(s, 2 H); MS-FAB *m/e* (no parent ion), 309 (100); IR (KBr, cm^{-1}) 3436 (b), 1595, 1578, 1424, 1386, 1234. Anal. ($C_{25}H_{16}N_5O_2ClF$-Na·$0.4H_2O$) C, H, N.

Biological Assays. Experimental details for the determination of LTD_4 antagonist activity in the isolated guinea pig ileum are as reported in ref. 1a and 4o.

Acknowledgment. We thank the members of the Physical Chemistry Department for providing analytical data.

Registry No. **2**, 107813-59-2; **3**, 138813-28-2; **3a**, 138786-13-7; **4**, 138786-14-8; **4a**, 138786-15-9; **5**, 138786-16-0; **5a**, 138786-17-1; **10**, 78265-34-6; **11**, 22115-41-9; **12**, 53020-08-9; **13**, 59961-15-8; **14**, 25109-86-8; **15**, 138786-18-2; **15a**, 138786-19-3; **16**, 138786-20-6; **17**, 138786-21-7; **17**·Na, 138786-22-8; **17a**, 138786-23-9; **18**, 138786-24-0; **18**·Na, 138786-25-1; **18a**, 138786-26-2; **19**, 138786-27-3; **19**·Na, 138786-28-4; **19a**, 138786-29-5; **20**, 138786-30-8; **20**·Na, 138786-31-9; **20a**, 138786-32-0; **21**, 138786-33-1; **21**·Na, 138786-34-2; **21a**, 138786-35-3; **22**, 138786-36-4; **22**·Na, 138786-37-5; **22a**, 138786-38-6; **23**, 138786-39-7; **23**·Na, 138786-40-0; **23a**, 138786-41-1; **24**, 138786-42-2; **24**·Na, 138786-43-3; **24a**, 138786-43-3; **24b**, 138813-29-3; **25**, 138786-45-5; **25**·Na, 138786-46-6; **25a**, 138786-47-7; **26**, 138786-48-8; **26**·Na, 138786-49-9; **26a**, 138786-50-2; **26b**, 138786-51-3; **27**, 138786-52-4; **27**·Na, 138786-53-5; **27a**, 138786-54-6; **28**, 138786-55-7; **28**·Na, 138786-56-8; **28a**, 138813-30-6; **29**, 138786-57-9; **29**·Na, 138786-58-0; **29a**, 138813-31-7; **30**, 138786-59-1; **30**·Na, 138786-60-4; **30a**, 138786-61-5; **31**, 138786-62-6; **31**·Na, 138813-32-8; **32**, 138786-63-7; **32**·Na, 138786-64-8; **32a**, 138786-65-9; 2-[(triphenylphosphonio)methyl]quinoline chloride, 99651-30-6; 3-cyanobenzaldehyde, 24964-64-5; 3-cyanophenol, 873-62-1; 2-(bromomethyl)-7-chloroquinoline, 115104-25-1; 7-chloroquinaldine, 4965-33-7; methyl 2-(bromomethyl)benzoate, 2417-73-4; 2-(3-methoxy-2-methylphenyl)-4,4-dimethyl-2-oxazoline, 72623-17-7; methyl 4-(bromomethyl)-3-methoxybenzoate, 70264-94-7; 3-isochromanone, 4385-35-7; 2-(bromomethyl)benzothiazole, 106086-78-6; 5-fluoro-2-methylbenzoic acid, 33184-16-6; methyl 2-[(3-cyanophenyl)methyl]-5-fluorobenzoate, 138786-66-0; [2-(bromomethyl)phenyl]acetic acid, 13737-35-4.

Analogues of Natural Phloroglucinols as Antagonists against Both Thromboxane A_2 and Leukotriene D_4

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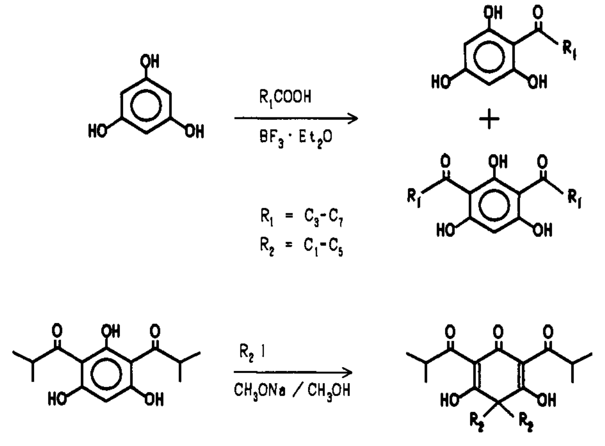
Antagonists against both thromboxane A_2 and leukotriene D_4 were prepared from phloroglucinol. These compounds showed almost the same activity as the chinesins which were isolated from *Hypericum chinense* L. The correlation between the structures and activity was studied in the synthesized and naturally occurring phloroglucinol derivatives.

Introduction

The plants belonging to the Guttiferae family are well-known folk medicines in Japan, having anodynic, staunching, and antiphlogistic properties. Previously, we found new antibacterial compounds, chinesin I (**1**), chinesin II (**2**),[1] otogirin (**3**), and otogirone (**4**)[2] from these plants (Figure 1). Compounds **1** and **2** were isolated from flowers of *Hypericum chinense* L. Compounds **3** and **4** were found in roots and flowers of *Hypericum erectum*, respectively. These compounds are derivatives of phloroglucinol, showing antimicrobial activity against Gram-positive microorganisms.[3] They also showed marked antiviral activity against both an RNA virus with envelope (vesicular stomatitis virus) and a DNA virus with envelope (herpes simplex virus type I).[3] Furthermore, we found that these compounds showed antagonistic activity against both thromboxane A_2 (TxA_2) and leukotriene D_4 (LTD_4) as evaluated by measuring the contraction of guinia pig trachea smooth muscle.[2] Especially, chinesins (a 3:1 mixture of **1** and **2**) and **4** showed strong activity in comparison with **3**.

Some allergic diseases involved with the IgE antibody are developed with chemical mediators such as histamine, leukotriene, and thromboxane. Leukotoriene mediates asthma,[4] psoriasis,[5] myocardial infarction,[6] endotoxin shock,[7] and heart anaphylaxis,[8] and thromboxane promotes platelet aggregation, blood vessel contraction, and bronchial contraction.[9,10] Antagonists against these chemical mediators are expected to be possible antiallergic agents.

Scheme I



† Laboratory of Bio-organic Chemistry.
‡ Pharmaceuticals Research Laboratories.

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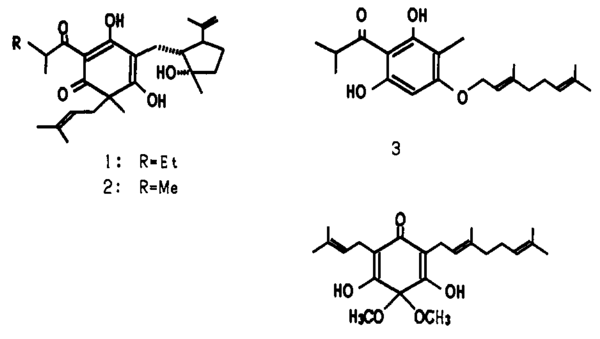
0022-2623/92/1835-1209$03.00/0



1: R=Et
2: R=Me

3

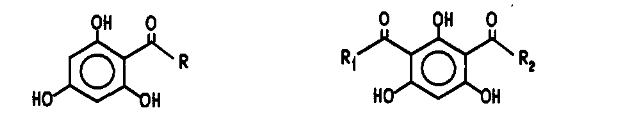
4

Figure 1. Natural phloroglucinol derivatives isolated from *Hypericum chinense* L. and *H. erectum*.

Desirable antiallergic compounds may widely inhibit these chemical mediators. Although a number of antagonists against LTD_4[11-18] or TxA_2[19-21] have been synthesized, it is interesting that the compounds isolated from Guttiferae are effective antagonists against both of them.

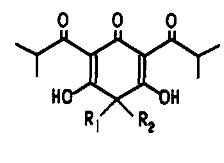
Table I. Effects of Natural Phloroglucinol Derivatives and Standard Inhibitors on U-46619- and LTD_4-Induced Contraction of Guinea Pig Trachea in the Magnus Test

		U-46619		LTD_4	
compounds	concn (M)	% inhibn (N)	IC_{50} (M)	% inhibn (N)	IC_{50} (M)
1 + 2 (3:1)	1×10^{-7}	23.6 (2)	7.2×10^{-7}	12.0 (2)	4.5×10^{-7}
	3×10^{-7}	31.6 (3)		37.8 (2)	
	1×10^{-6}	83.2 (2)		71.1 (2)	
3	1×10^{-4}	50.0 (2)		50.9 (2)	
	2×10^{-5}	10.8 (2)		12.6 (2)	
4	2×10^{-5}	94.9 (2)		63.6 (2)	
Ibudilast	1×10^{-5}			64.5 (3)	
PTA_2	3×10^{-8}	85.4 (3)			



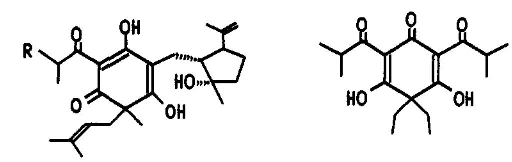
5: R=CH(CH3)2
6: R=CH2CH2CH3
7: R=CH2CH2(CH3)2
8: R=CH(CH3)CH2CH3
9: R=CH2CH2CH2CH2CH3

10: R1=R2=CH3
11: R1=R2=CH2CH3
12: R1=R2=CH(CH3)2
13: R1=R2=CH2CH2CH3
14: R1=R2=CH2CH(CH3)2
15: R1=R2=CH(CH3)CH2CH3
16: R1=R2=CH2CH2CH2CH2CH3
17: R1=R2=CH2CH2CH2CH2CH2CH2CH3



18: R1=R2=CH3
19: R1=R2=CH2CH3
20: R1=R2=CH2CH2CH3
21: R1=R2=CH2CH2CH2CH3
22: R1=R2=CH2CH2CH2CH2CH3

Figure 2. Synthesized phloroglucinol derivatives.



Chinesin I R=Et
Chinesin II R=Me

2,6-Di-isobutyryl-4,4-diethyl-cyclohexane-1,3,5-trione

Figure 3. Chemical structures of chinesins and phloroglucinol derivatives which show antagonistic activity against both TxA_2 and LTD_4.

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Chinesins possess one acyl chain and three alkyl chains at benzene carbons and have no aromaticity because of disubstitution of methyl and isopentenyl groups on the same carbon of the ring. Compound **4** also possesses geminally disubstituted groups at the benzene ring, and it shows stronger activity than **3**. It is therefore suggested that the structures of side chains and the number of substituent groups at the benzene ring is closely correlated with the inhibitory activity of phloroglucinols.

In the present investigation, 18 phloroglucinol derivatives were synthesized and their antagonistic activity against LTD_4 and TxA_2 were evaluated.

Chemistry

2-Acyl- and 2,4-diacylphloroglucinols and 2,6-diacyl-4,4-dialkylcyclohexane-1,3,5-triones were prepared according to Scheme I. 2-Acylphloroglucinols were synthesized by treating phloroglucinol with corresponding carboxylic acid in the presence of BF_3–Et_2O (boron trifluoride–diethyl ether) complex. 2,4-Diacylphloroglucinols were synthesized in the same way and product ratios of the monoacyl and the diacyl derivatives were controlled by the amount of carboxylic acids.

A diacylphloroglucinol was dissolved in a solution of sodium methoxide and methanol, and corresponding alkyl halides were added to yield 2,6-diacyl-4,4-dialkylcyclohexane-1,3,5-triones with monoalkylated products.

Biological Test

Magnus Test. The compounds were dissolved in dimethyl sulfoxide and diluted with Tyrode's solution. The tracheal chain of guinea pigs (300–500 g) was suspended in a 30-mL organ bath containing Tyrode's solution maintained at 37 °C and gassed continuously with 95% O_2 and 5% CO_2. After a 60-min equilibration, isotonic contractions were elicited by 5×10^{-8} M LTD_4 or U-46619 (a stable form of TxA_2) under a tension of 0.5 g. The test compounds were added to the organ bath 20 min prior to challenge with LTD_4 or U-46619. The inhibitory activity was calculated by the contraction in the presence and absence of the test compounds. Ibudilast[22] and PTA_2[23] were used as standard antagonists against LTD_4 and U-46619, respectively. The 50% inhibitory concentration (IC_{50}) was calculated by the method of least squares.

Results and Discussion

Table I shows the antagonistic activity of natural phloroglucinol derivatives and standard compounds against U-46619 and LTD_4. The activity of 2-acyl- and 2,4-diacylphloroglucinols (structures in Figure 2) against U-46619 and LTD_4 were also shown in Table II. Most of the monoacyl derivatives inhibit just TxA_2, and they are much weaker than chinesins; on the other hand, diacylphloroglucinols which possess acyl chains composed of four or five carbons inhibited both TxA_2 and LTD_4. Compound **14** showed good activity among the 2-acyl- and 2,4-diacylphloroglucinols approaching the activity of chinesins.

Table II. Effects of Mono- and Diacylphloroglucinols on U-46619- and LTD_4-Induced Contraction of Guinea Pig Trachea in the Magnus Test

		U-46619		LTD_4	
compds	concn (M)	% inhibn (N)	IC_{50} (M)	% inhibn (N)	IC_{50} (M)
5	3×10^{-6}	11.0 (2)	6.6×10^{-6}		
	1×10^{-5}	70.4 (1)		32.0 (2)	
6	1×10^{-5}	27.3 (2)		22.4 (2)	
7	3×10^{-6}	22.7 (2)	4.7×10^{-6}		
	1×10^{-5}	96.6 (1)		30.5 (2)	
8	3×10^{-6}	13.8 (2)	6.0×10^{-6}		
	1×10^{-5}	76.3 (1)		25.4 (2)	
9	1×10^{-5}	56.9 (2)	8.9×10^{-6}	62.3 (2)	7.6×10^{-6}
	2×10^{-5}	100.0 (2)		93.9 (2)	
10	2×10^{-5}	44.3 (4)		39.1 (4)	
11	2×10^{-7}			30.8 (1)	1.2×10^{-5}
	2×10^{-6}			42.5 (2)	
	2×10^{-5}	53.4 (4)		97.1 (1)	
12	2×10^{-7}			28.9 (1)	1.2×10^{-6}
	2×10^{-6}			48.5 (2)	
	2×10^{-5}	53.6 (3)		92.3 (2)	
13	2×10^{-7}	8.4 (2)	2.7×10^{-6}	12.5 (1)	1.4×10^{-6}
	2×10^{-6}	57.0 (2)		59.4 (2)	
	2×10^{-5}	72.5 (1)		87.0 (1)	
14	2×10^{-7}	22.6 (2)	1.5×10^{-6}	29.9 (2)	5.6×10^{-7}
	2×10^{-6}	42.0 (2)		78.2 (2)	
	2×10^{-5}	100.0 (2)		100.0 (1)	
15	2×10^{-6}	34.7 (2)	1.7×10^{-6}	45.5 (2)	1.4×10^{-6}
	2×10^{-5}	98.0 (2)		80.1 (2)	
16	2×10^{-5}	25.9 (2)		43.3 (2)	
17	2×10^{-5}	15.1 (2)		17.4 (2)	

Table III. Effects of 2,6-Diacyl-4,4-dialkylcyclohexane-1,3,5-trione on U-46619- and LTD_4-Induced Contraction of Guinea Pig Trachea in the Magnus Test

		U-46619		LTD_4	
compds	concn (M)	% inhibn (N)	IC_{50} (M)	% inhibn (N)	IC_{50} (M)
18	2×10^{-7}	38.5 (2)	5.8×10^{-7}	36.8 (2)	5.7×10^{-7}
	2×10^{-6}	61.2 (2)		60.7 (2)	
	3×10^{-6}	80.2 (2)		87.0 (2)	
19	3×10^{-7}	49.0 (2)	3.1×10^{-7}	44.9 (2)	3.5×10^{-7}
	1×10^{-6}	84.2 (2)		87.8 (2)	
20	3×10^{-6}	25.3 (2)	6.9×10^{-6}	28.5 (2)	5.9×10^{-6}
	2×10^{-5}	81.7 (1)		88.5 (1)	
21	3×10^{-6}	58.6 (2)	1.8×10^{-6}	66.5 (2)	1.2×10^{-6}
	2×10^{-5}	91.6 (1)		100.0 (1)	
22	3×10^{-6}	17.0 (1)	5.6×10^{-6}	58.3 (2)	2.3×10^{-6}
	2×10^{-5}	80.4 (1)		95.0 (1)	

Table III shows the effects of 4,4-dialkyl derivatives of diacylploroglucinols (structures in Figure 3) on U-46619- and LTD_4-induced contraction of guinea pig trachea. Cytotoxicity was observed in the 4,4-diethyl derivative of **14** in the concentration of more than 2×10^{-5} M; meanwhile, that of **12** showed weaker cytotoxicity. Thus, the activity was examined with dialkyl derivatives of **12**. These compounds showed marked inhibitory activity against both TxA_2 and LTD_4, and the activity of **19** was almost the same level as that of the chinesins.

Chinesins and **19** possess at least one branched acyl chain composed of four or five carbons, and both of them have a β,β'-triketone moiety (Figure 3). As some compounds having a β,β'-triketone moiety showed lower activity (IC_{50} of 2-carbamoylcyclohexane-1,3-dione, 2-carbamoyl-5-methylcyclohexane-1,3-dione, 2-carbamoyl-5,5-dimethylcyclohexane-1,3-dione, 2-acetyl-5-phenylcyclohexane-1,3-dione, 5-phenylcyclohexane-1,3-dione, and 2,6-diacetyl-4,4-dimethylcyclohexane-1,3,5-trione against U-46619 and LTD_4 were higher than 2×10^{-5} M), the β,β'-triketone moiety may not be essential for the inhi-

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bitory activity. On the other hand, it is interesting that the phloroglucinol derivatives show good activity by introducing the dialkyl chains on the same carbon of the six-membered ring. Marked inhibitory activity against both TxA_2 and LTD_4 was found alike in the natural phloroglucinol derivatives and synthesized analogues which possess disubstituted alkyl chains on the same carbon of the six-membered ring.

Experimental Section

NMR spectra were measured with a JEOL GX-270 spectrometer in $CDCl_3$ solution containing tetramethylsilane as an internal standard. IR and UV spectra measured on JASCO IR-810 spectrometer and a JASCO UVIDEC-460 UV-vis spectrophotometer.

Synthesis of 2-Acylphloroglucinols. 2-Methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, 2-methylpentanoic acid, or hexanoic acid (8.0 mmol) was dissolved in BF_3–Et_2O complex (5.0 mL) at room temperature. Anhydrous phloroglucinol (4.0 mmol) was added to this complex, and the mixture was heated on a steam-bath for 24 h. After cooling, the reaction mixture was cooled, it was added dropwise to aqueous potassium acetate (2.6 g/50 mL). After filtration, the filtrate was dissolved with AcOEt and dried over $MgSO_4$. Evaporation of the dried AcOEt and purification with silica-gel column chromatography (hexane–AcOEt) gave **5**–**9** respectively (yield: 14–40%). Diacylphloroglucinol was also isolated, and the reaction conditions was not optimized. Compounds **5**–**9** were identified with ^{1}H- and ^{13}C-NMR, IR, and UV spectra.

Synthesis of 2,4-Diacylphloroglucinols. Acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, hexanoic acid, or octanoic acid (12 mmol) was dissolved in BF_3–Et_2O complex (5.0 mL) at room temperature. Anhydrous phloroglucinol (4.0 mmol) was added to this complex. The reaction and separation was accomplished by the procedure described earlier to yield **10**–**17** (yield: 7.7–96.5%). Monoacylphloroglucinol was also isolated and the reaction condition was not optimized. Compounds **10**–**17** were identified with ^{1}H-NMR, ^{13}C-NMR, IR, and UV spectra.

Alkylation of 2,6-Diisobutyrylphloroglucinol. Anhydrous diisobutyrylphloroglucinol (7.5 mmol) was dissolved in a solution of sodium (1.0 g) in methanol (33 mL) followed by slow addition of methyl, ethyl, propyl, butyl, or pentyl iodide (185 mmol). After the addition was complete, stirring was continued for 15 min at room temperature. Then 2 M hydrochloric acid was added, and the reaction mixture was extracted with AcOEt. The combined AcOEt extracts were washed with water, dried over $MgSO_4$, and concentrated. Purification by column chromatography (over silica-gel, hexane–AcOEt–AcOH 5–20:1:0.1) gave **18**–**22**, respectively (yield: 17.9–34.1%). The monoalkyl derivative was also isolated, and the reaction condition was not optimized.

Registry No. **1**, 110383-37-4; **2**, 110383-38-5; **3**, 137251-97-9; **4**, 137201-18-4; **5**, 35458-21-0; **6**, 2437-62-9; **7**, 26103-97-9; (±)-**8**, 98498-56-7; **9**, 5665-89-4; **10**, 2161-86-6; **11**, 3145-11-7; **12**, 3133-29-7; **13**, 3098-40-6; **14**, 2999-10-2; **15**, 139409-36-2; **16**, 3118-34-1; **17**, 3118-46-5; **18**, 35932-10-6; **19**, 139409-37-3; **20**, 139426-54-3; **21**, 139409-38-4; **22**, 139409-39-5; TxA_2, 57576-52-0; LTD4, 73836-78-9; $HO_2CCH(CH_3)_2$, 79-31-2; $HO_2C(CH_2)_3H$, 107-92-6; $HO_2CCH_2CH(CH_3)_2$, 503-74-2; $HO_2CCH(CH_3)(CH_2)_3H$, 97-61-0; $HO_2C(CH_2)_5H$, 142-62-1; CH_3CO_2H, 64-19-7; $HO_2C(CH_2)_2H$, 79-09-4; $HO_2C(CH_2)^7H$, 124-07-2; MeI, 74-88-4; EtI, 75-03-6; PrI, 107-08-4; BuI, 542-69-8; pentyl iodide, 628-17-1; phloroglucinol, 108-73-6.

Antimycobacterial Activity of a Series of Pyrazinoic Acid Esters

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A series of pyrazinoic acid esters has been prepared and evaluated for in vitro antimycobacterial activity. Several of the pyrazinoate esters have substantially better activity than the first-line antituberculous agent pyrazinamide against susceptible isolates of *Mycobacterium turberculosis* as well as activity against pyrazinamide-resistant isolates. The minimal inhibitory concentrations (MICs) were lower for each organism and at each pH than the MICs for pyrazinamide. The esters have activity against *Mycobacterium bovis* and *Mycobacterium kansasii*, two species resistant to pyrazinamide, but not against *Mycobacterium avium* complex.

The use of nicotinamide-related compounds for the therapy of tuberculosis followed the demonstration by Chorine[1] and confirmation by McKenzie[2] that nicotinamide was effective for the treatment of murine tuberculosis. Many nicotinamide analogues, including pyrazinamide, were subsequently synthesized and tested for antituberculous activity.[3,4] Pyrazinamide was the most active of the analogues. Although it is active in vitro against most isolates of *Mycobacterium tuberculosis* at concentrations below 50 μg/mL, pyrazinamide is unusual because of its narrow spectrum of activity. *Mycobacterium bovis* and nontuberculous mycobacteria are usually resistant.[5] Other interesting features of this agent are its requirement for a low pH for activity[6,7] and its unique in vivo sterilizing activity.[8] The mode of action of pyrazinamide is not known. Although the mechanism of resistance has not

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