Cancer Chemopreventive 3-Substituted-4-oxoretinoic Acids

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The introduction of substituents at position 3 of methyl 4-oxoretinoate can be effected in good yields by alkylating the lithium dienolate. A second substituent can be introduced also, but the resulting 3,3-disubstituted-4-oxoretinoates were isolated in lower yields. Evidence was obtained for a slower rate of alkylation at the α-position (carbon 14) of the ester group. Some of these 4-oxoretinoic acid analogues showed high activity in assays *in vivo* for the inhibition of ornithine decarboxylase activity and carcinogen-induced papillomas in mouse skin.

Introduction

Various methods for preventing the development of cancers are of much current interest and are being extensively investigated. One of the methods for achieving the goal of cancer prevention is through chemoprevention, *i.e.*, the administration of natural products or synthetic compounds to prevent or reverse the malignant transformation of cells.[1-14] Compounds of the vitamin A group, many derivatives and analogues of these classical retinoids,[1-11] and certain retinoid analogues[15] that appear to differ greatly in structure from the classical retinoid structure are active in bioassays that are believed to be predictive for cancer chemopreventive activity. Retinoic acid (RA, **2a**, Chart 1) and retinol have cancer chemopreventive activity, but they are quite toxic at pharmacologic doses. 4-Oxoretinoic acid (4-oxo-RA, **3a**), a metabolite of RA, has exhibited significant activity in several bioassays.[16] Although evidence has been presented that 4-oxo-RA and its metabolic precursor, 4-hydroxy-RA, are initial metabolites in the catabolic deactivation of RA,[17] the reported biological activities constituted the rationale for the synthesis of analogues of 4-oxo-RA.[8] It was postulated that 3-substituted-4-oxoretinoic acids, as analogues of 4-oxo-RA, might also exert chemopreventive effects and that a substituent at position 3 might hinder catabolic degradation.

Chemistry

The synthesis of such retinoids might be achieved by beginning with 2,6,6-trimethylcyclohexen-4-one derivatives and constructing the side chain with known synthons. Because of the sensitivity of the conjugated double-bond side chain, most retinoids that retain the 2,6,6-trimethylcyclohexenyl group have been synthesized by employing such routes, and analogues in which the trimethylcyclohexenyl group is replaced by other groups have been synthesized by similar stepwise routes.[18] Relatively few retinoids modified in the cyclohexenyl group have been synthesized by modification of the intact retinoid structure. In a preliminary communication,[19] we described a method for the direct introduction of substituents at position 3 of a 4-oxo-RA ester.

Many examples of base-catalyzed alkylations of α,β-unsaturated cyclohexenones (*e.g.*, **1**) at either the α- or the α′-position have been described. It has been demonstrated that alkylation at the α′-position can be effected by employing kinetically controlled conditions to form the dienolate anion.[20-22] α-Alkylation of lithium enolates of α,β-unsaturated esters has also been reported.[23,24] Therefore, it would be expected *à priori* that alkylation of methyl 4-oxo-RA (**3b**) could occur at the α′-position of the cyclohexenone group or at the α-position (carbon 14), the γ-position (carbon 20), or a more remote position from the ester group. The lithium dienolate of **3b** was formed with lithium hexamethyldisilazide in tetrahydrofuran at −78 °C; alkylation with methyl iodide, ethyl iodide, or cinnamyl bromide led to the isolation of the 3-methyl (**4a**), 3-ethyl (**4b**), or 3-cinnamyl (**4f**) derivatives[19] (Chart 1). Subsequently, the 3-propenyl (**4c**), 3-propynyl (**4d**), 3-benzyl (**4e**), and 3-isopropyl (**4g**) derivatives were obtained similarly. Optimization of yields of most of these derivatives (**4**) was not attempted; however, methyl 3-methyl-4-oxoretinoate (**4a**) that assayed 90–95% by HPLC could be isolated in yields of 70–85%, and specimens purified further (98–99% by HPLC) were obtained in yields of 60–70%. Even though cinnamyl bromide was not easily separable from **4f**, the latter retinoid was isolated in yields of 53–63% (purity, 98–99.5% by HPLC). The formation of small amounts of dialkylation or trialkylation products during alkylations of **3b** was observed by mass and ^{1}H-NMR spectral analyses and high-pressure liquid chromatography. Deliberate methylation of **4a** under similar conditions produced methyl 3,3-dimethyl-4-oxoretinoate (**5a**) and several other components, as explained further below; from the mixture, **5a** could be isolated in modest yields.[19] 3,3-Di(2-propynyl)-4-oxo-RA methyl ester (**5b**) and the 3-cinnamyl-3-methyl derivative (**5c**) were obtained, respectively, from **4d** and **4f**. Alkaline hydrolysis of **4a–f** and **5a** in 90% ethanol afforded the corresponding 4-oxoretinoic acid analogues (**6**).

During further investigations of the methylation of **4a** with 4 equiv of methyl iodide and 1.1 equiv of the base, the total crude solids from reaction mixtures were analyzed by HPLC and ^{1}H-NMR spectrometry. In addition to **4a** and **5a**, two unidentified compounds were shown to be present in considerable amounts. One of these components and **4a** were present in approximately equal amounts in a small fraction that was isolated, after **5a** and other components had been separated. Analysis of the ^{1}H-NMR spectrum of this fraction, as well as analyses of the NMR spectra of total crude

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Chart 1

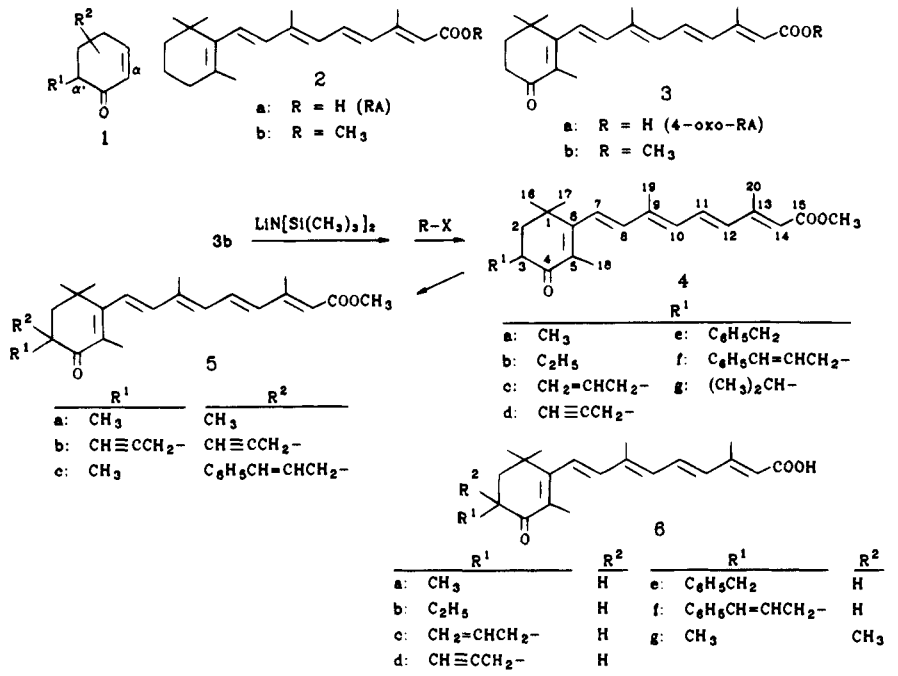
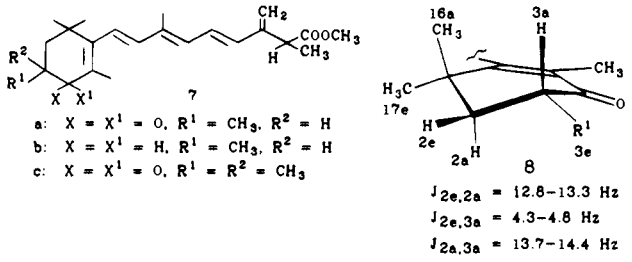


Chart 2



products, showed that one of the previously unidentified components was a product (**7a**, Chart 2) of α-methylation of the ester group.[19] These analyses of total crude reaction products suggested that a fourth component might be a trimethylation product (**7c**), but this structure has not been unequivocally confirmed. Similar investigations of total crude products of the methylation of **3b** showed that **7a** is also one of the minor components, and alkylation of C14 was demonstrated further by subjecting methyl retinoate (**2b**) to similar methylation conditions and showing that the 14-methyl derivative **7b** was formed.[19] Alkylation at C14 is consistent with prior demonstrations of α-alkylation of lithium enolates of α,β-unsaturated esters.[23,24]

The structures of **4a–g**, **5a–c**, and **7a,b** were confirmed by ^{1}H-NMR studies. The chemical shifts of the side-chain protons of the isolated, purified, and bioassayed specimens of **4–6** are typical of these signals from methyl retinoate and other retinoic acid derivatives; therefore, the side chains of these compounds were unaltered. The dramatic simplification of the NMR spectra of the cyclohexenyl region in proceeding from methyl retinoate (**2b**) → **3b** → **4a** → **5a** is demonstrated in Figure 1. The cyclohexenyl regions of the 3-monosubstituted-4-oxoretinoic acids **6a–f** and esters **4a–g** were assigned by comparison of coupling constants and chemical shift positions and confirmed by NOE difference spectroscopy (*cf.* Experimental Section). The 1,2-*trans* diaxial relationship of H-3a and H-2a is shown

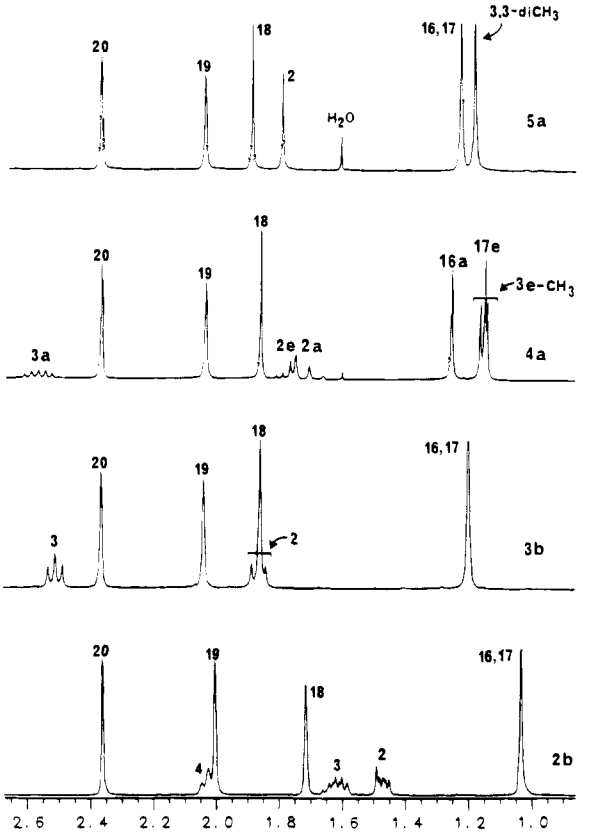


Figure 1. ^{1}H-NMR spectra (300 MHz) of the cyclohexenyl region of methyl esters **2b**, **3b**, **4a**, and **5a**, ppm downfield from TMS.

by the large coupling constants of $^3J_{2a,3a}$ = 13.7–14.4 Hz (*cf.* **8**, Chart 2), and the 1,2-*cis* relationship of H-3a and -2e is shown by coupling constants of $^3J_{2e,3a}$ = 4.3–4.8 Hz. The assignments of 16a-CH_3 and 17e-CH_3 were confirmed by NOE difference spectroscopy. The irradiation of the signals produced by the methyl group 16a-CH_3 resulted in an enhancement of signals corresponding to H-2e (% NOE = 1.7–3.1) and H-3a (% NOE =

Table 1. Mouse-Skin Bioassays

retinoid	ODC assay,[a] average % of ODC in TPA-induced control mice[c] (no. of tests)	papilloma assay[b] average no. of tumors per mouse, % of control group[c] (no. of tests)
3-methyl-4-oxo-RA (**6a**)	26 (4)	29 (4)
3-methyl-4-oxo-RA methyl ester (**4a**)	28 (4)	27 (3)
3-ethyl-4-oxo-RA (**6b**)	40 (3)	57 (3)
3-ethyl-4-oxo-RA methyl ester (**4b**)	31 (3)	68 (2)
3-(2-propenyl)-4-oxo-RA (**6c**)	68 (2)	47 (1)
3-(2-propenyl)-4-oxo-RA methyl ester (**4c**)	56 (2)	42 (1)
3-(2-propynyl)-4-oxo-RA (**6d**)	47 (1)	64 (1)
3-(2-propynyl)-4-oxo-RA methyl ester (**4d**)	34 (2)	40 (1)
3-benzyl-4-oxo-RA (**6e**)	34 (5)	38 (3)
3-benzyl-4-oxo-RA methyl ester (**4e**)	56 (3)	78 (2)
3-cinnamyl-4-oxo-RA (**6f**)	23 (3)	39 (2)
3-cinnamyl-4-oxo-RA methyl ester (**4f**)	31 (2)	20 (2)
3,3-dimethyl-4-oxo-RA (**6g**)	26 (3)	25 (2)
3,3-dimethyl-4-oxo-RA methyl ester (**5a**)	22 (3)	22 (3)
4-oxo-RA (**3a**)	17 (3)	39 (3)
4-oxo-RA methyl ester (**3b**)	18 (3)	25 (3)
RA (**2a**)	8 (30)	16 (8)
13-*cis*-RA	22 (4)	72 (2)
4-HPR	60 (6)	89 (2)

[a] Based on nmol of CO_2/30 min/mg of protein. ODC was induced with 17 nmol of TPA, and 17 nmol of retinoid was applied; method of Verma *et al.*[26,27] [b] Initiation by a single application of 51 μg of DMBA; promotion with 10 nmol of TPA, application of 17 nmol of retinoid; method of Verma *et al.*[28] [c] Average of the results from the stated number of tests.

3.4−10.9); therefore, H-3a and 16a-CH_3 are in a 1,3-diaxial relationship. Similarly, the irradiation of signals produced by the methyl group 17e-CH_3 resulted in an enhancement of the signals corresponding to H-2e (% NOE = 1.0−1.9) and H-2a (% NOE = 3.2−5.1); therefore, 17e-CH_3 is in a quasi-equatorial position. The NOE results and the *J*-values indicate that the cyclohexenyl region of the monosubstituted-4-oxoretinoids (**4a**−**f** and **6a**−**f**) adopts similar conformations (**8**) with a 1,3-diaxial relationship between 16a-CH_3 and H-3a and, consequently, the quasi-equatorial position of the group 3e-R^1.

Biological Evaluations[25]

Both the 4-oxo-RA analogues **6** and the methyl esters **4** were tested for inhibition of induction by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) of ornithine decarboxylase (ODC) activity in mouse skin. The method was that of Verma *et al.*[26,27] The results listed in Table 1 show the inhibition of TPA-induced ODC activity as a percentage of the ODC activity in groups of control mice. There was little, if any, significant difference in activities of the 4-oxo-RA analogues and the corresponding methyl esters. The 3-methyl (**4a** and **6a**), 3-cinnamyl (**4f** and **6f**), and 3,3-dimethyl (**5a** and **6g**) derivatives appeared to be the most effective; the average percentages of control groups in two to four experiments were in the range 22−31%.

These 4-oxoretinoids were tested by the method of Verma *et al.*[28] for inhibition of the development of mouse-skin papillomas initiated by DMBA and promoted by TPA. In Table **1**, averages of the number of papillomas per mouse are reported as percentages of the average number of papillomas per mouse that appeared in the control groups. In this assay, also, there was little, if any, difference in the effectiveness of the 4-oxo-RA analogues and most of the corresponding methyl esters. Again, the most effective retinoids were the 3-methyl, 3-cinnamyl, and 3,3-dimethyl derivatives; the averages of two to four assays of these derivatives were 22−39% (Table 1).

These three 4-oxo-RA analogues and the methyl esters, therefore, are comparable in effectiveness in the Verma−Boutwell assays[26−28] to the parent 4-oxo-RA (**3a**) and its methyl ester (**3b**) (Table 1), and these assay results demonstrate, at least, that substituents at position 3 do not necessarily negate activity. In addition, the results of the same assays of three well-known retinoids—RA (**2a**), 13-*cis*-retinoic acid (13-*cis*-RA), and *N*-(4-hydroxyphenyl)retinamide (4-HPR)—that are being used clinically are included in Table 1. The better 4-oxo-RA analogues appear to be somewhat less effective than RA, are as effective as 13-*cis*-RA in the ODC assay, and are more effective than 4-HPR in both assays.

In the hamster trachea organ culture assay of Sporn and co-workers,[29,30] retinoids **4a**, **5a**, and **6a** were active;[8] 3,3-dimethyl-4-oxo-RA methyl ester (**5a**, ED_{50} 3×10^{-10} M) was as active as 13-*cis*-retinoic acid (3.3×10^{-10} M). In addition, 3-(2-propynyl)-4-oxo-RA (**6d**, ED_{50} 2×10^{-10} M) demonstrated comparable activity, and 3-benzyl-4-oxo-RA (**6e**, ED_{50} 1.1×10^{-8} M) and its methyl ester (**4e**, ED_{50} 1.1×10^{-8} M) were modestly active.

Experimental Section

General Methods. All operations involved in the preparation, isolation, purification, and transfer of retinoids were performed in an atmosphere, or under a current, of nitrogen or argon. All such operations were also performed in dim light or photographic dark-room light and, insofar as possible, with containers wrapped with aluminum foil or black cloths. The THF solution of lithium hexamethyldisilazide was purchased from Aldrich Chemical Co., Milwaukee, WI. All retinoids were stored in an atmosphere of argon or nitrogen in hermetically sealed containers at −20 or −80 °C. Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (UV) were determined with ethanol solutions and recorded with a Perkin-Elmer Lambda 9 UV−visible−NIR spectrophotometer. Mass spectral (MS) data were taken from low-resolution, electron-impact spectra determined at 70 eV with a Varian/MAT Model 311A spectrometer: M = molecular ion; some of the other peaks are identified as probable fragments, *e.g.*, M minus a fragment. ^{1}H-NMR spectra were determined at 300.635 MHz with a Nicolet NT 300NB NMR spectrometer; the solvent was $CDCl_3$. Chemical shifts (δ) are given in parts per million downfield from tetramethylsilane, the internal reference. Assignments of chemical shifts are designated by the position numbers shown on structure **4**. Multiplicity of the chemical shifts and the position numbers are given parenthetically with each chemical shift; a = axial, e = equatorial. The NOE difference experiments were conducted on non-degassed solutions of $CDCl_3$. To minimize the effects of magnetic perturbations with the sample nonspinning, eight FID's (free reduction decays) were acquired with the decoupler set at a desired frequency and eight FID's were recorded with a decoupler off-resonance. The process was repeated until 400 FID's had been accumulated. Subsequent substraction of the two spectra afforded the net enhancement. High-pressure liquid chromatography (HPLC) was performed with Waters Associates components systems and a Hewlett-Packard Model 3380-S integrator or a Hewlett-Packard Model 1084B system. HPLC was performed on columns packed with octadecylsilylated silica (Spherisorb ODS), 5 μm particle size; unless indicated otherwise, the eluting solvent was 85:15 acetonitrile−1% aqueous ammonium

acetate, isocratic, 1 mL/min flow rate; and elution was monitored by UV absorption at 340 nm. Deactivated alumina for column chromatography was prepared by thoroughly mixing activated neutral Al_2O_3 (Brockman No. 1) and water with the ratio of 10:1.

Methyl 4-Oxoretinoate (3b). A mixture of methyl retinoate (**2b**; 28.8 g, 92 mmol), petroleum ether (4 L, bp 35–60 °C), and manganese dioxide[31] (360 g) at room temperature was stirred with a motor-driven stirrer for 22 h, and the MnO_2 was then collected on a sintered-glass funnel. Because TLC (developing solvent, $CHCl_3$) showed that unchanged **2b** remained in the filtrate, the MnO_2 was washed repeatedly with small portions of petroleum ether until TLC indicated that the washings contained little, or no, **2b**. A mixture of the filtrate, the washings, and a second portion of MnO_2 (300 g) was stirred for 5 h, and the MnO_2 was separated and washed with petroleum ether as before. Each portion of MnO_2 was washed repeatedly with MeOH until TLC (developing solvent, 9:1 pentane–ethyl acetate) indicated that the washings contained little, or no, **3b**. The petroleum ether filtrate and all of the MeOH washings of the two portions of MnO_2 were combined, and this solution was concentrated under reduced pressure to a syrup (28 g). A solution of the syrup in ether (25 mL) was placed in a freezer (−20 to −25 °C) overnight, and a solid precipitate was collected on a filter, washed with cold petroleum ether, and dried *in vacuo*: weight, 10.2 g; HPLC, 85% **3b**. A solution of the solid in warm ethyl acetate (20 mL) was diluted with pentane (30 mL), and this solution was poured onto a column (64 × 6.4 cm) of deactivated alumina. Elution of the adsorbed material with pentane–ethyl acetate (9:1) was monitored by TLC, and portions of effluent that contained **3b** were combined and concentrated under reduced pressure to a yellow crystalline solid: yield, 8.0 g (26.7%); mp 95–96 °C (lit.[32] mp 93 °C); HPLC, 98.7%; UV λ_{max} (ϵ) = 362 (52 000), 286–287 (12 900), 232 nm (8000); ^{1}H-NMR δ 1.19 (s, 16 and 17), 1.85 (s, 18), 1.86 (t, 2), 2.04 (d, $J_{10,19}$ = 0.9 Hz, 19), 2.37 (d, $J_{14,20}$ = 1.0 Hz, 20), 2.53 (t, 3), 3.71 (s, OCH_3), 5.82 (unresolved m, 14), 6.26 (d, $J_{10,11}$ = 11.5 Hz, 10), 6.32 (s, 7 and 8), 6.36 (d, $J_{11,12}$ = 15.1 Hz, 12), 6.98 (dd, 11).

General Procedure for the Synthesis of Methyl 3-Substituted-4-oxoretinoates 4. All alkylations were carried out in oven-dried glassware and under an argon atmosphere. A Firestone valve was employed to maintain a positive argon pressure when reactants were being added to the cold reaction solution. A solution of methyl 4-oxoretinoate (**3b**) in anhydrous tetrahydrofuran (2.5–5 mL/mmol) was added from an addition funnel during 5–10 min to a cold solution, maintained at −78 °C, of lithium hexamethyldisilazide (1–1.3 equiv) in anhydrous THF. The solution of lithium hexamethyldisilazide had been prepared by diluting a 1 M THF solution of the base with 1–3 mL of anhydrous THF/mmol of the base. The solution of **3b** and the base was stirred at −78 °C for 30 min, and the alkylating agent (2 equiv) was added. The reaction mixture was stirred for 30 min at −78 °C, allowed to warm slowly to room temperature, and then, typically, stirred overnight. Most of the THF and the excess alkylating agent (if sufficiently volatile) were evaporated under reduced pressure, a saturated solution of ammonium chloride was added to the residue, and the aqueous mixture was extracted (3×) with ether or ethyl acetate. The ether or ethyl acetate solution was dried ($MgSO_4$), and the organic solvent was evaporated under reduced pressure. The residual crude methyl 3-substituted-4-oxoretinoate **4** was purified by gravity chromatography, flash chromatography, preparative TLC, recrystallization, or sequences of these techniques.

The 3-methyl (**4a**), 3-ethyl (**4b**), 3-cinnamyl (**4f**), and 3,3-dimethyl (**5a**) derivatives were prepared and characterized as previously described.[19] Subsequently, compounds **4a**,**f** were obtained by beginning the workup soon after the reaction mixtures had warmed to room temperature rather than continuing the stirring overnight.

Methyl (±)-4-oxo-3-(2-propenyl)retinoate (4c) was prepared, according to the general procedure, from **3b** and allyl bromide. The crude product was purified by flash chromatography (on silica gel with 9:1 pentane–ethyl acetate as eluting solvent) followed by recrystallization from ether–pentane: mp 95–96 °C; HPLC, 99.4–100% (85:15 acetonitrile–1% aqueous ammonium acetate); MS peaks at *m/z* 368 (M), 353 (M − CH_3), 336 (M − CH_3OH), 321 (M − CH_3 − CH_3OH); UV λ_{max} (ϵ) 361 (53 500), 285 (12 800), 231 nm (8300); ^{1}H NMR δ 1.15 (s, 17e), 1.23 (s, 16a), 1.64 (t, $J_{2a,2e}$ = 13.3 Hz, $J_{3a,2a}$ = 14.2 Hz, 2a), 1.80 (dd, $J_{2e,3a}$ = 4.8 Hz, 2e), 1.86 (s, 18), 2.03 (s, 19), 2.10 (m, $-CH_2CH{=}CH_2$), 2.36 (d, $J_{14,20}$ = 1.0 Hz, 20), 2.52 (m, 3a), 2.74 (m, $-CH_2CH{=}CH_2$), 3.72 (s, OCH_3), 5.04 and 5.07 (m, $-CH_2CH{=}CH_2$), 5.80 (m, $-CH_2CH{=}CH_2$), 5.81 (unresolved m, 14), 6.25 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.32 (s, 7 and 8), 6.36 (d, $J_{11,12}$ = 15.1 Hz, 12), 6.98 (dd, 11). Anal. ($C_{24}H_{32}O_3$) C, H.

From one such experiment, 288 mg (HPLC, 99.4%) of recrystallized **4c** was obtained from 1.092 g of **3b**. Trituration of the filtrate residue with pentane afforded 179 mg of **4c** (HPLC, 99.6%): total yield, 38%.

Methyl (±)-4-oxo-3-(2-propynyl)retinoate (4d) was prepared, according to the general procedure, from **3b** (1.51 g, 4.6 mmol) and 2-propynyl bromide (820 mg, 6.9 mmol). The crude product was purified by flash chromatography on a column of silica gel with pentan–ethyl acetate as the eluting solvent. Fractions containing **4d** (determined by TLC) were combined and concentrated under reduced pressure to a yellow solid (0.81 g) that was recrystallized from ether–pentane: yield, 536 mg (32%); mp 117–119 °C; MS peaks at *m/z* 366 (M), 351 (M − CH_3), 334 (M − CH_3OH), 391 (M − CH_3OH − CH_3), 307 (M − $COOCH_3$); HPLC, 98.5–100% (85:15 acetonitrile–1% aqueous ammonium acetate); UV λ_{max} (ϵ) 362 (53 400), 286 (12 900), 231 nm (8000); ^{1}H NMR δ 1.19 (s, 17e), 1.27 (s, 16a), 1.81 (t, $J_{2a,2e}$ = 13.2 Hz, $J_{2a,3a}$ = 14.4 H, 2a), 1.86 (s, 18), 1.98 (t, J = 2.7 Hz, $-C{\equiv}CH$), 2.03 (d, $J_{10,19}$ = 0.9 Hz, 19), 2.07 (dd, $J_{2e,3a}$ = 4.7 Hz, 2e), 2.32 (m, $-CH_aH_bC{\equiv}CH$), 2.36 (d, $J_{14,12}$ = 1.1 Hz, 20), 2.66 (m, 3a), 2.82 (m, $CH_aH_bC{\equiv}CH$), 3.72 (s, OCH_3), 5.82 (unresolved m, 14), 6.26 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.34 (s, 7 and 8), 6.36 (d, $J_{11,12}$ = 15.1 Hz, 12), 6.98 (dd, 11). Anal. ($C_{24}H_{30}O_3$) C, H.

Methyl (±)-4-oxo-3-(phenylmethyl)retinoate (4e) was prepared, according to the general procedure, from **3b** and benzyl bromide and purified by column chromatography: yield, 313 mg from 390 mg of **3b** (63%); HPLC, 97.5%. A specimen was recrystallized from ether–hexane: mp 93–95 °C; MS peaks at *m/z* 418 (M), 403 (M − CH_3), 386 (M − CH_3OH), 371 (M − CH_3OH − CH_3); ^{1}H NMR δ 1.09 (s, 17e), 1.13 (s, 16a), 1.62 (t, $J_{2a,2e}$ = 13.2 Hz, $J_{2a,3a}$ = 14.4 Hz, 2a) 1.65 (dd, $J_{2e,3a}$ = 4.8 Hz, 2e), 1.88 (s, 18), 2.02 (d, $J_{10,19}$ = 0.9 Hz, 19), 2.36 (d, $J_{14,20}$ = 1.1 Hz, 20), 2.48 (dd, J = 9.2 Hz, J = 13.9 Hz, $C_6H_5CH_aH_b-$), 2.73 (m, 3a), 3.49 (dd, J = 4.1 Hz, J = 13.9 Hz, $C_6H_5CH_aH_b-$), 3.72 (s, OCH_3), 5.82 (unresolved m, 14), 6.25 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.30 (m, $J_{7,8}$ = 16.3 Hz, 7), 6.33 (m, 8), 6.35 (d, $J_{11,12}$ = 15.0 Hz, 12), 6.98 (dd, 11), 7.17–7.32 (aromatic CH). Anal. ($C_{28}H_{34}O_3$) C, H.

Methyl (±)-3-Isopropyl-4-oxoretinoate (4g). Methyl 4-oxo-RA (**3b**) was treated with isopropyl iodide according to the general procedure, and the crude product was purified by preparative TLC on silica gel (developing solvent, 8:2 hexane–ethyl acetate). A pure specimen of **4g** was obtained from the leading band and identified by mass spectral analysis: TLC, 1 spot; MS peaks at *mz* 370 (M of $C_{24}H_{34}O_3$), 355 (M − CH_3), 338 (M − CH_3OH), 323 (M − CH_3 − CH_3OH).

Methyl 3,3-Di(2-propynyl)-4-oxoretinoate (5b). Methyl 3-(2-propynyl)-4-oxoretinoate (**4d**) in THF was treated with 1.5 equiv of propynyl bromide in toluene according to the general procedure, but the reaction mixture was not stirred overnight at room temperature. The crude product, which was shown by TLC to contain both the starting material and the desired dispropynyl derivative, was purified by preparative TLC on plates of silica gel (developing solvents successively: pentane, 90% pentane–ethyl acetate, 1:1 pentane–ethyl acetate, and ethyl acetate). The dipropynyl derivative was extracted from the middle band, and an additional dipropynyl derivative was obtained in the same way by TLC of the material extracted from the leading band of the first preparative TLC: HPLC of both specimens, 96–97%. A specimen was purified further by crystallization from ether–pentane: UV λ_{max} (ϵ) 362 (52 500), 288 (15 500), 230–200 nm (11 500); HPLC, 99.2% (85:15 acetonitrile–1% aqueous ammonium acetate, isocratic); ^{1}H

NMR δ 1.27 (s, 16a and 17e), 1.90 (s, 18), 2.03 (d, $J_{10,19}$ = 0.8 Hz, 19), 2.05 (t, J = 2.7 Hz, H−C≡C), 2.15 (s, 2a and 2e), 2.36 (d, $J_{14,20}$ = 1.1 Hz, 20), 2.54 (m, H−C≡CCH_aH$_b$), 2.60 (m, HC≡CH$_a$$H_b$), 3.72 (s, OCH$_3$), 5.82 (unresolved m, 14), 6.27 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.33 (m, $J_{7,8}$ = 16.3 Hz, 8), 6.36 (d, $J_{11,12}$ = 15.0 Hz, 12), 6.38 (m, $J_{7,8}$ = 16.3 Hz, 7), 6.98 (dd, 11). Anal. Calcd ($C_{27}H_{32}O_3$): C, 80.15; H, 7.97. Found: C, 79.18; H, 8.04.

Methyl (±)-3-Cinnamyl-3-methyl-4-oxoretinoate (5c). Methyl 3-methyl-4-oxoretinoate (**4a**) was treated with 1,2 equiv of lithium hexamethyldisilazide and 2 equiv of cinnamyl bromide in anhydrous THF according to the general procedure. After the temperature of the reaction mixture had been allowed to rise to room temperature during 1 h, additional lithium hexamethyldisilazide (*ca.* 1 equiv) was added. The mixture was cooled to −78 °C, stirred for 30 min, allowed to warm to room temperature, and stirred overnight. The crude product was obtained, as usual, by the addition of saturated ammonium chloride solution and extraction with ether and purified by the use successively of TLC, flash chromatography, and preparative HPLC: UV λ_{max} (ϵ) 361 (50 700), 292 (sh), 285 (14 400), 252 nm (23 600); ^{1}H NMR δ 1.21 (s, 3-CH$_3$), 1.22 (s, 17e), 1.24 (s, 16a), 1.68 (d, $J_{2a,2e}$ = 14.3 Hz, 2a), 1.90 (s, 18), 1.95 (d, 2e), 2.03 (d, $J_{10,19}$ = 0.8 Hz, 19), 2.36 (d, $J_{14,20}$ = 1.1 Hz, 20), 2.42 (m, J = 7.7 Hz, J = 1.1 Hz, J = 13.8 Hz, −CH_a-CH$_b$CH=), 2.49 (m, J = 7.3 Hz, J = 13.8 Hz, −CH$_a$$H_b$CH=), 3.72 (s, OCH$_3$), 5.82 (unresolved m, 14), 6.16 (m, CH$_2$CH=CH), 6.25 (d, $J_{10,11}$ = 11.3 Hz, 10), 6.39 (d, J = 15.7 Hz, CH$_2$-CH=CH), 6.35 (s, 7 and 8), 6.35 (d, $J_{11,12}$ = 15.0 Hz, 12), 6.98 (dd, 11), 7.12−7.38 (m, aromatic CH). Anal. ($C_{31}H_{38}O_3$) C, H.

3-Substituted- and 3,3-Disubstituted-4-oxoretinoic Acids 6. General Procedure. 3-Substituted-4-oxoretinoic acid esters **4** and methyl 3,3-dimethyl-4-oxo-RA (**5a**) were hydrolyzed in basic solutions to the corresponding 4-oxoretinoic acids as illustrated by the following general procedure. A solution of the ester in 90% ethanol and containing sodium hydroxide or potassium hydroxide (1.2 mol of base/mol of ester) was heated at 60 °C for about 1 h under an atmosphere of argon or nitrogen. (The course of the hydrolysis was monitored by TLC.) The reaction mixture was cooled to room temperature and then extracted with hexane, pentane, or ether to remove any neutral material. The aqueous layer was then acidified to about pH 3, and the resulting mixture was extracted with ethyl acetate or ether. The organic extract was dried (*e.g.*, MgSO$_4$), filtered, and concentrated *in vacuo* to a solid residue. The residue was purified by recrystallization from an organic solvent or acidification of a basic solution.

(±)-3-Methyl-4-oxoretinoic Acid (6a). The crude product, obtained according to the general procedure, was recrystallized from ethyl acetate: mp 210−211 °C; HPLC, 100% (85:15 acetonitrile−1% aqueous ammonium acetate); MS peaks at *m/z* 328 (M), 313 (M − CH$_3$), 295 (M − CH$_3$ − H$_2$O); ^{1}H NMR δ 1.14 (s, 17e), 1.15 (d, J = 6.4 Hz, 3-CH$_3$), 1.24 (s, 16a), 1.71 (t, $J_{2a,2e}$ = 12.8 Hz, $J_{2a,3a}$ = 13.7 Hz, 2a), 1.76 (dd, $J_{2e,3a}$ = 4.3 Hz, 2e), 1.85 (s, 18), 2.03 (s, 19), 2.37 (s, 20), 2.56 (m, 3a), 5.84 (unresolved m, 14), 6.26 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.33 (s, 7 and 8), 6.38 (d, $J_{11,12}$ = 15.1 Hz, 12), 7.03 (dd, 11), 10.9 (broad, COOH). Anal. ($C_{21}H_{28}O_3$) C, H.

(±)-3-Ethyl-4-oxoretinoic Acid (6b). The crude product, obtained according to the general procedure, was recrystallized from ethyl acetate: mp 214−216 °C; HPLC, 99−100% (85:15 acetonitrile−1% aqueous ammonium acetate); MS peaks at *m/z* 342 (M), 327 (M − CH$_3$), 309 (M − CH$_3$ − H$_2$O), 298, 283; ^{1}H NMR δ 0.94 (t, CH_3CH$_2$−), 1.16 (s, 17e), 1.23 (s, 16a), 1.39 (m, CH$_3$CH_aH$_b$−), 1.66 (t, $J_{2a,2e}$ = 13.2 Hz, $J_{2a,3a}$ = 14.2 Hz, 2a), 1.80 (dd, $J_{2e,3a}$ = 4.8 Hz, 2e), 1.85 (s, 18), 1.97 (m, CH$_3$CH$_a$$H_b$−), 2.04 (s, 19), 2.36 (m, 3a), 2.37 (d, $J_{14,20}$ = 1.1 Hz, 20), 5.84 (unresolved m, 14), 6.26 (d, $J_{10,11}$ = 11.3 Hz, 10), 6.34 (s, 7 and 8), 6.38 (d, $J_{11,12}$ = 15.1 Hz, 12), 7.02 (dd, 11). Anal. ($C_{22}H_{30}O_3$) C, H.

(±)-4-Oxo-3-(2-propenyl)retinoic Acid (6c). The crude product, obtained according to the general procedure, was recrystallized from ethyl acetate; mp 195−196 °C; HPLC, 100% (85:15 acetonitrile−1% aqueous ammonium acetate); MS peaks at *m/z* 354 (M), 339 (M − CH$_3$), 321 (M − CH$_3$ − H$_2$O); ^{1}H NMR δ 1.15 (s, 17e), 1.23 (s, 16a), 1.65 (t, $J_{2a,2e}$ = 13.2 Hz, $J_{2a,3a}$ = 14.4 Hz, 2a), 1.80 (dd, $J_{2e,3a}$ = 4.7 Hz, 2e), 1.86 (s, 18), 2.04 (d, $J_{10,19}$ = 0.9 Hz, 19), 2.10 (m, −CH_aH$_b$CH=CH$_2$), 2.37 (d, $J_{14,20}$ = 1.1 Hz, 20), 2.52 (m, 3a), 2.74 (m, −CH$_a$$H_b$CH=CH$_2$), 5.04 and 5.06 (m, −CH$_2$CH=C$H_2$), 5.80 (m, −CH$_2CH$=CH$_2$), 5.84 (unresolved m, 14), 6.26 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.34 (s, 7 and 8), 6.38 (d, $J_{11,12}$ = 15.1 Hz, 12), 7.03 (dd, 11). Anal. ($C_{23}H_{30}O_3$) C, H.

(±)-4-Oxo-3-(2-propynyl)retinoic Acid (6d). The crude product, obtained according to the general procedure, was recrystallized from ethyl acetate: mp 211−213 °C; HPLC, 98.7% (85:15 acetonitrile−1% aqueous ammonium acetate); MS peaks at *m/z* 352 (M), 337 (M − CH$_3$), 319 (M − CH$_3$ − H$_2$O); ^{1}H NMR δ 1.19 (s, 17e), 1.27 (s, 16a), 1.81 (t, $J_{2a,2e}$ = 13.2 Hz, $J_{2a,3a}$ = 14.4 Hz, 2e), 1.86 (s, 18), 1.98 (t, J = 2.7 Hz, HC≡C−), 2.04 (s, 19), 2.06 (dd, $J_{2e,3a}$ = 4.8 Hz, 2a), 2.33 and 2.83 (m, H−C≡C−CH_2−), 2.37 (d, $J_{14,20}$ = 1.1 Hz, 20), 2.66 (m, 3a), 5.85 (unresolved m, 14), 6.27 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.35 (s, 7 and 8), 6.39 (d, $J_{11,12}$ = 15.1 Hz, 12), 7.03 (dd, 11). Anal. ($C_{23}H_{28}O_3$) C, H.

(±)-4-Oxo-3-(phenylmethyl)retinoic Acid (6e). The crude product, obtained according to the general procedure, was recrystallized twice from ethyl acetate: mp 209−210 °C; HPLC, 99−100% (85:15 acetonitrile−1% aqueous ammonium acetate); MS peaks at *m/z* 404 (M), 389 (M − CH$_3$), 371 (M − CH$_3$ − H$_2$O); ^{1}H NMR δ 1.09 (s, 17e), 1.13 (s, 16a), 1.61 (t, $J_{2a,2e}$ = 13.2 Hz, $J_{2a,3a}$ = 14.4 Hz, 2a), 1.65 (dd, $J_{2e,3a}$ = 4.8 Hz, 2e), 1.88 (s, 18), 2.03 (s, 19), 2.37 (d, $J_{14,20}$ = 0.7 Hz, 20), 2.48 (dd, J = 13.9 Hz, J = 9.2 Hz, C$_6$H$_5$CH_aH$_b$), 2.74 (m, 3a), 3.49 (dd, J = 4.0 Hz, J = 13.9 Hz, C$_6$H$_5$CH$_a$$H_b$), 5.84 (unresolved m, 14), 6.26 (d, $J_{10,11}$ = 11.4 Hz, 10), 6.33 (s, 7 and 8), 6.38 (d, $J_{11,12}$ = 15.1 Hz, 12), 7.02 (dd, 11), 7.14−7.34 (aromatic CH). *Anal.* ($C_{27}H_{32}O_3$) C, H.

(±)-3-Cinnamyl-4-oxoretinoic Acid (6f). The crude product, obtained by a procedure similar to the general procedure, was recrystallized from ethyl acetate: mp 197−198 °C; HPLC, 98−99% (85:15 acetonitrile−1% aqueous ammonium acetate); MS peaks at *m/z* 430 (M), 415 (M − CH$_3$), 397 (M − CH$_3$ − H$_2$O), 386, 374; ^{1}H NMR δ 1.14 (s, 17e), 1.23 (s, 16a), 1.71 (t, $J_{2a,2e}$ = 13.3 Hz, $J_{2a,3a}$ = 14.0 Hz, 2a), 1.83 (dd, $J_{2e,3a}$ = 4.9 Hz, 2e), 1.87 (s, 18), 2.04 (s, 19), 2.31 (m, CH_aH$_b$CH=CH−), 2.37 (d, $J_{14,20}$ = 0.9 Hz, 20), 2.61 (m, 3a), 2.85 (m, −CH$_a$$H_b$CH=CH), 5.84 (unresolved m, 14), 6.21 (m, CH$_2$CH=CH), 6.25 (d, $J_{10,11}$ = 11.3 Hz, 10), 6.34 (s, 7 and 8), 6.38 (d, $J_{11,12}$ = 15.2 Hz, 12), 6.42 (d, J = 15.9 Hz, CH$_2$CH=CH), 7.03 (dd, 11), 7.15−7.40 (aromatic CH). Anal. ($C_{29}H_{34}O_3$) C, H.

3,3-Dimethyl-4-oxoretinoic Acid (6g). The crude product, obtained by a procedure similar to the general procedure, was recrystallized from ethanol−ethyl acetate and then from ethyl acetate: mp 226−228 °C; HPLC, 100% (acetonitrile−1% aqueous ammonium acetate); MS peaks at *m/z* 342 (M), 327 (M − CH$_3$), 309 (M − CH$_3$ − H$_2$O); ^{1}H NMR δ 1.18 (s, 2 CH$_3$ groups at position 3), 1.22 (s, 17e and 16a), 1.79 (s, 2a and 2e), 1.88 (s, 18), 2.04 (d, $J_{10,19}$ = 0.8 Hz, 19), 2.37 (d, $J_{14,20}$ = 1.0 Hz, 20), 5.84 (unresolved m, 14), 6.27 (d, $J_{10,11}$ = 11.3 Hz, 10), 6.36 (s, 7 and 8), 6.38 (d, $J_{11,12}$ = 15.1 Hz, 12), 7.03 (dd, 11). Anal. ($C_{22}H_{30}O_3$) C, H.

Ornithine Decarboxylase Assay. Assays for the reduction by 4-oxoretinoids of ornithine decarboxylase activity induced by 12-*O*-tetradecanoylphorbol 13-acetate were performed by the procedures of Verma *et al.*[26,27]

Antipapilloma Assay. In this assay, reported by Verma *et al.*,[28] tumors were initiated with a single application of 51.2 μg of DMBA in 0.2 mL of acetone to the shaved skin of each CD-1 female mouse. Two weeks later, 2 nmol of TPA in acetone was administered twice per week to the initiated area for the duration of the experiment (12−15 weeks). At the end of an experiment, the number of tumors on each mouse was counted and the average number of tumors per mouse was calculated for the treated and control groups (10−15 mice per group). These averages from each of the stated number of experiments were averaged and used to calculate the percentages listed in Table 1.

Hamster Trachea Organ Culture Assay. The 4-oxoretinoids were evaluated for their capacity to reverse squamous metaplasia and keratinization in organ cultures of tracheas obtained from vitamin A-deficient hamsters. The methods and procedures were those described by Sporn and co-workers.[30,31]

The ED_{50}-values were estimated from best-fitting straight lines obtained by standard methods of linear regression analysis, using data derived from typical experiments. Compounds **4a**,**e**, **5a**, and **6a**,**d**,**e** were evaluated in this assay.

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