The Antagonistic Effects of Khellactones on Platelet-Activating Factor, Histamine, and Leukotriene D_4 [1]

Yoko AIDA,[b] Toshio KASAMA,[b] Naoki TAKEUCHI,*[,a] and Seisho TOBINAGA[a]

Showa College of Pharmaceutical Sciences,[a] Machida, Tokyo 194, Japan and Research Laboratory of Biological Sciences, Kodama Ltd.,[b] Matsudo, Chiba 271, Japan. Received November 17, 1994; accepted January 19, 1995

Khellactones of *Peucedanum praeruptorum* DUUN., including praeruptorins A (=Pd-Ia, 2) and B (=Pd-II, 11), had an antagonistic effect specifically on platelet aggregation induced by platelet activating factor (PAF) among various aggregating agents examined, and represent a new class of PAF antagonists. We examined the effects of twenty compounds on PAF-induced platelet aggregation and on histamine- and leukotriene D_4 (LTD_4)-induced contractions in isolated guinea pig ileum. Compounds 2, (±)-*cis*-3′,4′-diacetylkhellactone (3), (±)-*cis*-4′-acetyl-3′-crotonoylkhellactone (5), (±)-*cis*-4′-acetyl-3′-tetrolylkhellactone (6), (±)-*cis*-4′-acetyl-3′-tigloylkhellactone (7), (±)-*cis*-4′-acetyl-3′-(2″-methylbutyryl)khellactone (8), (±)-*cis*-3′,4′-ditigloylkhellactone (10), and 11 all strongly inhibited PAF-induced platelet aggregation. (±)-*cis*-4′-Acetyl-3′-(2″-methyl-2″-dodecenoyl)khellactone (9), (±)-*cis*-4′-ethyl-3′-tigloylkhellactone (13), (±)-*cis*-4′-ethyl-3′-[*N*-(2″-triethylammonio)ethylcarbamoyl]khellactone iodide (16), (±)-*trans*-3′,4′-diacetylkhellactone (18), (±)-*trans*-4′-acetyl-3′-crotonoylkhellactone (19), (±)-*trans*-4′-acetyl-3′-valerylkhellactone (20), (±)-*trans*-4′-acetyl-3′-isovalerylkhellactone (21), and (±)-*trans*-4′-acetyl-3′-tigloylkhellactone (22) were weakly inhibitory. Most of the compounds exhibited noncompetitive antagonist actions on histamine- and LTD_4-induced contractions. The potencies of the antagonistic effects on histamine action were in the order $7=22\geqq2=8=10>6=11=13\geqq5>19=9$ and those on LTD_4 action were in the order $6=22=2>10=8>7=9=11\geqq13$. Thus, compounds with potent PAF-antagonistic activities have the following features: *cis* isomers of khellactone at the C-3′ and C-4′ positions are more favorable than *trans* isomers, and the acyl moiety at the C-3′ position of khellactone must be of an appropriate molecular size. In the case of histamine- and LTD_4-antagonistic activities, both isomers show similar effects and acyl moieties of appropriate size are required at the C-3′ and C-4′ positions. These results are of interest in regard to the medicinal uses of *Peucedanum* species as a herbal drug.

Key words *Peucedanum praeruptorum*; khellactone; PAF antagonist; histamine antagonist; LTD_4 antagonist; antagonist effect

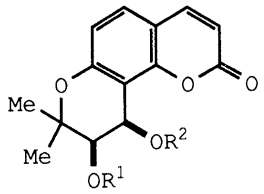
Among the naturally occurring khellactones, various biological activities of khellactones, namely, piscicidal,[2] *in vitro* uterine relaxation,[2] vasodilation,[2,3] hypotension,[2] calcium antagonism,[4] inhibition of platelet aggregation,[5] histamine release,[2,3] and acetylcholine-, barium chloride-, and serotonin-induced spasms of isolated rat and rabbit intestines,[2,3,6] have been reported. We found that natural (constituent of *Peucedanum japonicum* THUNB.) and synthetic prenyl coumarins have calcium- and histamine-antagonistic activities and cerebral blood flow-increasing effects.[7] Here we report that a natural khellactone (a mixture **2** and **11**) derived from *Peucedanum praeruptorum* DUUN. (a plant called Zenko), corresponding to praeruptorins A (=Pd-Ia)[8] and B (=Pd-II),[8] has a specific antagonistic effect on platelet activating factor (PAF)[9] among several platelet-aggregating agents examined. We also analyzed the structure–activity relationship of compounds derived from khellactone and their inhibitory effects on PAF-induced platelet aggregation and on contractions of isolated guinea pig ileum induced by histamine and LTD_4, with the aim of finding a new drug for treatment of allergy and inflammation in humans.

Materials and Methods

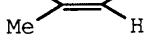
Materials Natural khellactone, a mixture of **2** and **11** in a ratio of 3:2, was separated from hexane extracts of the roots of *Peucedanum praeruptorum* DUUN. Compounds **2** and **11** were isolated from natural khellactone by silica gel column chromatography.[8] (±)-*cis*-Khellactone (**1**),[10,11] (±)-*cis*-4′-acetylkhellactone (**4**),[10,12] (±)-*cis*-4′-ethylkhellactone (**12**),[13] (±)-*trans*-4′-acetylkhellactone (**17**),[11] and (±)-trans-4′-ethylkhellactone (**24**)[13] were prepared from seselin.[14] (±)-*cis*-3′,4′-Diacetylkhellactone (**3**)[10,11,15] and (±)-*trans*-3′,4′-diacetylkhellactone (**18**) were prepared by the reactions of **1** and **17** with acetic anhydride in pyridine in yields of 43.8 and 94.1%, respectively. (±)-*cis*-4′-Acetyl-3′-crotonoylkhellactone (**5**), (±)-*cis*-4′-acetyl-3′-tetrolylkhellactone (**6**), (±)-*cis*-4′-acetyl-3′-(2″-methylbutyryl)khellactone (**8**), and (±)-*cis*-4′-acetyl-3′-(2″-methyl-2″-dodecenoyl)khellactone (**9**) were prepared by the reactions of **4** with crotonoic acid, tetrolic acid, 2-methylbutyric acid, and 2-methyl-2-dodecenoic acid in the presence of dicyclohexylcarbodiimide and 4-pyrrolidinopyridine in methylene chloride with refluxing, in yields of 71.9, 56.5, 65.2, and 83.7%, respectively. Compound **7** was prepared from **4** by the method of Bal-Tembe *et al.*[10] (±)-*cis*-3′,4′-Ditigloylkhellactone (**10**) and (±)-*cis*-4′-ethyl-3′-tigloylkhellactone (**13**) were prepared by the reactions of **1** and **12** with tigloyl chloride in benzene under reflux in yields of 64.0 and 65.1%, respectively. (±)-*trans*-4′-Acetyl-3′-crotonoylkhellactone (**19**), (±)-*trans*-4′-acetyl-3′-valerylkhellactone (**20**), (±)-*trans*-4′-acetyl-3′-isovalerylkhellactone (**21**), (±)-*trans*-4′-acetyl-3′-tigloylkhellactone (**22**), and (±)-*trans*-4′-acetyl-3′-decanoylkhellactone (**23**) were prepared by reactions of **17** with crotonoyl chloride, valeryl chloride, isovaleryl chloride, tigloyl chloride, and decanoyl chloride in benzene under reflux in yields of 53.5, 93.1, 80.5, 74.8, and 81.3%, respectively. (±)-*trans*-4′-Ethyl-3′-tigloylkhellactone (**25**) was prepared by the reaction of **24** with tigloyl chloride in benzene under reflux in a yield of 50.7%. (±)-*cis*-4′-Ethyl-3′-[*N*-(2″-triethylammonio)-ethylcarbamoyl]khellactone iodide (**16**) was prepared from **12** as described below. That is, the reaction of **12** with 2-chloroethylisocyanate in pyridine and chloroform gave (±)-*cis*-3′-[*N*-(2″-chloro)ethylcarbamoyl]-4′-ethylkhellactone (**14**) (75.0% yield), which was transformed to (±)-*cis*-3′-[*N*-(2″-diethylamino)ethylcarbamoyl]-4′-ethylkhellactone (**15**) in a yield of 83.3%. The conversion of **15** to **16** was established by refluxing with ethyl iodide in a 62.5% yield.

Agents Natural khellactone, compounds **1**—**3**, **5**—**11**, **13**, **16**—**23**, and **25**, and etizolam (Yositomi Ltd.) were dissolved in dimethyl sulfoxide (DMSO) and diluted with water as required for *in vitro* or *in vivo* studies. Other agents used were diphenhydramine hydrochloride (diphenhydramine; Wako Pure Chemical Industries, Ltd.), verapamil (Eisai

* To whom correspondence should be addressed.

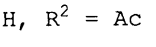


1: $R^1 = R^2 = H$

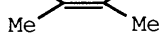
2: $R^1 =$  $R^2 = Ac$

3: $R^1 = R^2 = Ac$

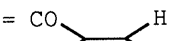
4: $R^1 = H$, $R^2 = Ac$

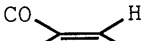
5: $R^1 =$  $R^2 = Ac$

6: $R^1 = COC{\equiv}CMe$, $R^2 = Ac$

7: $R^1 =$  $R^2 = Ac$

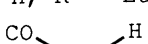
8: $R^1 = COCHMeCH_2Me$, $R^2 = Ac$

9: $R^1 =$  $R^2 = Ac$

10: $R^1 = R^2 =$ 

11: $R^1 = R^2 =$ 

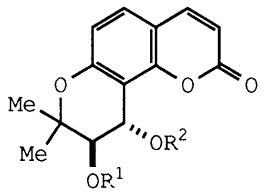
12: $R^1 = H$, $R^2 = Et$

13: $R^1 =$  $R^2 = Et$

14: $R^1 = CONH(CH_2)_2Cl$, $R^2 = Et$

15: $R^1 = CONH(CH_2)_2NEt_2$, $R^2 = Et$

16: $R^1 = CONH(CH_2)_2N^+Et_3\ I^-$, $R^2 = Et$



17: $R^1 = H$, $R^2 = Ac$

18: $R^1 = R^2 = Ac$

19: $R^1 =$  $R^2 = Ac$

20: $R^1 = CO(CH_2)_3Me$, $R^2 = Ac$

21: $R^1 = COCH_2CHMe_2$, $R^2 = Ac$

22: $R^1 =$  $R^2 = Ac$

23: $R^1 = CO(CH_2)_8Me$, $R^2 = Ac$

24: $R^1 = H$, $R^2 = Et$

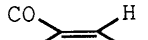
25: $R^1 =$  $R^2 = Et$

Chart 1

Ltd.), ketotifen (Sankyo Ltd.), histamine dihydrochloride (histamine; Wako Pure Chemical Industries, Ltd.), and LTD_4 (Salford Ultrafine Chemicals and Research Ltd.). LTD_4 was obtained as a stock solution (0.005 mg/ml) in 20% ethanol–phosphate buffer solution (pH 6.9). The stock solution of LTD_4 was stored at $-80\,^{\circ}C$ and diluted with water as required on each experimental day. Diphenhydramine, verapamil, ketotifen, and histamine were dissolved or diluted with water. Aggregating agents such as adenosine diphosphate (ADP; Aldrich), PAF (Sigma Chemical Company), sodium arachidonate (AA; Sigma Chemical Company), and collagen (Sigma Chemical Company) were used. ADP and AA were dissolved in 0.9% saline, PAF was dissolved in 0.2% bovine serum albumin, and collagen dissolved in SKH Horm buffer.

Animals Male albino rabbits weighing 2 to 3 kg and male Hartley strain guinea pigs weighing 350 to 500 g (Japan Laboratory Animal Inc.), male Wistar strain rats weighing 220 to 300 g, and male ddY strain mice weighing 16 to 30 g (Japan SLC Inc.) were acclimatized for at least one week after purchase. Animals were housed in a room controlled to maintain the temperature and relative humidity at $22\pm3\,^{\circ}C$ and $55\pm10\%$, respectively, with 10 to 15 air changes per hour and artificial lighting between 07:00—19:00. Animals were given free access to diet and tap water.

Methods **1) Effects of Natural Khellactone on Platelet Aggregation *in Vitro*** One volume of a 3.8% sodium citrate solution was added to 9 volumes of blood of rabbits and centrifuged at 1000 rpm for 10 min to obtain platelet-rich plasma (PRP). The remaining blood was further centrifuged at 3000 rpm for 10 min to obtain platelet-poor plasma (PPP). The degree of platelet aggregation was assessed by measuring the difference in light transmission between PPP and PRP with an Aggrecorder (Kyoto Daiichi Kagaku). A test drug dissolved in DMSO (2.5 μl) was added to PRP (222.5 μl) and the inhibitory effect on platelet aggregation was assessed. ADP (final concentration 10 μM), PAF (final concentration 0.01 μg/ml), AA (final concentration 300 μM), and collagen (final concentration 30 μg/ml), 25 μl each, were used as the platelet-aggregating agents.

2) Effects of Natural Khellactone on PAF-Induced Hypotension Rats were immobilized in a dorsal position under urethane anesthesia (1.2 g/kg i.p.), then a tracheal catheter was inserted and an airway secured. After insertion of a polyethylene catheter into the right carotid artery, blood pressure was measured through a pressure transducer and heart rate was measured through a pulse meter connected to the pressure transducer. In addition, catheters were inserted into the left jugular and left femoral veins. The jugular vein was used for administering the test drug and the femoral vein for administering PAF. For assessment of the antagonistic effects on PAF, the test drug was administered intravenously after confirming an initial blood pressure response to PAF (1 μg/kg), and 5 min later PAF was readministered.

3) Effects of Natural Khellactone on PAF-Induced Acute Death PAF (1 μg/kg) was rapidly administered in 2—3 s *via* the tail vein at 30 and 60 min after administering the test drug i.p. to mice and the protective effect against acute death was assessed.

4) Effects of Compounds on Isolated Ileum Guinea pigs were killed and the ileum was isolated. Tubular specimens 15—20 mm long were prepared and suspended in 10 ml Magnus tubes perfused with Tyrode solution at 37 °C. The specimens were subjected to a 0.5 g load and the response was recorded through an isometric transducer. The solution was aerated with 95% O_2: 5% CO_2 gas mixture. Histamine and LTD_4 were used as agonists and each test drug were added to the Magnus tube at 5 min before the agonists were added.

5) Statistical Analysis of Results Student's *t*-test and the chisquare test was used for the statistical analysis of the antagonistic effects on PAF-induced hypotension and the protective effects on PAF-induced acute death. The effects on the isolated ileum specimens were evaluated by the van Rossum method.

Results

1) Effects of Natural Khellactone on Platelet Aggregation *in Vitro* Preliminary investigations of the platelet aggregation-inhibitory effects of natural khellactone from *Peucedanum praeruptorum* DUUN. using various inducing

agents showed that the inhibitory effect on PAF-induced platelet aggregation was much stronger than that on ADP-, AA-, or collagen-induced aggregation (Table 1). Thus, natural khellactone specifically inhibited PAF-induced platelet aggregation. The control drug etizolam also specifically inhibited PAF-induced aggregation. The potency of natural khellactone was about 1/50 of that of etizolam.

2) Effects of Natural Khellactone on PAF-Induced Hypotension The results of the antagonistic effects of natural khellactone on PAF-induced hypotension are shown in Fig. 1. DMSO used in this experiment had no effect on the persistent hypotensive response induced by the administration of PAF (1 μg/kg i.v.). A significant antagonistic effect was evident 15—40 min after administration with natural khellactone (1.0 mg/kg i.v.); it appeared immediately after PAF administration and lasted until 60 min at doses of 3.0 and 10.0 mg/kg i.v. However, there was no significant difference between the effects with 3.0 and 10.0 mg/kg. Etizolam exhibited significant antagonistic effects on PAF at 0.3—3.0 mg/kg i.v., dose-dependently. Etizolam did not immediately inhibit the fall of blood pressure after the administration of PAF, but shortened the duration of the blood pressure fall. The pressure recovered to nearly its initial level at about 10 min after administration of PAF when etizolam was given at 3.0 mg/kg i.v. Consequently, though simple comparisons of potency were impossible, the antagonistic effect of natural khellactone on PAF-induced hypotension was 1/3—1/10 of that of etizolam in terms of the dose at which the potency was manifest.

Table 1. Effects of Natural Khellactone and Etizolam on Rabbit Platelet Aggregation

Compound	IC_{50} (mM)			
	ADP	PAF	AA	Coll
Natural khellactone	0.9<	0.09	0.9<	0.74
Etizolam	0.9<	0.0016	0.9<	0.9<

ADP, adenosine diphosphate (10 μM); PAF, platelet activating factor (0.01 μg/ml); AA, sodium arachidonate (300 μM); Coll, collagen (30 μg/ml); natural khellactone, a mixture of **2** and **11** in a ratio of 3:2.

3) Effects of Natural Khellactone on PAF-Induced Acute Death The results of administering PAF (75 μg/kg i.v.) 30 min after administration of the test drugs are shown in Table 2. No difference from the control group was observed with natural khellactone at 10—300 mg/kg i.p. On the other hand, PAF-induced acute deaths were dose-dependently prevented by etizolam at 3—30 mg/kg i.p. and ketotifen at 3—100 mg/kg i.p.

4) Investigations of Structure–Activity Relationship The inhibitory effects of compounds on PAF-induced platelet aggregation and on histamine- and LTD_4-induced contractions of the isolated ileum, shown in Table 3 and

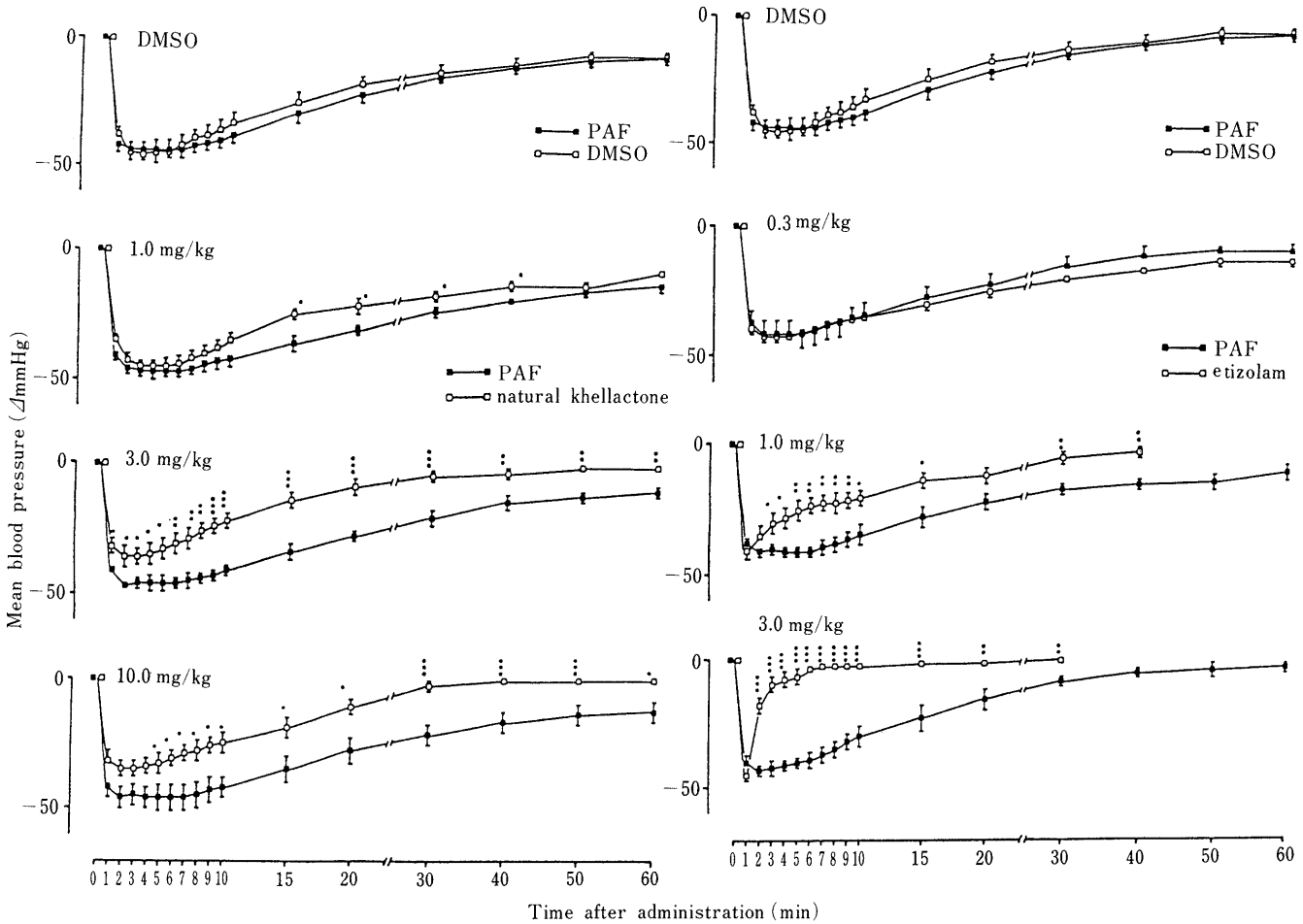


Fig. 1. Effects of Natural Khellactone (Mixture of **2** and **11** in a ratio of 3:2) on PAF-Induced Hypotension in Anesthetized Rats

Significantly different from the control at *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$.

Table 2. Effects of Natural Khellactone on PAF-Induced Acute Death in Mice

Compound (mg/kg, i.p.)		No. of survivors	No. of animals	Survival (%)
Control	0	4	31	13
Natural	10	2	10	20
khellactone	30	0	10	0
	100	1	10	10
	300	0	10	0
Etizolam	3	5	19	26
	10	9	10	90***
	30	9	10	90***
Ketotifen	3	0	10	0
	10	3	10	30
	30	5	10	50**
	100	10	10	100***

Agents were administered intraperitoneally 30 min before PAF administration. Natural khellactone, a mixture of **2** and **11** in a ratio of 3 : 2. Significantly different from the control at **, $p<0.01$; ***, $p<0.001$.

Figs. 2, 3, and 4, were investigated from the viewpoint of the structure–activity relationship.

(1) Effects of Compounds on PAF-Induced Aggregation: As described in 1)—3) above, PAF antagonistic effects were observed both *in vitro* and *in vivo* with natural khellactone. When the platelet aggregation-inhibitory effects of **2** and **11** were investigated *in vitro*, **2** having an angelyl group at the C-3′ position and an acetyl group at the C-4′ position of khellactone was about 0.5-fold weaker than **11** having two angelyl groups in antagonistic effect on PAF (Table 3).

When the *cis* and *trans* isomers at the C-3′ and C-4′ positions of khellactone were compared, the activities of the *cis* isomers are stronger than those of the *trans* isomers: **3** and **18** ($p<0.05$); **5** and **19**; **7** and **22** ($p<0.05$); **13** and **25** (no effect). On the other hand, the compounds having an acyl moiety such as the angelic acid and the tiglic acid moiety at both C-3′ and C-4′ positions, that is, **11** and **10**; **2** and **7**, were similarly potent. Similarly, the compound in which R^1 was an unsaturated acyl group (**7**) exhibited activity similar to that of a compound with saturated R^1 (**8**), but increased unsaturation resulted in poor activity (**6**; $p<0.05$). In the cases of R^1 = a bulky acyl group and R^2 = alkyl group, that is, **9** (no effect), **13** ($p<0.05$), and **25** (no effect), these compounds were less potent than the corresponding compounds such as **7** and **22**. Further, compound **16** with R^1 = –CONH-$(CH_2)_2N^+Et_3$, which constitutes PAF itself in part, did not show the expected activity.

(2) Effects of Compounds on Histamine-Induced Contractions: Histamine showed dose-dependent contraction curves in the range of $10^{-8}-3\times10^{-5}$ M, and all the compounds tested at $10^{-6}-3\times10^{-5}$ M exhibited a noncompetitive antagonistic effect, as shown in Figs. 2 and 3 and Table 3. Although antagonistic effects were not observed at the dose of 10^{-6} M **2** and **11**, clear noncompetitive antagonistic effects were observed at the dose of 10^{-5} M or more. At the dose of 10^{-6} M **9** and **10**, the maximum contractions were slightly inhibited, and dose-dependent antagonistic effects were observed at 10^{-5} and 3×10^{-5} M. The antagonistic effects were not observed

Table 3. Effects of Synthetic Khellactones on Rabbit Platelet Aggregation and Histamine- and LTD_4-Induced Contraction in the Isolated Guinea Pig Ileum

Compound	Platelet aggregation (IC_{50} mM)	Isolated ileum (pD'_2)	
	PAF	Histamine	LTD_4
1	>0.9	—	—
2	0.09	5.08	4.84
3	0.13	>4.00	>4.00
5	0.09	4.57	>4.00
6	0.18	4.89	4.87
7	0.05	5.14	4.40
8	0.05	5.04	4.64
9	0.80	4.20	4.38
10	0.04	5.08	4.70
11	0.05	4.84	4.34
13	0.50	4.80	4.28
16	0.43	—	—
17	>0.9	—	—
18	0.54	>4.00	>4.00
19	0.55	4.28	>4.00
20	0.48	—	—
21	0.61	—	—
22	0.36	5.14	4.85
23	>0.9	—	—
25	>0.9	—	—
Etizolam	0.0016	—	—
Diphenhydramine	—	8.66[a]	—
Verapamil	—	—	6.93

a) pA_2.

with **6**, **7**, **8**, **13**, or **22** at the dose of 10^{-6} M, but were seen dose-dependently at 10^{-5} M or more. In addition, **5** and **19** at the dose of 10^{-5} M or more and **3** and **18** at the dose of 3×10^{-5} M exhibited slight antagonistic effects. Consequently, the inhibitory potencies of the compounds on histamine-induced contractions were in the order **7** = **22** ≧ **2** = **8** = **10** > **6** = **11** = **13** ≧ **5** > **19** = **9**. The compounds having the tiglic acid moiety and the 2-methylbutanoic acid moiety at the C-3′ position and the acetic acid moiety at the C-4′ position in khellactone, such as **7**, **22**, and **8**, and having the acetic acid moiety at both the C-3′ and C-4′ positions, such as **10**, exhibit potency similar to or somewhat stronger than that of **2**. The compounds having the acetic acid moiety at both the C-3′ and C-4′ positions, such as **3** and **18**, were less potent than the corresponding compounds such as **7**, **10**, and **19**. On the other hand, the *cis* isomer at the C-3′ and C-4′ positions of khellactone afforded activity similar to that of the corresponding *trans* isomers: **3** and **18**; **5** and **19**; **7** and **22**. With an antihistamine, diphenhydramine, dose-dependent competitive effects were observed at 10^{-9} M or more. Virtually no effect was observed with the solvent.

(3) Effects of Compounds on LTD_4-Induced Contraction: At concentrations of $10^{-12}-3\times10^{-9}$ M, LTD_4 exhibited dose-dependent contraction curves. When each test drug was investigated at concentrations of $10^{-5}-3\times10^{-5}$ M, practically all of them exhibited noncompetitive effects, as shown in Figs. 4 and 5 and Table 3. Antagonistic effects were observed with **2** and **11**, and **6**, **7**, **8**, and **22** exhibited dose-dependent antagonistic effects at the dose of 10^{-5} M or more. The maximum contractions

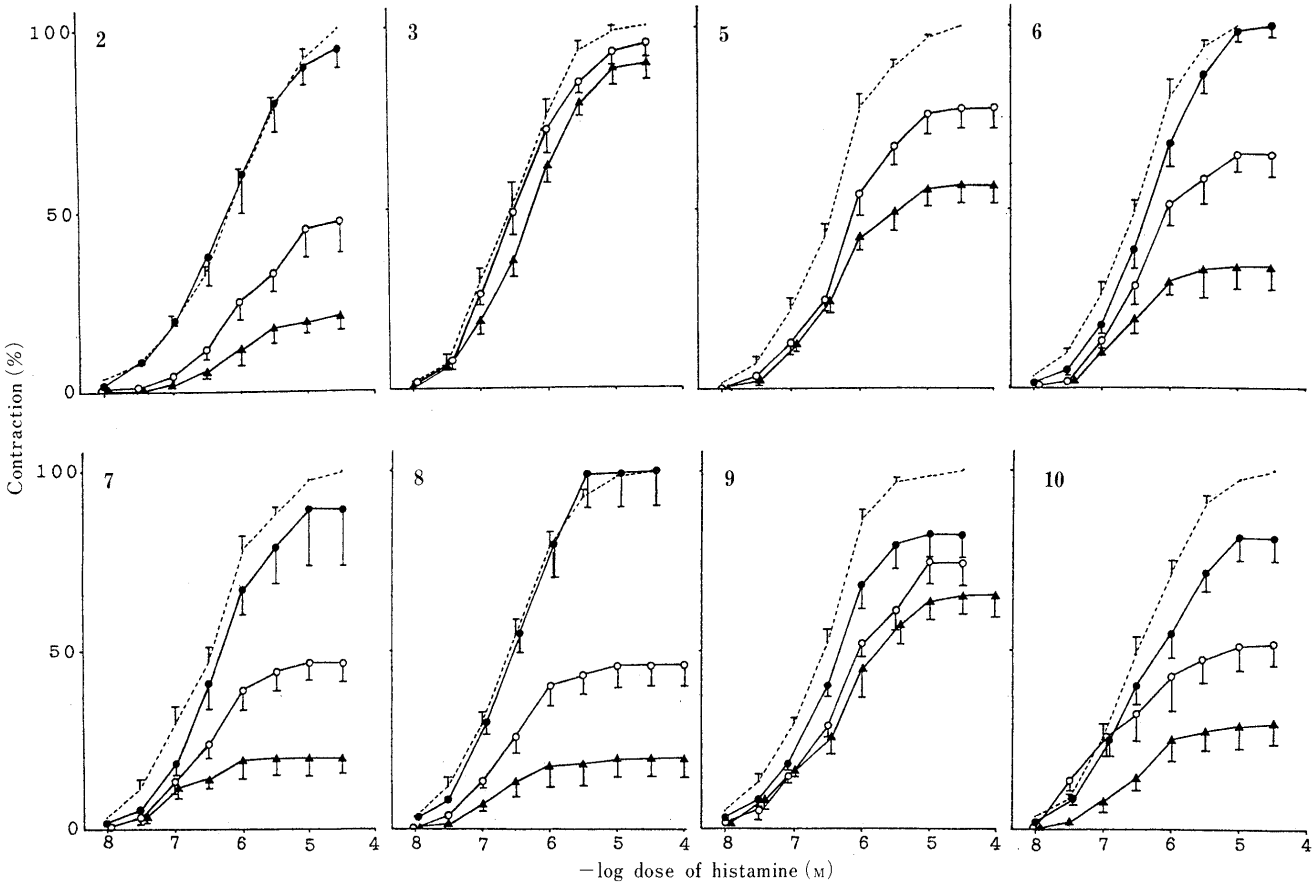


Fig. 2. Effects of **2**, **3**, **5**, **6**, **7**, **8**, **9**, and **10** on Histamine-Induced Contraction in the Isolated Guinea Pig Ileum
– – –, control; ●—●, 10^{-6}; ○—○, 10^{-5}; ▲—▲, 3×10^{-5} M. Each point represents the mean ± S.E. of 4 to 8 experiments.

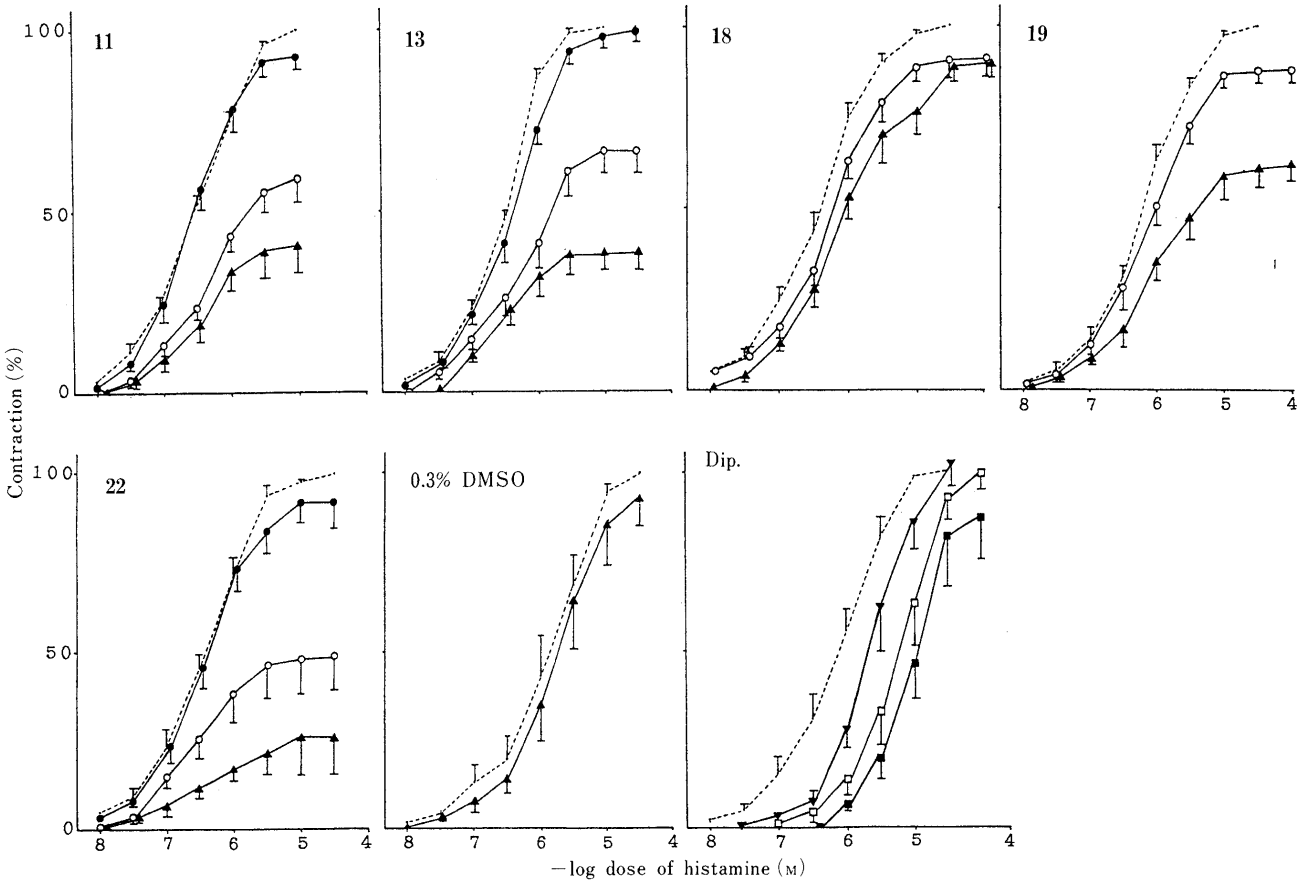


Fig. 3. Effects of **11**, **13**, **18**, **19**, **22**, 0.3% DMSO, and Diphenydramine (Dip.) on Histamine-Induced Contraction in the Isolated Guinea Pig Ileum
– – – –, control; ▼—▼, 10^{-9}; □—□, 10^{-8}; ■—■, 10^{-7}; ●—●, 10^{-6}; ○—○, 10^{-5}; ▲—▲, 3×10^{-5} M. Each point represents the mean ± S.E. of 4 to 8 experiments.

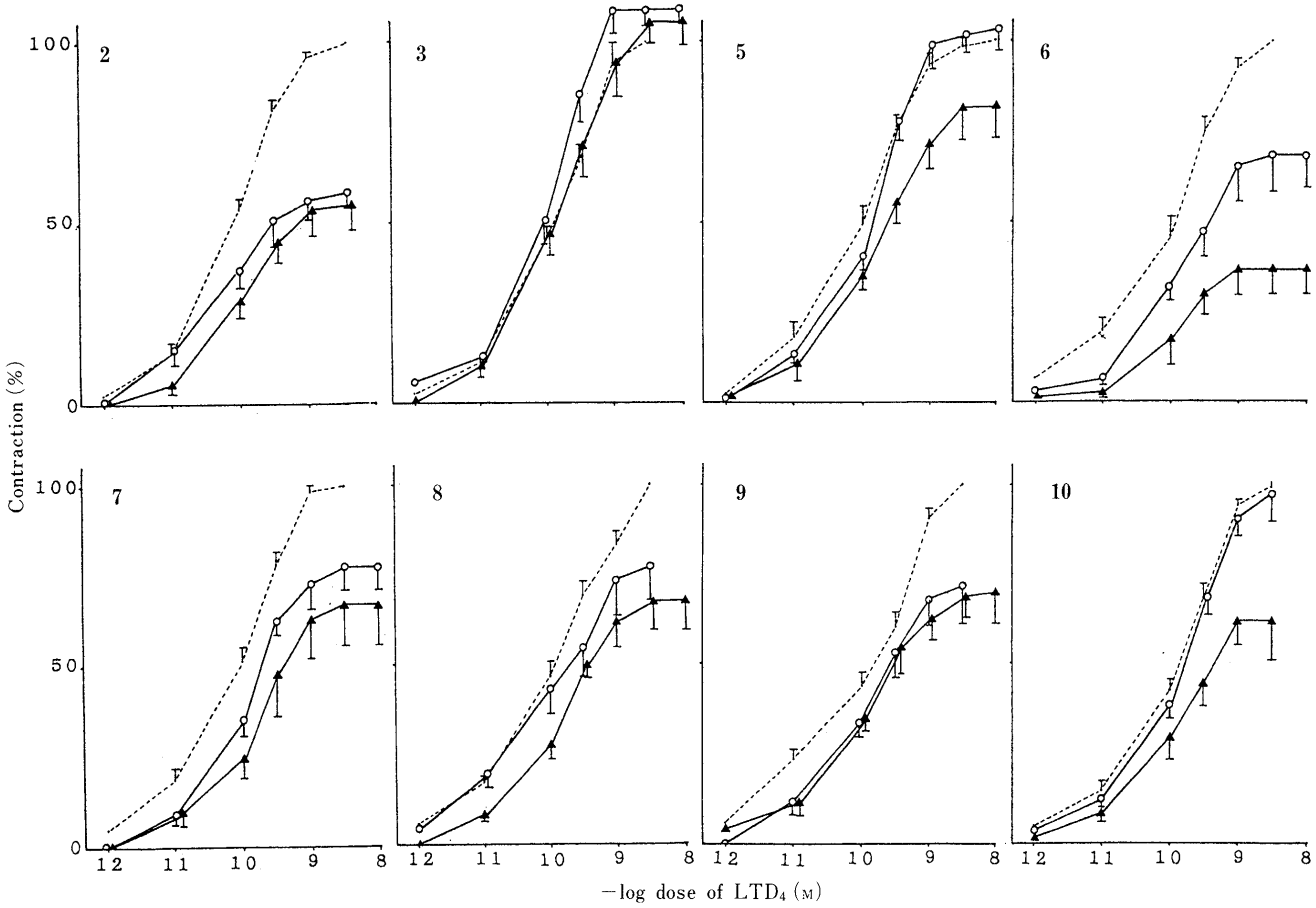


Fig. 4. Effects of **2**, **3**, **5**, **6**, **7**, **8**, **9**, and **10** on LTD_4-Induced Contraction in the Isolated Guinea Pig Ileum

----, control; ●—●, 10^{-6}; ○—○, 10^{-5}; ▲—▲, 3×10^{-5} M. Each point represents the mean±S.E. of 4 to 8 experiments.

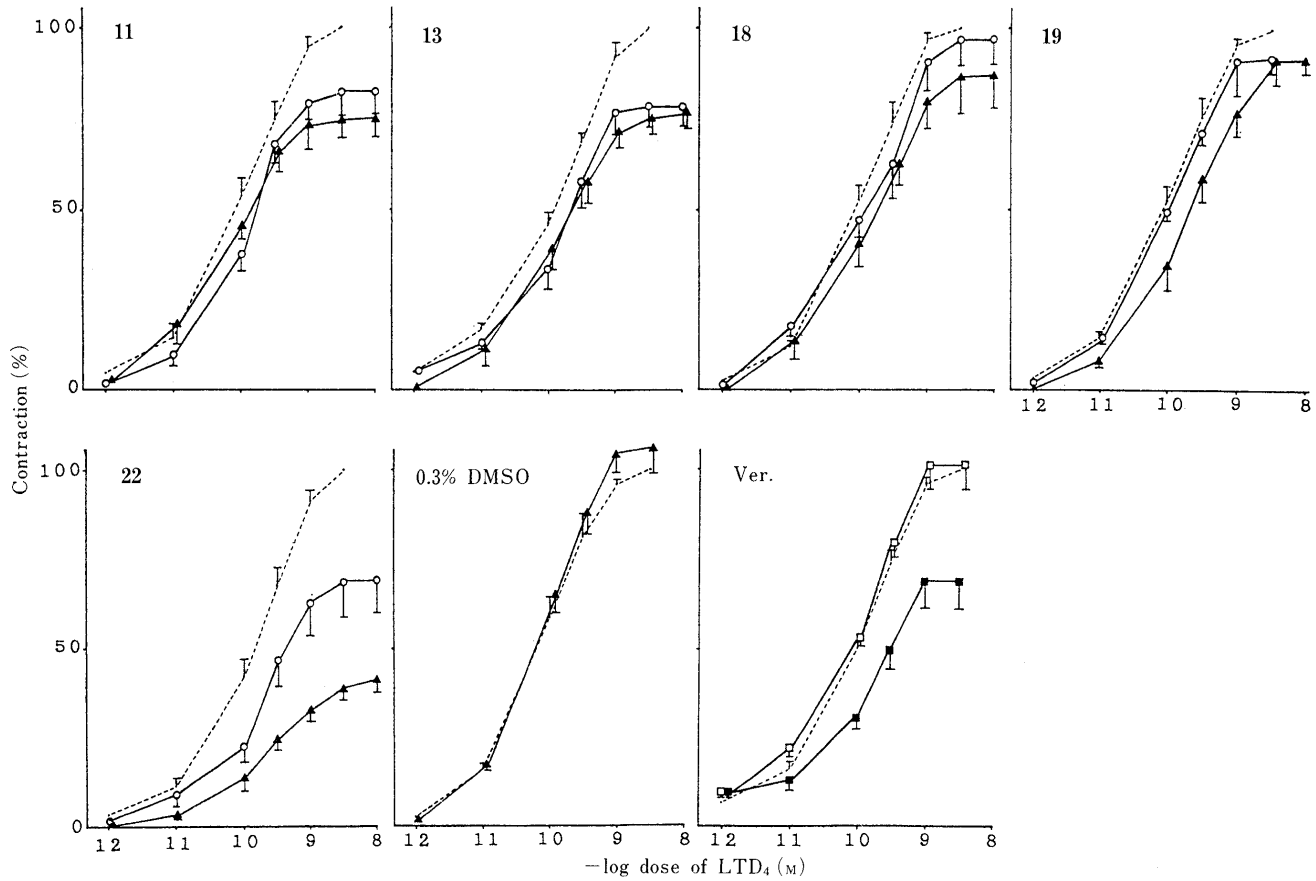


Fig. 5. Effects of **11**, **13**, **18**, **19**, **22**, 0.3% DMSO, and Verapamil (Ver.) on LTD_4-Induced Contraction in the Isolated Guinea Pig Ileum

----, control; □—□, 10^{-8}; ■—■, 10^{-7}; ●—●, 10^{-6}; ○—○, 10^{-5}; ▲—▲, 3×10^{-5} M. Each point represents the mean±S.E. of 4 to 8 experiments.

of LTD_4 were slightly inhibited at 10^{-5} and 3×10^{-5} M **9** and **13** and 3×10^{-5} M **5**, **10**, **18**, and **19**, but **3** did not exhibit an effect even at 3×10^{-5} M. Consequently, the inhibitory potencies of the test compounds on LTD_4-induced contractions were in the order **6**=**22**=**2**>**10**=**8**>**7**=**9**=**11**≧**13**. This result shows that the presence of an unsaturated acyl group such as the tetrolic acid moiety, the tiglic acid moiety, and the 2-methylbutanoic acid at the C-3′ position and the presence of the acetic acid moiety at the C-4′ position in khellactone were related to enhanced activity, that is, **6**, **22**, and **8**. The activity of the compound having two acetic acid moieties at the C-3′ and C-4′ positions was reduced. In comparisons of potency based on the *cis* and *trans* isomers at the C-3′ and C-4′ positions of khellactone, the *trans* isomer **22** was more active than the *cis* isomer **7**, but other *cis* and *trans* isomers (**3** and **18**; **5** and **19**) showed no potency differences. With the calcium antagonist verapamil at the dose of 10^{-7} M, a noncompetitive effect was observed and at the dose of 10^{-6} M, LTD_4-induced contractions were completely inhibited. DMSO had no significant effect on induced contractions.

Discussion

Extracted components of *Peucedanum praeruptorum* DUUN. and *Peucedanum decursiyum* MAXIM. show calcium-antagonistic effects and inhibition of histamine release and platelet aggregation.[2-6,8] We have confirmed inhibition of contraction and increased blood flow in an isolated blood vessel with the extracted components of *Peucedanum japonicum* THUNB.[7] In the present study, we first investigated the antagonistic effect of the natural khellactone from the roots of *Peucedanum praeruptorum* DUUN. on rabbit platelet aggregation induced by PAF, ADP, AA, and collagen, and found that the natural khellactone inhibited platelet aggregation by PAF more strongly than that by the other aggregating agents (Table 1). Thus, the natural khellactone showed a specific PAF-antagonistic activity. It is well established that PAF, histamine, and leukotrienes significantly increase during an allergic episode[16] and that, when PAF is administered intravenously, it results in a persistent hypotensive effect and acute death.[17] Administration of PAF in mice and rats has effects on blood pressure, smooth muscle, platelets, *etc.*.[18] We studied the effects of natural khellactone on PAF-induced hypotension in rats and PAF-induced acute death in mice. We also investigated the structure–activity relationship of related compounds on PAF-induced platelet aggregation and histamine- and LTD_4-induced contractions of smooth muscle.

First, significant antagonistic effects of natural khellactone and etizolam on PAF-induced hypotension were observed (Fig. 1). The natural khellactone inhibited the maximum response of PAF-induced hypotension and the subsequent persistent hypotensive response as well. However, etizolam did not inhibit the maximum hypotensive response but hastened the recovery from persistent hypotension. The antagonistic patterns of natural khellactone and etizolam on PAF-induced hypotension were apparently different.

Next, etizolam and ketotifen significantly inhibited PAF-induced acute death in mice, though natural khellactone did not prevent it (Table 2). These facts suggested that either the absorption of natural khellactone is poor or there are species differences in PAF-antagonistic activities.

The analysis of the structure–activity relationship of the compounds (Table 3) yielded the following results. Strong inhibitory effects against PAF were observed with compounds **2**, **5**, **7**, **8**, **10**, and **11** and against histamine and LTD_4 with **2**, **7**, **8**, **10**, and **22**. These facts suggest that compounds having an angelic acid or tiglic acid moiety at the C-3′ position and an angelic acid, tiglic acid, or acetic acid moiety at the C-4′ position of khellactone are active. In addition, the *cis* isomers at the C-3′ and C-4′ positions of khellactone were more active than the corresponding *trans* isomers.

The present results, showing specific inhibition of PAF-induced platelet aggregation and antagonistic effects against histamine and LTD_4 by **2** and **11** are of interest in regard to the medical uses of the *Peucedanum* species as a herbal drug for bronchial disorders, stomach pain, *etc.* These results may lead to new series of PAF, histamine, and LTD_4 antagonist compounds from natural sources.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded with a Hitachi 260-10 spectrometer with polystyrene calibration at 1601 cm^{-1}. ^{1}H-NMR spectra were taken on a JEOL JNM-FX 100 spectrometer with tetramethylsilane as an internal standard. HREI-MS were obtained on a JEOL JMS-D 300 spectrometer. Wakogel C-200 and Merck Kieselgel G nach Stahl were used for column chromatography and thin layer chromatography, respectively.

(±)-*cis*-3′,4′-Diacetylkhellactone (3) Acetic anhydride (3 ml) was added to a solution of **1**[10,11] (50 mg) in dry pyridine (1 ml) and the mixture was allowed to stand overnight at room temperature, then poured into ice-water and extracted with chloroform. The organic layer was washed with saturated $NaHCO_3$, diluted HCl, and water, then dried and evaporated. The residue was subjected to silica gel column chromatography. The chloroform eluate gave 25 mg (43.8%) of **3** as colorless flaky crystals (ether–hexane), mp 165—166 °C (lit.[15] mp 160—162 °C).

(±)-*trans*-3′,4′-Diacetylkhellactone (18) Compound **18** was prepared from **17**[11] as described for compound **3** to afford colorless flaky crystals (ether–hexane), mp 157—158.5 °C, in 94.1% yield. IR ν (Nujol) cm^{-1}: 1730, 1720, 1610, 1490. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, s, –Me), 1.45 (3H, s, –Me), 2.11 (3H, s, –Me), 2.13 (3H, s, –Me), 5.29 (1H, d, J=4.3 Hz, methine H), 6.22 (1H, d, J=4.3 Hz, methine H), 6.25 (1H, d, J=9.6 Hz, olefinic H), 6.82 (1H, d, J=8.5 Hz, aromatic H), 7.38 (1H, d, J=8.5 Hz, aromatic H), 7.62 (1H, d, J= 9.6 Hz, olefinic H). HREI-MS m/z Calcd for $C_{18}H_{18}O_7$ (M^+): 346.1052. Found: 346.1070.

(±)-*cis*-4′-Acetyl-3′-crotonoylkhellactone (5) 4-Pyrrolidinopyridine (1.3 mg), crotonoic acid (30 mg), and dicyclohexylcarbodiimide (103 mg) were added to a solution of **4**[10,12] (50 mg) in dry methylene chloride (13 ml) and the mixture was refluxed overnight. The reaction mixture was washed with diluted HCl, saturated $NaHCO_3$, and water, then dried and evaporated. The residue was subjected to silica gel column chromatography. The chloroform eluate gave 44 mg (71.9%) of **5** as colorless prisms (ether–hexane), mp 159—162 °C. IR ν (Nujol) cm^{-1}: 1740, 1720, 1650, 1605, 1485. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, s, –Me), 1.45 (3H, s, –Me), 1.88 (3H, dd, J=6.8, 1.5 Hz, –Me), 2.12 (3H, s, –Me), 5.38 (1H, d, J=5.0 Hz, methine H), 5.86 (1H, dd, J=15.6, 1.5 Hz, olefinic H), 6.23 (1H, d, J=9.5 Hz olefinic H), 6.58 (1H, d, J=5.0 Hz, methine H), 6.81 (1H, d, J=8.5 Hz, aromatic H), 7.02 (1H, dd, J=15.6, 6.8 Hz, olefinic H), 7.36 (1H, d, J=8.5 Hz, aromatic H), 7.60 (1H, d, J=9.5 Hz, olefinic H). HREI-MS m/z Calcd for $C_{20}H_{20}O_7$ (M^+): 372.1209. Found: 372.1211.

(±)-*cis*-4′-Acetyl-3′-tetrolylkhellactone (6) Compound **6** was pre-

pared from **4** and tetrolic acid as described for compound **5** to afford colorless prisms (ether–hexane), mp 195—196 °C, in 56.5% yield. IR ν (Nujol) cm^{-1}: 2240, 1750, 1730, 1705, 1605. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, s, –Me), 1.47 (3H, s, –Me), 1.98 (3H, s, –Me), 2.17 (3H, s, –Me), 5.37 (1H, d, J=5.1 Hz, methine H), 6.24 (1H, d, J=9.5 Hz, olefinic H), 6.58 (1H, d, J=5.1 Hz, olefinic H), 6.81 (1H, d, J=8.7 Hz, aromatic H), 7.36 (1H, d, J=8.7 Hz, aromatic H), 7.60 (1H, d, J=9.5 Hz, olefinic H). HREI-MS m/z Calcd for $C_{20}H_{18}O_7$ (M^+): 370.1053. Found: 370.1095.

(±)-*cis*-4′-Acetyl-3′-(2″-methylbutyryl)khellactone (8) Compound **8** was prepared from **4** and 2-methylbutyric acid as described for compound **5** to afford colorless prisms (ether–hexane), mp 135—136 °C, in 65.2% yield. IR ν (Nujol) cm^{-1}: 1730, 1600. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7.2 Hz, –Me), 1.16 (3H, d, J=7.1 Hz, –Me), 1.23—1.92 (2H, m, methylene H), 1.40 (3H, s, –Me), 1.45 (3H, s, –Me), 2.13 (3H, s, –Me), 2.42 (1H, q, J=7.1 Hz, methine H), 5.32 (1H, d, J=4.6 Hz, methine H), 6.24 (1H, d, J=9.6 Hz, olefinic H), 6.57 (1H, d, J=4.6 Hz, methine H), 6.80 (1H, d, J=8.5 Hz, aromatic H), 7.36 (1H, d, J=8.5 Hz, aromatic H), 7.60 (1H, d, J=9.6 Hz, olefinic H). HREI-MS m/z Calcd for $C_{21}H_{24}O_7$ (M^+): 388.1522. Found: 388.1567.

(±)-*cis*-4′-Acetyl-3′-(2″-methyl-2″-dodecenoyl)khellactone (9) Compound **9** was prepared from **4** and 2-methyl-2-dodecenoic acid as described for compound **5** to afford a colorless oil in 83.7% yield. IR ν (neat) cm^{-1}: 1740, 1610, 1490. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.0 Hz, –Me), 1.26 (14H, s, methylene H), 1.42 (3H, s, –Me), 1.47 (3H, s, –Me), 1.81 (3H, s, –Me), 2.10 (3H, s, –Me), 2.15 (2H, m, methylene H), 5.37 (1H, d, J=4.9 Hz, methine H), 6.23 (1H, d, J=9.5 Hz, olefinic H), 6.48—6.89 (1H, m, olefinic H), 6.62 (1H, d, J=4.9 Hz, methine H), 6.81 (1H, d, J=8.4 Hz, aromatic H), 7.36 (1H, d, J=8.4 Hz, aromatic H), 7.60 (1H, d, J= 9.5 Hz, olefinic H). HREI-MS m/z Calcd for $C_{29}H_{38}O_7$ (M^+): 498.2768. Found: 498.2770.

(±)-*cis*-3′,4′-Ditigloylkhellactone (10) Tigloyl chloride (360 mg) was added to a solution of **1** (200 mg) in dry benzene (12 ml) and the mixture was refluxed overnight under a nitrogen atmosphere. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ and water, then dried and evaporated. The residue was subjected to silica gel column chromatography. The chloroform eluate gave 208 mg (64.0%) of **10** as a colorless oil. IR ν (neat) cm^{-1}: 1720, 1645, 1605, 1490. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, s, –Me), 1.49 (3H, s, –Me), 1.76 (6H, d, J=7.3 Hz, –Me) 1.80 (6H, s, –Me), 5.40 (1H, d, J=4.9 Hz, methine H), 6.21 (1H, d, J=9.5 Hz, olefinic H), 6.65—7.05 (2H, m, olefinic H), 6.66 (1H, d, J=4.9 Hz, methine H), 6.81 (1H, d, J=8.7 Hz, aromatic H), 7.37 (1H, d, J=8.7 Hz, aromatic H), 7.60 (1H, d, J=9.5 Hz, olefinic H). HREI-MS m/z Calcd for $C_{24}H_{26}O_7$ (M^+): 426.1678. Found: 426.1678.

(±)-*cis*-4′-Ethyl-3′-tigloylkhellactone (13) Compound **13** was prepared from **12**[13] and tigloyl chloride as described for compound **10** to afford colorless flaky crystals (ether–hexane), mp 98—99.5 °C, in 65.1% yield. IR ν (Nujol) cm^{-1}: 1730, 1715, 1645, 1600, 1485. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, t, J=8.0 Hz, –Me), 1.42 (3H, s, –Me), 1.45 (3H, s, –Me), 1.88 (3H, d, J=8.0 Hz, –Me), 1.94 (3H, s, –Me), 3.68—4.37 (2H, m, methylene H), 4.98 (1H, d, J=5.2 Hz, methine H), 5.26 (1H, d, J=5.2 Hz, methine H), 6.26 (1H, d, J=9.1 Hz, olefinic H), 6.83—7.12 (1H, m, olefinic H), 6.89 (1H, d, J=8.0 Hz, aromatic H), 7.37 (1H, d, J=8.0 Hz, aromatic H), 7.67 (1H, d, J=9.1 Hz, olefinic H). HREI-MS m/z Calcd for $C_{21}H_{24}O_6$ (M^+): 372.1571. Found: 372.1561.

(±)-*trans*-4′-Acetyl-3′-crotonoylkhellactone (19) Compound **19** was prepared from **17** and crotonoyl chloride as described for compound **10** to afford colorless prisms (ether–hexane), mp 179—181 °C, in 53.5% yield. IR ν (Nujol) cm^{-1}: 1745, 1730, 1715, 1650, 1605, 1490. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, s, –Me), 1.35 (3H, s, –Me), 1.91 (3H, dd, J=7.2, 2.0 Hz, –Me), 2.11 (3H, s, –Me), 5.42 (1H, d, J=4.6 Hz, methine H), 5.90 (1H, dd, J=15.5, 2.0 Hz, olefinic H), 6.26 (1H, d, J=4.6 Hz, methine H), 6.30 (1H, d, J=9.0 Hz, olefinic H), 6.90 (1H, d, J=8.1 Hz, aromatic H), 6.96 (1H, dd, J=15.5, 7.2 Hz, olefinic H), 7.46 (1H, d, J=8.1 Hz, aromatic H), 7.61 (1H, d, J=9.0 Hz, olefinic H). HREI-MS m/z Calcd for $C_{20}H_{20}O_7$ (M^+): 372.1209. Found: 372.1210.

(±)-*trans*-4′-Acetyl-3′-valerylkhellactone (20) Compound **20** was prepared from **17** and valeryl chloride as described for compound **10** to afford a colorless oil in 93.1% yield. IR ν (neat) cm^{-1}: 1755, 1730, 1720, 1645, 1605, 1490. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6.0 Hz, –Me), 1.37 (3H, s, –Me), 1.42 (3H, s, –Me), 1.12—1.85 (4H, m, methylene H), 2.18 (3H, s, –Me), 2.37 (2H, m, methylene H), 5.35 (1H, d, J=4.2 Hz, methine H), 6.23 (1H, d, J=9.1 Hz, olefinic H), 6.25 (1H, d, J=4.2 Hz, methine H), 6.83 (1H, d, J=7.5 Hz, aromatic H), 7.40 (1H, d, J=7.5 Hz, aromatic H), 7.60 (1H, J=9.1 Hz, olefinic H). HREI-MS m/z Calcd for $C_{21}H_{24}O_7$ (M^+): 388.1522. Found: 388.1552.

(±)-*trans*-4′-Acetyl-3′-isovalerylkhellactone (21) Compound **21** was prepared from **17** and isovaleryl chloride as described for compound **10** to afford colorless needles (ether–hexane), mp 134—135.5 °C, in 80.5% yield. IR ν (neat) cm^{-1}: 1750, 1725, 1605, 1495. ^{1}H-NMR ($CDCl_3$) δ: 0.94 (6H, d, J=6.2 Hz, –Me), 1.28—1.78 (1H, m, methine H), 1.39 (3H, s, –Me), 1.44 (3H, s, –Me), 2.12 (3H, s, –Me), 2.18—2.26 (2H, m, methylene H), 5.35 (1H, d, J=4.7 Hz, methine H), 6.24 (1H, d, J=9.4 Hz, olefinic H), 6.26 (1H, d, J=4.7 Hz, methine H), 6.82 (1H, d, J=8.2 Hz, aromatic H), 7.40 (1H, d, J=8.2 Hz, aromatic H), 7.65 (1H, d, J=9.4 Hz, olefinic H). HREI-MS m/z Calcd for $C_{21}H_{24}O_7$ (M^+): 388.1522. Found: 388.1538.

(±)-*trans*-4′-Acetyl-3′-tigloylkhellactone (22) Compound **22** was prepared from **17** and tigloyl chloride as described for compound **10** to afford colorless prisms (ether–hexane), mp 160.5—161.5 °C, in 74.8% yield. IR ν (Nujol) cm^{-1}: 1750, 1715, 1650, 1610, 1495. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, s, –Me), 1.45 (3H, s, –Me), 1.72 (3H, d, J=5.9 Hz, –Me), 1.19 (3H, s, –Me), 2.13 (3H, s, –Me), 5.35 (1H, d, J=4.9 Hz, methine H), 6.25 (1H, d, J=9.5 Hz, olefinic H), 6.29 (1H, d, J=4.9 Hz, methine H), 6.73—6.96 (1H, m, olefinic H), 6.83 (1H, d, J=8.7 Hz, aromatic H), 7.38 (1H, d, J=8.7 Hz, aromatic H), 7.62 (1H, d, J=9.5 Hz, olefinic H). HREI-MS m/z Calcd for $C_{21}H_{22}O_7$ (M^+): 386.1363. Found: 386.1740.

(±)-*trans*-4′-Acetyl-3′-decanoylkhellactone (23) Compound **23** was prepared from **17** and decanoyl chloride as described for compound **10** to afford colorless flaky crystals (ether-hexane), mp 79—80 °C, in 81.3% yield. IR ν (Nujol) cm^{-1}: 1745, 1735, 1725, 1605, 1490. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=5.2 Hz, –Me), 1.16—1.86 (14H, m, methylene H), 1.38 (3H, s, –Me), 1.43 (3H, s, –Me), 2.18 (3H, s, –Me), 2.22—2.53 (2H, m, methylene H), 5.37 (1H, d, J=4.5 Hz, methine H), 6.28 (1H, J=9.0 Hz, olefinic H), 6.30 (1H, d, J=4.5 Hz, methine H), 6.86 (1H, d, J=8.3 Hz, aromatic H), 7.42 (1H, d, J=8.3 Hz, aromatic H), 7.68 (1H, d, J=9.0 Hz, olefinic H). HREI-MS m/z Calcd for $C_{26}H_{34}O_7$ (M^+): 458.2305. Found: 458.2370.

(±)-*trans*-4′-Ethyl-3′-tigloylkhellactone (25) Compound **25** was prepared from **24**[13] and tigloyl chloride as described for compound **10** to afford colorless flaky crystals (ether–hexane), mp 143—144.5 °C, in 50.7% yield. IR ν (Nujol) cm^{-1}: 1720, 1705, 1645, 1605, 1485. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, J=6.9 Hz, –Me), 1.49 (3H, s, –Me), 1.53 (3H, s, –Me), 1.73 (3H, d, J=6.2 Hz, –Me), 1.79 (3H, s, –Me), 4.08 (2H, m, methylene H), 4.62 (1H, d, J=3.0 Hz, methine H), 5.25 (1H, d, J= 3.0 Hz, methine H), 6.25 (1H, d, J=9.2 Hz, olefinic H), 6.40—7.06 (1H, m, olefinic H), 6.83 (1H, d, J=8.3 Hz, aromatic H), 7.37 (1H, d, J= 8.3 Hz, aromatic H), 7.65 (1H, d, J=9.2 Hz, olefinic H). HREI-MS m/z Calcd for $C_{21}H_{24}O_6$ (M^+): 372.1571. Found: 372.1568.

(±)-*cis*-3′-[*N*-(2″-Chloro)ethylcarbamoyl]-4′-ethylkhellactone (14) 2-Chloroethylisocyanate (2.9 g) was added to a solution of **12** (300 mg) in dry pyridine (6 ml) and chloroform (3 ml) and the mixture was stirred overnight at 50 °C, then poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water, then dried and evaporated. The residue was recrystallized from chloroform to give 307 mg (75.0%) of **14** as colorless prisms, mp 203—205 °C. IR ν (Nujol) cm^{-1}: 3330, 1725, 1690, 1600, 1520, 1490. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz, –Me), 1.42 (3H, s, –Me), 1.53 (3H, s, –Me), 3.52—3.70 (2H, m, methylene H), 3.74—4.24 (4H, m, methylene H), 4.88—5.44 (1H, m, –NH–), 4.98 (1H, d, J=4.7 Hz, methine H), 5.18 (1H, d, J=4.7 Hz, methine H), 6.28 (1H, d, J=9.2 Hz, olefinic H), 6.75 (1H, d, J=8.1 Hz, aromatic H), 7.37 (1H, d, J=8.1 Hz, aromatic H), 7.66 (1H, d, J=9.2 Hz, olefinic H).

(±)-*cis*-3′-[*N*-(2″-Diethylamino)ethylcarbamoyl]-4′-ethylkhellactone (15) Diethylamine (843 mg) was added to a solution of **14** (45 mg) in *N*,*N*-dimethylformamide (2 ml) and the mixture was stirred overnight at 65 °C, then poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water, then dried and evaporated. The residue was recrystallized from ether–hexane to give 41 mg (83.3%) of **15** as colorless prisms, mp 95—96.5 °C. IR ν (Nujol) cm^{-1}: 3340, 1725, 1695, 1600, 1520, 1480. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (6H, t, J=7.5 Hz, m, –Me), 1.12 (3H, t, J=7.5 Hz, –Me), 1.41 (3H, s, –Me), 1.50 (3H, s, –Me), 2.13—2.75 (4H, m, methylene H), 3.03—3.48 (2H, m, methylene H), 3.60—4.33 (4H, m, methylene H), 4.94 (1H, d, J=4.6 Hz, methine H), 5.12 (1H, d, J=4.6 Hz, olefinic H), 5.22—5.54 (1H, m, –NH–), 6.25 (1H, d, J=9.6 Hz, olefinic H), 6.73 (1H, d, J=8.9 Hz, aromatic H), 7.33 (1H, d, J=8.9 Hz, aromatic H), 7.61 (1H, d, J=9.6 Hz, olefinic H).

HERI-MS *m/z* Calcd for $C_{25}H_{32}N_2O_6$ (M^+): 472.2208. Found: 472.2200.

(±)-*cis*-4′-Ethyl-3′-[*N*-(2″-triethylammonio)ethylcarbamoyl]khellactone Iodide (16) A solution of **15** (200 mg) in iodoethane (2.5 ml) was refluxed for 2 h and then the mixture was allowed to stand at room temperature. The separated crystals were collected and washed with ether to yield 165 mg (62.5%) of **16** as colorless prisms, mp 235—237 °C. IR ν (Nujol) cm^{-1}: 3250, 1730, 1720, 1600, 1500, 1485. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (12H, t, J=7.2 Hz, –Me), 1.40 (3H, s, –Me), 1.50 (3H, s, –Me), 3.07—4.16 (12H, m, methylene H), 3.87—4.14 (1H, m, –NH–), 4.99 (2H, s, methine H), 6.26 (1H, d, J=9.3 Hz, olefinic H), 6.76 (1H, J=7.9 Hz, aromatic H), 7.40 (1H, d, J=7.9 Hz, aromatic H), 7.73 (1H, d, J=9.3 Hz, olefinic H).

References and Notes

1) For a preliminary communication of part of this work, see Takeuchi N., Kasama T., Mayuzumi K, Tamaru K., Fukaya H., Ohno T., Tobinaga S., *Chem. Pharm. Bull.*, **36**, 4221 (1988).
2) Thompson E. B., Aynilian G. H., Dobberstein R. H., Cordell G. A., Fong H. H. S., Farnsworth N. R., *J. Natural Products*, **42**, 120 (1979), and references cited therein.
3) Smith E., Hosansky N., Bywater W. G., van Tamelen E. E., *J. Am. Chem. Soc.*, **79**, 3534 (1957).
4) Kozawa T., Sakai K., Uchida M., Okuyama T., Shibata S., *J. Pharm. Pharmcol.*, **33**, 317 (1981); Suzuki T., Kobayashi Y., Uchida M. K., Sakakibara I., Okuyama T., Shibata S., *J. Pharmacobio-Dyn.*, **8**, 257 (1985).
5) Okuyama T., Kawasaki C., Shibata S., Hoson M., Kawada T., Osada H., Noguchi T., *Planta Medica*, **52**, 132 (1986).
6) Willette R. E., Soine T. O., *J. Pharm. Sci.*, **51**, 149 (1962).
7) Takeuchi N., Kasama T., Aida Y., Oki J., Maruyama I., Watanabe K., Tobinaga S., *Chem. Pharm. Bull.*, **39**, 1415 (1991).
8) Chen Z. X., Huang B. S., She Q. L., Zeng G. F., *Acta Pharm. Sin.*, **14**, 486 (1979); Okuyama T., Shibata S., *Planta Medica*, **42**, 89 (1981).
9) "Biologically Active Ether Lipids," ed. by Braquet P., Mangold H. K., Vargaftig B. B., (Progress in Biochemical Pharmacolgy, Vol. 22, ed. by R. Paoletti), Karger, Basle, 1988.
10) Bal-Tembe S., Bhedi D. N., de Souza N. J., Rupp R. H., *Heterocycles*, **26**, 1239 (1987).
11) Schroeder H. D., Bencze W., Halpern O., Schmid H., *Chem. Ber.*, **92**, 2338 (1959).
12) Bohlmann F., Franke H., *Chem. Ber.*, **104**, 3229 (1971).
13) Hata K., Kozawa M., Ikeshiro Y., Yen K.-Y., *Yakugaku Zasshi*, **88**, 513 (1968).
14) Hlubucek J., Ritchie E., Tayler W. C., *Aust. J. Chem.*, **24**, 2347 (1971).
15) Shanbhag S. N., Maheshwari M. L., Bhattacharyya S. C., *Tetrahedron*, **23**, 1235 (1967).
16) Meslier N., Braunseein G., Lacronique J., Dessangen J. F., Rakosihanaka F., Devillier P., Lockhart A., Mencia-Huerta J. M., Boushey H. A., *Am. Rev. Respir. Dis.*, **137**, 617 (1988); Miadoma A., Tedeschi A., Arnoux B., Sala A., Zanussi C., Benveniste J., *Am. Rev. Respir. Dis.*, **140**, 142 (1989).
17) Blank M. L., Snyder F., Byers L. W., Brooks B., Muirhead E. E., *Biochem. Biophys. Res. Commun.*, **90**, 1194 (1979); Prewitt R. R., Leach B. E., Byers L. W., Brooks B. B., Lands W. E. M., Murihead E. E., *Hypertension*, **1**, 299 (1979); Murihead E. E., Byers L. M., Desiderio Jr. D., Smith K. A., Prewitt R. L., Lands W. E. M., *ibid.* **3**, Suppl. I, 107 (1981); Tanaka S., Kasuya Y., Masuda Y., Shigenobu K., *J. Pharm. Dyn.*, **6**, 866 (1983).
18) Namm D. H., Tadepalli A. S., High J. A., *Thrombosis Res.*, **25**, 341 (1982); Tarashita I., Imura Y., Nishikawa K., *Biochem. Pharmacol.*, **34**, 1491 (1985); Saeki S., Masugi F., Ogihara T., Otsuka A., Kumahara Y., Watanabe K., Tamura K., Akashi A., Kumagai A., *Life Sci.*, **37**, 325 (1985); Chang S., Feddersen C. O., Menson P. M., Voelkel N. F., *J. Clin. Invest.*, **79**, 1498 (1987).