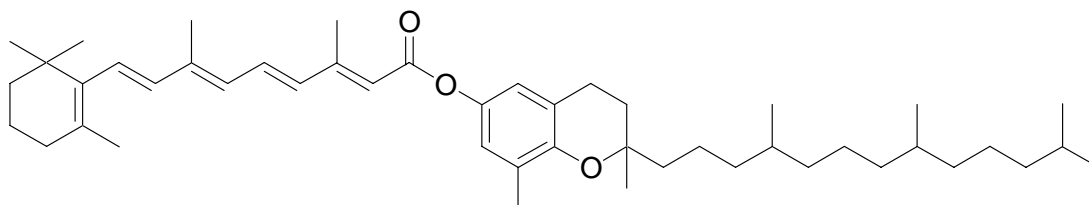


Vitamin-derived Ester with Excellent Anti-aging Properties

NIKKOL Tocoretinate-10



INCI name: Caprylic/Capric Triglyceride (and) Tocopheryl Retinoate

NIKKOL Tocoretinate-10 is a caprylic/capric triglyceride solution containing 10% of an ester of retinoic acid and a natural vitamin E that consists mainly of *d-δ*-tocopherol. *D-δ*-tocopherol has the strongest anti-oxidant property among all types of vitamin E, while retinoic acid is known for its effect in reducing wrinkles caused both by intrinsic aging and photoaging. NIKKOL Tocoretinate-10 is proved to be a safe ingredient with excellent promotional effects in collagen synthesis and active oxygen scavenging activities. NIKKOL Tocoretinate-10 is, therefore, an extremely useful ingredient for anti-aging cosmetics aiming mainly at wrinkle reduction.

Properties of NIKKOL Tocoretinate-10

- Reduces skin wrinkles.
- Promotes collagen synthesis.
- Scavenges active oxygen species.
- Does not release retinoic acid due to the resistance to breakdown by esterase.
- Low skin irritation potential.

Composition/Description of NIKKOL Tocoretinate-10

- Composition: Caprylic/Capric Triglyceride 89 (w/w%)
d-δ-Tocopheryl retinoate 10
d-δ-Tocopherol (as anti-oxidant) 1

- Description: Yellow to brown liquid with a faint characteristic odor

Effects of NIKKOL Tocoretinate-10 and its components

1. Singlet Oxygen Scavenging Ability

Various types of skin damage is caused by UV exposure. One such damage is the production of active oxygen species, which decrease collagen synthesis and promote the production of enzymes causing collagen breakdown.

Singlet oxygen scavenging ability of *d*- δ -Tocopheryl retinoate, a main component of NIKKOL Tocoretinate-10, was evaluated through the spin-trapping method using Electron Spin Resonance (ESR). As shown in Figure 1, *d*- δ -Tocopheryl retinoate has this ability, which is dependant on its concentration.

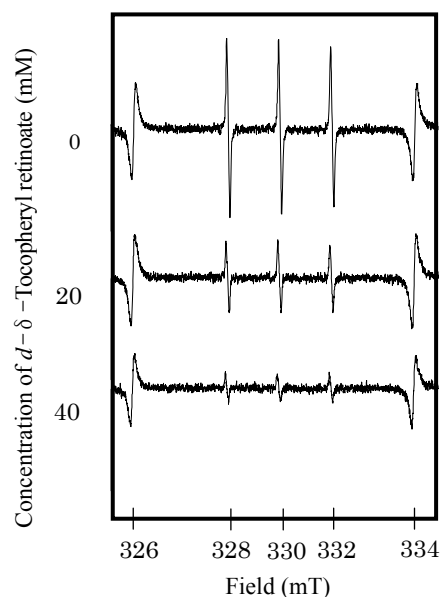


Fig. 1 Singlet Oxygen Scavenging Ability

2. Prevention of Cell Damage Caused by Lipid Peroxide

Active oxygen species produce lipid peroxides that cause cell damage and promote aging. The inhibitory effect of *d*- δ -Tocopheryl retinoate on cell damage was evaluated with human keratinocytes. *D*- δ -Tocopheryl retinoate, *d*- δ -tocopherol and retinoic acid, at different concentrations, were added to human keratinocytes, respectively. After a 24-hour incubation, *tert*-butyl peroxide, a lipid peroxide, was exposed to each sample for 5 hours, and then cell survival rate of each sample was determined through a NR assay. As shown in Figure 2, *d*- δ -Tocopheryl retinoate inhibited cell damage caused by *tert*-butyl peroxide at the same level as *d*- δ -tocopherol and retinoic acid.

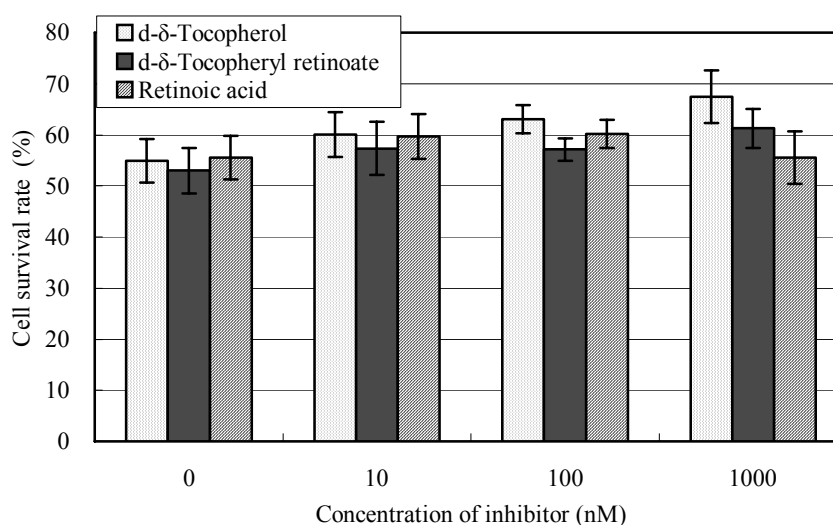


Fig. 2 Prevention of cell damage caused by lipid peroxide

3. Promotion of Hyaluronic Acid Synthesis

NIKKOL Tocoretinate-10 contains an ester of retinoic acid that has been reported to promote the production of hyaluronic acid, a natural moisturizing factor in the skin. In this experiment, *d*- δ -Tocopheryl retinoate was added to human keratinocytes, at various concentrations. After a 24-hour incubation, the amount of hyaluronic acid released in the medium was measured through the ELISA method. As shown in Figure 3, *d*- δ -Tocopheryl retinoate significantly increased the amount of hyaluronic acid in the medium. This demonstrates NIKKOL Tocoretinate-10 can promote hyaluronic acid synthesis.

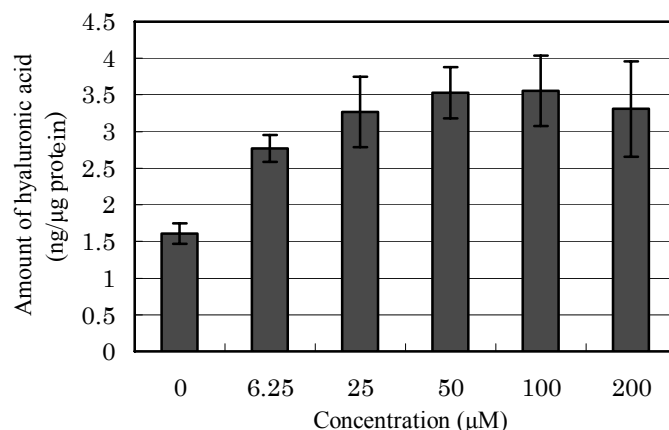
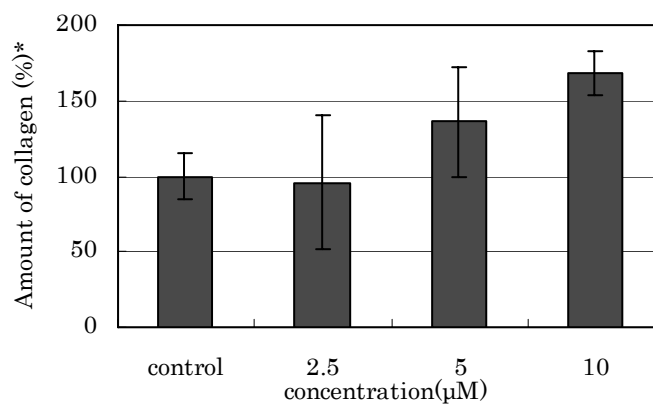


Fig. 3 Ability to promote hyaluronic acid synthesis

4. Promotion of Collagen Synthesis

D- δ -Tocopheryl retinoate was added to human fibroblasts, at various concentrations. After a 48-hour incubation, the amount of collagen released in the medium was measured through the ELISA method. The result was indicated in percent figures, providing the amount of collagen released from a control without *d*- δ -Tocopheryl retinoate represents 100%. As shown in Figure 4, *d*- δ -Tocopheryl retinoate increased the amount of collagen released in the medium. This means that NIKKOL Tocoretinate-10 promotes collagen synthesis.



*:Providing the amount of collagen released from the reference (containing no *d*- δ -Tocopheryl retinoate) represents 100%.

Fig. 4 Ability to promote collagen synthesis

5. Inhibition of Production of Collagen Degrading Enzyme

D- δ -Tocopheryl retinoate (TR) was added at various concentrations to human keratinocyte HaCaT cells. As a control, human keratinocyte HaCaT cells without *d*- δ -Tocopheryl retinoate were used. After a 24-hour cultivation, cells were irradiated by UVA rays and incubated for another 24 hours. Then, the expression of two types of collagen degrading enzymes, MMP-2 and MMP-9, released in the medium was determined by zymography (Figure 5). Furthermore, MMP-9 expression was determined by the RT-PCR (Reverse Transcription-Polymerase Chain Reaction) method (Figure 6). These results show that *d*- δ -Tocopheryl retinoate (TR) inhibited the production of UVA-induced MMP.

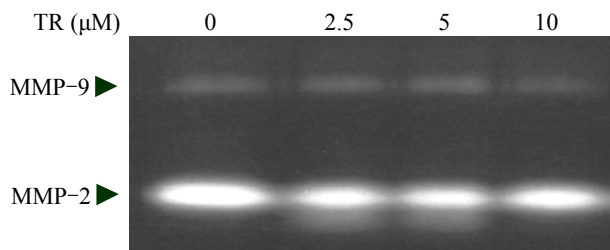


Fig. 5 MMP-2 and MMP-9 expression detected by zymography

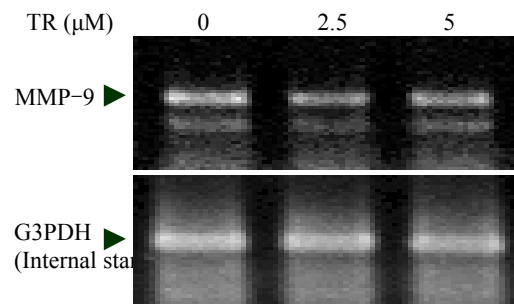


Fig. 6 MMP-9 expression detected by RT-PCR

6. Reduction of Skin Wrinkles

In 9 volunteers, a cream containing 1% of NIKKOL Tocoretinate-10 was applied to the outer corner of one eye and a placebo cream without NIKKOL Tocoretinate-10 was applied to the outer corner of the other eye twice a day for 90 days. On the 90th day, the wrinkle depth and the area where wrinkles were found were measured. As a result, wrinkle depth and wrinkle area were significantly reduced in 5 and 6 of the 9 volunteers, respectively. NIKKOL Tocoretinate-10 was proved to be effective in reducing skin wrinkles. Figure 7 shows photos of a volunteer's skin taken before and after the application of creams.

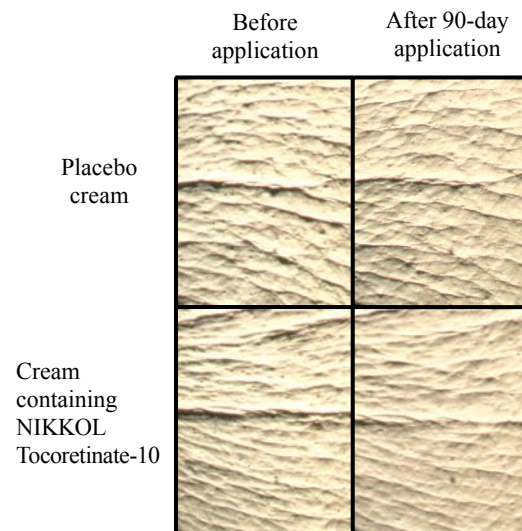


Fig. 7 Reduction of wrinkles at the corner of eyes

Stability of NIKKOL Tocoretinate-10

1. Stability over time

NIKKOL Tocoretinate-10 was stored in a dark place for 6 months at 5°C, room temperature (about 23°C), and 45°C, and the content of tocopheryl retinoate in it was measured at after a half-month, 1 month, 2 months, 3 months and 6 months. As shown in Table 1, the content of Tocopheryl Retinoate in NIKKOL Tocoretinate-10 hardly changed after being stored for 6 months at 5°C and room temperature.

Table 1 Stability of NIKKOL Tocoretinate-10

| Tocopheryl Retinoate content (%) / Storage Temperature | Initial value | 0.5 month | 1 month | 2 months | 3 months | 6 months |
|--|---------------|-----------|---------|----------|----------|----------|
| 5°C | 10.0 | 10.2 | 10.8 | 10.8 | -- | 10.1 |
| approx. 23°C (room temp.) | 10.0 | 10.6 | 10.7 | 10.8 | - | 10.0 |
| 45°C | 10.0 | 10.2 | 10.5 | 10.1 | 9.6 | 9.0 |

2. Resistance to breakdown by esterase

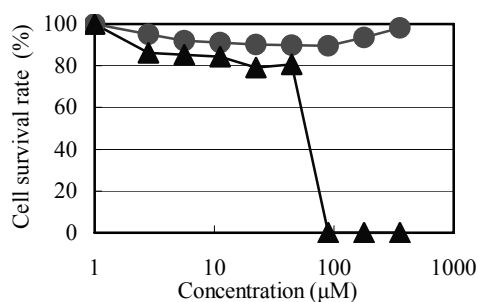
Pig liver esterase was added to *d*- δ -Tocopheryl retinoate and then retinoic acid released in the *d*- δ -Tocopheryl retinoate sample was detected. As a result, the amount of retinoic acid in the sample was under the lower detection limit. This proves that *d*- δ -Tocopheryl retinoate was hardly broken down by the enzyme esterase.

Safety of NIKKOL Tocoretinate-10 and its components

1. Cytotoxicity test

D- δ -Tocopheryl retinoate and retinoic acid, at various concentrations, were individually added to human keratinocytes (a) and human fibroblasts (b). Twenty-four hours after addition, the cell survival rate in each sample was measured through a NR assay. As shown in Figure 8, cell survival rate in samples containing *d*- δ -Tocopheryl retinoate were considerably high while retinoic acid showed strong cytotoxicity in both keratinocytes and fibroblasts.

(a) Human keratinocytes



(b) Human fibroblasts

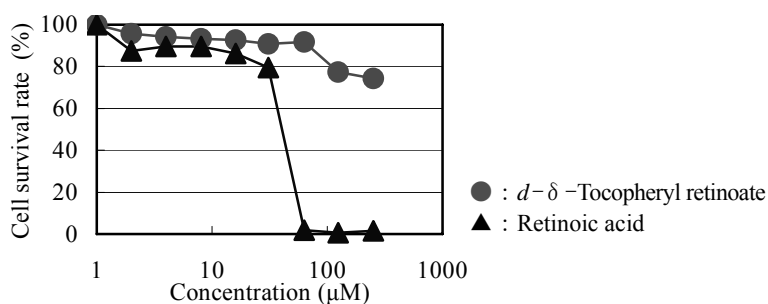


Fig. 8 Cytotoxicity test

2. Safety Data

- Primary skin irritation*: No irritation (2.5% , human, closed patch)
- Eye irritation**:
- Sensitization*:
- Phototoxicity**:
- Photo Sensitization*:
- Mutagenicity**:

*: Examination was conducted with a caprylic/capric triglyceride solution containing *d*- δ -tocopherol at the specified concentration.

** : Examination was conducted with undiluted *d*- δ -tocopherol.

Instructions for Use of NIKKOL Tocoretinate-10

Please note the following instructions when storing and making preparations with NIKKOL Tocoretinate-10 because prolonged exposure to light, oxygen and heat may cause breakdown of NIKKOL Tocoretinate-10.

- Store this product in a tightly closed container in the fridge and use quickly after opening.
- Avoid heating for long periods and avoid contact with air as much as possible when making preparations.
- Place the preparation in a light-resistant container with a narrow opening to minimize contact with air and light.
- It is recommended to use this product for cosmetics used at night. Do not use this product in preparations to be exposed to direct sunlight, for example sunscreens or makeup preparations.
- After using a day treatment preparation containing this product, it is required to apply another preparation with UV-blocking properties to avoid UV damage to this product in the treatment preparation.
- The recommended usage of NIKKOL Tocoretinate-10 is 1.0 – 2.5%. Avoid exceeding maximum recommended usage.

Formulations

Eye Treatment Gel

| | | |
|---|--|------------------|
| A | NIKKOL Tocoretinate-10 | 2.0 (wt%) |
| | NIKKOL Lecinol S-10 (Hydrogenated Lecithin) | 1.0 |
| | NIKKOL Olive Squalane | 10.0 |
| | NIKKOL Pentarate-408 (Pentaerythrityl Tetraethylhexanoate) | 6.0 |
| | Propylparaben | q.s. |
| B | Pemulen TR-1 (Acrylates/C10-30 Alkyl Acrylate | |
| | Crosspolymer, Noveon) (2% aq. sol.) | 15.0 |
| | Xanthan Gum (2% aq. sol.) | 10.0 |

| | | |
|---|----------------------------------|------------|
| | Water | 14.5 |
| C | Sodium Hyaluronate (1% aq. sol.) | 1.0 |
| | 1,3-Butylene Glycol | 7.0 |
| | Methylparaben | q.s. |
| | Water | q.s. 100.0 |
| D | Arginine | 0.2 |
| | Water | 4.5 |

Procedure

Heat A, B and C separately to 80°C to dissolve homogeneous. Add C to B and mix. While stirring BC, add A and keep stirring to cool. At 50°C, add D to ABC and cool to 35°C while stirring.

Eye Contour Cream 1

| | | |
|---|---|------------------|
| A | NIKKOL Tocoretinate-10 | 2.0 (wt%) |
| | NIKKOL Lecinol S-10 (Hydrogenated Lecithin) | 0.5 |
| | NIKKOL Decaglyn 1-M (Polyglyceryl-10 Myristate) | 3.0 |
| | NIKKOL Selachyl Alcohol (Oleyl Glyceryl Ether) | 2.0 |
| | NIKKOL N-SPV (Cetyl Palmitate) | 4.0 |
| | NIKKOL Olive Squalane | 10.0 |
| | NIKKOL Trifat S-308 (Triethylhexanoin) | 8.0 |
| | Cetanol | 6.0 |
| | Propylparaben | q.s. |
| B | Methylparaben | q.s. |
| | Glycerin | 3.0 |
| | Xanthan Gum (2% aq. sol.) | 10.0 |
| | Water | q.s. 100.0 |

Procedure

Heat A and B separately to 80°C to dissolve homogeneous. While stirring A with a homomixer, add B to emulsify. Keep stirring the mixture to cool to 35°C.

Eye Contour Cream 2

| | | |
|---|--|------------------|
| A | NIKKOL Tocoretinate-10 | 1.0 (wt%) |
| | NIKKOL MGS-DEXV (Glyceryl Stearate and Glyceryl Stearate SE and PEG-10 Stearate) | 0.5 |
| | NIKKOL BC-25TX (Ceteth-25) | 1.5 |
| | NIKKOL Olive Squalane | 8.0 |
| | NIKKOL Triester F-810 (Caprylic/Capric Triglyceride) | 7.0 |
| | Cetanol | 5.0 |
| | Propylparaben | q.s. |
| B | Methylparaben | q.s. |
| | 1,3-Butylene Glycol | 1.5 |

| | | |
|---|---------------------------|------------|
| | Water | q.s. 100.0 |
| C | Xanthan Gum (2% aq. sol.) | 10.0 |
| | Water | 10.0 |
| | Disodium EDTA | 0.2 |

Procedure

Heat A and B separately to 80°C to dissolve homogeneous. While stirring A with a paddle mixer, add B to emulsify. Keep stirring AB to cool and add C at 50°C. Cool the mixture to 35°C while stirring.

Skin Lotion

| | | |
|---|--|------------------|
| A | NIKKOL Tocoretinate-10 | 0.2 (wt%) |
| | NIKKOL HCO-40 (PEG-40 Hydrogenated Castor Oil) | 1.5 |
| | 1,3-Butylene Glycol | 5.0 |
| | Diplopylene Glycol | 5.0 |
| | Methylparaben | q.s. |
| B | Sodium Hyaluronate (1% aq. sol.) | 5.0 |
| | Water | q.s. 100.0 |

Procedure

Heat A to 70°C to dissolve homogeneous. While stirring A at room temperature, add B in small portions to solubilize.

We do not guarantee the stability of the final products of formulations in this brochure though the stability was checked at 45°C for 4 weeks.

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