polar lipids, bearing in mind that the latter consisted mainly of ceramides plus oxidized cholesterol derivatives and glycosylceramides.

The low amount of lipid extract obtained with acetone can be attributed to the low swelling effect of this pure solvent. Higher extraction percentages were obtained with the acetone/methanol az. and pure methanol; our analysis showed similar percentages of polar lipids and total lipids. However, the best results were obtained with the chloroform/methanol az. and the chloroform/methanol/water az. These gave the highest percentages of lipids extracted, especially the polar lipid fraction. Although IWL are believed to account for greater than 1.5% owf (percentage on wool fiber basis) (17), similar low values, i.e., 0.5 (15) and 0.6% owf (16), have been obtained by other authors when Australian Merino wool cleaned with *t*-butanol and heptane was extracted using the Soxhlet method and a chloroform/methanol az.

The total amount of lipids extracted is directly related to the

TABLE 1
Quantification of Internal Lipids from Australian Merino Wool Fibers, Soxhlet-Extracted with Different Solvent Systems[a]

Extraction procedure	Total extract (% owf)	FFA (% ole)	Cholesterol (% ole)	Polar lipids (% ole)	Total lipids analyzed (% owf)
Acetone	0.23	27.5	18.8	20.9	0.15
Methanol	0.43	20.2	17.2	20.8	0.25
Acetone/methanol az.	0.34	22.7	22.4	21.3	0.23
Chloroform/methanol az.	0.52	21.5	21.8	24.0	0.35
Chloroform/methanol/water az.	0.58	16.1	21.5	29.3	0.39

[a]Expressed in percentage on wool fiber (% owf) or percentage on lipid extract (% ole). Abbreviation: az., azeotrope.

TABLE 2
Quantification of Internal Lipids (in % owf) from Different Wool Fibers, Soxhlet-Extracted with Chloroform/Methanol[a]

Wool	Total extract (% owf)	FFA (% owf)	Cholesterol (% owf)	Polar lipids (% owf)	Total lipids analyzed (% owf)
Ro. N.Z.	1.037	0.338	0.183	0.287	0.808
Me. S.A.	0.916	0.205	0.227	0.268	0.700
Me. A.	1.012	0.237	0.256	0.263	0.756
Me. R.	1.415	0.188	0.180	0.494	0.862
Me. Sp.	0.947	0.190	0.201	0.302	0.693

[a]Ro. N.Z., Romney wool from New Zealand; Me. S.A., Merino wool from South Africa; Me. A., Merino wool from Australia; Me. R., Merino wool from Russia; Me. Sp., Merino wool from Spain. For other abbreviation see Table 1.

swelling properties of the solvent systems used. Furthermore, some differences were obtained in the acetone extraction for the lipid fractions with the highest amount of FFA and in the chloroform/methanol and chloroform/methanol/water extractions for the fractions with the highest amount of polar lipids. Therefore, this analysis showed it is possible to avoid chlorinated solvents by using methanol or acetone/methanol and still obtain similar compositions, but with lower lipid contents extracted than with chloroform.

To determine the lipid composition of wool fibers of different origins, Soxhlet extraction was performed with a chloroform/methanol az. since a high amount of lipid extract with a rich polar lipid fraction was obtained with this azeotrope. The potential industrial applications of this study prompted us to use raw wool samples industrially cleaned with sodium carbonate and a nonionic polyoxyethylene surfactant without any other prior laboratory cleaning procedure. Determination of the amount of extract and the general quantification of the main lipids were performed following the TLC–FID procedure described above. The results are listed in Table 2.

The different cleaning procedures exerted a considerable influence on the total amount of internal lipid extract [i.e., 1.0% owf (Table 2) and 0.52% owf (Table 1) for Merino A with chloroform/methanol az. extraction]. The amounts of extracts obtained from industrially cleaned fibers varied between 0.9 and 1.4% depending on their origin, and the total lipids analyzed accounted for 0.69 to 0.86%. The highest amounts of FFA were found in the coarsest wool studied (Romney from New Zealand), and the highest amounts of polar lipids were found in the Russian Merino wool extracts.

Since our main aim was to obtain lipid extracts rich in ceramides, with a relative percentage of lipids similar to that of the SCL, it was possible to compare the composition of lipids extracted from different wool types (see Fig. 1). Russian and Spanish Merino wool lipid extracts were fairly similar to those of SCL. The different lipid composition of the New Zealand Romney, which was the only one with a higher amount of FFA than cholesterol or polar lipids, should be pointed out.

To optimize the SFE of IWL, two wool samples with very different lipid fraction compositions were assayed: the New Zealand Romney wool fibers, with a very high concentration of FFA, and the Spanish Merino wool fibers, with a composition similar to the SC lipids. In addition, the effects of temperature, pressure, and type and percentage of modifiers were evaluated. The wool samples were extracted twice, as described in the Experimental Procedures section, under the different experimental conditions shown in Table 3. The total amount extracted was determined, and the amount of total lipids was quantified by TLC–FID analyses of the different extracts (Table 3).

The total extraction percentage was very low in all cases in which MeOH was used as a modifier. Increases in temperature or in the percentage of MeOH did not raise the total extraction percentage. However, SFE using EtOH as a modifier led to a much higher total percentage extracted, with the amount of lipid analyzed similar to that obtained with Soxhlet extraction. The amount of each component extracted was analyzed by TLC–FID, and the results obtained for each kind of fiber were compared with those obtained *via* Soxhlet extraction with the chloroform/methanol az. (Figs. 2 and 3).

The resemblance between the SFE extracts obtained from the two kinds of wool fiber under different experimental conditions should be mentioned in each case. They were lower in FFA and even lower in cholesterol but higher in polar lipids than the extracts obtained with Soxhlet extraction using

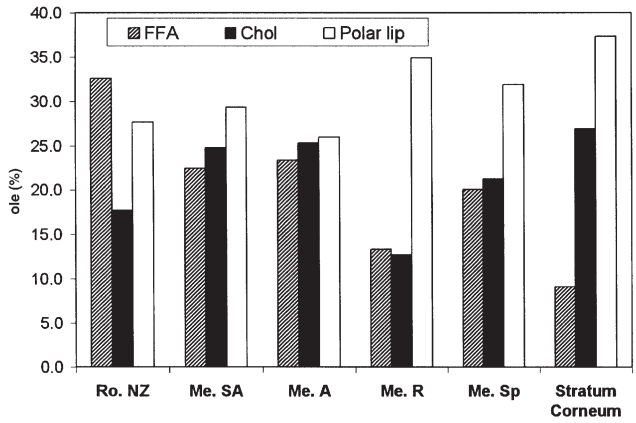

FIG. 1. FFA, cholesterol, and polar lipids (on total lipid extract, ole) obtained with chloroform/methanol Soxhlet extraction from different kinds of wool fibers [Romney from New Zealand (Ro. NZ), and Merino from South Africa (Me. SA), Australia (Me. A), Russia (Me. R), and Spain (Me. Sp)] compared with human stratum corneum. Chol, cholesterol; Polar lip, polar lipids.

TABLE 3
Quantification of Total Extract and Total Lipids Analyzed (in % owf) from Wool Fibers Extracted with Supercritical Fluid Extraction (SFE)[a]

Experimental SFE conditions	Total extraction (%)	Total lipids analyzed (%)
Ro. N.Z. 60ºC, 360 bar, 10% MeOH	0.225	0.143
Ro. N.Z. 100ºC, 360 bar, 10% MeOH	0.355	0.226
Ro. N.Z. 160ºC, 360 bar, 10% MeOH	0.311	0.188
Ro. N.Z. 100ºC, 360 bar, 20% MeOH	0.210	0.125
Me. Sp. 60ºC, 360 bar, 10% MeOH	0.295	0.147
Me. Sp. 100ºC, 360 bar, 10% MeOH	0.290	0.202
Me. Sp. 60ºC, 100 bar, 20% EtOH	1.917	0.881
Me. Sp. 60ºC, 360 bar, 20% EtOH	1.523	0.564

[a]For other abbreviations see Tables 1 and 2.

$CHCl_3$/MeOH. It may therefore be concluded that SFE with CO_2 using different modifiers and experimental conditions had considerable specificity for the polar lipid fraction. The changes in extraction conditions played a greater role in opening the fiber and in increasing the total amount of lipids extracted than in favoring extraction of a particular lipid. The best results were obtained using SFE with CO_2 in the presence of EtOH (10%) under mild conditions of temperature (60°C) and pressure (100 bar).

An evaluation of the tenacity of these two kinds of wool fiber under some of the aforementioned conditions was performed, as described in the Experimental Procedures section. Tenacity (see Table 4) is important in determining the possible effects of these extraction procedures on the subsequent processing of wool. The small decrease in tenacity in all fibers subjected to the two extraction methods does not rule out their subsequent use in textile processing.

In light of our findings, it may be concluded that the Spanish and Russian Merino wool fibers had the highest content of ceramide derivatives, resembling those of the SCL from human skin. Two different extraction methods were optimized. The best conditions for obtaining the highest amount of lipid extract were: (i) Soxhlet extraction with the chloroform/methanol az. (79:21, vol/vol; solvent ratio 1:30) for 5 h and (ii) CO_2 SFE with 20% ethanol at 60°C and 100 bar. The mechanical characteristics of the two methods could account for the different compositions of lipid extracts obtained from the wool fibers. Furthermore, these results do not invalidate their subsequent use in industrial textile processing.

The isolation of lipid extracts enriched in ceramides prompted us to concentrate on the qualitative and quantitative analysis of different ceramide components for comparison with the ceramides of SC origin. SC ceramides make up about 40 to 65% of the total lipids in the SC and consist of seven heterogeneous classes of compounds or families. Ceramides assigned a higher number are more polar than those with a lower number. They are derivatives of sphingosine or phytosphingosine in amide linkage with nonhydroxy, α-hydroxy, or ω-hydroxy acids. Each ceramide family consists of a number of compounds that differ from each other by the length and degree of saturation of the FA attached to the sphingoid base. The most

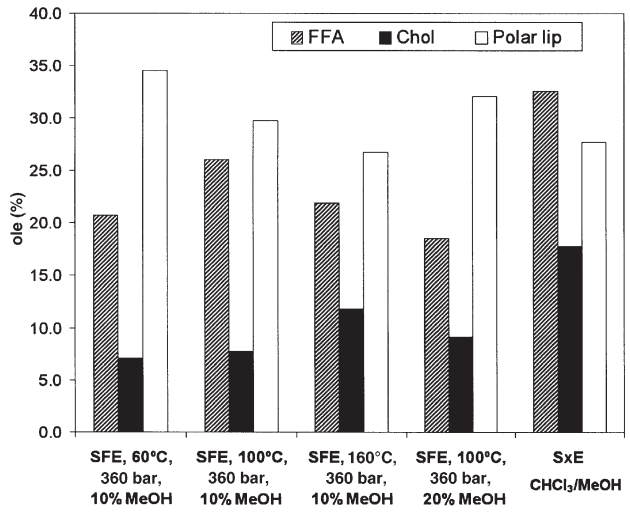

FIG. 2. Percentages of FFA, cholesterol, and polar lipids (ole) of the extracts obtained with supercritical fluid extraction (SFE) under different experimental conditions and chloroform/methanol Soxhlet extraction (SxE) of New Zealand Romney wool. For other abbreviations see Figure 1.

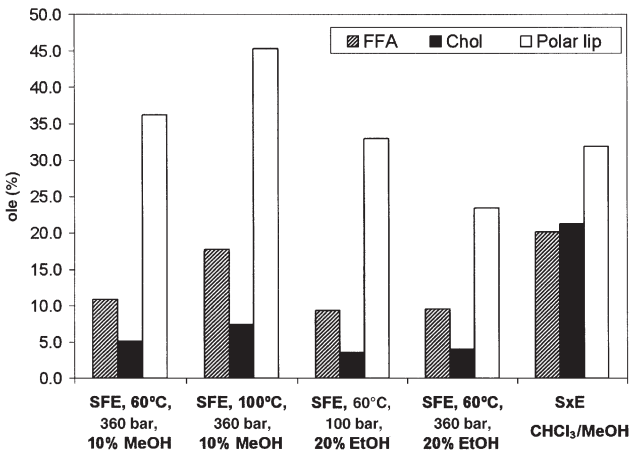

FIG. 3. Percentages of FFA, cholesterol, and polar lipids (ole) of the extracts obtained with SFE under different experimental conditions and chloroform/methanol SxE of Spanish Merino wool. For abbreviations see Figures 1 and 2.

TABLE 4
Tenacity (cN/tex) of Unextracted Wool Fibers and Fibers Extracted Under Different Conditions[a]

Experimental extraction conditions	Tenacity cN/tex (CV%)
Ro. N.Z., unextracted	13.64 (7.3)
Ro. N.Z., Sox. E., $CHCl_3$/MeOH	12.18 (12.4)
Ro. N.Z., SFE, 100°C, 360 bar, 10% MeOH	9.47 (13.17)
Merino Sp., unextracted	8.91 (11.98)
Me. Sp., Sox. E., $CHCl_3$/MeOH	7.11 (10.52)
Me. Sp., SFE, 100°C, 360 bar, 10% MeOH	7.89 (26.35)

[a]Sox. E, Soxhlet extraction. For other abbreviations see Tables 2 and 3.

widely used method for measuring ceramide content is HPTLC separation followed by densitometric quantification (18,19). TLC–FID was used in the present work and in earlier works to study the lipid composition of wool extracts, although different types of ceramides could not be distinguished and appeared as a single peak. Therefore, several mobile phases were tested, and the TLC–FID method was optimized for separation and quantification of the different ceramides. A number of tests were performed to determine the mobile phase combination with the best ceramide resolution and the smallest number of developments. The best results were obtained using two mobile phases. The first phase was used to separate polar and nonpolar lipids, and after a partial scan of 85% of the chromarod length, the nonpolar lipids were quantified and eliminated because they were burned. The second mobile phase was used to separate and quantify the resolved ceramides and other polar lipids.

The chloroform/methanol extract obtained from Spanish Merino wool was analyzed using this procedure. The nonpolar chromatogram (Fig. 4) contained six peaks of increasing polarity, which could be identified as paraffin wax, sterol esters, TG, FFA, DG, and sterols (mainly desmosterol and cholesterol). The polar chromatogram (Fig. 5) contained 10 peaks of increasing polarity, some of which were identified as ceramides I and II; 7-hydroxycholesterol; ceramides III, IV, and VI; glycosylceramides; and cholesterol sulfate. A complete quantification of all these compounds was performed. The results listed in Table 5 are compared with a SCL analysis published elsewhere (2).

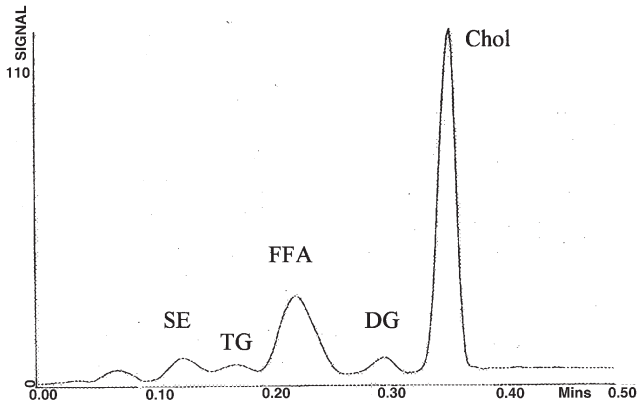

FIG. 4. TLC–FID chromatogram of internal wool lipid (IWL) extract developed with the nonpolar mobile phase, *n*-hexane/diethyl ether/formic acid (53:17:0.3, by vol). SE, sterol esters; for other abbreviation see Figure 1.

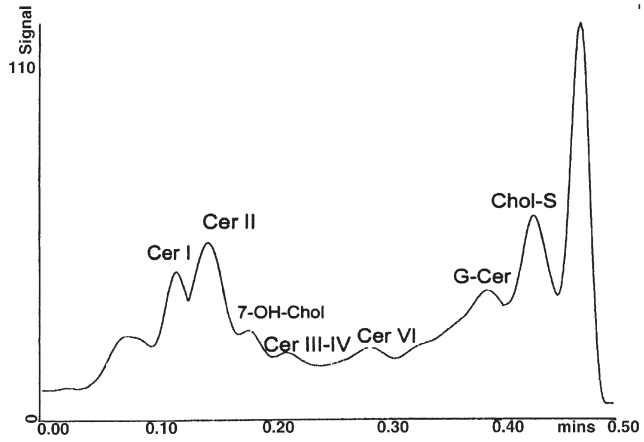

FIG. 5. TLC–FID chromatogram of IWL extract developed with the polar mobile phase, chloroform/*n*-hexane/methanol/acetone (55:5:3:7, by vol). Cer, ceramides; 7-OH-Chol, 7-hydroxycholesterol; G-Cer, glycosylceramides; and Chol-S, cholesterol sulfate. For other abbreviation see Figure 4.

As deduced from the lipid analyses above, the main components are FFA (≅22%) and cholesterol (≅25%). However, the percentage of ceramides is high (≅22%), with ceramide II being predominant. The lipids from other keratinized tissues such as human SC also consist of the same kinds of compounds, especially ceramides. The main differences were the higher amounts of FFA, cholesterol sulfate, and glycosylceramides in the IWL extract.

In sum, the IWL composition, especially of ceramides, which resemble those in the human SC, allows us to consider wool fiber as an alternative natural source of human-resembling ceramides. Indeed, liposomes made up of IWL have been applied topically, and their ability to reinforce skin barrier integrity and to improve the skin's water-holding capacity has

TABLE 5
The Lipid Composition of Internal Wool Lipid (IWL) Extract and Human Stratum Corneum Lipids (SCL)[a]

Component	IWL (%)	SCL[a] (%)
Cholesterol esters	3.6	10.0
TG	3.0	0.0
DG	2.6	—
FFA	22.3	9.1
Cholesterol	24.5	26.9
Ceramide I	2.7	3.2
Ceramide II	7.8	8.9
Ceramide III/IV	6.2	11.0
Ceramide VI(B)	5.4	12.3
Glycosylceramides	7.2	0.0
Cholesterol sulfate	9.8	1.9
7-Hydroxycholesterol	3.1	—

[a]From Reference 2.

been demonstrated (9). The ability of IWL liposomes and SC liposomes (modeling SCL using synthetic ceramides) to repair human skin has been evaluated. The greater repair effect of IWL highlights their suitability for use in new pharmaceutical or cosmetic products designed for skin care.

This work presents two methods for obtaining IWL with a high amount of ceramides without causing damage to the wool fiber. This new source of ceramides should be regarded as an alternative to other animal or vegetable sources used in the cosmetic and dermopharmaceutical fields.

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REFERENCES

1. Coderch, L., C. Soriano, A. de la Maza, P. Erra, and J.L. Parra, Chromatographic Characterization of Internal Polar Lipids from Wool, *J. Am. Oil Chem. Soc. 72*:715–720 (1995).
2. Schaefer, H., and T.E. Redelmeier, *Skin Barrier: Principles in Percutaneous Penetration*, Karger, Basel, 1996, pp. 55–58.
3. Elias, P.M., Lipids and the Epidermal Permeability Barrier, *Arch. Dermatol. Res. 270*:95–117 (1981).
4. Yang, L., M. Mao-Qiang, M. Tljebini, P.M. Elias, and K.R. Feingold, Topical Stratum Corneum Lipids Accelerate Barrier Repair After Tape Stripping, Solvent Treatment and Some but Not All Types of Detergent Treatment; *Br. J. Dermatol. 133*:679–685 (1995).
5. Mao-Qiang, M., K.R. Feingold, C.R. Thornfeldt, and P.M. Elias, Optimization of Physiological Lipid Mixtures for Barrier Repair, *J. Invest. Dermatol. 106*:1096–1101 (1996).
6. Körner, A., S. Petrovic, and H. Höcker, Cell Membrane Lipids of Wool and Human Hair Form Liposomes, *Tex. Res. J. 65*:56–58 (1995).
7. Coderch, L., A. de la Maza, A. Pinazo, and J.L. Parra, Physicochemical Characteristics of Liposomes Formed with Internal Wool Lipids, *J. Am. Oil Chem. Soc. 73*:1713–1718 (1996).
8. Fonollosa, J., M. Martí, A. de la Maza, M. Sabés, J.L. Parra, and L. Coderch, Thermodynamic and Structural Aspects of Internal Wool Lipids, *Langmuir 16*:4808–4812 (2000).
9. de Pera, M., L. Coderch, J. Fonollosa, A. de la Maza, and J.L. Parra, Effect of Internal Wool Lipid Liposomes on Skin Repair, *Skin Pharmacol. Appl. Skin Physiol. 13*:188–195 (2000).
10. Moore, D.J., and M.E. Rerek, Recent Biophysical Studies of Ceramides and Their Role in Skin Barrier Function, *Proc. Cos. Sci. Conf. (Barcelona) I*:36–43 (2000).
11. Chen, H., R. Mendelsohn, M.E. Rerek, and D.J. Moore, Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry Studies of Fatty Acid Homogeneous Ceramide 2, *Biochim. Biophys. Acta 1468*:293–303 (2000).
12. Gale, D.J., R.I. Logan, and D.E. Rivett, Detection of Desmosterol in the Internal Lipids of Wool Fibers, *Text. Res. J. 57*:539–542 (1987).
13. Alzaga, R., E. Pascual, P. Erra, and J.M. Bayona, Development of a Novel Supercritical Fluid Extraction Procedure for Lanolin Extraction from Raw Wool, *Anal. Chim. Acta 381*:39–48 (1999).
14. International Wool Textile Organisation, IWTO Test Method (E)-5-73 (E), Appendix B, IWTO, Brussels.
15. Leaver, I.H., D.M. Lewis, and D.J. Westmoreland, Analysis of Wool Lipids Using Thin-Layer Chromatography with Flame Ionization Detection, *Text. Res. J. 58*:593–600 (1988).
16. Logan, R.I., D.E. Rivett, D.J. Tucker, and H.F. Hudson, Analysis of the Intercellular and Membrane Lipids of Wool and Other Animal Fibres, *Ibid. 59*:109–113 (1989).
17. Leeder, J.D., The Cell Membrane Complex and Its Influence on the Properties of the Wool Fibre, *Wool Sci. Rev. 63*:3–35 (1986).
18. Ponec, M., and A. Weerheim, Retinoids and Lipid Changes in Keratinocytes, *Methods Enzymol. 190 (Retinoids, Pt. B)*:30–41 (1990).
19. Melnik, B.C., J. Hollmann, E. Erler, B. Verhoeven, and G. Plewig, Microanalytical Screening of All Major Stratum Corneum Lipids by Sequential High-Performance Thin-Layer Chromatography, *J. Invest. Dermatol. 92*:231–234 (1989).

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