Serotonergic Activity-Guided Phytochemical Investigation of the Roots of *Angelica sinensis*

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Serotonin receptor (5-HT_7) binding assay-directed fractionation of a methanol extract of the dried roots of *Angelica sinensis* led to the isolation and identification of 21 compounds including a new phenolic ester, angeliferulate (**1**), and three new phthalides, 10-angeloylbutylphthalide (**2**), sinaspirolide (**3**), and ansaspirolide (**4**), along with 17 known compounds, *p*-hydroxyphenethyl *trans*-ferulate (**5**), *Z*-ligustilide (**6**), *Z*-butylidenephthalide (**7**), senkyunolide I (**8**), *Z*-6-hydroxy-7-methoxydihydroligustilide (**9**), *N*-butylbenzenesulfonamide (**10**), 11(*S*),16(*R*)-dihydroxyoctadeca-9*Z*,17-diene-12,14-diyn-1-yl acetate (**11**), (3*R*,8*S*)-falcarindiol (**12**), heptadeca-1-en-9,10-epoxy-4,6-diyne-3,8-diol (**13**), oplopandiol (**14**), 8-hydroxy-1-methoxy-, *Z*-9-heptadecene-4,6-diyn-3-one (**15**), imperatorin, ferulic acid, vanillin, stigmasterol, sucrose, and 1,3-dilinolenin. This is the first report of a sulfonamide (**10**) identified from a higher plant source, although its presence needs further investigation. Biosynthetic pathways for dimeric phthalides **3** and **4** are proposed. Compounds **5**, **7**, **11**, **12**, **15**, and imperatorin exhibited affinity toward 5-HT_7 receptors in a competitive binding assay.

Angelica sinensis (Oliv.) Diels (Apiaceae) is a herbaceous perennial plant mainly distributed in the northwest of China. The root is variously called Dang Gui or Dong Quai, among others. It has been traditionally applied to the treatment of gynecological disorders (e.g., menstrual disorders, amenorrhea, dysmenorrhea, anemia, premenstrual syndrome, menopause) for thousands of years in Asia, with the earliest record of its use in the *Divine Husbandman's Classic of the Materia Medica (Shen Nong Ben Cao Jing)* published during the period of the Han Dynasty (A.D. 25−225).[1−3] Dang Gui has been regarded as "lady's ginseng" or "female's ginseng", implicating its popularity and importance for various women's ailments. It was first introduced into the West in 1899 by Merck in the form of a liquid extract named "Eumenol" [4] and is presently marketed in the United States as a dietary supplement, with numerous products being marketed worldwide.

It was generally presumed that Dang Gui has estrogenic activity on the basis of its broad applications for female ailments. However, there is no evidence of estrogenicity in both *in vitro* and clinical studies,[5,6] which indicates that Dang Gui may act through an alternative mechanism rather than being a phytoestrogen. Indeed, preliminary testing of extracts demonstrated that the methanol extract of Dang Gui has serotonergic activity, suggesting that it may contain serotonergic ligands that act on serotonin receptors and, thus, exhibit pharmacological effects related to improvement of symptoms of moods, behaviors, and hot flashes for premenstrual and menopausal women.

Previous phytochemical studies on Dang Gui have resulted in the isolation and identification of a variety of constituent classes, including phthalides, polysaccharides, lipids, polyacetylenes, aromatic compounds, terpenes, amino acids, trace elements, and vitamins.[7−13] The root contains up to 0.65% volatile oil, with alkylphthalides being the major components, of which *Z*-ligustilide is the most abundant, with up 45%−60%.[9,11,14−16] Other alkylphthalides identified so far include the phthalide monomers *E*-ligustilide, butylidenephthalide, butylphthalide, senkyunolides A−I, senkyunolide K, *Z*-6-hydroxy-7-methoxydihydroligustilide, *Z*-6,7-epoxyligustilide, sedanenolide, neocnidilide, 3-butylidene-7-hydroxyphthalide, 11-angeloylsenkyunolide F, and *E*-6,7-dihydroxydihydroligustilide.[11,14−18] In addition to these phthalide monomers, several dimeric phthalides, angelicide,[9,11] *E*-232,[19] *Z,Z*′-3.3′,8.8′-diligustilide, *Z,Z*′-6.8′,7.3′-diligustilide, levistolide A,[11] and *Z*-3′,8′,3′a,7′a-tetrahydro-6,3′,7,7′a-diligustilide-8′-one[20] have also been reported. These dimers were thought to be derived through cycloaddition reactions, with or without further oxidation or ring cleavage of various phthalide monomers.[19,21,22]

The long-term aim of our research is to investigate a potential mechanism underlying Dang Gui's use in a wide range of gynecological indications, particularly those associated with PMS and menopause, and to chemically and biologically characterize the crude extract in order to provide a standardized clinical formulation. Toward this end, the bioactive phytochemicals of Dang Gui were investigated guided by the 5-HT_7 receptor-binding assay. The present paper describes the isolation and identification of 21 compounds including a new phenolic ester, angeliferulate (**1**), and three new phthalides, 10-angeloylbutylphthalide (**2**), sinaspirolide (**3**), and ansaspirolide (**4**), along with 17 known compounds, as well as the serotonergic (5-HT_7) effect of these isolates.

Results and Discussion

In our initial biological study, a methanol extract of the dried roots of Dang Gui exhibited serotonergic activity with a 59.0 ± 4.0% inhibition on [^{3}H] LSD binding to the 5-HT_7 receptor. The serotonergic active methanol extract was successively partitioned with petroleum ether (PE), chloroform ($CHCl_3$), and *n*-butanol (*n*-BuOH), and the partitions, along with aqueous mother liquor, were evaluated for their serotonergic activity in the same bioassay. The bioactivity was localized in the PE and $CHCl_3$ partitions. Bioassay-guided chromatographic fractionation of the PE and $CHCl_3$ extracts afforded 21 compounds. Four of these were new compounds (**1**−**4**), one phenolic ester and three phthalides. The new phenolic ester was named angeliferulate (**1**), and the three new phthalides were given the trivial names 10-angeloylbutylphthalide (**2**), sinaspirolide (**3**), and ansaspirolide (**4**) to reflect the nature of their cyclic arrangements, respectively. The remaining 17 isolates were identified as known compounds by comparison of their spectroscopic data with those reported previously in the literature or with those of previously authenticated reference samples. Compound **1** was obtained as a pale yellow oil. The IR spectrum of **1** exhibited characteristic absorption bands at 3385, 1701, 1600, and 1515 cm^{-1}, suggesting the presence of a hydroxyl, an α,β-unsaturated ester carbonyl, and an aromatic moiety, respectively. The HRESIMS spectrum showed a deprotonated molecule of *m*/*z* 403.1380 (calcd for 403.1393), corresponding to a molecular formula of $C_{21}H_{24}O_8$

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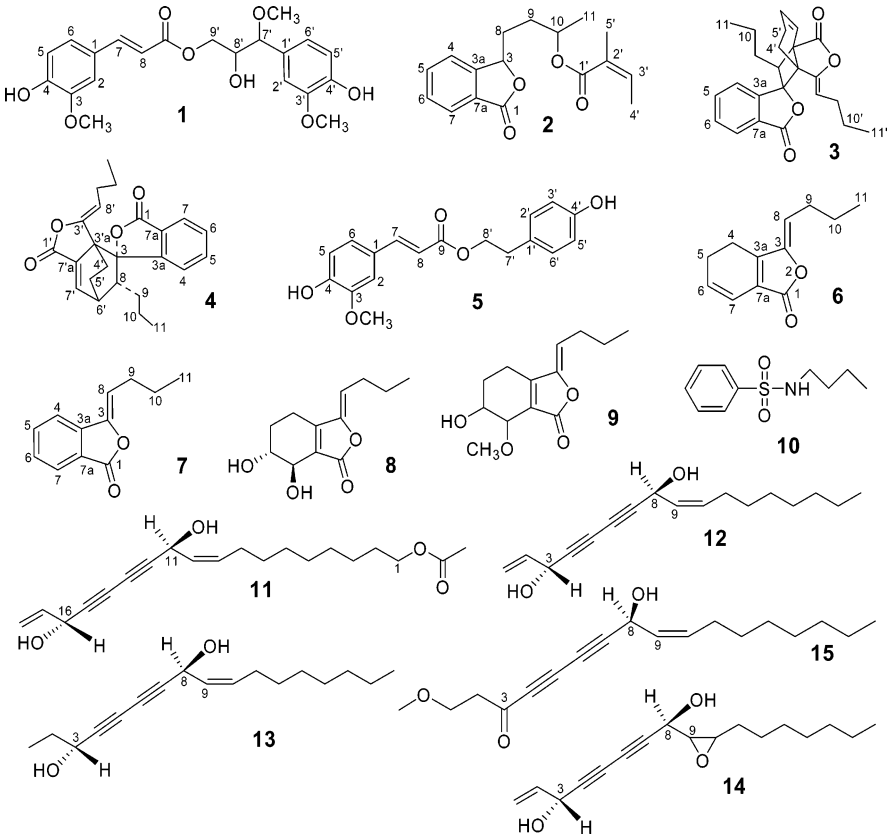
Chart 1



containing 10 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectral data of **1** were found to be similar to those of *p*-hydroxyphenethyl *trans*-ferulate (**5**), which in turn was identified by comparison of its NMR data with literature data.[8,23,24] A fragment ion of *m*/*z* 193 in the CID product ion spectrum suggested the presence of a feruloyl unit.[25,26] The ^{1}H NMR spectrum of **1** displayed signals at δ 7.588 (1H, d, J = 16.0 Hz) and 6.322 (1H, d, J = 16.0 Hz), indicating the presence of two *trans*-coupled olefinic protons, and signals at δ 3.929 (3H, s), 3.889 (3H, s), and 3.278 (3H, s) attributable to three methoxyl groups. In addition, two sets of 1,3,4-trisubstituted phenyl units (ABX system) were revealed by signals at δ 7.061 (1H, dd, J = 8.2, 2.0 Hz), 7.030 (1H, d, J = 2.0 Hz), 6.915 (1H, d, J = 8.2 Hz), 6.905 (1H, d, J = 8.6 Hz), 6.810 (1H, d, J = 2.0 Hz), and 6.806 (1H, dd, J = 8.6, 2.0 Hz) and confirmed by the COSY spectrum. The observed downfield chemical shift of the methylene and methine protons [δ 4.148 (dd, J = 13.0, 3.1 Hz, H-9′a), 3.981 (m, H-9′b), 3.983 (m, H-8′), and 4.116 (d, J = 11.8 Hz, H-7′)] indicated their attachment to the oxygen-bearing carbons (C-9′, C-8′, and C-7′, respectively). The presence of an ester carbonyl group was revealed by a ^{13}C NMR signal at δ 167.4 (C-9). The linkage among the different moieties and the substitution patterns were fully assigned by the following HMBC correlations: H-7 → C-2, C-6, and C-9; H-8 → C-1; H-7′ → C-2′ and C-6′; H-9′ → C-9 and C-7′. On the basis of the aforementioned data, compound **1** was identified as a previously unreported phenolic ester, 3-methoxyl-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxypropyl-3-(4-hydroxy-3-methoxyphenyl)-2*E*-propenoate, and the trivial name of angeliferulate was suggested.

Compound **2** was obtained as a pale yellow oil. The presence of an α,β-unsaturated lactone ring was suggested by its IR spectrum (λ_{max} at 1765 and 1699 cm^{-1}).[27] Its molecular formula was determined as $C_{17}H_{20}O_4$ by HRESIMS. The ESIMSMS experiment resulted in the most abundant fragment ion at *m*/*z* 189, which is consistent with that of butylphthalide.[11] The ^{1}H and ^{13}C NMR data of **2** were observed to be similar to those of *Z*-butylidenephthalide (**7**), a compound identified by direct comparison of its spectral data with those reported in the literature.[28] Thus, **2** was determined to be a phthalide. The ^{1}H NMR spectrum of **2** exhibited signals of four aromatic protons at δ 7.912 (1H, dd, J = 7.6, 1.2 Hz), 7.685 (1H, ddd, J = 7.7, 7.6, 1.2 Hz), 7.542 (1H, ddd, J = 7.7, 7.6, 0.8 Hz), and 7.427 (1H, dd, J = 7.6, 0.8 Hz, H-4), suggesting the presence of a 1,2-disubstituted aromatic ring. Two oxygen-bearing methine signals at δ 5.490 (1H, dd, J = 7.2, 3.9 Hz) and 5.000 (1H, m) were coupled with four methylene protons at δ 2.205/1.776 and 1.823/1.721, as well as a methyl group at δ 1.265 (3H, d, J = 6.4 Hz), respectively, as supported by the analysis of the COSY spectrum and their coupling constants. One vinyl proton at δ 6.055 (1H, qq, J = 7.3, 1.5 Hz) was observed to be coupled with two vinylic methyls at δ 1.975 (3H, dq, J = 7.3, 1.5 Hz) and 1.885 (3H, dq, J = 1.5, 1.5 Hz). The ^{13}C NMR spectrum displayed 17 carbons, including two ester carbonyl carbons (δ 170.9, 168.0) and two vinyl carbons (δ 137.9, 128.4). The presence of an angelic acid unit was indicated by a spin system composed of proton signals at δ 6.055 (1H, qq, J = 7.3, 1.5 Hz), 1.975 (1H, dq, J = 7.3, 1.5 Hz), and 1.885 (1H, dq, J = 1.5, 1.5 Hz),[29] which was confirmed by an ESIMSMS fragment ion at *m*/*z* 83. The connectivity between the butylphalide and the angelic acid moieties was established by the HMBC correlations between the protons at δ 5.000 (1H, m, H-10) and the quaternary carbon signal at δ 168.0 (C-1′). Thus, compound **2** was elucidated as an angeloyl ester of butylphthalide and named 10-angeloylbutylphthalide.

The HRESIMS spectrum of **3** exhibited a protonated molecule of *m*/*z* 379.1922 (calcd for 379.1909), suggesting the molecular formula of $C_{24}H_{26}O_4$, with 12 degrees of unsaturation. The strong bands at 1776 and 1700 cm^{-1} in the IR spectrum indicated the existence of both an α,β-unsaturated and a saturated lactone ring, respectively.[19] The ^{1}H NMR and COSY spectrum suggested the presence of a butyl side chain [δ 3.016 (1H, dd, J = 7.8, 7.8 Hz, H-8), 1.790 (1H, dddd, J = 14.1, 10.2, 7.8, 5.4, H-9a), 1.510 (1H, dddd, J = 18.0, 10.2, 7.8, 5.4, H-9b), 1.220 (1H, m, H-10a), 1.010 (1H, m, H-10b), 0.776 (3H, t, J = 7.4, H-11)] and a butylidene side chain [δ 4.406 (1H, t, H-8′), 2.246 (1H, m, H-9′a), 2.147 (1H,

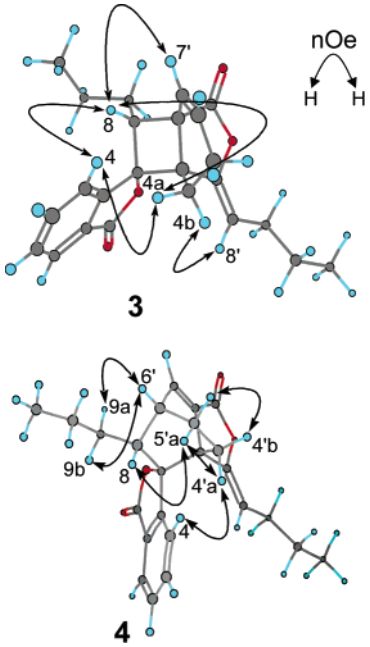


Figure 1. Key NOESY correlations for compounds **3** and **4**. The NOESY spectrum established the relative configuration of compound **3**. The Z-configurations of butyl and butylidene side chains were indicated by correlations between H-4 and H-8, as well as H-4′b and H-8′, respectively. In addition, the correlations between H-4 and H-4′a, H-8 and H-4′a, and H-8 and H-7′ suggested that these protons are on the same face of the molecule. Key NOESY correlations of **4** were between H-4 and H-4′a, as well as H-8 and H-5′a. This not only suggested that the butyl side chain possesses a Z-configuration but also defined the relative spatial positions of two six-membered rings as shown in this model.

m, H-9′b), 1.399 (1H, m, H-10′a), 1.256 (1H, m, H-10′b), 0.885 (3H, t, J = 7.4, H-11′)]. Two olefinic signals attributable to an isolated double bond [δ 6.113 (1H, ddd, J = 9.8, 6.4, 2.0, H-6′) and 5.994 (1H, dd, J = 9.8, 2.8 Hz, H-7′)] and four aromatic protons [δ 7.901 (1H, dd, J = 7.4, 1.2 Hz, H-7), 7.710 (1H, td, J = 7.6, 1.2 Hz, H-5), 7.586 (1H, dd, J = 7.6, 7.4 Hz, H-6), 7.577 (1H, brd, J = 7.6 Hz, H-4)] were also observed. The ^{13}C NMR spectrum of **3** exhibited the presence of 24 carbons with two ester carbonyl units [δ 173.9 (C-1′), 168.5 (C-1)] and displayed a resonance pattern very similar to a combination of those of the phthalide monomers *Z*-ligustilide (**6**) and *Z*-butylidenephthalide (**7**), except for the substitution of the vinyl carbons of the two double bonds in **6** and **7** with a tertiary and three quaternary carbons in **3** (^{13}C δ 88.5, 53.0, 54.2, and 48.6). These data suggested **3** to be a phthalide dimer composed of one ligustilide and one butylphthalide unit. This conclusion was confirmed by the presence of two prominent fragment ions of *m*/*z* 191 and 189 in the product ion spectrum. The ^{1}H NMR of **3** displayed an olefinic proton of a butylidene side chain at δ 4.406 (1H, t, H-8′), and the upfield shift of this signal indicated that it was not affected by the deshielding effect of the neighboring lactone oxygen and suggested the butylidene side chain in the ligustilide moiety to have a *Z*-configuration.[29] The linkage of the two monomers was established by the HMBC correlations (H-4′ → C-3, H-8 → C-1′, and H-8 → C-7′). Consequently, compound **3** was determined as a dimeric phthalide forming a cyclobutane ring at C-3 and C-8 of a butylphthalide molecule with C-3′a and C-7′a of a *Z*-ligustilide moiety. The NOESY spectrum established the relative configuration of compound **3**. The correlations between H-4 and H-8, and H-4′b and H-8′, excluded the *E*-configurations of the two side chains. The presence of cross-peaks between H-4 and H-4′a, H-8 and H-4′a, and H-8 and H-7′ established that these protons are in the same plane (Figure 1). Thus, **3** was determined to be a new 3−3′a, 8−7′a dimeric phthalide of *Z*-butylidenephthalide and *Z*-ligustilide and was trivially named sinaspirolide.

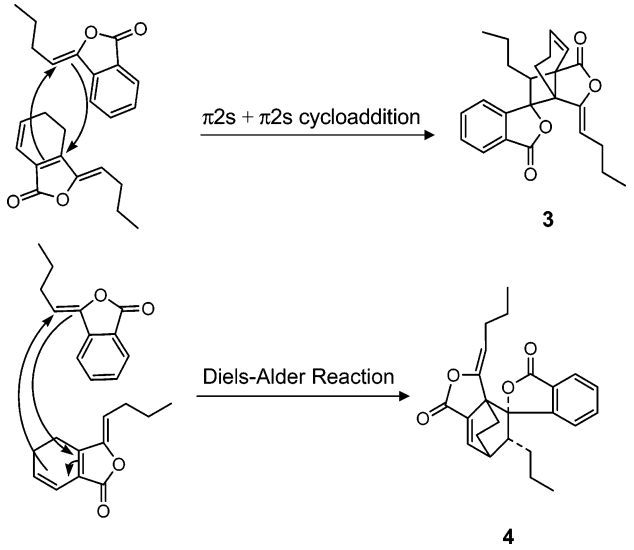
The spectroscopic data (NMR, IR, and MS) of **4** were found to be similar to those of **3**. The HRESIMS spectrum of compound **4** with a protonated molecule of *m*/*z* 379.1923 (calcd for 379.1909) suggested the same molecular formula ($C_{24}H_{26}O_4$) as **3**, and the product ion spectrum of **4** also contained the same two prominent fragment ion peaks at *m*/*z* 191 and 189 found in **3**, which indicated **4** to be another dimeric phthalide. The ^{1}H and ^{13}C NMR data of **4** were very similar to those of **3**, and the major difference between the two was that the two vicinal olefinic protons at δ 6.113 (1H, ddd, J = 9.8, 6.4, 2.0, H-6′) and 5.994 (1H, dd, J = 9.8, 2.8 Hz, H-7′)] of **3** were not present in the ^{1}H NMR spectrum of **4**. Instead, a doublet of an olefinic proton coupled with a methine proton [δ 3.246 (1H, dd, J = 6.6, 1.3 Hz, H-6′)] was observed at δ 7.469. The ^{13}C spectrum of **4** showed signals for a sp^2 quaternary carbon at 133.9 and a conjugated lactone carbonyl carbon at δ 163.9, as compared to an olefinic carbon at δ 131.6 and a nonconjugated lactone carbonyl carbon at δ 173.9 observed in **3**. Comparative analysis indicated that the double bond between C-6′ and C-7′ in **3** was shifted to C-7′ and C-7′a in **4**, which was confirmed by its HMBC correlations (H-4′ → C-3; H-8 → C-5′ and C-7′; H-9 → C-6′). Therefore, compound **4** was identified as a dimeric phthalide possessing a 3−3a′, 8−6′ linkage, which most likely originated from the Diels−Alder reaction with the butylidene side chain of a butylidenephthalide molecule as the dienophile. In addition, the fact that the olefinic proton of H-8′ resonated at a markedly high field (δ 3.214), which is due to a shielding effect caused by its spatially neighboring aromatic ring, supported the geometry of the butylidene side chain to be the *Z*-configuration. The overall relative configuration was established on the basis of the following key NOESY correlations: H-4 and H-4′a, H-8 and H-5′a, H-4′a and H-5′a, H-4′b and H-5′b, H-9a and H-6′, and H-9b and H-6′ (Figure 1). To indicate both the *ansa*-type and the spirocyclic partial structures of **4**, we assigned the name of ansaspirolide to this previously unreported structure.

In addition to the four new compounds described above, 17 known compounds were identified by comparison of their chemical and spectral data with literature data and/or with those of authentic samples as *p*-hydroxyphenethyl *trans*-ferulate (**5**),[8,23,24] *Z*-ligustilide (**6**),[21,28,30,31] *Z*-butylidenephthalide (**7**),[28] senkyunolide I (**8**),[24,31] *Z*-6-hydroxy-7-methoxydihydroligustilide (**9**),[11,21] imperatorin,[32,33] ferulic acid, vanillin, *N*-butylbenzenesulfonamide (**10**),[34] stigmasterol,[35] sucrose, 11(*S*),16(*R*)-dihydroxyoctadeca-9*Z*,17-diene-12,14-diyn-1-yl acetate (**11**),[36] (3*R*,8*S*)-falcarindiol (**12**),[37] heptadeca-1-en-9,10-epoxy-4,6-diyne-3,8-diol (**13**),[38] oplopandiol (**14**),[39] 8-hydroxy-1-methoxy-, *Z*-9-heptadecene-4,6-diyn-3-one (**15**),[40,41] and 1,3-dilinolenin, respectively.

Imperatorin was reported from *A. archangelica*, *A. dahurica*, and *A. sylvestris* previously,[42] but not heretofore isolated from *A. sinensis*. *N*-Butylbenzenesulfonamide (**10**) was first isolated as an antifungal antibiotic from a greenhouse soil sample,[34] and this is the first report of a sulfonamide from a higher plant. However, whether **10** is a natural constituent of *A. sinensis* or an environmental contaminant sequestered during plant growth is in need of further investigation.

Compounds **11**−**15** are polyynes, typical constituents found in the seven dicotyledonous families Araliaceae, Campanulaceae, Asteraceae, Pittisporaceae, Oleaceae, Santalaceae, and Apiaceae.[43] Of these five polyynes, 8-hydroxy-1-methoxy-, *Z*-9-heptadecene-4,6-diyn-3-one (**15**) was synthesized previously,[40,41] but this is the first report of this compound as a natural product. 11(*S*),16(*R*)-Dihydroxyoctadeca-9*Z*,17-diene-12,14-diyn-1-yl acetate (**11**) has been isolated from the Apiaceae and Araliaceae previously[39] and displayed inhibitory activity on 5-lipoxygenase (5-LO) and cyclooxygenase (COX-1) with IC_{50} values of 24 and 73 μM, respectively.[36] (3*R*,8*S*)-Falcarindiol (**12**), previously isolated from

Scheme 1. Postulated Biosynthetic Pathways of Dimers **3** and **4**



Oplopanax horridus and *Aegopodium podagraria*, exhibited marked antimicrobial, antibacterial, antifungal, and antiproliferative activities.[39,44,45] Heptadeca-1-en-9,10-epoxy-4,6-diyne-3,8-diol (**13**) was originally isolated from *Panax quinquefolius* and exhibited cytotoxic activity against L1210 leukemia cells, and the *cis* configuration of the epoxide ring in the structure of **13** was assigned by the coupling constant of $J = 4.4$ Hz between H-9 and H-10.[38] The antibacterial and anti-TB active oplopandiol (**14**) was first obtained from *Oplopanax horridus*.[39] Compounds **11**, **13**, and **14**, along with1,3-dilinolenin, are reported here from *A. sinensis* for the first time.

Compounds **3** and **4** are dimeric derivatives of the two monomers ligustilide and butylphthalide, formed via cycloaddition reactions as postulated in Scheme 1. Compound **3** may be regarded as a product directly formed by dimerization of two monomers via a Diels−Alder reaction, whereas compound **4** is probably generated by an alternative cycloaddition mechanism that forms the cyclobutane ring.[21]

All of the isolates were evaluated for competitive binding activity in the 5-HT_7 receptor binding assay. Compounds **5**, **11**, and **12** exhibited inhibitory effects on [^{3}H] LSD binding to the 5-HT_7 receptors with IC_{50} values of 47.6 ± 1.2, 118.1 ± 25.7, and 117.5 ± 12.0 μM, respectively. Meanwhile, imperatorin and **15** displayed 57 ± 1% and 69.1 ± 1% inhibition on [^{3}H] LSD binding to the 5-HT_7 receptors at a concentration of 100 μM, respectively (Table 1). These compounds may at least partially contribute to the serotonergic activity of Dang Gui and provide a rationale for its traditional use in the treatment of women's complaints, including PMS and menopausal symptoms. It remains to be clarified whether these 5-HT_7 ligands are specific binding or nonspecific binding, as well as agonists or antagonists to 5-HT_7 receptors.

Experimental Section

General Experimental Procedures. Optical rotation, $[\alpha]_D$, values were measured on a Perkin-Elmer 241 polarimeter at 20 °C. UV λ_{max} values were determined from the HPLC-PDA chromatograms. IR spectra were taken on a JASCO FT/IR-410 spectrometer. ^{1}H NMR and ^{13}C NMR data were recorded on Bruker Avance-500, DPX-400, Avance-360, and/or DPX-300 spectrometers using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to TMS signals. COSY, HSQC, HMQC, NOESY, and HMBC experiments were performed using standard Bruker pulse sequences. The digital resolution in 1D ^{1}H NMR was always better than 0.1 Hz equivalent to 0.0002 ppm (e.g., 16K real data points, 4 ppm spectral width). High-resolution mass spectra (HRMS) and tandem mass spectra (MS-MS) were obtained on a Micromass (Manchester, UK) Q-Tof-2 quadrupole/time-of-flight mass spectrometer equipped with an electrospray ionization source (ESI). The spectrometer was operated in either positive or negative ion mode. Tandem mass spectra were acquired at a collision energy of 20 eV using Ar as the collision gas at a pressure of 2.0×10^{-5} mbar. Vacuum liquid chromatography (VLC) was carried out on Merck silica gel 60 (65−400 mesh). Reversed-phase medium-pressure column chromatography (MPLC) was performed on a Merck Lobar Lichroprep RP_{18} column, equipped with a fluid pump and an autocollector. Semipreparative HPLC was conducted on a Waters 600 system aided by a photodiode array (PDA) detector, a Waters 717 plus autosampler, and Millennium32 Chromatography Manager (Waters Corp.) using a Watrex GROM-Sil 120 ODS-4 HE column (5 μm, 20 × 300 mm) at a flow rate of 6 mL/min. Droplet countercurrent chromatography (DCCC) was carried out using an EYELA instrument (Model DCC-3000; Tokyo Rikakikai Co. Ltd, Japan) equipped with 300 glass columns (i.d. 3.4 × 400 mm), a Shimadzu LC-610 solvent pump, and an autocollector (flow rate at 0.4 mL/min, 25 min per fractions). Analytical thin-layer chromatography (TLC) was carried out on Merck TLC plates (250 μm thickness, KGF Si gel 60 and KGF RP-18 Si gel 60), and compounds were visualized by spraying the dried plates with H_2SO_4−EtOH (1:19) or *p*-anisaldehyde−H_2SO_4−EtOH (1:1:48) followed by heating at 110 °C.

Plant Material. Dried roots of *Angelica sinensis* (Oliv.) Diels were purchased from Kiu Shun Trading Ltd., Vancouver, Canada, in 2000, and identified by us by using a series of comparative macroscopic, microscopic, TLC, HPLC, and PCR analysis with Dang Gui reference plant materials obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China, Beijing, People's Republic of China (Lot # 927-200110).[46] A reference sample has been deposited at the UIC/NIH Center for Botanical Dietary Supplements Research (BC165), Chicago, IL.

Cell Culture Conditions. The human 5-HT_7-transfected Chinese hamster ovary (CHO) cell line was generously provided by David Sibley (National Institutes of Health, Bethesda, MD) and cultured with Ham's F-12 medium, containing FBS (10%), 1 mM MEM sodium pyruvate, 50 mg/mL gentamycin, and 50 units/mL each of penicillin and streptomycin.

Membrane Preparation. Cells (human 5-HT_7 CHO) were plated into dishes (150 mm × 10 mm) and cultured to confluence in order to collect membranes as previously described.[47] A hypotonic buffer (15 mM Tris, 1.25 mM $MgCl_2$, and 1 mM EDTA, pH 7.4) was added to the dishes and incubated at 4 °C for 15 min, the cells were scraped from the dishes, and the lysate was centrifuged. The hypotonic buffer was removed and the membrane pellet resuspended in TEM buffer (75 mM Tris, 1 mM EDTA, 12.5 mM $MgCl_2$, pH 7.4). The cell membranes were homogenized and centrifuged twice at 12000*g* for 20 min. The pellets were dissolved in TEM buffer and were stored at −80 °C. Protein concentrations were determined according to the Lowry method using bovine serum albumin as the standard.

Serotonin Receptor Binding Assays. Initial radioligand binding studies were performed by Panlabs (Bothell, WA) as previously described for serotonin receptor subtypes 1A, 1B, 1D, 2A, 2B, 2C, 3, 5A, 6, and 7.[48−54] Assays were performed in-house with minor modifications using human recombinant CHO cell membrane and [^{3}H] LSD (5 nM) in an incubation buffer (75 mM Tris-HCl, 1.25 mM $MgCl_2$, 1 mM EDTA, pH 7.4).[54] After a 1 h incubation at 37 °C, the mixture for receptor was filtered over 934-AH Whatman filters that had been presoaked in 0.5% polyethylenimine (PEI) and washed twice in ice-cold 50 mM Tris buffer (pH 7.4) using a 96-well Tomtec-Harmester (Orange, CT). Each filter was dried, suspended in Wallac microbeta plate scintillation fluid (Perkin-Elmer Life Sciences, Boston, MA), and counted with a Wallac 1450 Microbeta liquid scintillation counter (Perkin-Elmer Life Sciences, Boston, MA). 5-Hydroxytryptamine (serotonin, 5-HT) (250 nM) was used to define nonspecific binding, which accounted for <10% of the total binding. The percent inhibition of [^{3}H] ligand bound to each 5-HT receptor was determined as $[1 - (dpm_{sample} - dpm_{blank})/(dpm_{DMSO} - dpm_{blank})] \times 100$, and the extracts and fractions displaying inhibition percentage over 50% were regarded as active and subjected to further fractionation in our experiment. The IC_{50} values were determined for the isolates with percent inhibition greater than 50%. The data represent the average ± SD of at least triplicate determinations.

Extraction and Isolation. The milled roots (8.0 kg) of Dang Gui were macerated in 20 L of MeOH for 24 h and then percolated exhaustively with the same solvent (total 40 L). The MeOH percolate was evaporated to a volume of ca. 2700 mL (E_0). After 200 mL of the MeOH percolate was evaporated to dryness for use in bioassay evaluation, to the remaining 2500 mL was added 500 mL of water and

Table 1. Competitive Binding Activities to 5-HT$_7$ Receptors of the Isolates from *Angelica sinensis*

isolate	inhibition %[a] (100 μM)	IC$_{50}$[a] (μM)	isolate	inhibition %[a] (100 μM)	IC$_{50}$[a] (μM)
1	31 ± 6		**11**	49 ± 4	118.1 ± 25.7
2	21 ± 6		**12**	61 ± 8	117.5 ± 12.0
3	24 ± 6		**13**	5 ± 4	
4	19 ± 0		**14**	36 ± 16	
5	71 ± 7	47.6 ± 1.2	**15**	69 ± 1	>150
6	33 ± 8		imperatorin	57 ± 2	
7	46 ± 10	126.6 ± 20.8	stigmasterol	0 ± 3	
8	0 ± 1		ferulic acid	41 ± 4	
9	36 ± 13		vanillin	0 ± 11	
10	0 ± 4		1,3-dilinolenin	0 ± 13	

[a] IC$_{50}$ values represent the concentration (μM) that shows 50% inhibition of binding activities. All data represent average ± SD of triplicate determinations. Positive control: serotonin (IC$_{50}$ = 2.5 ± 0.7 nM).

it was then partitioned against petroleum ether (PE, 2000 mL × 3). The PE extract (E$_1$, 268 g; 5-HT$_7$ 69.0 ± 3.0% inhibition at 100 μg/mL) was obtained in vacuo by rotary evaporation. The aqueous-methanol mother liquid was evaporated in vacuo to remove MeOH, and the resulting aqueous extract was successively partitioned with $CHCl_3$ (2000 mL × 3) and *n*-BuOH (2000 mL × 3). The $CHCl_3$ (E$_2$, 57 g; 5-HT$_7$ 51.0 ± 3.0% inhibition at 100 μg/mL) and *n*-BuOH extracts (E$_3$, 39 g; 5-HT$_7$ 27.0 ± 1.0% inhibition at 100 μg/mL) were acquired after being dried in vacuo, respectively. The aqueous mother liquid was lyophilized to afford an aqueous extract (E$_4$, 158 g; 5-HT$_7$ 28.0 ± 9.0% inhibition at 100 μg/mL). The MeOH extract (E$_0$; 5-HT$_7$ 59.0 ± 4.0% inhibition at 100 μg/mL) and its partitions (E$_1$−E$_4$) were evaluated for their serotonergic activities in the 5-HT$_7$ receptor binding assay.

Bioassay results showed that partitions E$_1$ and E$_2$ exhibited serotonergic activities with 69.0 ± 0% and 51.0 ± 3.0% inhibitory effects on [^{3}H] LSD binding to the 5-HT$_7$ receptors at a concentration of 100 μg/mL, respectively. Therefore, both the PE (E$_1$) and the $CHCl_3$ partitions (E$_2$) were selected for bioassay-guided fractionation. The pooled E$_1$ and E$_2$ were subjected to flash column chromatography eluting with a stepwise gradient solvent system of PE−EtOAc (10:0 → 0:10) and EtOAc−MeOH (10:0 → 0:10) to afford 13 primary pooled fractions (F1−F13).

Primary fraction F5 (7.0 g) was chromatographed over silica gel (600 g, 60−200 mesh, 9 × 30 cm column) using the vacuum liquid chromatography (VLC) technique eluting with gradients of PE−EtOAC−MeOH (100:0:0 → 0:0:100) to give 14 secondary fractions (F5-1 to F5-14). Stigmasterol (20 mg) was precipitated from secondary fraction F5-4 at room temperature. The secondary fraction F5-5 (4.5 g) was further separated on a VLC column packed with silica gel (70 g, 230−400 mesh, 1.5 × 10 cm) and eluting with a PE−EtOAc stepwise gradient (100:0 → 0:100) to afford seven tertiary fractions, F5-5-1 to F5-5-7. Further purification of F5-5-2 (500 mg) by reversed-phase preparative HPLC (75% aqueous MeOH → 99% MeOH, 6 mL/min) resulted in the isolation of **2** (3.0 mg, t_R = 33 min), **3** (2.2 mg, t_R = 55 min), **10** (1.9 mg, t_R = 18.5 min), **12** (90.0 mg, t_R = 73 min), **13** (1.4 mg, t_R = 46 min), **14** (1.6 mg, t_R = 91 min), and **15** (0.7 mg, t_R = 94 min). Fraction F5-5-3 was also subjected to reversed-phase preparative HPLC (6 mL/min) using an isocratic condition (MeOH−H_2O, 78:22) to afford imperatorin (2.0 mg, t_R = 31 min), ferulic acid (1.3 mg, t_R = 14 min), and vanillin (2.0 mg, t_R = 13 min).

Primary fraction F3 (43.2 g) was initially fractionated by VLC (6 × 30 cm) on silica gel (220−400 μm, 500 g), eluting with a gradient of PE−EtOAc starting from 100% PE to 50% PE in EtOAC to afford 13 secondary fractions (F3-1 to F3-13). Secondary fractions F3-7 (3.6 g) and F3-8 (6.3 g) were combined based on their TLC profiles and subjected to preparative RP-18 MPLC (3 × 25 cm, 80 g) eluted with a MeOH−H_2O (70% MeOH → 98% MeOH) gradient to afford 1,3-dilinolenin (2.3 mg). The tertiary fraction F3-7-10 was further purified by preparative HPLC eluted with isocratic 70% aqueous MeOH to afford compounds **6** (50.0 mg, t_R = 44 min), **7** (30.0 mg, t_R = 48 min), and **8** (3.8 mg, t_R = 14 min).

The primary fraction F7 (4.0 g) was chromatographed by VLC over a silica gel column (500 g, 100−230 mesh) with a stepwise gradient of hexane−$CHCl_3$−EtOAC−MeOH (20:80:0:0 → 0:0:0:100) to give nine pooled secondary fractions (F7-1 to F7-9). Fraction F7-5 (1.6 g) was subjected to an ascending mode of DCCC with a flow rate at 0.4 mL/min, using a two-phase solvent system of PE−EtOAc−MeOH−H_2O (6:4:6:4). The pooled tertiary fraction F7-5-8 (150 mg) was further purified by RP-C18 HPLC using a gradient of MeCN−H_2O (60% MeCN → 98% MeCN in H_2O) to afford compound **4** (1.0 mg, t_R = 48 min). Fractions F7-7 (400 mg) and F7-8 (400 mg) were rechromatographed over a RP-C18 Lobar column (40−63 μm) and eluted with a gradient of MeOH−H_2O (50% → 100% MeOH), which led to the tertiary fractions F7-7-5 (39.0 mg) and F7-8-5 (60.0 mg), respectively. F7-7-5 was further purified by RP-C18 HPLC eluting with isocratic MeCN−H_2O (70:30) to give compound **5** (11.7 mg, t_R = 42 min). F7-8-5 was purified by RP-C18 HPLC with a MeCN−H_2O gradient (50% MeCN → 95% MeCN in H_2O) to afford compound **9** (2.0 mg, t_R = 44 min). F7-8-8 (37.9 mg) was purified by preparative TLC (1 mm, 20 cm × 20 cm) developed with CH_2Cl_2−EtOAc (7:3) and monitored under UV$_{254}$ nm to yield compound **11** (8.7 mg, R_f = 0.6).

Primary fraction F10 (5.6 g) was chromatographed over silica gel (500 g, 220−400 mesh, 7 × 31 cm) using the VLC technique eluting with a gradient of CH_2Cl_2−EtOAC−MeOH (100:0:0 → 0:0:100) to afford secondary fraction F10-10 (120 mg), which was subjected to reversed-phase preparative HPLC (55% MeOH in H_2O, 6 mL/min) to yield compound **1** (10.0 mg, t_R = 32 min).

Sucrose was crystallized directly from the MeOH crude extract (E$_0$) at room temperature.

Angeliferulate (1): pale yellow oil; $[\alpha]_D^{20}$ +2 (*c* 0.2, CH_2Cl_2); UV (LC-PDA) λ_{max} 326 nm; IR λ_{max} (CH_2Cl_2) 3385 (broad) (OH), 2920, 1701 (CO_2R), 1600, 1515 (Ph), 1430, 1267, 1159, 1034 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.588 (1H, d, J = 16.0 Hz, H-7), 7.061 (1H, dd, J = 8.2, 2.0 Hz, H-6), 7.030 (1H, d, J = 2.0 Hz, H-2), 6.915 (1H, d, J = 8.2 Hz, H-5), 6.905 (1H, d, J = 8.6 Hz, H-5′), 6.810 (1H, d, J = 2.0 Hz, H-2′), 6.806 (1H, dd, J = 8.6, 2.0 Hz, H-6′), 6.322 (1H, d, J = 16.0 Hz, H-8), 4.148 (1H, dd, J = 13.0, 3.1 Hz, H-9′a), 4.116 (1H, d, J = 11.8 Hz, H-7′), 3.983 (1H, m, H-8′), 3.981 (1H, m, H-9′b), 3.929 (3H, s, OCH_3-3), 3.889 (3H, s, OCH_3-3′), 3.278 (3H, s, OCH_3-7′); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 167.4 (C-9), 148.4 (C-4), 147.2 (C-3′), 147.1 (C-3), 146.3, (C-4′), 145.7 (C-7), 129.6 (C-1′), 127.2 (C-1), 123.6 (C-6), 121.1 (C-6′), 115.3 (C-8), 115.1 (C-5′), 114.8 (C-5), 109.6 (C-2), 109.5 (C-2′), 84.7 (C-7′), 74.1 (C-8′), 65.0 (C-9′), 57.0 (OCH_3-7′), 56.3 (OCH_3-3′), 56.0 (OCH_3-3); HRESIMS [M − H]$^-$ 403.1380 *m*/*z* calcd for $C_{21}H_{24}O_8$ (−3.3 ppm); ESIMSMS product ions *m*/*z* (% base peak) 371 (100), 193 (83), 341 (37), 175 (19), 403 (18), 327 (17).

10-Angeloylbutylphthalide (2): pale yellow oil; $[\alpha]_D^{20}$ −16 (*c* 0.1, CH_2Cl_2); UV (LC-PDA) λ_{max} 226, 274 nm; IR λ_{max} (CH_2Cl_2) 2918, 1765, 1699, 1235, 1162, 1061 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.912 (1H, dd, J = 7.6, 1.2 Hz, H-7), 7.685 (1H, ddd, J = 7.7, 7.6, 1.2 Hz, H-5), 7.542 (1H, ddd, J = 7.7, 7.6, 0.8 Hz, H-6), 7.427 (1H, dd, J = 7.6, 0.8 Hz, H-4), 6.055 (1H, qq, J = 7.3, 1.5, H-3′), 5.490 (1H, dd, J = 7.2, 3.9 Hz, H-3), 5.000 (1H, m, H-10), 2.205 (1H, m, H-8b), 1.975 (3H, dq, J = 7.3, 1.5, H-4′), 1.885 (3H, dq, J = 1.5, 1.5, H-5′), 1.823 (1H, m, H-9b), 1.776 (1H, m, H-8a), 1.721 (1H, m, H-9a), 1.265 (2H, d, J = 6.4 Hz, H-11); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 170.9 (C-1), 168.0 (C-1′), 149.9 (C-3a), 137.9 (C-3′), 134.5 (C-5), 129.6 (C-6), 128.4 (C-2′), 126.5 (C-7a), 126.2 (C-7), 122.0 (C-4), 81.3 (C-3), 70.4 (C-10), 31.5 (C-9), 31.3 (C-8), 21.0 (C-5′), 20.4 (C-11), 16.1 (C-4′); HRESIMS [M + Na]$^+$ 311.1297 *m*/*z* calcd for $C_{17}H_{20}O_4$ (4.4 ppm); ESIMSMS product ions *m*/*z* (% base peak) 189 (100) 171 (83) other product ions 153 (8), 145 (16), 133 (10), 83 (6).

Sinaspirolide (3): pale yellow oil; $[\alpha]_D^{20}$ +24 (*c* 0.1, CH_2Cl_2); UV (LC-PDA) λ_{max} 280 nm; IR λ_{max} (CH_2Cl_2) 2929, 1776, 1700, 1080, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.901 (1H, dd, J = 7.4, 1.2 Hz, H-7), 7.710 (1H, td, J = 7.6, 1.2 Hz, H-5), 7.586 (1H, dd, J = 7.6,

7.4 Hz, H-6), 7.577 (1H, brd, $J = 7.6$ Hz, H-4), 6.113 (1H, ddd, $J = 9.8$, 6.4, 2.0, H-6′), 5.994 (1H, dd, $J = 9.8$, 2.8 Hz, H-7′), 4.406 (1H, t, H-8′), 3.016 (1H, dd, $J = 7.8$, 7.8 Hz, H-8), 2.246 (1H, m, H-9′a), 2.151 (1H, m, H-5′a), 2.147 (1H, m, H-9′b), 2.091 (1H, m, H-4′a), 1.990 (1H, m, H-5′b), 1.790 (1H, dddd, $J = 14.1$, 10.2, 7.8, 5.4, H-9a), 1.634 (1H, ddd, $J = 12.4$, 3.7, 2.0, H-4′b), 1.510 (1H, dddd, $J = 18.0$, 10.2, 7.8, 5.4, H-9b), 1.399 (1H, m, H-10′a), 1.256 (1H, m, H-10′b), 1.220 (1H, m, H-10a), 1.010 (1H, m, H-10b), 0.885 (3H, t, $J = 7.4$, H-11′), 0.776 (3H, t, $J = 7.4$, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9 (C-1′), 168.5 (C-1), 148.2 (C-3′), 146.8 (C-3a), 134.0 (C-5), 131.6 (C-6′), 130.2 (C-6), 126.8 (C-7a), 126.4 (C-7), 124.8 (C-7′), 123.3 (C-4), 106.5 (C-8′), 88.5 (C-3), 54.2 (C-3′a), 53.0 (C-8), 48.6 (C-7′a), 27.7 (C-9), 27.4 (C-9′), 23.3 (C-4′), 22.8 (C-10′), 21.2 (C-5′), 20.6 (C-10), 14.1 (C-11), 13.8 (C-11′); HRESIMS $[M + H]^+$ 379.1909 *m*/*z* calcd for $C_{24}H_{27}O_4$ (3.3 ppm); ESIMSMS product ions *m*/*z* (% base peak) 191 (100) 189 (16).

Ansaspirolide (4): colorless oil; $[ga]_D^{20}$ +3 (*c* 0.1, CH_2Cl_2); UV (LC-PDA) λ_{max} 277 nm; IR λ_{max} (CH_2Cl_2) 2922, 1772, 1700, 1466, 1270, 1079, 1021 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.886 (1H, d, $J = 7.5$ Hz, H-7), 7.760 (1H, t, $J = 7.5$ Hz, H-5), 7.608 (1H, t, $J = 7.5$ Hz, H-6), 7.535 (1H, d, $J = 7.5$ Hz, H-4), 7.469 (1H, d, $J = 6.6$, H-7′), 3.246 (1H, dd, $J = 6.6$, 1.3 Hz, H-6′), 3.214 (1H, $J = 8.6$, 6.8 Hz, H-8′), 2.261 (1H, dt, $J = 6.9$, 1.3 Hz, H-8), 2.218 (1H, m, H-4′a), 2.045 (1H, m, H-9′a), 1.904 (1H, m, H-5′a), 1.739 (1H, m, H-9′b), 1.560 (1H, m, H-5′b), 1.503 (1H, m, H-4′b), 1.206 (1H, m, H-10b), 1.176 (2H, m, H-9), 1.015 (1H, m, H-10′a), 1.085 (1H, m, H-10′b), 1.002 (1H, m, H-10a), 0.736 (3H, t, $J = 7.0$, H-11), 0.679 (3H, t, $J = 7.3$, H-11′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.9 (C-1), 163.9 (C-1′), 149.4 (C-3a), 147.8 (C-3′), 140.7 (C-7′), 133.9 (C-7′a), 133.9 (C-5), 129.8 (C-7a), 129.8 (C-6), 125.7 (C-7), 122.1 (C-4), 107.4 (C-8′), 90.7 (C-3), 52.0 (C-3′a), 52.0 (C-8), 38.3 (C-6′), 32.6 (C-9), 26.9 (C-9′), 25.4 (C-4′), 25.1 (C-5′), 22.3 (C-10′), 21.4 (C-10), 14.0 (C-11), 13.5 (C-11′); HRESIMS $[M + H]^+$ 379.1923 *m*/*z* calcd for $C_{24}H_{26}O_4$ (3.6 ppm); ESIMSMS product ions *m*/*z* (% base peak) 189 (100) 191 (83).

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