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NAPHTHALENE—A CONSTITUENT OF *MAGNOLIA* FLOWERS

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Key Word Index—*Magnolia*; Magnoliaceae; herbivore deterrents; plant-insect interaction; GC-mass spectrometry; benzenoid; naphthalene.

Abstract—Ether extracts of petals, gynoecia and leaves of nine *Magnolia* taxa were analysed using GC-mass spectrometry. The extracts of flowers and gynoecia of five taxa (*M. denudata*, *M. liliiflora*, *M. tomentosa*, *M. p.* var. *praecocissima* and var. *borealis*) contained naphthalene as their main component. The extracts of *M. salicifolia* characteristically contained several phenylpropanoids including eugenol methyl ether. Two well known sesquiterpene lactones, dehydrocostuslactone and porthenolide, were found in extracts of flowers and leaves of two taxa.

INTRODUCTION

Magnolia (Magnoliaceae) is an extant archaic taxon (angiosperm) based on its floral characters [1]. The plants usually bear a large number of odoriferous flowers which are pollinated by beetles [2]. Insect pollination has been considered a major factor responsible for the ascendency of the angiosperms (coevolution). In the process, floral scent acts as an attractant for flower-visiting insects and is important for flower–insect interactions and the key to the evolution of the early angiosperms [3, 4]. Investigations of floral scent have been carried out on several *Magnolia* taxa and allied genera, e.g. *Liriodendron* and *Michelia*, in North America and Japan [2, 5, 6], and their evolutionary and ecological implications have been discussed [3]. *Magnolia* taxa emit various volatile compounds such as fatty acid derivatives, mono- and sesquiterpenoids, and benzenoids and attract a wide range of insects including Diptera, Hymenoptera and Coleoptera. Pellmyr and Thien [3] suggested that olfactory attractants serve as chemical cues which may have originated from general herbivore deterrents in the leaves of the insect-pollinated plants. Therefore, it is important to understand the biosynthetic relationship between pollinator attractants and insect deterrents, although effects of structural difference of these compounds on insect behaviour remain unclear. It is also necessary to investigate the phytochemical similarity or difference between reproductive organs (floral parts) and vegetative organs (leaves) as a prelude to discuss plant–insect coevolution and development of floral functions.

In this study, we analysed the chemical composition of ether extracts of petals, gynoecia (including attached stamens) and leaves of nine *Magnolia* taxa (*M. denudata* Desr., *M. liliiflora* Desr., *M. tomentosa* Thunb. (=*M. stellata*), *M. praecocissima* var. *praecocissima* Koidz. (=*M. kobus*), and var. *borealis* (Sargent) Koidz., *M. salicifolia* (Sieb. et Zucc.) Maxim., *M. obovata* Thunb., *M. sieboldii* ssp. *japonica* Ueda and *M. grandiflora* L. (Magnoliaceae) [7–10]) using GC-mass spectrometry. *M. tomentosa*, *M. p.* var. *praecocissima* and var. *borealis*, *M. salicifolia*, *M. obovata* and *M. s.* ssp. *japonica* are native to Japan, *M. denudata*, *M. liliiflora* are Chinese native species, and *M. grandiflora* in a North American native species. Six taxa, that is, *M. denudata*, *M. liliiflora*, *M. tomentosa*, *M. p.* var. *praecocissima*, *M. p.* var. *borealis* and *M. salicifolia*, belong to subgenus *Yulania* and their flowers opened in late-March to mid-April before or at leaf emergence. The other *Magnolia* taxa examined belong to subgenus *Magnolia* and their flowers opened in May to July after leaf emergence [7].

Although many phytochemical investigations of *Magnolia* and other genera have been reported, almost all of these studies deal with nonvolatile compounds, such as sesquiterpene lactones [11, 12] and lignans [13, 14] obtained from leaf and bark concretes, and essential oils [15–21]. In the present study, we report the phytochemical relationship between reproductive and vegetative organs of *Magnolia* taxa.

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Table 1. Chemical compositions of the ether extracts of nine *Magnolia* taxa by GC-mass spectrometry

		Subgenus *Yulania*																		Subgenus *Magnolia*								
		M.d.			*M.l.*			*M.t*			*M.p.p.*			*M.p.b.*			*M.s.*			*M.o*			*M.s.j.*			*M.g.*		
	Identification	P	G	L	P	G	L	P	G	L	P	G	L	P	G	L	P	G	L	P	G	L	P	G	L	P	G	L
Fatty acid derivatives																												
Total (number of compounds)		55.2 (13)	25.9 (9)	44.8 (6)	9.0 (7)	31.3 (10)	72.9 (7)	25.4 (6)	22.2 (9)	8.1 (3)	19.2 (7)	12.6 (6)	19.5 (2)	11.8 (3)	20.6 (6)	42.1 (5)	13.3 (8)	16.3 (7)	11.9 (5)	21.1 (8)	20.3 (10)	20.6 (3)	0.0 (0)	16.2 (6)	17.4 (2)	3.3 (1)	26.9 (8)	1.8 (1)
Benzenoids																												
Veratrole	MS*	–	–	–	–	–	–				–	–	–	–			–	–	–	2.8	7.2	–		–	–	–	–	–
Naphthalene	RT	10.0	12.4		30.0	22.8	–	15.4	10.0		26.5	19.2	–	36.5	–	–	–		–	–	–	–	–	–	–				–
Acid derivatives																												
Benzoic acid	RT	–	–	–	–	–	–		–	–	–	–	–	–			–	–	–	13.7	–	–	–	–	–	–	–	–
Pentyl benzoate	MS*	–	–	–	–	–	–	–	–			–	–	–		–	–		–	1.3	–	–	–	–	–			–
Cinnamic acid	MS*	–	–	–			–		–	–	–				–	–	–	–	–	2.9		–	–	–	–			
Aldehydes																												
Hydroxybenzaldehyde	MS*	–			–	–	–	–	–			–	–	–		–	14.7			–		–	–	–	–			–
Hydroxybenzaldehyde	MS*	–	–	–			–	–	–		–			–		–	18.6	–	–			–		–	–	–		
Veratraldehyde	MS*	–	–	–	–	–			–	–		–	–	–			4.3	3.5	–	–	–			–	–		–	–
Asaraldehyde	MS*	–			–	–	–	–				–	–	–		–	5.9	8.2	–	–				–				–
Alcohols																												
Benzyl alcohol	RT		–	–	–	–	–	4.5	–	–	2.3	–	–	–			0.7	0.6	–	–	–			–			–	
Phenethyl alcohol	RT	5.2	3.9		34.6	–	–	–	2.8			5.1		–		–		4.6		–			–	–				–
4-Methoxybenzyl alcohol	RT	–	–	–			–		–		–					–	3.6	–				–		–	–	–	–	
Phenylpropanoids																												
5 (1 Propenyl)-1,3-benzodioxole	MS*	–			–		–	–	–			–	–	–		–	–	3.0	12.5	–		–		–				–
Eugenol	MS*	–	–	–			–	0.8	1.1	–	–	–			–	–	–	–	0.2			–	–	–	–	–	–	–
iso-Eugenol methyl ether	MS*		–		–	–				–	–	–	–	–			0.7	3.5	4.5		–			–	–	–	–	–
Eugenol methyl ether	MS*	–					–		–								8.6	32.4	58.5									
Asarone	MS*	–	–	–			–	–	–	–	–	–		–	–	–	6.3	19.7			–	–	–	–	–	–	–	–
Obovatol	NMR	–	–	–	–	–	–	–	–	–	–	–	–	–			–	–	–	13.3	32.8	32.4		–	–		–	–
Total		15.2	16.3	0.0	64.6	22.8	0.0	20.7	13.9	0.0	28.8	24.3	0.0	36.5	0.0	0.0	63.4	75.5	75.7	34.0	40.0	32.4	0.0	0.0	0.0	0.0	0.0	0.0
Monoterpenoids																												
Normal monoterpenes																												
Camphene	RT			–			–	–	–		1.4	1.2		–		–	–	–	–			–		–	–	–	–	
Sabinene	RT	3.2	9.0	–	–		–			–		–		–	2.0			–		–	–	–	–		–			7.5
β-Pinene	RT	2.5	6.5		–	–	–	–	–		2.0	–	–	–	2.0	–	–			–	–	–	–	–	–			–
β-Myrcene	RT		9.2	5.4	6.8	17.1		4.6	3.7	–	1.7	0.9			3.1		–	–	–	–			–	–	–	–	–	–
Limonene	MS†	0.8	3.7	–	12.1	8.2	–	14.4	12.5	–	13.5	15.4	27.8	28.2	23.6		–	–	–	–	0.8	–			–	–	–	–
cis-β-Ocimene	MS*	–	–			–	–	–	–	1.4		–	–	–	–	–	–		0.7	–	–	–	–	1.4	–			
trans-β-Ocimene	MS*	–	–	–			–	–	–	–	–	–		–	–	–	–	–	–	0.4		–	–	–	–	–	–	–
p-Cymene	RT		–	1.6	–	–	–	1.8		–	3.5	1.4	–	4.7	8.7		–	–	–		–				–	–	–	–

Oxygenated monoterpenes																													
1,4-Cineole	RT	–	–	–	0.8		–	–	–	–	–		–	–	–	–	–	–		–	–	–	–	–		–	–	–
1,8-Cineole	RT	4.9	11.2		2.4	1.1	–	–	12.0		–	–	–	5.1	7.6			–	–	–									
Linalool oxide (furanoid)	MS*		–	–	–	–	–		–	–	–	–			–	–	–	–	0.5		–	–	–	–		–	–	–
Linalool	RT	–	–	–	–		–	–	–	–	–			–	0.6	–	–	–	0.6	–	–	–	–			–	–	–
Linalool oxide (furanoid)	MS*	–	–		–	–	–	–	–		–	–	–	–	–		–	–	5.2	1.6	–		–	–	–	–	–	–
Fenchone	MS*		–	–	–	–	–	12.5	14.9	–	–	–		–	–	–	–	–	–		–	–	–		–	–	–	–
Borneol	RT	–	–	–	–		–	–	–	–	1.5	7.0	–	–	–	–	–	–	–	1.3	–	–	–	–	–	–	–	–
Camphor	RT	–	–		–	–	–	6.5	8.1		15.7	18.8	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–
Linalool oxide (pyranoid: alcohol)	MS*		–	–	–	–	–		–	–	–	–	–			–	–	–	10.9		–	–	–	–	–	–	–	–
α-Terpineol	RT	–	–	–	–			–	1.6	–	–			–	2.2	–	–	–	–	–	–	–	–	–	–	–	–	–
Geraniol	RT	–	–		–	–	–	–	–			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bornyl acetate	MS†		–	–	–	–	–		–	–	3.9	6.8	–	–		–	–	–	–	–	–	–	–	–	–	2.9	–	–
Total		11.4	39.6	7.0	22.1	26.4	0.0	39.8	52.8	1.4	43.2	51.5	27.8	38.0	49.8	0.0	0.0	0.0	1.1	17.2	3.7	0.0	0.0	1.4	0.0	2.9	0.0	7.5
Sesquiterpenoids																													
Normal sesquiterpenes																													
Bicyclogermacrene	MS†		–	–	–	–	–	–		–	–	–	–	–		–	–	–	0.7	–	–		–	–	–	5.3	–	17.6
β-Elemene	RT	–	–	1.0	–			–	–	–	–	–		0.8	3.9	4.0	–	–			4.8	–	14.6	23.7	16.0	9.3	5.9	18.3
β-Caryophyllene	MS†	–	0.5	8.0	–	1.4	–	2.3	1.3	7.8	2.6	2.8	8.9	0.7	2.4	5.3		0.3	1.0	3.1	13.3	28.5	1.8	–	–	–	–	2.6
α-Caryophyllene	MS†		–	0.7	–	–	–			–	–	–	–	–		–	–	–	–	–	1.0	5.3	–	–	–	–		–
γ-Cadinene	MS†	2.2	7.1	0.9	1.3	8.0	1.6	6.2	5.4	1.7	2.3	4.4	5.1	0.8	2.1	1.0	–	4.8	4.9		1.8	–	–	–	–	1.6	–	5.6
α-Farnesene	MS†	–	–		2.3	4.9	11.4	0.5	0.5	–		–	–	–	0.9	–			3.1	–	–	–	–	–	–	–	–	–
δ-Cadinene	MS*			–	0.6	0.8	–	–		–	–	–	–	–		–	–	–	–	–	5.0	1.5	–	–	–	–	–	–
Oxygenated sesquiterpenes		–	–	–	–			–	–	–	–	–			–	–	–	–	–	–	–	–	–	–	–	–	–	–
trans Nerolidol	RT		0.8	11.3		0.6	5.7	1.1	–	7.8		0.7	–	–	–	8.4			–	–	–	–	–	–	–	–	–	–
Elemol	RT		0.5	–	–	–	–	–	0.7	11.1	–	–		3.7	4.8	25.5	–		–	–			–	–	–	–	–	–
Farnesol	MS*	–	–	–	–	–		–	–	12.9	–	–	32.0		–	–	–	–	–		–	–	–	–	–	4.5	–	–
9-Oxonerolidol	NMR	–	–	–		–	–	2.1	–	44.7	1.9		–	–	–	10.5	–		–	–	–	–	–	–	–	0.6	–	0.9
Phytol	MS†			18.9	–	–	5.9	–		3.0	–	–	4.0	–	–		–	–	0.4	–	–		–	–	0.9	–	–	
Total		2.2	8.9	40.8	4.2	15.7	24.6	12.2	7.9	89.0	6.8	7.9	50.0	6.0	14.1	54.7	0.0	5.1	10.1	3.1	25.9	35.3	16.4	23.7	16.9	21.3	5.9	45.0
Sesquiterpene lactones																													
Dehydrocostuslactone	NMR	–	–	–	–	–			–	–	–	–	–		–	–	–	–	–		–	–	80.6	52.6	64.6		–	–
Parthenolide	NMR	–	–	–		–	–	–	–	–			–	2.3	4.4	–	–		–	–	–	–	–		–	47.9	63.1	24.6
Total		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.6	52.6	64.6	47.9	63.1	24.6
Unknowns																													
Total		15.9	9.2	7.4	0.0	4.4	2.6	1.9	3.2	1.6	2.1	3.7	2.8	5.3	12.1	3.3	23.1	3.0	1.4	24.7	9.8	11.8	3.1	6.2	1.0	23.8	4.2	21.0
(number of compounds)		(9)	(5)	(3)	(0)	(3)	(1)	(2)	(3)	(1)	(2)	(2)	(1)	(3)	(5)	(1)	(9)	(5)	(3)	(8)	(6)	(5)	(2)	(4)	(1)	(7)	(2)	(3)

M.d. = *Magnolia denudata*, *M.l.* = *M. liliflora*, *M.t.* = *M.t.* = *M. tomentosa*, *M.p.p.* = *M. praecocissima* var. *praecocissima*, *M.p.b.* = *M. p.* var. *borealis*, *M.s.* = *M. salicifolia*, *M.o.* = *M. obovata*, *M.s.j.* = *M. sieboldii ssp. japonica*, *M.g.* = *M. grandiflora*, P = petal, G = gynoecium, L = leaf.

The numbers are relative abundance on the total ion chromatogram of GC-mass spectral analyses (– = not detected or present in <0.1%).

*,†Identified by comparison of mass spectra of the NBS library and the original library, respectively; RT = identified by comparison of retention times and mass spectra with the authentic compounds.

RESULTS AND DISCUSSION

The *Magnolia* taxa investigated in this study belong to the two subgenera, *Yulania* and *Magnolia* (Table 1) [7]. The flowers of subgenus *Yulania* have white, cream or purple (only *M. liliiflora*) flimsy petals. They emit a faint and sweet scent and anthesis occurs before or at the leaf emergence. The flowers of subgenus *Magnolia* have white or cream fleshy petals that open after the leaf emergence and emit a pleasant fruity scent.

Table 1 shows the relative and total amounts of components in ether extracts of various *Magnolia* taxa. Fatty acid derivatives, hydrocarbons, alcohols and esters, are commonly present and exhibit large variations in relative amounts (total; 0–75%) in all taxa examined. Benzenoids, especially naphthalene (**1**), which was identified by comparison with the mass spectrum, retention time and co-injected GC-mass spectrum of the authentic compound, was exclusively present as the main component, together with benzyl alcohol or phenethyl alcohol in the extracts of only the floral parts of *M. denudata* (petal; 10%, gynoecium; 12%), *M. liliiflora* (30%, 23%), *M. tomentosa* (15%, 10%), *M. praecocissima* var. *praecocissima* (27%, 19%) and *M. p.* var. *borealis* (37%, 0%). However, these compounds were not found in the gynoecium extract of *M. p.* var. *borealis* and in the extracts of their leaves and in any part of the other taxa. These five taxa belong to subgenus *Yulania* together with *M. salicifolia*. Therefore, although the extracts of *M. salicifolia*, containing characteristically several phenylpropanoids, lack naphthalene, the fact that naphthalene has been observed only in the floral extracts of subgenus *Yulania* suggests that the formation of naphthalene may be one of the specific physiological characters that distinguish subgenus *Yulania*.

Many essential-oil studies of *Magnolia* taxa have been reported [15–21], which show that the leaves, branchlets, flower buds and flowers have a very wide distribution of mono- and sesquiterpenoids and other compounds. However, naphthalene was not detected, and chemical compositions of extracts of several taxa were differed from each other. Although analyses of flower and leaf extracts of *M. denudata* and *M. liliiflora* showed almost the same compositions with earlier studies [16, 17] except for naphthalene and phenethyl alcohol, the results obtained in earlier studies and this study are somewhat controversial as to *M. tomentosa*, *M. p.* var. *praecocissima* and *M. p.* var. *borealis* [18–21]. For example, from *M. p.* var. *borealis* leaves, high amounts of limonene, camphor and α-terpineol were reported [21], but in this analysis, sesquiterpenes such as elemol and 9-oxonerolidol were most abundant. Likewise, in *M. salicifolia* flowers and leaves citral-a (geranial) and citral-b (neral), methylchavicol and other phenylpropanoids were reported as major components [15], but we could not find any of these components in our analysis. Instead, various phenylpropanoids, such as hydroxybenzaldehyde, eugenol methyl ether and asarone were detected.

This contradiction, especially in the accumulation of naphthalene, between the previous reports and this study may be due to different extraction procedures and/or different growth stages of materials examined (all *Magnolia* taxa examined in this study are protogynous). Results obtained from the same taxa are often different, depending on the extraction procedures. Extraction of essential oils in the previous studies was performed by steam distillation immediately after collecting. However, in this study, materials that include both female and male flowers were simply soaked in ether, without drying, and placed in darkness for about six months. It may be argued that the occurrence of naphthalene in the ether extracts originate from other materials or the environment. The suggestion, however, may be indirectly refuted because of the relatively high amounts of naphthalene in some extracts and absence in the extracts of other taxa (Table 1). Moreover, a preliminary examination showed that naphthalene accumulates in extracts with increasing time of extraction. It remains a substantial problem whether naphthalene exists in the floral parts *in situ*. If naphthalene exists free in the cell, it would be detected in the essential oils extracted by steam distillation. Therefore, there is probably a precursor present which can release or produce naphthalene during extraction.

The presence of naphthalene in floral parts of several *Magnolia* taxa is very important to understanding the floral biology and evolutionary aspects of the taxa. It may function as protection of tissue against chewing insects and is a factor in speciation of the taxa. Their UV absorption may function as markers for insect attraction [22, 23]. It is, therefore, supposed that taxa of subgenus *Yulania*, (except for *M. salicifolia*) examined might attract insects to pollinate by the UV absorption of accumulated naphthalene in the floral parts and floral scent [6], but prevent the insects attracted from feeding on the floral parts.

The *Magnolia* taxa examined in this study, generally contained various secondary compounds such as benzenoids (including phenylpropanoids) and terpenoids (Table 1). The extracts of *M. salicifolia* (belonging to subgenus *Yulania* but not containing naphthalene) had several phenylpropanoids which were characteristic of the examined taxa. Interestingly, although eugenol methyl ether (**2**) was predominant in the leaf (59%) and gynoecium (32%) extract, in the petal extract two isomeric aldehydes, hydroxybenzaldehydes (15%, 19%), whose hydroxyl group position could not be determined and which were probably derived from phenylpropanoids, were more predominant than eugenol methyl ether (9%). These differences in chemical compositions between leaf and floral parts might reflect ecological and/or physiological differences which function in reproductive and vegetative organs respectively, and possibly be significant in understanding the evolutionary process of organs of *M. salicifolia* in connection with its biotic and nonbiotic environment. Although anatomical characters of *M. salicifolia* are similar to those of naphthalene-containing taxa, this study emphasizes its phytochemical specificity among taxa examined as has already been reported [15].

Generally, benzenoids and monoterpenoids were predominant in the extracts of the floral parts, while sesquiterpenoids tended to accumulate in the leaf. This suggests that floral organs may have evolved with the development of biosynthetic pathways of benzenoids and monoterpenoids which are commonly present in the floral scent emitted by various plants [24].

Obovatol (**3**), a phenylpropanoid dimer, is a characteristic compound of the extracts of all parts of *M. obovata* (13–32%), as it was found only in this species [13]. Two well known sesquiterpene lactones, dehydrocostuslactone (**4**) and parthenolide (**5**), which are distributed in some species [25] were found in the extracts of *M. sieboldii* ssp. *japonica* (53–81%) and *M. grandiflora* (25–63%), respectively.

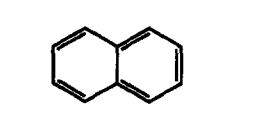
EXPERIMENTAL

Plant materials and sampling. The plant materials, *M. tomentosa*, *M. praecocissima* var. *praecocissima*, *M. p.* var. *borealis*, *M. salicifolia*, *M. obovata* and *M. sieboldii* ssp. *japonica* were collected in their natural habitats in Japan 1994. Those of the other species, *M. denudata*, *M. liliiflora* and *M. grandiflora* were collected from plants cultivated in Kyoto Univ. campus or the Botanical Garden of Kyoto Univ. in the same season. Petals and gynoecia which in most cases have many slender stamens attached were collected from female and/or early male flowers. New leaves were also collected from the same individuals. When the large flowers, *M. denudata*, *M. liliiflora*, *M. obovata* and *M. grandiflora*, were treated, many stamens had naturally detached from the gynoecia. These materials were immediately soaked in diethyl ether after collecting and then extracted for about 6 months in darkness in the laboratory of Tokushima Bunri University.

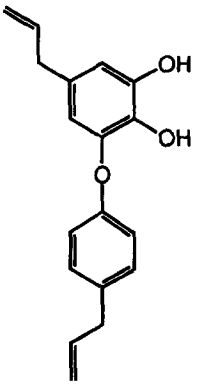
GC-MS analysis. A non-aq. portion of the extract was concentrated by air flow and analysed by GC-MS. GC-MS analysis was performed with a Hewlett-Packard 5971A mass selective detector to which a Hewlett-Packard 5890 SERIES II gas chromatograph was connected. A fused silica capillary column (DB-17, 0.25 mm i.d. × 30 m, 0.25 μm) was used. Ion-source temp. was 185°, and the ionization energy was 70 eV. The injection temp. was 250°. The oven temp. was kept at 50° for the first 5 min, programmed 5° min^{-1} to 150°, and finally raised to 10° min^{-1} to 250°, kept for 10 min. He was used as carrier gas.

Identification and data choice. Identification of compounds detected were made by comparison of their mass spectra with those in computer libraries, or with GC-retention times and mass spectra of authentic compounds. NMR was used to identify unknown major components detected in the GC-MS. A large number of peaks were detected in the GC-MS of each extract. Therefore, we chose peaks whose area percentage indicated more than about 0.1% in the total ion chromatogram and other minor peaks were ignored.

Preparation for NMR analysis. The ether extracts were fractionated by silica gel CC, TLC and HPLC to obtain pure compounds using a solvent system of hexane–EtOAc 400 MHz NMR spectroscopy was used and the spectra of compounds were identical to those in literature.



1



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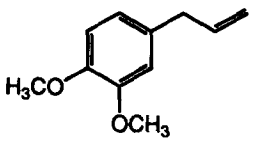
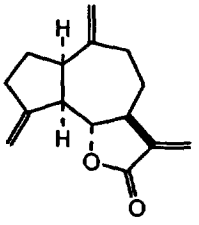
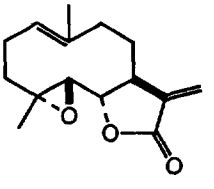


2



4



5

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