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Synthesis and in vitro biological activity of retinyl polyhydroxybenzoates, novel hybrid retinoid derivatives

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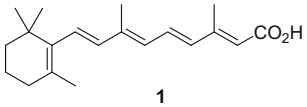
ABSTRACT

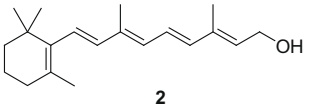
A new hybrid derived from retinol was designed to improve the stability and anti-oxidant activity of retinol and also to add whitening properties besides its usual anti-aging properties. A variety of polyhydroxybenzoates of retinol were prepared either by base-catalysis or by direct esterification of retinol and screened for such desirable properties by analyzing the in vitro biological activity of the hybrids. Some of the retinol derivatives enhanced their thermal stability and decreased photosensitivity, and exhibited an activity in collagen synthesis similar to that of retinol. In addition, the retinyl gallate **6** showed higher activities in free radical scavenging and melanogenesis inhibition than retinol. Thus, owing to its excellent stabilities, retinyl gallate **6** may be conveniently used not only as an additive for cosmetics for prevention and improvement of skin aging and whitening but also as medicine for the treatment of skin troubles.

The irreversible photo-aging of skin, which is a principal cause of skin aging,[1,2] activates cell surface growth factors and cytokine receptors. More specifically, UV irradiation stimulates the transcription factor activator protein 1 (AP-1), which regulates the expression of matrix-metalloproteinase (MMP) to degrade skin collagen. Thus, photoaging is associated with increased AP-1 activity, increased MMP expression, enhanced collagen degradation, and decreased collagen synthesis, all of which result in changes within the matrix of the dermis and at the dermal–epidermal junction.[3–7]

Retinoids bind to nuclear receptors to activate the transcription of specific DNA sequences, which results in modulation of gene expression. Thanks to this ability to modulate genes involved in cellular differentiation and proliferation, retinoids are good candidates for both treating and preventing the photoaging process. Indeed, retinoids such as *trans*-retinoic acid (RA) **1**[8–14] and more tolerable retinol **2**[15] have been the agents of choice to repair the skin damaged by chronological aging or photoaging. However, the fat-soluble RA causes side effects such as skin irritation, skin dryness, wounds, and scraping[16–19] attributed to the carboxyl end group in RA.[20–22] Many efforts have been made to modify the structure of RA. Unfortunately, however, these retinoids are unstable towards light, oxygen, heat, peroxides, acid or water.[23]



1
trans-retinoic acid (RA)



2
retinol

Herein we tried to modify retinol to enhance the stability of the parent retinoid by attaching a chemically stable moiety for intramolecular energy transfer, by which the retinoid part would remain intact. (Scheme 1)[24] Additionally, while maintaining the usual collagen-enhancing properties of retinoids, one might be able to add, by proper choice of the attachment G, such new biologically favorable properties as potent anti-oxidant activity and whitening activity for the reasons stated below, which cannot be expected from the unmodified retinoids.

The formation of advanced glycosylation end-products (AGEs) that darkens the skin with their color produces dimerized imidazolium products. It is thus one of the causes of skin aging triggered by reactive oxygen species (ROS) besides the usual chronological production (Scheme 2)[25–28]. Consequently, it follows that blocking the formation of ROS may contribute to slowing of the skin aging processes.

Moreover, the formation of melanin, which is the main source of skin pigmentation, is the result of 3 consecutive enzymatic oxidation reactions (Scheme 3)[29–31]. Thus, appropriate anti-oxidants are expected to provide tools for reducing the rate of skin aging and also of pigmentation.

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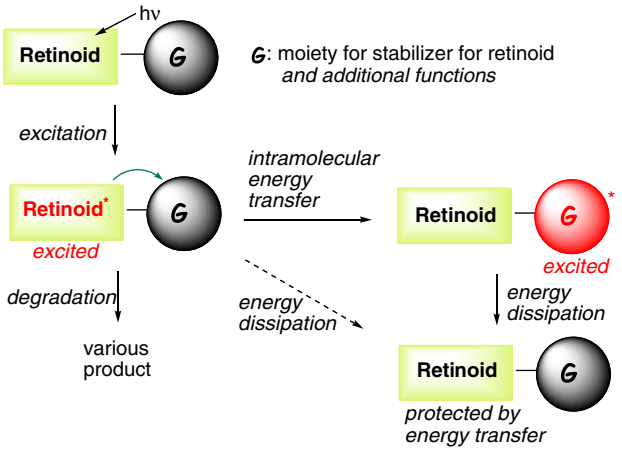
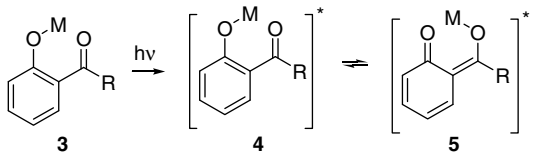


Scheme 1. Schematic explanation of internal energy transfer to the modifying group G for stabilization of retinol.

After a considerable amount of work, polyhydroxybenzoates were selected as the stabilizing attachment G: the choice was based on the many reports that polyhydroxybenzoates, especially the *o*-hydroxyaroyl groups, dissipate the excess energy by excited-state intramolecular proton-transfer (Scheme 4; M = H).[32,33]

Furthermore, the salicylic acid derivatives[34] and/or other polyhydroxybenzoates[35,36] are known to capture ROS by themselves, and thus known to be potent anti-oxidants. Moreover, these benzoates can bind metals (Scheme 4, M = metal) and the resulting chelates behave as superoxide dismutase and catalase[37–39] and thus behave as a scavenger of Fenton-active Fe which produces the biologically damaging hydroxyl radical. All these indications point to the possibility that the hybrid between retinol and polyhydroxybenzoic acid could be used for treatment of skin aging and pigmentation at the same time.



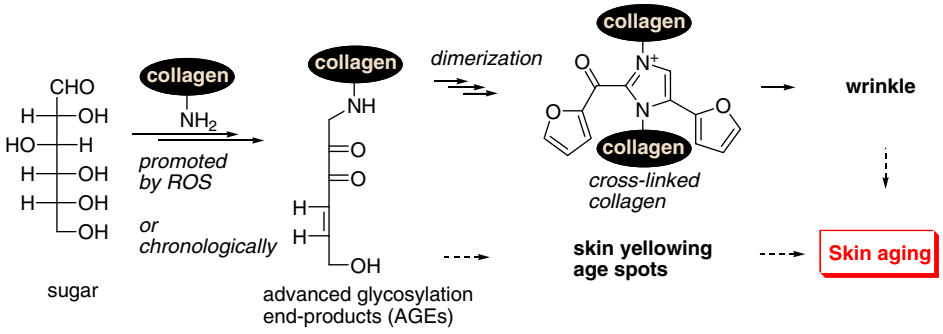
Scheme 4. Energy dissipation by excited-state intramolecular transfer of proton and metal.

The retinyl salicylates **4** were prepared in 25–40% yields by the base-catalyzed reaction (90 °C, 1–2 h) of retinol **2** with cyanomethyl salicylates **3**, which were prepared from the corresponding salicylic acid and $ClCH_2CN$ in the presence of potassium carbonate in dimethyl acetamide at reflux.[40] On the other hand, the corresponding gallate **6** was prepared in 41% yield directly from retinol **2** and the carboxylic acid **5** in THF in the presence of DCC and a catalytic amount of DMAP (Scheme 5).

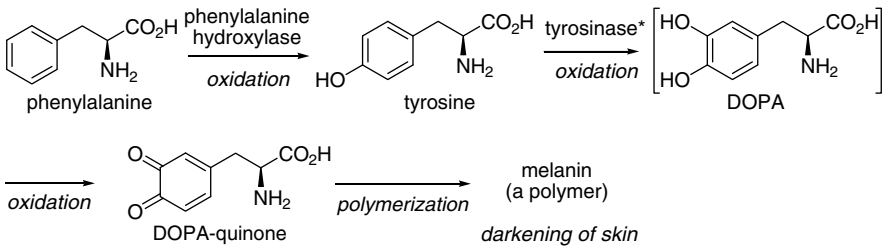
Thus, the following 13 retinyl polyhydroxybenzoates, **6**–**18**, were prepared by these routes (Fig. 1), among which salicylates **7** and **8** have already been reported to possess an analgetic, anti-inflammatory, anti-thrombotic and anti-pyretic effect.[41]

The 13 retinyl polyhydroxybenzoates, **6**–**18**, were screened for formation of procollagen (anti-aging activity) and % inhibition of melanin formation (whitening activity). In order to evaluate the activities of the compounds, the relative activities were calculated as % of the references, retinol and resveratrol, respectively.

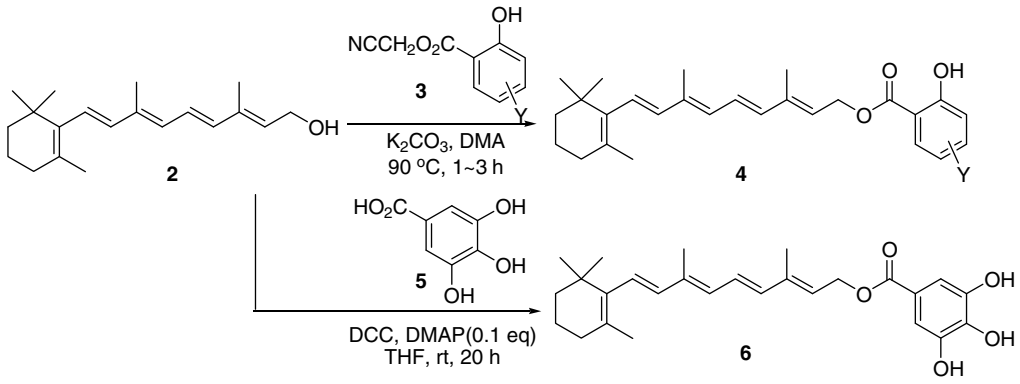
As can be seen in Table 1, all compounds except for **10** and **17** showed an acceptable level of cell toxicity, and the salicylates **7** and **8** exhibited activities inferior to the rest of the compounds. Thus, by and large, both the anti-aging and the whitening activities of each compound increased at the increase of the electron density of the aromatic ring (and thus the increase of oxidizability), which supports our initial assumption that anti-oxidant activity is essential for anti-aging and whitening of the skin.



Scheme 2. Generation of AGE and cross-linked collagen by reactive oxygen species.



Scheme 3. The route to melanin by three consecutive oxidation reactions and polymerization starting from phenylalanine.



Scheme 5. Preparation of retinyl polyhydroxybenzoates.

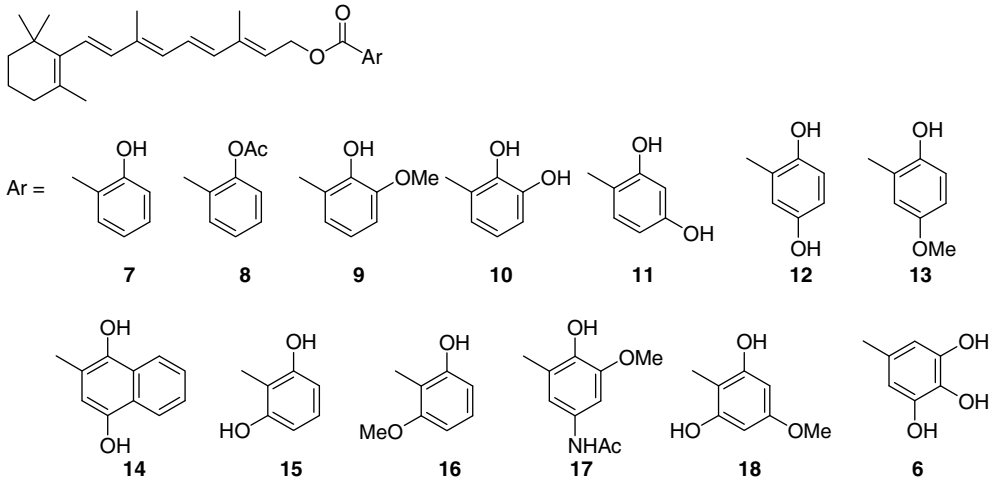


Figure 1. Representative retinyl polyhydroxybenzoates prepared in this study.

Table 1
Relative activities of the retinyl polyhydroxybenzoates by crude screening (relative activities) for anti-aging and whitening activities

Compound	Cell toxicity, IC_{50}, mM	Collagen assay[a] Ref: Retinol (0.0005%)	Melanogenesis inhibition[b] Ref: Resveratrol (0.001%)
7	0.15	46% (0.002%)	34% (0.002%)
8	0.15	61% (0.002%)	32% (0.002%)
9	0.027–0.057	116% (0.0005%)	22% (0.001%)
10	<<0.01	66% (0.0001%)	105% (0.001%)
11	0.12	26% (0.001%)	41% (0.001%)
12	0.014~0.028	55% (0.0005%)	95% (0.001%)
13	0.057~0.115	81% (0.001%)	30% (0.001%)
14	0.42~0.85	37% (0.0005%)	18% (0.001%)
15	0.014~0.034	102% (0.0005%)	112% (0.001%)
16	0.23	54% (0.005%)	28% (0.001%)
17	<<0.01	15% (0.001%)	—
18	0.014–0.028	55% (0.0005%)	95% (0.001%)
6	0.035	91% (0.0002%)	73% (0.0002%)

[a] Expression rate of procollagen as % of the reference, retinol. The values in parentheses refer to weight % concentration.
[b] Percentage inhibition rate of melanin formation as % of the reference, resveratrol. The values in parentheses refer to weight % concentration.

From these results, the six retinyl esters, **9**, **10**, **12**, **15**, **18** and **6**, were selected, despite some cell toxicity of **10**, and each of them was scrutinized closely for the both activities of anti-aging and whitening.

Although the precise experimental set-ups for close examinations were different, the trends of the compounds in anti-aging activities (formation of procollagen type I and inhibition of MMP-1) (Table 2) and whitening activities (inhibition rate of tyrosinase and melanin formation) (Table 3) were almost the same as in the

Table 2
Effects of 6 anti-aging candidates on cell viability, procollagen type I and MMP-1 in normal human dermal fibroblasts

Sample	Final concn	Percentage of negative control[a]		
		Cell viability	Procollagen type I[c]	MMP-1[d]
9	10 μM	125.9	70.0	16.9
10	1 μM	71.8	NT[e]	NT[e]
12	10 μM	91.8	78.7	−32.2
15	1 μM	88.1	85.5	−63.0
18	10 μM	92.6	89.2	−17.4
6	5 μM	84.2	115.9	−19.3
L-ascorbic acid[b]	200 μM	95.4	127.5	NT
Retinoic acid[b]	10 μM	105.2	NT	59.2
(−)Epigallocatechin gallate[b]	10 μM	95.2	NT	46.7
DMSO[a]	1%	100	100	0

[a] Dimethyl sulfoxide (DMSO) was used as a negative control.
[b] Positive controls.
[c] Expression rate of procollagen type I.
[d] Inhibition rate of MMP-1 expression.
[e] NT, not tested.

Table 3
Effects of 6 whitening candidates on cell viability, tyrosinase activity and melanin formation in cultured B16/F10 melanoma cells

No.	Concn (μM)	Cell viability[a]	Tyrosinase activity[b]	Melanin formation[c]
DMSO[d]	0	100	0	0
9	5 μM	107.1	38.1	16.2
10	5 μM	97.0	36.7	18.6
12	5 μM	99.7	31.3	25.8
15	5 μM	102.5	45.4	12.7
18	5 μM	102.0	41.9	9.5
6	5 μM	83.9	49.6	27.5
Melasolv[e]	10 ppm	95.4	46.9	39.3

[a] Percentage of negative control.[d]
[b] Percentage inhibition rate of tyrosinase activity.
[c] Percentage inhibition rate of melanin formation.
[d] Dimethyl sulfoxide (DMSO) was used as a negative control.
[e] Trade name for thymyl 3,4,5-trimethoxycinnamate (Amore-Pacific) which was used as a positive control.

crude experiments (Table 1). Thus, among the three compounds (**15**, **18**, and **6**) which showed good activities in anti-aging and whitening, the gallate **6** seemed to be the best candidate.

Subsequently, retinyl gallate **6** was compared with other commonly accepted agents for skin troubles.[42,43] First of all, it is less cell-toxic than retinol, and its free radical scavenging activity was truly outstanding as expected. Although the degree of promotion of collagen synthesis by **6** was less than a half, it was more effective in inhibiting elastase, which cleaves elastin, an elastic fiber that determines the mechanical properties of connective tissue. Moreover, retinyl gallate **6** was shown to be potentially a good whitening agent as its activities toward inhibition of tyrosinase and melanogenesis were better than that of arbutin but less than that of resveratrol (Table 4).

To compare the time-dependent thermal stability of retinyl gallate **6** with retinol **2**, the phase stability in a thermohygrostat (humidity: 58%) at 40 °C *without exclusion of atmospheric oxygen* was examined for 4 weeks by a quantitative HPLC analysis. As shown in Figure 2, almost a half of retinyl gallate **6** decomposed after 4 weeks under this condition, while the thermal stability of retinol was much less than that of retinyl gallate with almost 70% of the material decomposed. These results clearly indicate that the new hybrid retinyl polyhydroxylbezoate **6** is much more stable than retinol itself (Fig. 2).

Retinyl gallate **6** has shorter absorption maxima at 260 and 295 nm than the other retinyl polyhydroxybenzoates (λ_{max} 325–345 nm), gallic acid (λ_{max} 275 nm) and retinol (λ_{max} 323 nm) (Fig. 3). Consequently, retinyl gallate **6** is able to absorb light in the more damaging UVB region (wavelength 290–315 nm) and thus able to protect the skin from photo-aging, although such utility would seem to have a rather slim chance in reality because the concentration of the active ingredient as an anti-aging and/or whitening agent is going to be rather low (~100 μM).

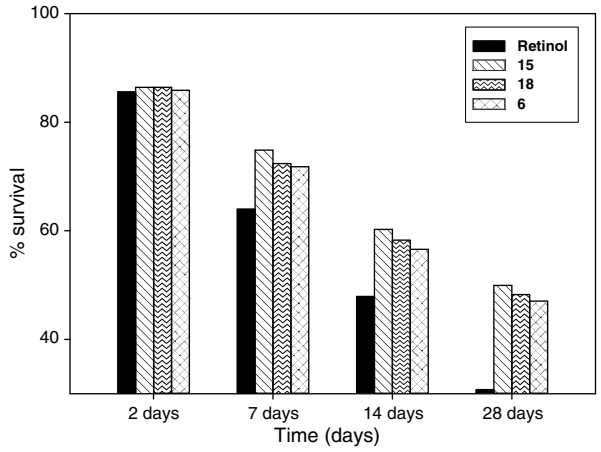


Figure 2. Thermal stability test at 40 °C.

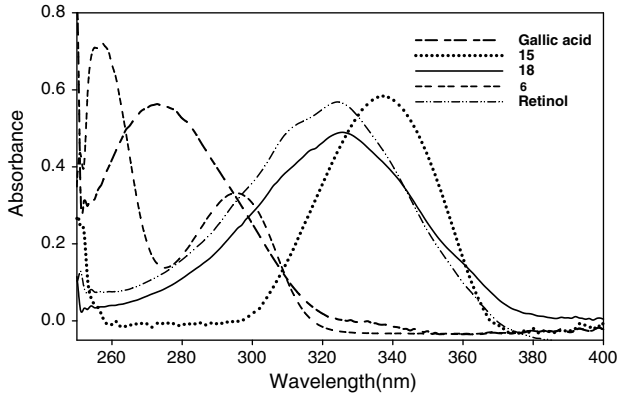


Figure 3. UV spectra of retinoids (concentration: 2.5×10^{-4} M (MeOH)).

In conclusion, we have discovered retinyl gallate **6** as a new class of retinyl polyhydroxybenzoate anti-oxidant that exhibits not only retinoid activity but also melanin inhibition effect.

Acknowledgments

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Table 4
Summary of biological activity screening results of retinyl gallate **6**

Activities	Concn (μM)	**6**	Retinol	Ursolic acid	Resveratrol	Arbutin	Ascorbic acid
Cell toxicity (IC_{50}, μM)		35	50	3.5	900	—	—
Free radical scavenging activity (%)	5	31.1	—	—	5.6	—	19.1
	10	62.8	—	—	16.7	—	38.6
Increase of collagen synthesis (%)	5	13.5	33.4	—	—	—	—
Elastase inhibitory activity (%)	5	12.3	—	10.3	—	—	—
	10	15.3	—	14.1	—	—	—
Tyrosinase inhibitory activity (%)	5	2.5	—	—	36.2	−1.5	—
Inhibition of melanogenesis (%)	5	18.6	—	—	25.5	5.5	—

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