rabbit small intestine as well as transport studies and photoaffinity labeling experiments were performed as described previously.[16,17]

Stability Study. 33 (2.5 mg) was dissolved in 0.1 mL of methanol, and 29 mg of NaCl and the amount of phosphate buffer pH 7 were added to yield 5 mL of test solution; 0.5 mL was removed, added to 0.5 mL of acetonitrile containing 0.1% trifluoroacetic acid, and taken as the reference (T = 0). The remaining test solution was then incubated with 4.5 mg of α-chymotrypsin at 37 °C. Aliquots of 0.2 mL each were taken at intervals and quenched by addition of 0.2 mL of acetonitrile (0.1% TFA). Analyses of the incubation were carried out by reversed-phase HPLC on nucleosil C-18 (7 μm) eluted using 60% MeOH/40% H_2O/0.1% ammonium acetate. Peak detection was by UV absorbance at 254 nm with quantification using a Gilson Data Master. $T_{1/2}$ turned out to be about 4 h.

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Base-Catalyzed Isomerization of Retinoic Acid. Synthesis and Differentiation-Inducing Activities of 14-Alkylated *all-trans*-, 13-*cis*-, and 20,14-*retro*-Retinoic Acids

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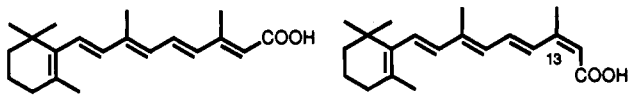
Retinoic acid (1) is isomerized regioselectively by excess amounts of lithium diisopropylamide (LDA) to give 20,14-*retro*-retinoic acid (3). Alkylation of the intermediate dianion of retinoic acid gave 14-alkylated derivatives of 3. By isomerization of the alkylated retro isomers under basic conditions, several 14-alkyl-*all-trans*- and -13-*cis*-retinoic acids were synthesized. The retinoidal activities of these derivatives were examined, based on the ability to induce differentiation of human promyelocytic leukemia cell line HL-60. 20,14-*retro*-Retinoic acid (3) is 1/50 as active as retinoic acid (1). Although 14-methyl-20,14-*retro*-retinoic acid (4) is as active as 3, the introduction of a 14-methyl group into *all-trans*- and 13-*cis*-retinoic acid resulted in decreased activity. Introduction of bulkier alkyl groups at the C-14 position caused the disappearance of the activity.

Retinoic acid (all-trans, 1) plays fundamental roles in cell differentiation and proliferation[1,2] and is a morphogen in chicks[3] and amphibia.[4] Elucidating its mechanism of action is currently considered to be one of the most important problems in biology. Recently, it has been established that retinoic acid (1) binds to its specific nuclear receptor(s) in order to regulate specific gene expressions,[5] being similar in that respect to the steroid hormones, thyroid hormone, and vitamin D_3. Now, retinoic acid (1) is regarded as an internal "hormone" rather than a vitamin.

In contrast to the recognition of the significant biological action of retinoic acid (1), its chemical behavior has not been systematically studied. Retinoic acid (1) has an unique structure, consisting of a conjugated pentaenoic acid system, and is expected to undergo various types of reactions. Among them, isomerization of retinoic acid (1) is one of the more interesting reactions from the viewpoint of pure chemistry. It is also useful in connection with the synthesis of biologically related derivatives, which might be useful clinically.[6] Retinoic acid (1) is isomerized photochemically and thermally to give a number of products including 13-*cis*-retinoic acid (2, Chart I),[7] but acid- and base-catalyzed isomerizations have scarcely been studied. We previously reported that retinoic acid (1) isomerizes under acidic conditions to give complex mixtures consisting of annulated isomers.[8] In this paper, we report the regioselective isomerization of retinoic acid catalyzed by LDA, which forms 20,14-*retro*-retinoic acid (3) in good yield. Furthermore, using the anionic intermediate of this reaction, several derivatives with an alkyl group at the C-14 position of *all-trans*-, 13-*cis*-, and 20,14-*retro*-retinoic acid were synthesized. The retinoidal activity of these compounds was examined, on the basis of ability to induce differentiation of human promyelocytic leukemia cell line HL-60 to mature granulocytes.[9,10] This ability of retinoids can be measured very sensitively and

Chart I



1 (all-trans) 2 (13-cis)

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† Faculty of Pharmaceutical Sciences.
‡ Institute of Applied Microbiology.

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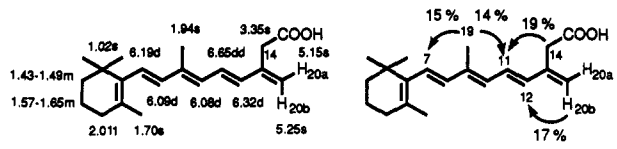
Chart II



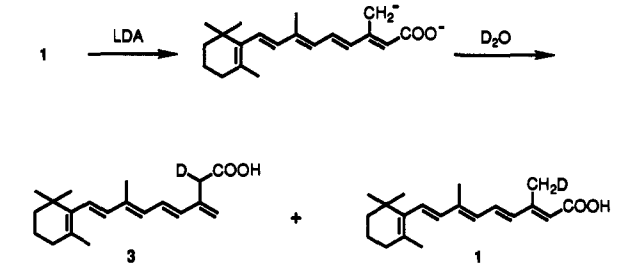
20,14-*retro*-retinoic acid (3)

quantitatively and seems to correlate well to activities mediated by the specific retinoic acid receptors.[1] The morphological changes of the cells were examined after Wright–Giemsa staining, and the Nitro-blue tetrazolium (NBT) reduction assay was employed as a functional marker of differentiation.[11] These two indexes of differentiation correlated well.[12-14] The ED_{50} values of active compounds were calculated from the NBT reduction assay data. Relative activity was defined as the ratio of ED_{50} of retinoic acid to ED_{50} of a test compound, both values having been obtained *in concurrent experiments* (Table III).

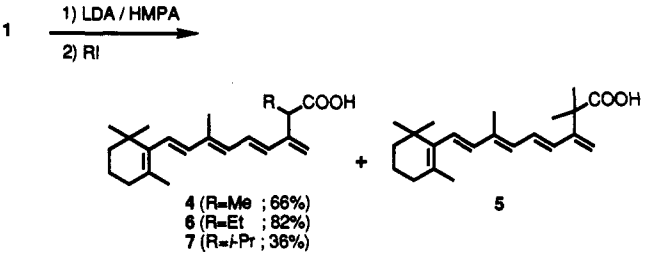
Chemistry

Isomerization of Retinoic Acid (1) by LDA. When retinoic acid (1) was treated with 5 molar equiv of LDA in THF at −78 °C, the solution turned immediately orange and then red after 5 min. When the solution was poured into ice water and neutralized with 1 N HCl(aq), a single product **3** was obtained in 57% yield, with a recovery of 1 (29%). Compound **3** is an isomer of 1, as deduced from elemental analysis and high-resolution mass spectroscopy. The UV absorption spectrum (95% EtOH) of **3** (λ_{max} (log ϵ): 322 (4.68), 310 (4.63), and 239 nm (4.16)) indicates a conjugated pentaene structure, not conjugated with a carboxyl group (1: 346 (4.37), 248 nm (3.40)). Table I gives the ^1H NMR chemical shifts of **3**, compared with those of 1. The shifts to higher field of the olefinic proton signals (H_7–H_{11}) of **3** also suggest the absence of conjugation between the pentaene system and the carboxyl group. Besides the signals of five olefinic methine protons, two methyls ($H_{16,17}$) on aliphatic carbon, and two methyls ($H_{18,19}$) on olefinic carbon, there were signals due to an *exo*-methylene (H_{20}: 5.15 ppm, s, 1 H, and 5.25 ppm, s, 1 H) and an aliphatic methylene (H_{14}: 3.35 ppm, s, 2 H) adjacent to a carboxyl group. The stereochemistry of the conjugated olefin (all-trans form) was determined from the ^1H–^1H coupling constants ($J_{7,8}$ = 16 Hz, $J_{10,11}$ = 11 Hz, and $J_{11,12}$ = 15.5 Hz), and NOE enhancements between H_7 and H_{19} (15%) and between H_{11} and H_{19} (14%) (Chart II). Therefore, the structure of **3** was determined as *all-trans*-20,14-*retro*-retinoic acid. Furthermore, the large NOE enhancements between H_{11} and H_{14} (19%) and between H_{12} and H_{20a} (17%) indicate that **3** exists in 12-s-trans form in $CDCl_3$ solution (Chart II).

Scheme I



Scheme II



Though the reaction of 1 with LDA was very rapid, 1 could not be converted completely into **3** even with excess amounts of LDA, at higher temperature, or after a longer time; treatment of 1 with 20 molar equiv of LDA at −78 °C (5 min) resulted in **3** (61%) and 1 (29%). Under similar conditions but at higher temperature (−60 °C), **3** was obtained in 68% yield with recovery of 1 in somewhat lower yield (15%), and stirring for 60 min at the same temperature resulted in **3** (70%) and 1 (11%). On the other hand, methyl retinoate (1′) could be isomerized to methyl *all-trans*-20,14-*retro*-retinoate (3′) by LDA (5 equiv) at −78 °C in a better yield (87%, with 5% of recovered 1′).[15]

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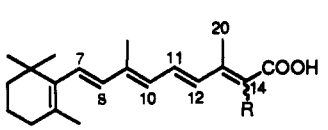
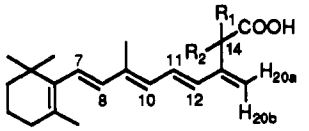
Table I. 400-MHz ^1H NMR Chemical Shifts of Retinoic Acid Derivatives[a]



1, 2, 8–13



3–7

		7	8	10	11	12	14	20a	20b
1	(R = H, all-trans)	6.29	6.14	6.16	7.05	6.31	5.80	2.37	
2	(R = H, 13-cis)	6.29	6.17	6.27	7.03	7.74	5.66	2.11	
3	(R_1 = R_2 = H)	6.19	6.09	6.08	6.65	6.32	3.35	5.15	5.25
4	(R_1 = H, R_2 = Me)	6.19	6.09	6.07	6.76	6.27	3.56	5.17	5.24
5	(R_1 = R_2 = Me)	6.17	6.08	6.06	6.81	6.15	-	5.10	5.34
6	(R_1 = H, R_2 = Et)	6.19	6.10	6.07	6.79	6.27	3.31	5.17	5.26
7	(R_1 = H, R_2 = *i*-Pr)	6.19	6.10	6.09	6.83	6.30	3.03	5.24	5.30
8	(R = Me, all-trans)	6.27	6.15	6.23	6.98	6.70	-	2.26	
9	(R = Me, 13-cis)	6.22	6.14	6.22	6.89	7.44	-	2.04	
10	(R = Et, all-trans)	6.27	6.15	6.22	6.98	6.69	-	2.23	
11	(R = Et, 13-cis)	6.22	6.14	6.21	6.88	7.33	-	2.05	
12[b]	(R = *i*-Pr, all-trans)	6.27	6.17	6.23	6.88	6.76	-	1.97	
13[b]	(R = *i*-Pr, 13-cis)	6.24	6.13	6.10	6.78	6.52	-	1.97	

[a] Chemical shifts are expressed in ppm relative to Me_4Si in $CDCl_3$. [b] Solvent: CD_3OD.

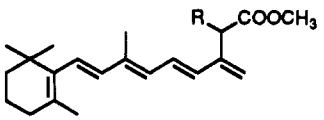
Formation of a dianion of retinoic acid (1) was confirmed by quenching the reaction mixture with deuterium oxide (Scheme I). When 1 was treated with 20 molar equiv of LDA in THF at −60 °C for 5 min and then the mixture was poured into deuterium oxide, the protons exchanged with deuterium by the treatment were H_{14} (86%) of 3 and H_{20} (18%) of recovered 1. This shows that even large excess amounts of LDA cannot abstract an allylic proton other than H_{20}. The incomplete deuterium exchange of recovered 1 indicated that 1 could not be completely converted to the dianion under these conditions, and the nondeuterated one would have been formed mostly by the protonation of the carboxylate.

Alkylation of Retinoic Acid (1). For the purpose of synthesis of some derivatives with an alkyl group on the retinoic acid skeleton, alkylations of the dianion formed from retinoic acid (1) were examined. The dianion, generated by the reaction of 1 with 20 molar equiv of LDA for 5 min at −60 °C, was reacted with 5 molar equiv of methyl iodide for 1 h at −60 °C. Methylation proceeded only at the C-14 position of 1, and 14-methyl-20,14-*retro*-retinoic acid (4) and 14,14-dimethyl-20,14-*retro*-retinoic acid (5) were obtained in 42% and 1% yields, respectively, with recovery of 1 (6%) and 3 (22%). Addition of HMPA to the solution of the intermediate dianion at −78 °C increased the ratio of alkylated compounds (4, 66%; 5, 2%, Scheme II). The structures of 4 and 5 were determined by ^1H NMR (Table I). Though several quenching methods after methylation were examined, the formation of 14-methylretinoic acid (8 and 9) was not found under these reaction conditions.

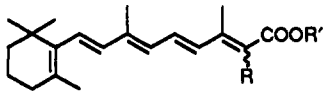
14-Ethyl-20,14-*retro*-retinoic acid (6) and 14-isopropyl-20,14-*retro*-retinoic acid (7) were similarly synthesized. The intermediate dianion was stirred with 8 molar equiv of ethyl iodide for 45 min at −70 °C to give 6 in 82% yield. The bulkier molecule isopropyl iodide (8 equiv) was reacted with the dianion for 30 min at −60 °C and for 30 min at −50 °C, then another 7 molar equiv of isopropyl iodide was added and allowed to react for another 30 min. As a result, 7 was obtained in 36% yield with recovery of 1 (5%) and 3 (27%).

Isomerization of 14-Alkyl-20,14-*retro*-Retinoic Acids. Since 14-alkylretinoic acid (and its 13-cis isomer) was not obtained by the alkylation of retinoic acid (1), isomerizations of 14-alkyl-20,14-*retro*-retinoic acids were studied. Under various basic conditions examined, such as MeONa/MeOH, *t*-BuOK/18-crown-6/*t*-BuOH, or aqueous KOH/EtOH, 4 did not isomerize. Since carboxylate formation seemed to make the proton abstraction difficult, isomerizations were studied after esterification.

Table II. Isomer Distribution of 14-Alkyl-20,14-*retro*-retinoic Acids under Basic Conditions



4′, 6′, 7′



8′–13′ (R′ = CH_3), 8–13 (R′ = H)

	compound	condn[a]	products (%) esters 4′	8′	9′	acids 4	8	9
4′	(R = methyl)		4′	8′	9′	4	8	9
		A	2	8	88	1	0	0
		B	0	0	0	13	20	39
6′	(R = ethyl)		6′	10′	11′	6	10	11
		A	3	18	74	5	0	0
		B	0	0	0	51	21	26
7′	(R = isopropyl)		7′	12′	13′	7	12	13
		A	64	<1	3	0	0	0
		B	0	9	9	78	0	0
		C	7	20	30	8	0	0

[a] (A) MeONa/MeOH/reflux; (B) *t*-BuOK/*t*-BuOH/18-crown-6/reflux; (C) NaH/DMF/50 °C.

Thus, 4 was esterified to methyl 14-methyl-20,14-*retro*-retinoate (4′) by CH_2N_2 treatment. Under basic conditions, 4′ was isomerized into all-trans- and 13-cis-conjugated pentaenoic acid structures (Table II). When 4′ was refluxed with MeONa (10 equiv) in dry MeOH for 7 h, the major isomer was methyl 13-*cis*-14-methylretinoate (9′) obtained in 57% yield, accompanied by the all-trans isomer 8′ (5%). On the other hand, with the use of *t*-BuOK (3 equiv) and 18-crown-6 (2 equiv) in dry *t*-BuOH at reflux, 4′ was isomerized and hydrolyzed[16] to give 8

(16) (a) Chang, F. C.; Wood, N. F. Reactions of Potassium tert.-Butoxide in Dimethylsulfoxide. III. Hydrolysis of Hindered Carboxylic Esters. *Tetrahedron Lett.* **1964**, *40*, 2969–2973. (b) Mandai, T.; Yanagi, T.; Araki, K.; Morisaki, Y.; Kawada, M.; Otera, J. Novel Synthesis of Acetylenes and Polyenes via Desulfonylation Reaction. *J. Am. Chem. Soc.* **1984**, *106*, 3670–3672.

(20%) and 9 (39%). Considering these results and the structure of the intermediate anion formed from 4', generation of the 13-cis compound seems to be kinetically preferred in the base-catalyzed isomerization of 4'. The yield of the 13-trans isomer 8 was probably increased by thermal isomerization of 9. Structures of 8 and 9 were determined by ^{1}H NMR analysis (Table I). Differences in chemical shifts of the H_{12} and H_{20} protons between 8 and 9 correlate very well to those of 1 and 2. The NOE enhancement (8%) between H_{12} and the methyl protons (2.06 ppm) at the C-14 position in 8 supports its 13-trans structure.

Methyl 14-ethyl-20,14-*retro*-retinoate (6') and methyl 14-isopropyl-20,14-*retro*-retinoate (7') were similarly isomerized under basic conditions (Table II). With *t*-BuOK in anhydrous *t*-BuOH, the major product was the retro acid (6), and the isomerization efficiency was low (14-ethylretinoic acid 10, 21%; 13-*cis*-14-ethylretinoic acid 11, 27%). Since the retro compound (6) could no longer be isomerized to 10 or 11 under these conditions, the hydrolysis seems to proceed more rapidly than the isomerization of 6' by *t*-BuOK. Isomerization of 6' by MeONa gave a mixture of isomers (all-trans:13-cis = 1:4), which was hydrolyzed by a 20% aqueous solution of KOH at reflux to give the all-trans isomer (10, 13%) and its 13-cis isomer (11, 45%). Their structures, with regard to cis or trans stereochemistry at the 13-position, were determined by the observations of NOE enhancements from ethyl methylene protons to H_{12} (8%) in 10 and H_{20} (2%) in 11.

The reactivity of the bulkier 14-isopropyl compound (7') was lower than those of the above compounds (5', 6') when *t*-BuOK or MeONa was used. A DMF solution of 7' heated with NaH (5 equiv) as a base at 50 °C for 90 min to give methyl 14-isopropylretinoate (all-trans, 12', 20%), its 13-cis isomer (13', 30%), and 7' (7% recovery). When the mixture of the three esters was treated with a 20% aqueous solution of KOH at reflux, only the retro compound 7' was hydrolyzed, and the methyl retinoates were resistant to hydrolysis. When 18-crown-6 (3 equiv) was added to the reaction mixture, 12' and 13' could be hydrolyzed to give 12 (36%) and 13 (46%). Their structures, with regard to cis or trans stereochemistry at the 13-position, were determined by the observations of NOE enhancements from the isopropyl methine proton to H_{12} (18%) in 12 and H_{20} (5%) in 13. While chemical shift differences of H_{12} and of H_{20} between 10 and 11, in the ethyl derivatives, correlate to those between all-*trans*- (1) and 13-*cis*-retinoic acid (2), in the isopropyl derivatives, those between 12 and 13 do not correlate (Table I). This fact indicates that these compounds may have different stereochemical conformations of the single bond (C_{12}–C_{13}), probably due to the steric effects of the bulky isopropyl group.

Biological Results

Table III shows the differentiation-inducing activities of retinoic acid, its isomers, and their alkylated derivatives on human promyelocytic leukemia cell line HL-60. Among these isomers, retinoic acid (1) has the highest activity in this assay. 13-*cis*-Retinoic acid (2) is one-fourth as active as 1. This was reproducible. The role of 2 in cellular modulation has been ambiguous, since it is not clear whether it is active as it is, or whether it is active after isomerization to 1. 20,14-*retro*-Retinoic acid (3) is $^1/_{50}$ as active as retinoic acid (1). The low, but significant activity of compound 3 is noteworthy, from the standpoint of structure–activity relationships of retinoids. This is the first example of a compound bearing CH_2CO_2H that shows retinoidal activity: the unsaturated carboxylic acid moiety is not always necessary,[17] though a possibility of a partial isomerization of 3 to 1 cannot unambiguously be eliminated. Thus, the carboxylic acid group can be an olefinic (α,β-unsaturated), an aliphatic (β,γ-unsaturated) or an aromatic one.[12-14] The effect of the conformational difference between 1 and the 12-s-trans structure of 3 (Chart II) is also interesting.

Table III. Differentiation-Inducing Activity of Retinoic Acid Derivatives

compound	ED_{50}, M	rel act.[a]
retinoic acid (RA, 1)	2.4×10^{-9} [b]	100
13-*cis*-RA (2)	5.9×10^{-9}	27
20,14-*retro*-RA (3)	5.2×10^{-8}	1.9
14-methyl-20,14-*retro*-RA (4)	5.5×10^{-8}	1.8
14,14-dimethyl-20,14-*retro*-RA (5)	$>10^{-6}$ [c]	$<10^{-2}$
14-methyl-RA (8)	1.3×10^{-8}	20
13-*cis*-14-methyl-RA (9)	6.3×10^{-8}	4.2
14-ethyl-RA (10)	6.8×10^{-7}	0.23
13-*cis*-14-ethyl-RA (11)	inactive[c]	
14-isopropyl-RA (12)	inactive	
13-*cis*-14-isopropyl-RA (13)	inactive	

[a] Relative activity was defined as the ratio of ED_{50} (retinoic acid) to ED_{50} (test compound), both values having been obtained in concurrent experiments, multiplied by 100. Therefore, this is not the simple ratio of the ED_{50} values shown in the table. [b] The deviation (σ_{n-1}) of retinoic acid is estimated to be 1.8×10^{-9} M (n = 90). [c] "Inactive" means there was no activity at 10^{-6} M. "$>10^{-6}$ M" means there was slight activity at 10^{-6} M.

Compound 4, a monomethylated analogue of 3 at the C-14 position, is as active as 3. However, the dimethyl derivative 5 is only slightly active at 10^{-6} M. In contrast to the similar potential of differentiation-inducing activity in 3 and 4, the introduction of an alkyl group at the C-14 position of *all-trans*- and 13-*cis*-retinoic acid caused a remarkable decrease of the activity: compounds 8 and 9, with a methyl group, are one-fifth as active as 1 and 2, respectively. Further, compound 10 with an ethyl group is less active than 1 by 3 orders of magnitude. The corresponding 13-cis isomer 11 and compounds with the bulkier isopropyl group (12 and 13) did not exhibit the activity at concentrations below 10^{-6} M. Thus, the activity was dramatically decreased by the introduction of an alkyl group near the carboxyl group of retinoic acid. The steric bulkiness of the alkyl group would change the binding affinities of the acid group to specific receptors[18] (not CRABP[1,5]) or give partial agonist(s) and antagonist(s).[19] Considering the importance of the orientation and distance between methyl group(s) on the cyclohexenyl ring and the carboxyl group for the retinoidal activities,[2,13] the conformation of the whole molecule might also be significantly changed by the introduction of an alkyl group.

Summary

The isomerization of retinoic acid by a strong base was observed. The isomer, 20,14-*retro*-retinoic acid, is biologically active, though its activity is less than that of retinoic acid. This shows that the carboxylic acid moiety of retinoids can be located on a saturated carbon (with an

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olefin) as well as an olefinic or aromatic carbon. The steric and biological effects of alkylation of the 14 carbon atom next to the important carboxylic acid were also discussed.

Experimental Section

General. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within ±0.3% of the theoretical values. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-DX300 spectrometer and were within ±3 mmu of the theoretical values. NMR spectra were recorded on JEOL JMN-GX400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane (Chart I).

All chemical reactions and handling were done under shielding from light.

Synthesis of Retinoic Acid (1) and 13-*cis*-Retinoic Acid (2).[20] A 10% aqueous solution (400 g) of KOH was added to a solution of retinyl palmitate (20.7 g, 40 mmol; a gift from Riken Bitamin K.K.) in 200 mL of *i*-PrOH and 200 mL of EtOH. The mixture was stirred for 75 min at 90 °C. After cooling, the mixture was poured into 1 kg of ice water and extracted twice with 700 mL of *n*-hexane. The organic layer was washed with water and brine and then dried over $MgSO_4$. The solvent was removed under vacuum to give retinol (10.2 g, 90%) as an orange oil.

Active MnO_2 (104 g, available from Aldrich Chemical Co.), NaCN (25.9 g, 530 mmol), and AcOH (12 mL, 210 mmol) were added to a solution of retinol (10.2 g, 36 mmol) in 1.2 L of EtOH. The mixture was stirred for 62 h at 20 °C. The emulsion was separated by centrifugation (3000*g*/10 min) and decanted to obtain the supernatant. The precipitates were washed with 600 mL of EtOH (once) and 600 mL of CH_2Cl_2 (twice). These extracted solutions were concentrated to 800 mL and washed with 1 L of water. The aqueous solution was reextracted with CH_2Cl_2 three times. The organic layer was washed with brine and dried over $MgSO_4$. The crude mixture was chromatographed on silica gel (CH_2Cl_2–*n*-hexane, 2:3, CH_2Cl_2 only) to give ethyl retinoate (orange oil, 6.7 g, 58%) with recovered retinol (1.8 g, 18%).

A 5% aqueous solution (100 g) of KOH was added to a solution of ethyl retinoate (6.0 g, 18 mmol) in 100 mL of EtOH. The mixture was heated at reflux for 30 min. After cooling, it was poured into ice-cooled dilute HCl and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The crude mixture was recrystallized to give retinoic acid (1; 4.2 g, 77%). 1: yellow needles (from CH_2Cl_2); mp 172–173 °C. Anal. ($C_{20}H_{28}O_2$) C, H.

13-*cis*-Retinoic acid was obtained as a byproduct (>6%) in the synthesis of 1, and it was purified by silica gel column chromatography (AcOEt–*n*-hexane, 1:3 to 3:1) and recrystallized. 2: orange needles (from MeOH); mp 162.5–164 °C. Anal. ($C_{20}H_{28}O_2$) C, H.

Isomerization of 1: Synthesis of 20,14-*retro*-Retinoic Acid (3). *n*-BuLi (2.1 mL, 1.6 M solution in *n*-hexane, 3.3 mmol) was added to a solution of 0.48 mL of diisopropylamine (3.4 mmol) in 8 mL of THF at 0 °C under Ar, and the mixture was stirred for 15 min. Then the mixture was cooled at −60 °C and a solution of 50 mg of retinoic acid (1; 0.17 mmol) in 2 mL of THF was added over 5 min. After being stirred for another 5 min, the mixture was poured into 50 mL of ice water over 5 min, and the whole was stirred for 5 min. To this solution was added 50 mL of ice-cooled 1 N HCl(aq), and the mixture was extracted with AcOEt. The organic layer was washed with water and brine and dried over Na_2SO_4 and $MgSO_4$. The crude mixture was purified by silica gel column chromatography (AcOEt–*n*-hexane, 1:3 to 2:1) to give 3 (34 mg, 68%) and recovered 1 (7.5 mg, 15%). 3: pale yellow prisms (from MeCN); mp 60–62 °C; HRMS ($C_{20}H_{28}O_2$) 300.2104 (+1.5 mmu). Anal. ($C_{20}H_{28}O_2 \cdot {}^1/_2H_2O$) C, H.

Isomerization and Methylation of 1: Synthesis of 14-Methyl-20,14-*retro*-retinoic Acid (4) and 14,14-Dimethyl-20,14-*retro*-retinoic Acid (5). *n*-BuLi (2.1 mL, 1.6 M solution in *n*-hexane, 3.3 mmol) was added to a solution of 0.48 mL of diisopropylamine (3.4 mmol) and 0.58 mL of HMPA (3.3 mmol) in 8 mL of THF at 0 °C under Ar, and the mixture was stirred for 15 min. The solution was cooled at −78 °C, and a solution of 1 (50 mg, 0.17 mmol) in 2 mL of THF was added over 5 min. The mixture was stirred for 5 min, and then 118 mg of MeI (0.83 mmol) in 0.5 mL of THF was added dropwise over 1.5 min. After being stirred for 1 h, the mixture was poured into 50 mL of ice water over 5 min and stirred for another 5 min. Then 50 mL of ice-cooled 1 N HCl(aq) was added, and the whole was extracted with AcOEt. The organic layer was washed successively with water and brine and dried over Na_2SO_4 and $MgSO_4$. The crude mixture was chromatographed on silica gel (AcOEt–*n*-hexane, 1:5 to 2:1) to give the desired 4 (35 mg, 66%) and 5 (1 mg, 2%), as well as nonalkylated 3 (6 mg, 12%) and recovered 1 (4.5 mg, 9%). 4: pale yellow oil; HRMS ($C_{21}H_{30}O_2$) 314.2247 (+0.1 mmu). 5: pale yellow oil; HRMS ($C_{22}H_{32}O_2$) 328.2408 (+0.6 mmu).

Esterification of 4: Synthesis of Methyl 14-Methyl-20,14-*retro*-retinoate (4′). A solution of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide in ether was added dropwise to a solution of KOH in ethanol–water at 65 °C to give a solution of CH_2N_2 in ether. 4 (1.07 g, 3.3 mmol) was esterified with CH_2N_2 (ca. 5 mmol) in 110 mL of ether at 0 °C for 20 min to give 4′ (861 mg, 77%).

Isomerization of 4′. Method A: MeONa/MeOH. A solution of MeONa, prepared from Na (269 mg, 12 mmol) in 9 mL of dry MeOH, was added to a solution of 4′ (384 mg, 1.2 mmol) in 30 mL of dry MeOH, and then the mixture was heated at reflux for 4.5 h. The mixture was poured into an ice-cooled 3% aqueous solution (350 mL) of citric acid and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$, providing a crude mixture (377 mg, 98%). The mixture consisted of 8′ (8%), 9′ (88%), recovered 4′ (2%), and hydrolyzed 4 (1%). These products were not isolated, and each yield was determined by NMR spectrum of this mixture.

Method B: *t*-BuOK/*t*-BuOH. *t*-BuOK (373 mg, 3.3 mmol) and 18-crown-6 (586 mg, 2.2 mmol) were added to a solution of 4′ (364 mg, 1.1 mmol) in 40 mL of dry *t*-BuOH, and then the mixture was heated at reflux for 3.5 h. The mixture was poured into an ice-cooled 3% aqueous solution (350 mL) of citric acid and extracted with CH_2Cl_2. The organic layer was washed successively with water (twice) and brine (once) and dried over $MgSO_4$. The crude mixture was purified by silica gel column chromatography (AcOEt–CH_2Cl_2, 1:6 to 1:5) followed by preparative TLC (AcOEt–CH_2Cl_2, 1:5) to give 8 (51 mg, 20%) and 9 (99 mg, 39%) with recovered 4 (33 mg, 13%). 8: yellow plates (from MeOH); mp 130–131 °C; HRMS ($C_{21}H_{30}O_2$) 314.2241 (−0.5 mmu). Anal. ($C_{21}H_{30}O_2 \cdot {}^1/_3H_2O$) C, H. 9: pale orange plates (from MeOH); mp 119–120 °C; HRMS ($C_{21}H_{30}O_2$) 314.2276 (+3.0 mmu). Anal. ($C_{21}H_{30}O_2 \cdot {}^1/_3H_2O$) C, H.

Synthesis of 14-Ethylretinoic Acid (10) and 13-*cis*-14-Ethylretinoic Acid (11). *n*-BuLi (26.4 mL, 2.5 M solution in *n*-hexane, 67 mmol) was added to a solution of 9.5 mL of diisopropylamine (68 mmol) and 11.6 mL of HMPA (67 mmol) in 300 mL of THF at 0 °C under Ar, and the mixture was stirred for 15 min. The solution was cooled at −60 °C, and a solution of 1 (2.0 g, 6.7 mmol) in 20 mL of THF was added over 5 min. After stirring for 5 min to form the intermediate dianion, the mixture was cooled at −70 °C. Then 8.3 g of EtI (53 mmol) was added dropwise over 2 min, and the mixture was stirred for 45 min. It was poured into 500 mL of ice water over 5 min, and the whole was stirred for another 5 min. Then 200 mL of ice-cooled 1 N HCl(aq) was added to this, and the mixture was extracted with AcOEt. The organic layer was washed successively with water (twice) and brine (once) and dried over Na_2SO_4 and $MgSO_4$. The crude mixture was chromatographed on silica gel (AcOEt–*n*-hexane, 1:5 to 2:1) to give 6 (1.8 g, 82%).

Compound 6 (1.07 g, 3.3 mmol) was esterified with CH_2N_2 (ca. 5 mmol) in 110 mL of ether at 0 °C for 20 min to give 6′ (859 mg, 77%). A solution of MeONa prepared from Na (1.17 g, 51 mmol) in 38 mL of dry MeOH was added to a solution of 6′ (581 mg, 1.7 mmol) in 20 mL of dry MeOH, and the mixture was heated at reflux for 7 h. The mixture was poured into an ice-cooled 3%

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aqueous solution (300 mL) of citric acid and extracted with AcOEt. The organic layer was washed successively with water (twice) and brine (once) and dried over Na_2SO_4 and $MgSO_4$. After evaporation, the crude residue (585 mg, 100%) was given (6:6′:10′:11′, 5:3:18:74).

A 700-mg portion of the mixture was hydrolyzed with 10% KOH(aq) in EtOH at reflux to give **10** (93 mg, 13%) and **11** (323 mg, 45%) with recovered **6** (74 mg, 10%). First **11** was recrystallized from AcOEt, and then the residue was separated by silica gel column chromatography (AcOEt–*n*-hexane, 1:4 to 1:2) and recrystallized. **10**: yellow needles (from CH_2Cl_2–*n*-hexane); mp 132–134 °C. Anal. ($C_{22}H_{32}O_2$) C, H. **11**: yellow plates (from AcOEt); mp 139.5–141 °C. Anal. ($C_{22}H_{32}O_2$) C, H.

Synthesis of 14-Isopropylretinoic Acid (12) and 13-*cis*-14-Isopropylretinoic Acid (13). *n*-BuLi (41.7 mL, 1.6 M solution in *n*-hexane, 67 mmol) was added to a solution of 9.5 mL of diisopropylamine (68 mmol) and 11.6 mL of HMPA (67 mmol) in 85 mL of THF at 0 °C under Ar, and the mixture was stirred for 15 min. The solution was cooled at −60 °C, and a solution of **1** (2.0 g, 6.7 mmol) in 15 mL of THF was added over 5 min. The mixture was stirred for 5 min to form the intermediate dianion, and then 9.1 g of *i*-PrI (53 mmol) was added dropwise over 2 min. The mixture was stirred for 30 min, warmed at −50 °C, and stirred again for 30 min. Another 7.9 g of *i*-PrI (46 mmol) was added, and the reaction mixture was stirred for another 30 min, poured into 500 mL of ice water over 5 min, and stirred for another 5 min. Then 200 mL of ice-cooled 1 N HCl(aq) was added, and the whole was extracted with AcOEt. The organic layer was washed successively with water (twice) and brine (once) and dried over Na_2SO_4 and $MgSO_4$. The crude mixture was chromatographed on silica gel (AcOEt–*n*-hexane, 1:5 to 1:2) to give the desired **7** (825 mg, 36%), nonalkylated **3** (540 mg, 27%), and recovered **1** (100 mg, 5%).

Compound **7** (762 mg, 2.2 mmol) was esterified with CH_2N_2 (ca. 5 mmol) in 56 mL of ether at 0 °C for 30 min to give **7′** (703 mg, 89%). NaH (287 mg, 60%, 7.2 mmol) was washed twice with 1 mL of *n*-hexane and suspended in 20 mL of dry DMF. A solution of **7′** (512 mg, 1.4 mmol) in 30 mL of dry DMF was added to the suspension under Ar, and the mixture was heated at 50 °C for 90 min. The mixture was poured into an ice-cooled 3% aqueous solution (150 mL) of citric acid and extracted with AcOEt. The organic layer was washed successively with water and brine and dried over Na_2SO_4 and $MgSO_4$. After evaporation, the crude residue (330 mg, 65%) was given (7:7′:12′:13′, 13:10:30:46).

A 920-mg portion of the mixture was hydrolyzed with 20% KOH(aq) in EtOH without 18-crown-6 at reflux to give acid **7** (280 mg, 32%) with recovered **12′** and **13′** (490 mg, 53%). The esters of **12′** and **13′** (490 mg, 1.4 mmol) were hydrolyzed with 20% KOH(aq) in EtOH in the presence of 2 molar equiv of 18-crown-6 at reflux to give **12** (45 mg, 9%) and **13** (87 mg, 18%) with recovered esters (164 mg, 33%). They were separated by silica gel column chromatography (AcOEt–*n*-hexane, 1:20 to 1:1), and **13** was recrystallized. **12**: colorless powder (crystallized from AcOEt–*n*-hexane); mp 95–97 °C; HRMS ($C_{23}H_{34}O_2$) 342.2543 (−1.6 mmu). **13**: pale yellow prisms (from *n*-hexane); mp 149–150.5 °C; HRMS ($C_{23}H_{34}O_2$) 342.2557 (−0.2 mmu). Anal. ($C_{23}H_{34}O_2 \cdot {}^1/_5H_2O$) C, H.

Cells and Culture. The human promyelocytic leukemia cell line HL-60 was provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) and was maintained in continuous suspension culture. The cells were cultured in plastic flasks in RPMI1640 medium, supplemented with 5% fetal calf serum (FCS) and antibiotics (penicillin G and streptomycin), at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Test compounds were dissolved in ethanol at 0.2 mM and added to the cells, which were seeded at about 8×10^4 cells/mL; the final ethanol concentration was kept below 0.5%. Control cells were given only the same volume of ethanol. Retinoic acid, as a positive control, was always assayed at the same time. The cells were incubated for 4 days and stained with Wright–Giemsa. Differential counts were then performed under a light microscope on a minimum of 200 cells. Nitro-blue tetrazolium (NBT) reduction was assayed as described.[11] Cells were incubated for 20 min at 37 °C in RPMI1640 medium (5% FCS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 200 ng/mL). The percentage of cells containing blue-black formazan was determined on a minimum of 200 cells. The results of these two evaluations were always in good agreement.

Relative activities were calculated as the ratio of the ED_{50} of retinoic acid to the ED_{50} of the test compound obtained in concurrent experiments, multiplied by 100.

Registry No. **1**, 302-79-4; **1** (deuterium labeled), 138093-49-9; **2**, 4759-48-2; **3**, 138093-35-3; **3** (deuterium labeled), 138093-50-2; **4**, 138093-36-4; **4′**, 138093-43-3; **5**, 138093-37-5; **6**, 138093-38-6; **6′**, 138093-44-4; **7**, 138093-39-7; **7′**, 138093-45-5; **8**, 138093-40-0; **8′**, 138093-46-6; **9**, 138231-97-7; **9′**, 138232-00-5; **10**, 138093-41-1; **10′**, 138093-47-7; **11**, 138231-98-8; **11′**, 138232-01-6; **12**, 138093-42-2; **12′**, 138093-48-8; **13**, 138231-99-9; **13′**, 138232-02-7; retinyl palmitate, 79-81-2; *all-trans*-retinol, 68-26-8; ethyl *all-trans*-retinoate, 3899-20-5.