Evaluation of Retinoid Lactones as Topical Therapeutic Agents in Dermatology

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Purpose. Optimization of the therapeutic ratio of analogs of the topically active 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid, δ-lactone (1) relative to antihyperproliferation and antihyperkeratinization *vs.* toxicity. ***Methods.*** Nine analogs of 1, in which variations were made in the lipophilic cyclohexenyl moiety or in the lactone ring, were evaluated for topical activity against hyperkeratinization, inhibition of TPA-induced DNA synthesis and for skin irritation. ***Results.*** Although more potent lactones than the parent lactone 1 were identified, none possessed the favorable therapeutic ratio associated with 1. ***Conclusions.*** The δ-lactone 1 possesses unique molecular features responsible for its desirable therapeutic ratio as an antihyperproliferative and antihyperkeratotic agent. In view of its very low systemic retinoid toxicity and the absence of any systemic toxicity, this lactone may be a good candidate for use in the topical treatment of acne.

KEY WORDS: retinoids; lactones; antihyperproliferation; antihyperkeratinization.

INTRODUCTION

Retinoic acids and some of their derivatives are known to be active in reversal of keratinization and inhibition of ornithine decarboxylase activity (1). Unfortunately, these desirable effects are accompanied by toxicity and teratogenicity in this group of compounds (1). Our investigation of a series of 12-substituted retinoic acids revealed that 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid δ-lactone (1) was effective as an inhibitor of hyperkeratinization and of epidermal ornithine decarboxylase activity while exhibiting neither systemic toxicity nor skin irritation (2,3). An investigation of analogs of lactone 1, with variations in the lipophilic cyclohexenyl moiety (2–4) and in the lactone ring (5–10) was, therefore, undertaken. The analogs were evaluated for topical activity against hyperkeratinization and compared to 1 and to retinoic acid (11).

MATERIALS AND METHODS

Chemistry

All syntheses were performed under dim red lights and argon gas. 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 1:1 ethyl acetate (EtOAc) and 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, a septumless Waters U6K injector, an automated gradient controller, and a Waters 490 UV detector in the maxplot mode recording at 20-nm intervals between 220 and 440 nm. Anhydrides (16) and lactones (1–10) were analyzed on SiO_2 using 40% and 20% t-butyl methyl ether in hexane, respectively. Monoesters (17) were analyzed on C-18 using 20% (1% NH_4OAc in H_2O, solvent A) and 80% (3:1, CH_3CN/A, solvent B); hydroxy acid (18) utilized a gradient of 70–100% B over 5 min. The flow rate was 2 ml/min. UV spectra were recorded using a Varian 2290 spectrophotometer and mass spectrometry was performed in the electron impact (EI) mode on a MS9 spectrometer. Unless otherwise noted, the compounds were stable in EtOH in the dark for at least 48 h.

5-(4′-Methoxy-2′,3′,6′-trimethylphenyl)-3-methylpenta-2,4-dienal (12b). This aldehyde was prepared by a modification of the published procedure starting from commercially available 2,3,5-trimethylphenol (4). Methylation with CH_3I in the presence of base (4) afforded 2,3,5-trimethylanisole in 72% yield. Formylation with $SnCl_4/Cl_2CHOCH_3$ (5) gave 4-methoxy-2,3,6-trimethylbenzaldehyde (4) in 95% yield which was condensed with acetone (4) to give 4-(4′-methoxy-2′,3′,6′-trimethylphenyl)-but-3-ene-2-one in quantitative yield. Wittig-Horner elaboration with triethylphosphonoacetate (6) afforded ethyl 3-methyl-5-(4′-methoxy-2′,3′,6′-trimethylphenyl)-2,4-pentadienoate (83% yield) which was reduced to 5-(4′-methoxy-2′,3′,6′-trimethylphenyl)-3-methylpenta-2,4-dienol with $LiAlH_4$ (99% yield) and reoxidized with MnO_2 to 5-(4′-methoxy-2′,3′,6′-trimethylphenyl)-3-methylpenta-2,4-dienal. After cleanup-chromatography (10% t-butyl methyl ether in hexane), the product was obtained as a mixture of geometric isomers (44% yield). Preparative HPLC on a SiO_2 Prep-Pak column eluting with 5% t-butyl methyl ether in hexane at 300 ml/min gave 20% pure *trans*-aldehyde, 16% pure *cis*-aldehyde and 8% *cis*/*trans* mixture.

2-*cis*-4-*cis*-3,7-Dimethyl-4-carboxy-9-(4′-methoxy-2′,3′,6′-trimethylphenyl)nona-2,4,6,8-tetraenoic Acid (15). To a stirred solution of *trans*-5-(4′-methoxy-2′,3′,6′-trimethylphenyl)-3-methylpenta-2,4-dienal (12b) (23.0 g, 0.094 mol) and diethyl β-methylglutaconate (14) (18.9 g, 0.094 mol) in MeOH (175 ml) was added dropwise 3M KOH/MeOH (94.3 ml). After stirring at room temperature for 6.5 h TLC (50% acetone in hexane) indicated the disappearance of the starting aldehyde 12b. More 3M KOH/MeOH (188.6 ml) was added and the reaction mixture was refluxed 45 min, then stirred overnight at room temperature, diluted with H_2O (800 ml) and washed with Et_2O (2 × 100 ml). Evaporation of the solvent, after drying over anhydrous Na_2SO_4, gave recovered 12b (9.6 g). The aqueous phase was acidified to pH 1 with 10% H_2SO_4 and extracted with Et_2O (4 × 200 ml) and

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EtOAc (200 ml). The combined organic extract was dried over anhydrous Na_2SO_4 and evaporated to a residue which was dissolved in abs. EtOH (600 ml). Treatment with 10% KOH/EtOH resulted in immediate precipitation. The solid was collected on a medium porosity glass funnel and washed with cold EtOH and Et_2O. The wet solid (*ca.* 40 g) was suspended in H_2O (500 ml); the suspension was acidified to pH 1 with 10% H_2SO_4 and extracted with Et_2O (8 × 300 ml). The extract was washed with H_2O and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded **15** as a yellow powdery solid (13.3 g, 66% yield), pure by HPLC with mp 196–199°C (dec. to red gum) and λ_{max} 341 nm (ϵ 25,000 MeOH), ^1H and ^{13}C NMR (dioxane-d_8) confirm the structure; m/z calcd for $C_{22}H_{26}O_5$, 370.1780; found, 370.1782.

2-*cis*-3,7-Dimethyl-4-carbomethoxy-9-(4′-methoxy-2′,3′,6′-trimethylphenyl)nona-2,4,6,8-tetraenoic Acid (17b). A stirred solution of **15** (13.27 g, 0.036 mol) in dry THF (700 ml) was treated with DCC (7.41 g, 0.036 mol). TLC (50% acetone in hexane) indicated immediate reaction. The reaction mixture turned very dark and a precipitate formed. The reaction was monitored by TLC and DCC (0.2 g, 0.001 mol) was added after 1.25 h and 3.5 h. After 4 h the reaction appeared complete (TLC). The solid was removed by filtration through a medium porosity glass filter and was washed with THF. The combined filtrate and washings were evaporated to give 6.3 g (50% yield) of the anhydride **16b** as a very dark solid. TLC of the solid (15.4 g) indicated that it also contained the anhydride **16b**. The ^1H NMR spectra of both samples indicated the presence of **16b** contaminated with DCU. The impure batches were each subjected to saponification (7). The first batch of impure **16b** (6.3 g) was dissolved in MeOH (200 ml) and 1M KOH/MeOH (48 ml) was added causing immediate lightening of the solution. After 4.5 h the reaction was acidified to pH 1 with 5% HCl and the solution was extracted with Et_2O (4 × 250 ml). After drying overnight over anhydrous Na_2SO_4 the solvent was evaporated and the residue was treated with a small amount of THF. The insolubles (DCU) were removed by filtration and the THF was evaporated. The second portion of impure **16b** was similarly treated. The combined residues were recrystallized from EtOAc to give 8.35 g (60% yield) of pure **17b** with mp 168–170°C (dec. to dark red gum) and λ_{max} 347 nm (ϵ 24,500 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) confirm the structure; m/z calcd for $C_{23}H_{28}O_4$, 384.1937; found, 384.1940.

2-*cis*-4-*cis*-3,7-Dimethyl-4-hydroxymethyl-9-(4′-methoxy-2′,3′,6′-trimethylphenyl)nona-2,4,6,8-tetraenoic Acid, δ-Lactone (2). A solution of the half ester **17b** (6.88 g, 0.018 mol) in anhydrous ($LiAlH_4$) THF (225 ml) was cooled to −25°C and a 1 M solution of $LiAlH_4$ in THF (20.2 ml, 0.020 mol) was added dropwise, maintaining the temperature below −24°C. After 2 h at −24°C the temperature was allowed to rise to −15°C and stirring was continued for 3 h, maintaining the temperature between −15°C and −10°C. HPLC at this time indicated that there was no **17b** left. The reaction was quenched with saturated aqueous NH_4Cl, left to stir overnight at room temperature, and then filtered over celite. The filtrate was extracted with Et_2O. The solids were added to the aqueous phase, which was acidified to pH 5 with 5% HCl. The process was repeated, with the filtrate being acidified to pH 1, and then repeated again. After evaporation of the organic extracts the residues from the pH 8 and pH 5 extracts were dissolved in cyclohexane and set up to reflux with Dean Stark traps. When no more H_2O was accumulating in the traps, the solvents were evaporated and the residues were recrystallized from hot EtOAc. Most of the lactone product (**2**) was obtained from the pH 8 extract, although some was obtained from the pH 5 extract as well. Byproducts were the diacid **15b** and the diol (from over reduction). A total of 1.6 g (27%) of the pure lactone **2** with mp 169–170°C and λ_{max} 369 nm, 259 nm (ϵ 24,500, 8,000 MeOH) was recovered. ^1H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{22}H_{26}O_3$, 338.1882; found, 338.1884.

3-(1′,2′,3′,4′-Tetrahydro-1′,1′,4′,4′-tetramethyl-6′-napthyl)propenal (12c). To a slurry of NaH (10.0 g, 0.42 mol) in dry THF (700 ml) was added, dropwise, a solution of triethylphosphonoacetate (48.4 g, 0.216 mol) in dry THF (100 ml). After stirring the slightly turbid solution for 0.5 h a solution of 6-acetyl-1,2,3,4-tetrahydro-1,1;4,4,-tetramethylnaphthalene (obtained as a gift from Givaudan) (50.0 g, 0.216 mol) in dry THF (200 ml) was added. After refluxing for 4 h and stirring overnight at room temperature, the reaction mixture was diluted carefully with H_2O (300 ml) and brine (600 ml). The layers were separated, the organic layer was washed again with brine (300 ml) and the aqueous phase was extracted with Et_2O (3 × 400 ml). The combined organic extract was dried over anhydrous Na_2SO_4 overnight and evaporated to yield a yellow oil (64.7 g). Purification by medium pressure chromatography on SiO_2, eluting with hexanes, followed by 1% t-butyl methyl ether in hexanes, gave the starting material (9.23 g), the product, ethyl 3-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthyl)propenoate as a mixture of *cis*/*trans* isomers (24.4 g), and overlap fractions (27.8 g). The mixture of esters (24.4 g, 0.081 mol) was dissolved in dry THF (400 ml) and added dropwise to an ice-cold solution of $LiAlH_4$ (3.08 g, 0.081 mol) in dry THF (1350 ml). After stirring 0.5 h the reaction was quenched by the sequential addition of H_2O (3 ml), 15% NaOH (3 ml) and H_2O (9.2 ml). The precipitated solids were removed by filtration and washed copiously with Et_2O. The combined organic phase was dried over anhydrous Na_2SO_4 overnight and evaporated. The oily alcohol, 3-(1′,2′,3′,4′,-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthyl)propenol (21.5 g, 100% yield) had m/z calcd for $C_{18}H_{26}O$, 258.1984; found, 258.1985. Stirring a solution of the alcohol (21.5 g, 0.081 mol) in hexane (100 ml), with MnO_2 (73 g, 0.84 mol) for 60 h gave, after removal of the oxidant and the solvent, 3-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethylnaphthyl)propenal (20.4 g, 96%) as an orange colored oil. Purification by medium pressure chromatography, eluting with 15% Et_2O in hexanes gave 5.39 g of the pure *trans*-3-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethylnaphthyl)propenal (**12c**) with λ_{max} 299 nm, 232 nm (ϵ 19,400, 12,700 MeOH). ^1H NMR (dioxane-d_8) confirms the structure; m/z calcd for $C_{18}H_{24}O$, 256.1827; found, 256.1825.

2-*cis*-4-*cis*-3-Methyl-4-carbomethoxy-7-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthyl)octa-2,4,6-trienoic Acid (17c). To a stirred, ice-cold solution of the **12c** (7.51 g, 0.029 mol) in dry THF was added rapidly a solution of **13a** (3.68 g, 0.029 mol) in dry THF (200 ml) followed by pyridine (70 μl). After stirring for a week at room temperature, the reaction mixture still contained a substantial amount of the

starting materials. A small amount of molecular sieves (4Å) was added and stirring was continued at room temperature. After 24 h the reaction was complete. The sieves were removed by filtration through celite and the volatiles were evaporated. Cyclohexane was added to the residue and the volatiles were again evaporated. The azeotroping was repeated once more, this time concentrating the mixture to 75 ml. The precipitated solid (**16c**) which had mp 137–139°C was collected (5.85 g, 55%). ^{1}H NMR (dioxane-d_8) confirms the structure.

To a slurry of **16c** (6.4 g, 0.018 mol) in MeOH (300 ml) was added 1M KOH/MeOH (70 ml) which caused dissolution. After stirring overnight at room temperature the reaction seemed complete by HPLC. The mixture was cooled in an ice-bath and acidified to pH 1 with 5% HCl (*ca.* 200 ml). The turbid solution was extracted with Et_2O (4 × 250 ml). The combined extract was dried over anhydrous Na_2SO_4 overnight. Evaporation of the solvent afforded a solid (5.20 g) which was recrystallized from hot EtOAc to give pure 17c (3.54g, 50%) with mp 181–182°C and λ_{max} 327 nm, 242 nm (ϵ 31,100, 9,260 MeOH). ^{1}H NMR (dioxane-d_8) confirms the structure; m/z calcd for $C_{25}H_{32}O_4$, 396.2300; found, 396.2303.

2-*cis*-4-*cis*-3-Methyl-4-hydroxymethyl-7-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthyl)octa-2,4,6-trienoic Acid, δ-Lactone (3). A solution of 17c (3.54 g, 0.0089 mol) in dry THF (125 ml) was cooled to −30°C and a 1 M solution of $LiAlH_4$ in THF (10.0 ml, 0.010 mol) was added dropwise, maintaining the temperature below −23°C. Monitoring by HPLC revealed that the reaction was not complete after 2 h at −20°C so it was allowed to warm to −10°C. The reaction was still not complete after 3.5 h so it was left at −10°C overnight. After a total of 24 h the reaction was quenched with saturated NH_4Cl, filtered through a celite pad and worked up as described for **17b**. After recrystallization of the product obtained by Dean Stark dehydration/cyclization from EtOAc, 0.7 g (23%) pure **3** with mp 148°C and λ_{max} 352 nm (ϵ 18,400 MeOH) was obtained. ^{1}H and ^{13}C NMR (dioxane-d_8) are in Tables I and II; m/z calcd for $C_{24}H_{30}O_2$, 350.2246; found, 350.2244.

2-*cis*-3-Methyl-4-carboxy-7-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)octa-2,4,6-trienoic Acid Anhydride (16d). To prepare 3-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)propenal (**12d**), the procedure described for **12c** was followed. The required starting ketone, 6-acetyl-1,2,3,4,-tetrahydronaphthalene was prepared by the portionwise addition of $AlCl_3$ (148 g, 1.1 mol) to a stirred solution of 1,2,3,4,-tetrahydronaphthalene (132 g, 1.0 mol) and acetyl chloride (86.6 g, 1.1 mol) in nitroethane (600 ml) which had been cooled to 5°C, maintaining a temperature of 10–15°C. After stirring at room temperature for 3 h the orange reaction mixture was poured over ice resulting in HCl evolution and in a color change to dark green. The aqueous phase was separated and discarded. The organic phase was washed with H_2O, dried over anhydrous $MgSO_4$ and evaporated. The residue was dissolved in petether (800 ml) and the solution was washed with 1M KOH, dried and evaporated. The oil was vacuum distilled; the fraction collected at 84°C (250 μ) contained pure ketone (75 g, 43%). This material was used in the Wittig reaction, as described for **12c**. The Wittig product, ethyl 3-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)propenoate, was obtained in 50% yield, as a mixture of *cis/trans* isomers; the alcohol, 3-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)propenol, was obtained in 90% yield, as was the aldehyde, 3-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)propenal. Final purification to obtain the pure *trans* aldehyde **12d** was by medium pressure chromatography, eluting with 5% t-butyl methyl ether in hexanes. ^{1}H NMR (dioxane-d_8) confirms the structure. A solution of *trans*-3-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)propenal (**12d**) (5.0 g, 0.025 mol) and **13a** (3.15 g, 0.025 mol) in Et_2O (400 ml) containing 400 μl of pyridine was refluxed for a week in a soxhlet extractor containing 4Å molecular sieves in the thimble. The precipitated solid was collected by filtration and the residue was recrystallized from warm EtOAc affording 5.85 g (76%) of **16d** with mp 171–172°C and λ_{max} 412 nm (ϵ 23,600 MeOH). ^{1}H and ^{13}C NMR (dioxane-d_8) confirm the structure; m/z calcd for $C_{20}H_{20}O_3$, 308.1412; found, 308.1418.

2-*cis*-3-Methyl-4-carbomethoxy-7-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)-octa-2,4,6-trienoic Acid (17d). Following the procedure described for **17b** the anhydride **16d** (5.50 g, 0.0179 mol) was saponified to give 17d (4.14 g, 68%) with mp 178–180°C and λ_{max} 328 nm (ϵ 30,900 MeOH). ^{1}H and ^{13}C NMR (dioxane-d_8) confirm the structure; m/z calcd for $C_{21}H_{24}O_4$, 340.1674; found, 340.1676.

2-*cis*-3-Methyl-4-hydroxymethyl-7-(1′,2′,3′,4′-tetrahydro-6′-naphthyl)-octa-2,4,6-trienoic Acid, δ-Lactone (4). A solution of 17d (3.22 g, 0.001 mol) in dry THF (distilled from benzophenone ketyl, 65 ml) was cooled to −20°C and a 1 M solution of $NaEt_3BH$ in THF (37.9 ml, 0.038 mol) was added dropwise. Monitoring by HPLC showed the reaction to slow down substantially after 4 h although starting material was still present. Therefore more (4.0 ml, 0.004 mol) reagent was added. After 45 min the starting material was consumed. The reaction mixture was treated with saturated aqueous NH_4Cl, stirred 1 h and acidified to pH 1 with 5% HCl. Extraction with Et_2O and solvent evaporation after drying gave a residue (**18d**) which was set up to dehydrate as described for **3**. After 6 h HPLC indicated no **18d** remaining. The precipitated solid **4** was collected, washed with hexanes and dried under vacuum (2.08 g, 74%). The solid had mp 149–150°C and λ_{max} 352 nm (ϵ 26,400 MeOH). ^{1}H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{20}H_{22}O_2$, 294.1620; found, 294.1621.

11-*cis*,13-*cis*-12-Carbomethoxy-14-methylretinoic Acid (17e). The preparation of the target compound required the anhydride **13b**, which was prepared as follows. Condensation of ethyl 2-methylacetoacetate (20.8 ml, 0.147 mol) with ethyl cyanoacetate (17.8 ml, 0.148 mol) in the presence of NH_4OAc (231 g, 0.03 mol) in 1:1 HOAc and PhH (60 ml) by refluxing for 72 h using a Dean Stark trap, gave, after workup and short path distillation, ethyl α-cyano-β,γ-dimethylglutaconate (21.3 g, 61%) as an oil. ^{1}H NMR (90 MHz, $CDCl_3$) confirms the structure. Hydrolysis and decarboxylation by reflux in conc. HCl (125 ml) for 7 h, followed by stirring at room temperature overnight, gave α,β-dimethylglutaconic acid (8.0 g, 47%) mp 143–146°C. ^{1}H NMR (DMSO-d_6) confirms the structure. The diacid was dehydrated by treatment of a slurry (9.58 g, 0.061 mol) in CH_2Cl_2 (120 ml) with $(CF_3CO)_2O$ (8.56 ml, 0.061 mol). After stirring overnight it appeared that some solid was still present so additional $(CF_3CO)_2O$ (7 ml, 0.055 mol) was added. After stirring another 24 h the volatiles were evaporated; the residual oil (9.53 g, 100%) had ^{1}H NMR (dioxane-

Table I. Proton Chemical Shifts

	δ[a] and (J)[b] for proton number[c]					
	2	3	7	8	10	11
1	1.51 (m)	1.63 (m)	6.35 (16)	6.21 (16)	6.63 (12)	6.73 (12)
2[d]			6.82 (16.2)	6.32 (16.3)	6.76 (11.5)	6.66 (12.8)
3[d]	1.69	1.69			7.00 (12.1, 0.7)	6.75 (12.2)
4[d]	1.78 (m)	1.78 (m)			6.74 (12.1)	6.98 (12.6)
5	1.55 (m)	1.65 (m)	6.33 (16.1)	6.17 (16.1)	6.48 (12.4)	6.59 (12.4)
6	1.51 (m)	1.62 (m)	6.32 (16.0)	6.21 (16.1)	6.55	6.55
7	1.50 (m)	1.50 (m)	6.47 (16.8)	6.32 (17.5)	7.45	7.45
8	1.65 (m)	1.65 (m)	6.36 (16.0)	6.19 (16.1)	6.59 (12.1)	6.66 (12.5)
9	1.50 (m)	1.70 (m)	6.34 (16.0)	6.20 (16.0)	6.59 (12.2)	6.75 (12.2)
10	1.50 (m)	1.65 (m)	6.43 (16)	6.29 (16)	6.35 (12)	6.91 (12)

	δ[a] and (J)[b] for proton number[c]							
	14	1a	5a	9a	13a	12a	14a	12b
1	5.79	1.03	1.72	2.00	2.30	4.78		
2[d]	6.28			2.09	2.20	5.77		
3[d]	5.81	1.26		2.32 (0.7)	2.22	4.80		
4[d]	5.80			2.20	2.31	4.79		
5		1.02	1.69	1.92	1.95	4.66	2.22	
6		1.02	1.70	1.97	2.39	4.69	2.65	
7		1.05	1.74	2.12	7.19 (7.5, 0.5)	5.18		
8	5.76	1.02	1.70	1.99	2.27	5.00 (6.5)		1.46
9	5.90	1.02	1.69	1.99	2.27			1.57
10	6.49	1.05	1.72	2.03	2.05	5.24		

[a] ppm.
[b] Hz.
[c] In dioxane-d_8 at 250 MHz.
[d] The chemical shifts of the protons corresponding to the analogous position in lactone 1 are shown.

d_8) consistent with the structure **13b**. Condensation of **13b** (9.53 g, 0.068 mol) with **12a** (14.84 g, 0.068 mol) in Et_2O (200 ml) in the presence of pyridine (0.2 ml) was accomplished by gently refluxing the solvent through a soxhlet containing 4Å molecular sieves. After 24 h reflux the precipitated solid was collected, washed with hexanes and dried *in vacuo* to give **16e** (11.3 g, 49%) with λ_{max} 426 nm (ε 29,800 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) confirm the structure. Saponification of **16e** (11.1 g, 0.033 mol) as described for **16b** afforded (8.18 g, 67%) of 11-*cis*,13-*cis*-12-carbomethoxy-14-methylretinoic acid (**17e**) with mp 168–169°C and λ_{max} 336 nm (ε 34,860 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) confirm the structure; m/z calcd for $C_{23}H_{32}O_4$, 372.2300; found, 372.2296.

11-*cis*,13-*cis*-12-Hydroxymethyl-14-methylretinoic Acid, δ-Lactone (5). Reduction of **17e** (4.18 g, 0.011 mol) as described for the preparation of **2**, but at −40°C, afforded, after dehydration and purification, the lactone (**5**), 1.4 g (38%) with mp 113–114°C and λ_{max} 354 nm (ε 34,400 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{22}H_{30}O_2$, 326.2246; found, 326.2242.

11-*cis*,13-*cis*-12-Hydroxymethyl-13,14-tetramethylene-13-desmethyl-retinoic Acid, δ-Lactone (6). A mixture 60% ethyl and 40% methyl 2-cyclohexanonecarboxylate (47.0 ml, 0.303 mol) was condensed with ethyl cyanoacetate (35.6 ml, 0.335 mol) as described in the preparation of **17e**. Ethyl 2-cyano-2-(2-carboethoxycylcohexylidene)acetate was obtained in 56% yield (38.4 g). This product was hydrolyzed by refluxing in conc. HCl (230 ml) for 6 h. The precipitated white solid was collected by filtration, washed with cold H_2O and dried *in vacuo* to give 2-carboxy-1-cyclohexaneacetic acid as a fluffy white material with mp 159–164°C (18.1 g, 68%). This diacid was dehydrated by stirring it as a slurry (18.1 g, 0.099 mol) in CH_2Cl_2 (450 ml) with $(CF_3CO)_2O$ (13.8 g, 0.099 mol) for 5 h. The small amount of solid remaining was removed by filtration, the solution was diluted with t-butyl methyl ether and evaporated to leave an orange oil. This residue was dissolved in a small amount of hot Et_2O; after coming to room temperature pet-ether was added to opalescence and the solution was refrigerated. Eventually **13c** was obtained as clear needles (5.3 g, 33%). ^1H and ^{13}C NMR (dioxane-d_8) confirmed the structure. Condensation of **13c** (5.3 g, 0.032 mol) with **12a** (7.0 g, 0.32 mol) as described for **16e** gave **16f** (8.97 g, 77%) with ^1H NMR (dioxane-d_8) confirming the structure. The anhydride **16f** (6.8 g, 0.019 mol) was saponified under the conditions described for **16e** to give 11-*cis*,13-*cis*-12-carbomethoxy-13,14-tetramethylene-13-desmethylretinoic acid (**17f**) (5.5 g, 74%). ^1H and ^{13}C NMR (dioxane-d_8) confirm the structure. Reduction of the half ester **17f** (3.52 g, 0.0088 mol) followed by dehydration, as described in the preparation of lactone **4**, gave, after chromatographic purification and recrystallization, pure **6** (0.78 g, 12%) with mp 119–121°C and λ_{max} 362 nm (ε 25,600 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{24}H_{32}O_2$, 352.2402; found, 352.2406.

Table II. Carbon Chemical Shifts

	δ (ppm) for Carbon number[a]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	34.8	40.0	19.7	33.5	130.5	138.3	130.0	138.3	142.2	126.0	129.0	128.5	152.0	119.6
2[b]	128.8	136.1	122.9	157.0	110.4	130.4	128.9	138.8	142.3	126.5	134.2	128.8	151.9	119.8
3[b]	34.9	35.6	35.7	34.8	145.7	145.5	124.6	123.9	144.0	127.3	129.4	128.8	152.1	120.0
4[b]	29.6	27.4	22.7	30.0	138.0	140.6	122.2	137.6	143.8	127.1	129.5	128.7	152.2	119.8
5	34.8	40.2	19.7	33.5	130.4	138.4	130.3	138.4	141.0	126.1	129.4	127.3	145.8	126.4
6	34.8	40.2	19.8	33.5	130.2	138.6	129.5	138.6	141.1	126.8	129.3	127.2	147.4	126.5
7	34.9	40.3	19.8	35.6	132.6	138.4	129.2	138.4	146.1	122.4	127.9	131.3	146.1	136.7
8	34.8	40.2	19.8	33.5	130.5	138.5	129.9	138.5	142.1	126.3	128.2	133.1	150.3	119.5
9	34.8	40.1	19.7	33.5	130.4	138.5	129.6	138.5	141.8	126.9	126.7	137.0	150.3	120.0
10	34.8	40.3	19.7	33.7	131.2	138.9	132.5	138.1	143.0	126.7	125.0	128.6	143.1	128.0

	δ (ppm) for Carbon number[a]												
	15	1a	5a	9a	13a	12a	12b	14a	13b	14b	$ArCH_3$	$ArOCH_3$	5b
1	163.8	29.1	21.8	12.0	24.5	64.7							
2[b]	163.7	12.0	17.4	11.8	22.6	73.6					21.3	55.4	
3[b]	163.9	32.0	121.5	15.6	22.8	73.6							122.6
4[b]	163.9		123.7	15.6	23.8	73.6							129.8
5	164.7	29.2	21.8	12.0	13.4	73.1		19.3					
6	164.5	29.2	21.8	12.0	24.9	73.1		30.5	22.4	22.9			
7	165.4	29.2	21.8	12.3	124.0	69.0		135.4	138.9	124.8			
8	163.1	29.2	21.8	12.1	22.9	79.9	21.5						
9	163.0	29.2	21.9	12.5	23.6	83.6	28.4						
10	205.6	29.2	21.9	12.5	17.5	68.4	28.4						

[a] In dioxane-d_8 at 250 MHz.
[b] The chemical shifts of the carbons corresponding to the analogous position in retinoic acid are shown.

13,14-Benzo-13-desmethyl-12-carboxyretinoic Acid Anhydride (16g). A solution of **12a** (24.68 g, 0.113 mol) and 13d (18.34 g, 0.113 mol) in Et_2O (400 ml) containing 400 μl of pyridine was refluxed in a soxhlet extractor containing 4Å molecular sieves in the thimble for a week. The precipitated solid was collected by filtration (11.80 g, 29%). The filtrate was concentrated yielding additional solid (5.0 g, 12%). Evaporation of all the volatiles gave an oil which was dissolved in warm EtOAc. Two crops of crystals were collected from this procedure, 3.28 g (8%) and 2.78 g (7%). Examination of these solids by HPLC (SiO_2; 40% t-butyl methyl ether/hexane, 2 ml/min) revealed that the first three (49%) were the same compound with mp 119–121°C and λ_{max} 424 nm, 224 nm (ε 25,100, 26,432 MeOH). ^1H NMR (dioxane-d_8); m/z calcd for $C_{24}H_{26}O_3$, 362.1882; found, 362.1877. The fourth set of crystals was a faster eluting compound with mp 121–125°C and λ_{max} 413 nm,222 nm (ε 25,400, 23,900 MeOH). An additional crop of this material (0.97 g, 2%) was also collected. ^1H and ^{13}C NMR (dioxane-d_8) showed the compounds to be isomeric.

13,14-Benzo-13-desmethyl-12-hydroxymethylretinoic Acid, δ-Lactone (7). The anhydride **16g** was saponified as described for **16b**. Starting with the solid with λ_{max} 424 nm (20.0 g, 0.055 mol) gave, after crystallization, 12.5 g (58%) of a mixture of monomethyl esters. Starting with the anhydride with λ_{max} 413 nm (2.50 g, 0.007 mol) gave a similar product mixture (0.18 g, 7%). This mixture was reduced and the resulting mixture dehydrated as described for **17b**. The product mixture obtained contained mainly two lactones. After multiple recrystallizations a pure lactone (1.25 g, 13%) was isolated with mp 174–176°C and λ_{max} 380 nm (ε 26,000 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{24}H_{28}O_2$, 348.2089; found 348.2085.

11-*cis*,13-*cis*-12-Acetylretinoic Acid (20). A solution of **12a** (6.41 g, 0.029 mol), **19** (5.00 g, 0.029 mol) and 3 M KOH/MeOH (29.4 ml) in MeOH (75 ml) was stirred overnight at room temperature. Since TLC (50% acetone/hexane) showed the reaction to be incomplete stirring was continued for another 24 h. More 3M KOH/MeOH (29.4 ml) was added and the reaction was refluxed for 2 h. After cooling to room temperature the mixture was diluted with H_2O (350 ml) and washed with Et_2O. The aqueous phase was then acidified and extracted with Et_2O. Evaporation of the extract, after drying, afforded an oil (7.0 g, 70%) which could not be crystallized. Attempted crystallization as a potassium salt or as a di(*n*-butyl)amine salt failed as well. The oil was subjected to preparative HPLC [C_{18} PrepPak, 30% (1% NH_4OAc in H_2O), 70% CH_3CN, 250 ml/min]; this gave several apparently pure fractions which were combined and evaporated to a yellow amorphous solid, which could not be crystallized. It had mp <60°C and λ_{max} 344 nm (ε 15,700 MeOH). Analysis by NMR indicated the presence of *ca.* 10% of an isomer; the major component had ^1H and ^{13}C NMR (dioxane-d_8) consistent with the structure; m/z calcd for $C_{22}H_{30}O_3$, 342.2195; found, 342.2198. This compound isomerized at the rate of *ca.* 15%/h in abs. EtOH.

11-*cis*-13-*cis*-12-(1′-Hydroxyethyl)retinoic Acid, δ-Lactone (8). Reduction of the keto acid **20** (3.50 g, 0.0102 mol) with $NaEt_3BH$ (0.0256 mol) followed by dehydration as described for the preparation of lactone **4** afforded a mixture of two lactones in a 4:1 ratio. Fractional crystallization from EtOAc gave pure **8** (0.93 g, 29%) with mp 160–162°C and λ_{max} 366 nm (ε 32,900 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{22}H_{30}O_2$, 326.2246; found, 326.2236.

11-*cis*,13-*cis*-12-[2′-(2′-Hydroxypropyl)]retinoic Acid, δ-Lactone (9). To a solution of the keto acid **20** (3.80 g, 0.011 mol) in dry THF (300 ml) at −10°C was added dropwise 3 M MeMgBr in Et_2O (7.40 ml, 0.022 mol), maintaining the temperature below −5°C. Monitoring the reaction by HPLC (C_{18} Novapak, A 1% NH_4OAc, B 3:1 CH_3CN/A, 50- > 80% B over 10 min, 2 ml/min) indicated that it was not progressing after 2 h. Therefore, more MeMgBr (3.0 ml, 0.009 mol) was added. After 30 min more the reaction appeared 80% complete. After 1 more h dilute HCl was added to quench the reaction and stirring was continued overnight. The reaction mixture was then extracted with Et_2O and the combined extract was dried and evaporated. The residue was dissolved in cyclohexane and the solution was evaporated; this process was repeated 5 times until no hydroxy acid could be detected by HPLC. TLC (25% EtOAc/hexane) indicated the presence of origin material. Column chromatography (SiO_2, 10% EtOAc/hexane) afforded an oil (1.0 g, 27%) which, by ^1H NMR showed the presence of impurities and suggested a mixture of two isomeric lactones. A second chromatography (Size C Prepack, 25% EtOAc/hexane) gave a yellow foam (0.51 g) which was chromatographically pure and appeared to be pure by ^1H and ^{13}C NMR in dioxane-d_8. This lactone (**9**) had λ_{max} 364 nm, 258 nm (ε 20,370, 7,600 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{23}H_{32}O_2$, 340.2402; found, 340.2395.

13-*cis*-12-Hydroxymethylthionoretinoic Acid, δ-Lactone (10). A solution of lactone **1** (2.50 g, 0.008 mol) and Lawesson's reagent (1.62 g, 0.004 mol) in THF (from 4Å sieves, 250 ml) was refluxed and stirred for 6 h. Since TLC (25% EtOAc/hexane) indicated approximately half the starting material to be consumed, additional (0.83 g, 0.002 mol) reagent was added and reflux was continued. After 2 additional h the ratio of product to starting material was 2:1 so a second portion (0.82 g, 0.002 mol) of reagent was added. After another h the reaction appeared complete (TLC). The reaction volume was reduced to 100 ml by evaporation of the volatiles, SiO_2 was added and evaporation was continue to afford a powder. This material was added to the top of a SiO_2 (20 g) column (5 cm i.d.) and washed on with hexane. Elution with 10% Et_2O/hexane gave the product (1.68 g), contaminated with *ca.* 5% of **1**. Recrystallization from EtOAc/hexane gave 1.19 g (45% yield) of **10** as a red crystalline solid with mp 91–94°C and λ_{max} 420 nm (ε 24,710 MeOH). ^1H and ^{13}C NMR (dioxane-d_8) are shown in Tables I and II; m/z calcd for $C_{21}H_{28}OS$, 328.1861; found, 328.1864.

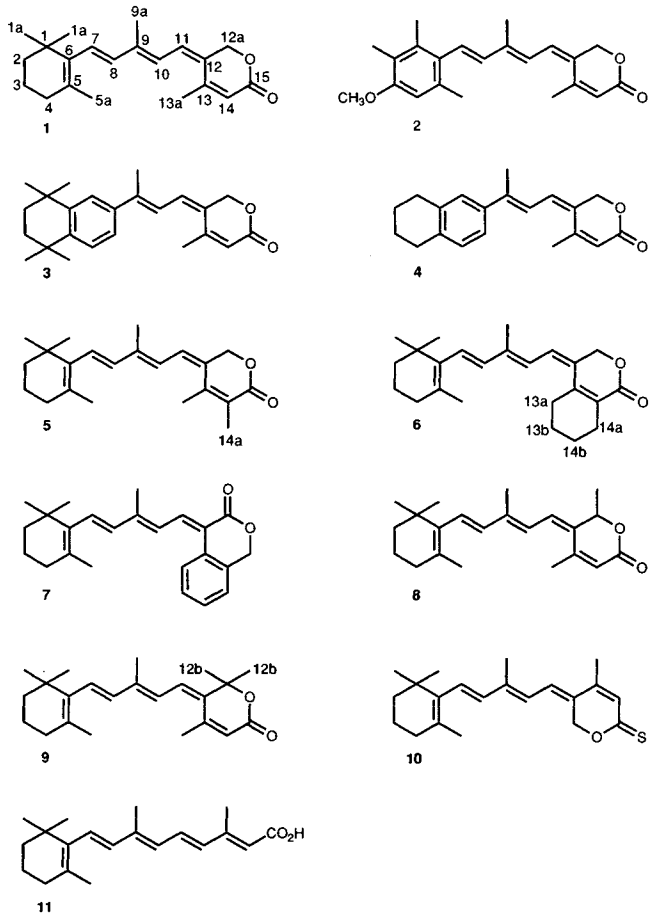
Biology

The materials used and the protocols pursued were as previously described (3).

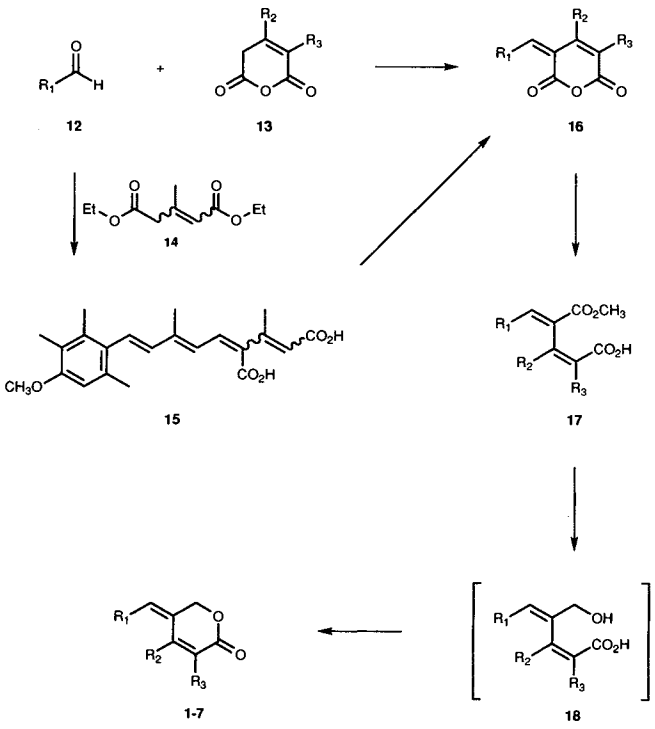
RESULTS

Chemistry

The compounds **1–9** were prepared following methodology closely related to that utilized previously for the synthesis of 12-substituted retinoids (8). A generalized pathway is shown in Scheme 1. For the lactones **1–7** the synthesis involved the reduction of an analog of 12-carbomethoxyretinoic acid (**17**) to the hydroxy acid **18** followed by *in situ* dehydration (7). The monoesters (**17**) were prepared by saponification of the appropriate anhydride precursor (**16**) (7). With the exception of the anhydride 2-*cis*-3,7-dimethyl-4-carboxy-9-(4′-methoxy-2′,3′,6′-trimethylphenyl)nona-2,4,6,8-tetraenoic acid anhydride (**16b**), the anhydrides (**16a, 16c–16g**) were obtained by condensation of the appropriate aldehyde (**12a, 12c, 12d**) with the appropriate derivative of glutaconic anhydride (**13a–13d**). In the case of the anhydride **16b**, the aldehyde *trans*-5-(4′-methoxy-2′,3′,6′-trimethylphenyl)-3-methylpenta-2,4-dienal (**12b**) (6) was condensed with diethyl β-methylglutaconate (**14**) and the resulting diacid **15** was converted to the anhydride **16b**. The required starting aldehyde **12b** was prepared by a slight modification of the literature procedure (4,6); the aldehydes **12c** and **12d** were prepared by acylation of the appropriate tetrahydronaphthalene followed by Wittig-Horner condensation with triethylphosphonoacetate, reduction to the allylic alcohol



Compounds 1–11



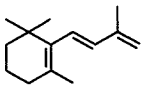
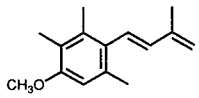
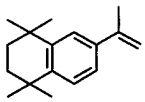
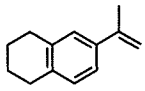
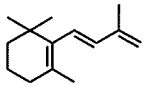
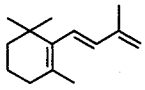
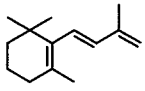
Scheme 1

and reoxidation. The required starting anhydrides **13b** and **13c** were prepared by condensation of ethyl cyanoacetate with ethyl α-methylacetylacetonate and 2-cyclohexanonecarboxylate, respectively, followed by decarboxylation, hydrolysis and cyclization with trifluoroacetic anhydride. The lactones **8** and **9** were both obtained (Scheme 2) from 11-*cis*,13-*cis*-12-acetylretinoic acid (**20**), which was prepared by condensation of *trans*-β-ionylideneacetaldehyde (**12a**) with ethyl 3-methyl-5-one-2-hexenoate (**19**). Thus, reduction of **20** with sodium triethylborohydride followed by azeotropic dehydration of the reaction product gave **8** while treatment of **20** with methylmagnesium bromide followed by azeotropic dehydration of the product afforded **9** (a mixture of isomers). The lactone **10** was prepared from **1** using the Lawesson reagent.

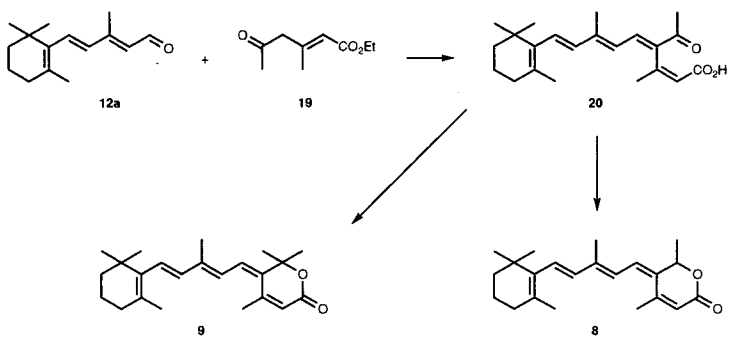
Biology

The compounds **1–10** were evaluated for topical activity against hyperkeratinization using the rhino mouse model (3). Several of the retinoids were also evaluated for inhibition of epidermal ornithine decarboxylase activity and for inhibition of DNA synthesis. Active retinoids were further evaluated for local irritation by repetitive application (3).

Utricle diameter of rhino mouse skin was assessed in whole mounts of the horizontal epidermal sheets; the results are shown in Table III. Compounds that reduced the size of the utricles also showed reductions in the size of surface cysts or pseudocomedones. Histological evaluation also showed that these compounds stimulated an increase in epidermal thickness, especially of the granular layer. Only one of the lactones in this series, the tetramethyltetrahydronaphthalene analog **3**, exhibited activity exceeding that of the lead compound 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid, δ-lactone (**1**). Lactone **3** was also more active than

Lactone No.	Substituents			Intermediate Numbers				
	R_1	R_2	R_3	12	13	16	17	18
1		CH_3	H	a	a	a	a	a
2		CH_3	H	b		b	b	b
3		CH_3	H	c	a	c	c	c
4		CH_3	H	d	a	d	d	d
5		CH_3	CH_3	a	b	e	e	e
6		$CH_2CH_2CH_2CH_2$		a	c	f	f	f
7		CH=CH–CH=CH		a	d	g	g	g

Scheme 1 (Legend)



Scheme 2

lactone 1 in the inhibition of DNA synthesis (Table IV) and exhibited irritation scores comparable to those obtained for retinoic acid (11) (Table V).

DISCUSSION

Structural modifications to the skeleton of the lead component, the lactone derived from 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid (1), were directed at the lipophilic head group as well as at the lactone ring. Replacement of the head group by the aryl and tetramethyltetrahydronaphthalene moieties was based on the high activity reported for retinoic acid analogs with these groups (9). Substitutions at the lactone rings were designed to vary the lipophilicity and the steric requirements of this portion of the molecule.

For the most part the reaction sequences used in the preparation of the target lactones were straightforward. The only aldehyde which failed to afford a retinoid anhydride product by direct condensation with β-methylglutaconic anhydride (**13a**) was *trans*-5-(4′-methoxy-2′,3′,6′-trimethylphenyl)-3-methylpenta-2,4-dienal (**12b**). Condensation with diethyl β-methylglutaconate did, however, afford the diacid 2-*cis*-4-*cis*-3,7-dimethyl-4-carboxy-9-(4′-methoxy-2′,3′,6′-trimethylphenyl)nona-2,4,6,8-tetraenoic acid (**15**). The anhydride **16b** could be obtained by treatment of the diacid **15** with dicyclohexylcarbodiimide or with trifluoroacetic anhydride; however, it seemed to be a labile material, suggesting that direct condensation of **12b** and **13a** might have proceeded to give the anhydride **16b**, but that the product did not survive the reaction conditions.

Based on the NMR spectra, the stereochemistry of most of the intermediates, and of the products, matches that obtained previously in the preparation of 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid, δ-lactone (1) (7). Thus, the spectra of the anhydrides **16a**, **16c–16f** were consistent with 13-*cis* stereochemistry; two isomers were obtained in the case of anhydride **16g**, presumably the 13-*cis*- and the 11-*cis*, 13-*cis*-isomers. As observed previously (7), the saponification of the 13-*cis*-anhydrides afforded a transient monoester which isomerized under the reaction conditions. The monomethyl esters **17a–f** isolated, as well as of the diacid **15** and the ketoacid **20** all had ^{1}H NMR spectra consistent with 11-*cis*,13-*cis* sterochemistry. In other words, they all possessed a highly deshielded (7.3–7.5 ppm) vinyl doublet for the proton at C-11 and a resonance of the proton at C-10 in the region of the C-7 and C-8 (6.0–6.4 ppm). The products obtained in the saponification of the 13,14-benzo-anhydride **16b** were more complex. Each anhydride gave a mixture of the same three monomethyl esters, but in different proportions. The major product of the reaction of the higher wavelength anhydride was the smallest component in the reaction of the lower wavelength anhydride and vice-versa; both mixtures contained about 35% of a third component, which was common to both reactions. Since at least three half esters were produced it is likely that positional as well as geometric isomers are involved. This may account for the fact that the compositions of these mixtures did not change with time.

Eight of the ten lactones produced could unambiguously be assigned 11-*cis*,13-*cis*-stereochemistry; the exceptions were 7 (the 13,14-benzo analog of 1) and **10** (the thiono analog of 1). Thus, in the lactones **2–9** there is almost no chemical shift difference between the protons at C-10 and C-11, as observed for 1 (7) (Table I). In addition, these lactones (with the exception of the 13,14-benzo-analog 7) all possess a carbon resonance in the range of 22–25 ppm for the 13a methyl group (7) (Table II). By contrast the chemical shift difference between the C-10 and C-11 protons in the lactone **10** is 0.56 ppm, and the chemical shift of the 13a methyl is 17.5 ppm. These values resemble those of 13-*cis*-12-hydroxymethylretinoic acid, δ-lactone (0.54 ppm and 18 ppm, respectively) (7). Since the protons at C-10 and C-12a are proximate in the 13-*cis*, but not in the 11-*cis*,13-*cis*-isomer, and the protons at C-11 and C-12a are proximate in the 11-*cis*,13-*cis*, but not in the 13-*cis*- configuration, the Nuclear Overhauser Effects of H-10 and H-11 were used to unambiguously establish the

Table III. Antihyperkeratotic Activity of Retinoids

Compound number	% Inhibition of utriculi[a,b]
1	38
2	7
3	51
4	0
5	22
6	<3
7	9
8	4
9	0
10	21
11	55–70

[a] Topical application of 0.1% solution to rhino mouse skin daily for 4 weeks.
[b] Percent reduction based on the vehicle control.

Table IV. Dose Response of Retinoids in the Inhibition of TPA Promoted DNA Synthesis

	% Inhibition of DNA synthesis using[a,b]						
Compound no.	5 mM	2.5 mM	0.5 mM	0.05 mM	0.005 mM	0.001 mM	0.0005 mM
1	28	23	5				
2				86		34	8
3	45	34	25	8		14	
11	83		66	45	25		

[a] Topical application of 100 μL of test solution to rhino mouse skin daily for 4 weeks.
[b] Percent reduction based on the vehicle control.

configuration. This experiment was carried out in deuteroacetone; in this medium the proton resonances of the 9a and 13a methyl groups were separated by 0.04 ppm. Irradiation of the most downfield methyl protons (2.03 ppm) resulted in enhancements in the integrated intensities of the signals at 6.91 (26%) and 6.49 (18%). Since the 6.49 ppm resonance is uniquely associated with H-14, the 2.03 ppm resonance is clearly assignable to the 13a methyl protons. It follows then that the 6.91 ppm signal arises from H-11 and since this signal is coupled to the signal at 6.35 ppm, the latter must be H-10. Irradiation of the 12a signal gave no signal enhancement at H-11 but enhanced the integrated intensity of the H-10 signal, confirming the 13-cis stereochemistry of **10**.

The structure of lactone **7** was open to question, even based on its preparation. Since the half esters produced in the saponification of the anhydrides **16g** were not separable, the mixture was used to prepare the lactone. The lactone mixture obtained, afforded, after extensive purification, the lactone **7**. A very small amount of a second isomer was also purified. These two lactones failed to interconvert upon heating or exposure to light (with or without iodine), suggesting that they were positional isomers. The H-10 and H-11 signals of the major isomer were identical, as observed for the lactones **1**, **3–6**, and **8–9**. In this case, however, it would be expected that the presence of the aromatic ring at 13,14 would lead to a chemical shift difference between these hydrogens. In other words, H-10 should be deshielded in the 13-*cis* configuration and H-11 should be deshielded in the 11-*cis*,13-*cis* isomer. Similar chemical shifts of H-11 and H-10 would be expected if this compound were a lactone derived from 12-carboxyretinol, since in this case deshielding by the carboxyl carbonyl would have a similar effect to deshielding by the aromatic ring. Irradiation of the methylene hydrogens had no effect on the H-11, H-10 signal, but sharpened some of the aromatic signals, confirming that the methylene group is benzylic rather than allylic. The sterochemistry of this lactone is not known. The minor isomer exhibited decoupling of the H-11 proton upon irradiation of the methylene hydrogens at C-12, suggesting that it was a lactone derived from 12-hydroxymethylretinoic acid. Its biological activity was not investigated due to the small amount obtained.

Replacement of the cyclohexenyl moiety in retinoic acid by an aryl substituent had been found to lead to compounds with improved therapeutic ratios in the antipapilloma test (9). Similarly it had been found that incorporation of the 5,6 double bond in retinoic acid derivatives in an aromatic ring coupled with replacement of the hydrogen atoms at C-4 by methyl groups afforded highly active retinoids in reversal of keratinization (10) with low therapeutic ratios (9). It was, therefore, surprising to find that although the tetramethyltetrahydronaphthyl analog **3** was more effective than the parent compound **1** in utricle reduction as well as in the inhibition of DNA synthesis, the 4′-methoxy-2′,3′,6′-trimethylphenyl analog **2** was essentially inactive in the reduction of utricles (Table III) but was highly active in the inhibition of DNA synthesis (Table IV). In fact, it was about twice as effective as retinoic acid (**11**) in the inhibition of DNA synthesis. Unfortunately the higher activity of the lactone **3** was accompanied by increased skin irritation (Table V). Thus this

Table V. Comparison of Toxicity and Antihyperkeratotic Activity of Retinoids

Treatment with compound no.	Treatment dose (%)	Rabbit[a] mean irritation scores[b]			Mouse[c]
		Erythema	Edema	Scaling	% Utricle reduction[d]
3	0.5	2.5 ± 0.3	1.1 ± 0.2	1.8 ± 0.3	NT[e]
	0.1	2.3 ± 0.5	1.1 ± 0.4	1.7 ± 0.3	51
11	0.5	2.6 ± 0.2	1.2 ± 0.4	2.0 ± 0.2	NT[e]
	0.1	2.9 ± 0.2	1.6 ± 0.3	2.3 ± 0.2	60
	0.01	1.6 ± 0.7	0.8 ± 0.5	1.2 ± 0.5	53
	0.001	1.1 ± 0.9	0.6 ± 0.6	1.0 ± 0.6	42

[a] Skin sites (4 × 4 cm) were treated with 0.2 mL/site of test solution daily for 4 weeks.
[b] Scores are determined by daily grading of the treated site, on a scale of 0–4: 0 = none, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe.
[c] Topical application of 0.1 mL of test solution daily for 4 weeks.
[d] Percent reduction based on the vehicle control.
[e] Not tested.

lactone did not retain the favorable therapeutic ratio observed for lactone 1 (3,11), exhibiting mean irritation scores comparable to those for retinoic acid (Table V). Replacement of the methyl substituents in the tetrahydronaphthyl moiety by hydrogen atoms (lactone 4) led to complete loss of activity (Table III).

CONCLUSION

The δ-lactone derived from 11-*cis*,13-*cis*-12-hydroxymethylretinoic acid (1) possesses unique molecular features associated with its favorable therapeutic ratio as an antihyperkeratotic and antihyperproliferative agent. None of the attempted modifications improved the overall profile of 1. In view of the very low systemic retinoid activity and the absence of any systemic toxicity of 1 (11), this lactone may be a good candidate for use in the topical treatment of acne.

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