

Self-tanning Based on Stimulation of Melanin Biosynthesis

Daniel Schmid, Esther Belser and Fred Zülfi

Mibelle AG Biochemistry, Buchs, Switzerland

KEY WORDS: *self-tanning, melanin, chaste tree, acetyl tyrosine, β -endorphin, UV protection*

ABSTRACT: *Cosmetic products used for a soft, healthy-looking tan are becoming popular. Commonly used self-tanning ingredients produce Maillard reaction-derived pigments that do not provide protection against UV. A new ingredient is described that confers tanning and protection by stimulation of melanin synthesis.*

In Western society, a healthy-looking tanned skin is desirable. As most people have become aware of the risks associated with UV exposure, self-tanning products now represent a continuously growing segment of the cosmetic market. Traditional sunless tanning products contain dihydroxyacetone (DHA) as the active ingredient. DHA reacts with the amino groups of the proteins in the stratum corneum (SC) in a Maillard reaction to produce pigments called *melanoidins*.

The use of DHA has several disadvantages. The resulting tan is likely to emerge streaky or uneven because with higher concentrations of DHA, as is generally known, DHA reacts with the outermost corneocytes. When they are not regularly shed, the remaining color appears uneven. Another common complaint about DHA-based products is the “chemical odor” that they emit. These problems are not relevant for products that contain only low concentrations of DHA.

A current trend exists toward moisturizing products containing a small amount of DHA, just enough to build up a slow, natural-looking tan. These tans develop nicely, but do not solve another problem linked to artificial tans; DHA-derived polymers absorb mainly in the visible light range and

only slightly in the UVA range. Unlike melanin, the natural skin pigment, melanoidins do not protect against UVB and consumers generally expect a tan to provide UV protection. In other words, the false tan may bear health risks. An ideal self-tanning ingredient should stimulate the synthesis of melanin in the skin.

β -endorphin is an endogenous opioid that induces an analgesic effect and a feeling of euphoria.

Regulation of Melanin Synthesis

Protection against UV rays is guaranteed by the tanning response in which UV radiation triggers the production of melanin in the melanocytes. These are specialized cells localized in the basal layer of the epidermis that synthesize melanin in organelles called melanosomes (see **Figure 1**). The melanosomes are transferred via dendrites to the neighboring keratinocytes where they

generate a protective screen around the cell nucleus.

Two key upstream components of the melanin cascade process are the α -melanocyte-stimulating hormone (α -MSH) and its receptor, the G-protein coupled melanocortin-1 receptor (MC1R). The expression of their genes is UV-inducible. Binding of α -MSH to MC1R increases the intracellular level of cAMP, which finally leads to an increased expression of the tyrosinase gene that encodes the rate-limiting enzyme in the synthesis of melanin (see **Figure 2**).

Melanin is a composition of mainly two substances, the black-brown eumelanin and the reddish-yellow pheomelanin. Tyrosine is hydroxylated to dihydroxyphenylalanine (DOPA) and to DOPA quinone by tyrosinase. After the generation of DOPA quinone, two separate pathways, which include several intermediate steps, lead to the formation of eumelanin and pheomelanin.

The Role of β -Endorphin in Melanin Formation

The peptide hormone α -MSH is a cleavage product of the precursor protein pro-opiomelanocortin (POMC). Another product from POMC is β -endorphin that comprises 31 amino acids. β -endorphin is an endogenous opioid that induces an analgesic effect and a feeling of euphoria in the central nervous system after binding to opiate receptors. POMC and its cleavage products are produced mainly in the hypothalamus and in the anterior lobe of the pituitary gland.

It was established several years ago that POMC and the enzymes for its processing are also expressed in peripheral tissues. It has been shown by Slominski et al. that the skin and the hair follicle

are both targets and a local source of POMC-derived peptides.¹ The involvement of α -MSH in skin pigmentation has been thoroughly investigated.

The role of β -endorphin in the skin is the subject of recently published articles. Expression of β -endorphin and its receptor, the μ -opiate receptor, has been detected in human epidermal keratinocytes and melanocytes as well as in hair follicle melanocytes. In keratinocytes, β -endorphin's action is related to differentiation and migration.² In melanocytes β -endorphin and its receptor appear to be closely associated with melanosomes. Treatment of cultured melanocytes with β -endorphin results in increased melanin synthesis.³ β -endorphin also stimulates formation of dendrites.⁴

The use of β -endorphin or compounds with similar activity could be a new approach for tanning or anti-graying hair products.

β -Endorphin-like Compounds in Chaste Tree Berries

Chaste tree (*Vitex agnus castus*), also known as monk's pepper, is a large shrub native to the Mediterranean area that produces aromatic berries with a bitter taste. Dried chaste tree berries have been used as a pepper substitute and as herbal medicine to treat inflammations. Today it is especially recommended by Wuttke et al. for the treatment of pre-menstrual syndrome (PMS), a term that refers to symptoms such as irritability, tension, anxiety and physical changes that some women experience in the two weeks before their period begins.⁵ Extracts of chaste tree berries are reported to bind to the opiate receptor.⁶

The highest affinity is found in the lipophilic fraction of an ethanol extract, but the exact molecular nature of the active compounds is not known. Very recently another research group found that the chaste tree berry extract exerts an agonistic action at the μ -opiate receptor and therefore acts like β -endorphin.⁷

Since anxiety, depression and sleeping problems are important symptoms of PMS and β -endorphins are known to induce feelings of pleasure and euphoria, the β -endorphin-like activity might also be involved in the beneficial effect

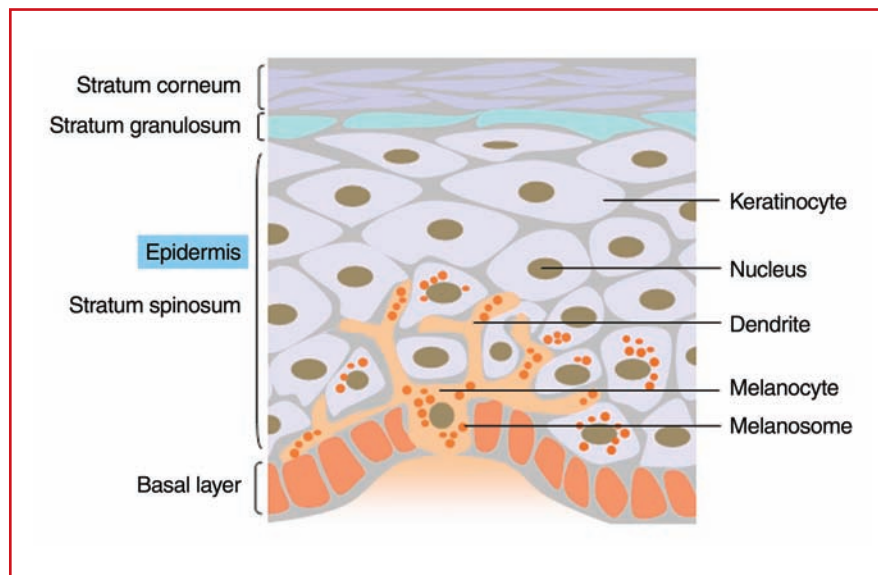


Figure 1. Distribution of melanin pigments in melanosomes from melanocytes to keratinocytes

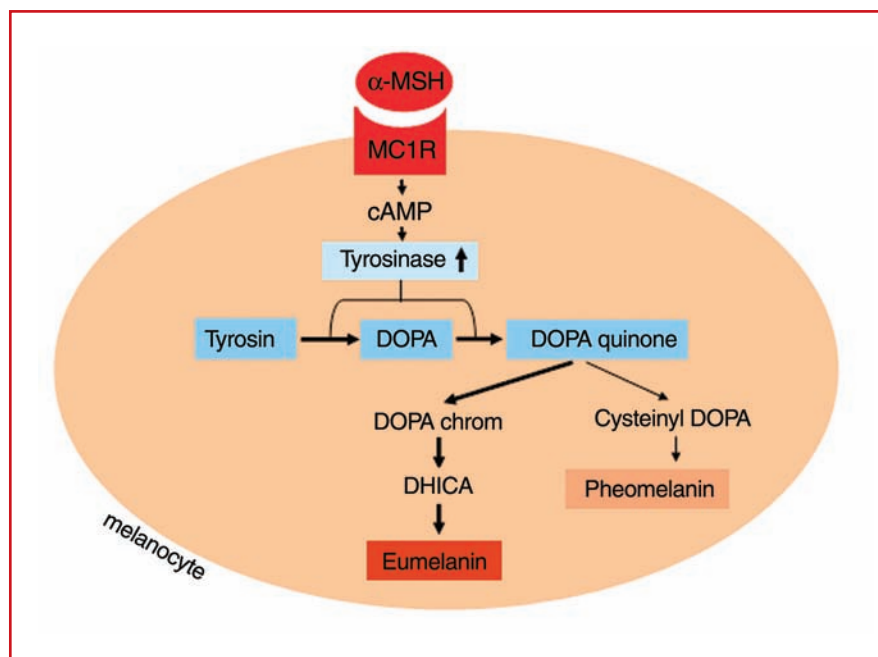


Figure 2. The synthesis of eumelanin is stimulated by α -MSH through its receptor MC1R.

of chaste tree extract in the treatment of PMS. And since β -endorphin stimulates melanin synthesis in melanocytes, chaste tree therefore could cause similar stimulation.

Self-tanning with Chaste Tree Berries Extract and Acetyl Tyrosine

Acetyl tyrosine is used in tanning bed products to stimulate the tanning process. It is a stable and water-soluble precursor of tyrosine, the substrate for tyrosinase that catalyzes the rate-limiting steps in synthesis of melanin. The greater the

quantity of substrate present, the higher the output of the synthesis reaction.

The stimulatory effect of acetyl tyrosine is only found when melanin synthesis is induced after exposure to UV-light or by downstream activators such as hormones like α -MSH or β -endorphin. Topical application of chaste tree berries extract should induce melanin synthesis through its β -endorphin-like constituents, independent of sun exposure. A cosmetic ingredient^a

^a MelanoBronze (INCI: Vitex agnus castus extract (and) acetyl tyrosine (and) glycerin (and) alcohol (and) water (aqua)) is a product of Mibelle AG Biochemistry.

containing chaste tree berries extract and acetyl tyrosine was prepared.

The individual contributions to pigmentation of acetyl tyrosine and the plant extract in the chaste berry (and) acetyl tyrosine ingredient with or without sunlight are shown in **Figure 3**.

When individuals are exposed to sunlight, the hormone α -MSH is produced that induces pigmentation (see top line in **Figure 3**). When at the same time the chaste berry (and) acetyl tyrosine ingredient is applied, pigmentation is accelerated because β -endorphin is an additional inducer and acetyl tyrosine provides additional substrate for the enzyme tyrosinase (see center line in **Figure 3**). When the chaste berry (and) acetyl tyrosine ingredient is applied, there is also tanning without exposure to sunlight because it contains the inducer β -endorphin and adds additional substrate for tyrosinase (see bottom line of **Figure 3**).

Methods and Results

The effect of chaste tree extract on melanin production of melanocytes in

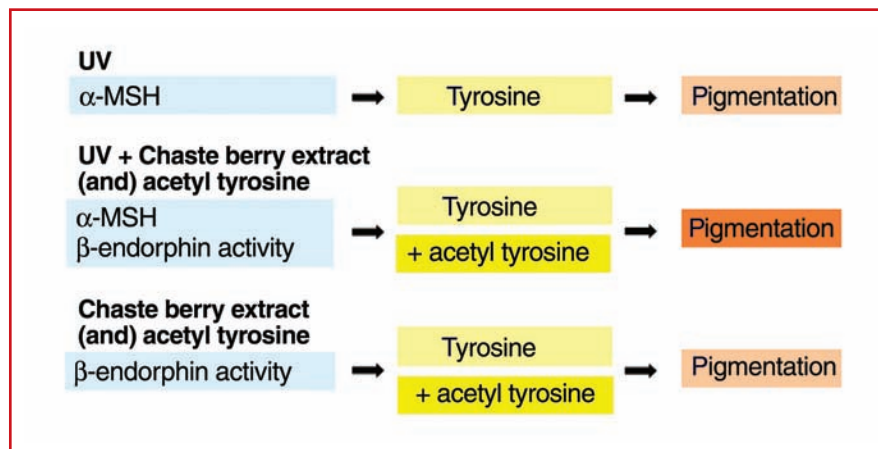


Figure 3. Chaste berry extract (and) acetyl tyrosine stimulates tanning even without sun exposure.

culture was studied. Dried berries were extracted using 40% ethanol. The final extract was obtained after cross-flow filtration through a 10 kDa membrane. The amount of the lipophilic flavonoid casticin was measured by HPLC-analysis. Casticin is a chemotaxonomic marker of the genus vitex and the most dominant substance in the fingerprint chromatogram of monk's pepper berries.⁸ Ten percent by weight of

dried berries were extracted giving a solution of 15 mg/L casticin. Melanin production and cell viability of normal human melanocytes (R6-NHEM-2) were measured 10 days after incubation with different concentrations of chaste berry extract. Cells were cultured in a standard medium at 37°C and 5% CO₂. After incubation, melanin was extracted with a solution of 0.5 M NaOH. The optical density was measured at 405 nm against melanin standards. Cell viability was analyzed by a standard MTT assay.

Melanin production of melanocytes in culture medium alone amounted to 37.1 µg/mL. Chaste berry extract was added in concentrations of 0.25%, 0.13% and 0.06%. This addition was found to increase melanin synthesis to 54.5, 41.6 and 36.2 µg/mL, respectively (**Figure 4**).

Chaste berry extract at 0.25% increased the melanin content by 47%, and 0.13% by 12%. Results of the MTT assay showed that cell viability did not change in the presence of the extract compared to the control. The experiment therefore demonstrated that the extract specifically induced melanin production.

The effect of an extract of chaste berry combined with acetyl tyrosine on skin pigmentation also was studied. The end concentration of acetyl tyrosine in the mixture was 6%. The positive result of the in vitro study with melanocytes does not necessarily mean that there will also be an effect on the skin. To reach the melanocytes, the β -endorphin-like compounds of chaste tree must be able to traverse the SC. Because it is believed that the actives belong to the class of lipophilic

polyphenols, penetration into deeper layers of the epidermis may be possible.

The *in vivo* efficacy of chaste berry extract (and) acetyl tyrosine was tested in two different studies, first with exposure of skin areas to UV radiation and then without UV exposure. The vehicle for the chaste berry extract (and) acetyl tyrosine was a cream containing glyceryl stearate, palmitic acid, stearic acid, stearyl alcohol, cetyl alcohol and phenoxyethanol.

In the first study, creams containing 2% and 5% of chaste berry extract (and) acetyl tyrosine, and a control cream containing only acetyl tyrosine in a concentration corresponding to the amount of 5% chaste berry extract (and) acetyl tyrosine, were compared with a placebo test cream. The test products were applied twice daily to the inside of the forearm of 20 women between 20 and 55 with a skin type of II or III. Skin tanning was initiated with UV radiation of 1.1 MED once daily for one week. Tanning was measured with a chromameter. After applying the placebo control cream, tanning reached a value of 1.83 after one week (Figure 5).

The use of β -endorphin or compounds with similar activity could be a new approach for tanning or anti-graying hair products.

With the creams containing 2% and 5% chaste berry extract (and) acetyl tyrosine, tanning could be clearly enhanced to values of 2.15 and 2.72, respectively. Tanning with the cream containing only acetyl tyrosine reached 2.3. The results of the 5% test product and of the cream with only acetyl tyrosine were significant compared to untreated skin.

This study showed that application of creams with chaste berry extract (and) acetyl tyrosine resulted in a significant and dose-dependent enhanced skin-tanning, compared with the placebo. Comparing the effects of 5% of chaste berry extract (and) acetyl tyrosine with the same con-

centration of acetyl tyrosine alone clearly demonstrated the distinct *in vivo* tanning efficacy of the chaste tree berries extract.

In the second study, creams containing 2 and 5% of chaste berry extract (and) acetyl tyrosine were compared with a placebo. The test products were applied twice daily for four weeks on the inner side of the forearm to 20 persons between 23 and 44 with a skin type of II or III. There was no exposure of the test areas to artificial UV light. Tanning was measured with a chromameter.

The tanning effect of 0.9, which was obtained with the placebo test product, is linked to seasonal variations (Figure 6).

After application of the test products containing 2% and 5% of chaste berry

extract (and) acetyl tyrosine, pigmentation was further increased to 1.22 and 1.4 respectively. The result of the 5% test product was significant compared to the control cream.

The use of a cream with 5% chaste berry extract (and) acetyl tyrosine for four weeks resulted in a significant sunless tanning effect. However, the delta chromameter values of 1.22 or 1.4 are small and hardly visible to the eye. The value of 0.9 of the control cream was a result of the study beginning in March. The spring sun induced a slight tan during the four-week study period. An effect in the same extent is received on top after four weeks' application of the 5% test product.

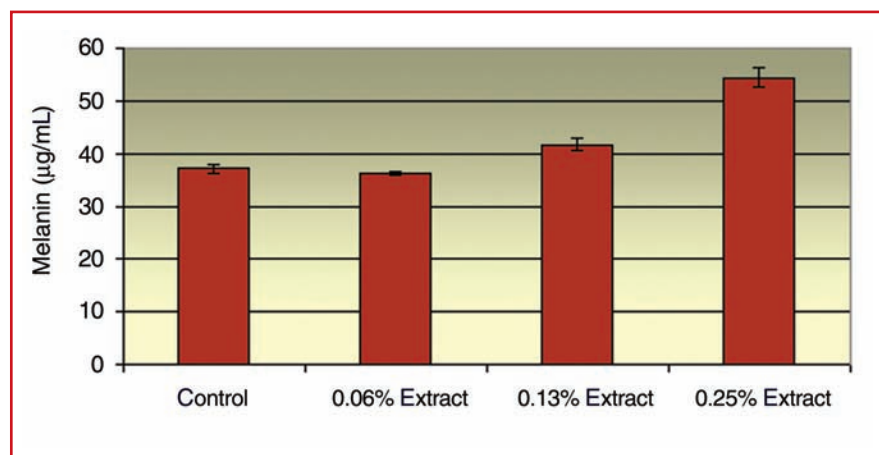


Figure 4. The effect of chaste tree extract on melanin production of melanocytes in culture

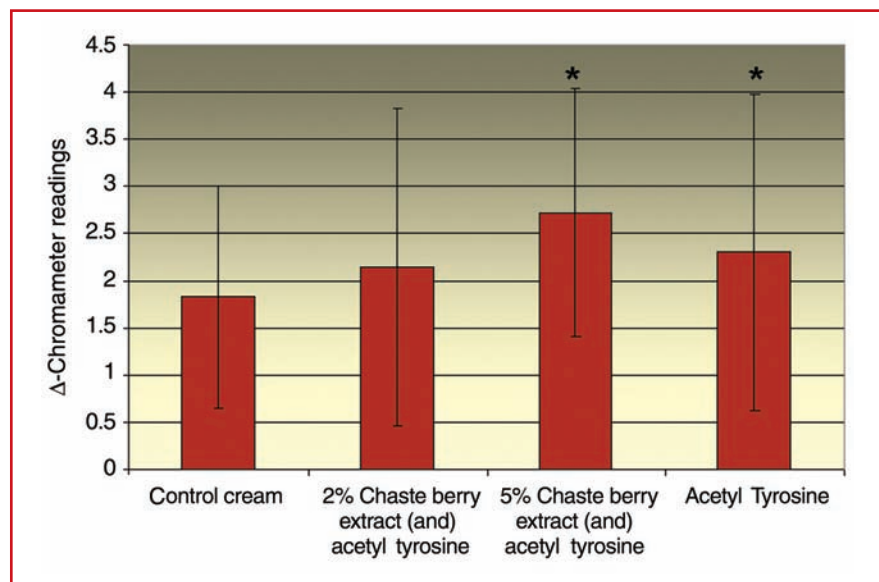


Figure 5. Chaste berry extract (and) acetyl tyrosine stimulates tanning after exposure to UV light. The chromameter readings represent the difference in lightness between the beginning and end of the study. An increase in tanning corresponds to a decrease in lightness. For clarity, the values have been turned into positives in the figure. * $p < 0.05$ versus untreated

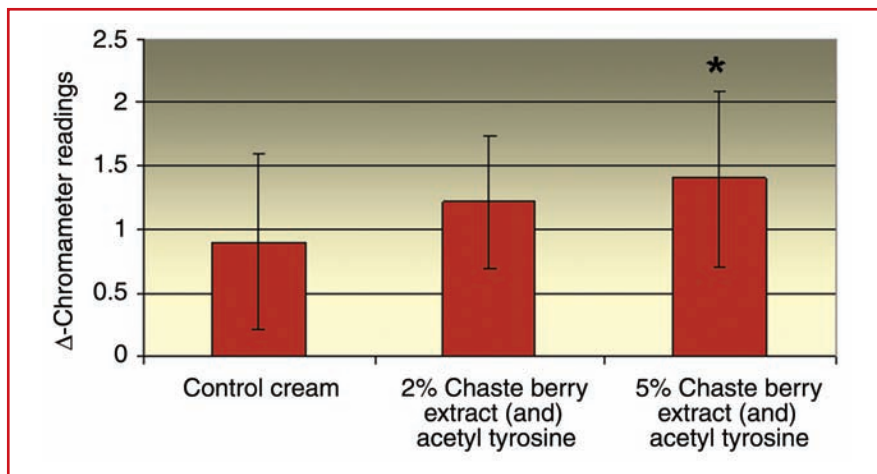


Figure 6. The sunless tanning effect of chaste berry extract (and) acetyl tyrosine * $p < 0.05$ versus control cream

For a noticeable tanning effect, day creams with chaste berry extract (and) acetyl tyrosine must be applied daily over a period of at least six to eight weeks. This corresponds to the intended use—means for a healthy sunless tan after long-term use. Since the tan received after the use of chaste berry extract (and) acetyl tyrosine is build up by melanin, the skin is also better protected against UV radiation.

Analysis of the MED (minimal erythema dose) before and after the four weeks' application of the test products showed a clearly higher MED in the zones treated with chaste berry extract (and) acetyl tyrosine than the untreated skin or those to which the placebo had been applied. Clearly, it is not known whether the higher MED is only the result of increased melanin production or also a consequence

of anti-inflammatory compounds in the chaste tree berries extract.

Conclusions

A combination of chaste berry extract (and) acetyl tyrosine was shown to slightly stimulate melanin synthesis in the skin, even without extra exposure to sun light. Applied on a daily basis as part of a day cream, this new ingredient combination gives a slight but measurable tanning effect. It can be also used during spring to build up early melanin for better protection in summer season, or in sun creams in order to accelerate melanin production for a faster development of self protection.

Reproduction of all or part of this article strictly is prohibited.

To get a copy of this article or others like it from a searchable database, log on to *Cosmetics & Toiletries* magazine's Article Archives at www.CosmeticsandToiletries.com/articles.

References

1. A Slominski, J Wortsman, T Luger, R Paus and S Solomon, Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress, *Physiol Rev* 80(3) 979–1020 (2000)
2. PL Bigliardi, S Büchner, T Ruffli and M Bigliardi-Qi, Specific stimulation of migration of human keratinocytes by μ -opiate receptor agonists, *J of Recept Signal Trans* 22 191–199 (2002)
3. S Kauser, KU Schallreuter, AJ Thody, C Gummer and DJ Tobin, Regulation of human epidermal melanocyte biology by beta-endorphin, *J Invest Dermatol* 120(6) 1073–1080 (2003)
4. S. Kauser, AJ Thody, KU Schallreuter, CL Gummer and DJ Tobin, β -Endorphin as a regulator of human hair follicle melanocyte biology, *J Invest Dermatol* 123 184–195 (2004)
5. W Wuttke, H Jarry, V Christoffel, B Spengler and D Seidlovai-Wuttke, Chaste tree (*Vitex agnus-castus*)—pharmacology and clinical indications, *Phytotherapy* 10(4) 348–357 (2003)
6. B Meier, D Berger, E Hoberg, O Sticher and W Schaffner, Pharmacological activities of *Vitex agnus-castus* extracts in vitro, *Phytotherapy* 7(5) 373–381 (2000)
7. DE Webster, J Lu, SN Chen, NR Farnsworth and ZJ Wang, Activation of the mu-opiate receptor by *Vitex agnus-castus* methanolextracts: Implication for its use in PMS, *J Ethnopharmacol* 106(2) 216–221 (2006)
8. E Hoberg, B Meier and O Sticher, Quantitative high performance liquid chromatographic analysis of casticin in the fruits of *Vitex agnus-castus*, *Pharmaceutical Biology* 39(1) 57–61 (2001)

C&T