The Requirement for a Retinoic Acid Lactone of 11-*cis*, 13-*cis*-Stereochemistry for Topical Dermatologic Activity

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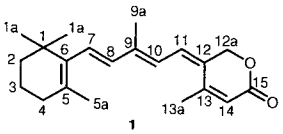
INTRODUCTION

Our evaluation of 12-substituted retinoids in several dermatological screens (1) demonstrated that the δ-lactone of 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid, (1), was effective in both the reduction of utricles and the inhibition of ornithine decarboxylase (ODC) in mouse skin. Additional investigation revealed that 1 had significant retinoid activity in several *in vivo* models (2) and produced no evidence of hypervitaminosis A (HVA) or systemic toxicity.

In order to establish whether these effects were unique to the lactone 1, two related lactones, the geometric isomer 2 and the positional isomer 3, along with the potential degradation products, the hydroxy acids 4 and 5, were investigated and compared to retinoic acid 6 and its 13-*cis*-isomer 7.



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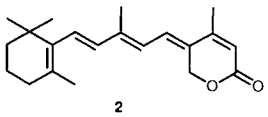

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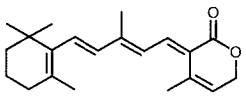

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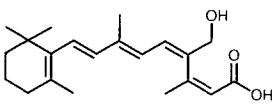

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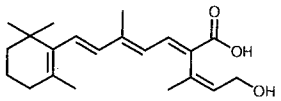

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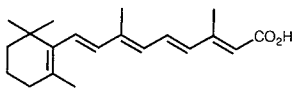

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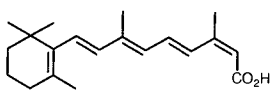

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MATERIALS AND METHODS

Chemistry

All syntheses were performed under dim red lights. Melting points were determined on a Thomas Hoover capillary tube apparatus. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as internal standard. Thin layer chromatography (TLC) was carried out on Whatman silica gel (SiO_2) 60 plates using 25% acetone/hexane with visualization by ultraviolet (UV) and/or iodine. High performance liquid chromatography (HPLC) was carried out using a system equipped with two M6000 Waters reciprocating pumps, septumless Waters U6K injector, automated gradient controller and Waters 490 UV detector in the maxplot mode recording at 20 nm intervals between 220 and 440 nm. UV spectra were recorded using a Varian 2290 spectrophotometer and mass spectrometry was performed in the electron impact (EI) mode on a MS9 spectrometer. Ethanol solutions of all the compounds were stable under dim red lights (or in the dark) and under argon for at least 24 h at room temperature and for at least 1 week when refrigerated (~10°C).

13-*cis*-12-Hydroxymethylretinoic Acid, δ-lactone (2). To a solution of 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid, δ-lactone (1) (3) (2.00 g, 0.064 mol) in t-butyl methyl ether (700 ml) was added a solution of I_2 (35 mg, 0.15 mmol) in t-butyl methyl ether (35 ml). This mixture was exposed to fluorescent laboratory lights and monitored by HPLC (SiO_2, 20% t-butyl methyl ether in hexane, 2 ml/min, UV 300, 340, 350 nm). After 15 min the mixture contained 95% 2 and 5% 1. After 30 min 1 was barely visible. The reaction mixture was washed with aqueous $Na_2S_2O_3$, followed by H_2O, dried and evaporated to give 2 as an orange solid (1.94 g, 97%), m.p. 100-103°C (lit. (3) 101-103°C), λ_{max} 370 nm (ε 28,000 EtOH) (lit. (3) 368 nm, ε 28,500 EtOH).

11-*cis*, 13-*cis*-12-Hydroxymethylretinoic Acid (4). To 3M potassium hydroxide/methanol (KOH/CH_3OH) (90 ml) was added 1 (3) (2.00 g, 0.006 mol) as a solid and the solution was stirred at room temperature for 1.5 h. TLC (SiO_2, 25% acetone/hexane) indicated the lactone to be consumed. The mixture was poured into H_2O (1000 ml) and the aqueous phase was washed with diethyl ether (Et_2O). The aqueous phase was then cooled in an ice-bath and acidified with 3 N hydrochloric acid (HCl). The acidified phase was extracted with Et_2O (4 × 200 ml). The extract was dried over anhydrous sodium sulfate (Na_2SO_4) and evaporated to give 4 as a pale yellow solid (1.82 g, 86%), m.p. 113–115°C (dec.), λ_{max} 300 nm (ε 24,400, CH_3OH), m/z calcd for $C_{21}H_{30}O_3$: 330.2195; found: 330.2196, ^1H NMR (dioxane-d_8), δ: 1.04 (s, 3, H-1a); 1.48 (m, 2, H-2 or H-3), 1.59 (m, 2, H-3 or H-2), 1.67 (s, 3, H-5a); 1.93 (s, 3, H-9a or H-13a); 1.95 (m, 2, H-4), 2.02 (s, 3, H-9a or H-13a); 4.19 (s, 2, H-12a); 5.88 (d, J = 12.3 Hz, 1, H-14); 5.95 (d, J = 16.0 Hz, 1, H-7); 6.21 (d, J = 12.0 Hz, 1, H-10 or H-11); 6.18 (d, J = 12.0 Hz, 1, H-10 or H-11); 6.43 (d, J = 16.1 Hz, 1, H-8); ^{13}C NMR (dioxane-d_8); δ: 166.8 (15), 156.3 (13), 144.6 (12), 138.9 (8), 138.5 (6), 136.4 (9), 129.3 (5), 127.0 (7), 126.4 (10), 120.1 (11), 119.4 (14), 40.2 (2), 34.8 (1), 33.4 (4), 29.2 (1a), 25.5 (13a), 21.8 (5a), 19.8 (3), 12.3 (9a).

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Biology

The compounds were evaluated topically in the rhino mouse model as a test for activity against hyperkeratinization, and for inhibition of ODC activity and DNA synthesis as antihyperproliferation models. The rhino mouse test and the inhibition of ODC activity assay have been previously described (1); the DNA synthesis assay was performed by treatment of hairless mouse skin with 12-O-tetradecanoylphorbol-13-acetate (TPA) (as in the ODC assay).

Animals. Nine to 14-week-old female hairless rhino mice ($hr^{rh}hr^{rh}$) were purchased from the Skin and Cancer Hospital, Temple University Health Sciences Center (Philadelphia, PA). Female hairless mice (HRS/J), age 6 to 8 weeks, were purchased from Jackson Laboratories (Bar Harbor, ME).

Animals were housed in accordance with the National Institutes of Health guidelines (U.S. Department of Health and Human Services, 1985). Animals had free access to food and water. The quarantine period was at least 7 days for rhino mice and at least 6 days for hairless mice.

Test Materials. Trans- and 13-*cis* retinoic acids (6 and 7, respectively) were obtained from the Eastman Kodak Company (Rochester, NY); TPA, L-[^{14}C]ornithine hydrochloride (specific activity 57 mCi/mmol) and [methyl-^{3}H]thymidine (specific activity 20 Ci/mmol) were purchased, respectively, from Sigma Chemical Company (St. Louis, MO), Amersham (Arlington Heights, IL) and DuPont/NEN (Boston, MA). Retinoid solutions in ethanol were prepared weekly and stored in amber vials under refrigeration. The vials were topped with argon gas to retard oxidation.

Retinoid Treatments. Retinoids were applied topically in an ethanol vehicle in all experiments unless otherwise indicated. The retinoid solutions were applied evenly to the dorsal skin of the animals at a dose of 2 $\mu l/cm^2$. All of the procedures were under yellow light to minimize photodegradation of the retinoids. During treatment, all animals were housed individually.

DNA Synthesis in Hairless Mice. Retinoids (0.1 ml) in acetone were applied topically to the dorsal skin of hairless mice 1 h before topical application of 17 nmol of TPA. The mice were given injections of 30 μCi [methyl-^{3}H]thymidine 1 h prior to sacrifice by CO_2 gas inhalation and epidermal DNA synthesis was determined following the published protocol (4). DNA synthesis was expressed as cpm/μg DNA.

RESULTS

Chemistry

The preparations of the lactones 1–3 and of the carboxy retinol 5 have been reported (3,5). However, since the preparation of 2 by the rapid reduction of the saponification product of 13-*cis*-12-carboxyretinoic anhydride was cumbersome and inefficient, 2 was more conveniently prepared by isomerization of the lactone 1 (Scheme 1). The hydroxy acid 4 was prepared by saponification of the lactone 1 (Scheme 1).

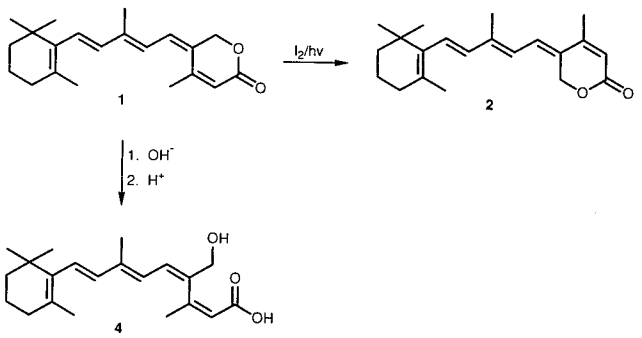
Biological

Evaluation of the retinoids 1–5 for activity against antihyperkeratinization and as antihyperproliferative agents (Table I) indicated that whereas the lactone 1 exhibited an activity profile resembling that of 13-*cis*-retinoic acid (7), the remaining retinoids, 2–4, had completely different activity profiles. In particular, neither the geometric isomer 2, nor the positional isomer 3, nor the metabolites of either lactone, 4 and 5, appeared to be as active as the lactone 1 in the inhibition of utricles. The potency of these retinoids in the inhibition of ODC activity and DNA synthesis diverged considerably. The lactone 3 was ineffective in ODC inhibition while being almost as active as 1 in the inhibition of DNA synthesis; the hydroxy acids 4 and 5 had opposite activities in the ODC assay.

DISCUSSION

The observed (1) activity of 11-*cis*-13-*cis*-12-hydroxyretinoic acid, δ-lactone (1) in antihyperkeratotic and antihyperproliferation assays, coupled with its lack of topical and systemic toxicity, prompted us to investigate the activity of related retinoids. In our early investigations of 12-substituted retinoids, we had noted (3) that although 11-*cis*,13-*cis*-12-carboxyretinoic acid anhydride isomerized spontaneously, and quantitatively, in the dark to the corresponding 13-*cis*-anhydride, the analogous lactones 1 and 3 did not undergo this spontaneous isomerization. Despite this observation, it seemed possible that isomerization might take place during light exposure on the animals' skin. We were, therefore, interested in investigating the effects of the 13-*cis* lactone 2. Since the previously reported preparation of this lactone (2) (3) was inefficient and inconvenient, the iodine-promoted isomerization, which had worked well in the selective isomerization of 12-carboxy-retinoates (6), was investigated. The isomerization proceeded rapidly and efficiently to the lactone 2, which was stable in absolute ethanol in the dark for at least 48 hours. The results of the bioassays indicated that this isomer was much less active than 1 in the reduction of utricles. Similarly, it appeared that the positional isomer of 1, 11-*cis*,13-*cis*-12-carboxyretinol, δ-lactone (3), was also substantially less active, suggesting that the unique combination of 11-*cis*,13-*cis*-stereochemistry and a terminal carboxyl moiety is required for dermatologic activity.

Possible break-down products of the lactones were also examined. The hydroxy-acids 4 and 5, which could arise from chemical or enzymatic hydrolysis, were also found to be inactive in an overall sense. Thus, although 5 has reasonable activity (54%) in the inhibition of TPA induced ODC,



Scheme 1

Table I. Activity of Retinoids

Number	Percent Inhibition of		
	Utricles[a,b]	ODC[c,d]	DNA Synthesis[d,e]
1	38	71	43
2	42[f]	NT[g]	NT[g]
3	10	0	32
4	17	−60	6
5	0	54	−9
6	55–70	97–99	86
7	33–51	84	50

[a] Topical application of 0.1% solution to rhino mouse skin daily for 4 weeks
[b] Percent reduction based on the vehicle control
[c] Topical application of 50 nmol retinoid to hairless mouse skin 1 h before 17 nmol TPA in acetone
[d] Percent inhibition based on acetone control
[e] Topical application of 500 nmol retinoid to hairless mouse skin 1 h before 17 nmol TPA in acetone
[f] 0.5% solution
[g] Not tested

relative to 71% for **1**, 84% for 13-*cis*-retinoic acid and 98% for retinoic acid, it has no activity in the inhibition of utricles. Conversely, the hydroxy-acid **4** has modest activity in utricle inhibition (17%) but actually stimulates TPA induced ODC. Neither **4** or **5** affect DNA synthesis.

These results indicate that the lactone **1** possesses some unique properties, suggesting its usefulness in the treatment of acne and/or psoriasis.

The results of this investigation, taken together with our previous results (1), indicate that effective antihyperproliferation and antihyperkeratinization without hypervitaminosis A requires a retinoid lactone of 11-*cis*,13-*cis*-stereochemistry with a terminal carboxyl functionality.

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