SYNTHESIS AND SKIN PERMEATION OF AMINOACIDIC ESTERS OF α-TOCOPHEROL

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Introduction

α-tocopherol (VE) is one of the most active and investigated lipophilic antioxidants present in the skin. α-tocopherol is involved in maintenance of skin physiological moisture and keratine turnover. But, since VE is very easily degradable by UV irradiation, it is commonly used as acetate and succinate esters. Natural Moisturizing Factor (NMF) plays a very important role in skin hydration. It consists of 16 aminoacids and other water soluble molecules.

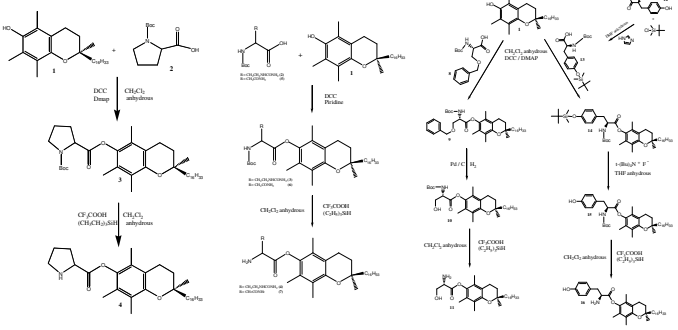
Objectives

We intended to syntesize new α-tocopherol esters (PV), that could be reconverted in the skin to the active α-tocopherol and that are able to release another active moiety to obtain a synergic effect in the skin. We focused our attention on amino acids such as L-proline, L-tyrosyne, L-serine, L-asparagine and L-citrulline.

The new derivatives (PV) were characterized and tested for sensitivity to in vitro enzymatic hydrolysis; lipophilicity was estimated using Log capacity factor. Skin accumulation and extent of metabolism were assessed using freshly excised rabbit ear skin.

Methodology

Synthesis



In vitro Hydrolysis

* Porcine Liver Esterases: 5 UI/ml.
* Reaction solution: 1:10 MeOH: diMethyl- β -Cyclodextrin (DMβCD) 5% in PB pH 8 @ 37°C.

HPLC analysis

* Supelco RP amide C16 column
* Flow rate: 2 ml/min
* UV detection @ 215 nm
* Mobile phase: CH_3CN/H_2O 95/5 (v/v)

Permeation experiments

* Franz type diffusion cells
* Membrane: rabbit ear skin
* Receptor: PBS pH 7.4 + 5% (w/w) DMβCD
* Temperature: 37°C
* Time: 2, 4, 6 hours
* Donor: 1 ml of saturated provitamins solution in EtOH: PG:H_2O (50:10:40, v/v/v) (0.7 mg/ml)
  1 ml of 1%VE and VEAc IPM solution
* Heat separation of epidermis and dermis
* Extraction with 2 ml of methanol @ room temp. for 60 min
* Centrifugation @ 11000 rpm for 10 min

Results

Physical-chemical properties of the new derivatives of α-tocopherol

Derivative	Molecular Weight (Da)	Water Solubility [a] (μg/ml)	Donor Solution [b] Solubility (mg/ml)	Log K' [c]	Half-life [d] (min)
VE	430.7	429 ± 3[f]	Miscible [e]	0.911 ± 0.009	-
VEAc	472.8	182 ± 6[f]	Miscible [e]	1.185 ± 0.004	48 ± 8 [f]
Proline (4)	527.8	891 ± 74 *	10.4 ± 1.0	1.188 ± 0.010	103 ± 9 *
Citrulline (7)	587.7	7115 ± 251 *	7.7 ± 0.7	0.678 ± 0.015	1013 ± 250 *
Asparagine (10)	562.8	1361 ± 18 *	5.4 ± 0.4	0.753 ± 0.002	341 ± 10 *
Tyrosine (15)	593.7	1322 ± 190 *	7.6 ± 0.7	0.862 ± 0.015	54 ± 7
Serine (19)	517.8	374 ± 23 *	5.4 ± 0.4	0.806 ± 0.017	223 ± 9 *

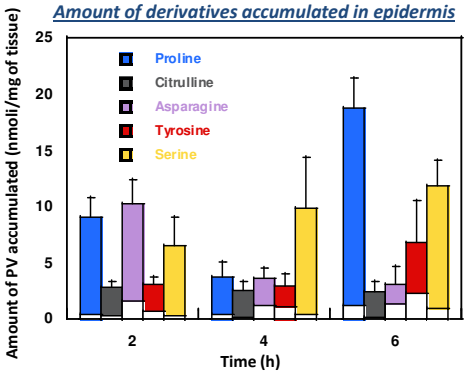
[a] in the presence of 5 % (w/v) dimethyl ß cyclodextrin at room temperature
[b] ethanol: propylene glycol: water (0.5: 0.1: 0.4, by volume)
[c] lipophilicity parameter calculated according to HPLC capacity factor
[d] calculated according to the first order equation
[e] in isopropyl myristate
[f] from reference (3)
* significantly different from acetate.

Total amount of the new derivatives of α-tocopherol (nmol/cm²) accumulated in the skin as function of time

Derivative	Time of contact		
	2 h	4 h	6 h
VE	17.5 ± 3.1	17.7 ± 3.6	15.8 ± 2.6
VEAc	8.0 ± 0.7	6.4 ± 0.8	9.9 ± 2.3
Proline	62.3 ± 9.0	68.7 ± 15.1	118.7 ± 17.7
Citrulline	10.3 ± 2.2	16.3 ± 5.2	14.6 ± 2.5
Asparagine	51.1 ± 14.1	28.6 ± 2.1	36.9 ± 6.6
Tyrosine	10.9 ± 2.0	8.2 ± 1.3	15.9 ± 4.0
Serine	32.6 ±5.8	31.3 ± 8.9	29.4 ± 5.6

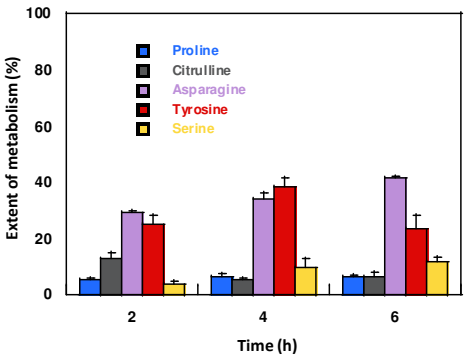
The coloured portion of the columns represents the amount of PV recovered, while the white part indicates the amount of VE originated after skin metabolism:

Amount of derivatives accumulated in epidermis

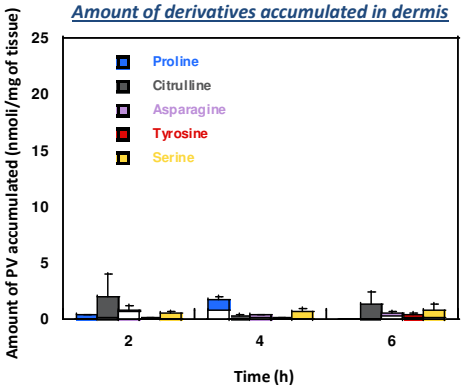


Extent of metabolism (E%) calculated according to:

$$E = \frac{VE}{VE+PV}\%$$



Amount of derivatives accumulated in dermis



References

1. Azzi A., Stocker A., Progr Lipid Res 2000; 39: 231-255.
2. Huang D., Ou B. et al., J Agric Food Chem 2002, 50: 1815-1821.
3. Ostacolo C., Marra F. et al., J Control Release 2004, 99: 403-413.

Conclusions

- The new α-tocopherol esters can be reconverted to the active α-tocopherol by esterases.
- The new derivatives are more hydrophilic than α-tocopherol acetate, this allows to use more hydrophilic vehicles.
- The new derivetives accumulated in the skin in a higher or similar extent compared to acetate ester and generated substantial amounts of α-tocopherol.