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PHTHALIDES FROM THE RHIZOME OF *LIGUSTICUM WALLICHII*

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Key Word Index—*Ligusticum wallichii*; Umbelliferae; senkyu; phthalide; 3-butylidene-7-hydroxyphthalide; *cis*-6,7-dihydroxyligustilide; *trans*-6,7-dihydroxyligustilide; wallichilide; 3-butyl-4,5-dihydro-3-hydroxyphthalide.

Abstract—Three phthalides, 3-butylidene-7-hydroxyphthalide, and *cis* and *trans*-6,7-dihydroxyligustilides, along with a dimeric phthalide wallichilide have been isolated from the hot water extract of the rhizome of *Ligusticum wallichii*. Their structures were established mainly on the basis of spectroscopic data. 3-Butyl-4,5-dihydro-3-hydroxy phthalide and adenosine were identified as active principles of the extract which are responsible for increase of coronary blood flow in the dog heart.

INTRODUCTION

'Chuan-Xiong' (Japanese name 'Senkyu') is the dried rhizome of *Ligusticum wallichii* Franch, and is well known in Chinese medicine as an important crude drug having haemodynamic and analgesic effects [1]. In China, it has been prescribed tentatively with other drugs in the treatment of angina pectoris [2]. Tetramethylpyrazine has already been identified as one of the active principles of *L. wallichii* and has been in clinical use against an ischemic cerebrovascular disease in China [3–5]. According to a detailed pharmacological evaluation using the dog heart [6, 7], the hot water extract of *L. wallichii* increases coronary blood flow. During the course of our continuing search for the active substances of *L. wallichii*, we have now isolated three phthalides, 3-butylidene-7-hydroxyphthalide (**1**), and *cis* and *trans*-dihydroxyligustilides (**2** and **3**), and a dimeric phthalide named wallichilide (**6**), the structures of which are described in this paper.

RESULTS AND DISCUSSION

The hot water extract of the rhizome of *L. wallichii* was extracted successively with benzene and ethyl acetate. From both extracts, a combination of column chromatography, prep. TLC and HPLC led to the isolation of compounds **1**, **2**, **3** and **6** along with the known phthalides ligustilide, butylidenephthalide, butylphthalide, senkyunolide and neocindilide.

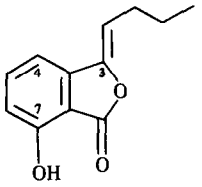
3-*Butylidene-7-hydroxyphthalide* (**1**)

Compound **1** had the molecular formula $C_{12}H_{12}O_3$ (M^+ 204.0791), and contained a hydroxyl (3600 cm^{-1}) group and a γ-lactone moiety (1750 cm^{-1}). Its mass spectrum exhibited fragment peaks at *m/z* 175 $[M-C_2H_5]^+$, 162 $[M-C_3H_6]^+$ and 147 $[M-C_2H_5-CO]^+$ indicating that **1** was butylidenephthalide substituted with a phenolic hydroxyl group. The ^1H NMR of **1** showed the presence of 1,2,3-substituted aromatic protons [δ6.91 (1H, *d*, *J* = 8.0 Hz), 7.13 (1H, *d*, *J* = 8.0 Hz), 7.55 (1H, *dd*, *J* = 8.0, 8.0 Hz)], but did not contain the lowfield proton around δ7.5 found in butylidenephthalide meaning that a hydroxyl group was located on C-7. This assignment was supported by an $AlCl_3$-induced bathochromic shift (224 → 230 nm and 330 → 340 nm) in the UV spectrum. Thus, the structure of **1** was established as 3-butylidene-7-hydroxyphthalide.

cis-6,7-*Dihydroxyligustilide* (**2**)

Compound **2**, λ_{max}^{MeOH} 272 nm, had the molecular formula $C_{12}H_{16}O_4$ (M^+ 224.1044). Its IR spectrum revealed the presence of hydroxyl (3400 cm^{-1}), γ-lactone (1765 cm^{-1}) and diene (1675, 1638 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of ligustilide (**5**) [9] except for the signals due to H-6 and H-7 and C-6 and C-7 (Tables 1 and 2). These spectral data suggested a close structural relationship between **2** and **5**. In place of the signals at δ5.97 (1H, *dt*, *J* = 9.5, 4.1 Hz, H-6), 6.25 (1H, *dt*, *J* = 9.5, 1.6 Hz, H-7) and δ130.0 (*d*, C-6), 117.4 (*d*, C-7) found in ligustilide (**5**), **2** contained signals due to methine protons at δ3.95 (1H, *ddd*, *J* = 9.8, 5.9, 3.1 Hz) and 4.50 (1H, *d*, *J* = 5.9 Hz), and carbon atoms bearing secondary hydroxyl groups at δ68.0 (*d*) and 71.9 (*d*), suggesting that in **2** the $\Delta^{6,7}$ double bond of **5** was replaced by a 6,7-dihydroxy group. This was supported by the formation of the diacetate **2a** and the dibenzoate **2b** on treatment of **2** with acetic anhydride and benzoyl chloride in pyridine respectively. In addition, the mass spectrum of **2** showed a fragment peak at *m/z* 180.0806 which was derived from a retro-Diels–Alder cleavage of the $[M-H_2O]^+$ ion at *m/z* 206, supporting the structure dihydroxylated at C-6 and C-7 for **2**. Thus, these data gave 6,7-dihydroxyligustilide a plane structure The configurations of the hydroxyl groups attached at C-6 and C-7 were determined by the complete assignment of the ^1H NMR signals of **2** by means of extensive decoupling

†To whom correspondence should be addressed.



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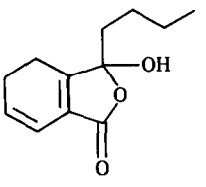

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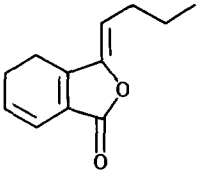

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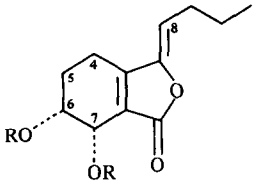
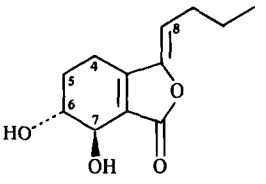


2 R = —H
2a R = —Ac
2b R = —COPh



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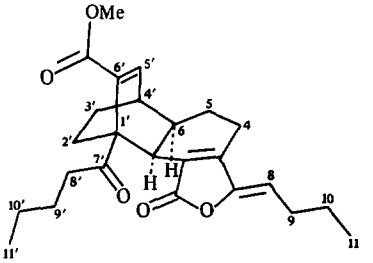


6

Table 1. ^{1}H NMR data of compounds 2, 3 and 5 (400 MHz, $CDCl_3$, TMS as int. standard)

H	2	3	5
4α	2.58 *dddd* (18.4, 5.9, 5.4, 1.1)*	2.38 *dddd* (18.4, 5.4, 5.4, 1.1)	2.57 *m*
4β	2.50 *dddd* (18.4, 8.1, 5.5, 1.9)	2.65 *dddd* (18.4, 7.6, 5.7, 1.4)	
5α	2.58 *dddd* (13 5, 9 8, 8.1, 5.9)	1.81 *dddd* (13.8, 7.6, 6 2, 2.4)	2.43 *m*
5β	2.12 *dddd* (13.5, 5.4, 5.5, 3.1)	2.14 *dddd* (13.8, 7 6, 5.7, 5.4)	
6	3.95 *ddd* (9.8, 5.9, 3.1)	4.07 *ddd* (7.6, 3.5, 2 4)	5.97 *dt* (9.5, 4.1)
7	4.50 *ddd* (5.9, 1.9, 1 1)	4.61 *ddd* (3 5, 1.9, 1.1)	6.25 *dt* (9.5, 1.6)
8	5.29 *t* (7.3)	5.31 *t* (7.7)	5.20 *t* (8.0)
9	2 36 *td* (7.3, 7.3)	2.36 *td* (7.7, 7.7)	2.43 *td* (8.0, 7.3)
10	1.50 *qt* (7 3, 7.3)	1.50 *qt* (7.7, 7.7)	1.48 *qt* (7.3, 7.3)
11	0 95 *t* (7.3)	0.95 *t* (7.7)	0.92 *t* (7 3)

*Coupling constants (J in Hz) are given in parentheses.

experiments (Table 1). Thus the J values of the vicinal couplings found for H-4 and H-5 ($J_{4\alpha,5\beta}$ = 5.4 Hz, $J_{4\alpha,5\alpha}$ = 5.9 Hz, $J_{4\beta,5\beta}$ = 5.5 Hz, $J_{4\beta,5\alpha}$ = 8.1 Hz) disclosed that the cyclohexene ring in **2** adopted a boat-like conformation. The J value between H-6 and 7 showed 5.9 Hz, a typical *cis*-coupling, indicating that the hydroxyl groups had to be *cis* to each other. An NOE for H-8 (8%) was observed upon irradiation at H-4α meaning that the $\Delta^{3,8}$ double bond had *Z*-geometry as in ligustilide (**5**). Thus, the structure of **2** was assigned to 3-(*Z*)-butylidene-4,5-dihydro-*cis*-6,7-dihydroxyphthalide.

trans-6,7-*Dihydroxyligustilide* (**3**)

Compound **3** had the same molecular formula, $C_{12}H_{16}O_4$, as **2**. Furthermore its IR, UV and mass spectra were very similar to those of **2**. The ^{1}H NMR spectrum of **3** was found to be almost identical with that of **2** except for the J value (3.5 Hz) between H-6 and H-7, which indicated that the hydroxyl groups attached at C-6 and 7 were in the *trans* configuration. Thus, the structure 3-(*Z*)-butylidene-4,5-dihydro-*trans*-6,7-dihydroxyphthalide was proposed for **3**.

Table 2. ^{13}C NMR data of compounds **2**, **5** and **6** (100.61 MHz, $CDCl_3$, TMS as int. standard)

Carbon	2	5	6
1	169.2 *s*	167.6 *s*	168.7 *s*
3	148.5 *s*	149.0 *s*	149.0 *s*
3a	153.0 *s*	147.2 *s*	153.5 *s*
4	19.1 *t*	18.6 *t*	19.0 *t*
5	26.7 *t*	22.6 *t*	25.8 *t*
6	71.9 *d*	130.0 *d*	38.4 *d**
7	68.0 *d*	117.4 *d*	38.5 *d**
7a	126.3 *s*	124.3 *s*	127.5 *s*
8	114.1 *d*	112.7 *d*	110.7 *d*
9	28.2 *t*	28.2 *t*	28.0 *t*
10	22.4 *t*	22.5 *t*	22.5 *t*
11	13.7 *q*	13.8 *q*	13.7 *q*
1′			57.4 *s*
2′			27.9 *t*
3′			28.5 *t*
4′			38.2 *d**
5′			138.8 *d*
6′			138.0 *s*
7′			208.1 *s*
8′			39.7 *t*
9′			28 5 *t*
10′			22.5 *t*
11′			14.0 *q*
COOMe			51.4 *q*
COOMe			169.3 *s*

*Assignments may be interchangeable.

Wallichilide (**6**)

Compound **6**, mp 163–164.5°, had the molecular formula $C_{25}H_{32}O_5$ (M^+ 412.2252, calc. 412.2250 and elementary analysis), showing that it contained ten unsaturations. Its IR spectrum contained absorption bands assignable to a γ-lactone group (1758 cm^{-1}), a conjugated ester (1715, 1620 cm^{-1}) and ketone (1690 cm^{-1}) groups. The ^{13}C NMR spectrum of **6** showed the presence of 25 carbon atoms comprised of three methyl groups including one methyl group bearing an O-atom, nine methylenes, three methines one tetrasubstituted carbon, two trisubstituted and one tetrasubstituted double bonds, and two ester carbonyls and one ketone carbonyl. The 400 MHz ^1H NMR spectrum exhibited signals due to two methyl groups [δ0.92, 0.93 (each 3H, *t*, *J* = 7.3, 7.8 Hz)], a methyl ester group [δ3.59 (3H, *s*)] and olefinic protons [δ5.06 (1H, *t*, *J* = 7.8 Hz), 6.70 (1H, *d*, *J* = 6.9 Hz)]. In addition extensive selective proton decoupling experiments (Table 3) disclosed the presence of the following partial structures: –C(–)–CH_2 (8′)–CH_2 (9′)–CH_2 (10′)–CH_3 (11′) (**A**), –C(–)=CH (8)–CH_2 (9)–CH_2 (10)–CH_3 (11) (**B**), –C(–)–CH_2 (2′)–CH_2 (3′)–CH (4′)–CH (5′)=C(–)–(**C**), –C(–)–CH_2 (4)–CH_2 (5)–CH (6)–CH (7)–C(–)–(**D**). The partial structure **D** showed homoallylic long-range coupling ($J_{7,4\alpha}$ = 1.4 Hz, $J_{7,4\beta}$ = 1.2 Hz), and the NOE for H-8 (7%) in **B** was observed upon irradiation at H-4β (δ2.12) in **D** suggesting that **B** and **D** originated from partial structure **E**. The presence of **E** was additionally supported by the observation of ^{13}C NMR shifts in **6** very similar to those in **2** (Table 2). On the other hand, the remaining

Table 3. ^1H NMR data of **6** (400 MHz, $CDCl_3$, TMS as int. standard)

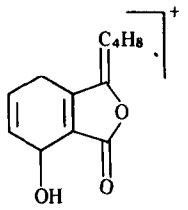
H	6	6 + Eu (fod)$_3$*	Shift value
4α	2.12 *dddd* (16.5, 11.9, 4.6, 1.4)†	2.27	−0.15
4β	2.23 *dddd* (16.5, 4.1, 2.7, 1.2)	—	—
5α	1.88 *dddd* (11.9, 11.0, 4.6, 2.7)	1.93	−0.05
5β	1.55 *dddd* (11.9, 11.9, 8.0, 4.1)	—	—
6	2.50 *dddd* (11.0, 8.8, 8.0, 1.0)	2.57	−0.07
7	3.32 *ddd* (8.8, 1.4, 1.2)	3.90	−0.58
8	5.06 t (7.8)	5.11	−0.05
9	2.31 *td* (7.8, 7.8)	2.32	−0.01
10	1.46 *qt* (7.8, 7.8)	1.47	−0 01
11	0.93 *t* (7.8)	0.93	0
2′α	2.13 *ddd* (12.2, 9.9, 5.3)	2.32	−0.19
2′β	1.63 *ddd* (12.2, 11.9, 3.4)	1.76	−0.13
3′α	1.92 *dddd* (12.2, 9.9, 3.4, 2.7)	1.98	−0.06
3′β	1.40 *dddd* (12.2, 11.9, 5.3, 2.3)	—	—
4′	2.59 *dddd* (6.9, 2.7, 2.3, 1.0)	2.67	−0.08
5′	6.70 *d* (6.9)	7 11	−0.41
8′	3.07 *ddd* (17.8, 8.5, 6.2)	3 29	−0.22
	2.55 *ddd* (17.8, 8.5, 6.2)	2 88	−0.33
9′	1.65 *m*	1.76	−0.11
10′	1.33 *qt* (7.3, 7.3)	1.37	−0.04
11′	0.92 *t* (7 3)	0.92	0
COOMe	3.59 *s*	3.69	−0.10

*0.2 mol.

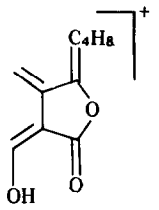
†Coupling constants (*J* in Hz) are given in parentheses.

group **A** and **C** might be converted to the partial structure **F** if the quaternary carbons in **C** were linked together. This assumption was supported by the following mass spectrum analysis: the interpretation of the mass spectrum of **6** with the help of high resolution allowed the assignment of prominent peaks at *m/z* 222 and 190 to fragment **7** and **8**, which was derived from a retro-Diels–Alder cleavage. The fragments **8** and **7** turned out to be ligustilide and its methyl ester respectively, which were clearly supported by the typical fragment **9**, **10** and **11** for ligustilide observed at *m/z* 161, 148 and 165 (Scheme 1). The results of the MS analysis were consistent with the partial structures **E** and **F** being bonded together. Thus, a bicyclo (2.2.2) octene skeletone was elucidated for the structure of wallichilide.

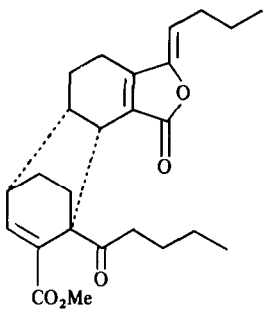
The presence of the small *J* value (1.0 Hz) between H-4′ and H-6 provided proof of $C_{4'}$–C_6 bond formation and indicated that $C_{1'}$–C_7 had to be connected. The bond linkage between **E** and **F** was established by comparison of the ^{1}H NMR in **6** to that on the addition of Eu (fod)$_3$ (Table 3). Namely, the chemical shift of H-7 resulted in a large downfield shift (−0.58) since a strong complex formation between Eu (fod)$_3$ and carbonyl groups caused a contact magnetic effect, whereas that of H-4 did not change, indicating that the carbonyl groups in **E** and **F** were placed in the same direction in space. This result can be explained only by the structure **6** tentatively proposed by the analyses of the ^{1}H, ^{13}C NMR and mass spectral data. The *cis* orientation for H-6 and H-7 were evident from the *J* value (8.8 Hz) and the presence of W-shaped long range coupling ($J_{7,4'}$ = 1 Hz) [10]. The $\Delta^{3,8}$ double bond had to take *Z*-configuration due to the NOE for H-8



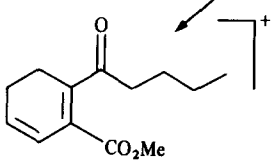
m/z 206



m/z 180 0806
($C_{10}H_{12}O_3$)



6 $C_{25}H_{32}O_5$
M^+ *m/z* obs 412 2252
calc 412 2250



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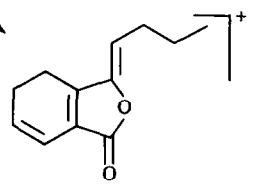
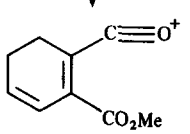
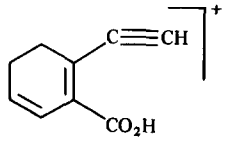
calc 222 1256



8 $C_{12}H_{14}O_2$
m/z obs. 190 1002
calc 190 0994



11 $C_9H_9O_3$
m/z obs 165.0349
calc 165.0552



10 $C_9H_8O_2$
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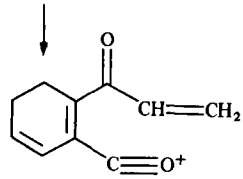
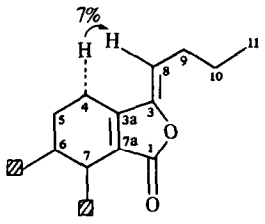
calc. 148 0524

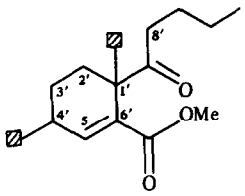


9 $C_{10}H_9O_2$
m/z obs 161 0593
calc 161 0602

Scheme 1 Mass spectral fragmentation of wallichilide (**6**).



E



F

(7%) upon irradiation at H-4β. Thus, on the basis of the spectral evidence in addition to the molecular formula, **6** was proposed for the structure of wallichilide.

In addition to **1**, **2**, **3** and **6** the rhizome of *L. wallichii* contained the previously known phthalides butylphthalide, butylidenephthalide, ligustilide, senkyunolide and neocnidilide, which were reported to be produced by the Japanese variety of 'Senkyu', *Cnidium officinale* [11, 12]. Wallichilide (**6**) has proved to be closely related to diligustilide which was recently reported by Kaouadji *et al.* [13]. Although we have not had an opportunity to compare both compounds, *trans*-6,7-dihydroxyligustilide (**3**) should be identical with ligustilidiol reported by Kaouadji *et al.* [14].

Finally, it is noted that we had already identified both adenosine [15–17] and 3-butyl-4,5-dihydro-3-hydroxyphthalide (**4**) as the substances of *L. wallichii* which can enhance strong coronary blood flow (6.5 ml/1 mg) in isolated blood-perfused dog heart preparations. We will report elsewhere on the detailed pharmacological properties of the compounds so far isolated from *L. wallichii*.

EXPERIMENTAL

L. wallichii rhizome was identified by Wang Pushan. A voucher specimen has been deposited at Peking Institute of Pharmaceutical Industries Mps. uncorr; ^{1}H NMR (400 MHz) and ^{13}C NMR (100.61 MHz): $CDCl_3$, TMS as int. standard. 70 eV; CC: silica gel (Merck 70–230 mesh); TLC: precoated silica gel plates F_{254} (Merck, 0 25, 1 and 2 mm). Spots were visualized by UV (254 nm) and 40% $CeSO_4$–H_2SO_4.

Extraction and isolation. (a) Air dried and powdered rhizome (5 kg) was extracted with H_2O (20 l.) at 80° and the H_2O extract extracted successively with C_6H_6 and EtOAc The C_6H_6 fraction (15 g) was chromatographed on silica gel using C_6H_6–EtOAc and divided into 23 fractions: fr. 1–6 (C_6H_6); fr. 7–13 (C_6H_6–EtOAc, 2·3). Fr. 5–6 (251 mg) were bulked and purified by CC on silica gel (*n*-hexane–Me_2CO, 9:1) followed by prep. TLC (n-hexane–Me_2CO, 4:1) to give 3-butylidene-7-hydroxyphthalide (**1**) (2.6 mg) and butylidenephthalide (22 mg). Fr. 9 (200 mg) was subjected to prep TLC (C_6H_6–EtOAc, 3 2) to afford senkyunolide (89 mg). Fr. 10 (4.2 g) was rechromatographed on silica gel (C_6H_6–EtOAc, 4.1) and the fourth fraction (1 g) purified by HPLC [Lichroprep Si 60 (type C), *n*-hexane–EtOAc (4 1), 9 ml/min] to give neocnidilide (21 mg). The EtOAc extract was evaporated to dryness and the residue (25 g) chromatographed on silica gel using C_6H_6–EtOAc and divided into 15 fractions: fr. 1–3 (C_6H_6–EtOAc, 3:2); fr 4–7 (C_6H_6–EtOAc, 2:3); fr. 8–15 (EtOAc). Fr. 3 (10 3 g) was rechromatographed on silica gel (C_6H_6–EtOAc, 2:3) and the second fraction (244 mg) purified by prep TLC (*n*-hexane–Et_2O, 4:1) to afford ligustilide (58 mg). The fifth fraction (512 mg) was purified by HPLC [Lichroprep RP-8 (type C), MeOH–H_2O–HOAc (200:40:1), 8 ml/min] and then prep. TLC (C_6H_6–EtOAc, 5.2) to give wallichilide (**6**) (30 mg). Fr. 9–15 (1.6 g) were bulked and subjected to HPLC [Lichroprep Si 60 (type C), EtOAc–C_6H_6–HOAc (3:2:0.025), 9 ml/min] to give *cis*-6,7-dihydroxyligustilide (**2**) (597 mg). (b) The dried crushed rhizome (1 kg) was extracted with EtOAc (10 l.) for 4 days. After filtering, the solvent was evaporated to leave a crude extract (68 g), which was chromatographed on silica gel (600 g) using C_6H_6–EtOAc and divided into nine fractions Fr. 9 (1 7 g) was subjected to HPLC [Lichroprep Si 60 (type C), C_6H_6–EtOAc (2:3), 9 ml/min] and then Sephadex LH-20 (MeOH as eluent) to give *trans*-6,7-dihydroxyligustilide (**3**, 22 5 mg). Fr. 5 (20.6 g) was purified by Sephadex LH-20 (MeOH as eluent) and then silica gel chromatography using C_6H_6–EtOAc (5:2) to afford **6** (15 mg)

3-Butylidene-7-hydroxyphthalide (**1**). Colourless amorphous powder, UV λ_{max}^{MeOH} nm: 221 (ε1140), 260 (ε6100), 326 (ε3600); +$AlCl_3$ 230, 340; IR ν_{max}^{KBr} cm^{-1}. 3600, 1755, 1620; MS *m/z* (rel. int): 204 [M]$^+$ (27), 186 (4), 175 (100), 162 (38), 147 (27), 134 (6), 119 (8), 77 (4), 65 (9); ^{1}H NMR: δ0.99 (3H, *t*, *J* = 7.3 Hz, H-11), 1.55 (2H, *m*, H-10), 2.44 (2H, *m*, H-9), 5.67 (1H, *t*, *J* = 7 3 Hz, H-8), 6.91 (1H, *d*, *J* = 8 0 Hz, H-6), 7 13 (1H, *d*, *J* = 8.0 Hz, H-4), 7.45 (1H, –OH, exchangeable with D_2O), 7.55 (1H, *dd*, *J* = 8 0, 8.0 Hz, H-5)

cis-6,7-Dihydroxyligustilide (**2**). Light yellow oil, UV λ_{max}^{MeOH} nm: 272 (ε10 500); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1765 (γ-lactone), 1675, 1638, 1250, 1030, 990; MS *m/z* (rel. int.) 224.1044 [M]$^+$ (calc. 224.1049 for $C_{12}H_{16}O_4$, 27), 206 (4), 195 (2), 180 0806 (calc. 180.0786 for $C_{10}H_{12}O_3$, 100), 165 (17), 151.0395 (calc. 151 0395 for $C_8H_7O_3$, 60), 138 (19), 123 (21), 95 (32), 55 (77), 43 (91); ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2.

cis-6,7-Diacetoxyligustilide (**2a**) The diacetate of **2** was prepared by treatment with Ac_2O and C_5H_5N The usual work-up yielded **2a** (25 mg) as an oil IR ν_{max}^{film} cm^{-1}: 1770 (γ-lactone), 1745 (ester), 1680 and 1645 (diene), 1420, 1370, 1240, 1030, 925; MS *m/z* (rel. int): 248 [M – 60]$^+$ (10), 224 (8), 206 (100), 177 (25), 164 (18), 105 (5), 91 (4), 77 (5), 55 (17); UV λ_{max}^{MeOH} nm 275 (ε6300), ^{1}H NMR: δ0.97 (3H, *t*, *J* = 7 3 Hz, H-11), 1.52 (2H, *qt*, *J* = 7 3, 7.3 Hz, H-10), 2 38 (2H, *td*, *J* = 7.3, 7.3 Hz, H-9), 2.07 and 2.09 (each 3H, *s*, –OAc), 2.51 and 2.59 (each 1H, *m*, H-4), 5.15 (1H, *m*, H-6), 5.34 (1H, *t*, *J* = 7 3 Hz, H-8), 5.73 (1H, *d*, *J* = 4.1 Hz, H-7).

cis-6,7-Dibenzoylligustilide (**2b**). A mixture of **2** (22.4 mg, 0.1 mmol), benzoyl chloride (30.9 mg, 0 22 mmol), C_5H_5N (1 ml) and 4-dimethylaminopyridine (12.2 mg, 0.1 mmol) was kept at room temp. overnight. Work-up as usual gave an oil, which was purified by prep. TLC to yield **2b** (6 mg) as a colourless powder. UV λ_{max}^{MeOH} nm· 230 (ε24 500), 273 (ε1900); MS *m/z* (rel. int.): 310 [M – 122]$^+$ (16), 255 (2), 206 (3), 190 (3), 167 (3), 149 (8), 136 (3), 122 (11), 105 (100), 77 (36); ^{1}H NMR: δ0.99 (3H, *t*, *J* = 7.3 Hz, H-11), 1.54 (2H, *qt*, *J* = 7 3, 7.3 Hz, H-10), 2.42 (2H, *dt*, *J* = 7.3, 7.3 Hz, H-9), 2.24 (1H, *dddd*, *J* = 13 8, 8.4, 6.2, 2.7 Hz, H-5α), 2.31 (1H, *dddd*, *J* = 13.8, 6 5, 5 7, 5.4 Hz, H-5β), 2 64 (1H, *dddd*, *J* = 18 4, 8 4, 5.7, 1.4 Hz, H-4β), 2 72 (1H, *dddd*, *J* = 18.4, 6.2, 5 4, 1.1 Hz, H-4α), 5.39 (1H, *t*, *J* = 7 3 Hz, H-8), 5 50 (1H, *ddd*, *J* = 6.5, 4 3, 2 7 Hz, H-6), 6.16 (1H, *ddd*, *J* = 4 3, 1 4, 1.1 Hz, H-7), 7.42 (3H, *t*, *J* = 7 0 Hz), 7 74 (3H, *t*, *J* = 7.0 Hz), 7 98 (2H, *d*, *J* = 7.0 Hz), 8 02 (2H, *d*, *J* = 7.0 Hz).

trans-*6,7-Dihydroxyligustilide* (**3**). Light yellow oil, IR ν_{max}^{film} cm^{-1} 3400 (OH), 1765 (γ-lactone), 1675 and 1638 (diene), 1410, 1270, 1190, 950; MS *m/z* (rel. int.)· 224 1044 [M]$^+$ (calc 224.1045, for $C_{12}H_{16}O_4$, 31), 206 (5), 195 (5), 180 (100), 165 (19), 151 (44), 138 (14), 123 (5), 95 (17), 55 (27); ^{1}H NMR: see Table 1.

Wallichilide (**6**). Colourless plate, mp 163–164.5°, UV λ_{max}^{MeOH} nm 272, IR ν_{max}^{KBr} cm^{-1}: 1758 (γ-lactone), 1715 (conj ester), 1690 (C=O), 1620 (C=C); MS *m/z* (rel. int) 412 [M]$^+$ (4), 380 (5), 296 (5), 267 (5), 222 (21), 190 (85), 165 (32), 161 (40), 148 (100), 120 (20), 105 (26), 77 (18), ^{1}H NMR and ^{13}C NMR. see

Tables 3 and 2 respectively. (Calc. $C_{25}H_{32}O_5$: C, 72.81; H, 7.76. Found: C, 72.51; H, 7.52.)

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REFERENCES

1. *The Chinese Pharmacopoeia* (1963), p. 30.
2. Hsiyuan Hospital of the Academy of Chinese Traditional Medicine, unpublished.
3. Peking Institute of Pharmaceutical Industries (1977) *Nat. Med. J. China* **57**, 420.
4. Bingzhang, W., Minggang, Y., Lei, P. and Zhanjiu, Y. (1979) *Acta Pharm. Sin.* **14**, 624.
5. Peking Pharmaceutical Industries Laboratories (1978) *Chin. Med. J.* **4**, 319.
6. Hashimoto, K and Kubota, K. (1973) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **278**, 135.
7. Endoh, M and Hashimoto, K. (1970) *Am. J. Physiol.* **218**, 1459.
8. Yamagishi, Y. and Kaneshima, H. (1977) *Yakugaku Zasshi* **97**, 237.
9. Mitsuhashi, H. and Nagai, U. (1963) *Tetrahedron* **19**, 1277.
10. Roll, D. D., Nist, B. J. and Huilfric, A. C. (1964) *Tetrahedron* **20**, 2851.
11. Mitsuhashi, H and Muramatsu, T (1964) *Tetrahedron* **20**, 1921.
12. Mitsuhashi, H. and Muramatsu, T (1967) *Chem. Pharm. Bull. (Tokyo)* **15**, 1606.
13. Kaouadji, M., Reutenauer, H., Chulia, A. J. and Marusura, A (1983) *Tetrahedron Letters* **24**, 4677.
14. Kaouadji, M., Baronnat, M. P and Marieotte, A.-M. (1983) *Tetrahedron Letters* **24**, 4675.
15. Hashimoto, K. and Kumakura, S. (1965) *Jpn J. Pharmacol.* **15**, 540.
16. Chiba, S. and Himori, N. (1975) *Jpn J. Pharmacol* **25**, 489
17. Chiba, S., Kubota, K. and Hashimoto, K. (1973) *Eur. J. Pharmacol.* **21**, 281.