Reviews

The Structural Diversity of Phthalides from the Apiaceae

John J. Beck*,† and Shen-Chieh Chou‡

United States Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, California 94710, and School of Pharmacy, University of Colorado Health Sciences Center, Denver, Colorado 80262

Received November 8, 2006

Phthalides, and their corresponding dihydro and tetrahydro analogues, are components of several genera of the plant family Apiaceae. These taxa have been reported as exhibiting a wide range of bioactivities against experimental models of several illnesses and physiological conditions, including microbial and viral infections, stroke, tuberculosis, and vasoconstriction. Many of these genera are purported to possess medicinal values, and of these several are considered to be traditional herbal medicines. This review provides an overview of the methods of investigation, the structural diversity, and the bioactivity of phthalides, dihydrophthalides, tetrahydrophthalides, and dimers from plants in the Apiaceae.

Introduction

Phthalides, and their corresponding dihydro, tetrahydro, and dimer analogues, are found as constituents of several genera within the family Apiaceae. Several of these plants have been reported to possess a variety of ethnobotanical applications. For instance, *Ligusticum* species are used among certain Hispanic cultures for the treatment of bronchitis, diarrhea, pneumonia, or colds.[1−3] In the Native American culture, *Ligusticum* and *Lomatium* species are employed for a multitude of other illnesses such as viral infections and tuberculosis.[4] In addition to the above ethnobotanical uses, a number of Asian cultures also have a rich history of species from *Angelica* and *Ligusticum* being used as traditional medicines for treatment of blood vessel diseases,[5] atherosclerosis,[6] anemia,[7] and stroke.[8] Beyond the ethnobotanical uses of some genera of the Apiaceae, the genus *Apium* is utilized for agricultural purposes. It has been reported that phthalides are the source of the "strong characteristic odor of celery".[9]

To date, 71 phthalides, including dihydro, tetrahydro, and hexahydro derivatives, as well as associated dimers, have been isolated from and/or implicated as being in 40 species of plants within the family Apiaceae and four plants from other families. The present review provides an overview of the methods of investigation, the structural diversity, and the bioactivity of phthalides, dihydrophthalides, tetrahydrophthalides, and dimers from plants in the Apiaceae. Included in this review are brief discussions on the biosynthesis, synthesis, and reactivity of phthalides, as well as phthalides from other plant families. The literature discussed is derived from readily accessible papers spanning the early 1960s to the end of 2006. This topic has not been the subject of review previously.

Methods of Investigation

The following sections provide succinct summaries of the methods employed for the extraction, isolation, and analytical techniques for the characterization and dereplication of known compounds. The citations provided are not inclusive and are meant only to serve as a representative reference. A more detailed dialogue of isolated compounds is provided in the Structural Diversity of Naturally Occurring Phthalide Derivatives section.

Extraction. With the majority of the phthalides discussed being relatively nonpolar, many of the extraction techniques have centered on the use of hexanes,[10,11] pentane,[12] or petroleum ether[13] as the initial extraction solvent. Additionally, many phthalides are components of essential oils of their plant of origin, in which case steam distillation has been regularly employed.[9,14] This latter method appears to have been the method of choice in the earlier decades covered by this review. For the more polar compounds, such as the diols or polyols, rhizomes or roots have been defatted and then extracted with chloroform,[15] or extracted with water followed by further removal with an organic solvent,[16] or extracted with methanol.[17,18]

These methods of extraction have remained widely utilized into the present decade with some small variations in the solvent type or makeup depending on the particular compounds it was desired to extract. The use of continuous extraction was originally noted in the literature in 1963 (with reference to use in the 1940s)[9] and was not noted again until the early 1980s during extraction of the first reported phthalide dimer.[19] The early to mid-2000s saw the refinement in extraction methods with the use of supercritical CO_2[20] or biomembranes[21] utilized for extraction of phthalides, and pressurized liquid extraction employed for their quantification.[22,23] The optimization of phthalide extraction procedures was also reported.[24]

Isolation. In many of the early investigations crude organic extracts were subjected to a series of basic aqueous partitions to remove the relevant acids and phenolics.[9,10] The resultant organic layer, termed the "neutral oil", was then subjected to distillation, and the various boiling point fractions were collected to yield the phthalides.[10,25] It was via this method that the isolation and identification of the chief phthalides included in this review were reported. It should be noted that some phthalides had been detected prior to these 1960 papers. References for these earlier findings are contained within the papers cited above.

Column chromatography is the benchmark method for the isolation of natural products and is prevalent throughout the literature on phthalides, with silica gel being the most widely used adsorbent.[10,26] Other separation media used for column chromatography of phthalides have included alumina,[19] LH-20,[27] polyamide,[28] and reversed-phase (C_{18}) silica gel.[29]

* To whom correspondence should be addressed. Tel: (510) 559-6154. Fax: (510) 559-6493. E-mail: jbeck@pw.usda.gov.
† U.S. Department of Agriculture.
‡ University of Colorado.

10.1021/np0605586 CCC: $37.00
Published on Web 05/04/2007

High-performance liquid chromatography (HPLC) is also a common method used for the isolation of phthalides, utilizing both normal-phase[30] and reversed-phase columns.[31] During the 1980s the use of HPLC for isolation of compounds became widespread and may have even been responsible for the increase of phthalide diversity reported in this decade.[16] Other methods that were utilized in the isolation process were medium-pressure liquid chromatography (MPLC),[32] vacuum-liquid chromatography (VLC),[33] preparative TLC (PTLC),[34] high-vacuum low-temperature distillation,[35] centrifugal circular TLC (CCTLC),[28] high-speed countercurrent chromatography (HSCCC),[36] and droplet-countercurrent chromatography (DCCC).[31]

Characterization. Until the advent and availability of modern analytical instrumentation, researchers in the early 1960s relied upon methods for the characterization of phthalides that included determination of melting points and mixed melting points[10] and boiling points[37] and chemical transformations that consisted of hydrolysis,[10] saponification,[10] hydrogenation,[38] derivatization,[37] ozonolysis,[37,38] and oxidation.[37] These methods were supplemented with data from infrared (IR) and ultraviolet (UV) spectrophotometry,[9,10,38] optical rotations,[10] refractive indexes,[10,25] gas chromatography (GC),[9] elemental analysis,[10,37] and mass spectrometric fragmentation patterns.[9] The use of nuclear magnetic resonance (NMR) spectrometry as a method of phthalide characterization was introduced in the 1960s.[37−39] Thin-layer chromatography (TLC) for phthalide detection was not reported in the literature until the late 1960s.[39]

Phthalide research in the 1970s witnessed the decline of chemical transformations for characterization and relied more upon data obtained from optical rotation, UV and NMR spectroscopic, and TLC methods.[14] The use of high-resolution mass spectrometry[14] and GC coupled to a mass selective detector started to become more evident during this period.[40−42] Interestingly, despite the availability of more sophisticated instrumentation, the 1970s did not lead to a large number of new compounds isolated and characterized from Apiaceae, with only one new phthalide being reported in the literature during this decade.[14] However, this downturn of new compounds was soon superseded during the 1980s by an increase in both the quantity and complexity of phthalides being isolated and characterized. There was a noticeable change in the format of how new compounds were being reported, with many papers being devoted to the assignment of proton and carbon NMR chemical shifts.[15,19] Use of X-ray crystallographic analysis was also reported for phthalides for the first time.[19] Other methods such as IR, UV, and GC-MS remained standard, but were no longer the principal characterization techniques.[43] This trend continued into the 1990s and 2000s with increases in the use of both two-dimensional NMR techniques and X-ray crystallographic analysis to facilitate structure elucidation.[26,44]

Dereplication. By the late 1980s and the early 1990s a large proportion of the currently known phthalides had been isolated and many papers began to focus on rapid analysis of compounds in plant material and medicinal herbal products with the goal of dereplicating known phthalides. The use of GC-MS[45] and HPLC[46] became prominent methods of ascertaining and standardizing phthalide content and did not require the isolation of pure compounds for NMR analysis. Another factor that added to this situation was the need to apply quality control to traditional Asian medicines that were becoming more prominent in the Western scientific literature.[8,47] Other areas of study that necessitated simple, rapid, and sensitive dereplication capabilities were investigations of chemical differences in plant anatomy[45] and geographical variations[48] or seasonal changes of plant metabolites.[49] By the early 2000s, the idea of dereplicating numerous phthalides at one time had become routine[50] and volatile phthalide components were being analyzed by LC-MS[51] and solid-phase microextraction (SPME).[52]

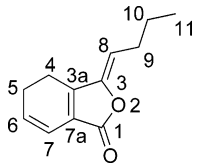

Figure 1. A common phthalide, (*Z*)-ligustilide (**20**), and its numbering.

Structural Diversity of Naturally Occurring Phthalide Derivatives

Figure 1 illustrates the most commonly accepted numbering system for the phthalides discussed herein. Figures 2−5 provide the structures and the relative configurations, where known, of the phthalides isolated during the period of this review, with the compounds in each figure listed in order of relative complexity. Figure 2 shows all true phthalides (aromatic rings), Figure 3 illustrates the structures of dihydrophthalides and the only hexahydrophthalide compound, Figure 4 shows tetrahydrophthalides, and Figure 5 presents the dimers from associated phthalides. Table S1 (Supporting Information) lists other phthalides that have been noted as possessing synonymous names and usage within the literature. Table S2 (Supporting Information) is a list of the 71 compounds isolated and/or discussed, the source plants from which they were isolated, and the literature citations in which they were discussed.

Examination of Table S2 (Supporting Information) supplies the reader with the plants most commonly investigated for phthalides and, through references, furnishes the decade of interest for that particular genus. For example, *Ligusticum officinale* (Makino) Kitag., synonymous with *Cnidium officinale* Makino,[17] and *Apium graveolens* L. var. *dulce* (P. Mill.) DC were heavily investigated in earlier decades and continue with fewer reports more recently. In contrast, *Ligusticum chuanxiong* Hort. was almost exclusively examined in the 1990s and 2000s.

Table S2 (Supporting Information) also illustrates compounds that have attracted the most attention in the literature. For example, (*Z*)-3-butylidenephthalide (**1**) and (*Z*)-ligustilide (**20**), the two main isolates from plants in the Apiaceae, are referenced 74 and 112 times, respectively. During the 1960s and 1970s several common phthalides (**1**, **5**, **20**, **25**, and **33**−**37**) were isolated from various plants. The following provides a discussion of the common phthalides isolated, as well as some of the problems encountered with structure elucidation and nomenclature during this time frame.

Common Phthalide Natural Products. The earliest paper of this review reports on the isolation of **1** and **20** (Figure 1) from *Angelica acutiloba* Kitagawa, with the latter being a newly reported isolate.[10] The correct structure for **20** was put forward by Mitsuhashi and co-workers over the course of two other reports,[38,53] with the problem being the location of the diene moiety in the dihydrophthalide ring. The final structure was confirmed after reaction of **20** with maleic anhydride and the use of NMR to assist in structure elucidation.[38]

An investigation by Mitsuhashi and co-workers[54] in the early 1960s described the phthalide content of the Apiaceae genera *L. acutiloba* var. *sugiyamae* Hikino, *L. acutilobum* Siebold & Zucc., *L. officinale*, *A. graveolens*, and *Levisticum officinale* Koch. The study illustrated the variation of phthalide content between different species and genera. The phthalides isolated from the various plants included **1**, 3-butylphthalide (**5**), **20**, (*Z*)-6,7-dihydroligustilide (**34**), neocnidilide (**35**), and cnidilide (**37**). Table S2 (Supporting Information) provides the specific constituent makeup for each plant.

During this time two other research groups investigated phthalides from celery stalks and celery seed oil, respectively.[9,25] Gold and Wilson[9] provided evidence that 3-alkyl and/or 3-alkylidenephthalide structures were responsible for the characteristic smell of celery (*A. graveolens*) and went on to describe the isolation of (*Z*)-isobutylidenephthalide (**4**), (*Z*)-isovalidenephthalide (**6**), (*Z*)-isobu-

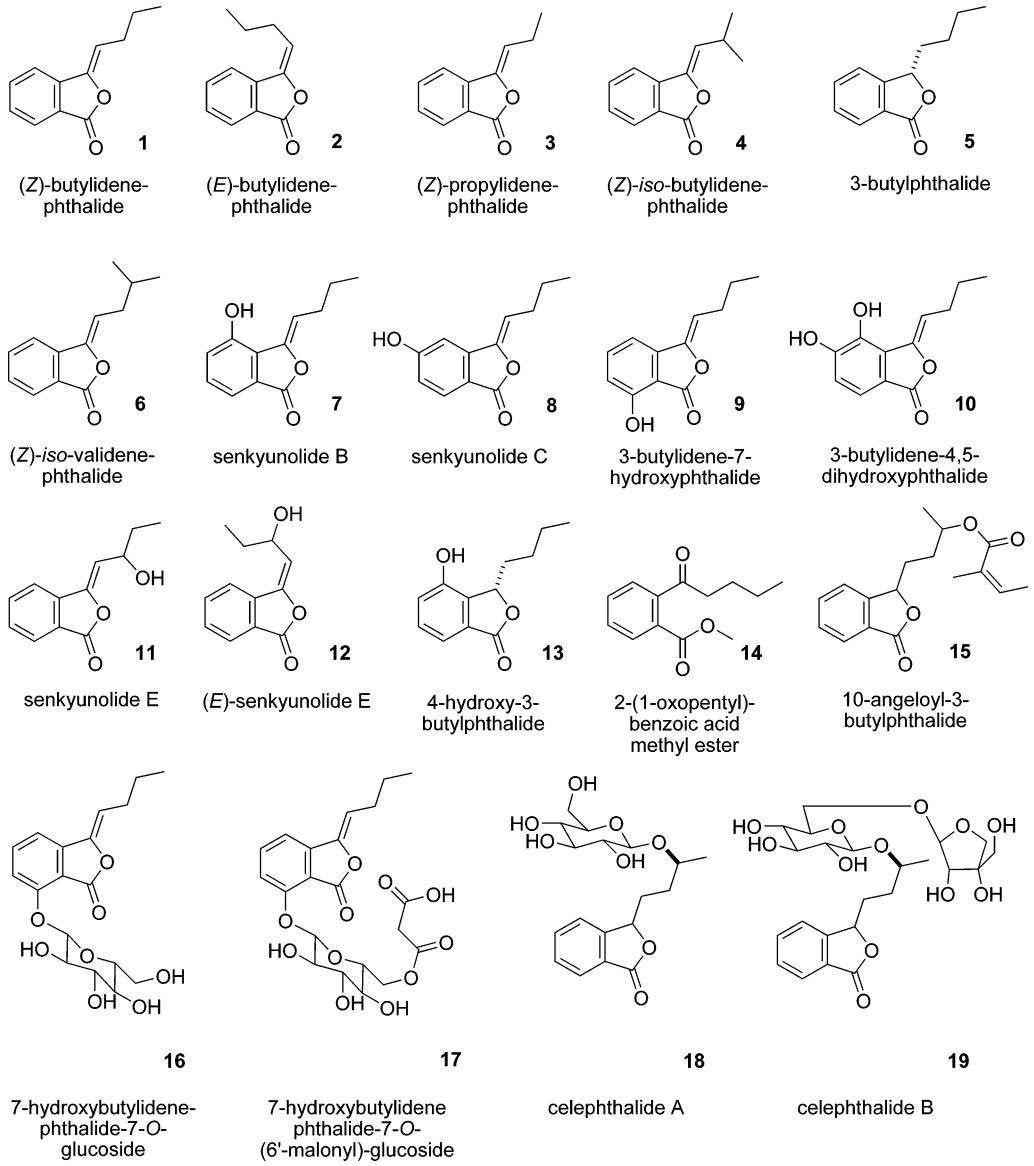

Figure 2. Phthalides **1**−**13** and **15**−**19** and one biogenetic precursor (**14**) from Apiaceae.

tylidene-3a,4-dihydrophthalide (**22**), and (*Z*)-isovalidene-3a,4-dihydrophthalide (**24**). The diene location of the two 3a,4-dihydrophthalide compounds was clarified in a later report by the same author who reinvestigated this plant.[55] Compound **22** was reported as having been detected via GC-MS from *L. officinal*[56] and a second time from *A. graveolens*,[41,57] although its definitive structure was questioned in the report on *L. officinale*. Compounds **6** and **24** were seen only in the earlier report from Gold and Wilson.[9] Moreover, despite a report that compound **4** was detected again in 2004, the actual compound was not isolated and confirmed by other structure elucidation methods. Because of the limited data for **4**, **6**, **22**, and **24**, more evidence might be required to establish the occurrence of these structures.

Characterization of some of the earlier phthalides proved somewhat difficult. For example, elucidation of the structure sedanolide (**36**) attracted considerable attention from Barton and de Vries.[25] In 1963 they considered a series of hydrolyses and reductions on fractions from *A. graveolens* since sedanolide was originally thought to have the 5,6,7,7a-tetrahydrophthalide skeleton. Chemical transformations were also performed on sedanolide by Mitsuhashi and co-workers, who investigated phthalides from the roots of *L. officinale*.[37] In 1985 Gijbels and co-workers[58] detected sedanolide from *A. graveolens*, then measured proton and carbon NMR data on sedanolide and several other phthalides in 1987.[43] The isomers of sedanolide (the *cis*- and *trans*-isomers, **36** and **35**, respectively) were also given the generic name neocnidilide.[54] By the early 1990s *cis*-neocnidilide and isocnidilide (**36**) appear to have become the accepted names for sedanolide. Interestingly, at that same time one report illustrated the wrong isomers for several phthalides, including the cnidilides, from celery volatiles.[35] Similar uncertainties were encountered in reports on the analogous structures of **35** and **37**.[37,59,60] A noteworthy feature of **37** is that it is the only mono- or diene phthalide with the alkene *not* in conjugation with the lactone carbonyl. Table S1 (Supporting Information) provides a complete list of phthalides that possess synonyms in the literature.[61]

In the 1970s, *A. graveolens* was investigated by three separate groups. Wilson[55] reported the known phthalide **5**, 3-butylhexahydrophthalide (**33**), and **36** by GC analysis of the essential oils. Work on the celery essential oil by Bjeldanes and Kim[14] confirmed the presence of **5** and reported the isolation of the new compound sedanenolide (**25**), which would eventually be named senkyunolide. Finally, investigation by Fehr[41,57] provided two reports on *A. graveolens* that utilized GC-MS to detect compounds **1**, **5**, **20**, and the ambiguous **22**.

Phthalides from Common Plants in the Apiaceae. With many of the major phthalides isolated previously discussed, attention may be focused on the major plants of the Apiaceae that have afforded

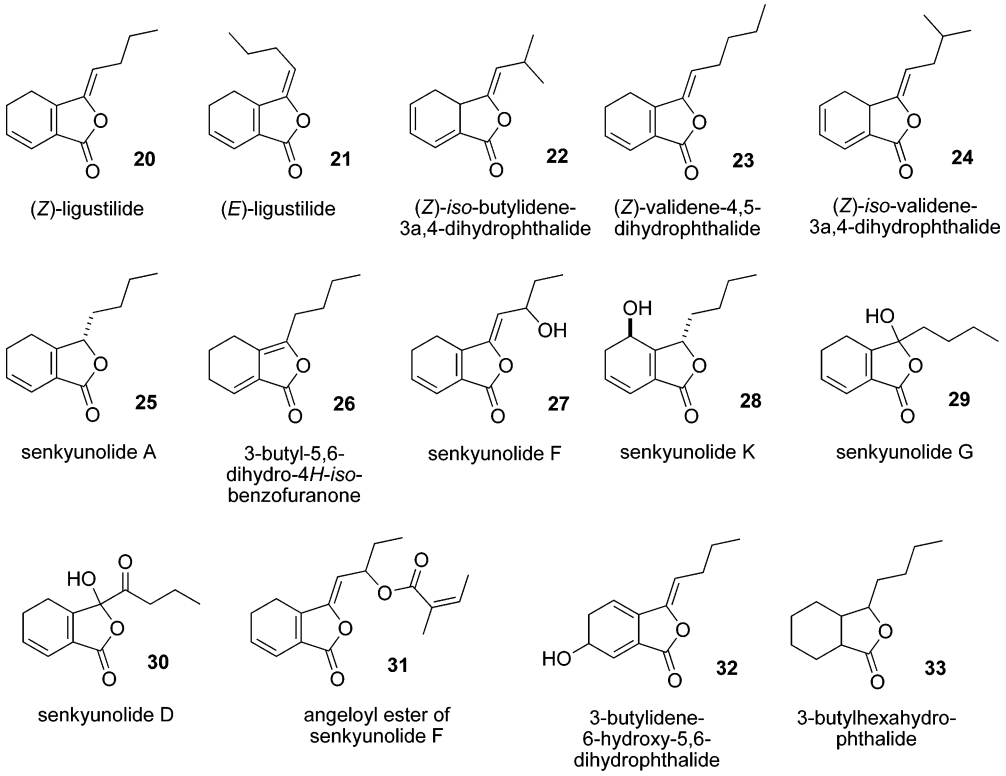

Figure 3. Dihydrophthalides **20**−**32** and the only hexahydrophthalide (**33**) from Apiaceae.

the greatest number of phthalides and therefore are the most referenced. These plants are common medicinal herbs and have yielded an unusually large number of phthalides. They are *Angelica sinensis* (Oliv.) Diels (28 phthalides isolated), *L. chuanxiong* (40 isolated), *L. officinale* (20 isolated), and *Ligusticum wallichii* Franch. (27 isolated). For all of these plants, the primary phthalides **1**, **5**, **20**, and **25** have been isolated and/or detected, and of the four plants, *L. officinale* has been investigated over the longest time period.

L. officinale is a medicinal herb commonly known as "Senkyu".[37] This species was investigated in the early 1960s due to its high yield of ligustilide.[38] A later study by Mitsuhashi and co-workers[17] revealed *L. officinale* to be among the first plants to yield the hydroxylated phthalide derivatives senkyunolide B (**7**), senkyunolide C (**8**), senkyunolide E (**11**), senkyunolide F (**27**), senkyunolide G (**29**), senkyunolide D (**30**), senkyunolide H (**39**), senkyunolide I (**43**), and senkyunolide J (**46**). In this report, compound **7** was originally thought to be 3-butylidene-7-hydroxyphthalide (**9**).[17] It was later renamed[62] senkyunolide B after a misinterpretation in NMR NOE signals was corrected and reassignment of the hydroxyl group from C-7 to the C-4 position was completed. Another interesting feature of this report was the agreement between two research groups to rename senkyunolide (**25**) to senkyunolide A so the subsequent senkyunolides B to J could be documented.[17] Other phthalides were isolated from *L. officinale* by Chan and co-workers,[63] namely, (*E*)-3-butylidenephthalide (**2**), (*E*)-ligustilide (**21**), and the dimer levistolide A (**64**).

L. wallichii, commonly known as the Chinese medicinal herb "Chuan-Xiong",[16] has afforded the next highest number of phthalides. Literature reports for phthalides from *L. wallichii* began in the late 1970s and early 1980s with reports of the isolation of the major phthalides **1**, **5**, and **20**. In the early 1980s Kaouadji and co-workers[28,64−67] investigated *L. wallichii* and reported the isolation of phthalides **8**, 3-butylidene-4,5-dihydroxyphthalide (**10**), (*Z*)-6,7-epoxyligustilide (**38**), ligustilidiol (**41**), and the dimers riligustilide (**60**), **64**, and (3′*R*)-*Z*-3′,8′-dihydro-6,6′,7,3a′-diligustilide (**67**) in addition to **1**, **2**, and **20**. During the same time, Fukuyama and co-workers[16] investigated *L. wallichii* and reported the isolation of phthalides **1**, **5**, **9**, **20**, **25**, **29**, **35**, **43**, *cis*-6,7-dihydroxyligustilide (**44**), and the dimer wallichilide (**69**). The report of compound **9** by this group appeared to be the correct structure for 3-butylidene-7-hydroxyphthalide. It should be noted that several research groups were actively investigating this and other Apiaceae plants during this time period and isolating similar compounds.[15−17,28,67,68] Finally, Mitsuhashi and co-workers investigated *L. wallichii* in the late 1980s and reported additional hydroxylated phthalides isolated from this medicinal herb.[68] Compounds included were **7**, **8**, **11**, **25**, **27**, senkyunolide K (**28**), **29**, **30**, **39**, **43**, **46**, senkyunolide M (**50**), senkyunolide L (**51**), and **64**. Compound **28** is the first and only dihydrophthalide reported to be hydroxylated on the six-membered ring, while compound **50** is the first phthalide to contain an alkanone side chain on the six-membered ring. Compound **51** was believed to be the result of residual HCl in the chloroform reacting with the phthalide epoxide **38**. Compound **51** was previously reported to be present in *L. wallichii*, but is now known not to be a naturally occurring phthalide.[28]

L. chuanxiong, also known as "Tousenkyu" and "Chaung-Xiong", has been purported to be among the most important of traditional Chinese medicines and is the plant with the largest number of reported phthalides isolated and identified.[69] Mitsuhashi, Naito, and co-workers[44,47,69,70] investigated *L. chuanxiong* from the early to mid 1990s and in four papers described the isolation of 30 phthalides, several of which were reported for the first time. They were **1**, **5**, **7**, **8**, **9**, **11**, (*E*)-senkyunolide E (**12**), 2-(1-oxopentyl)-benzoic acid methyl ester (**14**), **20**, **25**, **27**, **29**, **30**, **35**, **37**, **39**, **43**, senkyunolide N (**45**), **46**, senkyunolide Q (**49**), **50**, **51**, senkyunolide R (**52**), senkyunolide S (**53**), **56**, **60**, **61**, **64**, senkyunolide O (**65**), and senkyunolide P (**66**). Although compound **14** is not a true phthalide, its 4,5-dihydro analogue is a building block for dimer **69** and is thus implicated as a possible biosynthetic metabolite. Compounds **52** and **53** marked the first phthalide triols to be isolated from an Apiaceae plant. The phthalide content of *L. chuanxiong* was subsequently investigated by several other researchers, and in 2006, two separate groups each discovered new phthalides. Ding and co-workers[26] isolated chuanxiongnolide A (**70**), chuanxiongnolide B (**71**), 4-hydroxy-3-butylphthalide (**13**), and 3,6,7-trihy-

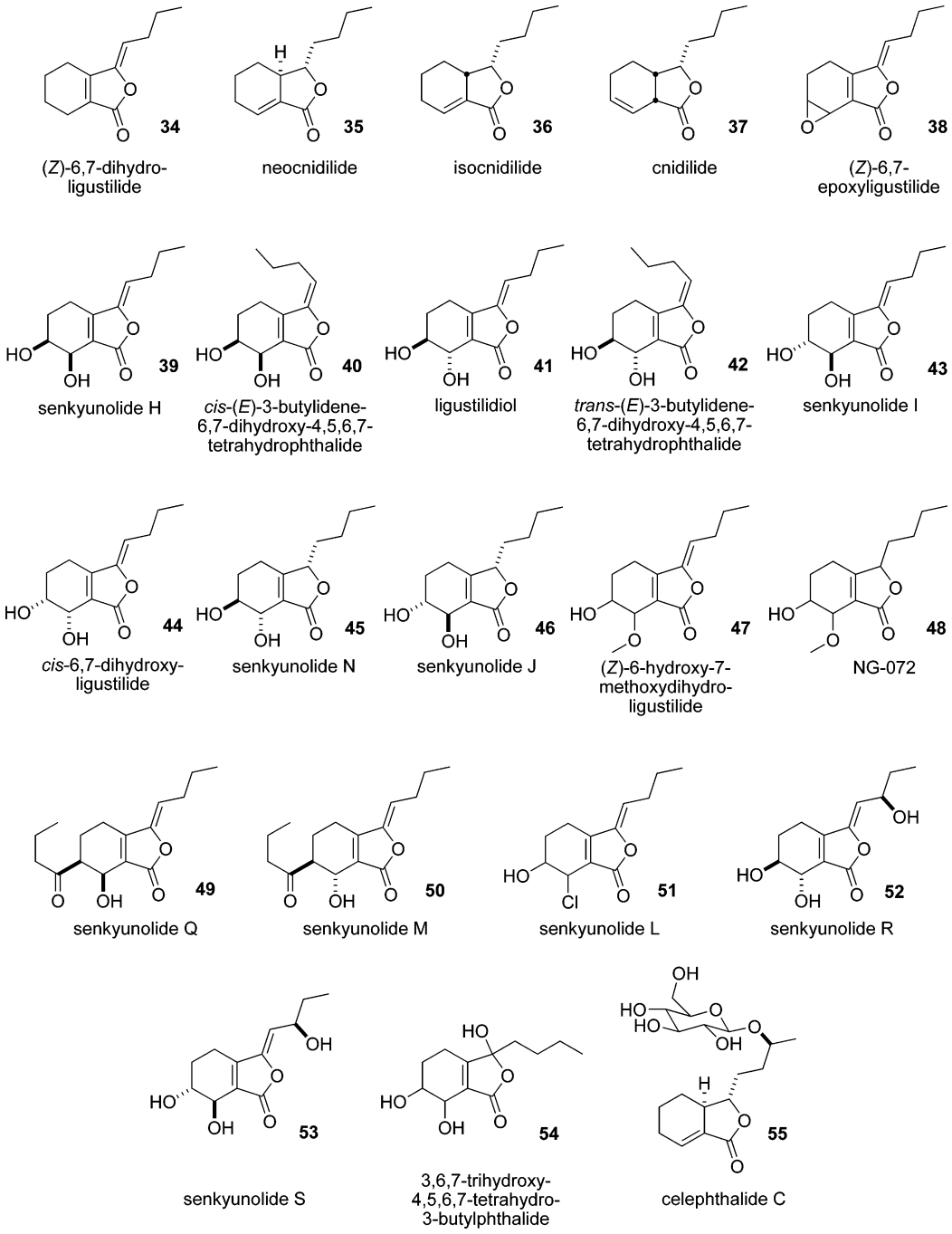

Figure 4. Tetrahydrophthalides **34**−**55** from Apiaceae.

droxy-4,5,6,7-tetrahydro-3-butylphthalide (**54**)[71] along with nine known phthalides. An interesting feature of the two dimers **70** and **71** was that they both required the racemic form of a new compound, 3-butylidene-6-hydroxy-5,6-dihydrophthalide (**32**), a phthalide that has not yet been isolated. Meanwhile, while performing bioassay-guided fractionation Yong and co-workers[32] isolated ligustilide along with the five corresponding dimers **56**, ansaspirolide (**58**), **60**, **64**, and 3,8-dihydro-diligustilide (**68**). Dimer **58** was isolated simultaneously from *A. sinensis*.[31] The *E*-isomer compounds **2** and **21**, along with several other phthalides, were detected in *L. chuanxiong* by HPLC analysis.[63]

A. sinensis, used widely as a traditional Chinese medicine and also known as "Danggui",[72] was included in the Western literature in 1990 with the isolation of the dimer E-232 (**57**). It was not until the late 1990s that more information on phthalides from this species began to appear. In 1998, Lin and co-workers[51] reported several phthalides detected by HPLC electrospray ionization mass spectrometry, in addition to the isolation of a number of phthalides. Included were **1**, **5**, **7**−**9**, **11**, **14**, **20**, **21**, **25**, **27**−**30**, **35**, **38**, **39**, (*Z*)-6-hydroxy-7-methoxy-6,7-dihydroligustilide (**48**), **57**, angelicolide (**61**), and **64**. However, the authors showed the structure for ligustilidiol (**41**) but named the structure as senkyunolide I (**43**). Also, they provided only a generic structure for either the *cis*- or *trans*-(*E*)-3-butylidene-6,7-dihydroxy-4,5,6,7-tetrahydrophthalide (**40** or **42**). Last, they reported a new compound (angelicide) as being an isomer of riligustilide (**60**) but provided no stereochemistry. In 2006, Pauli and co-workers[31] performed a bioactivity-guided investigation to yield the new phthalides 10-angeloyl-3-butylphthalide (**15**) and sinaspirolide (**62**) along with compounds **1**, **15**, **20**, **43**, **47**, and **58**. Last, Chan and co-workers[73] detected **2** and several other known compounds from *A. sinensis* while quantifying the constituents of a number of medicinal herbs.

Phthalides from Other Plants in the Apiaceae. Of the 71 phthalides isolated and/or discussed in the literature over a 46-

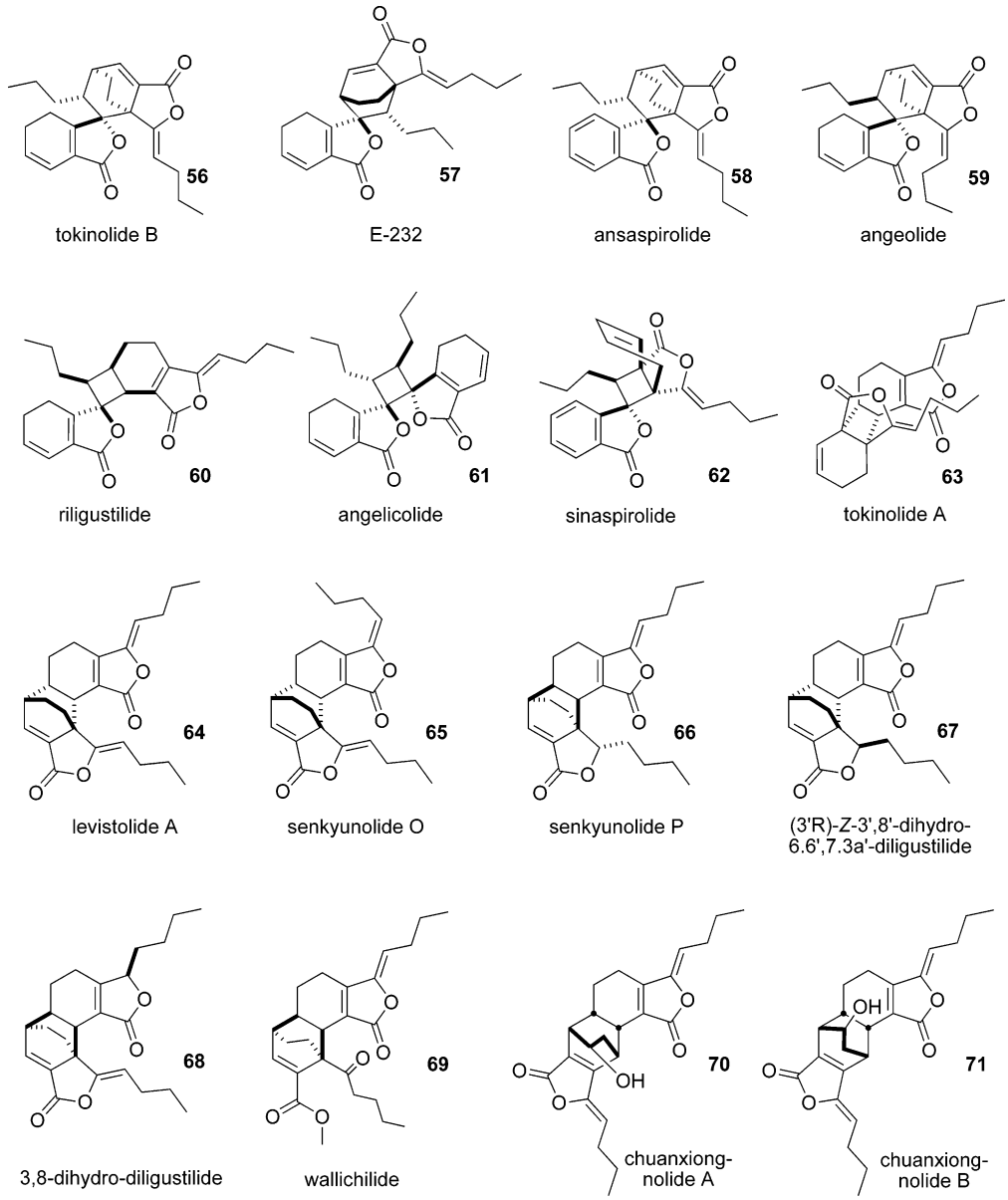

Figure 5. Dimers **56**−**71** from Apiaceae.

year period, all but 13 have been accounted for above. This section covers the isolation and/or discussion of these remaining phthalides.

In the early 1980s, Gijbels and co-workers[74−76] performed a series of investigations on *L. officinale* and published three reports. In addition to some major phthalides typically seen in other Apiaceae, the authors assigned tentatively two GC peaks to 3-propylideneph-thalide (**3**) and validene-4,5-dihydrophthalide (**23**), on the basis of their MS fragmentation patterns.[75] The validity of these compound identifications may be suspect, as they were never characterized beyond their MS fragmentation patterns.

In the early and late 1990s, the plant *Petroselinum crispum* (Mill.) Nyman was investigated by two different researchers, each isolating dissimilar phthalides. Nitz and co-workers[36] isolated 3-butyl-5,6-dihydro-4*H*-isobenzofuranone (**26**) in addition to several other known phthalides. The authors employed HSCCC to isolate this apparently unstable compound. In the second investigation, undertaken by Strack and co-workers[18] in 1999, cell suspension cultures of *P. crispum* were extracted and the compounds 7-hydroxybutylidenephthalide-7-*O*-glucoside (**16**) and 7-hydroxybutyl-idenephthalide-7-*O*-(6′-malonylglucoside) (**17**) obtained. In addition to these phthalides, compounds **8** and **9** were also isolated and characterized.

The genus *Angelica* has been found to produce some unusual phthalides. In 1982, Banerjee and co-workers[19] isolated and characterized by NMR and X-ray analysis the dimer angeolide (**59**) from *Angelica glauca* Edgeworth. Additionally, dimer **61** was isolated in the 1982 study, but was not fully characterized until 1984 by the same group.[77] Several more interesting phthalides were isolated in 1987 when Mitsuhashi and co-workers[78] investigated *A. acutiloba* and reported the new compounds senkyunolide F angeloyl ester (**31**), tokinolide B (**56**), and tokinolide A (**63**), as well as the known phthalides **11**, **27**, **39**, **43**, and **64**. The isolation of **31** would eventually corroborate the discovery of the second phthalide containing an angeloyl ester, **15**.[31]

Despite having been investigated carefully in the 1980s, *A. graveolens* was again studied in 2003, and three new phthalides were isolated and characterized, namely, celephthalides A (**18**), B (**19**), and C (**55**).[79] Compounds **18** and **19** were congruent with the structure of **15**, and compound **55** was the only nonaromatic phthalide to have a hydroxyl group at the C-10 position. Moreover, the skeleton of compound **55** closely resembled that of neocnidilide (**35**). Last, the phthalide NG-072 (**48**) was isolated from *A. graveolens* in 1991 and included in a patent filing by Maruhashi and co-workers.[80] Compound **48** was found to be the first of two

phthalides that contain a methoxy group at the C-7 position (cf. compound **47**). The remaining references for isolation or discussion of phthalides are included in Table S2 (Supporting Information).

Phthalides from Families Other than Apiaceae. It was noted that there are four plants not in the Apiaceae that contain at least one phthalide and are thus deserving of mention in this review. None of these plants are from similar families or from similar orders. All but one plant, *Acorus calamus* var. *angustatus* Bess (Araceae, class Liliopsida), are from the same class of Magnoliopsida.

A. calamus was analyzed by SPME GC-MS, and compounds **4** and **20** were reported in addition to two unidentified phthalides.[81] *Geum montanum* L. (Rosaceae) was investigated and compound **20** was identified as one of the major components detected by GC-MS.[82] *Perilla frutescens* var. *acuta* Kudo (Lamiaceae) was reported to contain compounds **4**, **20**, and **25** after analysis by GC-MS and GC aroma extract dilution analysis.[83] Last, compounds **40** and **42** were isolated from *Polygonum multiflorum* Thunb. (Polygonaceae).[29]

Biosynthesis of the Phthalides. There are few reports in the cited literature that specifically discuss the biosynthesis of the phthalides. In the 1960s, phthalide biogenesis was thought to occur via head-to-tail linkage of acetate units.[13] Results from this work suggested that acetate units were incorporated into ligustilide (**20**). It was not until the late 1980s that the topic of biosynthesis occurred again as stating that "alkylphthalides are known to be biosynthesized from polyketide precursors".[68] This assertion appears to be validated given that phenolics such as ferulic acid have been isolated together with phthalide compounds.[33]

Synthesis of Phthalides. Phthalides have structures that are relatively simple, and a number have been synthesized. Many of the reported syntheses were either in support of structure determination or to provide increased quantities of phthalides for bioactivity testing. The following are compounds synthesized and their corresponding references: compound **1**,[84,85] **5**,[86,87] **7**,[88] **8**,[88−90] **9**,[89] **11**,[88,91−93] **20**,[94−96] **34**,[90,97,98] **35**,[99−104] **36**,[100] **37**,[100] **56**,[105] **60**,[106] **64**,[105,107] **68**,[107] and **69**.[107]

Two structure−activity relationship studies have been reported for butylidenephthalide (**1**) analogues: one with an alkylidene side chain on hydroxylated phthalide ring moieties as a series of analogues for potency against an atherosclerotic plaque experimental model[108,109] and a second that sought increased antimicrobial activity among benzylidenephthalide analogues with varying electron density as an alternative to the alkyl side chain.[110]

Reactivity of Phthalides. Despite many phthalides occurring as achiral molecules, the presence of an α,*β*,*γ*,*δ*-unsaturated lactone, cross-conjugated alkene, and 1,3-diene moieties of the dihydrophthalides has provided a multitude of reactive sites and perhaps explains the large number of biological activities attributed to (*Z*)-ligustilide (**20**), for example.

The report of dimers **56**−**71** demonstrates the thermal and photochemical reactivity of ligustilide and other phthalides. For instance, dimers **56**−**59** illustrate that the 3,8-alkene unit undergoes 4+2 cycloaddition as the dienophile with the ligustilide 1,3-diene system. In dimers **60**−**62**, the 3,8-alkene reacts with other alkenes in a photochemical 2+2 cycloaddition. Dimer **63**, however, results from 2+2 cycloaddition involving the 3a,7a-tetrasubstituted alkene with the 6,7-alkene. The cross-conjugated 6,7-alkene is seen undergoing various 4+2 cycloadditions to afford dimers **64**−**71**. Delgado and co-workers[111−114] have performed a number of valuable investigations on the reactivity of phthalide dimers under varying conditions. The same group has also reported on the correct structure of compound **67**.[111] Moreover, neat ligustilide has been shown to undergo cyclizations if kept for extended periods or exposed to sunlight.[51]

Other studies have shown the chemical diversity of ligustilide as an electrophile. In 1994, compound **20** was found to undergo facile 1,6-conjugate addition of a sulfur nucleophile and, after rearrangement of the resulting enolate, a second 1,4-addition of a second sulfur nucleophile. In the same investigation it was demonstrated that a nitrogen nucleophile added to the lactone carbonyl and subsequent rearrangement afforded a 3-hydroxy lactam.[95] It should be noted that in this particular study compounds that retained the α,*β*,*γ*,*δ*-unsaturated lactone were active against certain microbes, while the compounds that lacked this unsaturation were inactive.

Bioactivity of Phthalides

Several plants of the Apiaceae are used as medicinal herbs. For instance, *Ligusticum porteri* Coult. & Rose, commonly known as "oshá" among the Hispano Americans of south-central Colorado, has been said to be "one of the most important herbal remedies of this once culturally and geographically isolated region".[1] Specific examples of the ethnomedical uses of *L. porteri* include the rhizomes in a tea to help alleviate diarrhea and stomachaches, or to treat bronchitis, colds, diabetes, pneumonia, and tuberculosis.[2] Likewise, *A. sinensis* has been reported as "one of the most commonly used traditional Chinese medicines in China" and has been employed to treat amenorrhea, anemia, cardiovascular disease, and hepatic fibrosis.[6] Affirmation of these medicinal qualities has been the numerous reports of phthalides from these plants that exhibited bioactivities as pure isolates. However, the vast majority of the types of bioactivity reported have been attributed to just two phthalides, (*Z*)-ligustilide (**20**) and (*Z*)-butylidenephthalide (**1**).

One of the first reports on the bioactivity of the phthalides was that of **20** in possessing anticholinergic activity.[10] Subsequent to that report, ligustilide was implicated 13 additional times as a bioactive component in different assays and from a variety of plants. Ligustilide was reported as being active in asthma,[94] bacteria,[33,95,115] brine shrimp,[11] fungi,[116] inflammation,[117] ischemic stroke,[8] oxidation,[118] and spasm[94] test systems. Moreover, compound **20** was purported to exhibit insecticidal,[119,120] multidrug-resistance modulation,[115] phytotoxic,[116] smooth muscle relaxant,[94] and vasodilation[5] activities. These purported bioactivities combined with the report that ligustilide is often the major phthalide isolate[95] lend credibility to the medicinal qualities of Apiaceae herbs.

Butylidenephthalide (**1**) is the next most commonly reported bioactive phthalide in a total of 11 different assays that included activity as an acaricidal,[30] antianginal,[121,122] antihypertensive,[123] antioxidative,[118] antiplatelet,[124] antispasmodic,[42,123,125] insecticidal,[119] and vasodialator.[5,126] Additionally, compound **1** was reported to inhibit calcium-induced contractions in depolarized guinea-pig ileum smooth muscle,[127] inhibit calcium release from calcium stores in isolated rat aorta,[128] selectively activate the central cholinergic neuronal system in rats,[129] and display serotonergic activities.[31] Although compound **1** has demonstrated a wide range of bioactivities in several experimental models, its exact mode of action remains unknown.[119,126]

Common structural characteristics of several other bioactive phthalides are those that contained the 3,8-dihydro moiety. For instance, senkyunolide A (**25**) has been demonstrated to exhibit antifungal, mosquitocidal, nematicidal, and topoisomerase I and II inhibitory activity.[130] Butylphthalide (**5**), neocnidilide (**35**), and cnidilide (**37**) exhibit insecticidal properties.[120] Compound **35** was found also to be an antifungal,[99] and the dihydroxy butylphthalides senkyunolides N (**45**) and J (**46**) were found to possess nematicidal[34] and topoisomerase I and II inhibitory activities.[131] Senkyunolide G (**29**) was found to increase coronary blood flow in dogs,[16] and a phthalide containing a methoxy group at position C-7, NG-072 (**48**), was patented for treatment of Alzheimer's disease.[80]

The remaining phthalides reported to possess bioactivity all contain an alkene between carbons 3 and 8. Senkyunolide H (**39**) was proposed as a prototype for a new antiatherosclerotic treatment,[132] and the *E*-isomers **40** and **42** were reported to inhibit

calcium ATPase.[29] Two dimers have also been found to be biologically active, E-232 (**57**), which inhibits nitrenpidine binding to calcium channels,[72] and dihydro-diligustilide (**68**), which has potent progesterone-like activity.[32]

Despite the numerous reports on the bioactivities of phthalides, investigations concerning the mode of action are few. One recent report exemplifies this by stating "although *L. chuanxiong* and its extracts have centuries of documented use..., its mechanisms of action were unclear",[32] and a second recent report stated "...the role of the phthalide lactones from *L. chuanxiong* in the therapeutic actions is not yet fully understood".[117]

There are, however, phthalide characteristics that have been implicated as being responsible for certain bioactivities. The 6,7-diol moiety of compounds **39**, **40**, **42**, **43**, and **46** has been reported as being responsible for calcium ion-ATPase inhibition[29] and competence inhibition of proliferation in primary cultures of mouse aorta smooth muscle.[132] Another investigation states "the five-membered lactone ring along with the butyl side chain in phthalides may be important for observed biological activities".[34] This idea was supported by an earlier report that showed compound **1** underwent 1,2-addition with cysteine followed by subsequent ring-opening. This metabolite was then excreted as a cysteine conjugate after metabolism in the hairless mouse.[156] Finally, as discussed in the previous section, the $\alpha,\beta,\gamma,\delta$-unsaturated lactone of compound **20** was required for the observed antimicrobial activity.[95]

Conclusions

Even with the rich history of their phthalide constituents and the valuable previous investigations of many researchers, there still exist some studies that could be undertaken on plants of the Apiaceae. For example, the complete biosynthetic pathway of any singular compound in this class has not yet been fully detailed; the isolation of dimers containing phthalide monomer components that have not been isolated reveals this gap in phthalide chemistry. For example, the formation of compounds **70** and **71** would require the so-far undiscovered monomer **32** for dimer formation, while compound **69** would require the dihydro form of compound **14** in order to undergo cycloaddition. Additionally, structures such as **15**−**19** bring into question the exact biogenetic order of formation, the role of hydroxylation, and the subsequent functionalization of the hydroxyl groups. Another interesting functional group, the 1-butanone moiety, appears in compounds **49** and **50**. Phthalides are responsible for numerous bioactivities; however their exact mode of action is not yet realized, although several reports have suggested possible bioactive sites. Investigations into the exact role phthalides play in the mode of action could potentially clarify some of these bioactivities. One last potential task is the complete structural characterization of compounds such as **4**, **6**, **22**, and **24**, whose structures were assigned tentatively in the literature from GC-MS data. As a whole, the genera of the family Apiaceae have offered a group of compounds whose diversity in number and structure, in addition to their wide array of bioactivities, has greatly contributed to the ethnobotanical applications of this family of plants.

Acknowledgment. The authors would like to thank B. C. Campbell, R. J. Molyneux, W. G. Trankle, and the reviewers for their insightful contributions. J.J.B. would also like to thank F. R. Stermitz for suggesting that a review of this topic would be valuable.

Supporting Information Available: Tables of synonyms and occurrence of phthalides in specific plants. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bye, R. A.; Linares, E.; Botanico, J. *J. Ethnobiol.* **1986**, *6*, 289−306.
(2) Linares, E.; Bye, R. A. *J. Ethnopharmacol.* **1987**, *19*, 153−183.
(3) Appelt, G. D. *J. Ethnopharmacol.* **1985**, *13*, 51−55.
(4) Willard, T. *Edible and Medicinal Plants of the Rocky Mountains and Neighbouring Territories*; Wild Rose College of Natural Healing, Ltd.: Calgary, 1979; pp 154−155.
(5) Liang, M.-J.; He, L.-C.; Yang, G.-D. *Life Sci.* **2005**, *78*, 128−133.
(6) Hou, Y.-Z.; Zhao, G.-R.; Yuan, Y.-J.; Zhu, G.-G.; Hiltunen, R. *J. Ethnopharmacol.* **2005**, *100*, 140−144.
(7) Wang, Y.-L.; Liang, Y.-Z.; Chen, B.-M.; He, Y.-K.; Li, B.-Y.; Hu, Q.-N. *Anal. Bioanal. Chem.* **2005**, *383*, 247−254.
(8) Chen, K. J.; Chen, K. *Chin. Med. J.* **1992**, *105*, 870−873.
(9) Gold, H. J.; Wilson, C. W. *J. Org. Chem.* **1963**, *28*, 985−987.
(10) Mitsuhashi, H.; Nagai, U.; Muramatsu, T.; Tashiro, H. *Chem. Pharm. Bull.* **1960**, *8*, 243−245.
(11) Delgado, G.; Reza-Garduno, R. G.; Rios, M. Y.; del Rio, F. *Planta Med.* **1992**, *58*, 570−571.
(12) Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Chromatographia* **1981**, *14*, 452−454.
(13) Mitsuhashi, H.; Nomura, M. *Chem. Pharm. Bull.* **1966**, *14*, 777−778.
(14) Bjeldanes, L. F.; Kim, I.-S. *J. Org. Chem.* **1977**, *42*, 2333−2335.
(15) Kaouadji, M.; Mariotte, A.-M. *Z. Naturforsch.* **1984**, *39c*, 872−875.
(16) Pushan, W.; Xuanliang, G.; Yixiong, W.; Fukuyama, Y.; Miura, I.; Sugawara, M. *Phytochemistry* **1984**, *23*, 2033−2038.
(17) Kobayashi, M.; Fujita, M.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1984**, *32*, 3770−3773.
(18) Hagemeier, J.; Batz, O.; Schmidt, J.; Wray, V.; Hahlbrock, K.; Strack, D. *Phytochemistry* **1999**, *51*, 629−635.
(19) Banerjee, S. K.; Gupta, B. D.; Sheldrick, W. S.; Hofle, G. *Liebigs Ann. Chem.* **1982**, 699−707.
(20) Dauksas, E.; Venskutonis, P. R.; Sivik, B.; Nillson, T. *J. Supercrit. Fluids* **2002**, *22*, 201−210.
(21) Dong, Z. B.; Li, S. P.; Hong, M.; Zhu, Q. *J. Pharm. Biomed. Anal.* **2005**, *38*, 664−669.
(22) Lao, S. C.; Li, S. P.; Kan, K. K. W.; Li, P.; Wan, J. B.; Wang, Y. T.; Dong, T. T. X.; Tsim, K. W. K. *Anal. Chim. Acta* **2004**, *526*, 131−137.
(23) Li, P.; Li, S. P.; Lao, S. C.; Fu, C. M.; Kan, K. K. W.; Wang, Y. T. *J. Pharm. Biomed. Anal.* **2006**, *40*, 1073−1079.
(24) Song, Z. H.; Ji, Z. N.; Lo, C. K.; Dong, T. T. X.; Zhao, K. J.; Li, O. T. W.; Haines, C. J.; Kung, S. D.; Tsim, K. W. K. *Planta Med.* **2004**, *70*, 1222−1227.
(25) Barton, D. H. R.; de Vries, J. X. *J. Chem. Soc.* **1963**, 1916−1919.
(26) Li, Y.-H.; Peng, S.-L.; Zhou, Y.; Yu, K.-B.; Ding, L.-S. *Planta Med.* **2006**, *72*, 652−656.
(27) Kaouadji, M.; Pouget, C. *J. Nat. Prod.* **1986**, *49*, 184−185.
(28) Kaouadji, M.; De Pachtere, F.; Pouget, C.; Chulia, A. J.; Lavaitte, S. *J. Nat. Prod.* **1986**, *49*, 872−877.
(29) Grech, J. N.; Li, Q.; Roufogalis, B. D.; Duke, C. C. *J. Nat. Prod.* **1994**, *57*, 1682−1687.
(30) Kwon, J.-H.; Ahn, Y.-J. *J. Agric. Food Chem.* **2002**, *50*, 4479−4483.
(31) Deng, S.; Chen, S.-N.; Yao, P.; Nikolic, D.; van Breemen, R. B.; Bolton, J. L.; Fong, H. H. S.; Farnsworth, N. R.; Pauli, G. F. *J. Nat. Prod.* **2006**, *69*, 536−541.
(32) Lim, L. S.; Shen, P.; Gong, Y. H.; Yong, E. L. *Phytochemistry* **2006**, *67*, 728−734.
(33) Chou, S.-C.; Everngam, M. C.; Sturtz, G.; Beck, J. J. *Phytother. Res.* **2006**, *20*, 153−156.
(34) Momin, R. A.; Nair, M. G. *J. Agric. Food Chem.* **2001**, *49*, 142−145.
(35) MacLeod, G.; Ames, J. M. *Phytochemistry* **1989**, *28*, 1817−1824.
(36) Nitz, S.; Spraul, M. H.; Drawert, F. *J. Agric. Food Chem.* **1992**, *40*, 1038−1040.
(37) Mitsuhashi, H.; Muramatsu, T. *Tetrahedron* **1964**, *20*, 1971−1982.
(38) Mitsuhashi, H.; Nagai, U. *Tetrahedron* **1963**, *19*, 1277−1283.
(39) Bohrmann, H.; Stahl, E.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1967**, *15*, 1606−1608.
(40) Yamagishi, T.; Kaneshima, H. *J. Pharm. Soc. Jpn.* **1977**, *97*, 237−243.
(41) Fehr, D. *Pharmazie* **1979**, *34*, 658−662.
(42) Ko, W. C.; Lin, S. C.; Yeh, C. Y.; Want, Y. T. *J. Formosan Med. Assoc.* **1977**, *76*, 669−677.
(43) Fischer, F. C.; Gijbels, M. J. M. *Planta Med.* **1987**, *49*, 77−80.
(44) Naito, T.; Ikeya, Y.; Okada, M.; Mitsuhashi, H.; Maruno, M. *Phytochemistry* **1996**, *41*, 233−236.
(45) Beauchamp, P. S.; Bottini, A. T.; Dev, V.; Melkani, A. B.; Timbrook, J. In *Food Flavors, Ingredients and Composition*; Gharalambous, G., Ed.; Elsevier Science: Amsterdam, 1993; pp 605−610.
(46) Zschocke, S.; Liu, J-H.; Stuppner, H.; Bauer, R. *Phytochem. Anal.* **1998**, *9*, 283−290.
(47) Naito, T.; Katsuhara, T.; Niitsu, K.; Ikeya, Y.; Okada, M.; Mitsuhashi, H. *Phytochemistry* **1992**, *31*, 639−642.
(48) Thappa, R. K.; Kaul, P.; Chisti, A. M.; Kapahi, B. K.; Suri, O. P.; Agarwal, S. G. *J. Essent. Oil Res.* **2005**, *17*, 361−363.

(49) Bylaite, E.; Venskutonis, R. P.; Roozen, J. P. *J. Agric. Food Chem.* **1998**, *46*, 3735−3740.
(50) Li, S.-L.; Chan, S. S.-K.; Lin, G.; Ling, L.; Yan, R.; Chung, H.-S.; Tam, Y.-K. *Planta Med.* **2003**, *69*, 445−451.
(51) Lin, L.-Z.; He, X.-G.; Lian, L.-Z.; King, W.; Elliot, J. *J. Chromatogr. A* **1998**, *810*, 71−79.
(52) Choi, H.-S.; Min, K.-C. *Food Sci. Biotechnol.* **2003**, *12*, 409−414.
(53) Mitsuhashi, H.; Nagai, U.; Muramatsu, T. *Chem. Pharm. Bull.* **1961**, *9*, 115−119.
(54) Mitsuhashi, H.; Muramatsu, T.; Nagai, U.; Nakano, T.; Ueno, K. *Chem. Pharm. Bull.* **1963**, *11*, 1317−1319.
(55) Wilson, C. W. *J. Food Sci.* **1970**, *35*, 766−768.
(56) Fehr, D. *Planta Med. Suppl.* **1980**, *40*, 34−40.
(57) Fehr, D. *Pharmazie* **1981**, *36*, 374−376.
(58) Gijbels, M. J. M.; Fischer, F. C.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Fitoterapia* **1985**, *56*, 17−23.
(59) Nagai, U.; Mitsuhashi, H. *Tetrahedron* **1965**, *21*, 1433−1440.
(60) Nagai, U.; Shishido, T.; Chiba, R.; Mitsuhashi, H. *Tetrahedron* **1965**, *21*, 1701−1709.
(61) Beck, J. J. Investigation of the Bioactive Constituents of Several Herbal Medicines. Ph.D. Dissertation, Colorado State University, Fort Collins, CO, 1996.
(62) Kobayashi, M.; Fujita, M.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1987**, *35*, 1427−1433.
(63) Lu, G.-H.; Chan, K.; Liang, Y.-Z.; Leung, K.; Chan, C.-L.; Jiang, Z.-H.; Zhao, Z.-Z. *J. Chromatogr. A* **2005**, *1073*, 383−392.
(64) Kaouadji, M.; Puech-Baronnat, M.; Mariotte, A. M. *Plant Med. Phytother.* **1983**, *17*, 147−156.
(65) Kaouadji, M.; Puech-Baronnat, M.; Mariotte, A. M. *Tetrahedron Lett.* **1983**, *24*, 4675−4676.
(66) Kaouadji, M.; Reutenauer, H.; Chulia, A. J.; Marsura, A. *Tetrahedron Lett.* **1983**, *24*, 4677−4678.
(67) Puech-Baronnat, M.; Kaouadji, M.; Mariotte, A. M. *Planta Med.* **1984**, *50*, 105−106.
(68) Kobayashi, M.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1987**, *35*, 4789−4792.
(69) Naito, T.; Katsuhara, T.; Niitsu, K.; Ikeya, Y.; Okada, M.; Mitsuhashi, H. *Heterocycles* **1991**, *32*, 2433−2442.
(70) Naito, T.; Niitsu, K.; Ikeya, Y.; Okada, M.; Mitsuhashi, H. *Phytochemistry* **1992**, *31*, 1787−1789.
(71) Compounds **13** and **54** were reported to have been isolated in two papers that the reviewers were unable to obtain: Xiao, Y. Q.; Li, L.; You, X. L.; Gu, K. Y. Y.; Ma, C. J. *Zhongguo Zhongyao Zazhi* **2002**, *27*, 519−522. Wen, Y. S.; He, Z. R.; Xue, K. F.; Cao, F. Y. *Zhongcaoyao* **1986**, *17*, 122−126.
(72) Hon, P.-M.; Lee, C.-M.; Choang, T. F.; Chui, K.-Y.; Wong, H. N. C. *Phytochemistry* **1990**, *29*, 1189−1191.
(73) Lu, G.-H.; Chan, K.; Chan, C.-L.; Leung, K.; Jiang, Z.-H.; Zhao, Z.-Z. *J. Chromatogr. A* **2004**, *1046*, 101−107.
(74) Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Planta Med. Suppl.* **1980**, 41−47.
(75) Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Planta Med.* **1982**, *44*, 207−211.
(76) Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Pharm. Weekbl.* **1982**, *117*, 501−502.
(77) Banerjee, S. K.; Gupta, B. D.; Sheldrick, W. S.; Hofle, G. *Liebigs Ann. Chem.* **1984**, 888−893.
(78) Tsuchida, T.; Kobayashi, M.; Kaneko, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1987**, *35*, 4460−4464.
(79) Kitajima, J.; Ishikawa, T.; Satoh, M. *Phytochemistry* **2003**, *64*, 1003−1011.
(80) Maruhashi, M.; Hanada, K.; Mizogami, K.; Nagakura, A. *Jpn. Kokai Tokkyo Koho* JP 04,334,378, 1992; *Chem. Abstr.* **1993**, *118*, 240924.
(81) Choi, H.-S. *J. Agric. Food Chem.* **2004**, *52*, 8099−8104.
(82) Vollmann, C.; Schultze, W. *J. Essent. Oil Res.* **1995**, *7*, 117−121.
(83) Choi, H.-S. *Food Sci. Biotechnol.* **2004**, *13*, 279−284.
(84) Mali, R.; Jagtap, P. *J. Chem. Res.* **1993**, *5*, 184−185.
(85) Ogawa, Y.; Maruno, M.; Wakamatsu, T. *Heterocycles* **1995**, *41*, 2587−2599.
(86) Kitayama, T. *Tetrahedron: Asymmetry* **1997**, *8*, 3765−3774.
(87) Martinez, M. M.; Onega, M. G.; Tellado, M. F.; Seijas, J. A.; Vazquez-Tato, M. P. *Tetrahedron* **1997**, *53*, 14127−14130.
(88) Bellina, F.; Ciucci, D.; Vergamini, P.; Rossi, R. *Tetrahedron* **2000**, *56*, 2533−2545.
(89) Ogawa, Y.; Maruno, M.; Wakamatsu, T. *Heterocycles* **1994**, *39*, 47−50.
(90) Li, S.; Wang, Z.; Fang, X.; Yang, Y.; Li, Y. *Synth. Commun.* **1997**, *27*, 1783−1791.
(91) Li, S.; Yan, F.; Wang, Z.; Li, Y. *Ind. J. Chem.* **1994**, *33B*, 1178−1179.
(92) Wang, Z.; Li, S.; Yan, F.; Li, Y. *Synth. Commun.* **1994**, *24*, 3135−3139.
(93) Eddine, A. C.; Daich, A.; Jilale, A.; Decroix, B. *Heterocycles* **1999**, *51*, 2907−2914.
(94) Li, S.; Wang, Z.; Fang, X.; Li, Y. *Synth. Commun.* **1993**, *23*, 2909−2913.
(95) Beck, J. J.; Stermitz, F. R. *J. Nat. Prod.* **1995**, *58*, 1047−1055.
(96) Ogawa, Y.; Maruno, M.; Wakamatsu, T. *Synlett* **1995**, 871−872.
(97) Lardelli, G.; Dijkstra, G.; Harkes, P. D.; Boldingh, J. *Rec. Trav. Chim. Pays-Bas* **1966**, *85*, 43−55.
(98) Li, S.; Fang, X.; Wang, Z.; Yang, Y.; Li, Y. *Synth. Commun.* **1993**, *23*, 2051−2054.
(99) Suzuki, H.; Tanaka, A.; Yamashita, K. *Agric. Biol. Chem.* **1987**, *51*, 3369−3373.
(100) Cocker, W.; Sainsbury, D. M. *Chem. Commun*. **1965**, 479−480.
(101) Tanaka, A.; Suzuki, H.; Yamashita, K. *Agric. Biol. Chem.* **1989**, *53*, 2253−2256.
(102) McClure, C. K.; Jung, K.-Y. *J. Org. Chem.* **1991**, *56*, 2326−2332.
(103) Jiao, X.-Z.; Xie, P.; Zu, L.-S.; Liang, X.-T. *Chin. Chem. Lett.* **2003**, *14*, 127−129.
(104) Jiao, X.-Z.; Xie, P.; Zu, L.-S.; Liang, X.-T. *J. Asian Nat. Prod. Res.* **2003**, *5*, 165−169.
(105) Ogawa, Y.; Mori, Y.; Maruno, M.; Wakamatsu, T. *Heterocycles* **1997**, *45*, 1869−1872.
(106) Quiroz-Garcia, B.; Figueroa, R.; Cogordan, J. A.; Delgado, G. *Tetrahedron Lett.* **2005**, *46*, 3003−3006.
(107) Rios, M. Y.; Delgado, G.; Toscano, R. A. *Tetrahedron* **1998**, *54*, 3355−3366.
(108) Mimura, Y.; Kobayashi, S.; Naitoh, T.; Kimura, I.; Kimura, M. *Biol. Pharm. Bull*. **1995**, *18*, 1203−1206.
(109) Mimura, Y.; Kobayashi, S.; Okabe, M.; Kimura, I.; Horikoshi, I.; Kimura, M. *Biol. Pharm. Bull*. **1995**, *18*, 1660−1664.
(110) Everngam, M. C.; Baig, N.; Heimbegner, J. L.; Poore, M. L.; Beck, J. J. *J. Undergrad. Chem. Res.* **2003**, *2*, 163−166.
(111) Delgado, G.; Reza-Garduno, R. G.; Toscano, R. A.; Bye, R.; Linares, E. *Heterocycles* **1988**, *27*, 1305−1312.
(112) Rios, M. Y.; Delgado, G.; Espinosa-Perez, G. *Tetrahedron Lett.* **1998**, *39*, 6605−6608.
(113) Quiroz-Garcia, B.; Hernandez, L.; Toscano, R. A.; Sterner, O.; Delgado, G. *Tetrahedron Lett.* **2003**, *44*, 2509−2512.
(114) Quiroz-Garcia, B.; Hernandez-Ortega, S.; Sterner, O.; Delgado, G. *Tetrahedron* **2004**, *60*, 3681−3688.
(115) Cegiela-Carlioz, P.; Bessiere, J-M.; David, B.; Mariotte, A.-M.; Gibbons, S.; Dijoux-Franca, M.-G. *Flavour Fragr. J.* **2005**, *20*, 671−675.
(116) Meepagala, K. M.; Sturtz, G.; Wedge, D. E.; Schrader, K. K.; Duke, S. O. *J. Chem. Ecol.* **2005**, *31*, 1567−1578.
(117) Liu, L.; Ning, Z.-Q.; Shan, S.; Zhang, K.; Deng, T.; Lu, X.-P.; Cheng, Y.-Y. *Planta Med.* **2005**, *71*, 808−813.
(118) Ka, M.-H.; Choi, E. H.; Chun, H.-S.; Lee, K.-G. *J. Agric. Food Chem.* **2005**, *53*, 4124−4129.
(119) Miyazawa, M.; Tsukamoto, T.; Anzai, J.; Ishikawa, Y. *J. Agric. Food Chem.* **2004**, *52*, 4401−4405.
(120) Tsukamoto, T.; Ishikawa, Y.; Miyazawa, M. *J. Agric. Food Chem.* **2005**, *53*, 5549−5553.
(121) Ko, W.-C.; Sheu, J.-R.; Tzeng, S.-H.; Chen, C.-M. *Planta Med.* **1998**, *64*, 229−232.
(122) Ko, W.-C.; Liao, C.-C.; Shih, C.-H.; Lei, C.-B.; Chen, C.-M. *Planta Med.* **2002**, *68*, 1004−1009.
(123) Ko, W.-C.; Chang, L.-D.; Wang, G.-Y. *Phytother. Res.* **1994**, *8*, 321−326.
(124) Teng, C.-M.; Chen, W.-Y.; Ko, W.-C.; Ouyang, C. *Biochim. Biophys. Acta* **1987**, *924*, 375−382.
(125) Ko, W. C. *Jpn. J. Pharmacol.* **1980**, *30*, 85−91.
(126) Chan, S. S.-K.; Choi, A. O.-K.; Jones, R. L.; Lin, G. *Eur. J. Pharmacol.* **2006**, *537*, 111−117.
(127) Ko, W.-C.; Sheu, J.-R.; Leu, Y.-R.; Tzeng, S.-H.; Chen, C.-M. *J. Pharm. Pharmacol.* **1997**, *49*, 1121−1125.
(128) Ko, W.-C.; Charng, C.-Y.; Sheu, J.-R.; Tzeng, S.-H.; Chen, C.-M. *J. Pharm. Pharmacol*. **1998**, *50*, 1365−1369.
(129) Hsieh, M.-T.; Wu, C.-R.; Lin, L.-W.; Hsieh, C.-C.; Tsai, C.-H. *Planta Med.* **2001**, *67*, 38−42.
(130) Momin, R. A.; Ramsewak, R. S.; Nair, M. G. *J. Agric. Food Chem.* **2000**, *48*, 3785−3788.
(131) Momin, R. A.; Nair, M. G. *Phytomedicine* **2002**, *9*, 312−318.
(132) Kobayashi, S.; Mimura, Y.; Naitoh, T.; Kimura, I.; Kimura, M. *Jpn. J. Pharmacol.* **1993**, *63*, 353−359.
(133) Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Fitoterapia* **1982**, *53*, 17−20.
(134) Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Sci. Pharm.* **1982**, *50*, 158−161.
(135) Gijbels, M. J. M.; Bos, R.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Planta Med.* **1983**, *47*, 3−6.
(136) Gijbels, M. J. M.; Fischer, F. C.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Sci. Pharm.* **1983**, *51*, 414−417.

(137) Gijbels, M. J. M.; Fischer, F. C.; Scheffer, J. J. C.; Baerheim Svendsen, A. *Planta Med.* **1984**, *50*, 110.
(138) Cichy, M.; Wray, V.; Hofle, G. *Liebigs Ann. Chem.* **1984**, 397−400.
(139) Sheu, S. J.; Ho, Y. S.; Chen, Y. P.; Hsu, H. Y. *Planta Med.* **1987**, *53*, 377−378.
(140) Fukuhara, K.; Fujimori, T.; Shigematsu, H.; Ohnishi, A. *Agric. Biol. Chem.* **1987**, *51*, 1449−1451.
(141) Segebrecht, S.; Schilcher, H. *Planta Med.* **1989**, *55*, 572−573.
(142) Van Wassenhove, F. A.; Dirinck, P. J.; Schamp, N. M.; Vulsteke, G. A. *J. Agric. Food Chem.* **1990**, *38*, 220−226.
(143) Van Wassenhove, F. A.; Dirinck, P. J.; Vulsteke, G.; Schamp, N. *Hortscience* **1990**, *25*, 556−559.
(144) Tang, J.; Zhang, Y.; Hartman, T. G.; Rosen, R. T.; Ho. C.-T. *J. Agric. Food Chem.* **1990**, *38*, 1937−1940.
(145) Abdel-Mogib, M.; Ayyad, S. N.; Metwally, M. A.; Dawidar, A. M. *Pak. J. Sci. Ind. Res.* **1992**, *35*, 93.
(146) Szebeni-Galambosi, Z.; Galambosi, B.; Holm, Y. *J. Essent. Oil Res.* **1992**, *4*, 375−380.
(147) Brandt, J. J.; Schultze, W. *J. Essent. Oil Res.* **1995**, *7*, 231−235.
(148) Kaul, P. N.; Mallavarapu, G. R.; Chamoli, R. P. *Planta Med.* **1996**, *62*, 80−81.
(149) Dung, N. X.; Cu, L. D.; Moi, L. D.; Leclercq, P. A. *J. Essent. Oil Res.* **1996**, *8*, 503−506.
(150) Bartschat, D.; Beck, T.; Mosandl, A. *J. Agric. Food Chem.* **1997**, *45*, 4554−4557.
(151) Gillespie, S. G.; Duszynski, J. N. *Planta Med.* **1998**, *64*, 392.
(152) Bedrossian, A.; Beauchamp, P. E.; Dev, V.; Kwan, S.; Munevar-Mendoza, E.; Okoreeh, E. K.; Moore, P. E. *J. Essent. Oil Res.* **1998**, *10*, 473−477.
(153) Matsumoto, K.; Kohno, S-I.; Ojima, K.; Tezuka, Y.; Kadota, S.; Watanabe, H. *Life Sci.* **1998**, *62*, 2073−2082.
(154) Tirillini, B.; Pellegrino, R.; Menghini, A.; Tomaselli, B. *J. Essent. Oil Res.* **1999**, *11*, 251−252.
(155) Baser, K. H. C.; Ozek, T.; Demirci, B.; Duman, H. *Flavour Frag. J.* **2000**, *15*, 45−46.
(156) Sekiya, K.; Tezuka, Y.; Tanaka, K.; Kumar, Prasain, J.; Namba, T.; Katayama, K.; Koizumi, T.; Maeda, M.; Kondo, T.; Kadota, S. *J. Ethnopharmacol.* **2000**, *71*, 401−409.
(157) Choudhury, S.; Rajkhowa, A.; Dutta, S.; Kanjilal, P. B.; Sharma, R. K. *J. Essent. Oil Res.* **2000**, *12*, 731−734.
(158) Li, H.-X.; Ding, M.-Y.; Yu, J.-Y. *J. Chromatogr. Sci.* **2002**, *40*, 156−161.
(159) Gong, F.; Liang, Y. Z.; Chau, F.-T. *J. Sep. Sci.* **2003**, *26*, 112−122.
(160) Chen, X.; Kong, L.; Su, X.; Fu, H.; Ni, J.; Zhao, R.; Zou, H. *J. Chromatogr. A* **2004**, *1040*, 169−178.
(161) Pala-Paul, J.; Garcia-Jimenez, R.; Perez-Alonso, M. J.; Velasco-Negueruela, A.; Sanz, J. *J. Chromatogr. A* **2004**, *1036*, 245−247.
(162) Huang, L.-F.; Li, B.-Y.; Liang, Y.-Z.; Guo, F.-Q.; Wang, Y.-L. *Anal. Bioanal. Chem.* **2004**, *378*, 510−517.
(163) Ding, M.-Y.; Lv, W.-F. *J. Liq. Chromatogr.* **2004**, *27*, 521−531.
(164) Zhang, H.; Shen, P.; Cheng, Y. *J. Pharm. Biomed. Anal.* **2004**, *34*, 705−713.
(165) Liu, L.; Cheng, Y.; Zhang, H. *Chem. Pharm. Bull.* **2004**, *52*, 1295−1301.
(166) Khetwal, K. S.; Pathak, S. K.; Sajwan, K.; Pandey, B.; Adhikari, A. *Ind. J. Chem.* **2004**, *43B*, 2452−2455.
(167) Asuming, W. A.; Beauchamp, P. S.; Descalzo, J. T.; Dev, B. C.; Dev, V.; Frost, S.; Ma, C. W. *Biochem. Syst. Ecol.* **2005**, *33*, 17−26.
(168) Zschocke, S.; Klaiber, I.; Bauer, R.; Vogler, B. *Mol. Diversity* **2005**, *9*, 33−39.
(169) Yan, R.; Li, S.-L.; Chung, H.-S.; Tam, Y.-K.; Lin, G. *J. Pharm. Biomed. Anal.* **2005**, *37*, 87−95.
(170) Santos, P. A. G.; Figueiredo, A. C.; Oliveira, M. M.; Barroso, J. G.; Pedro, L. G.; Deans, S. G.; Scheffer, J. J. C. *Plant Sci.* **2005**, *168*, 1089−1096.
(171) Deng, C.; Ji, J.; Wang, X.; Zhang, X. *J. Sep. Sci.* **2005**, *28*, 1237−1243.
(172) Shibano, M.; Okuno, A.; Taniguchi, M.; Baba, K.; Wang, N.-H. *J. Nat. Prod.* **2005**, *68*, 1445−1449.
(173) Yi, T.; Leung, K. S.-Y.; Lu, G.-H.; Zhang, H.; Chan, K. *Chem. Pharm. Bull.* **2005**, *53*, 1480−1483.
(174) Li, S.-L.; Lin, G.; Tam, Y.-K. *Planta Med.* **2006**, *72*, 278−280.
(175) Dong, T. T. X.; Zhao, K. J.; Gao, Q. T.; Ji, Z. N.; Zhu, T. T.; Li, J.; Duan, R.; Cheung, A. W. H.; Tsim, K. W. K. *J. Agric. Food Chem.* **2006**, *54*, 2767−2774.

NP0605586