THREE ADDITIONAL PHTHALIDE DERIVATIVES, AN EPOXYMONOMER AND TWO DIMERS, FROM *LIGUSTICUM WALLICHII* RHIZOMES

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ABSTRACT.—Among the apolar constituents of *Ligusticum wallichii* rhizomes (Umbelliferae), three phthalide derivatives were isolated from the *n*-hexane extract. Structures of Z-6.8',7.3'-diligustilide (**2**), Z'-3,8-dihydro-6.6',7.3'a-diligustilide (**3**), and Z-6,7-epoxyligustilide (**8**) were determined by spectroscopic evidence. Compounds **3** and **8** were previously unreported.

Umbelliferous plants, collected in the subfamily Apioidae and particularly those belonging to the subtribes Peucedanae and Apiae, are known to produce phthalide derivatives that are generally located in the underground parts (1). From the rhizomes of *Ligusticum wallichii* Franch., we have reported the isolation of the first Z-ligustilide dimer (**1**) (2), found later in two other umbelliferous plants, *Levisticum officinale* Koch. (3) and *Meum athamanticum* Jacq. (4). Formation of such a compound has been explained by coupling of two Z-ligustilide units through the Diels-Alder reaction (1,2). Besides Z,Z'-6.6',7.3'a-diligustilide (**1**), only two other dimeric phthalide derivatives, probably generated by the same biosynthetic pathways, have been described: angeolide (**4**) from *Angelica glauca* Edgew. (5) and wallichilide (**5**) from *L. wallichii* (6). On the other hand, riligustilide (**2**) was described in *Ligusticum acutilobum* (7); this dimer containing a cyclobutane ring is probably generated in a manner other than by the Diels-Alder reaction.

We have also reported the isolation of the first 6,7-dihydroxyligustilide derivative which has been deduced to be generated from Z-ligustilide via the 6-hydroxy derivative or the 6,7-epoxide (1,8).

Continuing with the investigation of the *n*-hexane extract of *L. wallichii* rhizomes, we have isolated three other phthalide derivatives, an epoxymonomer, and two dimers. We now report the identification of these minor constituents as Z-6.8',7.3'-diligustilide (**2**), Z'-3,8-dihydro-6.6',7.3'a-diligustilide (**3**), and Z-6,7-epoxyligustilide (**8**). Elucidation of these structures has been facilitated by the use of ¹H-nmr data relative to dimer **1** and monomers **6** and **7**.

During fractionation of the *n*-hexane extract of *L. wallichii* rhizomes by centrifugal circular tlc on silica gel, compound **2** was eluted before dimer **1**, and its purification was carried out by semi-preparative hplc on Lichrosorb 60. From the ir absorption at 1755 cm⁻¹ and ¹H-nmr data (Table 1), compound **2** was deduced to contain an α,β-unsaturated five ring lactone, a butylidene chain, and two endocyclic *cis*-ethylenic protons; these are three partial structures found in Z-ligustilide (**6**). Moreover, four fragment ions at *m/z* 396, *m/z* 379, *m/z* 191, and *m/z* 189 exhibited by the mass spectrum of **2** in the ci mode (NH₃ used as reacting gas), and two signals at δ 170.3 ppm and δ 168.5 ppm shown by the ¹³C-nmr spectrum of **2**, simultaneously indicated that this natural product could be a dimeric ligustilide derivative. Compared to dimer **1** generated from Z-ligustilide (**6**) by a Diels-Alder reaction through the cyclohexadiene rings (Scheme 1), the ¹H-nmr spectrum of **2** exhibited two protons at δ 3.46 ppm, broad d,

TABLE 1. ^{1}H-nmr Data of Dimer 1 at 250 MHz and Dimers 2 and 3 at 300 MHz ($CDCl_3$; δ ppm/TMS)

Atoms	Compounds		
	1	2	3
H-3	2.06, br ddd, J=17, 5, 2.5 Hz	2.47-2.70, m	5.11, dd, J=10, 3 Hz
H-4	2.20, m	2.47-2.70, m	2.14, m 2.22, m
H-5	1.53, m 1.87, m	2.02, m 2.16, br ddd, J=12, 12, 3 Hz	1.43-1.50, m 1.87, m
H-6	2.56, br ddd, J=9, 6.5, 2.5 Hz	2.47-2.70, m	2.47, m, J=8.8, 6.5, 2.5 Hz
H-7	3.27, br d, J=9 Hz	3.46, br d, J=7.5 Hz	3.02, br d, J=8.8 Hz
H-8	5.08, t, J=8 Hz	5.21, t, J=8 Hz	1.43-1.50, m 1.77, m
H-9	2.30, m, J=8, 7.5 Hz	2.33, m, J=8, 7.5 Hz	1.43-1.50, m
H-10	1.45, m, J=7.5, 7.5 Hz	1.50, m, J=7.5, 7.5 Hz	1.43-1.50, m
H-11	0.92, t, J=7.5 Hz	0.95, t, J=7.5 Hz	0.93, t, J=7.5 Hz
H-4'	1.56, br d, J=13 Hz 1.95, br dd, J=13, 3 Hz	2.47-2.70, m 2.47-2.70, m	1.43-1.50, m 1.87, m
H-5'	1.33, br dd, J=12, 4 Hz 1.87, m	2.47-2.70, m 2.47-2.70, m	1.43-1.50, m 1.77, m
H-6'	3.00, m, J=6.5, 2.5, 2.5, 2.5 Hz	5.93, dt, J=9.5, 4 Hz	2.95, m, J=6.5, 2.5, 2.5, 2.5 Hz
H-7'	7.36, d, J=6.5 Hz	6.17, dt, J=9.5, 1.5 Hz	7.31, d, J=6.5 Hz
H-8'	5.00, t, J=7.5 Hz	2.94, dt, J=8, 8 Hz	5.18, t, J=8 Hz
H-9'	2.18, m, J=7.5, 7.5 Hz	1.44, m, J=8, 8 Hz	2.33, m, J=8, 7.5 Hz
H-10'	1.45, m, J=7.5, 7.5 Hz	1.14, m, J=8, 7.5 Hz	1.43-1.50, m
H-11'	0.92, t, J=7.5 Hz	0.86, t, J=7.5 Hz	0.94, t, J=7.5 Hz

TABLE 2. ^{1}H-nmr Data of Z-Ligustilide (**6**), Sedanenolide (**7**)
and 6,7-Epoxyligustilide (**8**) at 250 MHz (CDCl$_3$; δ ppm/TMS)

Atoms	Compounds		
	6	**7**	**8**
H-3		4.93, dd, J=6.5, 4 Hz	
H-4	2.57, m	2.47, m	2.59, m
H-5	2.43, m	2.47, m	{2.06, m {2.14, m
H-6	5.97, dt, J=9.5, 4 Hz	5.91, dt, J=10, 3.5 Hz	4.33, ddd, J=5.5, 3.5, 2.5 Hz
H-7	6.25, dt, J=9.5, 1.5 Hz	6.21, dt, J=10, 1 Hz	4.61, br d, J=2.5 Hz
H-8	5.20, t, J=8 Hz	{1.88, m, J=14, 7, 4 Hz {1.56, m, J=14, 6.5 Hz	5.36, t, J=8 Hz
H-9	2.43, m, J=8.0, 7.5 Hz	1.39, m	2.38, m, J=8, 7.5 Hz
H-10	1.48, m, J=7.5, 7.5 Hz	1.39, m	1.58, m, J=7.5, 7.5 Hz
H-11	0.92, t, J=7.5 Hz	0.90, t, J=7.5 Hz	0.96, t, J=7.5 Hz

J=7.5 Hz (H-7), and δ ca. 2.57 ppm, m (H-6) suggesting that one ligustilide unit was affected via Δ^6 by condensation. As the cyclohexadiene ring was unchanged in the second ligustilide unit (H-6′: δ 5.93 ppm; H-7′: δ 6.17 ppm), it was simultaneously concluded that condensation of the two monomer units had not been carried out by a Diels-Alder reaction and that the side chain of the second unit was implicated in the dimerization through Δ^3. This analysis was in agreement with disappearance of an ethylenic CH (^{1}H δ 5.00-5.20 ppm; ^{13}C δ 108-112 ppm) in favor of a methine group (^{1}H δ 2.94 ppm; ^{13}C δ 32.3 ppm), and substitution of a quaternary ethylenic O-bound C-atom (δ 148-150 ppm) for a quaternary O-bound carbon (δ 92.0 ppm), two carbons which were respectively identified as C-8 and C-3 (Table 3). A cyclobutane ring, including C-6, C-7, C-3′, and C-8′, was, therefore, shown to arise consequent to dimerization. The 7.3′-link between the two monomer units could be unambigously defined according to the ^{1}H-nmr characteristics exhibited by H-7 (broad d, $J_{6,7}$=7.5 Hz) and ruling out any 7.8′-connection (Table 1). Compound **2** was consequently identified as Z-6.8′,7.3′-diligustilide, a natural product which did not give the expected molecular ion at m/z 380 in the mass spectrum taken in the ei mode. This is the second report of this com-

TABLE 3. ^{13}C-nmr Chemical Shifts of Z,Z'-6.6′,7.3′a-Diligustilide (**1**)
and Z-6.8′,7.3′-Diligustilide (**2**) at 75.5 MHz (CDCl$_3$; δ ppm/TMS)

Atoms	Compounds		Atoms	Compounds	
	1	**2**		**1**	**2**
C-1	168.4	168.5	C-1′	164.9	170.3
C-3	148.1	149.2	C-3′	150.5	92.0
C-3a	155.0	154.6	C-3′a	47.6	160.1
C-4	19.8	19.6	C-4′	31.1	21.0^e
C-5	29.0	26.2	C-5′	25.8	20.7^e
C-6	38.4	35.0^c	C-6′	41.5^a	128.7
C-7	41.6^a	44.0	C-7′	142.0	117.0
C-7a	126.6	122.3^d	C-7′a	134.3	122.5^d
C-8	112.1	112.2	C-8′	108.8	32.3^c
C-9	28.0	28.0	C-9′	27.5	20.0^e
C-10	22.3	22.4	C-10′	22.3	22.6
C-11	13.9^b	13.9	C-11′	13.8^b	14.1

$^{a-e}$Assignments with the same letter designation may warrant changing.

pound from a natural source. Named riligustilide, it was first isolated from *L. acutilobum* and was only studied by X-ray analysis (7).

Less concentrated than dimers **1** and **2**, compound **3** was also a dimeric phthalide derivative as indicated by the ^{1}H-nmr spectrum and the mass spectrum recorded in the ci mode. Contrary to dimer **2**, this compound was eluted after dimer **1** by centrifugal circular tlc, and its purification was also achieved by semi-preparative hplc. Except for a butylidene chain replaced with an *n*-butyl chain of which the nonequivalent protons were exhibited at δ 1.77 and 1.47 ppm, respectively, the ^{1}H-nmr spectrum of this compound was similar to that for dimer **1** (Table 1) and particularly signals relative to the four protons H-6, H-7, H-6′, and H-7′. The above results suggested that the two monomer units were linked by 6.6′- and 7.3′a-bonds, as observed in *Z,Z′*-6.6′,7.3′a-diligustilide (**1**), consequent to the Diels-Alder reaction occurring between a *Z*-ligustilide unit and another phthalide derivative unit containing an *n*-butyl chain and identified as sedanenolide (**7**) which H-3 was recorded at δ 5.11 ppm, dd, *J* = 10, 3 Hz. This proton δ-value indicated that during dimerization, *Z*-ligustilide was the diene and sedanenolide, the dienophile. Effectively, deshielding recorded here, for H-3, was in accord with an O-bound methine group in the α-position to an ethylenic carbon, as observed in the sedanenolide monomer (δ 4.93 ppm) (Table 2). Moreover this analysis was in agreement with shielding exhibited by H-7 (δ 3.02 ppm) in compound **3**, on account of a lack of a conjugated double bond, in comparison with the corresponding proton in compound **1** (δ 3.27 ppm). Thus, we assign this new structure as *Z′*-3,8-dihydro-6.6′,7.3′a-diligustilide; this is the first report of a dimeric phthalide derivative comprising a sedanenolide unit.
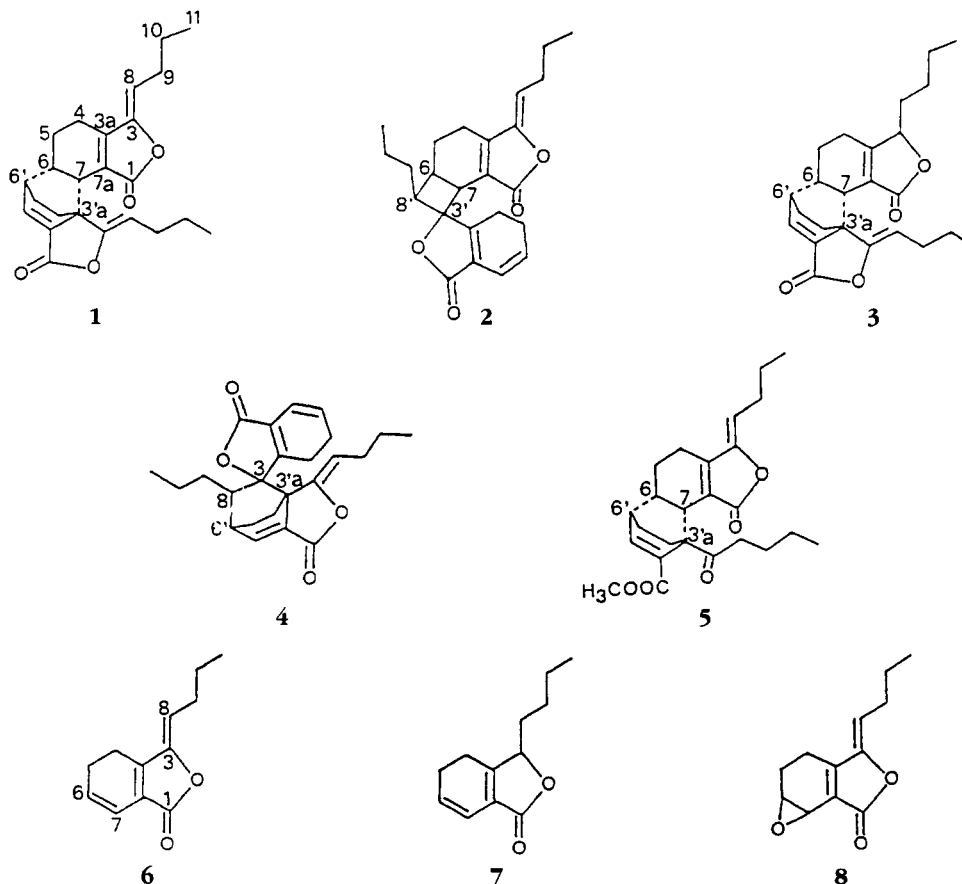
Compound **8** was the third molecule isolated from the *n*-hexane extract of *L. wallichii* rhizomes. More polar than dimers **1**, **2**, and **3**, it was eluted just before 6,7-dihydroxyligustilide derivatives (**9**). It gave $M^{+\cdot}$ at *m/z* 206 showing one more oxygen atom than *Z*-ligustilide ($M^{+\cdot}$ *m/z* 190) and its ¹H-nmr spectrum was, furthermore, similar to that of *Z*-ligustilide, except for two adjacent methine groups at δ 4.61 ppm, broad d, J=2.5 Hz and δ 4.33 ppm, ddd, J=5.5, 3.5, 2.5 Hz, instead of two *cis*-ethylenic protons in *Z*-ligustilide (H-6: δ 5.97 ppm; H-7: δ 6.25 ppm) (Table 2), indicating disappearance of Δ^6 in compound **8**. To accommodate the *Z*-ligustilide skeleton with the supplementary oxygen and deshielding of H-6 and and H-7, the two C-atoms C-6 and C-7 must be O-bound and included in an epoxide function. These data supported the assignment of **8** as the new compound *Z*-6,7-epoxyligustilide.
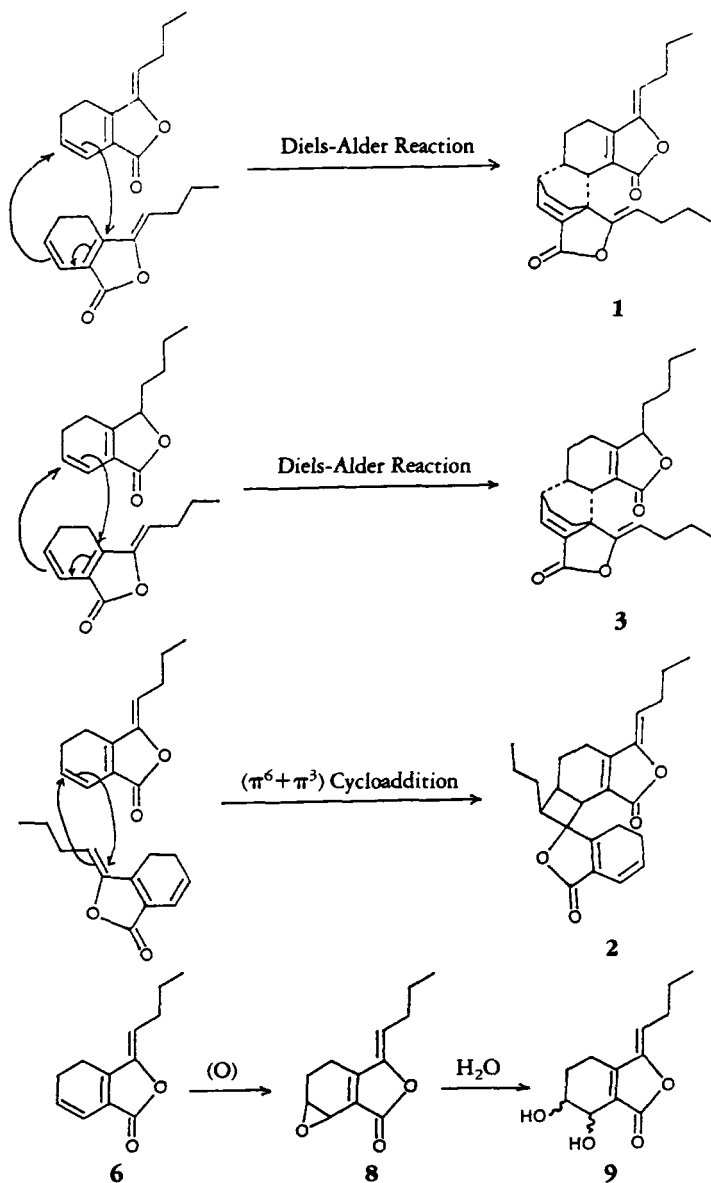
SCHEME 1. Postulated biosynthesis of dimers **1**, **2**, and **3**, and 6,7-dihydroxyligustilide derivatives (**9**)

The biogenesis of dimers **1** and **3** can be explained by coupling of a ligustilide unit with either a ligustilide or a sedanenolide unit, by the Diels-Alder reaction affecting the cyclohexadiene rings, while the biogenesis of dimer **2** may follow a different route based on the cycloaddition between the cyclohexadiene ring of a ligustilide unit and the side chain of another ligustilide unit (Scheme 1). On the other hand, occurrence of 6,7-epoxyligustilide (**8**) and the number of 6,7-dihydroxyligustilide derivatives it generated seem to correlate quite well, as reported in Scheme 1.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical tlc was carried out on silica gel 60F-254 plates (E. Merck). Polyamide CC6 (Macherey Nagel) was used for column chromatography. Silica gel 60 PF-254 containing gypsum (E. Merck) for preparative layer chromatography, was used for centrifugal circular tlc. Hplc was carried out on a Waters Model 6000A instrument equipped with a variable wavelength detector and Lichrosorb 60 column (25 cm). Uv spectra were measured in MeOH on a Beckman 25 spectrometer. Ir spectra (KBr) were taken on an Unicam SP1100 spectrometer. Nmr spectra (CDCl$_3$; δ ppm/TMS) were recorded on WM250 and AM300 Bruker spectrometers for ^{1}H and AM300 Bruker spectrometer for ^{13}C; extensive decoupling was used to verify assignments. Ei and ci (NH$_3$ used as reacting gas) mass spectra were taken on an AEI MS902 mass spectrometer (70 eV).

ISOLATION OF THE PHTHALIDE DERIVATIVES.—After isolation of dimer **1** (2), from the *n*-hexane extract (1 g) of *L. wallichii* rhizomes, by SiO$_2$ cc, dimers **2** and **3** have been detected but not isolated. Another portion of the *n*-hexane extract (5 g), corresponding to 100 g of dry material, was, therefore, subjected to centrifugal circular tlc (cctlc) on silica gel and eluted by a gradient from *n*-hexane-CHCl$_3$ (40:60) to CHCl$_3$. Combination of similar eluates, on the basis of tlc analysis on silica gel in *n*-hexane-CHCl$_3$ (20:80), *n*-hexane-CHCl$_3$-*i*PrOH-MeOH (95:3:1:1), CHCl$_3$, and CHCl$_3$-MeOH (98:2), provided eleven fractions. Dimers **1**, **2**, and **3** were concentrated in fraction 5. 6,7-Epoxyligustilide was found in fraction 9, and 6,7-dihydroxyligustilide derivatives were located in the last fraction.

Purification of dimeric compounds was performed by subjecting fraction 5 (982 mg) to cctlc on silica gel; elution by *n*-hexane-CHCl$_3$-*i*PrOH-MeOH (95:3:1:1) gave five fractions. Dimer **2** was found in fraction 2 (132 mg) and dimers **1** and **3** were observed in fraction 4 (248 mg). A further purification was needed and carried out by semi-preparative hplc on Lichrosorb 60 (*n*-hexane-*i*PrOH, 98:2) to obtain pure dimers **1** (19 mg; 0.019%), **2** (5 mg; 0.005%), and **3** (1.5 mg; 0.0015%).

6,7-Epoxyligustilide was purified in two steps by subjecting fraction 9 (435 mg) to polyamide cc (C$_6$H$_6$ to C$_6$H$_6$-MeOH, 90:10), then to semi-preparative hplc on Lichrosorb 60 (*n*-hexane-CHCl$_3$-*i*PrOH-MeOH, 95:3:1:1). This procedure yielded 1.5 mg of the epoxide (0.0015%).

Z-6.8′,7.3′-DILIGUSTILIDE (**2**).—A colorless oil; uv λ MeOH 278 nm; ir ν KBr 3005, 2990, 2960, 2900, 1755, 1670, 1630, 1460, 1430, 1375, 1300, 1270, 1155, 1100, 1060, 1020, 950, 940, 710 cm^{-1}; ms (ei) *m/z* (%) 190 (M$^+$/2; 100), 188 (52), 175 (10), 161 (55), 159 (88), 148 (69), 146 (20), 131 (9), 115 (10), 103 (12), 91 (15), 77 (25), 55 (50); ms (ci) *m/z* (%) 396 [(M-2H)NH$_4^+$; 12.5], 379 [(M-2H)H$^+$; 11], 356 (33), 339 (7), 279 (28), 206 (43), 191 (16), 190 (50), 189 (100), 183 (14), 159 (25), 149 (11), 148 (11), 105 (17), 91 (16); ^{1}H nmr (Table 1); ^{13}C nmr (Table 3).

Z′-3,8-DIHYDRO-6.6′,7.3′a-DILIGUSTILIDE (**3**).—A colorless oil; uv λ MeOH 273 nm; ir ν KBr 2970, 1750, 1645, 1630, 1550, 1465, 1430, 1370, 1340, 1270, 1040, 955, 910, 840 cm^{-1}; ms (ci) *m/z* (%) 383 (MH$^+$; 11), 360 (50), 339 (100), 325 (20), 271 (27), 270 (29), 193 (34), 192 (22), 107 (18), 91 (19); ^{1}H nmr (Table 1).

Z-6,7-EPOXYLIGUSTILIDE (**8**).—A colorless oil; uv λ MeOH 275 nm; ms *m/z* (%) 206 (M$^+$; 81), 177 (75), 164 (87), 149 (100), 121 (62); ^{1}H nmr (Table 2).

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