A Conformationally Defined 6-*s*-*trans*-Retinoic Acid Isomer: Synthesis, Chemopreventive Activity, and Toxicity

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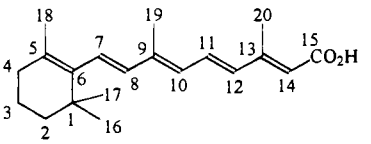
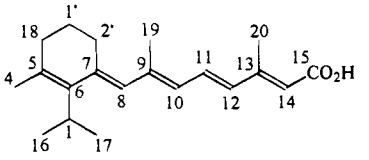
A conformationally defined retinoic acid analog (**1**) which contains a dimethylene bridge to maintain the 6-*s*-*trans* orientation for two terminal double bonds in the polyene chain was synthesized. A Reformatsky reaction was utilized to extend the polyene chain of the starting enone, which provided exclusively the 9*Z*-configuration for the intermediate aldehyde. A Horners–Emmons condensation with this aldehyde then produced retinoic acid analogs with both 9*Z*- and 9*Z*,13*Z*-configurations. An I_2-catalyzed isomerization of the intermediate 9*Z*-aldehyde yielded the *all-E*-aldehyde, which was olefinated as above to yield the (*all-E*)- and (13*Z*)-retinoic acid analogs of **1**. Each configurational isomer of **1** was evaluated for its ability to inhibit the binding of retinoic acid to CRABP (chick skin) and to inhibit the chemical induction of ornithine decarboxylase in mouse skin. In each assay (*all-E*)-**1** was the most active isomer, and this activity was comparable to or better than that for (*all-E*)-retinoic acid. (*all-E*)-**1** and (13*Z*)-**1** were both shown to be equally effective as (13*Z*)-retinoic acid in suppressing the proliferation of human sebaceous cells *in vitro*. (*all-E*)-**1** was further evaluated for its ability to prevent the induction of mouse skin papillomas and to induce signs of vitamin A toxicity in mice. The cancer chemopreventive activity of (*all-E*)-**1** was comparable to that of (*all-E*)-retinoic acid, and the toxicity was comparable to or slightly better than that of the natural vitamin.

It is well known that one form of vitamin A, retinoic acid (RA), plays an essential role in many diverse biological events[1] ranging from maintaining the correct differentiation state of a cell to acting as a morphogen.[2] Even though the biological mechanisms by which RA elicits its actions are not well understood, it has become increasingly evident that two families of nuclear retinoic acid receptors, RARs and RXRs, are important in the biological actions of this vitamin.[3] Since RA is capable of controlling gene expression, its use in the therapy of cancer, as either a chemopreventive or a chemotherapeutic agent, has been extensively explored in animal models.[4] Numerous studies have now shown that RA provides a promising new approach to the prevention and treatment of cancer. For example, RA has been used as a clinically effective treatment for promyelocytic leukemia (PML) and juvenile chronic myelogenous leukemia (JCML) in a majority of patients.[5] From the work of Davies[6] and Bollag,[7] extensive studies have also shown that it is active in the treatment of skin diseases,[8] including acne, psoriasis, and skin cancer.[9] Evidence is accumulating that these disorders may be due to the abnormal expression of retinoic acid receptors.[10]

Currently, the use of retinoic acid in therapy has been hampered by its high toxicity and teratogenicity, which may be mediated by RA receptors and binding proteins.[11] Several laboratories have tried to improve the therapeutic ratio by synthesizing retinoic acid analogs, collectively named retinoids,[12] including arotinoids,[13] retinobenzoic acids,[14] and bifunctional retinoids.[15] Structure–activity relationships have revealed that modifications in every region of the RA molecule alter the cancer chemopreventive activity. For example, the trimethylcyclohexenyl ring of RA is particularly important for its chemopreventive actions.[16] When this ring is absent or altered, the biological activities of the corresponding retinoids are usually diminished. For RA in solution, this ring adopts two rapidly interconverting low-energy conformational states relative to the polyene chain; a 6-*s*-*cis* conformer is dominant, and a 6-*s*-*trans* conformer is only a few kilocalories per mole above the low-energy state.[17] Furthermore, several different vitamin A-binding proteins have been reported[18] to select one conformation, but not both.



6-*s*-*trans* retinoic acid



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In order to examine the effects of each conformational state in interactions with proteins, cancer chemopreventive ability, and vitamin A toxicity, we have designed conformationally defined RA analogs.[19] In these, a dimethylene bridge was employed to rigidly create either a 6-*s*-*cis* or a 6-*s*-*trans* orientation. Here we report the synthesis and biological activities for one example of the 6-*s*-*trans* analogs, retinoid **1**, which is a

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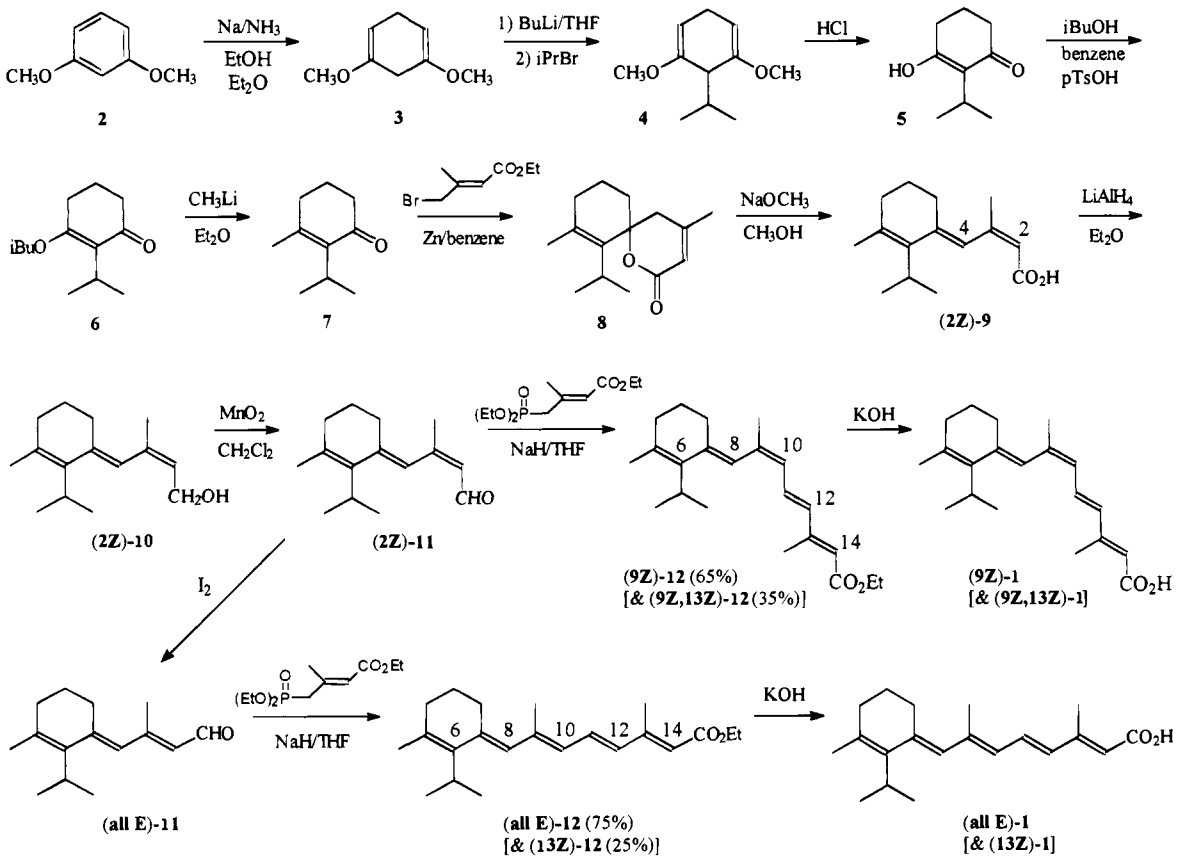
Scheme 1

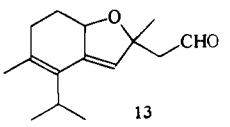


structural isomer of retinoic acid. We show that this retinoid exhibits similar biological activities *in vitro* and in animal models as RA (in terms of recognition by protein binding sites, suppression of skin diseases like acne, and prevention of the chemical induction of skin cancer) but has a slightly lower toxicity than the natural vitamin.

Chemistry. A retrosynthetic analysis for retinoid **1** suggested enone **7** as a suitable intermediate that could undergo olefination to introduce the polyene chain. The synthesis of this ketone was accomplished using an approach described for the synthesis of 2,3-dimethyl-2-cyclohexenone.[20] As shown in Scheme 1, 2-isopropyl-1,3-cyclohexanedione (**5**) was synthesized from 1,3-dimethoxybenzene (**2**) according to a literature method.[21] Thus compound **2** was treated under Birch reduction conditions to give diene **3**. This was alkylated under basic conditions with 2-bromopropane to provide the isopropyl derivative **4**, which was hydrolyzed in aqueous HCl to give dione **5**. This was converted to the isobutyl enol ether **6**, which was reacted with MeLi to give the desired ketone **7**. As we previously reported[22] for other hindered cyclohexanones, ketone **7** did not react with triethylphosphonosenecioate under Horner–Emmons conditions. A Reformatsky reaction between enone **7** and ethyl 4-bromo-3-methyl-2-butenoate[23] was then attempted, which gave δ-lactone **8** as the major product. Similar δ-lactone products have previously been observed for Reformatsky reactions between this bromo ester and other α,β-unsaturated aldehydes or ketones by us,[22] Gedye et al.,[24] Kogel et al.,[25] Eiter et al.,[26] and De Bruin et al.[27] Lactone **8** cleanly underwent eliminative ring opening in the presence of sodium methoxide to give the acid (2*Z*)-**9**. This acid was reduced to alcohol (2*Z*)-**10** using $LiAlH_4$, and the crude alcohol was immediately oxidized with MnO_2 to give aldehyde (2*Z*)-**11**.

Although the conversion of δ-lactone **8** to acid **9** was anticipated to produce the 2*Z*-isomer, this geometry was confirmed by NOE experiments. For acid **9** and aldehyde **11**, a significant steady-state NOE (25%) was observed between the C3 methyl protons and H2, verifying the 2*Z*-configuration for compounds **9**–**11**.

During the oxidation of (2*Z*)-**10** to (2*Z*)-**11**, it was observed that long exposure of the alcohol to MnO_2 at room temperature decreased the yield of aldehyde via conversion of the product to the cyclic ether **13**. A mechanism for the formation of **13** from (2*Z*)-**11** may be postulated. Hydroxylation of (2*Z*)-**11** at one of the allylic cyclohexene ring methylenes would produce an intermediate which could, via 1,4-addition, attack the carbon that is β to the aldehyde to produce **13**. Although we could not avoid the formation of this ether, aldehyde (2*Z*)-**11** was obtained in 70% yield using the procedure detailed in the Experimental Section.



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Further elaboration of the polyene side chain was then accomplished using a Horner–Emmons condensation between aldehyde (2*Z*)-**11** and triethylphosphonosenecioate (Scheme 1). This provided the ester **12** (80%) as a mixture of stereoisomers. The ^1H chemical shift assignments (Table 1) were made by selective decoupling experiments and COSY 2D experiments. The

Table 1. ^{1}H Chemical Shifts (300 MHz) of Four Isomers of Retinoids **1** and **12** in $CDCl_3$ (ppm, TMS)[a]

isomer	H1′	H2′	H1	H4	H8	H10	H11	H12	H14	H16	H18	H19	H20
(*all-E*)-**12**	1.58	2.44	3.01	1.77	5.97	6.05	6.90	6.20	5.71	1.15	2.08	1.96	2.33
(9*Z*)-**12**	1.57	2.03	3.05	1.75	5.84	5.99	6.57	6.16	5.69	1.18	2.08	1.86	2.22
(13*Z*)-**12**	1.56	2.43	3.01	1.77	5.95	6.15	6.90	7.71	5.58	1.15	2.07	1.95	2.04
(9*Z*,13*Z*)-**12**	1.56	2.05	3.04	1.74	5.83	6.07	6.58	7.63	5.56	1.17	2.07	1.86	1.92
(*all-E*)-**1**	1.63	2.48	3.06	1.82	6.02	6.10	6.99	6.27	5.78	1.20	2.12	2.01	2.37
(9*Z*)-**1**	1.61	2.10	3.10	1.80	5.91	6.04	6.68	6.22	5.75	1.23	2.10	1.91	2.26
(13*Z*)-**1**	1.63	2.50	3.06	1.82	6.02	6.21	6.98	7.70	5.64	1.20	2.12	2.01	2.11
(9*Z*,13*Z*)-**1**	1.61	2.10	3.09	1.80	5.91	6.15	6.68	7.65	5.62	1.23	2.10	1.92	2.00

[a] Retinoids **1** and **12** were numbered analogously to retinoic acid for easy comparison.

E/*Z*-stereochemical assignments for **1** and **12** were determined by (1) chemical shift differences according to Hope et al.,[28] (2) steady-state NOE measurements between methyl and vinyl protons in *Z*-configurations, (3) relative retention times on silica HPLC,[29] and (4) relative wavelength maxima and extinction coefficients in UV/vis spectra (see the Experimental Section). These experiments indicated that (9*Z*)-**12** and (9*Z*,13*Z*)-**12** were formed in a 2:1 ratio during this condensation. (Note that atom numbering consistent with that for retinoic acid is now being used.) After preparative HPLC separation of the 9*Z*- and 9*Z*,13*Z*-isomers of **12** on silica gel, the corresponding acids **1** were generated, without isomerization, by hydrolysis in KOH.[30] We have also reported similar success in hydrolyzing other retinoid esters without isomerization under these conditions.[29]

In order to produce the *all-E*- and 13*Z*-isomers of **1**, aldehyde (2*Z*)-**11** was thermally isomerized at room temperature, using I_2 as a catalyst, to give a 2:1 mixture of (2*Z*)-**11** and (*all-E*)-**11** (Scheme 1). Similar attempts to isomerize the 2*Z*-isomers of compounds **9** and **10** were unsuccessful. Following separation on silica gel, the 2*Z*-isomer was reisomerized. A Horner–Emmons condensation between (*all-E*)-**11** and triethylphosphonosenecioate generated the *all-E*- and 13*Z*-isomers of **12** in a 3:1 ratio. After HPLC separation on silica gel, the *all-E*- and 13*Z*-isomers of **1** were generated by hydrolysis of the corresponding isomers of **12**, again without interconversion of the stereoisomers. The configurational assignments were made using the procedures mentioned for the 9*Z*- and 9*Z*,13*Z*-isomers (see Table 1).

Biology. The four stereoisomers of retinoic acid analog **1** that were produced in Scheme 1 were first evaluated in a protein-binding assay developed by Sani and co-workers.[31] Each isomer of retinoid **1** was tested for its ability to inhibit the binding of (*all-E*)-RA to cytoplasmic retinoic acid-binding protein (CRABP) from chick skin in order to test their ability to recognize a RA-binding site within protein. Also, Trown and co-workers,[32] Sani,[33] and Hill and Grubbs[33b] have suggested that an empirical correlation exists between retinoid binding to CRABP and cancer chemoprevention in skin; retinoids which bind well to CRABP usually have high chemopreventive activity in the mouse skin antipapilloma assay, but the opposite was not necessarily true. While it is unlikely that retinoid binding to CRABP is responsible for cancer chemoprevention, the *in vitro* assay appears to provide a useful and rapid screen for identifying potential new chemopreventive retinoids in skin.

These isomers were also evaluated in an enzyme assay developed by Verma and Boutwell,[34] which measures the extent that retinoids inhibit the chemical induction of ornithine decarboxylase (ODC). Verma, Boutwell, and co-workers[35] also demonstrated that retinoids which were active in the ODC assay were active in the suppression of papilloma formation in mouse skin. This suggests that ODC production is a biochemical marker for the two-stage chemical induction of tumors in mouse skin. Dawson et al.[36] recently extended this correlation using a larger number of retinoids and suggested that the ODC assay be used as a rapid preliminary screen for cancer chemoprevention in skin.

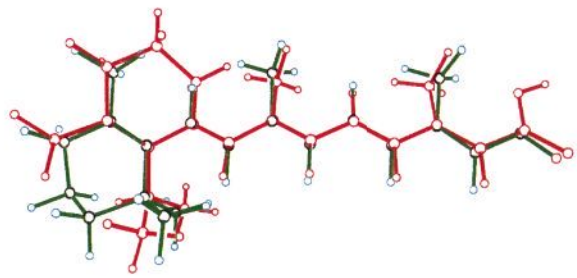

Figure 1. The structures of the 6-*s-trans* conformation of (*all-E*)-retinoic acid (green) and (*all-E*)-retinoid **1** (red) overlaid to maximize the overlap of the polyene chain carbons and carboxylic acid groups. The torsional angles of the *s-trans* bonds were set to 180° for each structure.

This approach was adopted in the evaluation of retinoid **1**; the activity of the tested retinoids in the CRABP-binding and ODC assays were used to select the most active isomer(s) for further evaluation. These isomers were next evaluated for their ability to inhibit the proliferation of human sebaceous cells *in vitro*.[37] A correlation exists been the activity of retinoids in this assay with their utility in acne prevention and treatment.[38] Also, retinoid **1** was evaluated in the antipapilloma assay, which directly measures the ability of retinoids to prevent chemically induced skin tumors. Finally, the most active isomer was evaluated for its toxicity in mice. The maximal tolerated dose (MTD), which is the largest dose that could be tolerated for a 29 day period without producing clinical signs of hypervitaminosis A, was determined.

Results and Discussion

The structure of 6-*s-trans*-(*all-E*)-retinoic acid (green) superimposed on the structure of (*all-E*)-retinoid **1** (red) is shown in Figure 1. These structures were not energy minimized but placed in conformations containing fully extended *s-trans* bonds in the polyene chain to clearly permit atom-to-atom comparisons. As shown, when the polyene chain and carboxylic acid groups are overlaid, the methyl group and isopropyl group substituents of the C5,C6 double bond on retinoid **1** can occupy similar

Table 2. Activities for Individual Isomers of Retinoid 1 and Retinoic Acid in the CRABP-Binding Assay, ODC Assay, Sebaceous Cell Assay, and Antipapilloma Assay

retinoid	CRABP-binding assay IC_{50} (μM)[a]	ODC assay (percent inhibition)[c]	sebaceous cell assay IC_{50} (nM)[d]	antipapilloma assay ED_{50} (nmol)[e]
(*all-E*)-retinoic acid	0.37	92[b]	100	2.9
(13*Z*)-retinoic acid	0.90	72[b]	100	
(*all-E*)-retinoid 1	0.47	98	100	3.0
(13*Z*)-retinoid 1	2.3	98	100	
(9*Z*)-retinoid 1	≥500	74		
(9*Z*,13*Z*)-retinoid 1	≥1000	53		

[a] The IC_{50} values were determined by a probit analysis. The standard deviation was less than ±0.1 μM for the *all-E* and 13*Z*-isomers. [b] The percent inhibition reported for (*all-E*)-RA and (13*Z*)-RA were means of 30 and 4 determinations as reported by Shealy and co-workers.[41] The standard deviation for the percent inhibition of (*all-E*)-RA was less than 1%. [c] The ODC assay for each isomer of 1 was performed at a single dose (17 nmol) of test retinoid in acetone. The positive TPA control yielded 9.81 nmol of CO_2/30 min/mg of protein, and the negative acetone control yielded 0.24 nmol of CO_2/30 min/mg of protein. (*all-E*)-RA yielded 0.70 nmol of CO_2/30 min/mg of protein in this assay. The percent inhibition was calculated as [1 − (ODC activity − acetone control)/(TPA control − acetone control)] × 100. [d] The IC_{50} values were determined graphically. The IC_{50} values for (13*Z*)-RA were determined over 100 times; the IC_{50} values reported for the isomers of 1 were determined twice. [e] The mean number of tumors in the TPA control group (X_{TPA}) of animals was 15.1 (20 animals). The mean number of tumors was determined for six doses of retinoid (X_{ret}) varying between 0.19 and 45.9 nmol. The ED_{50} values were determined by a probit analysis of $[1 - (X_{ret}/X_{TPA})] \times 100$ versus log[retinoid]. The standard error was less than ±1.5 nmol for each retinoid.

space as the C4 methylene and C1 *gem*-dimethyl groups of retinoic acid. The C1′ and C2′ methylene carbons of retinoid 1, which maintain the 6-*s-trans* orientation of the polyene chain, add extra steric volume near the C9 methyl group (C19) which is not present in retinoic acid. Therefore, besides this extra volume, retinoid 1 has a structure analogous to retinoic acid which rigidly maintains the 6-*s-trans* conformation.

Four isomers of retinoid 1 were evaluated in the CRABP-binding assay and in the ODC assay. The IC_{50} values are given in Table 2 and compared to those of (*all-E*)-RA and (13*Z*)-RA. As shown, (*all-E*)-1 was nearly as effective as (*all-E*)-RA in competing for the single ligand-binding site in CRABP-II from the skin.[39] Studies[31] have shown that many RA analogs with modifications at the ring (e.g., phenyl, dichlorophenyl) or retinoids without defined rings often lead to reduced binding affinities in the CRABP-binding assay (chick skin). The results displayed in Table 2 indicate that the dimethylene bridge introduced to maintain a 6-*s-trans* conformation in (*all-E*)-1 does not sterically interfere with the RA-binding site in CRABP. (The C1′ and C2′ methylene groups occupy space not in common with atoms of RA when the polyene chains are superimposed.)

The IC_{50} for (13*Z*)-1 was only 3-fold greater than that for (*all-E*)-1 (Table 2). These results are consistent with studies[33] on (13*Z*)-RA; this isomer competes nearly as well for the RA-binding site in CRABP-II (chick skin) as the *all-E*-isomer (Table 2). Interestingly, Napoli and co-workers[40a] have indicated that binding of (13*Z*)-RA to CRABP-II (cloned from mouse) is about 10-fold weaker than binding of (*all-E*)-RA. In comparison to the *all-E*- or 13*Z*-isomer, both (9*Z*)-1 and (9*Z*,13*Z*)-1 had considerably lower affinity for binding to chick skin CRABP-II; the IC_{50} values of these retinoids were at least 500-fold greater than that of (*all-E*)-1 (Table 2). This result is consistent with recent reports from several laboratories[40] showing that (9*Z*)-RA is a less effective binder to both CRABP-I and CRABP-II (mouse skin) than (*all-E*)-RA. Studies on the relative binding affinity of (9*Z*,13*Z*)-RA have not been reported.

These isomers were next evaluated in the ODC assay. As summarized by Shealy, Hill, and co-workers,[41] (*all-E*)-RA was very active in suppressing the production of ODC (92% inhibition) and (13*Z*)-RA was less active (72% inhibition) than (*all-E*)-RA (Table 2). Consistent with these data, both Verma et al.[42] and Dawson et al.[17] have shown that the (13*Z*)-RA is less active than (*all-E*)-RA in this assay. In contrast to this trend for RA, (*all-E*)-1 and (13*Z*)-1 were equally active in the ODC assay (Table 2), and the activity of each of these isomers was marginally better than that of (*all-E*)-RA. (In this experiment, the percent inhibition for (*all-E*)-RA was 94%.) The reason for the enhanced activity of (13*Z*)-1 over (13*Z*)-RA is unknown. Interestingly, (9*Z*)-1 also displayed good activity in suppressing ODC activity; this activity was similar to the average ODC value reported for (13*Z*)-RA by Shealy et al.[41] (Table 2). Even (9*Z*,13*Z*)-1 had modest activity in this assay.

Taken together, the data from the preliminary biological screens suggested that an *all-E*-structure in the polyene chain provided the highest activity, and this activity diminished with the introduction of *Z*-configurations within the polyene side chain. (*all-E*)-1 was essentially as active as (*all-E*)-RA in both assays, revealing that (1) rigidly constraining the C6−C7 bond to an *s-trans* conformation or (2) introducing two extra methylene groups (C1′ and C2′) between C7 and C18 of 6-*s-trans*-RA does not diminish activity.

The activity of both (*all-E*)-1 and (13*Z*)-1 were next evaluated for their ability to supress the proliferation of human sebaceous cells *in vitro* over a 12 day period. The IC_{50} values for both of these isomers were about 100 nM (Table 2), which was equal to the positive standard, (13*Z*)-RA. Previous studies have shown that retinoids known to be active against acne (13*Z*-RA) are also active in preventing proliferation of these cells *in vitro*, while retinoids which are inactive in acne[43] (temarotene) or active in psoriasis but inactive in acne[44] (etretinate) have poor activity. Doran, Shapiro, and co-workers[38] have recently shown a correlation between the effectiveness of retinoids in the treatment of acne and their activity in this *in vitro* assay. The high activity of (*all-E*)-1 and (13*Z*)-1 in this assay indicates that these retinoids may have utility in the treatment of acne.

(*all-E*)-1 was next evaluated for its ability to reduce the induction of tumors in the mouse skin antipapilloma assay (Table 2). The ED_{50} value of 3 nmol for (*all-E*)-1 was nearly the same as that of (*all-E*)-RA reported in this study and by Dawson et al.[29] (3.5 nmol). Due to

Table 3. Toxicity Comparison of (*all-E*)-1 and (*all-E*)-Retinoic Acid in Mice

retinoid dose level (mg kg^{-1} day^{-1})	day 29 body weight (g, mean ± SD)[a]	hypervitaminosis A[b]	
		mortality	skin scaling
	(*all-E*)-Retinoic Acid		
0 (corn oil)	28.6 ± 2.6	–	–
2.5	29.3 ± 1.0	–	–
10	30.5 ± 1.8	–	–
30	28.0 ± 1.6	0/6	6/6
	(*all-E*)-Retinoid 1		
0 (corn oil)	24.3 ± 1.3	–	–
15	25.0 ± 1.5	–	–
30	23.7 ± 0.9	–	–
60	21.8 ± 2.0	1/6	1/5

[a] The mean body weight and standard deviation were determined from groups of six animals. [b] The – indicates that no signs of toxicity were found in any animals within the group of six. When toxicity was present, the first number is the number of animals with the sympton of hypervitaminosis A and the second number is the total number of animals within the group.

the recent interest in (9*Z*)-RA and its interaction with RARs and RXRs, (9*Z*)-1 was also evaluated in this assay. Even though the data did not permit the determination of an ED_{50} for this isomer, no tumor reduction was apparent at doses of 5 nmol; doses greater than 15 nmol were required for 30% tumor suppression. Thus, (9*Z*)-1 is less effective as a chemopreventive agent than (*all-E*)-1, which is the same trend observed for these two isomers in the ODC assay and the CRABP-binding assay. A comparison of the chemoprevention effects of (*all-E*)-RA with (9*Z*)-RA has not previously been reported in the literature.

Since (*all-E*)-1 had equal activity to (*all-E*)-RA in several biological assays (Table 2), we next evaluated its subacute toxicity in mice. The maximum tolerated dose (MTD), the largest dose which does not produce clinical signs of hypervitaminosis A, was determined for 29 days of dosing. As shown in Table 3, both 2.5 and 10 mg kg^{-1} day^{-1} doses of (*all-E*)-RA for 29 days did not induce a significant decrease in weight gain (compared to controls) nor result in the appearance of clinical signs of hypervitaminosis A in any animal within the group. At doses of 30 mg kg^{-1} day^{-1} for (*all-E*)-RA, symptoms of hypervitaminosis appeared as skin scaling in all animals within the group (Table 3). This is consistent with extensive toxicology studies conducted by Lindamood et al.[45] on (*all-E*)-RA. Clinical signs of hypervitaminosis A appeared at 30 mg kg^{-1} day^{-1} (21 days of dosing). Skin scaling was one of the first signs observed, and it appeared in the majority of the animals tested. (Other signs like alopecia occurred later and were less prevalent.) Only mild signs of toxicity were reported previously for (*all-E*)-RA at doses of 10 mg kg^{-1} day^{-1} (21 or 56 days of dosing). These reports and those reported by Hixson and Denine[46] suggest an oral MTD of 10 mg kg^{-1} day^{-1} using these clinical signs of toxicity, which was well below the oral LD_{50} (1100 mg kg^{-1} day^{-1}).

The toxicity of (*all-E*)-1 was determined in a separate experiment using higher doses of the test compound. In contrast to (*all-E*)-RA, the 15 or 30 mg kg^{-1} day^{-1} doses of (*all-E*)-1 resulted in no signs of toxicity for any animal within the group (neither reduction in weight gain nor other clinical signs) when administered for 29 days (Table 3). Toxicity appeared at doses of 60 mg kg^{-1} day^{-1}; one animal had signs of skin scaling in a group of six, and one died. Even though this dose of (*all-E*)-RA was not tested in the present study, Hixson and Denine[46] showed that some mortality occurred for (*all-E*)-RA in this dosing range (3 out of 60 animals died in the 5–60 mg kg^{-1} day^{-1} doses of (*all-E*)-RA). The toxicity data clearly show a difference in toxicity between (*all-E*)-RA and (*all-E*)-1 at the 30 mg kg^{-1} day^{-1} doses, suggesting a comparable or slightly lower toxicity for this new retinoid. The lower toxicity (Table 3) and similar activity (Table 2) of (*all-E*)-1 indicates it has an improved therapeutic index (MTD/ED_{50}) as compared to (*all-E*)-RA in animal models.

In conclusion, we have synthesized one example of a new class of conformationally constrained 6-*s*-*trans* retinoids (1). We have shown that neither this conformational constraint nor the extra steric volume introduced by the dimethylene bridge utilized to maintain this conformation diminishes the activity of the retinoid 1 in several biological assays including the mouse skin antipapilloma assay. Even though (*all-E*) 1 lacks two methylene carbons found in the trimethylcyclohexenyl ring of RA (C2 and C3), it has essentially the same activity as (*all-E*)-RA with slightly less toxicity. Furthermore, the synthetic approach employed for this class of retinoids allows for the straightforward addition of other R-groups to the cyclohexenyl ring of 1 that would more fully mimic the steric volume of RA. Studies involving these more highly elaborated conformationally defined 6-*s*-*trans*-RA analogs are presently being pursued.

Experimental Section

Chemistry. General Methods. ^{1}H NMR spectra were obtained at 60 MHz on a Varian EM-360 spectrometer or at 300.1 MHz on a GE spectrometer (NT300) equipped with a 1180e Nicolet computer and 293c pulse programmer. NMR spectra were referenced internally to TMS. Steady-state NOE experiments were performed on degassed samples in sealed tubes. Irradiation prior to acquisition was for 10 s at a power sufficient to saturate the irradiated signal. NOE were calculated from integrating peaks in the difference spectrum obtained by subtracting the off-resonance spectrum. UV/vis spectra were recorded on a Perkin Elmer Lambda 6 or AVIV 14DS spectrophotometer in methanol solution (Fisher, Spectrograde). IR spectra were recorded using Beckman AccuLab-4 or Nicolet FT IR spectrometers. Electron-impact mass spectra were obtained on a Hewlett Packard 5985 GC/MS with an ultraperformance fused silica column. High-resolution mass spectra were measured on a VG Instruments 70S mass spectrometer. The reference compound was perfluorokerosene, and the mass spectrometer was tuned to a resolution of 6000 prior to analysis. HPLC separations were performed on a Rainin HPLC system using Gilson pumps and controller and a Hitachi model 100-40 variable wavelength detector. The column employed was a Whatman Partisil 10 M9/50 (500 × 9.4 mm i.d.). Preparative separations were obtained with 2 mL/min flow rates using the indicated solvent systems. TLC was performed on precoated 250 μm silica gel GF glass plates (Analtech, Inc.; 5 × 10 cm) using 10% acetone/hexane as eluent. Solvents and liquid starting materials were distilled prior to use. When necessary, reactions and purifications were conducted with deoxygenated solvents, under inert gas (N_2), and using subdued lighting. Triethylphosphonosenecioate was synthesized according to Iqbal et al.[23] Compound **5** was synthesized from 1,3-dimethoxybenzene according to Sardina et al.[21] as summarized in Scheme 1.

3-(Isobutyloxy)-2-isopropyl-2-cyclohexen-1-one (6). A solution of compound **5** (29.6 g, 0.192 mol), isobutyl alcohol (64.0 mL, 51.4 g, 0.687 mol), and *p*-toluenesulfonic acid (1.0 g) in benzene (400 mL) was refluxed overnight with azeotropic removal of water (Dean–Stark trap). The solution was cooled

to room temperature and extracted with ether (3 × 100 mL), and the combined ether layers were washed once with 5% sodium bicarbonate (100 mL). The ether was dried (Na_2SO_4) and removed under reduced pressure to yield enol ether **6** (38.6 g, 96.0% yield): bp 98 °C (0.2 mmHg); IR (neat) 1640 (C=O), 1600 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 267 nm (ϵ 9800); R_f 0.35; MS *m/z* 210 (M^+); ^1H NMR (300 MHz, $CDCl_3$) δ 3.7 (d, 2 H, OCH_2), 3.2 (m, 1 H, CH), 2.5 (t, 2 H, allylic CH_2), 2.2 (t, 2 H, CH_2), 2.0 (m, 3 H, CH_2 and CH). Anal. ($C_{13}H_{22}O_2$) C, H.

2-Isopropyl-3-methyl-2-cyclohexen-1-one (7). To a solution of enol ether **6** (38.6 g, 0.183 mol) in ether (250 mL) at 0 °C was added 1.4 M methyllithium (196 mL, 0.275 mol), and the resulting mixture was stirred for 90 min at 0 °C. The reaction was quenched with water, and the product was extracted with ether (3 × 600 mL). The ether was dried (Na_2SO_4) and concentrated under reduced pressure to provide ketone **7** (26.1 g, 94% yield) as a colorless oil: bp 56 °C (0.2 mmHg); IR (neat) 1650 (C=O), 1600 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 244, 204 nm; R_f 0.58; MS *m/z* 152 (M^+); ^1H NMR ($CDCl_3$) δ 3.0 (m, 1 H, CH), 2.3 (t, 4 H, CH_2), 1.9 (s, 3 H, CH_3), 1.8 (m, 2 H, CH_2), 1.2 (d, 6 H, CH_3). Anal. ($C_{10}H_{16}O$) C, H.

7-Isopropyl-4,8-dimethyl-1-oxaspiro[5.5]undec-3-en-2-one (8). Zinc dust (4.0 g, 0.061 mol) was activated by washing successively with 10% HCl (8 mL), water (4 × 15 mL), MeOH (15 mL), acetone (15 mL), ether (2 × 15 mL), and anhydrous benzene (3 × 15 mL). The activated zinc was suspended in dried benzene (10 mL), and the mixture was heated to reflux without stirring. About one-third of a solution containing ketone **7** (3.8 g, 0.025 mol) and ethyl bromosenecioate[22] (7.8 g, 0.038 mol) in benzene (5 mL) was added all at once. Vigorous boiling resulted, and a yellow-green color developed. The heating source was removed, and the remaining solution of reactants was added dropwise at a rate that maintained reflux. Heating at reflux was then resumed overnight. To the refluxing mixture was slowly added water (2 mL), which resulted in vigorous boiling, and the mixture was stirred for 2 h at room temperature. This mixture was filtered through a pad of Celite, the filter was washed with water and ether, and the filtrate was concentrated on a rotary evaporator. The residue was dissolved in ether (150 mL) and extracted with 6 N NaOH (2 × 75 mL) followed by water (2 × 75 mL). The ether was dried (Na_2SO_4) and concentrated under reduced pressure to yield a yellow oil (4.6 g), whose TLC indicated that it contained primarily product **8** and unreacted ketone **7**. This was distilled to give the starting ketone **7** (1.2 g): bp 42–47 °C (0.25 mmHg). To the distillation pot residue was added hexane, and the mixture was cooled to provide δ-lactone **8** (2.4 g, 60% yield) as a white solid: mp 91.5–92.5 °C (hexane); IR (neat) 1730 (C=O), 1690 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 228 nm; R_f 0.17; MS *m/z* 234 (M^+); ^1H NMR ($CDCl_3$) δ 5.8 (s, 1 H, vinyl), 2.7 (m, 1 H, CH), 2.6 (s, 2 H, CH_2), 1.9 (s, 3 H, CH_3), 1.7 (s, 3 H, CH_3), 2.2–1.6 (m, 6 H, CH_2), 1.2 (q, 6 H, CH_3). Anal. ($C_{15}H_{22}O_2$) C, H.

(2*Z*)-4-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3-methyl-2-butenoic Acid ((2*Z*)-9). Freshly cut sodium metal (400 mg, 17.4 mmol) was dissolved in methanol (20 mL). To this solution was added δ-lactone **8** (3.35 g, 14.3 mmol), and the resulting mixture was heated at reflux for 1.5 h, at which time TLC indicated completion of the reaction. The solvent was removed under vacuum, the residue was dissolved in ether (150 mL), and the solution was washed with water (2 × 100 mL). The combined water layers were acidified (pH ≤ 2) with concentrated HCl and then extracted with ether (2 × 125 mL). The combined ether layers were dried (Na_2SO_4) and concentrated to give a solid residue. This was triturated with hexane, cooled, and filtered to provide acid (2*Z*)-**9** (3.30 g, 98.5% yield) as a white solid: mp 114–116 °C; IR (neat) 1690 (C=O), 1590 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 232 (ϵ 8100), 307 nm (ϵ 9500); R_f 0.20; MS *m/z* 234 (M^+); ^1H NMR ($CDCl_3$) δ 6.5 (s, 1 H, vinyl), 5.7 (s, 1 H, vinyl), 3.1 (m, 1 H, CH), 2.2 (m, 4 H, CH_2), 2.1 (s, 3 H, CH_3), 1.8 (s, 3 H, CH_3), 1.6 (m, 2 H, CH_2), 1.2 (d, 6 H, CH_3). Anal. ($C_{15}H_{22}O_2$) C, H.

(2*Z*)-4-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3-methyl-2-butenol ((2*Z*)-10). A solution of acid (2*Z*)-**9** (2.25 g, 9.61 mmol) in anhydrous ether (24 mL) was cooled to 0 °C in an ice bath, and 1 M $LiAlH_4$/ether (13.0 mL, 13.0 mmol) was added dropwise with stirring. The mixture was stirred at 0 °C for an additional 45 min. The reaction mixture was cooled to −78 °C, and methanol (10 mL) followed by 10% H_2SO_4 (20 mL) was added dropwise. This was allowed to warm to room temperature, additional 10% H_2SO_4 (30 mL) was added, and the mixture was extracted with ether (50 mL). The ether layer was washed with brine (1 × 40 mL), dried (Na_2SO_4), and concentrated under vacuum to provide (2*Z*)-**10** (2.10 g, 100% yield) as a pale yellow oil. Alcohol (2*Z*)-**10** was carried on in this form without further purification: IR (neat) 3320 (OH), 1610 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 254 nm; R_f 0.24; MS *m/z* 220 (M^+); ^1H NMR ($CDCl_3$) δ 5.8 (s, 1 H, vinyl), 5.4 (t, 1 H, vinyl), 4.0 (d, 2 H, CH_2O), 3.0 (m, 1 H, CH), 2.1 (m, 4 H, CH_2), 1.8 (s, 8 H, CH_3 and CH_2), 1.2 (d, 6 H, CH_3). HRMS calcd for $C_{15}H_{24}O$, 220.1827; found, 220.1813.

(2*Z*)-4-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3-methyl-2-butenal ((2*Z*)-11) and 4-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3,6′-epoxy-3-methylbutanal (13). To a stirred solution of alcohol (2*Z*)-**10** (6.00 g, 27.3 mmol) in dry CH_2Cl_2 (250 mL) at 0 °C was added activated MnO_2 powder (25.0 g) in five portions over a period of 4 h. Activated, powdered 4 Å molecular sieves (3.0 g) were added, and this was stirred for an additional 8 h at 0 °C. MnO_2 (25.0 g) was again added in five portions during the next 4 h, powdered 4 Å molecular sieves (3.0 g) were added, and the mixture was stirred at 0 °C for an additional 8 h. The reaction mixture was then filtered through a pad of Celite, and the filter was washed extensively with CH_2Cl_2 (1 L). The filtrate was concentrated under vacuum to give an oily residue (5.61 g). This was placed on a flash silica gel column (5 × 15 cm; 600 g of silica) and eluted with 2.5% acetone/hexane to give the starting alcohol (2*Z*)-**10** (0.31 g), (2*Z*)-**11** (4.1 g, 69% yield), and cyclic aldehyde **13** (0.27 g, 4.0% yield). For (2*Z*)-**11**: IR (neat) 1670 (C=O), 1600 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 239, 307 nm; R_f 0.60; MS *m/z* 218 (M^+); ^1H NMR ($CDCl_3$) δ 9.5 (d, 1 H, CHO), 6.0 (s, 1 H, vinyl), 5.8 (d, 1 H, vinyl), 3.1 (m, 1 H, CH), 2.2 (t, 4 H, CH_2), 2.0 (s, 3 H, CH_3), 1.8 (s, 3 H, CH_3), 1.7 (m, 2 H, CH_2), 1.2 (d, 6 H, CH_3). HRMS calcd for $C_{15}H_{22}O$, 218.1671; found, 218.1656. For **13**: IR (neat) 1720 (C=O), 1670 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 241 nm; R_f 0.40; MS *m/z* 234 (M^+); ^1H NMR ($CDCl_3$) δ 9.7–9.5 (m, 1 H, CHO), 5.5 (s, 1 H, vinyl), 4.6 (m, 1 H, CH), 2.9 (m, 1 H, CH), 2.6 (m, 2 H, CH_2), 2.1 (m, 2 H, CH_2), 1.8 (s, 3 H, CH_3), 1.4 (d, 3 H, CH_3), 1.2 (m, 6 H, CH_3). Anal. ($C_{15}H_{22}O_2$) C, H.

(2*E*,4*E*)-4-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3-methyl-2-butenal ((*all-E*)-11). In a 500 mL round bottom flask containing (2*Z*)-**11** (2.76 g, 12.7 mmol) and 1:9 benzene/hexane (200 mL) at room temperature, under nitrogen and in the dark, was added a 1% I_2 solution (in hexane; 12 mL) in 4 mL portions during 30 min. The mixture was stirred for an additional 30 min at room temperature, saturated sodium thiosulfate (100 mL) was added, and stirring was continued for 15 min. This was extracted with ether (100 mL), and the ether layer was dried (Na_2SO_4) and concentrated under reduced pressure. The resulting yellow oil (2.69 g) was placed on a flash silica column (2.5 × 10 cm; 270 g of silica) and eluted with 1% acetone/hexane. Two components with R_f values of 0.45 and 0.60 were separated. The more polar band provided (*all-E*)-**11** (680 mg, 48% yield based on unrecovered starting aldehyde), while the less polar band was the starting aldehyde (2*Z*)-**11** (1.36 g). Recovered (2*Z*)-**11** was recycled as above to provide additional (*all-E*)-**11**: IR (neat) 1670 (C=O), 1580 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 236, 344 nm; R_f 0.45; MS *m/z* 218 (M^+); ^1H NMR ($CDCl_3$) δ 10.0 (d, 1 H, CHO), 6.0 (s, 1 H, vinyl), 5.9 (d, 1 H, vinyl), 3.0 (m, 1 H, CH), 2.4 (t, 2 H, CH_2), 2.2 (s, 3 H, CH_3), 2.1 (t, 2 H, CH_2), 1.8 (s, 3 H, CH_3), 1.7 (m, 2 H, CH_2), 1.2 (d, 6 H, CH_3).

General Procedure for the Horner–Emmons Condensations. NaH (1.2 equiv) was washed in dry THF three times to eliminate mineral oil. At 0 °C under N_2, freshly distilled triethylphosphonosenecioate[23] (1.1 equiv) in THF was added. After 30 min of stirring, HMPA (0.2 equiv) followed by either (*all-E*)- or (2*Z*)-**11** (1 equiv, final concentration 0.8 M) was added. After 90 min of reaction at room temperature, water was added and the reaction mixture was partitioned between ether and water. The ether layer was washed once with brine,

dried (Na_2SO_4), and concentrated at reduced pressure to give ester **12** as a mixture of mainly two configurational isomers. The mixtures were separated by HPLC on silica gel as described below. In this manner were prepared the following compounds:

(2*E*,4*E*,6*E*)- and (2*Z*,4*E*,6*E*)-Ethyl 8-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoate ((*all-E*)-12 and (13*Z*)-12). This preparation employed a suspension of NaH (20 mg, 0.50 mmol) in dry THF (2 mL), a solution of triethylphosphonosenecioate[23] (100 mg, 0.39 mmol) in dry THF (1 mL), HMPA (25 mg, 0.14 mmol), and a solution of (*all-E*)-11 (70 mg, 0.32 mmol) in dry THF (1 mL) to give ester **12** (84 mg, 80% yield) as a mixture of mainly two configurational isomers (*all-E* and 13*Z*).

(2*E*,4*E*,6*Z*)- and (2*Z*,4*E*,6*Z*)-Ethyl 8-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoate ((9*Z*)-12 and (9*Z*,13*Z*)-12). This preparation employed a suspension of NaH (47 mg, 1.2 mmol) in dry THF (4 mL), a solution of triethylphosphonosenecioate (240 mg, 0.91 mmol) in dry THF (2 mL), HMPA (60 mg, 0.33 mmol), and a solution of (2*Z*)-11 (165 mg, 0.76 mmol) in dry THF (2 mL) to give ester **12** (239 mg, 96% yield) as a mixture of mainly two configurational isomers (9*Z* and 9*Z*,13*Z*).

HPLC Separation of the C_{20} Ester Isomers 12. For the separation on silica gel of the reaction product mixture containing (*all-E*)- and (13*Z*)-**12**, prepared by the above Horner–Emmons condensation, 1.5% Et_2O in hexane was used. For the product mixture containing (9*Z*)- and (9*Z*,13*Z*)-**12**, 1.0% Et_2O in hexane was employed. In this manner were obtained the following pure isomers:

Ester (*all-E*)-12: retention time 38.9 min; IR (neat) 1710 (C=O), 1600 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 367 nm (ϵ 28 000); R_f 0.77; MS *m/z* 328 (M^+); ^1H NMR ($CDCl_3$) δ 6.90 (dd, 1 H, H11, J = 11.4 and 14.9 Hz), 6.20 (d, 1 H, H12, J = 15.0 Hz), 6.05 (d, 1 H, H10, J = 11.4 Hz), 5.97 (s, 1 H, H8), 5.71 (s, 1 H, H14), 3.01 (m, 1 H, H1), 2.44 (t, 2 H, H2′), 2.33 (s, 3 H, H20), 2.08 (t, 2 H, H18), 1.96 (s, 3 H, H19), 1.77 (s, 3 H, H4), 1.58 (m, 2 H, H1′), 1.15 (d, 6 H, H16 and H17, J = 7.2 Hz). HRMS calcd for $C_{22}H_{32}O_2$, 328.2402; found, 328.2387.

Ester (9*Z*)-12: retention time 36.3 min; IR (neat) 1710 (C=O), 1600 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 331 nm (ϵ 22 000); R_f 0.76; MS *m/z* 328 (M^+); ^1H NMR ($CDCl_3$) δ 6.57 (dd, 1 H, H11, J = 11.1 and 15.3 Hz), 6.16 (d, 1 H, H12, J = 15.6 Hz), 5.99 (d, 1 H, H10, J = 11.1 Hz), 5.84 (s, 1 H, H8), 5.69 (s, 1 H, H14), 3.05 (m, 1 H, H1), 2.22 (s, 3 H, H20), 2.05 (m, 4 H, H2′ and H18), 1.86 (s, 3 H, H19), 1.75 (s, 3 H, H4), 1.57 (m, 2 H, H1′), 1.18 (d, 6 H, H16 and H17, J = 7.2 Hz).

Ester (13*Z*)-12: retention time 30.4 min; IR (neat) 1710 (C=O), 1600 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 369 nm (ϵ 26 000; R_f 0.79; MS *m/z* 328 (M^+); ^1H NMR ($CDCl_3$) δ 7.71 (d, 1 H, H12, J = 15.3 Hz), 6.90 (dd, 1 H, H11, J = 11.4 and 15.3 Hz), 6.15 (d, 1 H, H10, J = 11.4 Hz), 5.95 (s, 1 H, H8), 5.58 (s, 1 H, H14), 3.01 (m, 1 H, H1), 2.43 (t, 2 H, H2′), 2.07 (t, 2 H, H18), 2.04 (s, 3 H, H20), 1.95 (s, 3 H, H19), 1.77 (s, 3H, H4), 1.56 (m, 2 H, H1′), 1.15 (d, 6 H, H16 and H17, J = 7.2 Hz).

Ester (9*Z*,13*Z*)-12: retention time 35.0 min; IR (neat) 1710 (C=O), 1610 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 334 nm (ϵ 20 000); R_f 0.76; MS *m/z* 328 (M^+); ^1H NMR ($CDCl_3$) δ 7.63 (d, 1 H, H12, J = 15.3 Hz), 6.58 (dd, 1 H, H11, J = 11.1 and 15.3 Hz), 6.07 (d, 1 H, H10, J = 10.8 Hz), 5.83 (s, 1 H, H8), 5.56 (s, 1 H, H14), 3.04 (m, 1 H, H1), 2.05 (m, 4 H, H2′ and H18), 1.92 (s, 3 H, H20), 1.86 (s, 3 H, H19), 1.74 (s, 3 H, H4), 1.56 (m, 2 H, H1′), 1.17 (d, 6 H, H16 and H17, J = 7.2 Hz).

General Procedure for Hydrolysis of the C_{20} Esters 12. The procedure was similar to that reported in the literature for the synthesis of α-ionylideneacetic acid.[30] To a solution of the C_{20} ester **12** (1 equiv) in methanol (final concentration 0.061 M) was added an aqueous solution of 2 M KOH (10 equiv). The solution was refluxed, and the reaction progress was monitored by TLC. After 1 h the hot solution was poured into a beaker of ice (40 g) and acidified with phosphoric acid until pH 2. The mixture was then extracted with Et_2O, which was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give the product. The following were synthesized by this method:

(2*E*,4*E*,6*E*)-8-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid ((*all-E*)-1). This preparation utilized a solution of KOH (102 mg, 1.82 mmol) in water (1 mL) and a warm solution of ester (*all-E*)-**12** (60 mg, 0.18 mmol) in methanol (3 mL) to provide acid (*all-E*)-1 (52.9 mg, 98% yield): IR (neat) 1680 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 365 nm (ϵ 36 000); R_f 0.16; MS *m/z* 300 (M^+); ^1H NMR ($CDCl_3$) δ 6.99 (dd, 1 H, H11, J = 11.4 and 15.0 Hz), 6.27 (d, 1 H, H12, J = 15.0 Hz), 6.10 (d, 1 H, H10, J = 11.4 Hz), 6.02 (s, 1 H, H8), 5.78 (s, 1 H, H14), 3.06 (m, 1 H, H1), 2.48 (t, 2 H, H2′), 2.37 (s, 3 H, H20), 2.12 (t, 2 H, H18), 2.01 (s, 3 H, H19), 1.82 (s, 3 H, H4), 1.63 (m, 2 H, H1′), 1.20 (d, 6 H, H16 and H17, J = 7.2 Hz). HRMS calcd for $C_{20}H_{28}O_2$, 300.2089; found, 300.2072.

(2*E*,4*E*,6*Z*)-8-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid ((9*Z*)-1). This preparation used a solution of KOH (126 mg, 2.25 mmol) in water (2 mL) and a warm solution of ester (9*Z*)-**12** (66 mg, 0.20 mmol) in methanol (3 mL) to give acid (9*Z*)-1 (55.2 mg, 92% yield): IR (neat) 1680 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 326 nm (ϵ 22 000); R_f 0.19; MS *m/z* 300 (M^+); ^1H NMR ($CDCl_3$) δ 6.68 (dd, 1 H, H11, J = 10.8 and 15.3 Hz), 6.22 (d, 1 H, H12, J = 15.3 Hz), 6.04 (d, 1 H, H10, J = 10.8 Hz), 5.91 (s, 1 H, H8), 5.75 (s, 1 H, H14), 3.10 (m, 1 H, H1), 2.26 (s, 3 H, H20), 2.10 (m, 4 H, H2′ and H18), 1.91 (s, 3 H, H19), 1.80 (s, 3 H, H4), 1.61 (m, 2 H, H1′), 1.23 (d, 6 H, H16 and H17, J = 7.2 Hz).

(2*Z*,4*E*,6*E*)-8-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid ((13*Z*)-1). This preparation employed a solution of KOH (56.5 mg, 1.01 mmol) in water (0.5 mL) and a warm solution of ester (13*Z*)-**12** (22 mg, 0.070 mmol) in methanol (2 mL) to give acid (13*Z*)-1 (21 mg, 99% yield): IR (neat) 1670 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 365 nm (ϵ 27 000); R_f 0.23; MS *m/z* 300 (M^+); ^1H NMR ($CDCl_3$) δ 7.70 (d, 1 H, H12, J = 15.0 Hz), 6.98 (dd, 1 H, H11, J = 11.4 and 15.0 Hz), 6.21 (d, 1 H, H10, J = 11.4 Hz), 6.02 (s, 1 H, H8), 5.64 (s, 1 H, H14), 3.06 (m, 1 H, H1), 2.50 (t, 2 H, H2′), 2.12 (t, 2 H, H18), 2.11 (s, 3 H, H20), 2.01 (s, 3 H, H19), 1.82 (s, 3H, H4), 1.63 (m, 2 H, H1′), 1.20 (d, 6 H, H16 and H17, J = 7.2 Hz).

(2*Z*,4*E*,6*Z*)-8-(2′-Isopropyl-3′-methyl-2′-cyclohexen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid ((9*Z*,13*Z*)-1). This preparation employed a solution of KOH (116 mg, 2.07 mmol) in water (1 mL) and a warm solution of ester (9*Z*,13*Z*)-**12** (34.0 mg, 0.104 mmol) in methanol (2 mL) to give acid (9*Z*,13*Z*)-1 (31 mg, 99% yield): IR (neat) 1650 (C=C) cm^{-1}; UV λ_{max} (CH_3OH) 325 nm (ϵ 18 000); R_f 0.18; MS *m/z* 300 (M^+); ^1H NMR ($CDCl_3$) δ 7.65 (d, 1 H, H12, J = 15.6 Hz), 6.68 (dd, 1 H, H11, J = 10.8 and 15.6 Hz), 6.15 (d, 1 H, H10, J = 10.8 Hz), 5.91 (s, 1 H, H8), 5.62 (s, 1 H, H14), 3.09 (m, 1 H, H1), 2.10 (m, 4 H, H2′ and H18), 2.00 (s, 3 H, H20), 1.92 (s, 3 H, H19), 1.80 (s, 3 H, H4), 1.61 (m, 2 H, H1′), 1.23 (d, 6 H, H16 and H17, J = 7.2 Hz).

Biology. Chick Skin CRABP-Binding Assay. Inhibition constants at 50% (IC_{50}) for retinoid binding to CRABP-II were determined according to a previously published method by Sani et al.[47] Briefly, CRABP-II (about 1 mg) isolated from chick embryo skin was incubated with 300 pmol of [^3H]-(*all-E*)-retinoic acid in the presence or absence of varying concentrations of the unlabeled retinoid (1-, 5-, 10-, 25-, and 50-fold mole excess). Free radioactively labeled retinoic acid was removed by adsorption on dextran-coated charcoal. A sucrose density gradient (5–20% sucrose) sedimentation was performed for 18 h at 180000*g*. The radioactive profiles of the 2S CRABP peak were obtained. The IC_{50} values were obtained from a probit analysis[48] of the specifically bound [^3H]-(*all-E*)-retinoic acid versus the log of molar concentrations of unlabeled retinoids. The standard deviations were calculated by standard procedures.[49]

Mouse Skin Ornithine Decarboxylase Assay. The ornithine decarboxylase (ODC) activity of the retinoids was determined according to the procedure of Verma and Boutwell[34] with the following modifications. Randomly bred female CD-1 mice (6–7 weeks old) were maintained on a regular laboratory chow diet containing 2500 IU of vitamin A/kg. The application of 0.2 mL of acetone (control) or 0.2 mL of an acetone solution

containing 10 nmol of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was made to the shaved backs of CD-1 mice 30–60 min after the single application (17 nmol) of (*all-E*)-retinoic acid or the test retinoid. Five hours after the application of TPA, the exposed skin from the backs of three animals were pooled and assayed as previously described. In order to improve the counting efficiency of radiolabeled product formed in the assay, the specific activity of [^{14}C]ornithine was increased 5-fold; the assay was terminated by the addition of citric acid at 45 min to ensure linear kinetics.

Human Sebaceous Cell Assay. Sebaceous cells were isolated from adult human sebaceous glands, derived from facial skin removed during cosmetic surgery, and cultured on a layer of mouse 3T3 fibroblasts as described previously.[37] Briefly, the top 0.4 mm section, containing the epidermis, of facelift tissue was removed with a keratome and discarded, and the second 0.4 mm section was used as the source of human sebaceous cells. This section was incubated in 10 mg/mL Dispase in DMEM with 10% fetal calf serum for 30 min at 37 °C. The tissue was then placed in 0.3% trypsin/1% EDTA in phosphate-buffered saline (PBS). The tissue was then scraped with a scalpel, and the released sebocytes were plated at a density of 10^4 cells/cm^2 onto a mitomycin C-treated layer of 3T3 fibroblasts. The 3T3 fibroblasts were plated at a density of 2×10^4 cells/cm^2.

Cells were plated in Iscove's medium containing 10% fetal calf serum and 4 μg/mL dexamethasone without the test compound and given fresh medium every 48 h. Stock solutions of retinoids were made up in DMSO and stored at −20 °C in the dark. During experimental use the solutions were brought to room temperature and used by diluting directly into complete medium. Retinoids were applied in three doses (10^{-8}, 10^{-7}, and 10^{-6} M) 48 h after initial plating of sebocytes. The experiments were done in triplicate. Colonies were monitored until the control cultures achieved 50% confluency, at which time sebocytes were counted. On the day of harvesting, after 12 days of growth, the cultures were rinsed with 0.03% EDTA in PBS to remove only the 3T3 fibroblasts, followed by incubation in 0.05% trypsin/0.03% EDTA. The isolated cells were centrifuged, suspended in PBS, and counted. The IC_{50} values were determined as the concentration of retinoid necessary to achieve a 50% reduction in absolute cell number compared to vehicle-treated controls. The error of this assay was estimated to be ±10 nM from over 100 analyses of (13*Z*)-RA.

Mouse Skin Antipapilloma Assay. The inhibition of mouse skin papilloma by retinoids was performed according to the procedure developed by Verma and Boutwell.[34] As described in the ODC assay, female CD-1 mice were used and maintained on regular laboratory chow containing 2500 IU of vitamin A/kg. For tumor initiation, 0.2 mL of an acetone solution containing 0.2 μmol (51.2 μg) of dimethylbenz[*a*]-anthracene (DMBA) was applied to the shaved dorsal skin of each animal. Two weeks after initiation, the mice received 0.2 mL applications of an acetone solution containing 10 nmol of TPA twice per week for 12 weeks. One hour prior to the TPA application, mice (groups of 10) were treated with either 0 (control), 0.19, 0.57, 1.7, 5.1, 15.3, or 45.9 nmol of retinoid in 5% DMSO and 95% acetone or untreated (groups of 20). The number of tumors were counted and averaged. The tumor suppression was defined as the ratio of the mean number of tumors with retinoid treatment, X_{ret}, to the number of tumors with only TPA application, X_{TPA}. The ED_{50} value (retinoid dose which inhibits 50% of skin papilloma) for this assay was determined by a probit analysis[48] of $(1 - X_{ret}/X_{TPA}) \times 100$ versus log[retinoid]. The standard deviations were calculated by standard procedures.[49]

Toxicity Determination in Mice. The maximum tolerated dose was determined by methods described by Lindamood and co-workers.[48] Groups of six female CD-1 mice, approximately 42 days of age on the day of dosing, were administered orally varying doses of the retinoid. This was performed in two separate experiments. In the first experiment, (*all-E*)-RA was given in 0 (control), 2.5, 10, and 30 mg kg^{-1} day^{-1} in corn oil. In the second experiment, (*all-E*)-1 was given in 0 (control), 15, 30, and 60 mg kg^{-1} day^{-1} in corn oil. In each experiment, the mice were dosed daily by oral gavage for 29 days. Body weights and clinical signs of hypervitaminosis A were recorded at 3 day intervals and at the termination of the study. The maximum tolerated dose is defined as the largest oral dose that can be administered without a significant decrease in weight gain (not more than 10% of controls) or without causing other signs of hypervitaminosis A in any animal within the group. In this assay,[45b] the MTD of (*all-E*)-RA is 10 mg kg^{-1} day^{-1}.

Molecular Modeling of Retinoids. Retinoid structures were generated with Sybyl version 6.0 (Tripos Inc., St. Louis, MO) running on a Silicon Graphics Indigo 2 workstation and plotted with a Tektronix Phaser 200e color printer. The structures were constructed with standard bond lengths and bond angles. The torsional angles for single bonds in the polyene chains were set to 180°.

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