J. Appl. Cosmetol. **24**, 55-61 (April/June 2006)

New Derivatives For Magnifying Skin Pigmentation

Giancarlo Guglielmini
Sinerga SpA, Pero (MI), Italy

Received: *May 2006. Presented at The VII ISCD World Congress "The New Frontier of Dermocosmetology: Efficacy, Stability, Safety", Rome, 4-6 November, 2004.*

Key words: *Tyrosine; Tanning agents; Skin pigmentation;*

Summary

The application of cosmetic products containing Tyrosine is based on the concept that L-Tyrosine is the primary substrate for tyrosinase, in order to induce melanogenesis. But it has been proved that pure tyrosine, per topic application, hasn't an acceptable bioavailability. Enhancing of cutaneous absorption could be reached by utilizing derivatives of Tyrosine in the perspective of magnifying tan. Aim of the present work was to obtain new functional derivatives from Tyrosine, with innovative and proved cosmetic efficacy.
In order to increase skin bioavailability *Caproyl Tyrosine* has been obtained via the condensation of tyrosine with capryc acid, a C_{10} saturated fatty acid, delivering the equivalent of about 10% pure tyrosine.

Riassunto

L'applicazione di prodotti cosmetici contenenti Tirosina è basata sul concetto che L-Tirosina è il materiale di partenza per la biosintesi della melanina ed è quindi il pigmento responsabile del colore dei capelli e della pelle.
Il substrato primario per la tirosinasi può essere limitante; infatti differenti esperienze provano che la tirosina pura, per applicazione topica, non ha una biodisponibilità accettabile.
Numerosi derivati della tirosina sono stati realizzati per aumentare l'assorbimento cutaneo della tirosina stessa e aumentare l'abbronzatura cutanea.
Scopo del presente lavoro è stato quello di ottenere un innovativo e funzionale derivato della tirosina con un' efficacia comprovata.
Nel Centro ricerche Sinerga si è ottenuto un derivato *Caproyl Tyrosine*, mediante la condensazione di Tirosina con Acido Caprico, un acido grasso C_{10} saturo, con Tirosina, il cui contenuto è pari al 10%.

INTRODUCTION

Tyrosinase, an enzyme containing copper, catalyzes the initial stages of melanogenesis. More precisely, it catalyzes the hydroxylation of tyrosine to DOPA and a further oxidation of DOPA to DOPA-quinone. The next steps, which lead to the synthesis of the different types of melanins, occur spontaneously without an enzymatic catalyst.[1]

Melanin biosynthesis depends not only the activity of tyrosinase, but also the bioavailability of tyrosine. In fact, it is well known that in a biologic synthesis, increasing the concentration of the starting substance also increases the quantity of the synthesized molecule. This is also true in the formation of melanin, where the UV rays stimulate the process of melanogenesis, reducing tyrosine in cells and at the same time leading to an insufficiency of tyrosine.[2]

The pigmentation of human skin could be the result of two different mechanisms: the melanin's biosynthesis at the level of melanocytes; and a simple reaction between skin proteins and specific reactive substances at the level of the stratum corneum.[3,4]

Almost certainly, tanning is a complex reaction. One report delineated a number of the paracrine factors made by keratinocytes that stimulate tanning normally in the skin. These include alpha-melanocyte stimulating hormone (a-MSH), adrenocorticotropin hormone (ACTH), and endothelin 1, all of which stimulate melanogenesis; factors such as basic fibroblast growth factor (bFGF), which can increase the number of melanocytes in skin; and agents such as nerve growth factor (NGF), which can preserve melanocytes in skin that might otherwise be lost.[5]

A second category of agents that have been identified are molecules involved in the intracellular signal transduction pathways that lead to tanning, such as cyclic AMP, which mediates MSH-induced tanning; protein kinase C (PKC) beta, which activates tyrosinase and enhances melanogenesis; nitric oxide, which is released by diols and stimulates tanning. DNA fragments released during the course of repair of UV-induced DNA photoproducts might also enhance tanning, according to this same report.[5]

Tyrosine and Derivatives

All these considerations led to possibility of cosmetic products containing L-tyrosine, using the concept of tanning magnifier as an agent that is able to enhance the coloring of the skin. Thus, attempts to induce melanogenesis by L-tyrosine are based on the concept that the primary substrate for tyrosinase may be the limiting factor in melanogenesis.[6,7] But different experiences have proved that pure tyrosine applied topically does not have acceptable bioavailability, and it is also an irritant ingredient. In fact it is soluble only at pH>11.4, a value at which keratin destruction occurs. At lower pH, L-tyrosine crystallizes, because it can not be absorbed. In fact it has a poor solubility in water (0.05 g/dl at RT). Other experiments validate that minimum concentration of L-tyrosine in the applied product must be more than 0.4%, the level at which one first notices an increase of melanogenesis.[2,8]

Several derivatives have been created to enhance cutaneous absorption of L-tyrosine itself. Among these are L-tyrosine copper salt, N-acetyl-L-tyrosine,[1] N-chloroacetyl-L-tyrosine, N-P-toluensulfonyl-L-tyrosine, L-tyrosine methylester hydrochloride and recently alpha-linoleoyl tyrosine.[9] As disclosed in a recent L'Oréal patent,[10] n-acyl amino acid esters (in particular isopropyl N-lauroyl sarcosinate) are considered tanning magnifiers or enhancing agents because some compositions containing them (with other cosmetic ingredients) are able to increase the level of tanning or skin browning.

The release of L-tyrosine is mediated by enzymes activated by UV rays. This fact was demonstrated by tests utilizing water-soluble derivatives marked with tritium, formulated in a cream at 1%, and applied on the skin of rats. Radiography revealed the presence of tritium in the basal layer and in the dermis.[8]

In the Sinerga R&D laboratories we sought to increase the skin bioavailability of tyrosine. First we obtained caproyl tyrosinic acid via the condensation of tyrosine with capric acid, delivering the equivalent of approximately 10% pure tyrosine.[11] The condensation product is a water-soluble lipoaminoacid, in which capric acid, a C_{10} saturated fatty acid, is able to produce a hydrophilic molecule with excellent affinity for the skin. From the condensation product we obtained two new forms:

- The lipophilic form has the INCI name Caproyl Tyrosine acid (and) Glyceryl Oleate (and) Sorbitan Isostearate.
- The hydrophilic form has the INCI name Potassium Caproyl Tyrosine (PCT) (Figure 1), an easy-to-use N-acyl derivative of tyrosine. It is a clear liquid that is completely water-soluble and compatible with traditional cosmetic ingredients, similar to other lipoaminoacids, oligopeptides and water-soluble amino acids. Its physical properties are described in Table I.

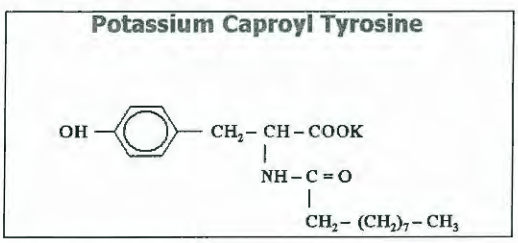

Fig. 1 Molecular formula.

Tolerability

The toxicological profile of PCT was obtained by an in vitro test with the aim of determining the ingredient's potential dermal irritation. This in vitro test, the Irritection Assay System, is a reliable substitute for the patch test normally used for this characterization.[12]

A standard concentration-dependent dose-response study was performed with the dermal Irritection test method. Results of the study indicated that the sample was classified as a non-irritating agent (score = 0.63).

Tab. I
Properties of potassium caproyl tyrosine

Aspect	clear liquid
Color	yellow
Odor	light
pH (c=10)	6.5 – 7.5
Solubility (water)	complete
Active substance (%)	28 – 32

Evaluation of the Pigmentation Efficacy After UV Irradiation

Furthermore it has been made the evaluation of the skin pigmenting properties of a cosmetic product after exposure to UV-radiation[13,14], on volunteers. The product (Potassium Caproyl Tyrosine), has been compared with a placebo and a control, untreated, area for three consecutive weeks, using a solar simulator.

MATERIALS AND METHODS

PCT was formulated at 5% concentration in a emulsion-gel, applied on the back of 12 volunteers (Fitzpatrick phototypes II, III, IV) for three consecutive weeks. UV irradiation was delivered using a solar simulator.(Multiport Solar UV Simulator Model 601, Solar Light Company, Philadelphia, Pennsylvania, USA).

The effect on the skin was evaluated with chromameter (Chromameter CR-300 Minolta, Minolta GmbH, Germany) up to 3 weeks after application.11 The parameters (L*, b* parameters, ITA° value), which are sensitive indexes of change in pigmentation intensity, are taken by

skin colorimetric measurements, before the product application and after several intervals. The Individual Typological Angle or ITA° value expresses the melanin index. It is calculated as a ratio obtained by complex calculations from L* and b* parameters. So, any change in the ratio might indicate a significant change in colorimetric values.

The assessment was performed on three selected areas on the volunteer's back. Each area was treated with a different sample (active, placebo or untreated), with a non-occlusive patch. A fourth area of the back was exposed to UVA-UVB rays in order to determine the minimum erythemal dose on unprotected skin (MEDu). Baseline colorimetric measurements were taken on Day 1 (T0) before irradiation and application. Measurements were also taken before application during the first week (T1-T3), the second week (T4-T8) and the third week (T9-T14). UVA-UVB irradiation corresponding to 50% MEDu was given after any application of the non-occlusive patch of the product sample or the placebo.

Variance analysis and Tukey test were carried out on the data to determine statistically significant differences among the set of values recorded at different times in the three areas.

RESULTS

Statistically significant differences among the set of values recorded at different times in the three areas have been evidenced (Table II):

- the area treated with the sample of PCT at 5% showed a highly significant decrease in ITA° values (T0 versus T3 p= 0.002 and T0 versus T14 p= 0.002)
- the area treated with the sample of Placebo did not show a significant decrease in ITA° values at any time
- the control area untreated did not show a significant decrease in ITA° values at any time

Tab. II
ITA° parameters

Time	PCT	Placebo	Control
T0	23.86 (7.9)	22.93 (8.8)	22.02 (7.2)
Week 1			
T1	20.14 (9.2)	21.72 (8.9)	20.55 (7.4)
T2	20.08 (9.3)	23.48 (7.7)	20.68 (6.3)
T3	18.68 (8.1)	20.27 (8.6)	21.10 (7.4)
Week 2			
T4	19.91 (8.8)	21.49 (8.6)	21.88 (7.0)
T5	22.58 (9.8)	23.28 (8.4)	21.14 (7.1)
T6	19.98 (8.8)	20.33 (8.1)	21.33 (6.3)
T7	21.18 (8.1)	20.10 (8.7)	22.06 (7.6)
T8	20.33 (7.3)	21.03 (8.6)	21.88 (7.5)
Week 3			
T9	21.10 (6.9)	20.22 (6.6)	21.54 (5.5)
T10	21.65 (7.3)	20.45 (6.8)	22.30 (5.4)
T11	21.50 (7.0)	20.24 (7.6)	22.53 (6.3)
T12	22.31 (6.3)	20.95 (7.7)	22.73 (6.3)
T13	20.29 (6.3)	19.93 (7.2)	22.16 (5.9)
T14	18.60 (6.1)	20.33 (6.9)	20.84 (7.1)

DISCUSSION

When the results are graphed (Figure 2), it is possible to understand the long-lasting effect of PCT. The process of melanogenesis is activated when UV rays are supplied. So when exogenous L- tyrosine (PCT) was supplied , endogenous L-tyrosine was activated too. Then melanogenesis occurs in two stages. The first is fast. The second is slow. But both need UV rays and copper to activate tyrosinase. So rapidly (T3) there is an increase of tanning, that is confirmed slowly (T14) too. Then a negative feed-back of the process could be generated; that means that cells don't produce L-tyrosine anymore because they realize that it is already present. That is an interpretation , not yet supported by in vitro results.
In the results, it can be noted[15] that the values recorded in the test areas treated with the two products are often lower than those recorded in the control area. That indicates a tendency toward a pigmentation increase. The difference of values is nevertheless not significant ($p<0.05$). PCT is significantly effective in increasing the skin tanning in comparison to the starting conditions, where a tan magnifying effect is requested, with significative efficacy, modulated in time. A similar level of significance could not be obtained in the comparison with the reference areas, probably because of the high standard deviation.
A similar level of statistical significance could not be obtained in the comparison with the control areas, probably because of the high standard deviation.

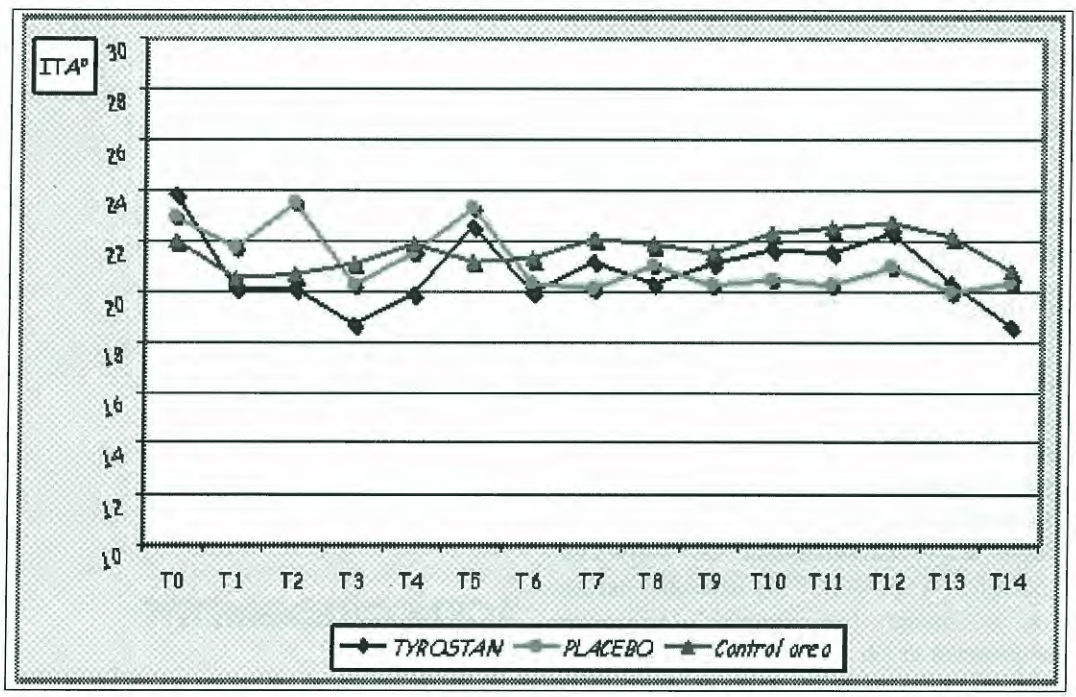

Fig. 2 Skin pigmentation results.

If baseline measurements are mathematically compared to final values it is possible to compare the tanning effects of PCT, placebo and control. The change in ITA° value from T0 to T14 is –5.3, –2.6 and –1.2 for PCT, placebo and control, respectively. Thus, application on volunteers of PCT for 3 weeks magnifies tanning or browning of the skin by about 50% in comparison to parallel application of placebo and about 77% in comparison to untreated areas.

Formulation Development

PCT is water-soluble, clear and easy to add to formulations where a tanning accelerator effect is requested, with significant efficacy, modulated in time. In formulating, the advice is to add it after the emulsifying phase at about 40°C and at any time during the cooling phase. To insure its stability, it is best to avoid its contact with strong oxidizing agents and alkaline solutions. Recommended percentage of use is 5%.
The cosmetic application as tanning magnifier has been developed in products for sun care during exposure, and for suntan maintenance after exposure and its stability over time has been confirmed in several formulations in the form of cleansers, gels and emulsions.[9]

CONCLUSIONS

Potassium caproyl tyrosine is a water-soluble, N-acyl derivative of tyrosine that increases the bioavailability of tyrosine, delivering the equivalent of approximately 10% pure tyrosine. Its effectiveness as a tanning magnifier was demonstrated with measurements of Individual Typological Angle (ITA°) on irradiated human skin. Its benign toxicological profile was demonstrated in vitro with the Irritection assay. Its use in cosmetic formulations, typically at 5%, has been demonstrated. The cosmetic application as tanning magnifier has been developed in products for suncare during exposure, and for suntan maintenance after exposure and its stability over time has been confirmed in several formulations in the form of cleansers, gels and emulsions.

ACKNOWLEDGMENTS

Dr. L. Rigano and Dr.ssa A. Bonfigli, ISPE Laboratories, Italy, provided in vivo evaluation of efficacy.

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Author Address:

Giancarlo Guglielmini
Scientific Support
Sinerga SpA
20016 Pero (MI), Italy
Email: guglielmini@sinerga.it