Biological Activity of (all-*E*)- β -Apo-12'-carotenoic Acid and the Geometrical Isomers on Human Acute Promyelocytic Leukemia Cell Line HL-60

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(Received July 17, 1995)

Summary (all-*E*)- β -Apo-12'-carotenoic acid and a series of the geometrical isomers were synthesized and their biological activities to inhibit growth and to induce differentiation of human acute promyelocytic leukemia cell line HL-60 were analyzed. It was found that (all-*E*)- β -apo-12'-carotenoic acid (I, Fig. 1) and (15'-*Z*)-apo-12'-carotenoic acid (II, Fig. 1) showed strong activity both in the inhibition of growth and the induction of differentiation. The biological activity of (13'-*Z*)- β -apo-12'-carotenoic acid (III, Fig. 1) was modest, while that of (13',15'-di-*Z*)- β -apo-12'-carotenoic acid (IV, Fig. 1) was negligible. (all-*E*)- β -Apo-12'-carotenoic acid and the geometrical isomers acted additively with (all-*E*)-retinoic acid, and synergetically with (9-*Z*)-retinoic acid. In accord with our previous report, biological activities of (all-*E*)- β -apo-14'-carotenoic acid (V, Fig. 1) and of (15'-*Z*)- β -apo-14'-carotenoic acid (VI, Fig. 1) were very weak. It was indicated that (all-*E*)- β -apo-12'-carotenoic acid, an intermediate metabolite of excentric cleavage pathway of β -carotene, *per se* may exert the biological function.

Key Words retinoic acid, retinoid, β -apo-12'-carotenoic acid, β -apo-14'-carotenoic acid, HL-60, excentric cleavage mechanism of β -carotene, vitamin A

Retinoic acid, one of the active metabolites of vitamin A, influences growth and differentiation of a variety of normal and malignant cells *via* the receptors (1, 2). Retinoids (analogs of retinoic acid) may have a potential as therapeutic anticancer agents, and are useful in the treatment of different skin disorders (1–3). Further, synthetic retinoids may be utilized for elucidating the mechanism of the biological activity of retinoic acid.

Human acute promyelocytic leukemia (HL-60) cells respond to retinoic acid with a marked decrease in the rate of cellular proliferation and phenotypic maturation into cells that resemble granulocytes (4, 5). (all-*E*)-Retinoic acid has been utilized clinically in the treatment of patients with acute promyelocytic

Fig. 1. Synthetic scheme of (all-*E*)-β-apo-12'-carotenoic acid and the geometrical isomers.

leukemia (6,7). Bioassay utilizing HL-60 has been widely carried out to estimate the biological activity of retinoids, since HL-60 respond rapidly and sensitively to them.

Analog of retinoic acid with elongated side chain may have interesting biological activities (8,9). However, synthesis of retinoic acid analogs with elongated chain length have not been carried out intensively. In this paper, we describe syntheses of (all-*E*)- β -apo-12'-carotenoic acid and its geometric isomers, and their biological effects on HL-60.

EXPERIMENTAL

Experiments were carried out under FLR40S Y-F/M colored fluorescent lamps (Matsushita Electric Industrial Co., Osaka) to avoid photoisomerization of retinoids (5). Syntheses of analogs of retinoic acid were carried out under argon. ¹H-NMR spectra were measured with a JEOL EX-400 spectrometer. High-resolution mass spectra were recorded with a JEOL DX-303 high-resolution mass spectrometer. Standard retinoic acid numbering was used for the NMR data of retinoids (see, Fig. 1, [I]). The configurations of the side chain-terminal double bond (C-14', C-13'-double bond) of (all-*E*)- β -apo-12'-carotenoic acid and the geometric isomers were deduced from the chemical shift of C-14'H: C-14'H of *Z*-isomer is expected to be observed at a lower field than that of the corresponding *E*-isomer due to the anisotropic effect of the carboxyl group (9,10).

Materials. (all-*E*)-5,9-Dimethyl-11-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10-undecapentaenoic acid [(all-*E*)- β -apo-14'-carotenoic acid] (V, Fig. 1) and (2*Z*,4*E*,6*E*,8*E*,10*E*)-5,9-dimethyl-11-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10-undecapentaenoic acid [(15-*Z*)- β -apo-14'-carotenoic acid] (VI, Fig. 1) were prepared as reported (9). 1-Ethoxycarbonyl-ethylidene-triphenylphosphorane (VII, Fig. 1) was prepared according to Isler et al. (11). (9-*Z*)-Retinoic acid was prepared as reported (5). (all-*E*)-Retinoic acid was purchased from Wako Pure Chemical Industries (Osaka).

Bioassay. Bioassay utilizing HL-60 was carried out as described previously (9). Additivity and synergy in this report was evaluated after the following formula (12):

$$F = A_c/A_s + B_c/B_s \begin{cases} = 1 & \text{for additivity} \\ < 1 & \text{for synergy,} \end{cases}$$

where the doses of A and B that produce some specific effects are A_s and B_s , and their doses in a combination that also have the same are A_c and B_c .

Synthesis. (all-*E*)- β -Apo-12'-carotenoic acid and the geometrical isomers were synthesized following the scheme shown in Fig. 1.

(all-*E*)-2,7,11-Trimethyl-13-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10,12-tridecahexaenoic acid [(all-*E*)- β -apo-12'-carotenoic acid] (I, Fig. 1) and (2*Z*,4*E*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,

10,12-tridecahexaenoic acid [(13'-Z)- β -apo-12'-carotenoic acid] (III, Fig. 1): A solution of dry ether (35 ml) containing 0.49 g of diazomethane was added dropwise at room temperature to a solution of 3.77 g of (all-E)- β -apo-14'-carotenoic acid (V, Fig. 1) dissolved in a mixture of toluene (30 ml) and methanol (10 ml). Methyl (all-E)- β -apo-14'-carotenoate (3.93 g), obtained after evaporation of the solvent, was dissolved in 9 ml of dry toluene, and then the solution was added dropwise to a solution of 0.45 g of LiAlH₄ in 70 ml of dry ether at -70°C, and was stirred continuously for 1 h at -30°C. To destroy excess LiAlH₄, 7 ml of ethyl acetate and 3 ml of saturated aqueous ammonium chloride solution were added successively, and the reaction mixture was maintained at room temperature. Precipitates were filtered off and washed with benzene. (all-E)- β -Apo-14'-carotenol (3.6 g) obtained after concentrating the filtrate *in vacuo* was dissolved in petroleum ether (36 ml), and then manganese dioxide powder (20 g) was added to the solution. The reaction mixture was kept for 15 h at room temperature in the dark. Manganese dioxide was filtered off and washed with petroleum ether. (all-E)- β -Apo-14'-carotenal (3.1 g) obtained after removal of petroleum ether under reduced pressure was dissolved in MeOH (15 ml) containing 1-ethoxycarbonyl-ethylidenetriphenylphosphorane (VII, Fig. 1) (3.6 g). The reaction mixture was kept for 18 h at room temperature in the dark to give a mixture of ethyl (all-E)- β -apo-12'-carotenoate and its (13'-Z)-isomer. Evaporation of MeOH *in vacuo* gave the residue which was chromatographed on silica gel [Wakogel C-200 (300 g)] with hexane-dichloromethane (4:1) as the eluent. Ethyl (13'-Z)- β -apo-12'-carotenoate (0.8 g) was eluted in the earlier fractions separated from the (all-E)-isomer (0.8 g). Obtained ethyl (13'-Z)- β -apo-12'-carotenoate was treated with 20% ethanolic KOH (20 ml) for 5 h at 70°C in the dark. The reaction mixture was acidified with 2 N HCl solution under cooling with ice, and then extracted with dichloromethane (50 ml $\times$ 2). The dichloromethane extract was dried over MgSO₄ and concentrated to give (13'-Z)- β -apo-12'-carotenoic acid (III, Fig. 1) (0.72 g). (all-E)- β -Apo-12'-carotenoic acid (I, Fig. 1) (0.72 g) was obtained by similar hydrolysis of ethyl (all-E)- β -apo-12'-carotenoate.

(all-E)-2,7,11-Trimethyl-13-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10,12-tridecahexaenoic acid [(all-E)- β -apo-12'-carotenoic acid] (I, Fig. 1): mp 158°C. UV $\lambda_{\max}$ (EtOH, CH₃COOH acidic) 407 nm (ϵ 67,000). HRMS calcd for C₂₅H₃₄O₂ 366.2550; found, 366.2561. ¹H-NMR (CDCl₃) δ 1.03 (6H, s, 1-gem Me), 1.45–1.48 (2H, m, 2-H₂), 1.59–1.65 (2H, m, 3-H₂), 1.72 (3H, s, 5-Me), 1.99–2.02 (11H, m, 4-H₂, 9-Me, 13-Me, and 13'-Me), 6.12–6.20 (3H, m, 7-H, 8-H and 10-H), 6.26 (1H, d, J = 13 Hz, 14-H), 6.36 (1H, d, J = 15 Hz, 12-H), 6.53 (1H, dd, J = 12 and 14 Hz, 15'-H), 6.76 (1H, dd, J = 12 and 15 Hz, 11-H), 6.95 (1H, dd, J = 12 and 13 Hz, 15-H), 7.42 (1H, d, J = 14 Hz, 14'-H).

(2Z,4E,6E,8E,10E,12E)-2,7,11-Trimethyl-13-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10,12-tridecahexaenoic acid [(13'-Z)- β -apo-12'-carotenoic acid] (III, Fig. 1): mp 166°C. UV $\lambda_{\max}$ (EtOH, CH₃COOH acidic) 399 nm (ϵ 63,000). HRMS calcd for C₂₅H₃₄O₂ 366.2550; found, 366.2556. ¹H-NMR (CDCl₃) δ 1.03

(6H, s, 1-*gem*Me), 1.45–1.48 (2H, m, 2-H₂), 1.59–1.65 (2H, m, 3-H₂), 1.72 (3H, s, 5-Me), 1.98–2.06 (11H, m, 3-H₂, 9-Me, 13-Me and 13'-Me), 6.12–6.23 (3H, m, 7-H, 8-H and 10-H), 6.29 (1H, d, *J* = 12 Hz, 14-H), 6.35 (1H, d, *J* = 15 Hz, 12'-H), 6.68 (1H, d, *J* = 13 Hz, 14'-H), 6.72–6.82 (2H, m, 11-H and 15-H), 7.41 (1H, dd, *J* = 13 and 15 Hz, 15'-H).

(2*Z*,4*Z*,6*E*,8*E*,10*E*,12*E*)-2,7,11-Trimethyl-13-(2,6,6-trimethyl)-1-cyclohexen-1-yl)-2,4,6,8,10,12-tridecahexaenoic acid [(13',15'-*di-Z*)- β -apo-12'-carotenoic acid] (IV, Fig. 1) and (2*E*,4*Z*,6*E*,8*E*,10*E*,12*E*)-2,7,11-trimethyl-13-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10,12-tridecahexaenoic acid [(15'-*Z*)- β -apo-12'-carotenoic acid] (II, Fig. 1): To synthesize II and IV from VI, a method similar to that described above for the syntheses of I and III was used. From 4.9 g of VI, 1 g of II and 1 g IV were obtained.

(2*Z*,4*Z*,6*E*,8*E*,10*E*,12*E*)-2,7,11-Trimethyl-13-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10,12-tridecahexaenoic acid [(13',15'-*di-Z*)- β -apo-12'-carotenoic acid] (IV, Fig. 1): mp 184°C. UV $\lambda_{\max}$ (EtOH, CH₃COOH acidic) 399 nm (ϵ 83,000). HRMS calcd for C₂₅H₃₄O₂ 366.2550; found, 366.2585. ¹H-NMR (CDCl₃) δ 1.04 (6H, s, 1-*gem*Me), 1.46–1.49 (2H, m, 2-H₂), 1.60–1.63 (2H, m, 3-H₂), 1.73 (3H, s, 5-Me), 1.99–2.02 (11H, m, 4-H₂, 9-Me, 13-Me, and 13'-Me), 6.13–6.32 (4H, m, 7-H, 8-H, 10-H, and 15-H), 6.69–6.81 (3H, m, 11-H, 14-H, and 14'-H), 7.88 (1H, t-like, *J* = 13 Hz, 15'-H).

(2*E*,4*Z*,6*E*,8*E*,10*E*,12*E*)-2,7,11-Trimethyl-13-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8,10,12-tridecahexaenoic acid [(15'-*Z*)- β -apo-12'-carotenoic acid] (II, Fig. 1): mp 170°C. UV $\lambda_{\max}$ (EtOH, CH₃COOH acidic) 404 nm (72,000). HRMS calcd for C₂₅H₃₄O₂ 366.2550; found, 366.2569. ¹H-NMR (CDCl₃) δ 1.03 (6H, s, 1-*gem*Me), 1.46–1.49 (2H, m, 2-H₂), 1.59–1.63 (2H, m, 3-H₂), 1.72 (3H, s, 5-Me), 1.99–2.08 (11H, m, 4-H₂, 9-Me, 13-Me, and 13'-Me), 6.12–6.37 (5H, m, 7-H, 8-H, 10-H, 12-H, and 14-H), 6.53 (1H, dd, *J* = 11 and 13 Hz, 15'-H), 6.76 (1H, dd, *J* = 11 and 15 Hz, 11'-H), 6.94 (1H, t, *J* = 13 Hz, 15-H), 7.41 (1H, d, *J* = 11 Hz, 14'-H).

RESULTS

We synthesized (all-*E*)- β -apo-12'-carotenoic acid and a series of the geometrical isomers (Fig. 1). Biological activities of these compounds on growth and differentiation of HL-60 cells are shown in Table 1. Interestingly, (all-*E*)- β -apo-12'-carotenoic acid (I, Table 1) and (15'-*Z*)- β -apo-12'-carotenoic acid (II, Table 1) showed considerably strong [approximately 60% of (all-*E*)-retinoic acid] activity both in the inhibition of growth and in the induction of differentiation. The biological activity of (13'-*Z*)- β -apo-12'-carotenoic acid (III, Table 1) was relatively modest [20% of (all-*E*)-retinoic acid], while that of (13',15'-*di-Z*)- β -apo-12'-carotenoic acid (IV, Table 1) was very weak. In contrast, as reported previously (9), both β -apo-14'-carotenoic acid (V, Table 1) and (15'-*Z*)- β -apo-14'-carotenoic acid (VI, Table 1) did not possess significant biological effect. The relation between the rate of differentiation of HL-60 and the exposure time to β -apo-12'-carotenoic

Table 1. Biological activities of (all-*E*)- β -apo-12'-carotenoic acid and the geometrical isomers on HL-60.

Compounds	Relative activity	
	Inhibition of growth	Induction of differentiation
(All- <i>E</i>)-retinoic acid	100	100
(All- <i>E</i>)- β -apo-12'-CA [I]	63.2	55.1
(15'- <i>Z</i>)- β -Apo-12'-CA [II]	52.0	50.3
(13'- <i>Z</i>)- β -Apo-12'-CA [III]	10.2	19.1
(13',15'-di- <i>Z</i>)- β -Apo-12'-CA [IV]	0.6	1.9
(All- <i>E</i>)- β -apo-14'-CA [V]	3.4	4.2
(15'- <i>Z</i>)- β -Apo-14'-CA [VI]	0.1	0.2

CA: carotenoic acid.

acids is shown in Fig. 2. Necessary exposure times required for the optimal differentiation of HL-60 were the same for (all-*E*)-retinoic acid, (all-*E*)- β -apo-12'-carotenoic acid and for (15'-*Z*)- β -apo-12'-carotenoic acid. The results indicated that (all-*E*)- β -apo-12'-carotenoic acid, an intermediate metabolite of the excentric cleavage pathway of β -carotene (13-15) (Fig. 3), possesses biological activity. Since (all-*E*)- β -apo-14'-carotenoic acid, a downstream metabolite of (all-*E*)- β -apo-12'-carotenoic acid in the excentric cleavage pathway (13-15) (Fig. 3), was shown to be inactive, it was assumed that (all-*E*)- β -apo-12'-carotenoic acid directly exerted its biological activity without being further metabolized.

Recent identification of (9-*Z*)-retinoic acid as a ligand for retinoid X receptors (RXRs) (16, 17) indicated intervention of the geometrical isomers of retinoic acid in the mechanism of action of vitamin A. We reported previously that (all-*E*)-retinoic acid and (9-*Z*)-retinoic acid acted synergistically on HL-60 cells (5). In the following, we analyzed the combined effects of (all-*E*)- β -apo-12'-carotenoic acid and of the geometrical isomers with (all-*E*)-retinoic acid and with (9-*Z*)-retinoic acid. The results are summarized in Table 2. (all-*E*)-Retinoic acid acted additively with (all-*E*)- β -apo-12'-carotenoic acid, (15'-*Z*)- β -apo-12'-carotenoic

Fig. 2. Analysis of necessary exposure time of HL-60 to retinoic acid or β -apo-12'-carotenoic acids required for inducing the differentiation.

Cells were incubated in growth medium containing (all-*E*)-retinoic acid ($0.5 \mu\text{M}$), (all-*E*)- β -apo-12'-carotenoic acid ($1 \mu\text{M}$) or (15'-*Z*)- β -apo-12'-carotenoic acid ($1 \mu\text{M}$) for the indicated period of time. Cells were removed of (all-*E*)-retinoic acid, (all-*E*)- β -apo-12'-carotenoic acid or (15'-*Z*)- β -apo-12'-carotenoic acid by washing, and were further incubated in fresh growth medium containing neither retinoic acid nor β -apo-12'-carotenoic acid. The rate of differentiation was determined after 6 days of incubation. Δ , (all-*E*)-retinoic acid; $\bullet$, (all-*E*)- β -apo-12'-carotenoic acid; $\circ$, (15'-*Z*)- β -apo-12'-carotenoic acid.

Fig. 3. The central cleavage mechanism and the excentric cleavage mechanism of β -carotene (13-15, 18, 19).

Table 2. Effect of combination of (all-*E*)- β -apo-12'-carotenoic acid and the geometric isomers with (all-*E*)-retinoic acid or with (9-*Z*)-retinoic acid on induction of differentiation of HL-60.

A) Combination of (all- <i>E</i>)- β -apo-12'-CA with (all- <i>E</i>)-RA.			B) Combination of (all- <i>E</i>)- β -apo-12'-CA with (9- <i>Z</i>)-RA.		
ED ₅₀ ($\times 10^{-8}$ M)		F	ED ₅₀ ($\times 10^{-8}$ M)		F
(all- <i>E</i>)- β -apo-12'-CA	(all- <i>E</i>)-RA		(all- <i>E</i>)- β -apo-12'-CA	(9- <i>Z</i>)-RA	
15	—	—	15	—	—
—	9.0	—	—	0.94	—
8.2	3.2	0.90	3.1	0.29	0.52
C) Combination of (15'- <i>Z</i>)- β -apo-12'-CA with (all- <i>E</i>)-RA.			D) Combination of (15'- <i>Z</i>)- β -apo-12'-CA with (9- <i>Z</i>)-RA.		
ED ₅₀ ($\times 10^{-8}$ M)		F	ED ₅₀ ($\times 10^{-8}$ M)		F
(15'- <i>Z</i>)- β -apo-12'-CA	(all- <i>E</i>)-RA		(15'- <i>Z</i>)- β -apo-12'-CA	(9- <i>Z</i>)-RA	
18	—	—	18	—	—
—	9.0	—	—	0.94	—
13.4	3.2	1.1	2.7	0.29	0.46
E) Combination of (13'- <i>Z</i>)- β -apo-12'-CA with (all- <i>E</i>)-RA.			F) Combination of (13'- <i>Z</i>)- β -apo-12'-CA with (9- <i>Z</i>)-RA.		
ED ₅₀ ($\times 10^{-8}$ M)		F	ED ₅₀ ($\times 10^{-8}$ M)		F
(13'- <i>Z</i>)- β -apo-12'-CA	(all- <i>E</i>)-RA		(13'- <i>Z</i>)- β -apo-12'-CA	(9- <i>Z</i>)-RA	
45	—	—	45	—	—
—	9.0	—	—	0.94	—
25.9	3.2	0.93	9.1	0.29	0.51
G) Combination of (13',15'-di- <i>Z</i>)- β -apo-12'-CA with (all- <i>E</i>)-RA.			H) Combination of (13',15'-di- <i>Z</i>)- β -apo-12'-CA with (9- <i>Z</i>)-RA.		
ED ₅₀ ($\times 10^{-8}$ M)		F	ED ₅₀ ($\times 10^{-8}$ M)		F
(13',15'-di- <i>Z</i>)- β -apo-12'-CA	(all- <i>E</i>)-RA		(13',15'-di- <i>Z</i>)- β -apo-12'-CA	(9- <i>Z</i>)-RA	
450	—	—	450	—	—
—	9.0	—	—	0.94	—
270	3.2	0.96	55	0.29	0.44

CA: carotenoic acid, RA: retinoic acid.

acid, (13'-Z)- β -apo-12'-carotenoic acid and with (13',15'-di-Z)- β -apo-12'-carotenoic acid. In contrast, (9-Z)-retinoic acid acted synergistically with (all-E)- β -apo-12'-carotenoic acid and the geometrical isomers. These results indicated that (all-E)- β -apo-12'-carotenoic acid, (15'-Z)- β -apo-12'-carotenoic acid, (13'-Z)- β -apo-12'-carotenoic acid and (13',15'-di-Z)- β -apo-12'-carotenoic acid acted not *via* RXRs, but *via* receptors for (all-E)-retinoic acid [retinoic acid receptors (RARs)].

DISCUSSION

β -Carotene can undergo oxidative cleavage to form retinoids by two pathways: (1) central cleavage pathway and (2) excentric cleavage pathway (Fig. 3). The central cleavage pathway involves oxidative cleavage of the central 15,15'-double

Compounds	HL60 Differentiation
(All-E)-RA	1 0 0
(All-E)- β -apo-12'-CA	5 5
(11-Z)-RA	9 5
(15'-Z)- β -apo-12'-CA	5 0
(13-Z)-RA	8 0
(13'-Z)- β -apo-12'-CA	2 0
(9,13-di-Z)-RA	1 0
(13',15'-di-Z)- β -apo-12'-CA	2

RA : Retinoic acid, CA : Carotenoic acid.

Fig. 4. Comparison of biological activities of geometrical isomers of retinoic acid and of β -apo-12'-carotenoic acid.

bond to form two molecules of retinal (18,19). The excentric cleavage pathway involves oxidative cleavage at any double bond in the polyene chain to form series of β -apocarotenoids (13–15). Wang et al. reported that the rate of formation of β -apocarotenoids was greater than that of retinoids in intestinal homogenates (13). However, the biological significance of the excentric cleavage pathway is unclear. The results presented in this paper suggested that an intermediate metabolite of the excentric cleavage pathway of β -carotene is biologically active. This suggests that the excentric cleavage pathway may not be disadvantageous, since β -apo-carotenoids could further be metabolized to give retinoic acid (15).

We reported previously the biological activities of geometrical isomers of retinoic acid on HL-60 (5). Activities of (13-*Z*)-retinoic acid and (9,13-di-*Z*)-retinoic acid corresponded respectively to approximately 4/5 and 1/10 of the level of (all-*E*)-retinoic acid (5). Activity of (11-*E*)-retinoic acid corresponded roughly to that of (all-*E*)-retinoic acid (20). The biological activities of geometrical isomers of retinoic acid and of β -apo-12'-carotenoic acid are summarized in Fig. 4. It is assumed that a strong correlation exists between the biological activity and the geometric structure of four conjugated double bonds neighboring the carboxyl group.

We reported previously that (all-*E*)-retinoic acid acted additively, while (9-*Z*)-retinoic acid acted synergistically with (11-*Z*)-retinoic acid, (13-*Z*)-retinoic acid and (9,13-di-*Z*)-retinoic acid (5,20). As described in this paper, similar biological effects were obtained by the combination of β -apo-12'-carotenoids with (all-*E*)-retinoic acid and with (9-*Z*)-retinoic acid (Table 2). These results also supported the assumption that the geometric structure of four conjugated side-chain double bonds neighboring the carboxyl group plays an important role in determining the biological activity.

We thank Dr. H. Oyamada of Tokyo Research Institute, Seikagaku Kogyo Co. Ltd. for the measurements of NMR spectra and for the assignments of the signals. We also thank Dr. T. Kitahara of the University of Tokyo for conducting high-resolution mass spectral analysis.

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