ADDITION OF METHYL THIOGLYCOLATE AND BENZYLAMINE TO (Z)-LIGUSTILIDE, A BIOACTIVE UNSATURATED LACTONE CONSTITUENT OF SEVERAL HERBAL MEDICINES. AN IMPROVED SYNTHESIS OF (Z)-LIGUSTILIDE

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ABSTRACT.—(Z)-Ligustilide [1] is a dihydrophthalide purported to be the active ingredient of *Ligusticum* plant species widely used as herbal medicines in the Orient and in Native American and Hispanic cultures. It readily underwent 1,6-conjugate addition with methyl thioglycolate in the presence of triethylamine. The methyl thioglycolate reaction also yielded a product from addition to the C-6–C-7 double bond and a diadduct from both 1,6-addition and addition to the C-6–C-7 bond. Reaction of 1 with benzylamine did not afford a 1,6-adduct, but yielded instead an N-benzyllactam, presumably formed by rearrangement from initial 1,2-addition to the carbonyl. An improved total synthesis of 1 was developed. (Z)-Ligustilide had weak antiviral properties and weak antimicrobial activity against Gram-positive, Gram-negative, and yeast microorganisms. The broad biological activity of 1 and its electrophilic reactivity are consistent with the use of *Ligusticum* species in folk medicine.

The most widely used herbal product in the southwestern United States and northern Mexico is the rhizome of *Ligusticum porteri* C.&R. of the Apiaceae (1). The root has been known in Hispanic cultures as "chuchupate" (from the Nahuatl term "chichipatli"), but is now more commonly denoted as "oshá" (2). Widespread and early (pre-Hispanic) use may be inferred from its vernacular names in Mescalero Apache, Zuni, Paiute, and Tarahumara cultures (3). The major component of *L. porteri* roots was shown to be (Z)-ligustilide [1], which was accompanied by several ligustilide dimers (4). Ligustilide, its dimers, and other phthalides have also been reported from rhizomes of several widely used Asian herbs of Chinese and Japanese traditional medicine, such as *L. wallichii, L. chuangxiong, Angelica sinensis,* and *A. acutiloba* (5–8). (Z)-Ligustilide is also the main essential oil component of roots of *Levisticum officinale,* lovage, a well-known European medicinal herb (9). Although (Z)-ligustilide is most often the major isomer isolated, (E)-ligustilide was reported to be a major component of *Angelica glauca* (10).

The medicinal uses of these herbs are so complex and varied (1–10) that it is difficult to differentiate them in order to focus on the validity of one or a few biological properties. The dried rhizomes of *Ligusticum chuangxiong* have been used in the Orient to treat headache, anemia, and irregularity of menstruation. In the San Luis valley of southern Colorado "oshá" is used for treating colds, sore throats, and stomachache, and to prevent infections in wounds (2). These uses, as well as employment of rhizome tinctures and teas at the first sign of virtually any ailment, have been confirmed by San Luis valley residents. Tinctured or chewed rhizome is stated by another source to be one of the best treatments for viral infections (1).

Reports of biological testing on total herb extracts are in the Chinese and Japanese traditional medicine literature, but only a few reports are available on the testing of pure components. Thus, 1 was reported (11) to be toxic (LC_{50} 20 ppm) in the brine shrimp bioassay (12). In a recent report on the first synthesis of 1 it was stated to have "antispasmodic, antiasthmatic, and smooth muscle relaxing activities" (13). An isolated dimer (the Diels-Alder adduct of the C-3–C-8 double bond with the cyclohexadiene moiety) was reported to be active in a calcium-channel radioreceptor assay (7). 6-Hydroxy-7-methoxydihydroligustilide, isolable from *Apium graveolens,* common celery,

was recently patented as being useful for the treatment of Alzheimer's disease (14). Celery isolates related to **1** were earlier reported to have sedative properties in mice (15). In the present work we report weak, but very broad, bioactivity in a number of screens.

To the best of our knowledge, the chemical reactivity of ligustilide {**1**} has not been investigated previously. Several {2 + 2} and {2 + 4} dimers have been isolated along with **1**, but there are no reports of the preparation of these compounds directly from **1**. As an unsaturated lactone, ligustilide {**1**} could also undergo 1,2- 1,4- and/or 1,6-conjugate addition reactions with nucleophiles (Figure 1). One or a combination of these reactions could be of importance in bioactivity or detoxification. Ligustilide [**1**] also contains a cross-conjugated system, which could play a role in modifying expected reaction pathways. As an initial study of ligustilide reactivity, we examined its reaction with the sulfur nucleophile methyl thioglycolate, which has been used as a model reactant with other biologically relevant electrophiles (16,17), and with the nitrogen nucleophile benzylamine. These reactions were performed with **1** isolated from *Ligusticum porteri*, but in order to more readily obtain material for future studies we also modified and improved the literature total synthesis (13).
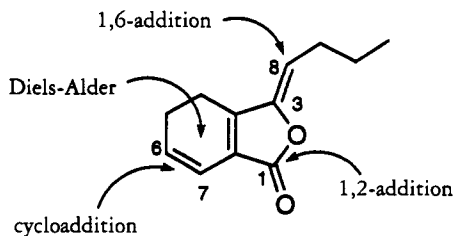

FIGURE 1.   (*Z*)-Ligustilide [**1**] and its reactive sites.

RESULTS AND DISCUSSION

Treatment of **1** with methyl thioglycolate in the presence of triethylamine and subsequent chromatography yielded a mixture of compounds, 33% of which was made up of two compounds separable only with difficulty. These proved to be the diastereomers of **2**, which resulted from 1,6-conjugate addition of methyl thioglycolate to **1** (Scheme 1). Some of the low yield could be attributed to reversion of **2** to ligustilide [**1**] and methyl thioglycolate in the presence of Si gel during chromatography (18). One diastereomer was obtained pure and was characterized spectroscopically (Table 1), while data for the other isomer resulted from subtraction of the resonances of the pure diastereomer from the mixture spectra (see Experimental). Of particular importance in the structural assignment for **2**, besides appearance of the [^1]H- and [^13]C-nmr resonances expected for the methyl thioglycolate moiety, were the disappearance of the ligustilide triplet δ 5.16 (H-8) vinyl proton resonance and appearance of the δ 5.20 one-proton doublet for the H-3 proton in **2**.

Another methyl thioglycolate adduct was assigned structure **3**, based upon the following data. Compound **3** lacked the ligustilide vinylic [^1]H-nmr resonances for H-6 and H-7 (δ 5.98 and 6.26, respectively) as well as the [^13]C-nmr resonances for C-6 and C-7, but retained the [^1]H-nmr resonance for H-8 and the [^13]C-nmr resonances for C-3 and C-8 (Table 1). Thus, addition had occurred at the C-6, C-7 bond. The regiochemistry of addition posed somewhat of a problem. Addition of either the sulfur anion or a mercaptyl radical might have been expected to take place at C-6, the end of the conjugated system. The resonance of the proton at the carbon bearing the sulfur adduct
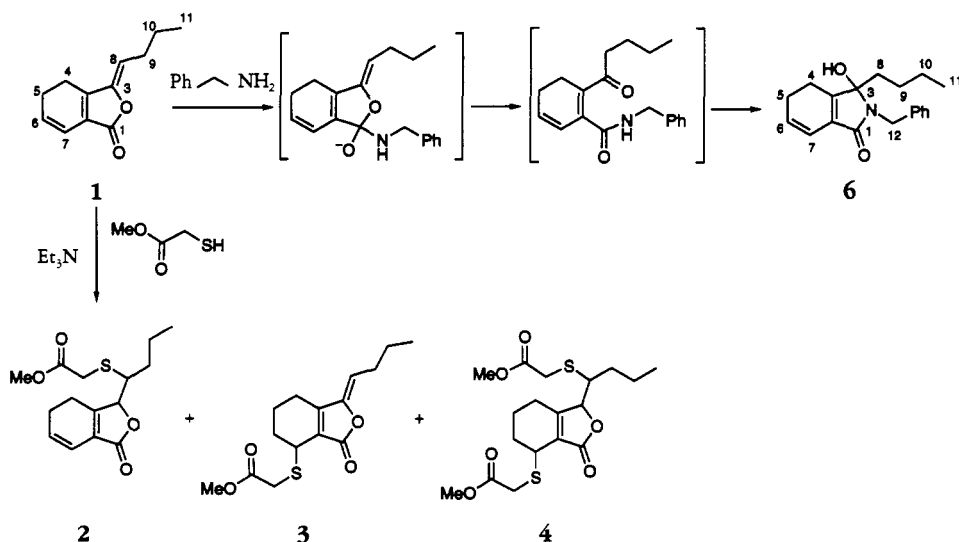
SCHEME 1.   Reaction of (Z)-ligustilide [1] with nucleophiles.

was, however, a broad singlet at δ 3.86. This chemical shift would be typical of a proton on a carbon bonded to a vinyl carbon and a sulfur (e.g., at C-7) (16,17), rather than a proton on a carbon bonded to two saturated carbon atoms (e.g., at C-6), about δ 3.1 (19). That the former was indeed the case was determined by nOe, HMQC (20), TOCSY (21), and HMBC (22) nmr experiments. Key correlations are shown in Figure 2. The TOCSY experiment identified the two separate coupled sp³ proton systems and allowed distinction of the C-9 methylenes from those at C-4. Irradiation of H-8 induced a strong nOe in a proton resonance at δ 2.24, which identified this as an H-4 resonance. This was correlated to H-7 by TOCSY and by HMBC through the π-system to the carbonyl at C-1. The H-7 key correlations (Figure 2) were also in accord with structure **3**.

An additional component of the reaction showed the presence of three carbonyl carbons and two OCH₃ groups in the nmr spectra and a fabms mol wt of 402, which was consistent with a diadduct, $C_{18}H_{26}S_2O_6$. Further analysis of the nmr spectra (Table 1) resulted in the assignment of structure **4**. The lack of the ¹³C-nmr resonances for C-6, C-7, C-3, and C-8 seen for **1**, and the appearance of ¹H-nmr resonances for H-3 and H-7, as observed in the spectra of **2** and **3**, were particularly diagnostic. The purification of the total adduct mixture yielded only a single stereoisomer of **4**, but the nmr data of the mixture suggested that more than one isomer may have been present originally. The stereochemistry of **4** was not elucidated.
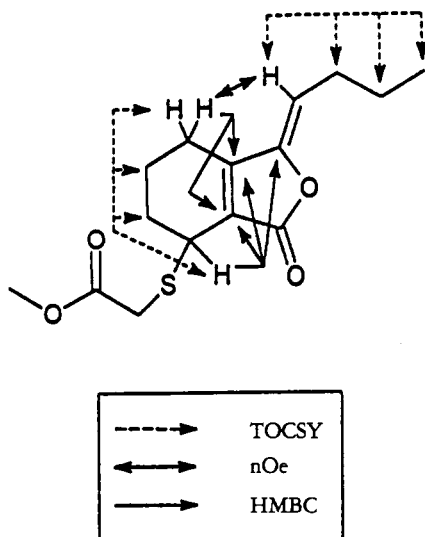
The addition of methyl thioglycolate at C-7 is reminiscent of so-called anti-Michael reaction products of α,β-unsaturated carbonyl compounds (24), but these concepts are unlikely to apply to the cross-conjugated C-6, C-7, C-7a, C-1 system of **1**. We have rationalized the C-7 adducts as arising from the rearranged intermediate **5** (Scheme 2).

Benzylamine reacted readily with ligustilide [1] to yield a product whose nmr spectral properties (see Experimental) retained characteristics of the cyclohexadiene moiety of **1**, but which lacked several of the expected resonances for a 1,6-adduct corresponding to **2**. In particular, six methylenes (rather than five) and no aliphatic methines (rather than two) were observed by DEPT ¹³C-nmr spectroscopy. A δ 92.2 ¹³C-nmr resonance consistent with a quaternary carbon bearing two electron-withdrawing atoms was now present. An oddity in the ¹H-nmr spectrum was that the methyl and neighboring methylene resonances of the side-chain now appeared at δ 0.54 and 0.64,

TABLE 1. Nmr Data for Ligustilide [1] and Methyl Thioglycolate Adducts 2–4.

| Position	Compound 1 ¹³C	1 ¹H	2 ¹³C	2 ¹H	3 ¹³C	3 ¹H	4 ¹³C	4 ¹H
1	167.1	—	170.6ᵃ	—	168.3	—	170.7ᵃ	—
3	148.6	—	85.0	5.20 d (2.4)	148.4	—	83.5	4.97 s
3a	147.1	—	158.5	—	151.8	—	163.2	—
4	18.5	2.57 m	22.2ᵇ	2.50 m	20.7	2.24 m, 2.42 m	20.3ᵇ	1.42 m
5	22.4	2.45 m	21.7ᵇ	2.50 m	17.8	1.78 m	17.9	1.85 m
6	129.9	5.98 dt (4.3, 9.7)	129.0	5.94 dt (3.7, 9.8)	28.9	1.84 m, 1.97 m	28.7	1.85 m, 2.00 m
7	117.1	6.26 dt (2.0, 9.7)	116.8	6.21 dt (2.0, 9.7)	36.2	3.86 br s	36.2	3.83 d (1.5)
7a	124.0	—	126.1	—	126.8	—	129.2	—
8	112.9	5.19 t (8.0)	47.4	3.18 m	112.4	5.16 t (7.9)	47.0	3.16 m
9	28.1	2.35 q (7.7)	20.2	1.45, 1.64 m	27.7	2.25 q (7.3)	23.9ᵇ	2.25 m, 2.50 m
10	22.4	1.47 sext. (7.4)	31.5	1.45 m	22.2	1.39 sext. (7.4)	33.3	1.59 m
11	13.8	0.93 t (7.4)	13.5	0.91 t (7.1)	13.6	0.85 t (7.4)	13.6	0.88 t (6.8)
Thioglycolyl CH₂			33.3	3.25 d (14.9), 3.40 d (14.9)	34.0	3.35 d (15.2), 3.56 d (15.2)	33.1	3.16 d (2.7)
CO			170.7ᵃ	—	170.7	—	171.4ᵃ	—
CH₃			52.4	3.72 s	52.3	3.66 s	52.4	3.72 s
CH₂'							34.2	3.38 d (15.2), 3.61 d (15.2)
CO'							170.9ᵃ	—
CH₃'							52.4	3.69 s

ᵃ·ᵇInterchangeable signals.

FIGURE 2.   Key nmr correlations for adduct **3**.

which suggested that side-chain protons were shielded by the π-electrons of the phenyl ring. All of these data were consistent with the assignment of structure **6** to the reaction product. This was confirmed by the ms fragmentation pattern (Scheme 3). Formation of **6** can be rationally attributed to an initial 1,2-addition of benzylamine to ligustilide, followed by rearrangement (Scheme 1).

(Z)-Ligustilide [**1**] has been synthesized from 2-formylbenzoic acid in four steps with an overall yield of 22% (13). Our synthesis (Scheme 4) involved three steps (30% yield) starting from commercially available phthalide and required shorter reaction times as well as less need for chromatographic purification of intermediates. The phthalide alcohol from the first step was obtained diastereomerically pure (stereochemistry not determined) in four hours and could be used in the next step without further
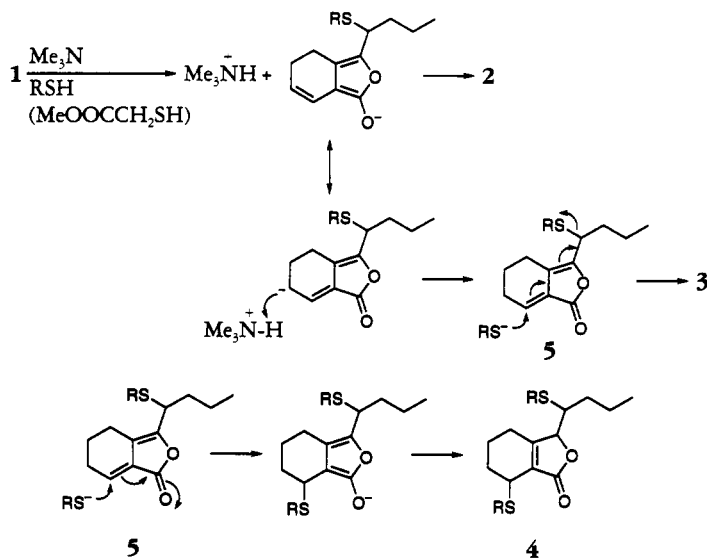
SCHEME 2.   Suggested mechanism for the formation of adducts **2**, **3**, and **4**.
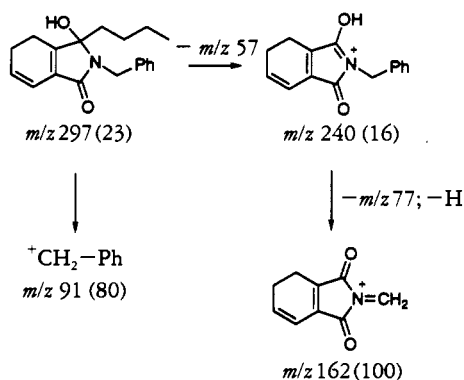
SCHEME 3.   Electron impact mass spectral fragmentation of **6**.

purification. In our hands, the use of *t*-butyl alcohol in the Birch reduction (13) did not yield consistent results, while isopropyl alcohol did. We explored several methods for the dehydration to **1**, but our best yield (Scheme 4) was only a slight improvement over the literature method which used pyridine and acetic acid.

(*Z*)-Ligustilide [**1**] and adduct **3** showed very weak but confirmed activity (10 mg/ml) against *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Sacharromyces cervevisiae*, and *Klebsellia pneumoniae*, while **2** and **6** were inactive. (*Z*)-Ligustilide also showed weak activity (one-half as potent as ribovarin) in an antiviral screen (J. Huffman, Utah State University, personal communication).

Claims about the effectiveness of *Ligusticum* rhizomes and related herbs in treatment of such a wide variety of ailments might raise suspicion as to the validity of the claims. We have found, however, that (*Z*)-ligustilide [**1**], a common component of these independently evolved herbal medicines, does have a broad range of bioactivities, even though they are quite weak. In addition, **1** has a number of electrophilic sites that accept nucleophiles in a manner consistent with mechanisms for the bioactivity of a number of drugs (25,26). The lack of specificity and low potency of (*Z*)-ligustilide are such that it would certainly not be of interest as a modern medicinal, but this should not affect the possible benefits of ligustilide-containing herbs employed as alternative medicines. Other components of these herbs could, of course, also contribute to any observed medicinal effects.

The difference in reactivity of ligustilide [**1**] toward a sulfur nucleophile (1,6-addition) and a nitrogen nucleophile (1,2-addition) is consistent with the hard and soft acid and base concept (27). Thus, the softer base (RS$^-$) reacts with the softer end of the

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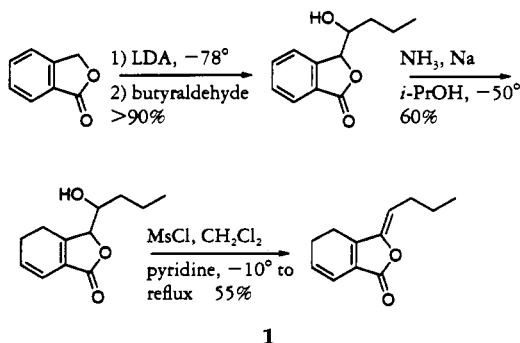
SCHEME 4.   Synthesis of (*Z*)-ligustilide [**1**].

conjugated system, while the harder base (RNH$_2$) reacts with the harder carbonyl group. In a related case, protoanemonin, **7**, underwent 1,6-conjugate addition with sulfur and oxygen nucleophiles, but both 1,2- and 1,6-addition with the nitrogen nucleophile piperidine (28,29). The addition of thiophenol to **8** has also been reported and was said to yield a single (cis) isomer, **9** (30). Our observed 1,6-addition of methyl thioglycolate to **1** was not stereospecific, since both diastereomers of **2** were found.
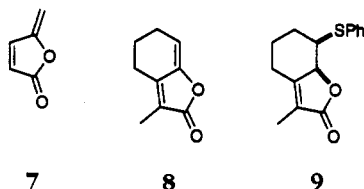
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Reactions were conducted in oven-dried glassware under an Ar atmosphere unless otherwise noted. Final solutions were dried over Na$_2$SO$_4$ prior to concentration. THF was distilled over Na/benzophenone, triethylamine was distilled over Na/KOH and stored over KOH, and benzylamine was distilled over Na. Phthalide (purchased from Aldrich Chemical Company) was recrystallized from H$_2$O and dried under high vacuum for 24 h. Methyl thioglycolate was purchased from Aldrich Chemical Company and used without further purification. Chromatography was carried out using 230–400 mesh Si gel. Tlc was performed on precoated Si gel 60 F254 plates using Me$_2$CO-petroleum ether (1:3) as eluent. Nmr spectra were measured on a Bruker AC300 spectrometer in CDCl$_3$ and internally referenced to residual CHCl$_3$ (7.24 ppm, ^{1}H; 77.00 ppm, ^{13}C); coupling constants are reported in Hz. Hrms were obtained from the Department of Chemistry, University of California, Riverside.

PLANT MATERIAL.—(Z)-Ligustilide [**1**] was isolated from *Ligusticum porteri* rhizomes obtained from Lawrence E. Sanchez (Rio Grande Herb Co., Albuquerque, NM) and from Food Co-op, Fort Collins, CO.

EXTRACTION AND ISOLATION.—Rhizomes (200 g) were milled in a blender, added to a foil-wrapped Erlenmeyer flask, and covered with hexanes (ca. 300–400 ml). The flask was allowed to stand for 24 h, after which time hexanes were decanted off and fresh hexanes added to the rhizomes. This process was repeated twice. The hexane portions were combined and concentrated *in vacuo*, resulting in a brown/yellow viscous liquid (15 g, 7.5% dry wt), which was chromatographed in aliquots of ca. 3 g using Me$_2$CO-petroleum ether (1:3) as eluent, R_f=0.54. The total (Z)-ligustilide [**1**] content was estimated to be ca. 6 g (3% of dry wt) from the weight of fraction residues and their purity based on ^{1}H-nmr spectra. ^{1}H- and ^{13}C-nmr spectra (Table 1) were essentially as reported previously (11).

[8-(METHYL THIOGLYCOLYL)-(3,8-DIHYDRO)]-LIGUSTILIDE [**2**], [7-(METHYL THIOGLYCOLYL)-(6,7-DIHYDRO)]-(Z)-LIGUSTILIDE [**3**] AND [7,8-BIS(METHYL THIOGLYCOLYL)-(3,6,7,8-TETRAHYDRO)]-LIGUSTILIDE [**4**].—To a stirred solution of pure **1** (324 mg, 1.7 mmol) in THF (5 ml) was added methyl thioglycolate (0.17 ml, 1.9 mmol) and Et$_3$N (0.28 ml, 2.0 mmol). The mixture was stirred at room temperature for 17 h in a darkened hood. The reaction mixture was quenched with concentrated aqueous HCl (ca. 4–5 drops), and the solution concentrated *in vacuo*. 2 M HCl (ca. 2 ml) was added to the residue and the aqueous portion extracted with EtOAc (3×5 ml). The combined organic extracts were dried, filtered, and evaporated to give a crude mixture of addition products (425 mg, 85%). Cc (Me$_2$CO-petroleum ether, 1:9) afforded recovered **1** (25 mg, 7.6%, R_f 0.54), **2** as a mixture of diastereomers (111 mg, 33%, R_f 0.35), **3** (40 mg, 8%, R_f 0.41), and **4** (113 mg, 17%, R_f 0.12).

Compound **2**.—One diastereomer, isolated pure by chromatography from the above mixture: hrms *m/z* (+50 eV ei) calcd for C$_{15}$H$_{20}$SO$_4$, 296.1082, found 296.1077; eims *m/z* 296 (M$^+$, 1), 190 (39), 161 (71), 101 (95), 83 (84), 55 (100); ^{1}H- and ^{13}C-nmr data, see Table 1. Resonances for the second diastereomer (present in the original mixture) were obtained by subtraction: ^{1}H nmr δ 0.91 (3H, t, *J*=7.1 Hz), 1.38–1.52 (3H, m), 1.58–1.70 (1H, m), 2.40–2.60 (4H, m), 3.13–3.23 (1H, m), 3.25 (1H, s), 3.40 (1H, d, *J*=14.9 Hz), 3.72 (3H, s), 5.15 (1H, br s), 5.94 (1H, dt, *J*=3.7 and 9.8 Hz), 6.21 (1H, dt, *J*=2.0 and 9.7 Hz); ^{13}C nmr δ 13.7, 20.3, 21.7, 22.2, 32.6, 33.2, 47.0, 52.5, 83.4, 116.7, 126.3, 129.1, 159.3, 170.6, 170.7.

Compound **3**.—Hrms *m/z* (+50 eV ei) calcd for C$_{15}$H$_{20}$SO$_4$, 296.1082, found 296.1078; eims *m/z* 296 (M$^+$, 25), 223 (100), 192 (57), 191 (40), 149 (82); ^{1}H- and ^{13}C-nmr data, see Table 1.

Compound **4**.—Fabms *m/z* 403 (M⁺+H, 54), 343 (17), 297 (35), 190 (100), 160 (44), 148 (25); ¹H- and ¹³C-nmr data, see Table 1.

N-BENZYL-3-BUTYL-3-HYDROXYPHTHALAMIDE [**6**].—To a stirred solution of **1** (210 mg, 1.1 mmol) in THF (2 ml) was added benzylamine (0.13 ml, 1.2 mmol) and stirring was continued at room temperature for 44 h. The solution was concentrated *in vacuo* to yield a mixture of white crystals and a yellow oil. Cc (gradient EtOAc/petroleum ether, 1:20, 1:12, 1:6, 1:3) of the crude material gave recovered **1** (120 mg, 57%) and **6** (133 mg, 41%) as a white solid, mp 148–149° (uncorrected); ¹H nmr δ 0.36–0.48 (1H, m, H-9), 0.54 (3H, t, *J*=6.9 Hz, H-11), 0.58–0.70 (2H, m, H-9, H-10), 0.84–0.92 (1H, m, H-10), 1.63–1.69 (2H, m, H-8), 2.23–2.32 (1H, m, H-4), 2.36–2.44 (2H, m, H-5), 2.49–2.61 (1H, m, H-4), 3.78 (1H, br s, exchangeable in D₂O, OH), 4.23 (1H, d, *J*=15.1 Hz, H-12), 4.69 (1H, d, *J*=15.1 Hz, H-12), 5.90 (1H, dt, *J*=4.0 and 9.6 Hz, H-6), 6.25 (1H, dt, *J*=1.7 and 9.7 Hz, H-7), 7.20–7.30 (3H, m, H-15, H-16), 7.38 (2H, d, *J*=8.1 Hz, H-14); ¹³C nmr δ 13.6 (C-11), 18.6 (C-4), 22.1 (C-10), 22.7 (C-5), 25.1 (C-9), 33.5 (C-8), 41.6 (C-12), 92.2 (C-3), 117.3 (C-7), 127.2 (C-16), 128.3 (C-15), 128.5 (C-6), 128.6 (C-14), 128.8 (C-13), 138.7 (C-7a), 152.5 (C-3a), 169.0 (C-1); *anal.*, calcd for C₁₉H₂₃NO₂: C, 76.7, H, 7.8, N, 4.7; found: C, 76.9, H, 8.1, N, 4.7; hrms *m/z* (+50 eV ei) calcd for C₁₉H₂₃NO₂, 297.1729, found 297.1740; eigcms *m/z* 297 (M⁺, 23), 240 (16), 162 (100), 91 (80).

3-HYDROXYBUTYLPHTHALIDE.—To a well-stirred solution of LDA (11 mmol) [prepared from diisopropylamine (1.5 ml, 11.0 mmol) and *n*-BuLi (2.5 M, 4.4 ml, 11.0 mmol)] in THF (12.5 ml) at −78° was slowly added a solution of phthalide (1.34 g, 10.0 mmol) in THF (25 ml) over a 20 min period, which resulted in the formation of a yellow precipitate. The mixture was allowed to stir at −78° for 10 min, then butyraldehyde (1.0 ml, 11.0 mmol) was added slowly. The solution was allowed to stir at −78° for 15 min and then quenched with ice chunks (ca. 2g). The resultant solution was stirred for 15 min and then removed from the −78° bath. H₂O (5 ml) was added and the mixture was concentrated *in vacuo* to yield a yellow aqueous residue. 0.1 M HCl (ca. 150 ml) was added to the residue until pH 3 and then extracted with EtOAc (3×60 ml). The combined organic layers were dried, filtered, and concentrated *in vacuo* to afford a light yellow oil (2.0 g, 98%); ¹H nmr δ 0.90 (3H, t, *J*=7.0 Hz), 1.30–1.40 (1H, m), 1.48–1.60 (3H, m), 3.86–3.92 (1H, m), 5.34 (1H, d, *J*=5.0 Hz), 7.46–7.66 (3H, m), 7.85 (1H, dt, *J*=0.9 and 7.7 Hz); ¹³C nmr δ 13.8, 18.7, 34.4, 72.5, 83.6, 123.1, 125.7, 126.5, 129.3, 133.9, 147.0, 170.6.

3-HYDROXYBUTYL-4,5-DIHYDROPHTHALIDE.—To a stirred solution of NH₃ (25 ml), 3-hydroxybutylphthalide (140 mg, 0.68 mmol) and *i*-PrOH (0.26 ml, 3.4 mmol) at −78° was added Na (250 mg, 10.9 mmol). The resulting dark-blue solution was warmed to −50° and allowed to stir for 5 h at −50° and then quenched with NH₄Cl until the blue color had dissipated. H₂O (ca. 20 ml) was carefully added and the NH₃ was removed *in vacuo*. The resulting aqueous residue was acidified to pH 1 (2 M HCl) and extracted with Et₂O (3×30 ml), and the combined organic extracts were washed with brine (ca. 2×20 ml) until the aqueous layer was neutral. The organic solution was dried, filtered, and concentrated *in vacuo* to yield a light-clear yellow liquid (84 mg, 60%, 14:1 mixture of product/starting material); ¹H nmr δ 0.86 (3H, t, *J*=7.1 Hz), 1.25–1.65 (4H, m), 2.30–2.40 (2H, m), 2.42–2.66 (2H, m), 3.70–3.82 (1H, m), 4.86 (1H, d, *J*=4.5 Hz), 5.85 (1H, dt, *J*=4.1 and 9.7 Hz), 6.09 (1H, dt, *J*=2.0 and 9.7 Hz); ¹³C nmr δ 13.7, 18.6, 22.0, 22.1, 34.3, 71.6, 85.6, 116.4, 125.2, 128.9, 160.0, 171.4.

(*Z*)-LIGUSTILIDE [**1**]—To a stirred solution of the dihydrophthalide (208 mg, 1 mmol) in CH₂Cl₂ (2 ml) at −10° was added pyridine (0.32 ml, 4 mmol), followed by slow addition of methanesulfonyl chloride (0.16 ml, 2 mmol). The solution was allowed to stir for 5 min at −10°, then removed from the bath and warmed to room temperature. Ar was passed over the solution to remove the CH₂Cl₂ and pyridine (3 ml) was added. The mixture was heated at reflux for ca. 1 h, cooled to 0°, acidified to pH 1 (2 M HCl), extracted with Et₂O (3×20 ml), washed with brine (10 ml), dried, filtered, and concentrated *in vacuo*. The crude product was chromatographed on Si gel (8 g) using Me₂CO-petroleum ether (1:4) as eluent to afford **1** (96 mg, 55%).

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