Studies of Hypolipidemic Agents. 1. Synthesis and Hypolipidemic Activities of Alkoxycinnamic Acid Derivatives

Toshio Watanabe,[1] Kimiaki Hayashi, Shigeki Yoshimatsu, Kazuo Sakai,

Products Formulation Research Laboratory, Tanabe Seiyaku Co., Kashima Yodogawa, Osaka 532, Japan

Shigeyuki Takeyama,* and Kohki Takashima

Pharmacological Research Laboratory, Tanabe Seiyaku Co., Toda, Saitama 335, Japan. *Received April 9, 1979*

More than 110 derivatives of alkoxycinnamic acids were synthesized and their hypolipidemic activities were evaluated in a screening system with rats. Cinnamic acids, α-methylcinnamic acids, and their various esters with a higher *p*-alkoxy substituent were found to possess hypolipidemic activities higher than or comparable to that of clofibrate. The proper length (C_{12}–C_{16}) and the para position of the alkoxy substituent seem to be essential for activity. Chloroethyl and methacryloxyethyl esters and monoglycerides of some of the active *p*-alkoxycinnamic acids were more active than the corresponding free acids.

Clofibrate [ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate], the most widely used hypocholesterolemic agent, and other related agents are relatively ineffective for Type IIa hyperlipidemia and they are not entirely devoid of untoward side effects.[2] In a series of studies to find more satisfactory hypolipidemic agents, we have synthesized many carboxylic derivatives and evaluated their hypocholesterolemic and hypotriglyceridemic activities. In the screening test with normal rats, a series of *p*-alkoxycinnamic acids and related compounds were found to exhibit marked activities in reducing serum cholesterol and triglyceride concentrations. Details of studies on the hypolipidemic property of one of these derivatives, 1-[*p*-(myristyloxy)-α-methylcinnamoyl]glycerol (LK-903), have been published.[3-5] In the present paper, we describe the preparation and some structure–activity relationships of these derivatives.

Chemistry. *p*-Alkoxycinnamic acids, *p*-alkoxy-α-methylcinnamic acids **1–13**, *m*-(myristyloxy)cinnamic acid (**18**), and *m*-methoxy-*p*-alkoxy-α-methylcinnamic acids **19** and **20** were prepared by alkylation of the respective hydroxy acids as described by Bennet et al.[6] (method A). *p*-Phenoxycinnamic acid (**14**) and *p*-phenoxy-α-methylcinnamic acid (**15**) were prepared by Perkin's reaction of the respective benzaldehydes (method B). *p*-Alkoxy-β-methylcinnamic acid (**16**) and *o*-alkoxy-α-methylcinnamic acid (**17**) were prepared by Reformatsky's reaction (method C). Several ester derivatives (**21–55** and **63–72**) were prepared by treating alkoxycinnamoyl chlorides with the respective hydroxy compounds (method D). Glycol monoester derivatives **56–62** were prepared from the respective β-chloroethyl or γ-chloropropyl esters by treating them with aqueous alcoholic silver nitrate according to the method described by Bevan et al.[7] (method E). Compounds **73** to **75** were prepared by acylation of the respective glycol monoesters **58** and **60** (method F). *p*-Alkoxycinnamic acid glycol diester derivatives **76–79** were prepared by employing 2 mol of the acid chloride (method D).

Scheme I[a]

method G

X–C6H4–CH=C(R1)COCl (I) —[HOCH2CHCH2 (Ip-protected)]→ X–C6H4–CH=C(R1)CO2CH2CHCH2 (Ip) → X–C6H4–CH=C(R1)CO2CH2CHCH2OH (OH) (II)

method H

I —[Bz-protected CH2CHCH2, OH]→ X–C6H4–CH=C(R1)CO2CH(CH2O)2Bz → X–C6H4–CH=C(R1)CO2CH(CH2OH)2

method I

II —[nR2—COCl]→ X–C6H4–CH=C(R1)CO2CH2CHCH2O2C—R2 (O—Y)

$n = 1$; Y = H
$n = 2$; Y = R^2CO

[a] Ip = isopropylidene; Bz = benzylidene.

The synthesis of monoglycerides was based on the well-established methods for the preparation of naturally occurring monoglycerides.[8] A series of 1-monoglycerides (**84–97** and **99–105**) were prepared by the reaction of 1,2-isopropylideneglycerol with an acyl chloride, followed by subsequent removal of the masking group (Scheme I,

(1) Present address: Faculty of Pharmaceutical Science, Josai University, Keyakidai, Sakado, Saitama 350-02, Japan.
(2) H. Meinertz and O. Faergeman in "Side Effects of Drugs", Annual 2, M. N. G. Dukes, Ed., Excerpta Medica, Amsterdam and Oxford, 1978, p 358.
(3) K. Takashima, K. Ohyama, T. Mori, and S. Takeyama, *Biochem. Pharmacol.*, 27, 2631 (1978).
(4) K. Takashima and S. Takeyama, *Biochem. Pharmacol.*, 27, 2637 (1978).
(5) K. Takashima, T. Mori, K. Ohyama, S. Nitta, and S. Takeyama, *Chem. Pharm. Bull.*, 26, 2454 (1978).
(6) G. M. Bennet and B. Jones, *J. Chem. Soc.*, 420 (1939).
(7) T. H. Bevan, T. Malkin, and D. B. Smith, *J. Chem. Soc.*, 1043 (1955).
(8) J. A. Anfinsen and E. G. Perkins, *J. Am. Oil Chem. Soc.*, 41, 779 (1964); L. Hartmen, *Chem. Ind. (London)*, 711 (1960).

0022-2623/80/1823-0050$01.00/0 © 1979 American Chemical Society

method G). A 2-monoglyceride (**106**) was prepared similarly by the use of 1,3-benzylideneglycerol instead of 1,2-isopropylideneglycerol (Scheme I, method H). Some of the masked glycerol derivatives (**80–83**) are shown in Table VI. In the case of 2-monoglyceride, boric acid in 2-methoxyethanol was preferable to formic acid in aqueous ethanol for removal of the masking group, because the latter gave rise to an isomerization of the monoglyceride. Thin-layer chromatography on silica gel impregnated with boric acid[9] was found to be particularly useful for separation of the 1- and 2-monoglycerides. The structures of the 1- and 2-monoglycerides were distinguished from each other by NMR spectroscopy, but they were indistinguishable by IR spectroscopy. The spectra of 1-monoglycerides had a peak at 3.5–4.0 ppm due to the protons attached to the carbon-2 and -3 atoms of glycerol. The carbon-1 proton gave a peak at 4.3 ppm. The spectra of 2-monoglycerides had a doublet peak due to the protons on the carbon-1 and -3 atoms at 3.8 ppm. The protons on carbon-2 gave a peak at 5.03 ppm.

The cis isomer of **97** (**98**) was prepared by cis/trans isomerization of the trans-ene 1-monoglyceride **97** induced by UV irradiation. Thin-layer chromatography showed that the equilibrium mixture contained the cis and trans isomers at a ratio of about 1:3. The cis-isomer **98** proved more soluble in organic solvents than the trans isomer and was obtained in a pure form by fractional recrystallization. The structure of the cis isomer was confirmed by IR and NMR. The two isomers **98** and **99** were distinguishable on TLC because of their different R_f values.

The di- and triglycerides were prepared by method I (Scheme I). The mixed 1,3-diglycerides **107–109** were prepared by using equivalent amounts of acid chlorides and 1-monoglycerides. The mixed triglycerides of alkoxycinnamic acid and other carboxylic acids (**110–115**) were also prepared from 1-monoglycerides and 2 equiv of acid chlorides. The triglycerides of alkoxycinnamic acids **116** and **117** were prepared from 1-monoglycerides (Table VII). Impurities were removed by silica gel chromatography. NMR spectroscopy was useful for distinguishing glyceride isomers. The characteristic bands of the proton on carbon-2 of 1,2- and 1,3-diglycerides gave pictures quite different from those of mono- and triglycerides. The structures of the 1,3-diglycerides obtained in our experiment were confirmed by the lack of the bands at 4.5–5.0 ppm of monoglycerides.

Biological Results. Since serum triglyceride levels in the rat were more variable than cholesterol levels, more emphasis was placed on hypocholesterolemic rather than hypotriglyceridemic activities in assessing the hypolipidemic activity of test compounds. The activities of cinnamic acids and α-methylcinnamic acids with a higher alkoxy or a phenoxy group at the para position are shown in Table I. There appears to be an optimal length of the *p*-alkoxy group, since greater activities were observed with compounds with an alkoxy group of C_{12} to C_{16} (**6**, **9**, and **11**) than with those with an alkoxy group of C_8, C_{10}, or C_{18}. Generally, α-methyl-substituted cinnamic acid derivatives were more active than the corresponding nonsubstituted ones (**3** vs. **4**, **5** vs. **6**, **8** vs. **9**, and **10** vs. **11**), whereas a β-methylcinnamic acid derivative (**16**) was inactive (Table II). Ortho or meta substitution with an alkoxy group (**17** and **18**) or blocking one of the meta positions with a methoxy group in *p*-alkoxy derivatives (**19** and **20**) abolished the activity (Table II). Table III lists the activities of various esters of para-substituted cinnamic acid derivatives. In general, the ester derivatives of the active acids were also active and the relationship of the activity to the length of the carbon chain of the *p*-alkoxy group ($C_8 \ll C_{10} < C_{12}, C_{14}, C_{16} \gg C_{18}$) was the same as in the parent acids, but the activities were different depending on the structure of the alcohol residue. The esters with lower alcohols (**21–23**), a higher alcohol (**24**), or phenol (**25**) showed only weak hypocholesterolemic activities, while their hypotriglyceridemic activities were significant. Although substitution at the ω-carbon of the lower linear alcohol with a diethylamino group (**28**) seems to have no potentiating effect, introduction of a pyridyl (**27**), methoxycarbonyl (**31**), or sodium sulfonyl group (**34**) in the aliphatic chain restored the activities of the lower alkyl esters to the levels of their corresponding parent acids. Activity was elevated by ω substitution with a chlorine atom in the aliphatic chain to levels higher than, or at least comparable to, those of the corresponding parent acids **40**, **41**, **43**, **44**, **46**, **47**, **49**, **50**, and **53**. A bromoethyl ester (**55**) was inactive. Displacement of the chlorine atom in the above chloroalkyl esters by a hydroxyl group (**57–61**) seemed to be somewhat detrimental in comparison with the corresponding chloralkyl esters, although some of them (**58**) may be more active than parent acids. Methacryl derivatives (**65–69**, **71**, **72**, and **75**) of the above hydroxyalkyl esters were more active than the corresponding hydroxyalkyl esters or the parent acids and comparable to the corresponding chloroalkyl esters. Esterification of the hydroxyalkyl esters with acrylic acid or crotonic acid resulted in a loss of activity (**73** and **74**). Ethylene or propylene glycol diesters of the cinnamic acids (**76–79**) were inactive (Table IV).

Like clofibrate, active cinnamate derivatives generally exerted greater effects on serum triglyceride than on serum cholesterol. Under the present screening conditions, 0.1% clofibrate in the diet depressed the serum cholesterol and triglyceride levels by about 20 and 40%, respectively. Similar magnitudes of depression in serum lipids were brought about by 0.05% or less concentrations of the active esters (e.g., **44**, **46**, **58**, **67**, and **69**) in the diet. The active free acids **4–6**, **9**, **11**, and **14** were at least as active as clofibrate. The augmented activities of the esters may probably be associated with their greater rates of intestinal absorption. It is likely that the active esters exert their effects only after hydrolysis.

These *p*-alkoxycinnamic acids can be regarded as analogues of naturally occurring long-chain fatty acids. The latter exist in other forms such as mono-, di- and triglycerides. Therefore, we were interested in comparing mono-, di- and triglyceride derivatives of the active cinnamic acids with the parent acids in their hypolipidemic properties. Monoglyceride derivatives of *p*-alkoxycinnamic acids or *p*-alkoxy-α-methylcinnamic acids generally showed greater activities than the parent acids (Table V). Some of them (**94**, **97**, and **100**) were two times as active as the parent acids, and their minimal effective concentrations in the diet were 0.02%, which corresponded to about 20 mg/kg of body weight/day. The relative potencies of the monoglycerides with various lengths of the *p*-alkoxy substituent were in the order of $C_8 < C_{10} \ll C_{12}–C_{16} \gg C_{18}$, and α-methylcinnamates **92**, **94**, **97**, and **100** were often more active than nonsubstituted cinnamates **91**, **93**, **96**, and **99** (Table V), reflecting the structure–activity relationship in the parent acids. Masking the remaining hydroxy groups of the monoglycerides with an isopropylidene group impaired their activities (Table VI). Triglyceride derivatives **116** and **117** were inactive (Table VII). Acylation of one or two more hydroxy groups in the monoglycerides

(9) B. Sedarevich, *J. Am. Oil Chem. Soc.*, 44, 381 (1967).

Table I

$R^1O-C_6H_4-CH=C(R^2)CO_2H$

no.	R[1a]	R[2]	prepn method[b]	% yield[c]	mp, °C	recrystn solvent	formula[d]	activity: dose, mg %	activity: days	activity: CHO[e]	activity: TG[f]
1	$CH_2=CH-CH_2$	H	A	74	178–179	EtOH	$C_{12}H_{12}O_3$	30	7	0	-16
2	C_8H_{17}	CH_3	A	57	73–74	EtOH	$C_{18}H_{26}O_3$	50	7	10[m]	8[m]
3	$C_{10}H_{21}$	H	A	65	127–128[h] 159–160	EtOH	$C_{19}H_{28}O_3$	50	7	9	18[p]
4	$C_{10}H_{21}$	CH_3	A	57	78–80	EtOH	$C_{20}H_{30}O_3$	50	7	16[p]	43[p]
5	$C_{12}H_{25}$	H	A	78	125–127[i] 157–158	EtOH	$C_{21}H_{32}O_3$	50	7	[illegible]	52[p]
6	$C_{12}H_{25}$	CH_3	A	70	81–82	EtOH	$C_{22}H_{34}O_3$	50	7	17[m]	31[m]
								20	7	7	32[p]
								10	7	14[p]	13
7	$C_{13}H_{27}$	CH_3	A	84	92–93 105	ligroin–benzene	$C_{23}H_{36}O_3$	50	7	13	-4
8	$C_{14}H_{29}$	H	A	61	123–124 126–127	EtOH	$C_{23}H_{36}O_3$	50	7	7	0
9	$C_{14}H_{29}$	CH_3	A	81	90–91	EtOH	$C_{24}H_{38}O_3$	100	7	16[m]	45[m]
								50	7	17 ± 2[n]	26 ± 8[n]
								20	7	12 ± 4[o]	38 ± 8[o]
10	$C_{16}H_{33}$	H	A	60	118–120[j]	EtOH	$C_{25}H_{40}O_3$	20	7	2[m]	-1[m]
								10	7	7	2
11	$C_{16}H_{33}$	CH_3	A	70	90–92[k]	EtOH	$C_{26}H_{42}O_3$	50	7	17[m]	25[m]
								20	7	9[m]	1[m]
12	$C_{18}H_{37}$	H	A	75	116–117	EtOH	$C_{27}H_{44}O_3$	50	7	0	9
13	$C_{18}H_{37}$	CH_3	A	67	97–98	EtOH	$C_{28}H_{46}O_3$	50	7	-12	-54
14	Ph	H	B	83	155–157[l]	MeOH	$C_{15}H_{12}O_3$	50	7	15[p]	34[p]
								20	7	14[m]	14[m]
15	Ph	CH_3	B	36	119–120	MeOH	$C_{16}H_{14}O_3$	20	7	8[m]	15[m]

[a] All alkyls larger than four carbons are normal. [b] The letter refers to the general methods described in the text. [c] No efforts were made to optimize yields. [d] All compounds were obtained with reasonable IR spectra and analyzed for C, H, and N, if present. [e] Percent decrease of cholesterol. [f] Percent decrease of triglyceride. [g] It is known that many *p*-alkoxycinnamic acids are mesomorphic and, therefore, double melting points were observed. [h] Lit. (ref 19) mp 133–144–163 °C; lit. (ref 20) mp 136–150.5–160 °C. [i] Lit. (ref 6) mp 132–145–153 °C; lit. (ref 20) mp 132–157–165 °C. [j] Lit. (ref 19) mp 200–202 °C; lit. (ref 6) mp 125–132 °C. [k] Lit. (ref 20) mp 120.5–157.5 °C. [l] Lit. (ref 18) mp 155 °C; lit. (ref 20) mp 118–159 °C. [m–o] Means ± SE of two, seven, and four experiments, respectively. [p] Statistically significant depression with $p < 0.05$.

with other common carboxylic acids usually weakened the activity, although hypotriglyceridemic activity was retained by some of the resulting glycerides (Table VII).

Discussion

Detailed pharmacological and pharmacokinetic studies have been undertaken on 1-[*p*-(myristyloxy)-α-methylcinnamoyl]glycerol (**97**, LK-903) in our laboratories.[3-5] We found that LK-903 gave higher blood concentrations after an oral dose than the corresponding parent acid [*p*-(myristyloxy)-α-methylcinnamic acid] **9**, and they both were transported by way of the lymphatic route like naturally occurring lipids (unpublished observation). Therefore, we concluded that one of the important factors that determine the activities of these drugs is absorbability from the intestinal tract. It has been established that monoglycerides and fatty acids are the predominant forms of the products of triglyceride digestion that are taken up by the intestinal mucosa.[10] Evidence is available indicating that α-monoglycerides are better utilized than free fatty acids in the synthesis of triglyceride by intestinal walls.[11] This may be related to the higher hypolipidemic activities of the 1-monoglycerides as compared to those of the free cinnamic acids, since we found that LK-903 and its free acid **9** were both incorporated into circulating di- and triglycerides after oral administration in the rat (unpublished observation). Similar incorporation of *p*-(alkoxyaryl)carboxylic acids into tissue lipids has been reported.[12]

(10) J. R. Senior, *J. Lipid Res.*, **5**, 495 (1964).
(11) J. M. Lohnson and J. L. Brown, *Biochim. Biophys. Acta*, **59**, 500 (1962).

Table II

no.	R^{1a}	R^2	R^3	prepn method[b]	% yield[c]	mp, °C	recrystn solvent	formula[d]	activity: dose, mg %	activity: days	activity: CHO[e]	activity: TG[f]
16	p-$C_{14}H_{29}$	CH_3	H	C	35	87–88	EtOH	$C_{24}H_{38}O_3$	50	7	5	41[h]
17	o-$C_{14}H_{29}$	H	CH_3	C	41	64–66	ligroin	$C_{24}H_{38}O_3$	50	7	−9	7
18	m-$C_{14}H_{29}$	H	CH_3	A	77	94–95	$CHCl_3$	$C_{24}H_{38}O_3$	50	7	−1[g]	−11[g]
19	m-CH_3, p-$C_{12}H_{25}$	H	CH_3	A	68	98–99	$CHCl_3$–ligroin	$C_{23}H_{36}O_4$	50	7	2	−31
20	m-CH_3, p-$C_{14}H_{29}$	H	CH_3	A	52	99–100	$CHCl_3$–ligroin	$C_{25}H_{40}O_4$	50	7	−6	−15

[a–f] See corresponding footnotes in Table I. [g] Mean of two experiments. [h] Statistically significant depression with $p < 0.05$.

Experimental Section

Biological Method. Male Sprague–Dawley rats (4 weeks of age) were purchased from Nihon CLEA Co., Tokyo, and maintained on commercial laboratory chow (Nihon CLEA CE-2 pellets) for at least 1 week before use. Grouping of rats (generally five rats per group), blood sampling, and calculation of the hypolipidemic effect were performed as described previously.[13]

Test compounds were mixed with Nihon CLEA CE-2 powder in a mortar and administered ad libitum to experimental groups generally for a period of 7 days. The concentration of a test compound in the diet is expressed in the tables as mg/100 g (mg %). This number is found to be approximately equal to the dose expressed as (mg/kg of body weight)/day calculated from the amount of daily food consumption. Control rats were fed CE-2 powder. After the experimental period, total serum cholesterol and triglyceride were determined by the methods of Zak et al.[14] and Ryan and Rasho,[15] respectively. The hypolipidemic activity of a test compound is expressed in the tables as percent depressions[13] of serum cholesterol and triglyceride compared to the mean lipid levels of the control group after the experimental period. The average mean levels of serum cholesterol and triglyceride of the control group in 25 experiments were 84 ± 1 and 79 ± 3 mg/100 mL, respectively. The result of statistical analysis for each depression is given in a footnote (significant depression when $p < 0.05$) for the test compounds whose activities were determined in a single experiment.

Chemistry. Melting points were determined in an electrically heated oil bath and were uncorrected. IR and UV spectra were taken on a Hitachi infrared spectrophotometer EPI-S and a Hitachi ESP-2U, respectively. NMR spectra were recorded on a Hitachi Perkin-Elmer R-204 instrument with tetramethylsilane as the internal standard. Solutions were dried over anhydrous $MgSO_4$. Evaporation was carried out under reduced pressure. p-Hydroxycinnamic acid,[16] p-hydroxy-α-methylcinnamic acid,[17] and p-phenoxycinnamic acid[18] (14) were prepared by the methods described in the literature.

p-(Myristyloxy)-α-methylcinnamic Acid (9; Method A). The following experiment illustrates the general procedure used to prepare the p-alkoxy acids of Tables I and II. A solution of 35.6 g (0.2 mol) of p-hydroxy-α-methylcinnamic acid, 60.9 g (0.22 mol) of myristyl bromide, and 26.9 g (0.48 mol) of KOH in 300 mL of 2-methoxyethanol was heated at 90–95 °C for 3 h. To the reaction mixture was added a solution of 11.2 g (0.2 mol) of KOH in 18 mL of H_2O, and the mixture was refluxed for an additional 1.5 h. After cooling the mixture, the precipitated potassium salt was collected by filtration, washed with cold EtOH, and converted to the free acid by treating with 150 mL of hot AcOH. After cooling the free acid, the crystals were collected by filtration, washed with H_2O, and dried. Recrystallization yielded 22.4 g of **9** (Table I).

p-Phenoxy-α-methylcinnamic Acid (15; Method B). The following experiment illustrates the general procedure used to prepare 14 and 15. A mixture of 27.7 g (0.14 mol) of p-phenoxybenzaldehyde, 21.8 g (0.175 mol) of propionic anhydride, and 13.5 g (0.14 mol) of sodium propionate was heated with occasional shaking at 130–135 °C for 30 h. The hot reaction mixture was poured into about 300 mL of ice–water, and the precipitate was filtered and washed with H_2O. The product was mixed with a solution of 10 g of KOH in 30 mL of EtOH and refluxed for 1.5 h. After cooling the solution, the precipitated salt was filtered,

(12) R. A. Parker, T. Kariya, J. M. Grisar, and V. Petrow, *J. Med. Chem.*, **20**, 781 (1977).
(13) K. Takashima, K. Izumi, H. Iwai, and S. Takeyama, *Atherosclerosis*, 17, 491 (1973).
(14) B. Zak, R. C. Dickman, E. G. White, H. Burnett, and P. J. Cherney, *Am. J. Clin. Pathol.*, **24**, 1307 (1954).
(15) W. G. Ryan and O. M. Rasho, *Clin. Chem.*, **13**, 769 (1967).
(16) C. G. Overberger, E. J. Luhrs, and P. K. Chien, *J. Am. Chem. Soc.*, 72, 1201 (1950).
(17) J. R. Johnson, *Org. React.*, 1, 251 (1952).
(18) E. Massarani, D. Nardi, L. Murai, and G. Cavallini, *Farmaco, Ed. Sci.*, 18(8) 582 (1963), *Chem. Abstr.*, **59**, 11465 (1963).
(19) R. Stoermer and Fr. Wodarg, *Ber. Dtsch. Chem. Ges.*, 61, 2323 (1928).
(20) G. W. Gray and B. Jones, *J. Chem. Soc.*, 1467 (1954).

Table III

$R^1O-C_6H_4-C(R^2)=C(R^3)-CO_2R^4$

no.	R^1 [a]	R^2	R^3	R^4	prepn method[b]	% yield[c]	bp (mmHg) or mp, °C	recrystn solvent	formula[d]	activity: dose, mg %	activity: days	activity: CHO[e]	activity: TG[f]
21	$C_{14}H_{29}$	H	CH_3	CH_3	D	85	62–64	Et_2O–MeOH	$C_{25}H_{40}O_3$	50	7	10	44[m]
22	$C_{14}H_{29}$	H	CH_3	C_2H_5	D	77	41–43	pet. ether–Et_2O	$C_{26}H_{42}O_3$	50	7	7	34
										20	7	6	37
23[l]	C_8H_{17}	H	H	$CH=CH_2$	D	45	48–49	pet. ether–Et_2O	$C_{19}H_{26}O_3$	100	7	–6	
24	$C_{16}H_{33}$	H	H	$C_{16}H_{33}$	D	69	76–78	Et_2O–MeOH	$C_{41}H_{72}O_3$	50	7	11	27
25	$C_{16}H_{33}$	H	CH_3	Ph	D	84	62	Et_2O–MeOH	$C_{32}H_{46}O_3$	50	7	12	46
26	C_8H_{17}	H	CH_3	CH_2Ph	D	84	42–44	Et_2O–MeOH	$C_{25}H_{32}O_3$	50	7	5	18
27	$C_{12}H_{25}$	H	CH_3	CH_2–pyridyl	D	48	62–63	ligroin	$C_{38}H_{39}O_3N$	50	7	24	60[m]
28	$C_{14}H_{29}$	H	CH_3	$CH_2CH_2N(CH_3)_2 \cdot HCl$	D	77	118–120	$CHCl_3$–MeOH	$C_{28}H_{48}O_3NCl$	50	7	8	35
29	C_4H_9	H	H	CH_2CH_2-c-$NC_5H_{10} \cdot HCl$	D	75	186	EtOH	$C_{20}H_{30}O_3NCl$	100	7	3	2
30	C_4H_9	H	H	CH_2CH_2-c-$N(CH_2CH_2)_2O \cdot HCl$	D	60	196–196.5	EtOH	$C_{19}H_{28}O_4NCl$	100	3	–9	–32
31	$C_{14}H_{29}$	H	CH_3	$CH_2CH_2CO_2CH_3$	D	41	56–58	Et_2O–MeOH	$C_{28}H_{44}O_5$	50	7	30[m]	37
										20	7	–1	7
32	C_4H_9	H	H	$CH_2CH_2SO_3Na$	D	88	230	50% MeOH	$C_{15}H_{19}O_6SNa$	100	7	–1	9
33	$C_{12}H_{25}$	H	CH_3	$CH_2CH_2SO_3Na$	D	81	300	80% EtOH	$C_{24}H_{37}O_6SNa$	50	7	27	63
34	$C_{16}H_{33}$	H	H	$CH_2CH_2SO_3Na$	D	54	300	80% EtOH	$C_{27}H_{43}O_6SNa$	100	7	17	47
35	$C_{16}H_{33}$	H	CH_3	$CH_2CH_2SO_3Na$	D	26	300	80% EtOH	$C_{28}H_{45}O_6SNa$	100	3	8	36[m]
36	CH_3	H	H	CH_2CH_2Cl	D	41	152–153 (1.5)		$C_{12}H_{13}O_3Cl$	100	7	0	
37	$CH_2=CH$-CH_2	H	H	CH_2CH_2Cl	D	72	54–55	EtOH	$C_{14}H_{15}O_3Cl$	100	7	–9	26
38	C_4H_9	H	H	CH_2CH_2Cl	D	80	55–56	EtOH	$C_{15}H_{19}O_3Cl$	100	7	0	–26
39	C_8H_{17}	H	H	CH_2CH_2Cl	D	65	54–55	EtOH	$C_{19}H_{27}O_3Cl$	100	7	5	
40	$C_{12}H_{25}$	H	H	CH_2CH_2Cl	D	85	52–53	EtOH	$C_{23}H_{35}O_3Cl$	50	7	16	46[m]
41	$C_{12}H_{25}$	H	CH_3	CH_2CH_2Cl	D	71	48–49	benzene	$C_{24}H_{37}O_3Cl$	50	7	21[m]	42[m]
42	$C_{14}H_{29}$	H	H	CH_2CH_2Cl	D	79	65–67	EtOH	$C_{25}H_{39}O_3Cl$	50	7	11	–1
43	$C_{16}H_{33}$	H	H	CH_2CH_2Cl	D	82	66–68	EtOH	$C_{27}H_{43}O_3Cl$	50	7	–4	43
										20	7	17	17
44	$C_{16}H_{33}$	H	CH_3	CH_2CH_2Cl	D		60–62	EtOH	$C_{28}H_{45}O_3Cl$	100	3	24[m]	48[m]
										50	3	20	26
										20	7	20[l]	5
45	$C_{18}H_{35}$[g]	H	H	CH_2CH_2Cl	D		72–74	EtOH–Me_2CO	$C_{29}H_{45}O_3Cl$	100	7	0	
46	Ph	H	H	CH_2CH_2Cl	D	73	58–59	EtOH	$C_{17}H_{25}O_3Cl$	20	7	18[i]	21[i]

47	Ph	H	CH_3	CH_2CH_2Cl	D	83	176-178 (0.04)	EtOH	$C_{18}H_{17}O_3Cl$	50	7	14	-6
										20	7	15[i]	22[i]
48	$PhCH_2$	H	H	CH_2CH_2Cl	D	64	159-161	benzene	$C_{18}H_{17}O_3Cl$	100	7	-3	
49	$C_{16}H_{33}$	H	H	$(CH_2)_3Cl$	D	66	46-48	EtOH	$C_{28}H_{45}O_3Cl$	100	7	22[i]	44[i]
										50	7	5	48
										20	7	1	38
50	$C_{16}H_{33}$	H	CH_3	$(CH_2)_3Cl$	D	81	57-59		$C_{29}H_{47}O_3Cl$	100	3	17	25
51	$C_{18}H_{37}$	H	H	$(CH_2)_3Cl$	D	92	51-53		$C_{30}H_{49}O_3Cl$	50	7	9	3
52	$C_{18}H_{37}$	H	CH_3	$(CH_2)_3Cl$	D	77	49-50		$C_{31}H_{51}O_3Cl$	50	7	0	11
53	Ph	H	H	$(CH_2)_3Cl$	D	72	208-210 (0.2)		$C_{18}H_{17}O_3Cl$	100	6	32[m]	54[m]
										20	7	17	-2
										10	7	4[i]	13[i]
54	C_4H_9	H	H	CH_2CH_2Br	D	87	46-47	MeOH	$C_{15}H_{19}O_3Br$	100	7	9	26[m]
55	$C_{16}H_{33}$	H	H	CH_2CH_2Br	D	73	70-72	EtOH	$C_{27}H_{43}O_3Br$	100	7	7	6
56	C_4H_9	H	H	CH_2CH_2OH	E	75	190-191 (1.5)		$C_{15}H_{20}O_4$	100	7	9	26
57	$C_{14}H_{29}$	H	H	CH_2CH_2OH	E	37	70-71	EtOH	$C_{25}H_{40}O_4$	50	7	10	24
58	$C_{16}H_{33}$	H	H	CH_2CH_2OH	E	32	68-69	EtOH	$C_{27}H_{44}O_4$	100	7	24[m]	57[m]
										20	7	18[i]	26[i]
59	$C_{16}H_{33}$	H	CH_3	CH_2CH_2OH	E	27	61-62	EtOH	$C_{28}H_{46}O_4$	100	3	13	59[m]
60	$C_{16}H_{33}$	H	H	$(CH_2)_3OH$	E	67	102-103	EtOH	$C_{28}H_{46}O_4$	100	3	16	16[m]
61	$C_{16}H_{33}$	H	CH_3	$(CH_2)_3OH$	E	58	48-49	EtOH	$C_{29}H_{48}O_4$	100	3	6	43[m]
62	$C_{18}H_{37}$	H	CH_3	$(CH_2)_3OH$	E	51	74-76	EtOH	$C_{31}H_{52}O_4$	50	7	13	14
63	C_4H_9	H	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	71	27	MeOH	$C_{19}H_{24}O_5$	100	3	14[m]	33
										50	7	9	17
64	C_8H_{17}	H	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	73	44-46	EtOH	$C_{23}H_{32}O_5$	100	7	16	-11
65	$C_{12}H_{25}$	H	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	42	34-35	EtOH	$C_{27}H_{40}O_5$	50	7	13	34[m]
66	$C_{12}H_{25}$	H	CH_3	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	55	oil[h]		$C_{28}H_{42}O_5$	50	7	17[m]	43[n]
67	$C_{16}H_{33}$	H	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	60	46-48	EtOH	$C_{31}H_{48}O_5$	100	7	27 ± 2[k]	65 ± 12[k]
										50	7	13[i]	42[i]
										20	7	16 ± 9[k]	38 ± 17[k]
										10	7	5	15
68	$C_{16}H_{33}$	CH_3	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	83	36-38	EtOH	$C_{32}H_{50}O_5$	100	7	11	12
69	$C_{16}H_{33}$	H	CH_3	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	65	46-48	EtOH	$C_{32}H_{50}O_5$	100	7	35[m]	62[n]
										50	3	25	27
										20	7	13[i]	12[i]
										10	7	3	10
70	$C_{18}H_{35}$[g]	H	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	68	oil[h]		$C_{33}H_{50}O_5$	100	5	-13	21
71	Ph	H	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	59	185-187 (0.5)		$C_{21}H_{20}O_5$	100	3	14	-15
72	$PhCH_2$	H	H	$(CH_2)_2O_2CC(CH_3)=CH_2$	D	55	64-65	EtOH	$C_{22}H_{22}O_5$	100	7	13	33
73	$C_{16}H_{33}$	H	H	$(CH_2)_2O_2CCH=CH_2$	F	67	45-46	EtOH	$C_{30}H_{46}O_5$	100	7	6	
74	$C_{16}H_{33}$	H	H	$(CH_2)_2O_2CCH=CHCH_3$	F	37	68-70	EtOH	$C_{31}H_{48}O_5$	100	7	3	11
75	$C_{16}H_{33}$	H	H	$(CH_2)_3O_2CC(CH_3)=CH_2$	F	60	61	EtOH	$C_{32}H_{50}O_5$	100	7	16	0
clofibrate										100	7	22 ± 3[j]	41 ± 6[j]
										50	7	14 ± 5[k]	35 ± 10[k]

[a-f] See corresponding footnotes in Table I. [g] $C_{18}H_{35}$ = oleyl. [h] Isolated by alumina chromatography. A single spot on TLC (benzene). [i-k] Means of two, eight, and three experiments, respectively. [l] Compound 23 was prepared by treatment of 39 with quinoline. [m] Statistically significant depression with $p < 0.05$.

Table IV

$R^1O-C_6H_4-CH=C(R^2)CO_2(CH_2)_nO_2CC(R^2)=CH-C_6H_4-OR^1$

no.	R^1 [a]	R^2	n	prepn[b] method	% yield[c]	mp, °C	recrystn solvent	formula[d]	dose, mg %	days	CHO[e]	TG[f]
									activity			
76	C_8H_{17}	H	2	D	30	96–99	Et_2O	$C_{36}H_{50}O_6$	50	7	4	9
77	$C_{14}H_{29}$	CH_3	2	D	32	102–103	$CHCl_3$–MeOH	$C_{50}H_{78}O_6$	50	7	−1	−9
78	$C_{16}H_{33}$	H	2	D	45	88–90	benzene	$C_{52}H_{82}O_6$	100	3	9[g]	−4
79	$C_{16}H_{33}$	H	3	D	43	77–79	EtOH	$C_{53}H_{84}O_6$	100	3	8	−2

[a–f] See corresponding footnotes in Table I. [g] Statistically significant depression with $p < 0.05$.

dissolved in about 300 mL of H_2O, and acidified with 20% HCl. The white solid was washed with H_2O and recrystallized, yielding 12.9 g of **15** (Table I).

***p*-(Myristyloxy)-β-methylcinnamic Acid (16; Method C).** A solution of 1.68 g (0.01 mol) of ethyl bromoacetate and 3.32 g (0.01 mol) of *p*-(myristyloxy)acetophenone in 20 mL of benzene was prepared. To about 5 mL of this solution was added 0.65 g (0.01 mol) of purified zinc dust. A spatula full of $HgCl_2$ was introduced, and the mixture was heated on a steam bath until reaction started. The remainder of the solution was added at such a rate that gentle refluxing took place. After the addition was complete, stirring and refluxing were continued for 2 h. The mixture was cooled to room temperature and poured into 20 mL of ice-cold 10% H_2SO_4 with vigorous stirring. The benzene layer was separated and the aqueous layer was extracted with two 10-mL portions of benzene. The combined benzene extracts were washed successively with H_2O, dilute aqueous ammonia, and H_2O. The benzene solution was dried and evaporated. The residual oil was dissolved in 20 mL of benzene and, after the addition of 1.2 g (0.11 mol) of P_2O_5, refluxed for 2 h. After cooling the mixture, the organic layer was separated, washed with H_2O, and dried. The residue obtained by evaporation of the benzene was dissolved in 15 mL of EtOH and to this was added 1.5 mL of 50% aqueous KOH solution. The mixture was refluxed for 1.5 h. After cooling the mixture, crystals of the potassium salt of **16** were collected and washed with dilute HCl and H_2O. Recrystallization yielded 1.3 g of **16** (Table II).

***o*-(Myristyloxy)-α-methylcinnamic Acid (17; Method C).** The treatment of a mixture of 9 g (0.03 mol) of o-(myristyloxy)benzaldehyde and 6 g (0.03 mol) of ethyl α-bromopropionate with 1.96 g (0.03 mol) of zinc dust and a trace of $HgCl_2$ as in **16** produced **17** (Table II).

Sodium *O*-*p*-Butoxycinnamoylisethionate (32; Method D). The following experiment illustrates the general procedure used to prepare **32–35**. To a solution of 2.2 g (0.01 mol) of *p*-butoxycinnamic acid in 16 mL of benzene was added 73 mg (0.001 mol) of DMF and 0.24 g (0.02 mol) of $SOCl_2$, and the mixture was warmed at 50 °C for 1 h. The reaction mixture was condensed to a crude oil of the acid chloride. This was dissolved in 20 mL of benzene and, after the addition of 1.5 g (0.01 mol) of sodium isethionate, refluxed for 4 h. The separated crystals were collected and washed with benzene. Recrystallization afforded 3.1 g of **31** (Table III).

γ-Chloropropyl *p*-Cetoxycinnamate (49; Method D). The following experiment illustrates the general procedure used to prepare **36–55**. *p*-Cetoxycinnamoyl chloride was prepared by a procedure similar to that used for **32**. A solution of 3.78 g (0.04 mol) of triethylene chlorohydrin in 30 mL of benzene was added to a stirred solution of 8.1 g (0.02 mol) of *p*-cetoxycinnamoyl chloride in 70 mL of benzene and further stirred under reflux for 3 h. After removal of volatile materials, crystallization of the residual oil afforded 6.1 g of **49** (Table III).

γ-Hydroxypropyl *p*-Cetoxycinnamate (60; Method E). The following experiment illustrates the general procedure used to prepare **56–62**. A solution of 6.1 g (0.012 mol) of $AgNO_3$ in 10 mL of H_2O was added to a solution of 5.6 g (0.012 mol) of **49** in 100 mL of EtOH and refluxed for 7 h. The insoluble materials were removed by filtration, and the filtrate was concentrated. Crystallization of the residual oil afforded 3.6 g of **60** (Table III).

β-(Methacryloxy)ethyl *p*-Cetoxycinnamate (67; Method D). The following experiment illustrates the general procedure used to prepare **63–72**. A solution of 8.1 g (0.02 mol) of *p*-cetoxycinnamoyl chloride in 50 mL of benzene was added dropwise to a solution of 3.3 g (0.025 mol) of β-hydroxyethyl methacrylate in 10 mL of benzene and refluxed for 1 h. The solvent was evaporated, and the residue was washed with H_2O. Recrystallization afforded 5.6 g of **67** (Table III).

Glycol Bis(*p*-cetoxycinnamate) (78; Method D). A solution of 4.1 g (0.01 mol) of *p*-cetoxycinnamoyl chloride in 20 mL of dioxane was added to a solution of 0.31 g (0.05 mol) of ethylene glycol in 10 mL of dioxane and stirred for 5 h under reflux. The mixture was evaporated, and the residue was crystallized to yield 1.8 g of **78** (Table IV).

1-[*p*-(Myristyloxy)-α-methylcinnamoyl]-2,3-isopropylideneglycerol (81) and 1-[*p*-(Myristyloxy)-α-methylcinnamoyl]glycerol (97; Method G). Oily crude acid

Table V

$R^1\text{-}C_6H_4\text{-}CH{=}C(R^2)CO_2CH_2CH(OH)CH_2OH$

no.	R[1a]	R[2]	prepn[b] method	% yield[c]	mp, °C	recrystn solvent	formula[d]	activity: dose, mg %	activity: days	activity: CHO[e]	activity: TG[f]
84	H	H	G	23	oil	AcOEt[g]	$C_{12}H_{14}O_4$	100	6	6	16
85	H	CH_3	G	37	oil	AcOEt[g]	$C_{13}H_{16}O_4$	100	3	10[m]	22
86	CH_3O	H	G	30	38-40	AcOEt[g]	$C_{13}H_{16}O_5$	100	6	4	31[m]
87	$CH_2{=}CHCH_2O$	H	G	37	55-57	AcOEt[g]	$C_{15}H_{18}O_5$	100	3	12	-14
88	C_4H_9O	H	G	39	45-57	AcOEt[g]	$C_{16}H_{22}O_5$	100	6	7	15
89	$C_8H_{17}O$	H	G	72	45-47	AcOEt,[g] benzene	$C_{20}H_{30}O_5$	100	6	7	15
90	$C_8H_{17}O$	CH_3	G	55	59-60	EtOH	$C_{21}H_{32}O_5$	50	7	8	21
91	$C_{10}H_{21}O$	H	G	45	oil	AcOEt[g]	$C_{22}H_{34}O_5$	50	7	-3	14
92	$C_{10}H_{21}O$	CH_3	G	55	62-63	AcOEt-pet. ether	$C_{22}H_{36}O_5$	50	7	10	52[m]
93	$C_{12}H_{25}O$	H	G	58	39-41	AcOEt[g]	$C_{24}H_{38}O_5$	50	7	17	38[m]
94	$C_{12}H_{25}O$	CH_3	G	31	58-60 83-84	EtOH	$C_{25}H_{40}O_5$	50	7	26 ± 5[j]	53 ± 15[j]
								20	7	16[i]	33[i]
								10	7	9	10
95	$C_{13}H_{27}O$	CH_3	G	55	84-86	EtOH	$C_{26}H_{42}O_5$	50	7	30[m]	53[m]
96	$C_{14}H_{29}O$	H	G	69	68-70	ligroin	$C_{26}H_{42}O_5$	50	7	26[m]	24
97	$C_{14}H_{29}O$	CH_3	G	72	86-87	ligroin	$C_{27}H_{44}O_5$	100	7	45[m]	52[m]
								50	7	25 ± 1[l]	43 ± 6[l]
								20	7	14 ± 3[k]	33 ± 6[k]
98[h]	$C_{14}H_{29}O$	CH_3	G	9.7	59-62	Et_2O-pet. ether	$C_{27}H_{44}O_5$	50	7	-2	9
99	$C_{16}H_{33}O$	H	G	65	86-88	AcOEt	$C_{28}H_{46}O_5$	100	7	26[m]	62[m]
								50	7	19[i]	17[i]
								20	7	9 ± 6[j]	8 ± 2[j]
								10	7	-4	11
100	$C_{16}H_{33}O$	CH_3	G	56	88-90	AcOEt	$C_{29}H_{48}O_5$	100	3	32[m]	68[m]
								50	7	15 ± 3[j]	28 ± 3[j]
								20	7	11[i]	5[i]
101	$C_{18}H_{37}O$	CH_3	G	44	94-95	AcOEt	$C_{31}H_{52}O_5$	50	7	-1	3
102	PhO	H	G		48-50	Et_2O	$C_{18}H_{18}O_5$	50	7	11	29[m]
								20	7	15[i]	26[i]
103	PhO	CH_3	G	50	oil	$CHCl_3$[g]	$C_{19}H_{20}O_5$	50	7	14	4
								20	7	7[i]	-1[i]
104	Ph	CH_3	G	60	111-112	AcOEt	$C_{19}H_{20}O_4$	50	7	16	-17
105	*p*-Cl-Ph	CH_3	G	35	105-107 117-119	AcOEt	$C_{19}H_{19}O_4Cl$	50	7	11[m]	18
106	$C_{14}H_{29}O\text{-}C_6H_4\text{-}CH{=}C(CH_3)CO_2CH(CH_2OH)CH_2OH$			74	81-83	ligroin	$C_{27}H_{44}O_5$	50	7	9	38[m]

[a-f] See corresponding footnotes in Table I. [g] Eluting system used for silica gel chromatography. [h] The cis isomer of 97. [i-l] Mean levels ± SE of 2, 3, 8, and 14 experiments, respectively. [m] Statistically significant depression with $p < 0.05$.

Table VI

$R^1O-C_6H_4-CH=C(CH_3)CO_2CH_2CHCH_2$ (O,O-isopropylidene)

no.	R^{1a}	% yield^c	mp, °C	recrystn solvent	formula^d	activity: dose, mg %	days	CHO^e	TG^f
80	$C_{12}H_{25}$	65	42-43	EtOH	$C_{28}H_{44}O_5$	50	7	16	41[g]
81	$C_{14}H_{29}$	70	49-50	EtOH	$C_{30}H_{48}O_5$	50	7	9	-2
82	$C_{16}H_{33}$	73	54-55	EtOH-AcOEt (4:1)	$C_{32}H_{52}O_5$	50	7	1	3
83	$C_{14}H_{29}O-C_6H_4-CH=C(CH_3)CO_2CH(CH_2O)_2CHC_6H_5$	78	84-86	Me_2CO-EtOH	$C_{34}H_{48}O_5$				

[a-f] See corresponding footnotes in Table I. [g] Statistically significant depression with $p < 0.05$.

Table VII

$R^1O-C_6H_4-CH=C(R^2)CO_2CH_2CH(OR^3)CH_2O_2CR^4$

no.	R^{1a}	R^2	R^3	R^4	prepn^b method	% yield^c	mp, °C	recrystn solvent	formula^d	activity: dose, mg %	day	CHO^e	TG^f
107	$C_{14}H_{29}$	CH_3	H	pyridyl	I	53	77-78	$CHCl_3$-EtOH	$C_{33}H_{47}O_6N$	50	7	4	33[h]
108	$C_{14}H_{29}$	CH_3	H	$C_{15}H_{31}$	I	40	75-77	Me_2CO	$C_{43}H_{74}O_6$	75.8	7	3[g]	-19[g]
109	$C_{14}H_{29}$	CH_3	H	X	I	36	63-65	Et_2O-pet. ether	$C_{51}H_{80}O_7$	50	7	6	6
110	C_8H_{17}	H	$C_{13}H_{27}CO$	$C_{13}H_{27}$	I	78	56-57	Et_2O	$C_{48}H_{82}O_7$	50	7	-7	10
111	$C_{12}H_{25}$	CH_3	PhCO	Ph	I	76	49-50	EtOH	$C_{39}H_{48}O_7$	50	7	3	47[h]
112	$C_{14}H_{29}$	CH_3	CH_3CO	CH_3	I	79	38-39	EtOH	$C_{31}H_{48}O_7$	50	7	4	41[h]
113	$C_{14}H_{29}$	CH_3	pyridyl-CO	pyridyl	I	63	83-84	EtOH	$C_{39}H_{50}O_7N_2$	50	7	7	17
114	$C_{14}H_{29}$	CH_3	$C_{15}H_{31}CO$	$C_{15}H_{31}$	I	64	68-70	Et_2O-MeOH	$C_{81}H_{120}O_7$	102	7	18[g]	5[g]
115	$C_{16}H_{33}$	H	C_3H_7CO	C_3H_7	I	83	52-53	MeOH	$C_{36}H_{58}O_7$	50	7	8	36[h]
116	$C_{12}H_{25}$	CH_3	Y-CO	Y	I	23	56-59	Et_2O-pet. ether	$C_{69}H_{104}O_9$	50	7	2	28[h]
117	$C_{16}H_{33}$	CH_3	Z-CO	Z	I	30	68-70	Et_2O-pet. ether	$C_{83}H_{128}O_9$	50	7	0	9

[a-f] See corresponding footnotes in Table I. [g] Mean of two experiments. [h] Statistically significant depression with $p < 0.05$. [i] X = $C_{14}H_{29}O$-Ph-CH=C(CH_3)–; Y = $C_{12}H_{25}O$-Ph-CH=C(CH_3)–; Z = $C_{16}H_{33}O$-Ph-CH=C(CH_3)–.

chloride was prepared from 3.75 g (0.01 mol) of **9** by a procedure similar to that for **32**. This was dissolved in 30 mL of benzene and heated to a refluxing temperature. To the boiling mixture was added portionwise 1.72 g (0.013 mol) of 1,3-isopropylideneglycerol under efficient stirring. During the addition period, the benzene was distilled slowly to remove the vapor of HCl produced from the reaction mixture. After the addition was complete, the mixture was heated for an additional 2 h under slow distillation of the benzene and condensed to afford the crude **81**, which was used usually for the subsequent reactions. This crude product was purified by column chromatography on silica gel with $CHCl_3$ as eluent. The fraction which had R_f 0.5 (silica gel F_{254} Merck, with the same solvent) was collected and crystallized to afford pure **81** (Table VI). A mixture of the above crude **81**, 6.18 g (0.1 mol) of H_3BO_3, and 25 mL of 2-methoxyethanol was heated on a boiling water bath for 1 h. After cooling the mixture, about 50 mL of H_2O was added, and the precipitated solid products were collected, washed with H_2O, and crystallized to afford **97** (Table V): TLC [silica gel G impregnated with 10% H_3BO_3, $CHCl_3$–Me_2CO (9:1)] R_f 0.14; UV (EtOH) λ_{max} 296 nm (ϵ 44065); IR (KBr) 3350 (OH), 1698 (ester C=O); NMR ($CDCl_3$) 7.67 ppm (olefinic proton).

2-[*p*-(Myristyloxy)-α-methylcinnamoyl]-1,3-benzylideneglycerol (83) and 2-[*p*-(Myristyloxy)-α-methylcinnamoyl]glycerol (106; Method H). To a solution of the acid chloride, prepared from 3.75 g (0.01 mol) of **9** in 25 mL of $CHCl_3$, were added 2.34 g (0.013 mol) of 1,3-benzylideneglycerol and 1.52 g (0.02 mol) of pyridine under cooling at 0–5 °C. The reaction mixture was allowed to stand for 12 h at room temperature and about 25 mL of $CHCl_3$ was added. The mixture was washed with H_2O and dried. The residue obtained by evaporation of the solvent was purified by column chromatography on silica gel with $CHCl_3$ as eluent and crystallized to afford **83** (Table VI). The benzylidene group of **83** was removed by treatment with H_3BO_3 in 2-methoxyethanol as described for the removal of the isopropylidene group (method G). The crude product was recrystallized to afford pure **106** (Table V).

Cis Isomer of 97 (98). Compound **97** (8.06 g, 0.018 mol) was dissolved in 500 mL of EtOH and irradiated with a xenon lamp for 40 h. After the solvent was evaporated, the residue was recrystallized several times, yielding 780 mg of **98** (Table V): TLC [silica gel G impregnated with 10% H_3BO_3, $CHCl_3$–Me_2CO (9:1)] R_f 0.22; UV (EtOH) λ_{max} 282 nm (ϵ 40990); IR (KBr) 3350 (OH), 1720 (ester C=O); NMR ($CDCl_3$) 6.75 ppm (olefinic proton).

Monopalmitate of 97 (108; Method I). To a mixture of 4.49 g (0.01 mol) of **97**, 1.58 g (0.02 mol) of pyridine, and 50 mL of $CHCl_3$ was added 2.75 g (0.02 mol) of palmitoyl chloride. After standing overnight, the solvent was evaporated and the residue was crystallized by adding a small amount of MeOH. These crude crystals were chromatographed on silica gel, $CHCl_3$–Me_2CO (9:1) as the eluent to yield **108** (Table VII): NMR ($CDCl_3$) 4.25 (m, 5 H protons attached to glycerol), 2.7 ppm (m, 1 H, OH).

Folate Analogues Altered in the C^9–N^{10} Bridge Region. 14. 11-Oxahomofolic Acid, a Potential Antitumor Agent

M. G. Nair,* Colleen Saunders, Shiang-Yuan Chen,

Department of Biochemistry, College of Medicine, University of South Alabama, Mobile, Alabama 36688

Roy L. Kisliuk, and Y. Gaumont

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111.
Received July 16, 1979

The chemical synthesis of 11-oxahomofolic acid (**2**) has been carried out using an unambiguous procedure. Reaction of methyl *p*-hydroxybenzoate with β-propiolactone gave 3-[*p*-(carbomethoxy)phenoxy]propionic acid (**7**), which was converted to 1-bromo-4-[*p*-(carbomethoxy)phenoxy]-2-butanone (**8**) by the Arndt–Eistert procedure. Protection of the carbonyl group of **8** as the oxime resulted in the formation of **10**, which on reaction with potassium phthalimide in the presence of crown-18 ether as a catalyst gave 1-phthalimido-4-[*p*-(carbomethoxy)phenoxy]-2-butanone oxime (**11**). Hydrazinolysis of **11** gave 1-amino-4-[*p*-(carbomethoxy)phenoxy]-2-butanone oxime (**4**), which was used as the key intermediate for the construction of 11-oxahomofolic acid (**2**) by modifications of the Boon and Leigh procedure. The dithionite reduction product of **2**, 7,8-dihydro-11-oxahomofolic acid, served as a substrate of *Lactobacillus casei* dihydrofolate reductase and exhibited a relative rate of 50% of the natural substrate under identical conditions. The catalytic reduction product of 11-oxahomofolic acid consisting of a mixture of diastereomers exhibited powerful antifolate activity against both MTX-sensitive and -resistant *L. casei* and *Streptococcus faecium*. The enzymatic reduction product of 7,8-dihydro-11-oxahomofolate having the "natural" configuration at C^6 exhibited good antifolate activity against both MTX-sensitive and -resistant strains of *L. casei* and *S. faecium*. This paper details the synthesis and preliminary biological evaluation of an antifol, which is a substrate of *L. casei* dihydrofolate reductase in its 7,8-dihydro form and the resulting enzymatic reduction product capable of inhibiting the growth of the same organism from which the enzyme was derived. Thus, 7,8-dihydro-11-oxahomofolic acid has been shown to be potentially capable of inducing a "lethal synthesis" in *L. casei*.

As early as 1961, Misra[1] and co-workers suggested the possibility of developing antitumor agents which might be capable of exhibiting selective toxicity against MTX-resistant tumor cells by exploiting the high levels of dihydrofolate reductase present in these cell lines. Ideally, the potential drug should be a substrate of this enzyme and the product thus formed should be capable of interfering with tetrahydrofolate utilization in such a manner that this process will lead to cytotoxicity.[2] If this concept could be realized in vivo, then it appeared possible to generate a cytotoxic drug preferentially in the resistant tumor cells as opposed to the normal ones.

This hypothesis was first put to experimental test when DeGraw and co-workers[3] synthesized homofolic acid (**1**). The 7,8-dihydro derivative of homofolic acid served as an

(1) Misra, D. K.; Humphreys, S. R.; Friedkin, M.; Goldin, A.; Crawford, E. J. *Nature (London)* **1961**, *189*, 39.

(2) Friedkin, M.; Crawford, E. J.; Plante, L. T. *Ann. N.Y. Acad. Sci.* **1971**, *186*, 209.

(3) DeGraw, J. I.; Marsh, J. P., Jr.; Acton, E. M.; Crews, O. P.; Mosher, C. W.; Fjuiwara, A. N.; Goodman, L. *J. Org. Chem.* **1965**, *30*, 3404.

0022-2623/80/1823-0059$01.00/0 © 1979 American Chemical Society