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Bakuchiol to Stabilize Retinol and Polyunsaturated Lipids

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KEY WORDS

retinol • bakuchiol • linoleic acid • squalene • xymenynic acid • antioxidant • stablization • lipid peroxidation • photo-oxidation

ABSTRACT

Bakuchiol, a meroterpene of plant origin, is examined here for its ability to stabilize retinol and polyunsaturated lipids. It was found to be approximately 60-fold more effective than natural tocopherol, under both photo-oxidative and singlet oxygen environments. An overview of retinol stabilization, lipid peroxidation and the unique role lipid peroxides play in biology also is included.

Structurally, bakuchiol (see **Figure 1**) belongs to the family of meroterpenes, which are terpenes having an aromatic ring in their structure. Bakuchiol possesses antioxidant,[1-4] anti-inflammatory,[5-8] anti-bacterial,[9] anti-tumor,[10, 11] hepatoprotective[12] and caspase-3-dependent apoptosis[13] properties. It has been shown to inhibit melanin production tenfold over arbutin, in a dose-dependent manner without strong cytotoxicity.[14]

Furthermore, bakuchiol protects mitochondria against oxidative stress,[3] maintains mitochondria membrane structure integrity,[15] and has been shown to protect against mitochondria genome damage.[16, 17] Bakuchiol's topical application has recently been reviewed by Chaudhuri.[18] Although bakuchiol has been known since 1973, and has

Figure 1. Structure of bakuchiol

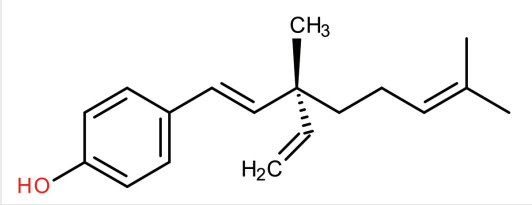
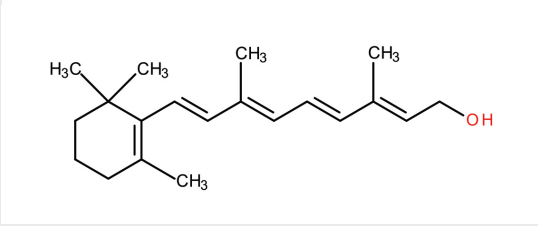


Figure 2. Structure of retinol



shown physiological properties beyond those described,[4, 18-21] its first commercial use in topical applications did not occur until 2007.[22] Here, the authors investigate its ability to stabilize retinol and polyunsaturated lipids, which are key ingredients for skin care.

Retinol Properties

Retinol or vitamin A (see **Figure 2**) has been around for well over eighty years. It was studied by Paul Karrer, a Swiss chemist who was awarded a Nobel Prize in Chemistry in 1937 for related investigations of vitamins A and B2, carotenoids and flavins. When applied to human skin, retinol penetrates and is sequentially oxidized to retinal, then retinoic acid, subsequently causing retinoic acid-like effects.[23] Tight regulation of retinoic acid activity in the skin is imperative for maintaining epithelial homeostasis. Compared to retinoic acid, retinol is more stable and induces less skin irritation.[24, 25] It also provides anti-aging effects and is therefore the preferred skin care ingredient over retinoic acid. However, retinol is not without flaws, and its widespread use, even with newer analogs, is still restricted due to undesirable side effects such as irritation, dryness, peeling, erythema and a burning sensation on the skin.[26, 27] Under certain circumstances, retinol treatments can also cause free radical generation and induce oxidative stress. Mei et al., for example, have shown in mouse lymphoma cells that retinol is mutagenic when exposed to UVA through a clastogenic mode of action.[28]

The relative susceptibilities of lipids to oxidation depend on the reaction milieu as well as their inherent structure.

Market Intelligence

According to a report by Canadean, Italian consumers desire makeup to cover age-related skin impurities. In fact, 19.4% of makeup consumption in Italy is driven by anti-aging needs. "Older generations will also pay greater attention to the ingredients they already know, such as collagen and retinol that protect skin from aging processes," added Veronika Zhupanova, analyst at Canadean.

Source: GCI (*GCImagazine.com*)

In addition, retinol esters such as retinyl palmitate (RP) are commonly used in skin care, and although these storage forms of retinol are required in many essential biological processes, they have been shown to be more photochemically labile than retinol.[29] The biological effects caused by photoexcitation of RP are not well-understood, but studies have shown that photo-irradiated RP is phototoxic and photoclastogenic.[30] Many attempts have been made to improve the stability of retinol with limited success. A few, selected from the literature, are as follows.

Stabilizing Retinol: Attempts

Tesoriere et al. showed a synergistic interaction between retinol and tocopherol against lipid peroxidation in phosphatidylcholine liposomes.[31] Peroxidation was evaluated using malondialdehyde production as an indicator of antioxidant activity; the data suggested that by limiting the auto-oxidation of retinol, alpha-tocopherol strongly promoted its antioxidant effectiveness.

Young and Gregoriadis reported that retinol in liposomes with tocopherol and oxybenzone exhibited a marginal improvement in stability.[32] Lee et al. incorporated retinol into multilamellar liposomes prepared from soybean phosphatidylcholine,[33] as well as into liposomes containing soybean phosphatidylcholine and cholesterol at various ratios. Retinol stability was enhanced by increasing the cholesterol content; further, results indicated that cholesterol in the liposomes increased the incorporation efficiency of retinol.[34]

Jee et al. reported on the stabilization of all-trans retinol (AR) by loading lipophilic antioxidants in solid lipid nanoparticles (SLNs).[35] This decelerated the degradation of AR, as compared with an AR solution dissolved in methanol; at 12 hr, the photostability of AR in SLNs was reported to be ~43%, whereas in methanol solution, it was only ~11%. In a different approach, Sapino et al. used two synthetic alkylcarbonates of γ-cyclodextrins to improve the stability and water-solubility of retinol.[36] These inclusion complexes increased the stability of retinol to both light and heat.

A pilot study was conducted by Akhavan and Levitt to assess retinol stability in a hydroquinone (4%) and retinol (0.3%) cream, in the presence of antioxidants and sunscreen. Results indicated approximately 10% degradation of retinol in 4 hr under simulated use conditions, including exposure to UV light, oxygen and body temperature.[37] Similarly, Bonda and Zhang showed an increase in retinol photostability when combined with ethylhexyl methoxycrylene,[38] although improving the stabilization of 0.1% retinol required inefficient levels of 4% to 5% ethylhexyl methoxycrylene[a].

Cho et al. described enhanced skin perme-

[a] *Solastay, Hallstar*

ation and UV/thermal stabilization of retinol emulsions by using polysorbate-20 [b] and biodegradable poly(ethylene oxide)-block-poly(ε-caprolactone)-block-poly(ethylene oxide) (PEO-PCL-PEO) triblock copolymers having a longer hydrophobic PCL block length. The results suggested that HLB and PCL block length are important factors to enhance the topical delivery of retinol into skin.[39]

One commercially available retinol [c] stabilized with BHT and BHA is reported to remain stable for 24 months when stored at 20°C in its original sealed containers; however, it rapidly degrades when stored at 40°C. Another blend of retinol (42.75% to 49.50%), diluted in polysorbate 20 and stabilized with BHT and BHA[d], has a similar stability profile.

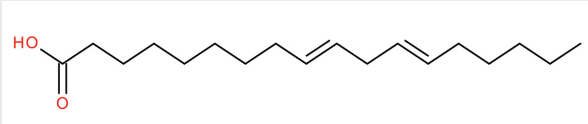
Polyunsaturated Lipids

Linoleic acid (LA) (see **Figure 3**) is an unsaturated omega-6 fatty acid that is a colorless liquid at room temperature. Chemically, linoleic acid is an 18-carbon chain carboxylic acid with two cis double bonds; the first double bond is located at the sixth carbon, and the second is at the ninth carbon from the methyl end.[40] LA is an essential fatty acid that the body cannot synthesize.

LA has been used in cosmetics and personal care products for its beneficial effects on skin. Research points to its anti-inflammatory, acne-reductive and moisture-retentive properties when applied topically on the skin.[41-43] Also, the lack of LA causes hyperkeratinization, barrier function disruption and bacterial proliferation.[44] LA has been reported to lighten UV-induced skin pigmentation[45] due to the post-translational degradation of tyrosinase,[46] and to improve robustness of cells.[47] One study was published on the effects of a combination of LA and vitamin C on more than 30,000 individuals, in which marked improvements in senile dryness (as a result of aging) and skin atrophy (thinness) were observed.[48]

A simple ester of LA, ethyl linoleate[e] (EL), is a more stable form of LA that is soluble in a wide range of solvents and emollients. EL is hydrolyzed to LA in vivo,[49] thereby providing all the skin benefits of LA. EL is reported to accelerate wound healing and has been clinically proven an effective anti-acne agent.[50]

Figure 3. Structure of LA



Suppliers Referenced

[b] Tween 20 (INCI: Polysorbate-20), **Croda**, *www.croda.com/pc*

[c] Retinol 50 C (INCI: Retinol (and) Polysorbate-20), **BASF**, *www.personal-care.basf.com*

[d] *Retinol GS50, DSM Nutritional Products*
[e] *Synovea EL, Sytheon Ltd.*

Squalene (see **Figure 4**) is a natural 30-carbon polyunsaturated lipid and an omega-2 oil. As a hydrocarbon and triterpene, it is a natural and vital part of the synthesis of all plant and animal sterols, including cholesterol, steroid hormones and vitamin D in the human body. It is a natural moisturizer; however, it is highly susceptible to aerial oxidation. Therefore squalane, which is a saturated form of squalene, is commonly used in personal care. The biological importance and applications of squalene and squalane were recently published by Kim and Karadeniz.[51]

Ximenynic acid (XA) (see **Figure 5**) is typically extracted from *Santalum album* (sandalwood tree). It is a yellow crystalline powder, which, due to the presence of a triple and double bond in the structure, is highly susceptible to aerial oxidation. Regarding XA, limited information is publicly available. This ingredient is supplied by two major manufacturers and one version claims to be stabilized using tocopherol. Traditionally, the *Santalum album* plant is used in ayurvedic treatments to make skin smoother and tauter.

Free fatty acids and squalene comprise sebum. Squalene is one of the most common lipids produced by human skin cells,[52] which as noted, is highly susceptible to peroxidation and photodegradation. By-products of these processes include squalene peroxides, which promote acne, roughening of the skin and wrinkling.[53, 54] Polyunsaturated free fatty acids degenerate and promote the peroxidation of nearby lipids, including squalene. Specific aspects of these processes are described next.

Lipid Peroxidation

While the mechanisms and sequence of events by which free radicals interfere with cellular functions are not fully understood, one of the most important oxidative events seems to be lipid peroxidation, which results in cell membrane damage. Lipid peroxidation refers to the oxidative degradation of lipids. It is the process by which free radicals "steal" electrons from the lipids in cell membranes, thereby resulting in cell damage.[55] This damage causes a shift in the net charge of the cell, changing the osmotic pressure and leading to swelling, eventually causing cell death.[56] The chemistry involved in initiating and propagating lipid peroxidation is summarized in **Figure 6**.

The propensity of polyunsaturated lipids to form lipid peroxides has attracted research attention in recent decades. One reason is due to the unique role that lipid-derived peroxides play in biology, both as modulators of enzymes and as intermediates in biosynthetic processes.[57] The primary products of autoxidation are peroxides or hydroperoxides, but these compounds are frequently unstable and decompose to aldehydes, ketones and other

reactive substances. A great diversity of aldehydes is formed when lipid hydroperoxides break down. Some of these aldehydes are highly reactive and may be considered as second toxic messengers, which disseminate and augment initial free radical events. The aldehydes studied most intensively thus far are 4-hydroxynonenal (4-HNE), 4-hydroxyhexanal and malondialdehyde.[58]

Both medium-chain aldehydes, obtained from polyunsaturated lipid oxidation, and retinoids exert potent biological activities. For example, 4-HNE reacts spontaneously with glutathione and with cysteine, histidine, and lysine residues in cellular proteins, causing a variety of cytotoxic and genotoxic effects.[59] High levels of all-*trans*-retinaldehyde are also cytotoxic. The finding that 4-HNE strongly inhibits the biosynthesis of retinyl esters and retinoic acid suggests that oxidative stress and lipid peroxidation can have deleterious consequences for the overall retinoid homeostasis in cells. Also, Xia et al. showed that retinyl palmitate acts as a photosensitizer, leading to the formation and induction of lipid peroxidation following irradiation with both UVA and UVB light.[60] Recently, Aldini et al. have shown detrimental effects of UVB radiation on 4-HNE metabolism and toxicity in human keratinocytes.[61]

Materials and Methods

Considering the propensity of polyunsaturated lipids to form lipid peroxides, and the instability issues and storage restrictions of retinol, the present authors sought to determine whether bakuchiol, which has broad-spectrum antioxidant properties, could stabilize retinol and other polyunsaturated lipids including LA, squalene and XA. The study also assessed the capability for bakuchiol to stabilize under photo-oxidative and singlet oxygen environments.

Retinol[c] was purchased commercially for the present study. This particular yellow oil forms crystals at low temperatures, is soluble in polysorbate 20, has an assay of approximately 50%, and is included a stabilizer system of ~3.5% BHT and ~1% BHA. Bakuchiol[f] having a minimum of 95% purity, as determined by quantitative HPLC analysis, was procured from the author's company. The LA[g] and squalene[h] purchased for this study had purities of ≥ 99% and ≥ 98%, respectively. The XA used[j] had a purity of ≥ 98%.

Stabilizing lipids under peroxidation: Lipid peroxidation was initiated by adding 100 µL of 100 mM 2,2'-azobis (2,4-dimethylvaleronitrile or AMVN), a lipophilic-free radical initiator, to the retinol or lipid substrates and bakuchiol at different concentrations. The mixtures were then incubated at 37°C overnight. Decomposition of the unstable peroxides resulted in the formation of malondialdehyde (MDA), which was quantified colorimetrically at 532 nm. MDA is a well-known index of lipid peroxidation; this protocol is detailed by Botsoglou et al.[62]

Stabilizing retinol under photo-oxidation: Retinol (50 µg/mL) was dissolved in ethanol in 5-mL vials, to which bakuchiol was added at different concentrations: 50 µg/mL, 100 µg/mL and 200 µg/mL. Test vials were placed in a photochemical reactor equipped with UVA and UVB lamps[k], to simulate daylight conditions. The samples were irradiated at 31°C for 5 min at a dose of 13 J/cm^2. Retinol was quantified by HPLC. The mobile phase consisted of 75% A (acetonitrile) and 25% B

Figure 4. Structure of squalene

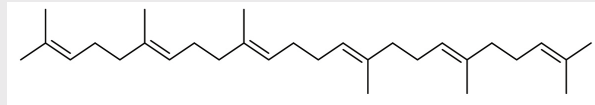


Figure 5. Structure of XA

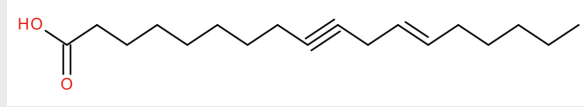
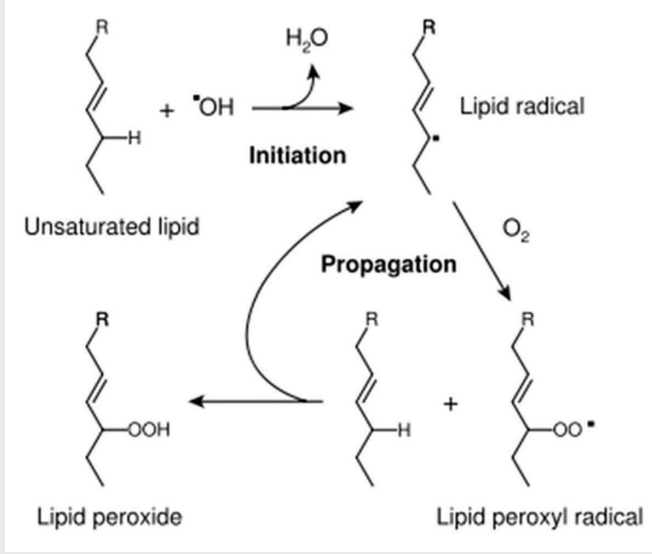


Figure 6. Initiation and propagation of lipid peroxidation

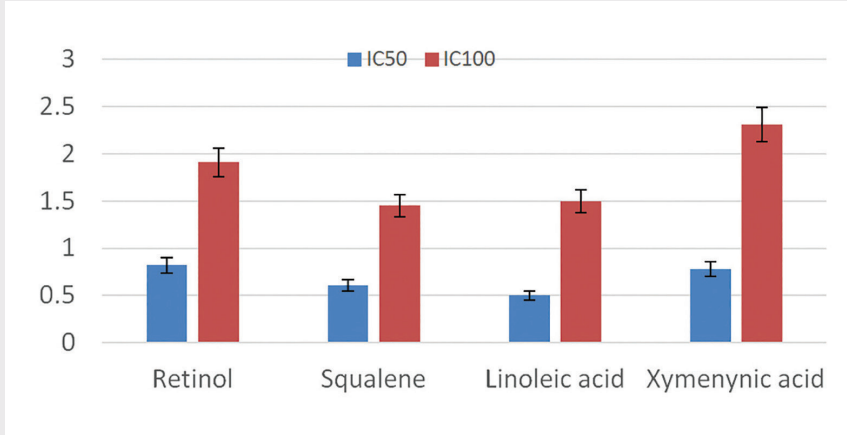


[f] *Sytenol A (INCI: Bakuchiol), U.S. Patents 8,529,967 and 8,859,0210; and pending U.S. and international patents; Sytheon Ltd.*
[g, h] *Sigma-Aldrich, St. Louis, MO*
[j] *Shanghai Tauto Biotech Ltd.*
[k] *Rayonet RPR-100, Southern New England Ultraviolet Company*
[m] *Luna C 18, 4.6 × 250 mm column, equipped with DAD detector at 280 nm, Phenomenex, Torrance*

(methanol), isocratically run at 1 mL/min for 10 min.

Stabilizing retinol under singlet oxygen ($^1O_2$): Retinol (50 µg/mL) was dissolved in ethanol in 5 mL vials, then bakuchiol was added at different concentrations: 50 µg/mL, 100 µg/mL and 200 µg/mL. Singlet oxygen was generated by the addition of H_2O_2 and lithium molybdate, resulting in a mixture with a pH of around 9. This mixture was then incubated at 37°C in the dark for 15 hr. During this period, singlet oxygen was generated and retinol was oxidized. Retinol was then quantified by HPLC[m]. Again, the mobile phase consisted of 75% A (acetonitrile) and 25% B (methanol), isocratically run at 1 mL/min; this protocol is described in *Tetrahedron Letters*.[63]

Figure 7. Inhibition of lipid peroxidation by bakuchiol (in µg/mL)



Results and Discussion

Lipids under peroxidation: Results demonstrated that bakuchiol significantly protected lipids from peroxidation (see **Figure 7**). It was a potent inhibitor of lipid peroxidation, with IC_{50} and IC_{100} ranging from 0.5 to 0.8 and 1.4 to 2.3 µg/mL, respectively. Comparing IC_{50} values, bakuchiol was also 50- to 60-fold more effective than natural tocopherol.

Note that the relative susceptibilities of lipids to oxidation depend on the reaction milieu as well as their inherent structure. The oxidation of linoleates has been discussed in some detail by Niki et al.[64] The peroxidation of squalene in its unsaturated form in sebum reportedly occurs more easily than the peroxidation of palmitoleic or oleic acid.[65] Squalene peroxidation occurs gradually by UV irradiation, with an increase in the formation of squalene peroxide, which has been reported to form even after small suberythematogenic doses of UVA (5 J/cm^2).[66]

Recently, Ryu et al. showed that the topical application of squalene peroxide in guinea pigs induces skin hyperpigmentation by increasing the release of prostaglandin E2 (PGE2) from keratinocytes.[67] Squalene peroxide was also associated with the initiation of the inflammatory cascade, triggering cytokines and the lipoxygenase pathway. Furthermore, it is also present in acne;[68] therefore, it is expected that bakuchiol could stabilize squalene and thereby protect skin from photo-induced damage, providing a reduction in hyperpigmentation and reducing the severity of acne.

A separate study of stabilized, 0.1% retinol-containing moisturizer was carried out in comparison with its vehicle in women having moderate facial photodamage. Results showed that, after eight weeks, the retinol moisturizer was significantly more efficacious than the vehicle in improving lines, wrinkles, pigmentation, elasticity, firmness and overall photodamage.[69] Bakuchiol has also been demonstrated clinically to have broad-spectrum anti-aging activity;[18] it would be interesting to evaluate whether a bakuchiol-stabilized retinol containing product would provide improvements, potentially synergistically, in the appearance of aged and/or photo-aged skin.

Rate constants for reactions of all-*trans* retinol and retinal with singlet oxygen were measured by Smith in a variety of solvents having different polarities.[70] The constants were found to increase with increasing solvent dielectric constant, which suggests a charge transfer mechanism is playing a part in the reaction. Further, the rate constant reaction of singlet oxygen with retinal was greater than with retinol, and since retinal has a lower ionization potential than retinol, these relative rates also support the hypothesis of charge transfer involvement in the reaction.

Retinol under photo-oxidation: A characteristic feature of retinoids is their instability to UV light. UVA (320 nm to 400 nm) and UVB (290 nm to 320 nm) have been shown to reduce retinol content in human skin.[71] Pathways for photodegradation of retinoids include photoisomerization, photodimerization and photo-oxidation.[72] Sunlight-induced photodegradation of retinyl esters proceeds much faster than that of retinol, and it has been suggested that Cellular Retinol Binding Protein-1 (CRBP-1) may protect retinol from photodegradation. However, studies using hairless mice treated topically with retinol before and after UVB exposure showed that retinol was depleted to a similar extent after UVB exposure of the pretreated animals as compared to untreated animals in spite of an induction CRBP-1. Sorg et al.[73] have suggested that UV light depletes epidermal retinol through a photochemical reaction rather than via oxidative stress. Generation of reactive oxygen spe-

cies accompanying irradiation of retinol, retinyl palmitate, and their corresponding photodecomposition products has been demonstrated by several authors.[74, 75] Membrane damage induced by the attack of singlet oxygen on the lipid or protein moieties can be highly deleterious.

Results demonstrated that bakuchiol dose-dependently protected retinol in vitro from photo-degradation. **Figure 8** summarizes the results obtained from this study. Use of 1:1 and 1:2 (w/w) showed moderate improvement in the stability of retinol 30% (46% to 60%) and 56% (46% to 72%), respectively, with bakuchiol. However, a four-fold increase in bakuchiol provided complete stabilization of retinol. It would be interesting to evaluate whether bakuchiol-treated skin could stabilize epidermal retinol under a photo-oxidative environment.

Retinol under singlet oxygen: Singlet oxygen is an oxygen molecule that has been excited from its ground triplet state to its first singlet state. After excitation, singlet oxygen decays within microseconds, which is due to interaction with molecules in its environment. Photochemical studies have demonstrated that excitation of retinol or its esters with UV light generates a number of reactive species including singlet oxygen and superoxide radical anion.[76] Singlet oxygen reacts with olefinic double bonds to produce hydroperoxides by a concerted *ene*-type mechanism. These reactive oxygen species have been shown to damage a number of cellular targets, including lipids and DNA. Consistent with the potential for damaging DNA, retinyl palmitate has been shown to be photomutagenic in an in vitro test system.

These results demonstrated that under a singlet oxygen environment, bakuchiol dose-dependently protected retinol in vitro from photo-degradation. **Figure 9** summarizes the results obtained from this study. Use of only two-fold excess of bakuchiol provided complete stabilization of retinol.

Conclusion

Bakuchiol, a meroterpene of plant origin, shows promise as a new agent that can complement and enhance the effectiveness of retinol and a range of polyunsaturated lipids, including squalene, LA and XA, due to the improved stability it imparts in different oxidative environments. Bakuchiol also has a wide range of beneficial skin properties, and its excellent safety profile, and photo- and hydrolytic-stability are advantages over retinol; thus, bakuchiol can be used throughout the day.[18] Topical formulations that include bakuchiol are likely to lead further improvements in the way aged or problem skin are treated now and in the future.

References

1. S Adhikari et al, Antioxidant activity of bakuchiol: Experimental evidences and theoretical treatments on the possible involvement of the terpenoid chain, *Chem Res Toxicol* 16 1062-1069 (2003)
2. H Haraguchi et al, Antioxidative components of *Psoralea corylifolia* (Leguminosae), *Phytother Res* 16 539-544 (2002)
3. H Haraguchi et al, Inhibition of mitochondrial lipid peroxidation by bakuchiol, a meroterpene from *Psoralea corylifolia, Planta Med* 66 569-571 (2000)
4. RK Chaudhuri and F Marchio, Bakuchiol in the management of acne-affected skin, *Cosm & Toil* 126 502-510 (2011)
5. ML Ferrandiz et al, Effect of bakuchiol on leukocyte functions and some inflammatory responses in mice, *J Pharm Pharmacol* 48 975-980 (1996)
6. CN Backhouse et al, Active constituents isolated from *Psoralea glandulosa*

Figure 8. Percent improvement of retinol (R) stabilization under photooxidative environment with bakuchiol (B)

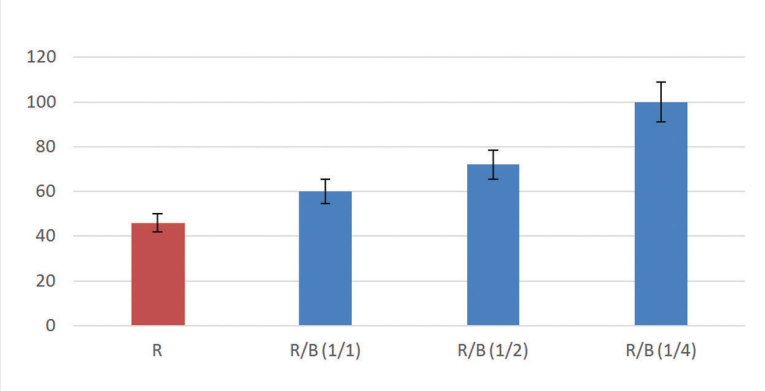
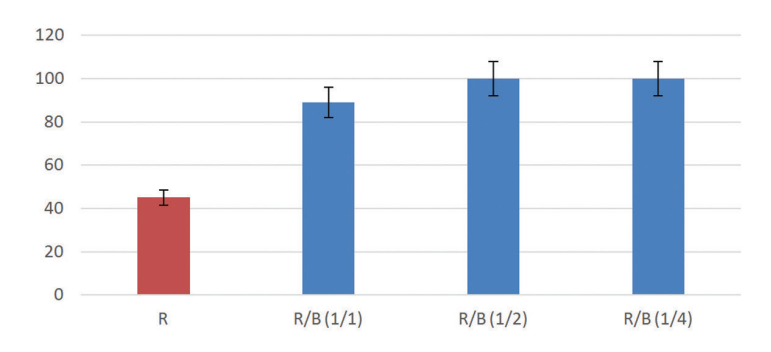


Figure 9. Percent improvement of retinol (R) stabilization under singlet oxygen environment with bakuchiol (B)



L. with anti-inflammatory and antipyretic activities, *J EtÚopharmacol* 78 27-31 (2001)

7. HO Pae et al, Bakuchiol from *Psoralea corylifolia* inhibits the expression of inducible nitric oxide synthase gene via the inactivation of nuclear transcription factor-κB in RAW 264.7 macrophages, *International Immunopharmacology* 1 1849-1855 (2001)
8. H Matsuda et al, Bioactive constituents from Chinese natural medicines. XXXIII. Inhibitors from the seeds of *Psoralea corylifolia* on production of nitric oxide in lipopolysaccharide-activated macrophages, *Biol Pharm Bull* 32 147-149 (2009)
9. H Katsura et al, In vitro antimicrobial activities of bakuchiol against oral microorganisms, *Antimicrobial Agents and Chemotherapy* 45 3009–3013 (2001)
10. U Pandey et al, Bioevaluation studies of 125I-Bakuchiol in tumor bearing animals, *BARC News Letter* 285 88-91 (2007)
11. Z Chen et al, Anti-tumor effects of bakuchiol, an analogue of resveratrol, on human lung adenocarcinoma A549 cell line, *Eur J Pharmacol* 643 170-179 (2010)
12. H Cho et al, Bakuchiol: A hepatoprotective compound of *Psoralea corylifolia* on tacrine-induced cytotoxicity in Hep G2 cells, *Planta Med* 67 750-751 (2001)
13. E Park et al, Bakuchiol-induced caspase-3-dependent apoptosis occurs through c-Jun NH2-terminal kinase-mediated mitochondrial translocation of Bax in rat liver myofibroblasts, *Eur J Pharmacol* 559 115-123 (2007)
14. O OÚo et al, Inhibitory effects of bakuchiol, bavachin and isobavachalcone isolated from *Piper longumon* melanin production in B16 mouse melanoma cells, *Biosci BiotecÚol Biochem* 74(7) 1504-1506 (2010)
15. Y Tsujimoto and S Shimizu, Role of the mitochondrial membrane permeability transition in cell death, *Apoptosis* 12(5) 835–840 (2007)
16. Z Wu et al, Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1, *Cell* 98(1) 115–124 (1999)
17. E Seo, YS Oh, D Kim, MY Lee, S Chae and HS Jun, Protective role of *Psoralea corylifolia* L. seed extract against hepatic mitochondrial dysfunction induced by oxidative stress or aging, *Evidence-Based Complementary and Alternative Medicine*, article ID 678028 (2013)
18. RK Chaudhuri, Bakuchiol: A retinol-Like functional compound, modulating multiple retinol and non-retinol targets, in *Cosmeceuticals and Active Cosmetics, 3rd Edition*, RK Sivamani, P Elsner and HI Maibach, eds, Taylor and Francis, Boca Raton (2015) *in press*
19. RK Chaudhuri, The miracle of retinol, *SPC* 23-24 (2010)
20. RK Chaudhuri, F Marchio and K Bojanowski, Retinol and retinol-like compounds in skin care, *Expression Cosmetique* 227-233 (2010)
21. RK Chaudhuri and K Bojanowski, Bakuchiol: A retinol-Like functional compound revealed by gene expression profiling and clinically proven to have anti-aging effects, *Intern J Cosmet Sci* 36(3) 221-230 (2014)
22. Commercial market introduction, *Sytheon Ltd.* (2007)
23. S Kang et al, Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation, *J Invest Dermatol* 105 549-556 (1995)
24. S Kang et al, Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation, *J Invest Dermatol* 105 549-556 (1995)
25. S Kang, EA Duell and GJ Fisher, Liarozole inhibits human epidermal retinoic acid 4-hydroxylase activity and differentially augments human skin responses to retinoic acid and retinol in vivo, *J Invest Dermatol* 107 183-187 (1996)
26. DE Hodak and NJ Lowe, Adverse effects of retinoids, *Med Toxicol Adverse Drug Exp* 3(4) 273-288 (1988)
27. S Kang et al, Long-term efficacy and safety of tretinoin emollient cream 0.05% in the treatment of photodamaged facial skin: A two-year, randomized, placebo-controlled trial, *Am J Clin Dermatol* 6 245-253 (2005)
28. N Mei, J Hu, Q Xia, M Moore and T Chen, Cytotoxicity and mutagenicity of retinol with ultraviolet A irradiation in mouse lymphoma cells, *Toxicol In vitro* 24(2) 439-444 (2010)
29. H Ihara, N Hashizume, N Hirase and R Suzue, Esterifcation makes retinol more labile to photolysis, *J Nutr Sci Vitaminol* 45 353-358 (1999)
30. N Mei, Q Xia, L Chen, MM Moore, PP Fu and T Chen, Photomutagenicity of retinyl palmitate by ultraviolet A irradiation in mouse lymphoma cells, *Toxicolog Sci* 88 142-149 (2005)
31. L Tesoriere, A Bongiorno, AM Pintaudi, R D'Anna, D D'Arpa and MA Livrea, Synergistic interactions between vitamin A and vitamin E against lipid peroxidation in phosphatidylcholine liposomes, *Arch Biochem Biophysics* 326(1) 5-63 (1996)
32. AM Young and G Gregoriadis, Photolysis of retinol in liposomes and its protection with tocopherol and oxybenzone, *Photochem Photobiol* 63(3) 344-352 (1996)
33. SC Lee, HG Yuk, DH Lee, KE Lee, YI Hwang and RD Ludescher, Stabilization of retinol through incorporation into liposomes, *J Biochem Mol Biol* 35(4) 358-63 (2002)
34. SC Lee, KE Lee, JJ Kim and SH Lim, The effect of cholesterol in the liposome bilayer on the stabilization of incorporated retinol, *J Liposome Res* 15(3-4) 157-166 (2005)
35. JP Jee, SJ Lim, JS Park JS and CK Kim, Stabilization of all-trans retinol by loading lipophilic antioxidants in solid lipid nanoparticles, *Eur J Pharm Biopharm* 63(2) 134-139 (2006)
36. S Sapino, ME Carlotti, R Cavalli, M Trota, F Trotta and D Vione, Effect of akyl-c-cyclodextrins on the stability of retinol, *J Incl Phenom Macrocycl Chem* 57 451–455 (2007)
37. A Akhavan and J Levitt, Assessing retinol stability in a hydroquinone 4% retinol 0.3% cream in the presence of antioxidants and sunscreen under simulated-use conditions: A pilot study, *Clinical Therapeutics* 30(3) 543–547 (2008)
38. C Bonda and J Zhang, Photostabilization of retinol and retinyl palmitate by ethylhexyl methoxycrylene, *Cosm & Toil* 126(1) 40-48 (2011)
39. HK Cho, JH Cho, SW Choi and IW Cheong, Topical delivery of retinol emulsions co-stabilized by PEO-PCL-PEO triblock copolymers: Effect of PCL block length, *J Microencapsul* 29(8) 739-746 (2012)
40. DJ Anneken, S Roth, R Christoph, G Fieg, U Steinberner and A Westfechtel, Fatty acids, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim, Germany (2006)
41. WE Diezel, E Schulz, M Skanks and H Heise, Plant oils: Topical application and anti-inflammatory effects (croton oil test), *Dermatologische Monatsschrift* 173(179) (1993)
42. C Letawe, M Boone and GE Pierard, Digital image analysis of the effect of topically applied linoleic acid on acne microcomedones, *Clinical & Exp Derm* 23(2) 56–58 (1998)
43. GL Darmstadt et al, Impact of topical oils on the skin barrier: Possible implications for neonatal health in developing countries, *Acta Paediatrica* 91(5) 546–554 (2002)
44. DT Downing, ME Stewart, PW Wertz and JS Strauss, Essential fatty acids and acne, *J Am Acad Dermatol* 13 221-225 (1986)
45. H Ando, A Ryu, A Hashimoto, M Oka and M Ichihasi, Linoleic acid and alpha-linolenic acid lightens ultraviolet-induced hyperpigmentation of the skin, *Arch Derm Res* 290 375-381 (1998)
46. H Ando et al, Fatty acid regulate pigmentation via proteosomal degradation of tyrosinase: A new aspect of ubiquitin-proteosome function, *Biochem J* 394 43-50 (2004)
47. M Butler, N Huzel, N Barnabé, T Gray and L Bajno, Linoleic acid improves the robustness of cells in agitated cultures, *CytotecÚology* 30(1-3) 27-36 (1999)
48. MC Cosgrove, OH Franco, SP Granger, PG Murray and AE Mayes, Dietary nutrient intakes and skin-aging appearance among middle-aged American women, *Am J Clin Nutr* 86(4) 1225-1231 (2007)
49. BL Hungund, Z Zheng and AI Barkai, Turnover of ethyl-linoleate in rat plasma and its distribution in various organs, *Alcohol Clin Exp Res* 19(2) 374-377 (1995)
50. A Charakida, M Charakida and AC Chu, Double-blind, randomized, placebo-controlled study of a lotion containing triethyl citrate and ethyl linoleate in the treatment of *Acne vulgaris, Brit J Dermatol* 157(3) 569-574 (2007)
51. SK Kim and F Karadeniz, Biological importance and applications of squalene and squalane, *Adv Food Nutr Res* 65 223-233 (2012)
52. M Spanova and G Daum, Squalene—Biochemistry, molecular biology, process biotecÚology and applications, *Eur J Lipid Sci TecÚolo* DOI: 10.1002/ejlt.01100203 (2011)

53. K Chiba, K Yoshizawa, I Makino, K Kawakami and M Onoue, Comedogenicity of squalene monohydroperoxide in the skin after topical application, *J Toxicological Sciences* 25 77–83 (2000)

54. K Chiba, T Sone, K Kawakami and M Onoue, Skin rougÚess and wrinkle formation induced by repeated application of squalene-monohydroperoxide to the hairless mouse, *Exper Dermatol* 8 471–9 (1999)

55. B Halliwell and S Chirico, Lipid peroxidation: Its mechanism, measurement and significance, *Am J Clin Nutr* 57(5) 715S-724S (1993)

56. RJ Nijveldt, E van Nood, DEC van Hoorn, PG Boelens, K van Norren and PA van Leeuwen, Flavonoids: A review of probable mechanisms of action and potential applications, *Am J Clin Nutr* 74(4) 418-425 (2001)

57. DA Pratt, KA Tallman and NA Porter, Free radical oxidation of polyunsaturated lipids: New mechanistic insights and the development of peroxyl radical clocks, *Acc Chem Res* 44(6) 458–467 (2011)

58. H Esterbauer, RJ Schaur and H Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, *Free Radical Biology and Medicine* 11(1) 81–128 (1991)

59. H Esterbauer, Cytotoxicity and genotoxicity of lipid-oxidation products, *Am J Clin Nutr* 57 779S–786S (1993)

60. Q Xia et al, Photo irradiation of retinyl palmitate in ethanol with ultraviolet light—Formation of photodecomposition products, reactive oxygen species and lipid peroxides, *Int J Envron Res Public Health* 3(2) 185-190 (2006)

61. G Aldini, P Granata, C Marinello, G Beretta, M Carini and RM Facino, Effects of UVB radiation on 4-hydroxy-2-trans-nonenal metabolism and toxicity in human keratinocytes, *Chem Res Toxicol* 20 (3) 416–423 (2007)

62. NA Botsoglou, DJ Fletouris, GE Papageorgiou, VN Vassilopoules, AJ Mantis and AG Trakatellis, Rapid, sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food and feedstuff samples, *J Agric Food Chem* 42 1931-1937 (1994)

63. V Nardello, S Bogaert, PA Alsters and JM Aubry, Singlet oxygen generation from $H_2O_2$2/MoO 4 2-: Peroxidation of hydrophobic substrates in pure organic solvents, *Tet letters* 43(48) 8731-8734 (2002)

64. E Niki, Y Yoshida, Y Saito and N Noguchi, Lipid peroxidation: Mechanisms, inhibition and biological effects, *Biochemical and Biophysical Research Communications* 338(1) 668-676 (2005)

65. K Ohsawa, T Watanabe, R Matsukawa, Y Yoshimura and K Imaeda, The possible role of squalene and its peroxide of the sebum in the occurrence of sunburn and protection from the damage caused by UV irradiation, *J Toxicol Sci* 9(2) 151-159 (1984)

66. ES Mudiyanselage, M Hamburger, P Elsner and JJ Thiele, Ultraviolet a induces generation of squalene monohydroperoxide isomers in human sebum and skin surface lipids in vitro and in vivo, *J Invest Dermatol* 120(6) 915-922 (2003)

67. A Ryu, K Arakane, C Koide, H Arai and T Nagano, Squalene as a target molecule in skin hyperpigmentation caused by singlet oxygen, *Biol Pharm Bull* 32(9) 1504-1509 (2009)

68. WP Bowe and AC Logan, Clinical implications of lipid peroxidation in *Acne vulgaris*: Old wine in new bottles, *Lipids Health Dis* 9 141 (2010)

69. S Tucker-Samaras, T Zedayko, C Cole, D Miller, W Wallo and JJ Leyden, A stabilized 0.1% retinol facial moisturizer improves the appearance of photodamaged skin in an eight-week, double-blind, vehicle-controlled study, *J Drugs Dermatol* 8(10) 932-936, (2009)

70. GJ Smith, Reaction of retinol and retinal with singlet oxygen, *Photochem Photobiol* 38(2) 119-121 (1983)

71. C Antille, C Tran, O Sorg, P Carraux, L Didierjean and JH Saurat, Vitamin A exerts a photoprotective action in skin by absorbing ultraviolet B radiation, *J Invest Dermatol* 121 1163-1167 (2003)

72. J Dillon, ER Gaillard, P Biski, CF Chignell and KJ Reszka, The photochemistry of retinoids as studied by steady-state and pulsed methods, *Photochem Photobiol* 63 690-686 (1996)

73. O Sorg, C Tran, P Carrau, L Didierjean, F Fason and JH Saurat, Oxidative stress independent depletion of epidermal vitamin A by UVA, *J Invest Dermatol* 118 513-518 (2002)

74. SH Cherng et al, Photodecomposition of retinyl palmitate in ethanol by UVA light-formation of photodecomposition products, reactive oxygen species and lipid peroxides, *Chem Res Toxicol* 18 129-138 (2005)

75. F Klamt, F Dal-Pizzol, EA Bernard and JC Moreira, Enhanced V-mediated free radical generation; DNA and mitochondrial damage caused by retinol supplementation, *Photochem Photobiol Sci* 2 856-860 (2003)

76. PP Fu et al, Photodecomposition of vitamin A and photobiological implications for the skin, *Photochem Photobiol* 83 409–424 (2007) C&T

This publication is dedicated to the memory of Dr. Boxin Ou who passed away in May 2015 at a very young age of 49. Boxin was an accomplished and well-known person in his area of expertise. He has been credited in developing widely used ORAC assay for antioxidant testing. Boxin was cited recently by Thompson Reuters as “the world’s most influential scientific minds 2014”. He was founding scientist of Brunswick Laboratories, Southborough, MA and founder of International Chemistry Testing Inc., Milford, MA. You may want to contact Dr. Tony Chang at tonyz@ichemtesting.com for your analytical tests in order to keep Boxin’s legacy alive.

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