reflux for 24 h in 20 mL of benzene in a flask equipped with a condenser and protected by a drying tube. Sufficient water to dissolve the carbonate was added, the layers were separated, and the aqueous layer was neutralized to yield 1.1 g of starting material. The benzene phase then was extracted with 10 mL of 10% aqueous NaOH and neutralized with HCl to give 0.82 g of crude product. After two crystallizations from petroleum ether, the substance was obtained with mp 88–89 °C and mp, with chlorination product (11) of 2-hydroxy-6-methoxyacetophenone, 88–89 °C.

Monomethylation of 3,5-Dichloro-2,6-dihydroxyacetophenone. 3,5-Dichloro-2,6-dihydroxyacetophenone[11] (1 g), anhydrous potassium carbonate (2 g), and dimethyl sulfate (0.57 g) were heated under reflux for 24 h in 25 mL of benzene, with protection from atmospheric moisture. Sufficient water then was added to dissolve the potassium carbonate and the resulting two phases were separated. Neutralization of the aqueous layer gave 920 mg of starting material. The benzene phase was extracted with 5 mL of 10% NaOH, and the sodium hydroxide solution was neutralized to yield 30 mg of 3,5-dichloro-2-hydroxy-6-methoxyacetophenone; mp, after one crystallization from petroleum ether (charcoal), 97–98 °C, mmp, with 2-hydroxy-6-methoxyacetophenone chlorination product (mp 98.5–100 °C), 97–99 °C.

E. ^{13}C NMR Spectral Data. Spectra were determined at 25.2 MHz on a Varian XL-100 instrument at normal probe temperature. The concentration of 4 was ca. 50 mg/3 mL of Me_2SO-d_6. The error in signal position, as indicated by the computer was ±2.5 Hz; 30 000 total transients were collected under block acquisition conditions. The approximate tipping angle was 50°. A 5-K spectral width was used, with a 0.4 s acquisition time and a 0.2 s pulse delay. Five watts of decoupling power were used with a band width of 1.5 K. The Me_2SO-d_6 peak at 40.4 ppm from Me_4Si was taken as standard.

Acknowledgment. This work was supported in part by a Grant (AI-01703) from the National Institutes of Health, U.S. Public Health Service. The XL-100 NMR instrument was purchased with funds from NSF Grant GP 10293. Both grants are gratefully acknowledged.

Registry No. 1, 491-78-1; 2, 548-58-3; 2 (bromo deriv), 87953-96-6; 3, 87953-83-1; 4, 87953-84-2; 4 (dibenzoate), 87953-85-3; 5, 87953-86-4; 6, 87953-87-5; 7, 87953-88-6; 8, 87953-89-7; 9, 87953-90-0; 10, 703-23-1; 11, 87953-91-1; 11 (benzoate), 87953-92-2; 12, 87953-93-3; 13, 87953-95-5; 14, 87953-94-4; 2,6-dihydroxyacetophenone, 699-83-2.

(11) Looker, J. H.; Edman, J. R.; Dappen, J. I. *J. Heterocycl. Chem.* **1964**, *1*, 141.

Synthesis and Characterization of *trans*-, 13-*cis*-, and 11-*cis*,13-*cis*-12-(Hydroxymethyl)retinols

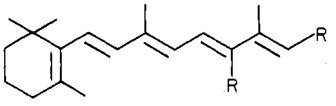
Anita H. Lewin,* Douglas H. Rector, Steven R. Parker, Mansukh C. Wani, and F. Ivy Carroll*

Chemistry and Life Sciences Group, Research Triangle Institute, Research Triangle Park, North Carolina 27709

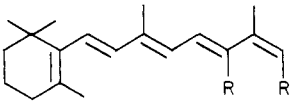
Received August 25, 1983

Whereas reduction of *trans*- and 11-*cis*,13-*cis*-12-carboxyretinoic acid dimethyl esters gives the trans and 11-cis,13-cis corresponding diols, reduction of 13-*cis*-12-carboxyretinoic acid dimethyl ester gives essentially no 13-cis diol with trans and 11-cis,13-cis diols being the major products. 12-(Hydroxymethyl)retinol with 13-cis stereochemistry is obtained by reduction of the configurationally locked retinoids 13-*cis*-12-carboxyretinol δ-lactone and 13-*cis*-12-(hydroxymethyl)retinoic acid δ-lactone.

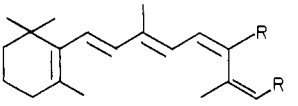
The demonstrated effectiveness of retinoids in preventing or delaying the progression of preneoplastic lesions to malignant, invasive carcinomas in epithelial tissues[1-3] and in bringing about regressions with skin papillomas,[2-4] carcinomas, and some types of murine melanomas[2,3,5] has generated substantial interest in the synthesis of novel retinoids. We have recently reported the synthesis, stereochemistry, and conformations of 12-carboxyretinoic acids[6,7] and of related anhydrides[7] and lactones.[8] We now report the methods for preparing *trans*-, 13-*cis*-, and 11-*cis*,13-*cis*-12-(hydroxymethyl)retinol (1–3), present chemical and spectral data establishing their structure and stereochemistry, and discuss their relative stability.



1, R = CH_2OH
4, R = CO_2CH_3



2, R = CH_2OH
5, R = CO_2CH_3



3, R = CH_2OH
6, R = CO_2CH_3

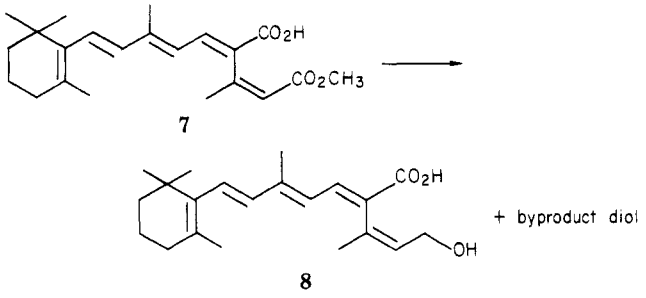
Results and Discussion

Lithium aluminum hydride reduction of retinoid esters has been shown to proceed with retention of the stereochemistry of the retinoid backbone.[9] It was, therefore, expected that reduction of the dimethyl esters of *trans*-, 13-*cis*-, and 11-*cis*,13-*cis*-12-carboxyretinoic acids (4–6,

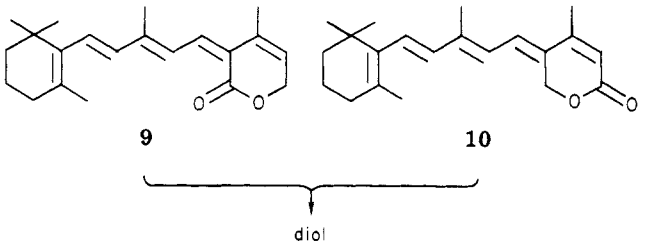
(1) Sporn, M. B.; Newton, D. L.; Smith, J. M.; Acton, M.; Jacobson, A. E.; Brossi, A. In "Carcinogens: Identification and Mechanism of Action"; Griffin, A. C., Shaw, C. R., Eds.; Raven Press: New York, 1979; p 441.
(2) Lotan, R. *Biochim. Biophys. Acta* **1980**, *605*, 33.
(3) Hill, D. L.; Grubbs, C. J. *Anticancer Res.* **1982**, *2*, 111.
(4) Sporn, M. B.; Newton, D. L. In "Inhibition of Tumor Induction and Development", Zedeck, M. S.; Lipkin, M., Eds.; Plenum Press: New York 1981.
(5) Bollag, W. *Cancer Chemother. Pharmacol.* **1979**, *3*, 207.
(6) Lewin, A. H.; Carroll, F. I.: Moreland, C. G. *J. Am. Chem. Soc.* **1981**, *103*, 6527.
(7) Lewin, A. H.; Whaley, M. G.; Parker, S. R.; Carroll, F. I.; Moreland, C. G. *J. Org. Chem.* **1982**, *47*, 1799.
(8) Lewin, A. H.; Rector, D. H.; Parker, S. R.; Wani, M. C.; Carroll, F. I. *J. Org. Chem.* **1983**, *48*, 222.
(9) Robeson, C. D.; Cawley, J. D.; Weister, L.; Stern, M. H.; Eddinger, C. C.; Chechak, A. J. *J. Am. Chem. Soc.* **1955**, *77*, 4111.

0022-3263/84/1949-0649$01.50/0

Scheme I



Scheme II



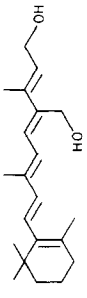
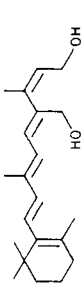
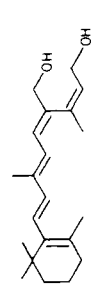
respectively) would produce the desired diols 1–3. On the other hand, since we had found that base treatment of the 13-cis diester 5 led to isomerization,[7] it was recognized that reduction of 5 may not be straightforward.

Reduction of the trans diester 4 gave a single product; the ^1H and ^{13}C NMR spectra were consistent with those expected for 1. In particular, the ^{13}C NMR shifts of the 9a and 13a methyl groups of 1 (12.4 and 16.4 ppm, respectively) were similar to the values recorded for other trans retinoids;[10,11] in the trans diester 4 the shifts are 12.5 and 15.5 ppm, respectively.[7] Lithium aluminum hydride reduction of 13-*cis*-12-carboxyretinoic acid dimethyl ester 5 gave a three component mixture. The major product was identical with the product obtained from the reduction of the trans diester 4; the minor products were also retinoid diols. In an attempt to avoid what appeared to be isomerization of the 13-cis system, the reaction was carried out at −70 °C. This led to the disappearance of one of the minor components. No change in this product composition was obtained under a varieity of workup conditions.

Somewhat to our surprise, we discovered that the minor product observed in the low-temperature reduction of the 13-cis diester 5 was identical with the byproduct obtained in the reduction of methyl 11-*cis*,13-*cis*-12-carboxyretinoate (7) to the hydroxy acid 8 (Scheme I). This retinoid diol had ^{13}C NMR shifts for the 9a and 13a methyl groups (12 and 23 ppm, respectively) consistent with the expected 11-cis,13-cis stereochemistry.[9] However, 13-cis stereochemistry could not be excluded.[7]

In order to preserve the 13-cis stereochemistry in the reduction step, it was decided to utilize a retinoid in which the 13-cis double bond was contained in a ring. Suitable systems for this purpose were available from our previous work,[8] i.e., the 13-cis δ-lactones 9 and 10 of 12-carboxyretinol and of 12-(hydroxymethyl)retinoic acid. In fact, since reduction of either lactone should give the same product, the lactone mixture produced in the reduction of the mixture of 13-cis half-esters obtained from methanolic saponification of 13-*cis*-12-carboxyretinoic anhydride[8] was reduced with excess lithium aluminum hydride (Scheme II). A diol, which was different from the diols

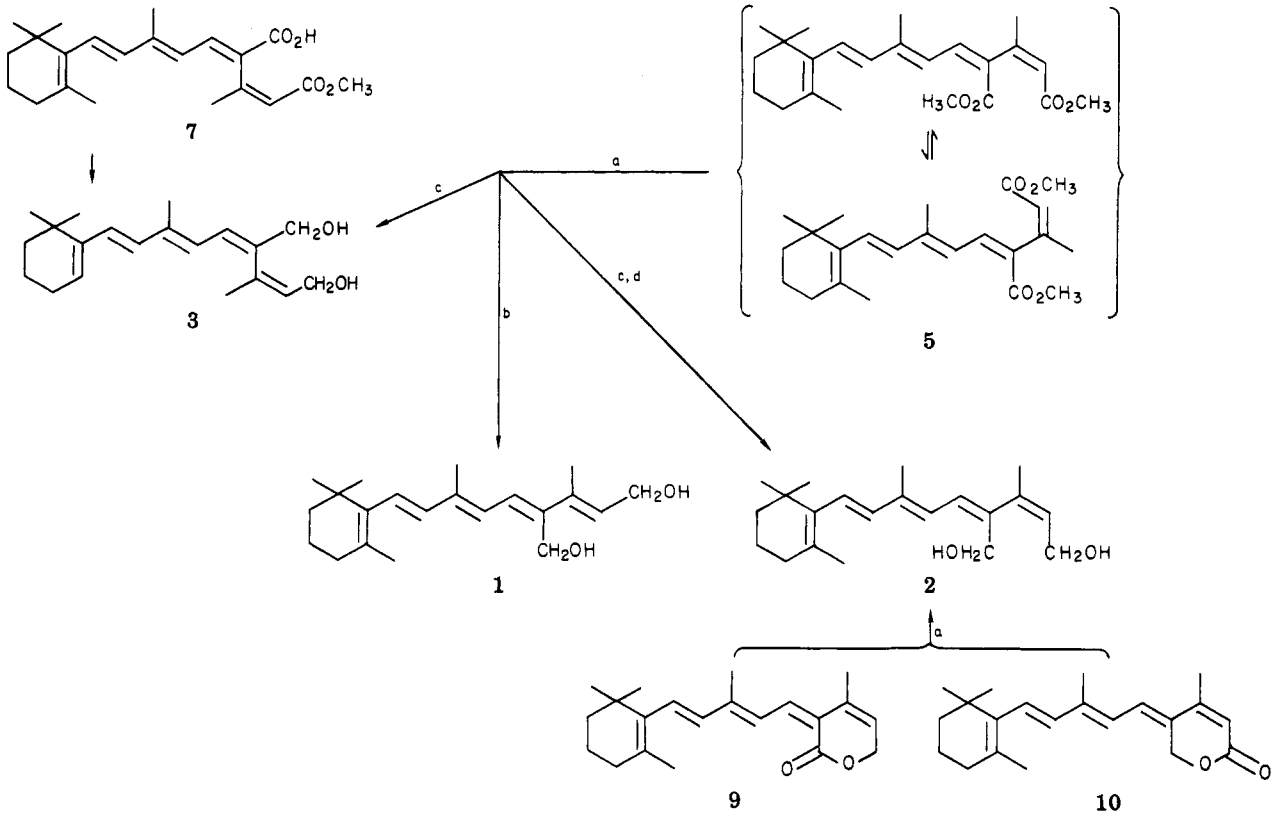
Table I. Carbon-13 and Proton Chemical Shifts (ppm) of Retinoids

no.	retinoid structure	proton and carbon shifts[a]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1a	5a	9a	13a	12a
1[b]		34.8	40.1	19.8	33.4	129.1	138.6	126.5	135.6	134.8	131.3	120.9	147.2	139.0	127.5	59.0	29.2	21.9	12.4	16.4	65.9
								6.09	*6.09*		*6.21*	*6.42*			*5.45*	*4.17*	*1.00*	*1.71*	*1.80*	*1.99*	*4.15*
11[c]								*6.09*	*6.24*		*6.21*	*6.51*			*5.39*	*4.70*	*1.02*	*1.70*	*d*	*d*	*4.63*
2[b]		34.7	40.1	19.8	33.6	129.4	138.6	126.2	138.6	137.3	128.9	125.6	140.7	141.6	127.3	59.7	29.2	21.8	12.2	23.2	65.6
								6.20	*6.20*		*6.29*	*6.41*			*5.55*	*4.29*	*1.03*	*1.72*	*1.92*	*1.92*	*4.02*
12[c]								*6.24*	*6.24*		*6.43*	*6.43*			*5.53*	*4.50*	*1.04*	*1.71*	*d*	*d*	*4.82*
3[b]		34.7	40.1	19.8	33.4	129.3	138.4	126.7	138.8	135.6	129.6	121.7	143.7	136.2	127.1	59.8	29.2	21.8	12.4	23.0	65.0
								6.12	*6.12*		*6.08*	*6.48*			*5.41*	*3.80*	*1.02*	*1.69*	*1.92*	*1.82*	*4.08*
13[c]								*6.16*	*6.26*		*6.04*	*6.53*			*5.64*	*4.34*	*1.02*	*1.70*	*d*	*d*	*4.70*

[a] Proton shifts in italics. [b] In dioxane-d_8. [c] In CD_3CN. [d] Obscured by solvent.

(10) Becker, R. S.; Berger, S.; Dalling, D. K.; Grant, D. M.; Pugmire, R. *J. Am. Chem. Soc.* **1974**, *96*, 7008.
(11) Englert, G *Helv. Chim. Acta* **1975**, *58*, 2367.

Scheme III[a]



[a] a, $LiAlH_4$/THF; b, major product; c, minor product; d, not produced at -70 °C.

obtained in the reduction of either trans or 11-cis,13-cis esters and which was identical with the third component observed in the reduction of the 13-cis ester at room temperature, was produced. This new diol also had ^{13}C NMR signals at 12 and 23 ppm for the 9a and 13a methyl groups, consistent with either 13-cis or 11-cis,13-cis stereochemistry.[10,11]

The stereochemical assignments of the diols produced in these reductions were aided by their isomerization behavior. They were unaffected by exposure to fluorescent laboratory lights for 5 days, but heating at 75 °C for 1 h led to isomerization of the diol produced in the reduction of the 13-cis lactones to the diol produced in the reduction of the trans diester **4**; the latter isomerized to the diol obtained from 11-cis,13-cis half-ester **7**. Similarly, treatment of the diacetate derivatives of these diols with dilute iodine solution in the dark caused the isomerization of the 13-cis lactone product and of the trans diester product to the 11-cis,13-cis half-ester product. The thermal isomerization data are consistent with a situation in which a high-energy species isomerizes to one which represents an energy minimum via a species of intermediate energy. Somewhat analogous results had been obtained in the saponification of 13-*cis*-12-carboxyretinoic acid dimethyl ester (**5**),[7] where both the trans diacid and the 11-cis,13-cis diacid were formed at 100 °C but the latter represented the energy minimum. By analogy, the lactone reduction product would be 13-*cis*-12-(hydroxymethyl)retinol (**2**), the product from trans diester **4** would be *trans*-12-(hydroxymethyl)retinol (**1**), and the product from 11-cis,13-cis half-ester **7** would be 11-*cis*,13-*cis*-12-(hydroxymethyl)-retinol (**3**).

The fact that **3** was obtained in the reduction of the 11-cis,13-cis half-ester **7** is consistent with this assignment, although it is inconslusive in itself. Obviously retention of the polyene stereochemistry cannot be assumed in these reductions since the diol **1** was produced in the reduction of both the trans diester **4** and the 13-cis diester **5**.

The isomerization results, taken together with the ^{13}C NMR data, support the assignments for the trans and 11-cis,13-cis diols (**1** and **3**).

The third diol would appear to be 13-*cis*-12-(hydroxymethyl)retinol (**2**). This conclusion is based primarily on its mode of formation, i.e., from a 13-cis lactone. The multiplicity of products obtained in the reduction of the 13-cis diester **5** is undoubtedly related to its propensity to isomerize, particularly under basic conditions.[7] Maintenance of the 13-cis double bond in a cycle until at least partial reduction had taken place was expected to help avoid isomerization, and therefore, the diol produced should have 13-cis stereochemistry.

It follows then that, in general, the low-temperature lithium aluminum hydride reduction of retinoid esters proceeds with retention of configurational integrity. The case of the 13-*cis*-12-carboxyretinoic acid dimethyl ester **5** is an exception due to the high energy associated with the 13-cis stereochemistry in 12-substituted retinoids (Sceme III).

Experimental Section

Melting points were determined on a Thomas Hoover capillary tube apparatus or on a Koffler hot stage, and they are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 267 grating spectrophotometer, ultraviolet spectra were recorded on a Cary 14 spectrophotometer, and mass spectra were obtained on an AEI MS-902 spectrometer. Proton NMR spectra were recorded on a Varian HA-100 spectrometer, and ^{13}C NMR spectra were determined on a JEOL JNM-PS-100 NMR instrument. ^{1}H and ^{13}C NMR data are summarized in Table I.

Analytical chromatography was carried out by using commercial silica gel F-254 for TLC and a Waters Associates high-pressure liquid chromatograph consisting of two constant flow pumps (M6000A) controlled electronically by a solvent programmer

Table II. HPLC Retention Times for Retinoids[a]

retinoids		eluant	retention time, min
no.	assigned structure		
1	*trans*-12-(hydroxymethyl)retinol[b]	A[c]	8.5
2	13-*cis*-12-(hydroxymethyl)retinol[b]	A[c]	6.2
3	11-*cis*,13-*cis*-12-(hydroxymethyl)retinol[b]	A[c]	7.0
11	*trans*-12-(hydroxymethyl)retinol diacetate[d]	B[e]	6.5
12	13-*cis*-12-(hydroxymethyl)retinol diacetate[d]	B[e]	8.5
13	11-*cis*,13-*cis*-12-(hydroxymethyl)retinol diacetate	B[e]	9.4

[a] On Radial Pak B.cartridge. [b] Detected at 300 nm. [c] 4% MeOH in 9:1 hexane/Et_2O. [d] Detected at 328 nm. [e] 5% Et_2O in hexane.

(Model 660), a septumless nonstop-flow high-pressure injector (Model U6-K), and a variable-wavelength UV detector (Model 450). The columns used were Waters Associates 3.9 mm × 30 cm μ-Porasil, μ-Bondapak C_{18}, and Radial Pak A and B cartridges in a Waters Associates radial compression module (Model 100).

All laboratory operations involving retinoids and related polyene systems were performed under dim red lights and in an inert atmosphere.

***all-trans*-12-(Hydroxymethyl)retinol (1). (a) Reduction of *all-trans*-12-Carboxyretinoic Acid Dimethyl Ester (4).** To a stirred slurry of 41 mg (1.08 mmol) in 13 mL of THF under argon was added a solution of 50 mg (0.134 mmol) of *all-trans*-12-carboxyretinoic acid dimethyl ester (4) in 2 mL of THF. The reaction was quenched with saturated aqueous NH_4Cl, diluted with H_2O, and extracted with EtOAc. HPLC analysis indicated this product to be identical with the product obtained from reduction of 13-*cis*-12-carboxyretinoic acid, dimethyl ester (5) described below.

(b) Reduction of 13-*cis*-12-Carboxyretinoic Acid Dimethyl Ester (5). Reduction of 1.9 g (5.1 mmol) of 13-*cis*-12-carboxyretinoic acid dimethyl ester (5) in 50 mL of THF with a slurry of 1.6 g (46 mmol) of $LiAlH_4$ in 450 mL of THF as described in (a) gave after similar workup 1.62 g of a crude mixture. HPLC analysis showed one major and two minor components, one of which was the 11-cis,13-cis diol 3. The mixture was eluted on a medium-pressure silica gel column (Merck, size B) by using 40% ethyl acetate in hexanes to give 560 mg (35%) of the major component 1 as an oil; IR (KBr) 3400 cm^{-1}; λ_{max} (MeOH) 303 nm (ε 20700); MS, *m/e* 316.241 (calcd for $C_{21}H_{32}O_2$, 316.240).

13-*cis*-12-(Hydroxymethyl)retinol (2). To a stirred suspension of 345 mg (9 mmol) of $LiAlH_4$ in THF was slowly added a solution of 345 mg (11 mmol) of a mixture of 13-*cis*-12-carboxyretinol δ-lactone (9) and 13-*cis*-12-(hydroxymethyl)retinoic acid δ-lactone (10) in 2 mL of THF at 0 °C. After being stirred at room temperature under N_2 for 1.75 h, the reaction mixture was cooled to 0 °C and cautiously quenched with saturated aqueous NH_4Cl, diluted with H_2O, acidified to pH 6 with 10% H_2SO_4, and extracted with EtOAc. The organic layers were back-washed with brine, dried, and evaporated to give 345 mg of an oil. HPLC showed it to consist mainly of one component. Purification by column chromatography (silica gel, 1:1 EtOAc/hexane) gave 285 mg of a yellow oil, which developed polar constituents (TLC) within 3 days. Rechromatography using 1% MeOH in 1:1 EtOAc/hexane gave 72 mg of pure 13-*cis*-12-(hydroxymethyl)retinol (2): λ_{max} (EtoH) 305 nm (ε 17415); MS, *m/e* 316.2405 calcd for $C_{21}H_{32}O_2$, 316.2401).

11-*cis*,13-*cis*-12-(Hydroxymethyl)retinol (3). To a cold (0 °C) solution of 228 mg (6.0 mmole) of $LiAlH_4$ in 18 mL of THF was slowly added a solution of 260 mg (0.75 mmol) of methyl 11-*cis*,13-*cis*-12-carboxyretinoate (7) in 2 mL of THF. After stirring for 0.5 h, usual workup gave a crude mixture (222 mg), which was eluted on a medium-pressure silica gel column (Merck, size B) by using 0.25% MeOH and 33% Et_2O in hexanes to give 11-*cis*,13-*cis*-12-(hydroxymethyl)retinol (80 mg, 33%); IR (KBr) 3400 cm^{-1}; λ_{max} (MeOH) 300 nm (Δ 46080); MS, *m/e* 316.241 (calcd for $C_{21}H_{32}O_2$, 316.240). Continued elution of the column gave 11-*cis*,13-*cis*-12-carboxyretinol (71 mg, 29%): IR (KBr) 1725 cm^{-1}; λ_{max} (MeOH) 327 nm (ε 26800); MS, *m/e* 330.219 (calcd for $C_{21}H_{30}O_3$, 330.219).

Diacetates of the Diols 1–3. For each preparation a small amount of the diol was stirred with acetic anhydride and pyridine for 1 h at room temperatures. The mixture was then evaporated to dryness, leaving a gum. HPLC showed only one component for each product. Proton NMR confirmed the presence of the acetoxy methyl groups. The structure of the diacetates 11–13 was confirmed by ^1H NMR (Table I).

Iodine-Promoted Isomerization of Retinoid Diacetates. To a solution of 1 drop of diacetate 11, 12, or 13 in 10 drops of hexane was added 1 μL of a 1% solution of I_2 in Et_2O. The solutions were kept in foil covered vials, and the isomerization was monitored by HPLC.

HPLC Analysis. The best separations were achieved by using Radial Pak B cartridges, although stainless steel packed columns gave similar results. The eluant used for the diols was 4% MeOH in 9:1 hexane/Et_2O with a flow rate of 2 mL/min; detection was at 300 nm. For the diacetate 5% Et_2O in hexane at 2 mL/min was used as eluant; detection was at 328 nm. The retention times are shown in Table II. The solvents were from Burdick and Jackson, and they were degassed prior to use. Analysis of the lactones has been described previously.[8]

Acknowledgment. This work was supported in part by National Cancer Institute Contract No. N01-CP-75932.

Registry No. 1, 88315-65-5; 1 diacetate, 88315-67-7; 2, 88335-98-2; 2 diacetate, 88335-99-3; 3, 88315-66-6; 3 diacetate, 88315-68-8; 4, 79985-66-3; 5, 80040-38-6; 6, 80009-89-8; 7, 83860-28-0; 8, 83803-36-5; 9, 83803-33-2; 10, 83803-32-1.