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Original Contribution

In vitro photostability and photoprotection studies of a novel 'multi-active' UV-absorber

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ABSTRACT

This paper reports on the synthesis and properties of a new UV-absorber (OC-NO) based on the most popular UV filter worldwide, ethylhexyl methoxycinnamate (OMC) in which the methoxy group has been replaced with a pyrrolidine nitroxide bearing antioxidant activity. This sunscreen active has therefore both UV-absorbing and antioxidant properties which could ideally address both the UV-B and UV-A skin photodamage. For broad-spectrum coverage, the combinations of OC-NO with two commonly used UV-A absorbers (BMDBM and DHHB) were also studied. The results obtained reveal that OC-NO: (a) is as photostable as OMC after UV-A exposure; (b) acts as free radical scavenger as demonstrated by EPR and chemical studies; (c) reduces UV-A and UV-A+BMDBM induced lipid peroxidation in liposomes and cells, measured as reduced TBARS levels and increased C11-BODIPY red fluorescence, respectively; (d) has comparable antioxidant activity to that of vitamin E and BHT commonly used in skin care formulations; (e) is non-cytotoxic to human skin fibroblasts as assessed with the MTT assay when exposed to increasing doses of UV-A; and (f) OC-NO+DHHB is a promising, photostable broad spectrum UV-filter combination that concomitantly reduces UV-induced free radical damage. These results suggest that nitroxide/antioxidant-based UV-absorbers may pave the way for the utilization of 'multi-active' ingredients in sunscreens thereby reducing the number of ingredients in these formulations.

Introduction

Human exposure to solar radiation has been shown to be strongly associated with skin damage, skin ageing and skin cancer, consequently sun protection, [1–4] through the application of sunscreens and improvement of the body defence systems are important issues. The majority of sunscreen formulations on the market contain UV-absorbers which have been primarily directed at protecting against UV-B-induced sunburn and DNA damage (dimer formation and [4–6] photoproduct formation) [5,6]. Less importance has instead been given to the protection against the indirect damage (e.g. dermal extracellular matrix protein degradation, fingerprint mutations) [7–9] resulting from reactive oxygen species (ROS) including free radicals, principally caused by the deeply penetrating UV-A rays [10]. Recent research has in fact revealed that free radical scavengers present in topical applications may be beneficial in delaying the ageing process and in reducing photodamage to skin promoted by excessive exposure to UV-A radiation [11–14]. Therefore new formulations should contain ingredients that address both the UV-B and the UV-A damage i.e. screening from 290-400 nm concomitantly with efficient antioxidant activity. Ideally, these could contain UV-absorbers with built-in antioxidant properties.

With this in mind, we previously synthesized and studied a new cinnamate derivative based on the most popular UV filter in sunscreens worldwide, ethylhexyl methoxycinnamate (OMC), to which a nitroxide moiety which has antioxidant properties was attached [15]. As part of our ongoing research on nitroxide-based sunscreens, here we report on the synthesis and properties of another OMC derivative bearing a nitroxide moiety which is more suitable for sunscreen applications than the aforementioned one. In fact, the new compound (OC-NO) (Fig. 1) retains both the cinnamate group responsible for the UV-B absorbing properties and the ethylhexyl moiety responsible for solubility in oil and water resistance: an essential feature for sunscreen formulations. The methoxy group has instead been replaced with a pyrrolidine nitroxide for conferring antioxidant activity [16].

The following were examined: radical scavenging properties, influence of UV-A exposure on its spectral stability, efficacy to prevent

Abbreviations: AIBN, azo-bisisobutyronitrile; BHT, butylated hydroxytoluene; BMDBM, butylmethoxy dibenzoylmethane; DHHB, diethylaminohydroxybenzoyl hexylbenzoate; HuDE, human dermal cells; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide; OC-NO, 4-(1-oxyl-2,2,5,5-tetramethyl-1,5-dihydro-1H-pyrrol-3-yl) methoxycinnamic acid ethylhexyl ester; OMC, 2-ethylhexyl-p-methoxycinnamate; PBS, phosphate-buffered saline; PC, phosphatidylcholine; ROS, reactive oxygen species; TBA, thiobarbituric acid; TBARS, TBA-reactive substances.

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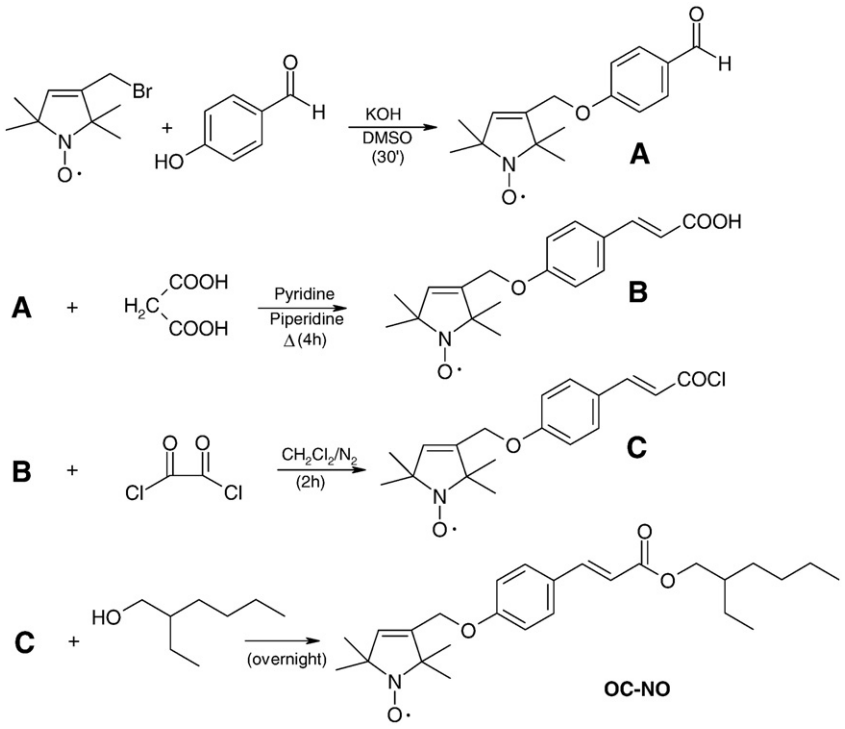

Fig. 1. Step-wise synthesis of 4-(1-Oxyl-2,2,5,5-tetramethyl-1,5-dihydro-1H-pyrrol-3-yl) methoxycinnamic acid ethylhexyl ester (OC-NO).

photo-oxidative damage to liposomes either when alone or in combination with two commonly used UV-A absorbers, comparison of antioxidant activity with that of frequently used antioxidants, effect on cell viability and cell membrane lipid peroxidation after UV-A exposure. UV-A exposure was chosen over UV-B principally because previous studies by Maier et al. showed that the majority of UV-absorbers were photostable at all UV-B wavelengths (290-320 nm), including OMC which is similar to OC-NO, while they were less so when exposed to UV-A (320-400 nm) [17]. Hence it was of interest to study the behaviour of OC-NO exposed to these latter wavelengths. Secondly, UV-A is the principal UV component of sunlight (>95%) and contributes to the direct and indirect production of free radicals and ROS in skin which leads to oxidative damage of both cellular and extra-cellular structures [9,10,18]. In fact, one of the best known effects of UV-A illumination is its ability to induce lipid peroxidation not only of the skin barrier lipids but also of cell membranes leading to impairment of skin barrier function and destruction of cell membrane structure and function [19–21]. In addition, with respect to UV-B, UV-A is 10 times more effective at promoting and propagating this damage. Unlike UV-B, UV-A penetrates further into the dermis of the skin where fibroblasts, the cells used in this study, reside. Thirdly, we previously showed that the results obtained under UV-A exposure for an experimental system similar to the one reported here, were comparable to those achieved under exposure to natural sunlight [15,22]. Gonzalez et al. also obtained qualitatively similar results in their studies when comparing the photostability of commercial sunscreens under exposure to natural sunlight with that under artificial UV radiation [23].

Materials and methods

Materials

L-α-phosphatidylcholine (P2772: Type XI-E), vitamin E, butylated hydroxytoluene (BHT), 2-ethylhexyl-*p*-methoxycinnamate (OMC), azo-*bis*isobutyronitrile (AIBN) as well as all other reagents and solvents were purchased from Sigma-Aldrich Chemical Co. (Milan, Italy). The UV-A filters, butylmethoxy dibenzoylmethane (BMDBM) and diethylamino hydroxybenzoyl hexylbenzoate (DHHB) (Fig. 2) were respectively purchased from BASF (Germany) and DSM (Netherlands). C11-BODIPY was obtained from Molecular Probes (Invitrogen).

Synthesis of OC-NO

4-(1-Oxyl-2,2,5,5-tetramethyl-1,5-dihydro-1H-pyrrol-3-yl) methoxycinnamic acid ethylhexyl ester (here abbreviated OC-NO) was synthesized stepwise as summarized in Fig. 1, starting from *p*-hydroxylbenzaldehyde which was reacted with 3-bromomethyl-2,2,5,5-tetramethyl pyrroline nitroxide according to the procedure described in the literature [24]. The substituted benzaldehyde thus obtained was condensed with malonic acid to give the corresponding cinnamic acid whose esterification with 2-ethylhexanol afforded the desired cinnamate, OC-NO [25]. Since 3-bromomethyl-2,2,5,5-tetramethyl pyrroline nitroxide is not a commercial product, this was first synthesized stepwise starting from 3-carboxy-2,2,5,5-tetramethyl pyrroline nitroxide (abbreviated as NO in Fig. 2) and ethylchloroformiate, *via* the intermediate formation of 3-hydroxymethyl-2,2,5,5-tetramethyl pyrroline nitroxide, according to the method described in the literature [26].

^{1}H NMR spectra were recorded at room temperature in $CDCl_3$ solution on a Varian Gemini 200 spectrometer (δ in ppm referred to tetramethylsilane). EPR spectra were recorded on a Bruker EMX EPR spectrometer (Bruker, Karlsruhe, Germany) equipped with an XL microwave frequency counter, Model 3120 for the determination of *g* factors. FT-IR spectra were recorded in the solid state on a Perkin Elmer MGX1 Spectrophotometer equipped with a Spectra Tech.

Radical scavenging activity

OC-NO (0.1 mmol) and the azo-initiator AIBN (0.1 mmol) were dissolved in THF, the resulting solution was thoroughly degassed with nitrogen and stirred overnight at 60°C. Evaporation of the solvent