Acetic Acid Writhing Test. This test was performed as described by Witkin et al.[15]

Hot Plate Test. This test was performed as described by Woolfe et al.[16]

Opioid-Type Withdrawal Jumping Precipitation Test.[17] Mice were made physically dependent on morphine as described.[1] Three days after implantation of the morphine pellet, eight mice (21-28 g body weight) per dose were administered the test compound and observed for withdrawal jumping. The number of jumps was counted individually for each mouse during a period of 60 min. The ED_{50} value for precipitation of withdrawal jumping was calculated by using the method of cubic splines and represents the dose at which the number of jumps was 50% of the control group value obtained with 0.1 mg/kg naloxone.[1]

Respiratory Activity Test. This test was performed as described earlier.[1]

Acetic Acid Writhing Antagonism Test. The test procedure corresponds to the acetic acid writhing test,[15] except that 20 min after the opioid agonist (morphine or U-50,488) was administered, the test compound was given.

Acknowledgment. We thank the Analytical Department of Hoffmann-La Roche & Co. Inc., Basle, for elemental analyses and Dr. K.-H. Ongania (Institute of Organic and Pharmaceutical Chemistry) for performing the mass spectra.

Registry No. 1, 92055-59-9; 2, 118111-54-9; 2·HBr, 118111-51-6; 3, 118111-52-7; 4, 118111-53-8; cyclopropylmethyl chloride, 5911-08-0.

(15) Witkin, L. B.; Heubner, C. F.; Galdi, F.; O'Keefe, E.; Spitaletta, P.; Plummer, A. J. *J. Pharmacol. Exp. Ther.* **1961**, *133*, 400.
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Studies on Hindered Phenols and Analogues. 1. Hypolipidemic and Hypoglycemic Agents with Ability To Inhibit Lipid Peroxidation

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A series of hindered phenols were investigated as hypolipidemic and/or hypoglycemic agents with ability to inhibit lipid peroxidation. 1,3-Benzoxathioles (**9** and **22**), phenoxypentanoic acid (**34**), phenoxypentanol (**35a**), phenoxynonanol (**35b**), phenylchloropropionic acid having a chromanyl group (**25**), and a thiazolidine compound (**27**) derived from **25**, all having a hindered phenol group, were prepared and examined. Compound **27** showed the expected biological properties in vivo and in vitro without any liver weight increase. Biological activities of the analogous thiazolidine compounds, **43-58**, were compared. Thus, (±)-5-[4-[(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methoxy]-benzyl]-2,4-thiazolidinedione (**27**) (CS-045) was found to have all of our expected properties and was selected as a candidate for further development as a hypoglycemic and hypolipidemic agent.

There have been many reports that described the relationship of lipid peroxides (LPO) to angiopathy.[1-4] On macroangiopathy, Glavind et al. showed a relationship of LPO with arteriosclerosis in 1952.[1] Several years later, Fukuzumi et al. confirmed the presence of LPO in the atherosclerotic aorta.[2] According to Yagi, the accumulation of the complex of LPO with protein was one of the pathogenic causes of arteriosclerosis.[3] On microangiopathy, which is associated with diabetic complications, Yagi et al. have reported that the average level of LPO in plasma is higher in diabetics than in normals.[4]

Concerning such angiopathy, some reports describe experimental trials for lowering the serum LPO level by vitamin E,[5,6] which is a type of hindered phenol and therefore plays a role as a radical trapping agent.[7] However, vitamin E has been reported to not improve the

Chart I

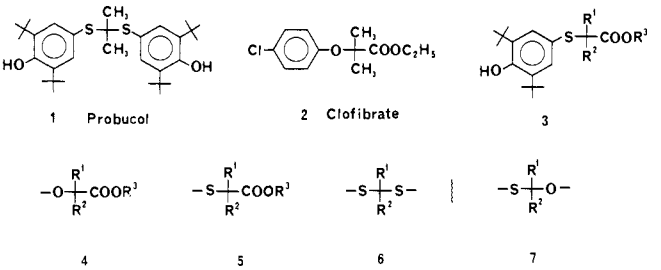


Chart II

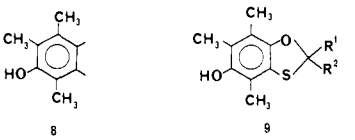
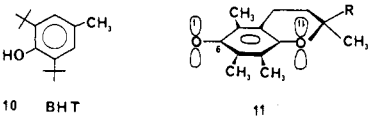


Chart III



(1) Glavind, J.; Hartmann, S.; Clemmesen, J.; Jessen, K. E.; Dam, H. *Acta Path. Microbiol. Scand.* **1952**, *30*, 1.
(2) (a) Fukuzumi, K.; Tanaka, T. *Oil Chem.* **1961**, *10*, 659. (b) Fukuzumi, K.; Iwata, Y. *Ibid.* **1963**, *12*, 93; *Chem. Abstr.* **1963**, *59*, 6652f. (c) Fukuzumi, K. *Ibid.* **1965**, *14*, 119; *Chem. Abstr.* **1965**, *64*. 2559d.
(3) Yagi, K. *J. Ther.* **1979**, *61*, 867.
(4) (a) Sato, Y.; Hotta, N.; Sakamoto, N.; Matsuoka, S.; Ohishi, N.; Yagi, K. *Biochem. Med.* **1979**, *21*, 104. (b) Nishigaki, I.; Hagihara, M.; Tsunekawa, H.; Maseki, M.; Yagi, K. *Biochem. Med.* **1981**, *25*, 373.

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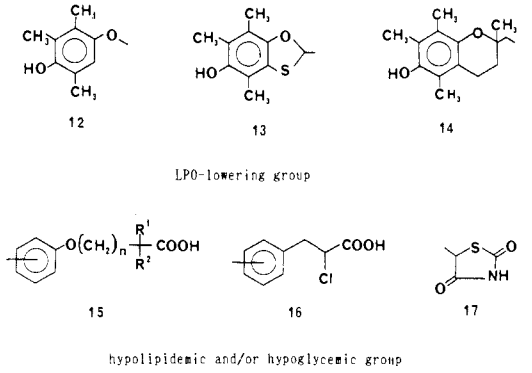
Table I. Inhibition of Lipid Peroxidation in Vitro (m-LPO)[a]

compd	IC_{50}, μg/mL[b]
9a: R = H, R′ = H	<0.1
9b: R = CH_3, R′ = H	<0.1
9c: R = $CH_3(CH_2)_2$, R′ = H	<0.1
10: [BHT]	0.1–0.3

[a] Reference 14. [b] Rat liver microsomal lipid peroxidation.

Chart IV



serum lipid level.[8]

Lowering of the lipid (cholesterol and/or triglyceride) level is generally effective on macroangiopathy such as arteriosclerosis.[9] However, a typical lipid lowering agent, 4,4′-(isopropylidenedithio)bis[2,6-di-*tert*-butylphenol] (**1**, probucol),[10] did not show the LPO lowering activity, in spite of the hindered phenolic structure, in a study using rat liver microsomes (Table III).

From this background, we attempted to establish a new type of drug for treating angiopathy, in other words, a drug having both hypolipidemic and/or hypoglycemic activity and LPO lowering activity. This paper compares the new types of hindered phenolic title compounds and describes how the most effective thiazolidine compound (**27**, CS-045) was found.

Design

Ethyl 2-(4-chlorophenoxy)-2-methylpropionate (**2**, clofibrate)[11] and the corresponding sulfur analogue,[12] ethyl 2-[(3,5-di-*tert*-butyl-4-phenyl)thio]-2-methylpropionate (**3**: R^1 = H, R^2 = CH_3), are well-known hypolipidemic agents.

Chart V

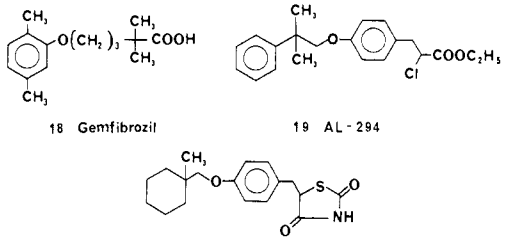
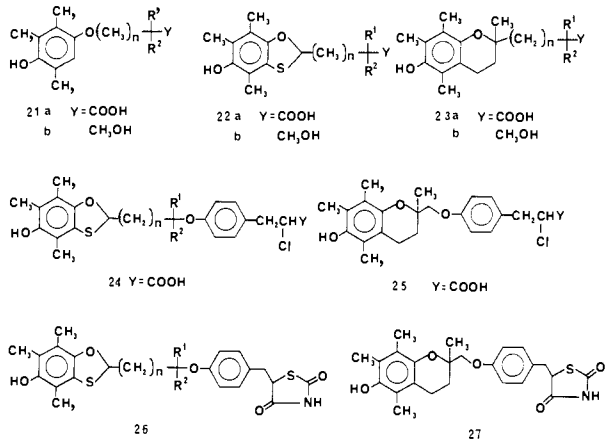


Chart VI



The substructures **4**, **5**, and **6** in drugs **1**, **2**, and **3** gave us the idea of using substructure **7**, which seems not to have been used in the field of hypolipidemic[9] or hypoglycemic[13] agents (Chart I). From the combination of **7** with a hindered phenolic group (**8**), a new type of hindered phenolic compound, 5-hydroxy-1,3-benzoxathiole (**9**), was designed (Chart II).

Unfortunately, compounds of type **9**[14] showed a small hypolipidemic effect in vivo[5] but showed higher ability to inhibit lipid peroxidation in vitro[14] than a typical antioxidant, 2,6-di-*tert*-butyl-4-hydroxyphenol (**10**, BHT) (Table I). The high LPO lowering activity of **9** is consistent with a hypothesis by Ingold et al.[15] that restriction of the lone-pair electrons of the oxygen atom of the chroman ring results in stabilization of the unpaired electron of the oxygen atom at the 6-position as shown in **11**. This hypothesis was based on the fact that the hydrogen-donating activity of vitamin E is about 200 times that of **10** (Chart III).

This finding of the effective new compound **9** and Ingold's hypothesis encouraged us to prepare agents having both an LPO-lowering group such as 4-hydroxy-2,3,5-trimethylphenoxy (**12**), 5-hydroxy-4,6,7-trimethyl-1,3-benzoxathiol-2-yl (**13**), or (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl (**14**) and a hypolipidemic and/or hypoglycemic group such as (carboxyalkoxy)phenyl (**15**), (2-carboxy-2-chloroethyl)phenyl (**16**), or 2,4-dioxothiazolidin-5-yl (**17**) (Chart IV). The latter groups are

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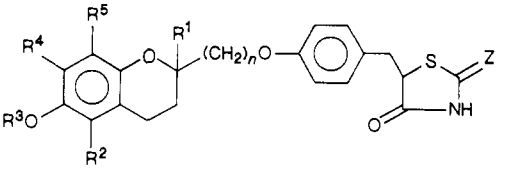
Table II. Thiazolidine Compounds



no.	R^1	R^2	R^3	R^4	R^5	n	Z	yield, %	crystn solvent	mp, °C	(formula) anal.
27	Me	Me	H	Me	Me	1	O	39[a]	benzene–acetone	184–186	($C_{24}H_{27}NO_5S$) C, H, N, S
43	Me	Me	H	Me	Me	1	NH	26[a]	acetone	205–207	($C_{24}H_{28}N_2O_4S$) C, H, N, S
44	Me	Me	Ac	Me	Me	1	O	78[a]	H_2O	ca. 90[g]	($C_{26}H_{29}NO_6S$) C, H, N, S
45	Me	Me	H	Me	Me	2	O	77[b]	MeOH–acetone	152–154	($C_{25}H_{29}NO_5S$) C, H, N, S
46	Me	H	H	*t*-Bu	H	1	O	82[b]	H_2O–EtOH	95–100[g]	($C_{25}H_{29}NO_5S$) C, H, N, S
47	Me	H	H	*t*-Bu	H	2	O	94[c]	*f*	70–72[g]	($C_{26}H_{31}NO_5S$) C, H, N, S
48	Me	H	Ac	*t*-Bu	H	2	NH	56[d]	*f*	175–178	($C_{28}H_{34}N_2O_5S \cdot {}^1/_2H_2O$) C, H, N, S[i]
49	Me	H	H	Me	H	1	O	87[c]	hexane–Et_2O	70–75[g]	($C_{22}H_{23}NO_5S$) C, H, N, S
50	Me	H	Ac	Me	H	1	NH	19[d]	*f*	170–175[g]	($C_{24}H_{26}N_2O_5S \cdot {}^1/_2H_2O$) C, H, N, S
51	Me	Me	PhCO	Me	Me	1	O	62[e]	*f*	198–200	($C_{31}H_{31}NO_6S$) C, H, N, S
52	Me	Me	3-PyCO	Me	Me	1	O	75[e]	hexane–EtOAc	196–198	($C_{30}H_{30}N_2O_6S$) C, H, N, S
53	Me	Me	PrCO	Me	Me	1	O	56[e]	benzene–EtOAc	147–150	($C_{28}H_{33}NO_6S$) C, H, N, S
54	Me	Me	Ac	MeO	MeO	2	NH	48[d]	*f*	ca. 110[g]	($C_{27}H_{32}N_2O_7S$) C, H, N, S
55	Me	Me	H	MeO	MeO	2	O	18[c]	*h*		($C_{25}H_{29}NO_7S$) C, H, N, S
56	H	Me	H	Me	Me	1	O	57[c]	*f*	158–159	($C_{23}H_{25}NO_5S$) C, H, N, S
57	Et	Me	H	Me	Me	1	O	54[b]	H_2O	57–63[g]	($C_{25}H_{29}NO_5S \cdot {}^1/_4H_2O$) C, H, N, S
58	*i*-Bu	Me	H	Me	Me	1	O	41[b]	*f*	68–73[g]	($C_{27}H_{33}NO_5S$) C, H, N, S

[a] See Experimental Section. [b] Obtained from phenylchloropropionate followed by hydrolysis. [c] Obtained from 2-iminothiazolidin-4-one. [d] Obtained from phenylchloropropionate. [e] Obtained from **27**. [f] Purified by column chromatography on SiO_2. [g] Softening point. [h] Obtained as a glassy substance. [i] S: calcd, 6.17; found, 5.74.

substructures of the compound **2**, 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid (**18**, gemfibrozil),[16] ethyl 2-chloro-3-[4-(2,2-dimethyl-2-phenylethoxy)phenyl]-propionate (**19**, AL-294),[17] and 5-[4-[(1-methylcyclohexyl)methoxy]benzyl]-2,4-thiazolidinedione (**20**, ciglitazone)[18] (Chart V), which are well-known or expected to be useful for therapeutic treatment of angiopathy, e.g., hyperlipemia, diabetes, and/or diabetic complications. Thus, the title compounds depicted in Chart VI were designed. In Charts II and VI, substituents on the benzene, 1,3-benzoxathiole, and chroman rings are formally expressed by a methyl group.

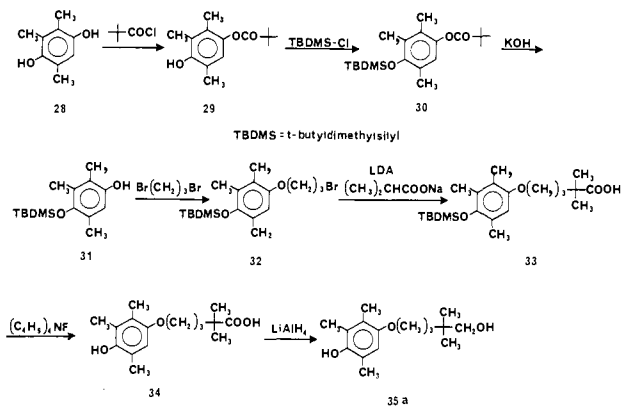
Chemistry

Compounds of type **21** were prepared, for example, as follows. Monoacylation of trimethylhydroquinone (**28**) and subsequent reaction with *tert*-butyldimethylchlorosilane gave silylated ester **30**. Removal of the pivaloyl moiety of **30** yielded monosilylated hydroquinone **31** followed by alkylation with 1,3-dibromopropane to give bromide **32**. Compound **32** was treated with a carbanion of sodium isobutyrate to form carboxylic acid derivative **33**, which was desilylated to give 5-(4-hydroxy-2,3,5-trimethylphenoxy)-2,2-dimethylpentanoic acid (**34**). Lithium aluminum hydride reduction of **34** gave 5-(4-hydroxy-2,3,5-trimethylphenoxy)-2,2-dimethylpentanol (**35a**). Analogue **35b**, 9-(4-hydroxy-2,3,5-trimethylphenoxy)-2,2-dimethylnonanol, was similarly given by using 1,7-dibromoheptane instead of 1,3-dibromopropane (Scheme I).

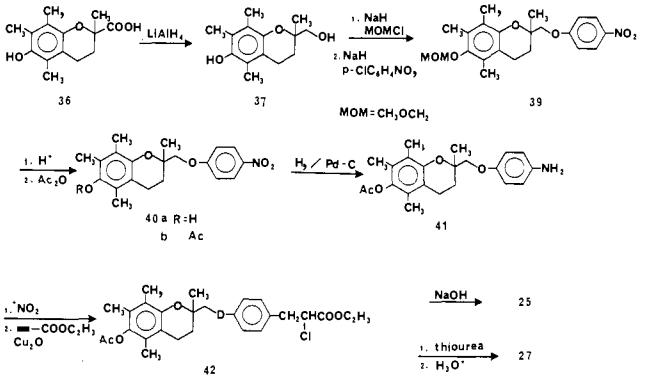
Preparation of (1,3-benzoxathiol-2-yl)alkanoic acid and alkanol (**22a,b**) has been described elsewhere.[14]

Compounds of type **23** were not prepared since the analogous compounds were recently reported to have hypolipidemic or cholagogous activities.[19]

Scheme I



Scheme II



Although preparation of the 1,3-benzoxathiole compounds **24** and **26** was unsuccessful because of the instability of the 1,3-benzoxathiole ring under aqueous acidic conditions being used in the general procedure described

(16) Creger, P. L.; Moersch, G. W.; Neuklis, W. A. *Proc. R. Soc. Med.* **1976**, *69*, Suppl 2, 3.
(17) Kawamatsu, Y.; Asakawa, H.; Saraie, T.; Mizuno, K.; Imamiya, E.; Nishikawa, K.; Hamuro, Y. *Arzneim.-Forsch.* **1980**, *30*, 751.
(18) Sohda, T.; Mizuno, K.; Imamiya, E.; Sugiyama, Y.; Fujita, T.; Kawamatsu, Y. *Chem. Pharm. Bull.* **1982**, *30*, 3580.
(19) (a) Kanehira, K.; Shiono, M.; Fujita, Y.; Nishida, T.; Yamahara, J. Jpn. Kokai Tokkyo Koho **1986**, JP61, 106, 571; *Chem. Abstr.* **1986**, *105*, P152928h. (b) Ejiri, K.; Kanehira, K.; Shiono, M.; Fujita, Y.; Yamahara,. J. Jpn. Kokai Tokkyo Koho 1986, JP61, 267, 570; *Chem. Abstr.* **1986**, *106*, P138257c.

Table III. Chemical and Biological Activities of the Phenols

compd	POV[a]	m-LPO[b] IC_{50}, μg/mL	ALLOXAN[c] (% decrease) dose, mg/kg	s-LPO	TG	CHOL	liver weight[d] (% increase)	other activities (% decrease)
34	0.71	>1.0						
35a	0.42	0.1–0.3	100	27.4	32.5	9.9		
			300	47.6***[i]	44.1**	21.7****	23.9*** (n = 6)[h]	TG (57.3****, n = 6)[e]
35b	0.84	<0.1					45.5**** (n = 6)	TG (60.5****, n = 6)[e]
25	1.02	0.1–0.3	100	46.3***	33.9	14.9	16.7*** (n = 6)	KK-MICE (33.4*, 150 mg/kg)[f,g]
27	0.85	0.1–0.3	50	36.0***	31.9	19.7		KK-MICE (49.0**, 150 mg/kg)[f]
			100	54.3**	57.5**	26.7***	−2.1 (n = 12)	(38.5*, 50 mg/kg)[f]
vitamin E	1.0	–	–	–	–	–	–	
Ac-vitamin E	–	>1.0	10	23.5	36.5	20.4		
			50	27.9	5.2	8.3		
			100	46.1***	27.3	10.7		
			300	56.6****	16.1	9.1		
probucol (1)	–	>1.0	100	5.8	−5.3	−1.1		
			200	−22.1	−25.2	−9.7	0.2 (n = 12)	
			300	6.1	−3.0	0.8		
clofibrate (2)	–	>1.0	50	2.8	1.1	7.4		
			100	26.6*	19.6	5.9		
			200	37.4***	26.8	8.5	37.2**** (n = 12)	TG (37.6****, n = 12)[e]
			300	37.6**	22.8	6.8		
gemfibrozil (18)	–	>1.0	100	12.3	−10.4	3.2		
			300	20.5	12.3	15.9	21.9**** (n = 12)	TG (39.4****, n = 12)[e]

[a] Relative peroxide value to vitamin E. [b] Rat liver microsomal lipid peroxidation. [c] In alloxan-induced hyperlipoperoxidemic and hyperlipidemic mice, po. [d] In Wistar-Imamichi rats (WI). [e] Hypotriglyceridemic activity (WI). [f] Hypoglycemic activity in KK-mice, po. [g] At 3 h after administration. [h] n = number of animals. [i] (*) $p < 0.05$, (**) $p < 0.02$, (***) $p < 0.01$, (****) $p < 0.001$.

below, the chroman compounds **25** and **27** were easily prepared as follows. Reduction of (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (**36**, Trolox) gave the corresponding alcohol (**37**).[20] A phenolic hydroxyl group of **37** was protected by the methoxymethyl (MOM) group and subsequent arylation of **38** with *p*-chloronitrobenzene yielded nitro compound **39**, which was converted to acetate **40b** via removal of the MOM group and reprotection of the phenol **40a** with acetic anhydride. Compound **40b** was hydrogenated to give amino derivative **41** followed by Meerwein arylation[21] to form the corresponding phenylchloropropionate (**42**). Compound **42** gave free carboxylic acid **25** by hydrolysis and also gave the corresponding thiazolidine derivative **27** by reaction with thiourea and subsequent complete hydrolysis (Scheme II).

The lead compound **27** was modified as explained below and analogues (**43–58**) listed in Table II were prepared. Compounds **43** and **44** were prepared by reaction of **42** with thiourea followed by partial hydrolysis. Compound **44** was also given by acetylation of **27**. Compounds **45–47**, **49**, and **55–58** were prepared from the corresponding chromancarboxylic acids[20] in a similar manner to that used to prepare **27**. Compounds **48**, **50**, and **54** were prepared by reaction of the corresponding phenylchloropropionates, analogues of **42**, with thiourea. Compounds **51–53** were prepared by acylation of **27** with the corresponding acylating agent.

Measurement of Chemical and Biological Activities

Inhibitory activity of the entitled phenols on peroxidation was chemically estimated by a relative peroxide value (POV) to vitamin E by using ethyl linoleate (Table III). In vitro activity was estimated by the IC_{50} value of the phenols in rat liver microsomal lipid peroxidation (m-LPO)[22,23] (Tables III and IV). In vivo activity was evaluated by the percent decrease of serum LPO level (s-LPO) by using a newly established method[5] in alloxan-induced hyperlipoperoxidemia in BALB/c mice (ALLOXAN in Tables III and IV).

This in vivo method could be also applied to evaluation of hypotriglyceridemic (TG) and hypocholesterolemic (CHOL) agents.[5] In this method, the activities (TG and CHOL) of compound **2** were not significant and those of compounds **1** and **18** were negative (Table III). Evaluation of the hypotriglyceridemic activity (TG) was also carried out in Wistar-Imamichi rats (WI) (male, 6-weeks old) as shown in Table III. The rats were treated with 0.2% test compounds in the powdered diet for 7 days.

Liver weight was observed in WI by the above-described method (Table III).

The hypoglycemic activity was evaluated by the percent decrease of serum glucose level when the test compounds were administered orally to genetically diabetic KK-mice, which is a model animal of NIDDM (non-insulin-dependent diabetes mellitus) (KK-MICE in Tables III and IV).

Results and Discussion

The phenolic 1,3-benzoxathioles **9a–c** and **22**[14] were not very potent except for the inhibition of peroxidation and of formation of SRS-A (slow reacting substance of anaphylaxis). These activities of **9a–c** and **22** will be reported elsewhere.

The phenoxypentanoic acid **34** had no activity except inhibition of peroxidation of ethyl linoleate (POV). In contrast to acid **34**, the corresponding alcohol **35a** had peroxidation-inhibiting activity (m-LPO), serum lipid peroxide and lipid lowering activity (ALLOXAN), and hypotriglyceridemic activity (WI). However, it increased liver weight. The analogous alcohol **35b**, modified by elongating the carbon chain of **35a**, similarly had peroxidation inhibiting activity (m-LPO) and hypotriglyceridemic activity (WI) and showed a large liver weight increase. The phenylchloropropionic acid **25** had the expected activities such as m-LPO, ALLOXAN, and KK-

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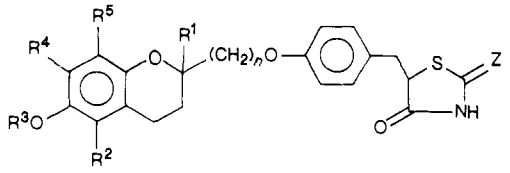
Table IV. Biological Activities of the Thiazolidine Compounds



no.	R^1	R^2	R^3	R^4	R^5	n	Z	m-LPO[a] IC_{50}, μg/mL	KK-MICE[b] 150 mg/kg	KK-MICE[b] 50 mg/kg	ALLOXAN[c] (% decrease) dose, mg/kg	s-LPO	TG	CHOL
											50	36.0***	31.9	19.7
27	Me	Me	H	Me	Me	1	O	0.1–0.3	49.0**[d]	38.5*	100	54.3**	57.5**	26.7***
43	Me	Me	H	Me	Me	1	NH	<0.1	−7.5		100	11.9	16.8	7.2
44	Me	Me	Ac	Me	Me	1	O	<0.1	42.0*	2.6	100	6.7	−4.0	4.0
45	Me	Me	H	Me	Me	2	O	<0.1	<20					
46	Me	H	H	*t*-Bu	H	1	O	<0.1	51.2*	28.5*	100	16.1	−48.6	5.6
47	Me	H	H	*t*-Bu	H	2	O	0.1–0.3	43.6*					
48	Me	H	Ac	*t*-Bu	H	2	NH	0.1–0.3						
49	Me	H	H	Me	H	1	O	0.3–1.0	9.5					
50	Me	H	Ac	Me	H	1	NH	0.3–1.0	6.3					
51	Me	Me	PhCO	Me	Me	1	O	0.1–0.3						
52	Me	Me	3-PyCO	Me	Me	1	O	<0.1	−14.5					
53	Me	Me	PrCO	Me	Me	1	O	<0.1	11.6					
54	Me	Me	Ac	MeO	MeO	2	NH	0.3–1.0						
55	Me	Me	H	MeO	MeO	2	O	0.3–1.0	12.0					
56	H	Me	H	Me	Me	1	O	<0.1	−5.9					
57	Et	Me	H	Me	Me	1	O	0.1–0.3	23.8*		100	17.6*	14.0	19.5
58	*i*-Bu	Me	H	Me	Me	1	O	0.1–0.3	31.6*		100	1.5	11.9	12.1
20	ciglitazone							>1.0	35.7*		100	18.7	7.9	10.8

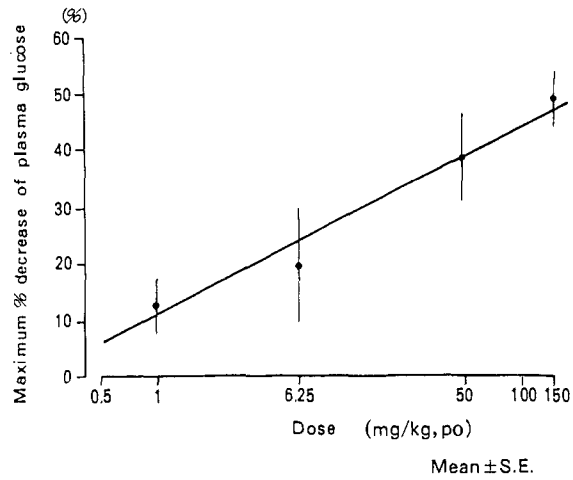
[a] See footnote *b*, Table III. [b] See footnote *f*, Table III. [c] See footnote *c*, Table III. [d] See footnote *i*, Table III.

MICE, while the hypoglycemic activity was rather shorter in duration. Unfortunately, compound **25** slightly increased liver weight. Finally, we found that the thiazolidine compound **27** has all of the desired activities with no liver weight increase. The s-LPO lowering activity of compound **27** at a dose of 100 mg/kg was approximately equal to that of vitamin E acetate at a dose of 300 mg/kg. Further, compound **27** was effective even at 50 mg/kg. Thus, compound **27** was selected as the lead compound.

We wished to compare the biological activities of the thiazolidine compounds analogous to **27**. We changed the substituents at the 2-, 5-, 7-, and 8-positions of the chroman ring (R^1, R^2, R^4, and R^5), protective group of phenolic hydroxyl group (R^3), number of methylene units (n), and the substituent at the 2-position of the thiazolidine ring (Z). Thus, the analogues **43–58** were prepared as described above and were examined (Table IV). Regarding m-LPO, all of the thiazolidine compounds in the table were very potent. Regarding KK-MICE, 2-iminothiazolidin-4-one derivatives (**43** and **50**) were not so potent as 2,4-dione derivatives. When the chroman ring had a *tert*-butyl group at the 7-position (R^4), the activity was also high (**46** and **47**). However, the activity relatively decreased when some part of compound **27** changed as follows: (i) R^1, the methyl group changed to a hydrogen atom (**56**), (ii) R^2 and R^5, two of the methyl groups changed to hydrogen atoms (**49**), (iii) R^4 and R^5, two of the methyl groups changed to methoxyl groups and the number of methylene units was 2 (**55**), or (iv) R^3, the phenolic hydroxyl group was blocked by acyl moieties (**50**, **52**, and **53**) except for the acetyl group (**44**). When the number of methylene units was 2, the activity tended to decrease (**45** and **55**). Lastly, when R^1 at the 2-position of the chroman ring was changed from a methyl group to another alkyl group, the activity was still positive (**57** and **58**).

The results on the substituents can be briefly summarized as follows: R^1, the length of the alkyl group at the 2-position of the chroman ring did not affect the activity so much; R^2, R^4, and R^5, trimethyl and 7-*tert*-butyl derivatives were very active compounds, but monomethyl and methyl dimethoxyl derivatives were not so active; R^3, free phenolic compounds and the acetate were active.

Chart VII. Hypoglycemic Effect of **27**



Because of their activities in the m-LPO, KK-MICE, and ALLOXAN (s-LPO, TG, and CHOL) tests, compounds **27**, **57**, and **58** were selected. Among them, compound **27** was further selected as the best because it was effective on s-LPO even at a dose of 50 mg/kg as stated above. The compound **20** was potent in KK-MICE but not so potent in m-LPO (>1.0) and ALLOXAN (not significant).

Hypoglycemic activity (KK-MICE) at various concentrations of **27** ranging from 1 to 150 mg/kg was determined. It showed the effect in a dose-dependent manner (Chart VII).[24] The statistically significant minimum effective dose was 1 mg/kg and ED_{25} was 6 mg/kg.[24]

In conclusion, compound **27** was selected as a candidate for further clinical studies.

Experimental Section

Mass spectra were recorded on a JEOL-JMS-01SG or JEOL-JMS-D300 mass spectrometer. Proton magnetic resonance (NMR)

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spectra were recorded on a 90-MHz Varian EM-390 spectrometer and are reported in parts per million (δ) downfield from the internal standard tetramethylsilane (Me_4Si); the abbreviation nd means that precise identification of the signal was not possible because of overlap by other signals or absorption of solvent. All NMR spectra were consistent with the structures assigned. Column chromatography was performed on Merck-60 silica gel with a reported solvent. TLC analyses were performed on Merck reagent silica gel 60 F_{254} (0.25 mm thickness). Spots were visualized either by ultraviolet (UV) light or by iodine. Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected.

2,3,6-Trimethyl-4-(pivaloyloxy)phenol (29). To a mixture of 3.5 g (0.023 mol) of **28**, 25 mL of CH_2Cl_2, and 6 mL (0.0742 mol) of pyridine was added dropwise 2.8 g (0.0232 mol) of pivaloyl chloride in CH_2Cl_2 (20 mL). After leaving it for one night at room temperature, 4.25 mL (0.0742 mol) of AcOH and 20 mL of water were added. The organic layer was separated, washed with water, and dried (Na_2SO_4). The solvent was removed by distillation and the residue was subjected to column chromatography with a solvent system of benzene/ethyl acetate (10:1) and was recrystallized from hexane to give 4.7 g (0.0199 mol) of **29** as light yellow prisms: yield 87%; mp 120–121 °C; MS, m/z 236 (M^+). Anal. ($C_{14}H_{20}O_3$) C, H.

4-[(*tert*-Butyldimethylsilyl)oxy]-2,3,5-trimethyl-1-(pivaloyloxy)benzene (30). To a mixture of 2.7 g (0.0114 mol) of **29**, 10 mL of DMF, and 1.9 g (0.0153 mol) of *tert*-butyldimethylchlorosilane was added gradually 2.3 g (0.0338 mol) of imidazole, and the resulting mixture was kept standing for one night at room temperature. The reaction mixture was poured into a mixture of ice and aqueous ammonia and was extracted with hexane. The extract was washed with water and dried (Na_2SO_4). The solvent was evaporated and the residue was subjected to column chromatography with benzene to give 3.9 g (0.0111 mol) of **30**: yield 98%; mp 48–49 °C; MS, m/z 350 (M^+). Anal. ($C_{20}H_{34}O_3Si$) C, H.

3-[4-[(*tert*-Butyldimethylsilyl)oxy]-2,3,5-trimethylphenoxy]propyl Bromide (32). Compound **30** (32.6 g; 0.093 mol) was dissolved in 60 mL of DMF. To the resulting solution was added 21 g (0.374 mol) of KOH in 100 mL of MeOH dropwise under a nitrogen atmosphere. After 2 days the reaction mixture was poured into a mixture of 300 g of ice and 1 L of water, extracted with hexane, washed with water, and dried (Na_2SO_4). The solvent was evaporated and the resulting residue was subjected to column chromatography with a solvent system of benzene/ethyl acetate (20:1) to give a light brown oil (**31**). This oil was dissolved in 207 g (1.02 mol) of 1,3-dibromopropane. The resulting solution was added dropwise to a mixture of 10.3 g (0.0975 mol) of Na_2CO_3, 10.3 g (0.0305 mol) of tetrabutylammonium hydrogen sulfate, 6.0 g (0.15 mol) of NaOH, and 100 mL of water with vigorous stirring under a nitrogen stream. After being stirred for 2 h at room temperature, the organic layer was separated and dried (Na_2SO_4). 1,3-Dibromopropane was evaporated and the resulting residue was subjected to column chromatography using hexane and then a 10:1 mixture of hexane and benzene to give 16.6 g (0.0428 mol) of **32**: yield 46%; mp 45–46 °C; MS, m/z 386 (M^+). Anal. ($C_{18}H_{31}BrO_2Si$) C, H; Br: calcd, 20.62%, found, 19.97%.

5-[4-[(*tert*-Butyldimethylsilyl)oxy]-2,3,5-trimethylphenoxy]-2,2-dimethylpentanoic Acid (33). According to a reported procedure,[25] 97.5 mg (2.23 mmol) of NaH (55% oil dispersion) was added to a mixture of 149.5 mg (6.69 mmol) of diisopropylamine and 1.5 mL of THF, and then 124 mg (1.41 mmol) of isobutyric acid in 1.5 mL of THF was added dropwise. The resulting solution was heated under reflux for 10 min. The reaction mixture was cooled to −5 to 0 °C, and 0.7 mL (1.61 mmol) of butyllithium solution (15 w/v % in hexane) was added dropwise. The reaction mixture was stirred for 15 min at −5 to 0 °C and then for 30 min at 30 to 35 °C. After the mixture was cooled to −5 to 0 °C again, 430 mg (1.11 mmol) of **32** in 1 mL of THF was added dropwise. The mixture was stirred for 30 min at 20 °C, for 30 min at 30 to 35 °C, and then for one night at room temperature. After evaporation of the solvent, 35 mL of water was added to the residue and the resulting aqueous solution was washed with ether. Into the aqueous solution were added 60 mL of hexane, 0.6 mL of concentrated HCl, and 50 mL of water. The mixture was stirred for 1 h at room temperature. The organic layer was separated, washed with water, and dried (Na_2SO_4). The solvent was evaporated and the residue was subjected to column chromatography with a solvent system of benzene/ethyl acetate (10:1) to give 310 mg (0.786 mmol) of **33**: yield 71%; mp 103–104 °C; MS, m/z 394 (M^+). Anal. ($C_{22}H_{38}O_4Si$) C, H.

5-(4-Hydroxy-2,3,5-trimethylphenoxy)-2,2-dimethylpentanoic Acid (34). A mixture of 116 mg (0.294 mmol) of **33**, 364 mg (1.16 mmol) of tetrabutylammonium fluoride trihydrate, 1 mL of THF, and 0.2 g (3.33 mmol) of AcOH was stirred for 3 h at room temperature. After evaporation of the solvent, water and ether were added to the residue. The organic layer was separated and dried (Na_2SO_4). The solvent was evaporated and the resulting powder was recrystallized from benzene and hexane to give 68 mg (0.243 mmol) of **34**: yield 83%; mp 87–89 °C; MS, m/z 280 (M^+); NMR ($CDCl_3$) δ 1.24 (6 H, s), 1.4–1.85 (4 H, nd), 2.14 (6 H, s), 2.19 (3 H, s), 3.7–3.95 (2 H, m), 6.49 (1 H, s), 7.1–8.3 (2 H, br). Anal. ($C_{16}H_{24}O_4$) C, H.

5-(4-Hydroxy-2,3,5-trimethylphenoxy)-2,2-dimethylpentanol (35a). To a mixture of 0.3 g (1.07 mmol) of **34** and 5 mL of THF was added 150.7 mg (3.98 mmol) of lithium aluminum hydride. After being stirred for 2 h at room temperature, the reaction mixture was poured into ice and water, acidified with dilute HCl, and extracted with benzene. The extract was dried (Na_2SO_4) and concentrated. The resulting residue was subjected to column chromatography with a solvent system of benzene/ethyl acetate (6:1) to give 0.26 g (0.976 mmol) of **35a**: yield 91%; mp 78–79 °C; MS m/z 266 (M^+); NMR ($CDCl_3$) δ 0.91 (6 H, s), 1.2–1.95 (4 H, m), 2.15 (6 H, s), 2.20 (3 H, s), 3.34 (2 H, s), 3.84 (2 H, t, J = 6 Hz), 4.42 (1 H, s), 6.51 (1 H, s). Anal. ($C_{16}H_{26}O_3$) C, H.

9-(4-Hydroxy-2,3,5-trimethylphenoxy)-2,2-dimethylnonanol (35b). This compound was prepared similarly to **35a**: yield 82%; mp 75.5–77 °C; MS, m/z 322 (M^+). Anal. ($C_{20}H_{34}O_3$) C, H.

[6-(Methoxymethoxy)-2,5,7,8-tetramethylchroman-2-yl]methanol (38). Compound **37** (16.1 g, 0.0681 mol) was dissolved in 70 mL of dry DMF. NaH (50% oil dispersion, 3.0 g, 0.0625 mol) (which had been washed with cyclohexane three times) was added gradually to the resulting solution at 5 to 10 °C with stirring. The mixture was reacted for 1 h at room temperature and then was ice-cooled to 3 to 5 °C, and 5.5 g (0.0683 mol) of chloromethyl methyl ether dissolved in 40 mL of dry benzene was added dropwise. After the whole of this had been added, the solution was reacted for 1 h at room temperature. The reaction mixture was then poured into ice–water and extracted with cyclohexane. The extract was washed four times with 5% aqueous NaOH solution and then with water. It was then dried (Na_2SO_4) and the solvent was evaporated, giving 16.4 g (0.0585 mol) of **38**: 86% yield as a yellowish glassy substance; MS, m/z 280 (M^+). Anal. ($C_{16}H_{24}O_4$) C, H.

6-(Methoxymethoxy)-2,5,7,8-tetramethyl-2-[(4-nitrophenoxy)methyl]chroman (39). NaH (50% oil dispersion, 6 g, 0.125 mol) was placed in a reaction container and washed with cyclohexane. Dry DMSO (100 mL) and then 19.0 g (0.0678 mol) of **38** dissolved in 20 mL of dry benzene were added, and the mixture was reacted for 20 min at 60 °C. Small portions of *p*-chloronitrobenzene [totaling 21.6 g (0.137 mol)] were added to this solution while being cooled with water to 30 °C; then the reaction was continued for 1 h at 60 °C. The reaction mixture was then poured into ice–water and extracted with ethyl acetate. The extract was washed with water and dried (Na_2SO_4). The solvent was evaporated, leaving a reddish brown crude oil. This oil was subjected to column chromatography, eluted first with a 1:1 by volume mixture of benzene and cyclohexane and then with benzene alone. Compound **39** (25.8 g, 0.0643 mol) was obtained from the portion eluted with benzene: 95% yield as a light yellowish liquid; MS, m/z 401 (M^+). Anal. ($C_{22}H_{27}NO_6$) C, H, N.

6-Hydroxy-2,5,7,8-tetramethyl-2-[(4-nitrophenoxy)methyl]chroman (40a). Compound **39** (32.8 g, 0.0817 mol) was dissolved in 300 mL of AcOH containing 5.3 g of 10% H_2SO_4 and the mixture was heated for 10 min at 60 °C. The reaction mixture

(25) Creger, P. L. *Organic Synthesis*; John Wiley & Sons, Inc.: New York, 1970; Vol 50, p 58.

was cooled and then poured into a mixture of 420 g of $NaHCO_3$ and 1 kg of ice and extracted with ethyl acetate. The extract was washed with water and dried (Na_2SO_4). The solvent was evaporated from the extract, leaving 27.4 g (0.0767 mol) of **40a**: 94% yield as a light yellowish powder: mp 114–116 °C; MS, m/z 357 (M^+). Anal. ($C_{20}H_{23}NO_5$) C, H, N.

6-Acetoxy-2,5,7,8-tetramethyl-2-[(4-nitrophenoxy)methyl]chroman (40b). Compound **40a** (20.4 g, 0.0571 mol) was dissolved in 60 mL of pyridine, and, while stirring, 30 mL (0.278 mol) of Ac_2O was added dropwise at 10 °C. The mixture was gradually restored to room temperature and then reacted for 1 h at 30 °C. The reaction mixture was cooled and then poured into ice–water and extracted with a 1:1 by volume mixture of benzene and cyclohexane. The extract was washed with 2% HCl and then with water and dried (Na_2SO_4). The solvent was removed by evaporation, giving 21.3 g (0.0533 mol) of **40b**: yield 94%; mp 136.5–138 °C; MS, m/z 399 (M^+). Anal. ($C_{22}H_{25}NO_6$) C, H, N.

6-Acetoxy-2-[(4-aminophenoxy)methyl]-2,5,7,8-tetramethylchroman (41). Compound **40b** (24.3 g, 0.0608 mol) was dissolved in a mixture of 200 mL of MeOH and 20 mL of benzene and reacted for 3 h under a hydrogen pressure of 45–55 lb/in.^{2} (3.1–3.8 bars) using Parr's hydrogenation apparatus in the presence of 7 g of 10% palladium-on-carbon. The palladium-on-carbon was removed by filtration from the reaction mixture and the solution was then washed with a mixture of 600 mL of acetone and 60 mL of concentrated HCl. The filtrate and the washings were combined and the mixture was neutralized with $NaHCO_3$. The solvent was then evaporated and the crude crystals obtained were dissolved in ethyl acetate. The ethyl acetate solution was washed with water and dried (Na_2SO_4). The ethyl acetate was then evaporated from the extract, and the crude substance obtained was washed with a 1:1 by volume mixture of benzene and cyclohexane, giving 22.0 g (0.0595 mol) of **41**: yield 98%; mp 138–140 °C; MS, m/z 369 (M^+). Anal. ($C_{22}H_{27}NO_4$) C, H, N.

Ethyl 3-[4-[(6-Acetoxy-2,5,7,8-tetramethylchroman-2-yl)methoxy]phenyl]-2-chloropropionate (42). Compound **41** (17.5 g, 0.0474 mol) was dissolved in a mixture of 130 mL of acetone and 30 mL of water, and 13 mL of concentrated HCl followed by 4.3 g (0.0623 mol) of $NaNO_2$ dissolved in 8.5 mL of water were added dropwise, with ice-cooling, to the mixture. Ethyl acrylate (37.3 mL) was added dropwise, and then 680 mg (4.75 mmol) of Cu_2O was added gradually to the resulting mixture while keeping its temperature at 40 to 43 °C. Generation of nitrogen terminated after about 30 min. Benzene was then added to the reaction mixture (which consisted of 2 layers) to extract the organic layer. The benzene extract was washed with brine and dried (Na_2SO_4). The solvent was then evaporated from the extract. The dark brownish oil thus obtained was subjected to column chromatography, eluted with a 1:1 by volume mixture of benzene and cyclohexane; then the proportion of benzene was progressively increased until it was eluted with benzene alone. Compound **42** (15.5 g, 0.0317 mol) was obtained (67% yield as a yellow oil) from the fractions eluted with a 2:1 by volume mixture of benzene and cyclohexane and with benzene alone: MS, m/z 488 (M^+). Anal. ($C_{27}H_{33}ClO_6$) C, H, Cl.

2-Chloro-3-[4-[(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methoxy]phenyl]propionic Acid (25). Compound **42** (0.48 g, 0.982 mmol) was dissolved in a mixture of 5 mL of 99.5% EtOH and 2 mL of THF. To this was added dropwise, under a nitrogen stream at 8 to 10 °C, a solution prepared by dissolving 133 mg (3.33 mmol) of NaOH in 1 mL of 99.5% of EtOH. When the whole of the solution had been added, the mixture was reacted for a further 18 h at 0 to 5 °C, after which it was neutralized by adding to it dropwise a solution prepared by dissolving 0.37 g (3.55 mmol) of concentrated HCl in 1 mL of 99.5% EtOH. The solvent was then evaporated from the mixture. The pale reddish oil thus separated was extracted with $CHCl_3$, and the extract was washed with water and dried (Na_2SO_4). The crude product obtained by distilling the $CHCl_3$ off was subjected to column chromatography with a solvent system of 10:1 benzene–ethyl acetate to give 0.37 g (0.883 mmol) of **25**: yield 89%; mp 148–149 °C; MS, m/z 418 (M^+); NMR ($CDCl_3$) δ 1.40 (3 H, s), about 2 (2 H, m), 2.10 (3 H, s), 2.15 (3 H, s), 2.6 (2 H, br t, J = 6 Hz), 3.05 (1 H, dd, J = 15 and 7.5 Hz), 3.30 (1 H, dd, J = 15 and 7.5 Hz), 3.83 and 3.98 (2 H, AB type, J = 9 Hz), 4.40 (1 H, t, J = 7.5 Hz), about 6 (2 H, br s), 6.85 (2 H, d, J = 9 Hz), 7.14 (2 H, d, J = 9 Hz). Anal. ($C_{23}H_{27}ClO_5$) C, H, Cl.

5-[4-[(6-Hydroxy-2,5,7,8-tetramethylchroman-2-yl)methoxy]benzyl]-2-iminothiazolidin-4-one (43) and 5-[4-[(6-Hydroxy-2,5,7,8-tetramethylchroman-2-yl)methoxy]benzyl]-2,4-thiazolidinedione (27). (a) A mixture of 9.6 g (0.0196 mol) of **42**, 1.8 g (0.0236 mol) of thiourea, and 11 mL of sulfolane was reacted for 80 min at 115 to 120 °C. Subsequently, a mixture of 90 mL of AcOH, 30 mL of concentrated HCl, and 15 mL of water was added to this, and the resulting mixture was heated further for 12 h at 85 to 90 °C. $NaHCO_3$ (27 g, 0.321 mol) was added to this reaction mixture and, once evolution of CO_2 had ceased, the solvent was evaporated. A 10:1 by volume mixture of benzene and ethyl acetate was added to the residue, and the organic solution was washed with dilute $NaHCO_3$ solution. The white powder produced was collected by filtration and washed with water. It was then recrystallized from acetone to give 2.2 g (4.99 mmol) of **43**: yield 26%; mp 205–207 °C; MS, m/z 440 (M^+); NMR (DMF-d_7 + D_2O) δ 1.37 (3 H, s), about 2 (2 H, m), 2.02 (3 H, s), 2.14 (6 H, s), 2.3–3.1 (2 H, nd), 3.42 (1 H, dd, J = 15 and 4.5 Hz), 4.60 (1 H, dd, J = 9 and 4.5 Hz), 6.93 (2 H, d, J = 9 Hz), 7.23 (2 H, d, J = 9 Hz). Anal. ($C_{24}H_{28}N_2O_4S$) C, H, N, S.

(b) The filtrate in step a above was washed with water and dried (Na_2SO_4). The solvent was then evaporated. The resulting crude product was purified by column chromatography by elution with a mixture of benzene and ethyl acetate, first in a volume ratio of 10:1 and then in a volume ratio of 50:7. Compound **27** (3.4 g, 7.70 mmol) was obtained from the fractions eluted with the latter mixture; yield 39%. The obtained crystals were dissolved in acetone and the solution was concentrated, followed by adding hot benzene to give analytically pure crystals: 184–186 °C; MS, m/z 441 (M^+); NMR (acetone-d_6) δ 1.39 (3 H, s), about 2 (2 H, m), 2.02 (3 H, s), 2.09 (3 H, s), 2.13 (3 H, s), 2.63 (2 H, br t, J = 6 Hz), 3.07 (1 H, dd, J = 15 and 9 Hz), 3.41 (1 H, dd, J = 15 and 4.5 Hz), 3.97 (2 H, AB type, J = 9 Hz), 4.70 (1 H, dd, J = 9 and 4.5 Hz), 6.90 (2 H, d, J = 9 Hz), 7.21 (2 H, d, J = 9 Hz). Anal. ($C_{24}H_{27}NO_5S$) C, H, N, S.

5-[4-[(6-Acetoxy-2,5,7,8-tetramethylchroman-2-yl)methoxy]benzyl]-2,4-thiazolidinedione (44). Compound **27** (0.725 g, 1.64 mmol) was dissolved in 4 mL of benzene and 400 mg of dry pyridine was added. Ac_2O (0.2 g, 1.85 mmol) was added dropwise at 5 to 10 °C and the mixture was reacted for 2 days at room temperature. The resulting white crystals were separated by filtration, washed with benzene, and vacuum-dried for 30 min at 90 °C, giving 0.74 g (1.32 mmol) of the benzene monoadduct of **44**; yield 80%. This substance was liquefied at 98–100 °C, solidified, and again liquefied at 176–178 °C: NMR ($CDCl_3$) δ 1.42 (3 H, s), 1.98 (3 H, s), about 2 (2 H, m), 2.03 (3 H, s), 2.09 (3 H, s), 2.31 (3 H, s), 2.63 (2 H, br t, J = 6 Hz), 3.03 (1 H, dd, J = 15 and 9 Hz), 3.42 (1 H, dd, J = 15 and 4.5 Hz), 3.84 and 3.98 (2 H, AB type, J = 9 Hz), 4.45 (1 H, dd, J = 9 and 4.5 Hz), 6.87 (2 H, d, J = 9 Hz), 7.15 (2 H, d, J = 9 Hz), 7.38 (6 H, s due to benzene), 8–8.5 (1 H, br s). Anal. ($C_{26}H_{29}NO_6S \cdot C_6H_6$) C, H, N, S.

In order to obtain the desired free **44**, 730 mg (1.30 mmol) of the benzene monoadduct obtained as described above was dissolved in 5 mL of acetone; the solvent was evaporated; the residue was solidified by adding water; and the white amorphous powder produced was vacuum-dried in a desiccator in the presence of P_2O_5 to give 0.61 g (1.26 mmol) of the title compound (**44**): yield 97%; softening at about 90 °C; MS, m/z 483 (M^+); NMR (acetone-d_6) δ 1.41 (3 H, s), 1.97 (3 H, s), 1.98 (3 H, s), about 2 (2 H, nd), 2.04 (3 H, s), 2.27 (3 H, s), 2.67 (2 H, br t, J = 6 Hz), 3.07 (1 H, dd, J = 15 and 9 Hz), 3.42 (1 H, dd, J = 15 and 4.5 Hz), 4.00 (2 H, AB type, J = 9 Hz), 4.71 (1 H, dd, J = 9 and 4.5 Hz), 6.91 (2 H, d, J = 9 Hz), 7.21 (2 H, d, J = 9 Hz). Anal. ($C_{26}H_{29}NO_6S$) C, H, N, S.

Measurement of POV. The test compound (10 mg) was dissolved in 2 g of ethyl linoleate and the solution was kept standing at 63 °C for 16 h. One gram of the solution was weighed accurately and was diluted by a 2:3 by volume mixture of $CHCl_3$ and AcOH. Saturated KI solution (1 mL) was added to the resulting solution and the solution was kept standing at room temperature for 5 min in a dark place. After 75 mL of water was added, the resulting aqueous solution was titrated with 0.01 N

$Na_2S_2O_3$. Relative peroxide value against vitamin E (POV) was calculated.

Inhibition of Lipid Peroxide Formation (m-LPO). This was investigated by a method similar to that described by Malvy et al.[22] (rat liver microsomes, ferrous sulfate/cysteine).

Effects on Hyperlipoperoxidemia and Hyperlipidemia (ALLOXAN). Male BALB/c mice were used at the age of 8 weeks. The animals were fasted for 18 h, after which 75 mg/kg of alloxan was administered intravenously. Each of the test compounds was administered orally at a dose of 300, 200, 100, 50, or 10 mg/kg body weight 30 min before and 24 and 30 h after administration of alloxan. Blood was collected from an incision in the cervical region 48 h after administration of alloxan. The collected amount of blood was 100 or 200 μL. Then whole blood was diluted 10 or 20 times with a saline solution and centrifuged (3000 rpm, 10 min) to determine lipid content.

LPO was measured by the TBA method.[5] CHOL and TG were measured according to the enzyme method. A Determiner TC (a registered trade mark of Kyowa Medix) kit was used to measure CHOL, and a Triglyceride Measuring Agent (GPO-*p*-chlorophenol color developing method) (Wako Pure Chemical Industries) kit was used for TG.

As a control, the procedure was repeated, except that no test compound was administered.

Effects on Hyperglycemia (KK-MICE). Male KK-mice were housed in individual cages at the age of 8 weeks. They were used for the experiment when their body weight was more than 40 g at the age of about 4–5 months.

Test compound was finely suspended in 0.5% (carboxymethyl)cellulose saline (vehicle). Each of the test compounds was administered orally at a dose of 150 or 50 mg/kg body weight 18 h before blood sampling. Blood was collected from the tail vein in a heparinized hematocrit tube and then centrifuged and plasma was separated to measure blood glucose. Plasma glucose level was determined by a glucose analyzer (Mitsubishi Kasei Co., Ltd, Model-101).

As a control, the same test was done simultaneously after administration of the vehicle.

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Studies on Bioactive Compounds. 13.[1] Synthesis and Lack of Growth-Inhibitory Properties of Cyclohexane-1,2,4-triol 1,2-Diesters, Which Resemble Ring C of the Phorbol Ester Molecule

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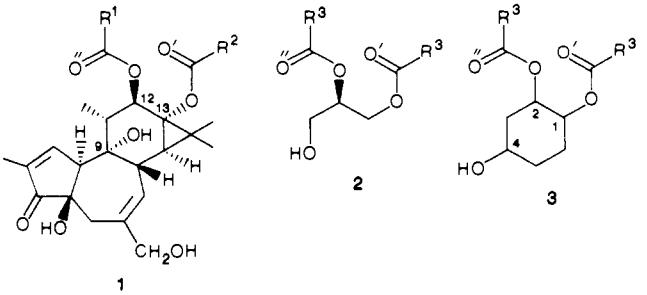
It has been suggested that ring C of biologically active phorbol esters is an essential structural feature of the pharmocophore which confers activity on these compounds. In this study the hypothesis has been tested that compounds which resemble ring C of the phorbol ester molecule mimic the ability of phorbol esters to inhibit cell growth at nontoxic concentrations. All four diastereoisomers of (±)-1,2-di-*O*-octanoylcyclohexane-1,2,4-triol have been prepared from cyclohexen-4-ol and tested for growth-inhibitory and cytotoxic properties. The phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate inhibited the growth of A549 human lung carcinoma cells by 50% at a concentration of 0.2 nM and exerted cytotoxicity at concentrations of >1 μM. Diacylglycerols are the physiological ligands and activators of protein kinase C, the receptor via which phorbol esters are thought to mediate their effects. The diacylglycerols 1-oleoyl-2-acetylglycerol and 1,2-dioctanoylglycerol and the cyclohexanetriol diesters inhibited the growth of A549 cells only at concentrations of 10^{-5} to 10^{-4} M, at which they were also cytotoxic. A computer-assisted analysis of the goodness of fit between the cyclohexanetriol diesters and ring C of the phorbol moiety revealed possible energetic grounds for conformational dissimilarities. The results suggest that activation of protein kinase C alone is probably not sufficient to reproduce phorbol ester induced growth arrest in A549 cells and that the cyclohexanetriol diesters may lack pivotal elements of the phorbol ester pharmacophore.

The multitude of recent studies on the mechanism by which tumor-promoting phorbol esters, of which 12-*O*-tetradecanoylphorbol 13-acetate (TPA, 1; see Scheme I) is the most potent derivative, exert their pleiotropic effects in biological systems have left many intriguing questions unanswered. There is now little doubt about the contention that the ability of these compounds to bind to their receptor, the ubiquitous calcium and phospholipid-dependent enzyme protein kinase C (pkC), plays a pivotal role in the generation of their biological effects.[2,3] However it is not clear whether the diverse responses to TPA, such as, for example, induction or mitogenesis,[4] inhibition of growth,[5] and induction or inhibition of differentiation,[6] are

Scheme I



1 2 3

R^1 = Me, R^2 = $Me(CH_2)_{12}$, R^3 = $Me(CH_2)_6$

(1) For paper 12, see: Cunningham, B. D. M; Lowe, P. R.; Threadgill, M. D. *J. Chem. Soc., Perkin Trans. 2*, in press.
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