Bioorganic & Medicinal Chemistry 16 (2008) 6891–6902

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Diarylheptanoids, new phytoestrogens from the rhizomes of *Curcuma comosa*: Isolation, chemical modification and estrogenic activity evaluation

Apichart Suksamrarn [a,*], Mathurose Ponglikitmongkol [b], Kanjana Wongkrajang [a], Anon Chindaduang [a,†], Suthadta Kittidanairak [b], Aroon Jankam [a,‡], Boon-ek Yingyongnarongkul [a], Narin Kittipanumat [a], Ratchanaporn Chokchaisiri [a], Pichit Khetkam [a], Pawinee Piyachaturawat [c]

[a] *Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand*
[b] *Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[c] *Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

ARTICLE INFO

Article history:
Received 24 March 2008
Revised 22 May 2008
Accepted 23 May 2008
Available online 28 May 2008

Keywords:
Diarylheptanoid
Curcuma comosa
Chemical modification
Estrogenic activity
Structure–activity relationship

ABSTRACT

Three new diarylheptanoids, a 1:2 mixture of (3*S*)- and (3*R*)-1-(4-methoxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol (**13a** and **13b**) and 1-(4-hydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-one (**15**), together with two synthetically known diarylheptanoids 1,7-diphenyl-(1*E*,3*E*,5*E*)-1,3,5-triene (**9**) and 1-(4-hydroxyphenyl)-7-phenyl-(4*E*,6*E*)-4,6-heptadien-3-one (**16**), and nine known diarylheptanoids, **2**, **8**, **10**–**12**, **14**, a 3:1 mixture of **17a** and **17b**, and **18**, were isolated from the rhizomes of *Curcuma comosa* Roxb. The absolute stereochemistry of the isolated compounds has also been determined using the modified Mosher's method. The isolated compounds and the chemically modified analogues were evaluated for their estrogenic-like transcriptional activity using RT-PCR in HeLa cell line. Some of the isolated diarylheptanoids and their modified analogues exhibited estrogenic activity comparable to or higher than that of the phytoestrogen genistein. Based on the transcriptional activation of both estrogenic targets, Bcl-xL and ERβ gene expression, the structural features for a diarylheptanoid to exhibit high estrogenic activity are the presence of an olefinic function conjugated with the aromatic ring at the 7-position, a keto group at the 3-position, and a phenolic hydroxyl group at the *p*-position of the aromatic ring attached to the 1-position of the heptyl chain.

1. Introduction

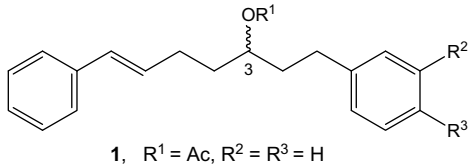
Phytoestrogens are naturally occurring compounds from plant with estrogenic-like biological activity.[1,2] They exert actions mainly by binding to specific estrogen receptors (ER) which exists in two forms, ERα and ERβ, and activate the transcription of specific target genes in the nucleus.[3,4] Phytoestrogens have several pharmacological effects in a large number of tissues including protection against development of cancer,[5,6] cardiovascular disease,[7,8] and osteoporosis[9] in menopausal women.[10] Other biological effects independent of the ER such as anti-oxidant and anti-inflammatory effects of phytoestrogens have also been ascribed.[10,11] Currently, the interest of using product containing phytoestrogens as alternatives to the steroid hormone to alleviate the menopausal symptom is dramatically increased as they have less adverse effects compared to the classical steroid hormone estrogen.

The rhizome of *Curcuma comosa* Roxb. (Zingiberaceae), commonly known as Waan Chak Modluk in Thai, has long been used in indigenous medicine in Thailand as an anti-inflammatory agent. It has been used widely for the treatment of postpartum uterine bleeding and as an aromatic stomachic. On the basis of local use, we investigated the biological effects of the extracts of the plant rhizomes and found that the hexane extract exhibited estrogenic-like activities by causing an increase in uterine weight and cornification of vaginal epithelium,[12,13] whereas the polar extracts showed a potent choleretic effect.[14] In an early chemical study, the methanol extract of its rhizomes was shown to have nematocidal activity and the diarylheptanoids **1**–**5** were isolated from the less polar part of the extract.[15] We have recently investigated the ethyl acetate and butanol extracts of this plant species and the known diarylheptanoids **3**, **6** and **7**, and a new phloracetophenone glucoside, 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl)acetophenone (**8**), were isolated. Compounds **6**, **7**, and the glucoside **8** exhibited choleretic activity in rats.[16] In the

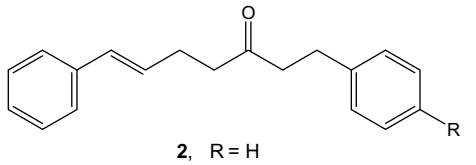
* Corresponding author. Tel.: +662 3190931; fax: +662 3191900.
E-mail addresses: s_apichart@ru.ac.th, asuksamrarn@yahoo.com (A. Suksamrarn).
† Present address: National Nanotechnology Center, National Science and Technology Development Agency, Pathumthani 12120, Thailand.
‡ Present address: Department of Chemistry, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand.

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.05.051

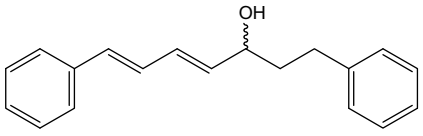
search for compounds with estrogenic activity, a number of plant species have been investigated for phytoestrogens by our group. This work deals with the isolation, absolute stereochemistry determination, chemical modification, and estrogenic activity evaluation of diarylheptanoids from the rhizomes of *C. comosa*. The diarylheptanoids are a new group of phytoestrogens which may have health benefit potential particularly for postmenopausal women.



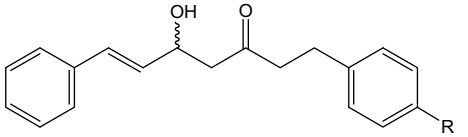
1, R^1 = Ac, R^2 = R^3 = H
3, R^1 = R^2 = R^3 = H
6, R^1 = R^2 = H, R^3 = OH
7, R^1 = H, R^2 = R^3 = OH



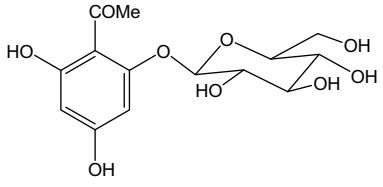
2, R = H
15, R = OH
20, R = OMe
24, R = OAc



4



5, R = H
31, R = OH



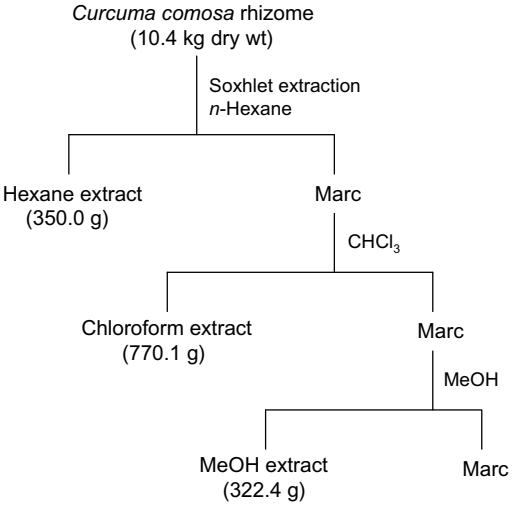
8

2. Results and discussion

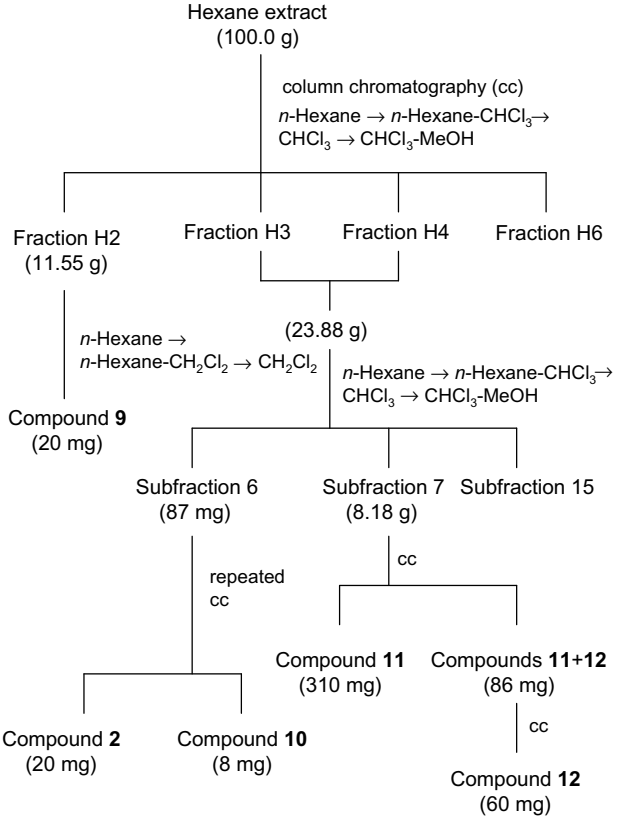
2.1. Structural elucidation of diarylheptanoids

Preliminary examination of the chemical constituents of *C. comosa* rhizomes collected from different locations in Thailand revealed that some of them contained a relatively high quantity of the major constituents and constituted additional number of minor diarylheptanoids which have not previously been reported in this plant species. The rhizomes of *C. comosa* from Kampaeng-saen district, Nakhon Pathom province were used for this study. The pulverized, dry rhizome was extracted successively with *n*-hexane, $CHCl_3$, and MeOH to yield the hexane, $CHCl_3$, and MeOH extracts, respectively (see Scheme 1).

The hexane extract of *C. comosa* rhizomes was subjected to column chromatography (Scheme 2) to yield five known diarylheptanoids **2**, **9**, **10**, **11**, and **12**. Compound **2** was identical to that isolated previously from this plant species.[15] Compound **9** has not previously been reported as a naturally occurring

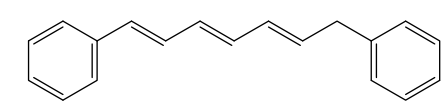


Scheme 1. Extraction of the pulverized, dry rhizome of *Curcuma comosa*.

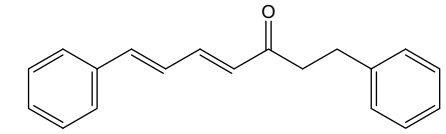


Scheme 2. Chromatographic separation of diarylheptanoids from the hexane extract.

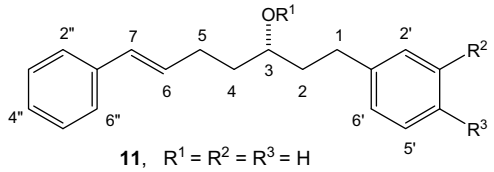
diarylheptanoid, but it has been synthesized for a conjugated carbanion study.[17] The spectroscopic data of compound **9** were consistent with those of the reported values.[17,18] Compound **10** was identical to the diarylheptanoid isolated from *C. xanthorrhiza* by spectroscopic (IR, ^{1}H NMR and mass spectra) comparison.[19]



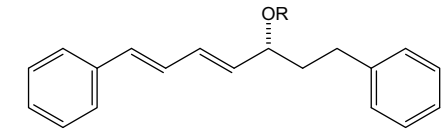
9



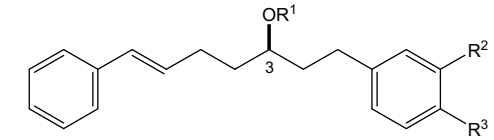
10



11, $R^1 = R^2 = R^3 = H$
13a, $R^1 = R^2 = H$, $R^3 = OMe$
17a, $R^1 = R^2 = H$, $R^3 = OH$
18, $R^1 = H$, $R^2 = R^3 = OH$
19, $R^1 = Me$, $R^2 = R^3 = H$
22, $R^1 = Ac$, $R^2 = R^3 = H$



12, R = H
23, R = Ac



13b, $R^1 = R^2 = H$, $R^3 = OMe$
17b, $R^1 = R^2 = H$, $R^3 = OH$

The diarylheptanoid **3** was isolated previously from a number of plant species.[15,16,20] From the previous isolation of this compound in *C. comosa*,[15] it was reported to exist as a racemic mixture. In this study, it was isolated as the enantiomer **11**, which showed identical ^{1}H NMR data as those reported for compound **3**[19] and exhibited low optical rotation ($[\alpha]_D^{27} - 2.6^\circ$ in EtOH). Compound **11** was obtained as the major component from this plant species. Determination of the absolute configuration at C-3 of this diarylheptanoid using the modified Mosher's method,[21,22] it was found to be *S* as indicated in the structure **11**. Thus, the diarylheptanoid **11** was subjected to esterification with (*R*)-(−)-MTPA chloride in dry pyridine[23] to yield the corresponding (*S*)-MTPA ester **11x**. Compound **11** was also subjected to esterification with (*S*)-(+)-MTPA chloride to yield the corresponding (*R*)-MTPA ester **11y**. The $\Delta\ \delta$ values ($\Delta\ \delta = \delta_{S-MTPA} - \delta_{R-MTPA}$) were determined as shown in Figure 1. Negative $\Delta\ \delta$ values were found for H-5, H-6, and H-7, while positive $\Delta\ \delta$ values were obtained for H-1. Following the MPTA rules,[21] these data indicated an *S* configuration at C-3 (see Fig. 1).

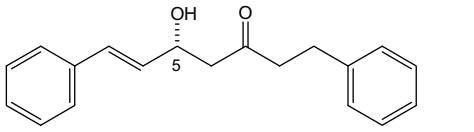
The closely related compound **12** was another optically active diarylheptanoid isolated from *C. comosa*. The ^{1}H NMR data of **12** were consistent with compound **4** isolated previously from this plant species[15] and other *Curcuma* species,[19] but the stereochemistry at C-3 was not reported. The absolute configuration of **12** was determined by the same analogy to that of compound **11** (see Fig. 1). The spatial arrangement of the groups at the asymmetric C-3 in **12** was the same as that of **11**. However, the presence of an additional double bond led to the assignment of the absolute configuration at C-3 of **12** to be *R*.

The diarylheptanoids isolated from the $CHCl_3$ extract included the already isolated compounds **11** and **12**, and compounds **13**–**16** (see Scheme 3).

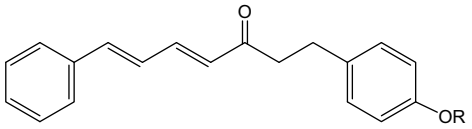
Compound **13** was a diarylheptanoid existed as a mixture of two C-3 enantiomers, **13a** and **13b**. The HR-TOFMS (ES^+) showed a pseudomolecular ion $[M+H]^+$ at *m*/*z* 297.1839, compatible with the molecular formula $C_{20}H_{24}O_2$. The IR absorption band at 3331 cm^{-1} indicated the presence of a hydroxyl group. Assignments of the ^{1}H and ^{13}C NMR data were achieved by COSY, DEPT, HMQC, and HMBC techniques. The presence of the vinylic function was evident from the double triplet signal at δ 6.20 (J = 15.8, 6.8 Hz, H-6) and the broad doublet signal at δ 6.39 (J = 15.8 Hz, H-7) in the ^{1}H NMR spectrum, which corresponded to the ^{13}C NMR at δ 130.26 and 130.27. The carbinolic and methoxy protons appeared as a multiplet signal and a singlet signal at δ 3.68 and 3.78, respectively. The ^{1}H NMR features of the heptyl chain were very similar to those of compound **11**. However, the ^{1}H NMR features of the aromatic protons of **13** were different from those of **11** by the presence of a *para*-disubstituted aromatic ring as evident from the two doublets (J = 8.2 Hz) at δ 6.81 (2H, H-3′ and H-5′) and δ 7.10 (2H, H-2′ and H-6′). Placement of the methoxyl group at the 4′-position was confirmed by HMBC correlation of the methoxy protons with C-4′ (see Section 4). The attachment of the 4-methoxyphenyl moiety to the 1-position was confirmed by the HMBC correlations of H-1 with C-1′ and C-2′/C-6′, H-2 with C-1′, and H-2′/H-6′ with C-1 (see Section 4). The attachment of the phenyl moiety to the 7-position was also confirmed by HMBC correlations of H-6 with C-1″, and H-7 with C-1″ and C-2″/C-6″.

Compound **13** exhibited low optical rotation ($[\alpha]_D^{27} + 2.4^\circ$ in EtOH). The absolute stereochemistry at C-3 and the enantiomeric ratio were determined by the modified Mosher's method.[21–23] Thus the diarylheptanoid **13a** and **13b** mixture was transformed to a 1:2 mixture of the corresponding (*S*)-MTPA esters **13ax** and **13bx**, and (*R*)-MTPA esters **13ay** and **13by**. Analysis of the ^{1}H NMR spectra of the two Mosher ester mixtures (Fig. 1) established the absolute configuration at C-3 of the diarylheptanoid mixture as *S* and *R* in a ratio of 1:2. The diarylheptanoid **13** was thus concluded as a 1:2 mixture of (3*S*)- and (3*R*)-1-(4-methoxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol (**13a** and **13b**).

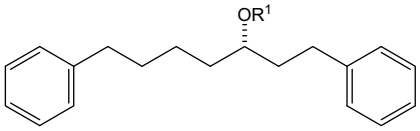
Compound **14** was a minor diarylheptanoid isolated from the $CHCl_3$ extract. The ^{1}H NMR data were consistent with the structure and were in agreement with those of reported values.[24] In the present work, the C-5 absolute configuration was determined to be *R* by the modified Mosher's method (see Fig. 1).



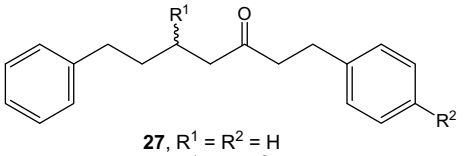
14



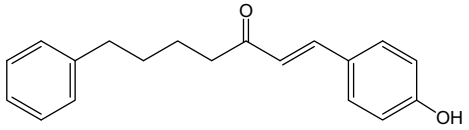
16, R = H
21, R = Me
25, R = Ac



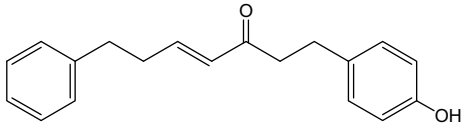
26, R^1 = H
28, R^1 = Ac



27, $R^1 = R^2$ = H
29, R^1 = H, R^2 = OH
32, $R^1 = R^2$ = OH



30



33

Compound **15** was isolated from *C. comosa* as colorless viscous oil. The HR-TOFMS (ES^+) at *m*/*z* 281.1525 $[M+H]^+$ established a molecular formula $C_{19}H_{20}O_2$. The IR absorption bands indicated the presence of a hydroxyl group and a saturated keto group at 3360 and 1697 cm^{-1}, respectively. The ^{13}C NMR resonance at δ 210.1 confirmed that this compound is a saturated ketone. The characteristic vinylic function was evident from the double triplet signal at δ 6.14 (*J* = 15.9, 6.7 Hz, H-6) and the doublet signal at δ 6.37 (*J* = 15.9 Hz, H-7). The placement of the keto group at the 3-position was deduced from the presence of two triplet signals (*J* = 7.3 Hz) of H-1 and H-2 at δ 2.84 and 2.72, respectively. The *p*-substituted nature of the phenolic hydroxyl group was evident from the two doublet signals (*J* = 8.2 Hz) at δ 6.76 (H-3′ and H-5′) and δ 7.01 (H-2′ and H-6′). The placement of the 4-hydroxyphenyl moiety at the 1-position was confirmed by the HMBC correlations of H-1 with C-1′ and C-2′/C-6′, H-2 with C-1′, and H-2′/H-6′ with C-1 (see Section 4). The attachment of the phenyl moiety to the 7-position was also confirmed by HMBC correlations of H-6 with C-1″, and H-7 with C-1″ and C-2″/C-6″. The structure of the diarylheptanoid **15** was concluded as 1-(4-hydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-one.

Compound **16** was obtained as prisms, mp 129–130 °C. The HR-TOFMS (ES^+) at *m*/*z* 279.1367 $[M+H]^+$ was compatible with the molecular formula $C_{19}H_{18}O_2$. The IR absorption bands at 3380 and 1673 cm^{-1} revealed the presence of a hydroxyl group and a conjugated keto group, respectively. The ^{13}C NMR of the conjugated keto group appeared at δ 200.1. The presence of α,β,γ,δ-unsaturated keto system was evident from the ^1H NMR signals at δ 6.26 (d, *J* = 15.6 Hz, H-4), δ 6.84 (dd, *J* = 15.6, 10.1 Hz, H-6), δ 6.92 (d, *J* = 15.6 Hz, H-7), and δ 7.33 (partially overlapping signal, H-5). The saturated H-1 and H-2 resonances appeared as a broad singlet at δ 2.86. The attachment of the phenyl group to the 7-position of the heptyl chain was confirmed from the HMBC correlations between H-6 and C-1″, H-7 and C-2″/C-6″, and H-2″/H-6″ and C-7, whereas that of the 4-hydroxyphenyl moiety to the 1-position was confirmed by the HMBC correlations between H-1/H-2 and C-1′, and H-2′/H-6′ and C-1 (see Section 4). The spectroscopic data of **16** were consistent with 1-(4-hydroxyphenyl)-7-phenyl-(4*E*,6*E*)-4,6-heptadien-3-one which has been synthesized recently.[25]

The MeOH extract was chromatographed (Scheme 4) and two phenolic diarylheptanoids were isolated. The first eluted component was shown to be a 3:1 mixture of *S* and *R* enantiomers, **17a** and **17b**, by the already mentioned modified Mosher's method (see Fig. 1). The ^1H NMR data of the two enantiomeric mixture were consistent with the reported data of compound **6**.[26] The second, more polar diarylheptanoid was shown to be **18** by comparison of the ^1H NMR data with those of reported values of compound **7**,[26] and determination of the absolute configuration at C-3 was shown to be *S* by the modified Mosher's method (see Fig. 1). The last and most polar compound was the phloracetophenone glucoside **8** isolated previously from this plant species by our group. The identity of this compound was based on ^1H NMR spectral comparison with the reported values.[16]

It was worth noting that, for diarylheptanoids with one asymmetric carbon at the 3-position and existed as only one enantiomer (compounds **11**, **12**, **14** and **18**), the spatial arrangement of functional groups at the asymmetric carbon was the same (*S* configuration in compounds **11** and **18** and *R* configuration in compounds **12** and **14**). For the existence of two enantiomers (compounds **13a** and **13b**, and compounds **17a** and **17b**), especially those with different ratios of *S* and *R* enantiomers (1:2 for **13a** and **13b**, and 3:1 for **17a** and **17b**), the present data did not permit a reasonable explanation of these observations.

2.2. Chemical modification of diarylheptanoids

A number of diarylheptanoids isolated from *C. comosa* rhizomes have been used for chemical modification to diarylheptanoid analogues for estrogenic evaluations (see Schemes 5 and 6). The identities of the modified compounds were achieved by spectroscopic (IR, ^1H and ^{13}C NMR, and mass spectra) techniques. In order to see the effects of hydrogen bonding on the estrogenic activity of the parent diarylheptanoids, the corresponding methyl ethers and acetates were prepared by standard methods. Starting from the alcohol **11**, and the phenols **15** and **16**, the methyl ether analogues **19**, **20**, and **21** were prepared by reacting the starting compounds with methyl iodide in DMF in the presence of silver oxide to yield, after column chromatographic purification, the required methyl ethers **19**, **20**, and **21** in 58, 86, and 82% yields, respectively (see Schemes 5 and 6). The spectroscopic (IR, ^1H and ^{13}C NMR, and mass spectra) data of **19**, **20** and **21** were in agreement with the structures (see Section 4). The alcohols **11** and **12**, and the phenols **15** and **16** were subjected to acetylation by the standard procedure,

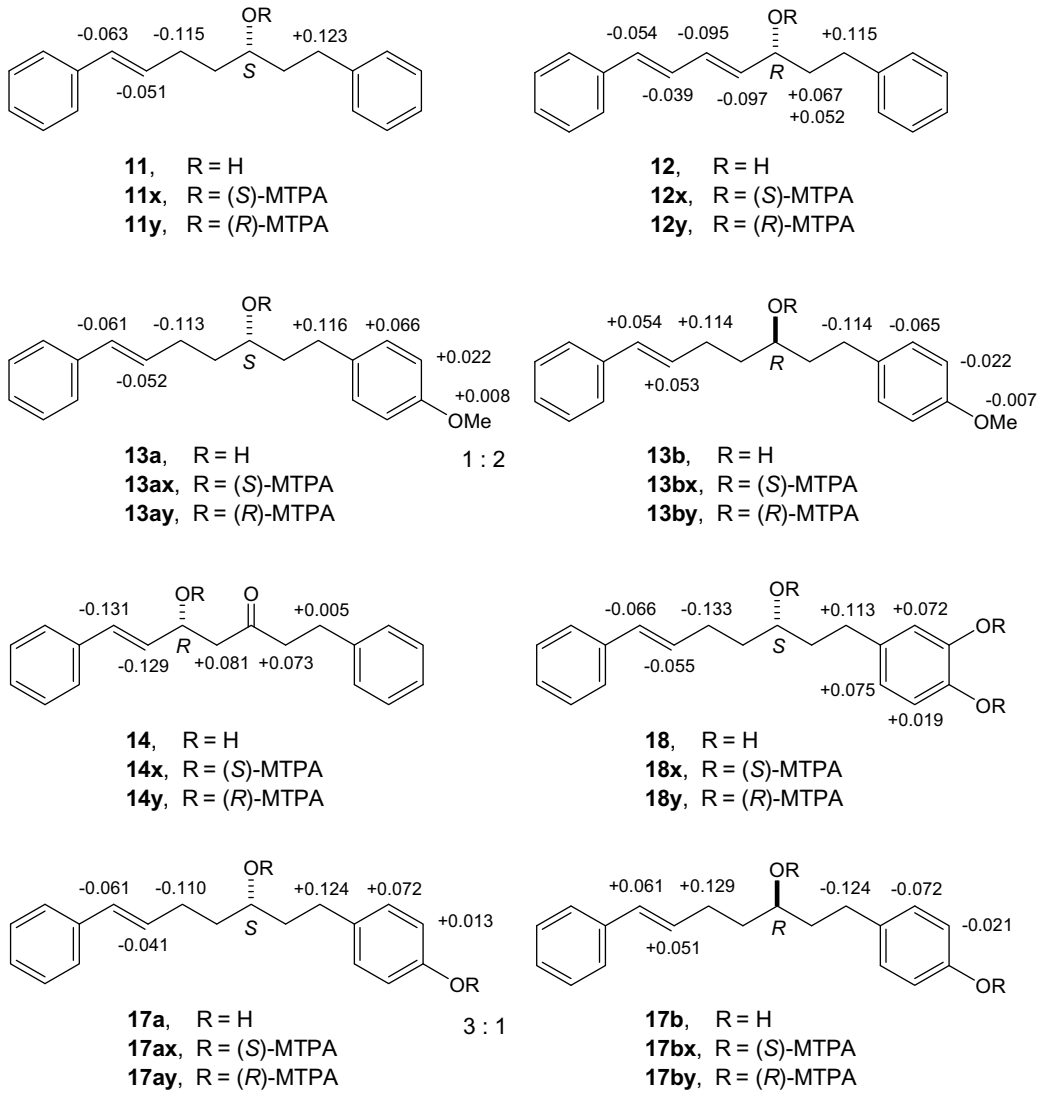
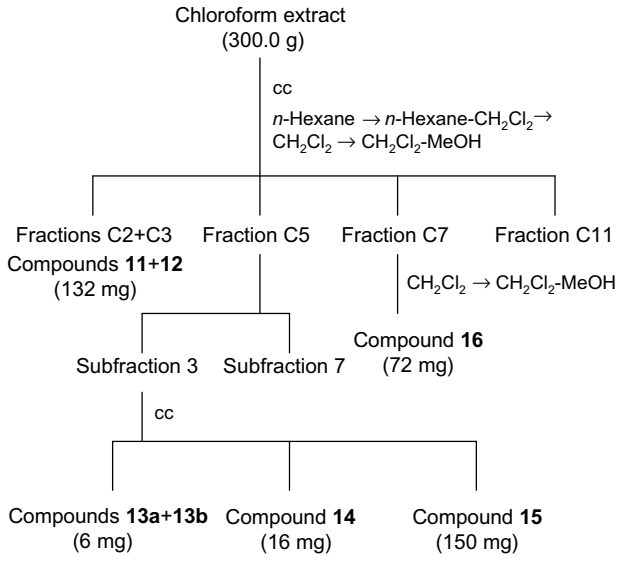


Figure 1. $\Delta\delta = (\Delta\delta_S - \Delta\delta_R)$ values obtained from the MTPA esters of compounds **11**, **12**, **13a** and **13b** mixture, **14**, **17a** and **17b** mixture, and **18** in $CDCl_3$.

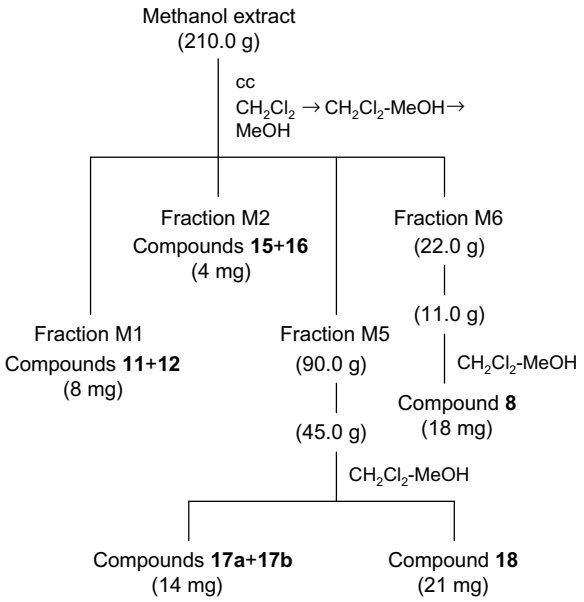


Scheme 3. Chromatographic separation of diarylheptanoids from the chloroform extract.

using acetic anhydride and dry pyridine, to afford the corresponding acetates **22**, **23**, **24** and **25** in 76, 79, 87 and 78% yields, respectively (see Schemes 5 and 6). The ^{1}H NMR data of **22** and **23** were consistent with the reported values,[15,19] whereas the spectroscopic (IR, ^{1}H and ^{13}C NMR and mass spectra) data of **24** and **25** were in agreement with the structures (see Section 4).

The contribution of the olefinic function at the 6-position of the alkyl chain of the diarylheptanoids was evaluated by comparison of the estrogenic activity of the parent compound **11** and its acetate **22**, and the parent compounds **2** and **15** with those of the corresponding dihydro analogues **26**, **28**, **27**, and **29**, which were prepared by hydrogenation reaction using palladium on charcoal as a catalyst (Schemes 5 and 6). The spectroscopic data of **26** and **28** were in agreement with the structures, whereas those of **27** and **29** were consistent with those reported previously.[27]

The natural diarylheptanoid **15** was chemically modified by placing the olefinic function at the 1-position of the molecule (see Scheme 6). Thus, the dihydro analogue **29** was subjected to dehydrogenation with DDQ in benzene at reflux to give the enone **30** in 47% yield. The presence of two doublets, J = 16.1 Hz, at δ 6.60 (H-2) and δ 7.70 (H-1) was compatible with the structure of **30**. The analogue with an olefinic moiety at the 4-position was also prepared. Thus. compound **15** was subjected to allylic hydroxylation



Scheme 4. Chromatographic separation of diarylheptanoids and phloracetophenone glucoside from the methanol extract.

using selenium dioxide in dioxane at reflux to yield the hydroxyl analogue **31**, which was hydrogenated to the corresponding dihydro analogue **32**, which in turn was dehydrated using *p*-toluenesulfonic acid as a catalyst to afford the enone **33** in 20% overall yield from compound **15**. The presence of an α,β-unsaturated double bond in **33** was evident from the ^{1}H NMR signals at δ 6.08 (d, J = 15.9 Hz, H-4) and δ 6.81 (dt, J = 15.9, 7.1 Hz, H-5). The modified compounds were evaluated for their estrogenic activity.

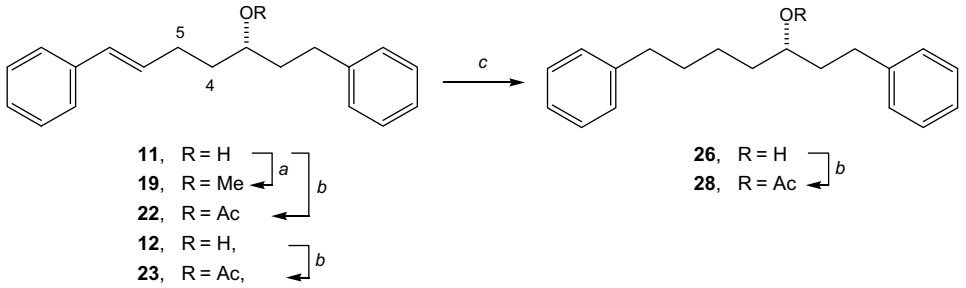
It should be noted that the magnitude of the optical rotations of the diarylheptanoids with two similar, saturated substituent groups is small (for example, −2.6 and −5.2° for compounds **11** and **18**). Without the preparation of the Mosher's salts, they might be mistaken for the existence of racemic mixtures. The magnitude of the optical rotations of the corresponding methyl ethers and acetate derivatives is only slightly higher than the parent alcohols (for example, −5.3 and −7.3° for the methyl ether **19** and the acetate **22** of the parent compound **11**). Larger ester group increased the magnitude of the optical rotation of the parent alcohol, for example, −19.8° and −47.4° for the benzoate and naphthalene-2-carboxylate esters, respectively (experimental data for the preparation of these latter two esters are not shown). The presence of different substituent groups, for example, the unsaturated substituent in compound **12**, led to a significant increase in the optical rotation (−64.2° for compound **12**).

2.3. Structures and estrogenic-activity relationships of diarylheptanoids

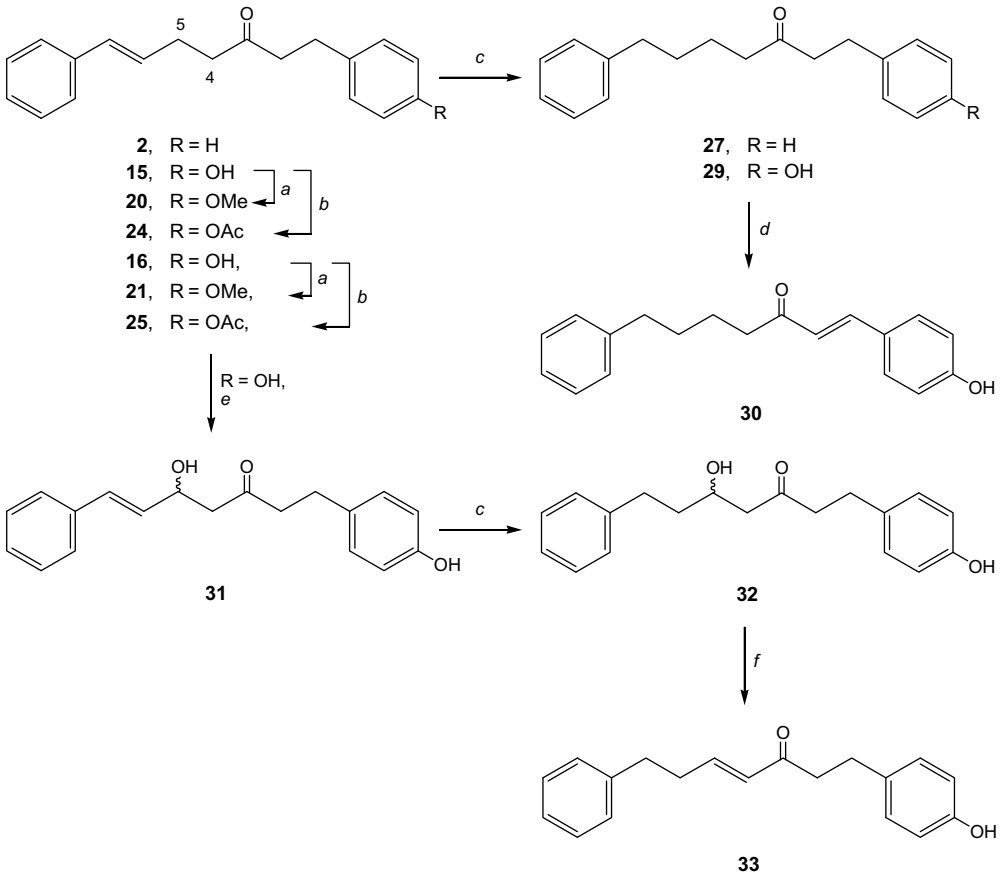
Among various in vitro determinations of estrogenic activity of plant compounds, the analysis of the transcriptional regulation of endogenous estrogen responsive genes in cell lines is crucial for the assessment of classical estrogenic-like action. There are a number of estrogen target genes whose estrogen responsive elements (ERE) have already been identified.[28–30] The estrogen receptors mediate the biological responses to estrogens by binding directly to ERE after forming hormone-receptor complex capable of activating gene transcription. Among these, anti-apoptotic Bcl-xL and estrogen receptor β (ERβ) genes were shown to respond to estrogens at both the transcriptional and the translational levels.[31–34] Although a wide range of concentrations (10^{-6} M to 10^{-10} M) of 17β-estradiol (E2), the most effective form of estrogens, could exert physiological effects, it was shown that 10^{-8} M gave the maximal result.[35] In this study, we measured the alteration of endogenous Bcl-xL and ERβ gene expression in human cervical cancer HeLa cell line by RT-PCR technique after exposure to various diarylheptanoids at the final concentrations of 10^{-8} M as compared to 10^{-8} M E2 which was used as a positive control. The diluent DMSO at the same final concentration of 0.01% was used as a negative control. Cytotoxicity test has been performed to ensure that the selected concentration of the pure compound (10^{-8} M) had no effect on cell viability (data not shown). Our data showed that most of diarylheptanoids investigated exhibited estrogenic activity by inducing gene expression although with different levels. The estrogenic activity of diarylheptanoids is expressed in percentage relative to the reference compound, E2, as summarized in Table 1. The explanation for the estrogenic activity of diarylheptanoids has not yet been established. However, we believed that the estrogenic activity could possibly be due to the ability of this class of compounds to adjust the alignment of the molecule to bind to the estrogen receptor.

Compound **9** is a polyconjugated olefin and is the only diarylheptanoid without oxygen function on the heptyl chain. It exhibited moderately high estrogenic activity (78% for Bcl-xL and 76% for ERβ) comparing to that of E2 (100%). The unconjugated ketone **2** also showed moderately high activity (74% for Bcl-xL and 85% for ERβ). The corresponding conjugated ketone **10**, however, was much less active (44% for Bcl-xL and 34% for ERβ). The result indicated that, in the case of the keto analogue, addition of one more extra double bond led to significant decrease in activity.

Addition of a phenolic hydroxyl group to the *p*-position of the phenyl group at the 1-position of the heptyl chain of compound **2** gave rise to the ketone **15**. Compound **15** exhibited the highest estrogenic activity (79% for Bcl-xL and 91% for ERβ) among the diarylheptanoids. Interestingly, addition of a phenolic hydroxyl group to the *p*-position of the phenyl group attached to the 1-position of the heptyl chain of compound **10** to the conjugated ketone



Scheme 5. Reagents: (a) MeI, Ag_2O, DMF; (b) Ac_2O, pyridine; (c) H_2, Pd–C, EtOH.



Scheme 6. Reagents and conditions: (a) MeI, Ag_2O, DMF; (b) Ac_2O, pyridine; (c) H_2, Pd–C, EtOH; (d) DDQ, benzene, reflux; (e) SeO_2, dioxane, reflux; (f) *p*-TsOH, benzene, 50 °C.

Table 1
Relative transcriptional activation activity of diarylheptanoids and genistein to 17β-estradiol (which was set as 100%) at the same concentration (10^{-8} M) on Bcl-xL and ERβ genes in HeLa cell line

Compound	Bcl-xL	ERβ
17β-Estradiol (E2)	100	100
Genistein	72.85 ± 6.84	75.51 ± 2.37
2	74.30 ± 0.54	85.45 ± 3.54
9	77.50 ± 3.54	76.46 ± 2.12
10	43.97 ± 8.23	33.72 ± 5.66
11	52.85 ± 2.91	42.48 ± 3.54
12	62.51 ± 9.19	75.42 ± 7.07
15	78.51 ± 0.37	90.56 ± 2.83
16	74.54 ± 0.62	87.75 ± 8.48
17	57.73 ± 7.58	52.50 ± 9.19
18	45.98 ± 7.36	51.50 ± 7.78
19	72.50 ± 0.71	74.97 ± 9.90
20	71.40 ± 0.57	58.51 ± 3.55
21	76.77 ± 3.64	72.00 ± 7.06
22	71.06 ± 9.70	75.50 ± 9.19
23	47.96 ± 3.19	58.36 ± 3.33
24	75.30 ± 4.96	84.00 ± 8.48
25	49.82 ± 9.79	68.45 ± 1.95
26	40.00 ± 9.90	37.03 ± 3.58
27	42.18 ± 1.20	57.50 ± 10.61
28	55.00 ± 7.07	58.97 ± 2.83
30	74.41 ± 8.36	70.33 ± 9.43
33	47.08 ± 10.71	50.86 ± 3.08

Means of intensities ± SD after normalization with those of internal standard are shown.

16 resulted in 2- to 3-fold increase in activity. The increment of estrogenic activity in going from the non-phenolic compound **2** to the phenolic compound **15**, especially from compound **10** to **16**, clearly was due to the influence of the extra phenolic hydroxyl group in **15** and **16**. Comparison of the structures of **15** and **16** with that of E2, the additional phenolic hydroxyl group could possibly act as either the 3- or 17-hydroxyl group of E2, and it might exert its binding ability to the receptor in similar fashion to that of E2.

Compound **11**, the hydroxyl analogue of the ketone **2**, on the other hand, was much less active than compound **2** (53% for Bcl-xL and 42% for ERβ). The finding implied that the polar hydroxyl group on the alkyl chain caused the decrease in estrogenic activity. This was further confirmed by the relatively less active alcohol **17** (58% for Bcl-xL and 53% for ERβ) comparing with the highly active corresponding ketone **15**. However, the same trend could not be applied to the alcohol **12**, which was more active than the ketone **10** (63% for Bcl-xL and 75% for ERβ). It should be noted that the presence of an extra hydroxyl group on the same aromatic ring as in compound **18** did not improve its activity (see Table 1).

To see whether it was the steric factor or electronic factor that made the alcohol **11** less active than the corresponding ketone **2**, the methyl ether **19** and the acetate **22** were prepared from the parent compound **11**. It was found that significant increase in activity was observed in both the methyl ether **19** and the acetate **22** (73% for Bcl-xL and 75% for ERβ, and 71% for Bcl-xL and 76% for ERβ, respectively). The results indicated that it was the electronic factor that caused decrease in estrogenic activity of the alcohol **11**. The hydrogen-bond forming ability of the hydroxyl group to the in-appropriate position of the receptor might lead to decrease in activity. The higher estrogenic activity of the ketone **2** than the corresponding alcohol **11** could possibly be due to the fact that the former functional group is located at a suitable position for the receptor to form a strong hydrogen bonding with the keto function of the diarylheptanoid. However, the same explanation could not

be applied to the dienol **12** and its acetate **23**, where the latter exhibited lower activity. The conflicting results on the low activity of the alcohol **11** and the high activity of the alcohol **12** need additional postulation. The lower activity of compound **11** than that of compound **12** could result from the too flexible heptyl chain of the former, while the additional conjugated double bond in **12** lowered the free rotation of the chain, so that the alignment of diarylheptanoid molecule **12** in the receptor was optimized. The lower activity of the conjugated dienone **10** than that of the non-conjugated enone **2** could possibly be due to the extended conjugation of the former resulted in a too rigid molecule to accommodate itself easily to the receptor. The explanation was further supported by the fact that, instead of a significant increase in activity in similar fashion to that in going from **11** to **12**, the activity of the conjugated dienone **16** was only comparable to the non-conjugated enone **15**.

In order to see whether the high estrogenic activity of the diarylheptanoids **15** and **16** was due to the presence of free hydroxyl group, compounds **15** and **16** were subjected to methylation and acetylation to the respective methyl ethers **20** and **21**, and the acetates **24** and **25**. As expected, the methyl ethers and the acetates were not more active than the parent compounds; some of them were even less active than their parent compounds (see Table 1). The results have demonstrated that the free phenolic hydroxyl group in the diarylheptanoids **15** and **16** contributed to high estrogenic activity in similar manner to that occurred in E2.

Next, we would also like to see whether the olefinic function adjacent to the aromatic ring attached to the 7-position contributed to estrogenic activity of the diarylheptanoids. Compounds **11**, **2** and **22** were hydrogenated to the corresponding dihydro analogues **26**, **27**, and **28** and it was found that all the saturated analogues were significantly less active than their parent compounds (see Table 1). The assay results indicated that the olefinic function conjugated with the 7-phenyl group contributed to high estrogenic activity.

We would also like to further investigate whether placement of the double bond function at the 1-position, instead of the 6-position, would affect estrogenic activity. The enone **30** was therefore synthesized and it was found that this analogue exhibited lower activity (74% for Bcl-xL and 70% for ERβ) than the parent compound **15** (79% for Bcl-xL and 91% for ERβ). Moreover, we have also synthesized the isomeric ketone **33** and this conjugated ketone was even much less active than compound **30** (47% for Bcl-xL and 51% for ERβ).

The results indicated that the diarylheptanoids **2**, **9**, **15**, **16**, **19**, **21**, **22** and **24** exhibited estrogenic activity comparable to the phytoestrogen genistein. Some of these diarylheptanoids (compounds **2**, **15**, and **16**) showed even higher activity than genistein. Compound **15** has been selected as a structure lead for further structural modification.

3. Conclusion

From the rhizomes of *Curcuma comosa* Roxb., three new diarylheptanoids, a 1:2 mixture of (3*S*)- and (3*R*)-1-(4-methoxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol (**13a** and **13b**) and 1-(4-hydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-one (**15**), together with two synthetically known diarylheptanoids 1,7-diphenyl-(1*E*,3*E*,5*E*)-1,3,5-triene (**9**) and 1-(4-hydroxyphenyl)-7-phenyl-(4*E*,6*E*)-4,6-heptadien-3-one (**16**), and nine known diarylheptanoids, **2**, **8**, **10**–**12**, **14**, a 3:1 mixture of **17a** and **17b**, and **18**, were isolated. The absolute stereochemistry of the isolated alcohols has also been determined using the modified Mosher's method. Compounds **2**, **11**, **12**, **15** and **16** were chemically modified for biological evaluation. The isolated diarylheptanoids and the modified analogues were subjected to estrogenic activity evaluation. Although all naturally occurring diarylheptanoids and their chemically modified analogues exhibited lower estrogenic activity than the natural hormone 17β-estradiol, some of them showed high activity. Induction of gene transcription by diarylheptanoids of both Bcl-xL and ERβ genes confirmed the novel activity of these compounds. The study on structure-activity relationship has led to a conclusion that, based on the transcriptional activation of both estrogenic targets, Bcl-xL and ERβ gene expression, the structural features for a diarylheptanoid from *C. comosa* to exhibit high estrogenic activity are the presence of an olefinic function conjugated with the aromatic ring at the 7-position, a keto group at the 3-position, and a phenolic hydroxyl group at the *p*-position of the aromatic ring attached to the 1-position of the heptyl chain to exhibit high estrogenic activity. The saturated heptyl-chain analogues, the 3-hydroxy analogues, and the analogues with an olefinic function at the 1- and 4-positions were relatively less active. Evaluations of these diarylheptanoids in vivo system are undergoing to establish their biological activities.

4. Experimental

4.1. General

Melting points were determined using an Electrothermal melting point apparatus and were uncorrected. IR spectra were recorded in KBr or as neat on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer operating at 400 and 100 MHz, respectively. Mass spectra were obtained using a Finnigan LC-Q mass spectrometer. High resolution mass spectra were obtained using a Bruker micrOTOF mass spectrometer. Unless indicated otherwise, column chromatography and TLC were carried out using Merck silica gel 60 (finer than 0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were detected under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

4.2. Plant material

The rhizomes of *C. comosa* were collected from Kampaengsaen district, Nakhon Pathom province, Thailand, in January, 2005. A voucher specimen (Apichart Suksamrarn, No. 052) is deposited at the Faculty of Science, Ramkhamhaeng University.

4.3. Extraction and isolation

The fresh rhizomes (80 kg) were sliced and oven-dried at 40 °C. The pulverized rhizome (10.4 kg) was extracted successively with *n*-hexane, $CHCl_3$, and MeOH in a Soxhlet extraction apparatus to yield the hexane (350.0 g), $CHCl_3$ (770.1 g), and MeOH (322.4 g) extracts, respectively (see Scheme 1). The hexane extract (100.0 g) was chromatographed (Merck silica gel, 0.063–0.200 mm, see Scheme 2), using a gradient of hexane, hexane–$CHCl_3$, $CHCl_3$, and $CHCl_3$–MeOH, to give 6 fractions (H1–H6). Fraction H2 (11.55 g) was chromatographed, using hexane, hexane–CH_2Cl_2, and CH_2Cl_2as eluent, to yield compound (**9**)[17,18] (20 mg) as an off-white solid. Fractions H3 and H4 (23.88 g) were combined and chromatographed, eluting with hexane–$CHCl_3$, $CHCl_3$, and $CHCl_3$–MeOH to afford 15 subfractions.

Subfraction **6** (87 mg) was chromatographed to give two impure compounds, which upon repeated column chromatography, yielded compound (**2**)[15] (20 mg) as colorless oil, and **10**[19,20] (8 mg) as pale yellow needles from MeOH, mp 62–63 °C. Subfraction **7** (8.18 g) was chromatographed, using similar eluting solvent system, to give compound **11** (310 mg), together with compound **12** contaminated with compound **11** (86 mg). Compound **11** was obtained as colorless needles from CH_2Cl_2–hexane, mp 46–48 °C (lit.[19] 47–49 °C), $[\alpha]_D^{27}$ − 2.6° (EtOH,*c* 0.30). Repeated column chro-

matography of compounds **11** and **12** mixture afforded pure **12**[15] (60 mg) which was recrystallized from CH_2Cl_2–hexane as white powder, mp 79–80 °C (lit.[19] 78–79 °C), $[\alpha]_D^{27} - 64.2^\circ$ (EtOH, *c* 0.30).

The $CHCl_3$ extract (300.0 g) was chromatographed (Merck silica gel, 0.063–0.200 mm, see Scheme 3), using a gradient of hexane, hexane–CH_2Cl_2, CH_2Cl_2, and CH_2Cl_2–MeOH (5% increment in the polar solvent for 250 mL of each proportion) to give 11 combined fractions (C1–C11).

Fractions C2 and C3 contained additional mixture of **11** and **12** (132 mg). Fraction C5 was chromatographed using CH_2Cl_2–MeOH under gradient condition to give seven combined subfractions. Subfraction 3, eluted by pure CH_2Cl_2, was further subjected to repeated column chromatography to yield a mixture of two enantiomers **13a** and **13b** (6 mg), compound **14** (16 mg, $[\alpha]_D^{27} + 3.4^\circ$ (EtOH, *c* 0.25), and compound **15** (150 mg). Fraction C7, upon repeated column chromatography using gradient elution with CH_2Cl_2 and CH_2Cl_2–MeOH, afforded compound **16** (72 mg).

4.3.1. Compounds 13a and 13b mixture

Colorless viscous oil; $[\alpha]_D^{27} + 2.4^\circ$ (EtOH,*c* 0.23); IR (neat) ν_{max}: 3331, 3026, 2945, 2915, 2831, 1610, 1512, 1453, 1439, 1336, 1302, 1243, 1180, 1081, 1035, 971 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.64 (m, 2H, H-4), 1.74 (m, 2H, H-2), 2.31 (m, 2H, H-5), 2.61 (m, 1H, H-1a), 2.73 (m, 1H, H-1b), 3.68 (m, 1H, H-3), 3.78 (s, 3H, OMe), 6.20 (dt, *J* = 15.8, 6.8 Hz, 1H, H-6), 6.39 (br d, *J* = 15.8 Hz, 1H, H-7), 6.81 (d, *J* = 8.2 Hz, 2H, H-3′, H-5′), 7.10 (d, *J* = 8.2 Hz, 2H, H-2′, H-6′), 7.16–7.32 (m, 5H, H-2″-H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.2 (C-5), 31.0 (C-1), 37.0 (C-4), 39.3 (C-2), 55.2 (OMe), 70.8 (C-3), 113.8 (C-3′, C-5′), 125.9 (C-2″, C-6″), 126.9 (C-4″), 128.2, 128.4 (C-3″, C-5″), 129.2 (C-2′, C-6′), 130.26, 130.27 (C-6, C-7), 134.0 (C-1′), 137.5 (C-1″), 157.7 (C-4′); HMBC correlations: H-1 (C-2, C-3, C-1′, C-2′/C-6′), H-2 (C-1, C-4, C-1′), H-3 (C-1, C-5), H-4 (C-2, C-3, C-5, C-6), H-5 (C-3, C-4, C-6, C-7), H-6 (C-4, C-5, C-7, C-1″), H-7 (C-5, C-6, C-1″, C-2″/C-6″), OMe (C-4′), H-2′/H-6′ (C-1, C-3′/C-5′, C-4′), H-3′/H-5′ (C-1′, C-4′); ESMS (+ve): *m*/*z* 297 $[M+H]^+$, 615 $[2M+Na]^+$; HR-TOFMS (ES^+): *m*/*z* 297.1839 $[M+H]^+$; calcd for $C_{20}H_{24}O_2$+H, 297.1849.

4.3.2. Compound 15

Colorless viscous oil; IR (neat) ν_{max}: 3360, 3025, 2919, 2860, 1697, 1611, 1595, 1514, 1445, 1365, 1263, 1224, 1173, 967, 832 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.46 (dt, *J* = 7.0, 6.8 Hz, 2H, H-5), 2.56 (t, *J* = 6.8 Hz, 2H, H-4), 2.72 (t, *J* = 7.3 Hz, 2H, H-2), 2.84 (t, *J* = 7.3 Hz, 2H, H-1), 6.14 (dt, *J* = 15.9, 6.7 Hz, 1H, H-6), 6.37 (d, *J* = 15.9 Hz, 1H, H-7), 6.76 (br d, *J* = 8.2 Hz, 2H, H-3′, H-5′), 7.01 (br d, *J* = 8.2 Hz, 2H, H-2′, H-6′), 7.16–7.33 (m, 5H, H-2″-H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 27.0 (C-5), 28.8 (C-1), 42.5 (C-4), 44.6 (C-2), 115.3 (C-3′, C-5′), 125.9 (C-2″, C-6″), 127.0 (C-4″), 128.4 (C-3″, C-5″), 128.7 (C-6), 129.3 (C-2′, C-6′), 130.7 (C-7), 132.7 (C-1′), 137.3 (C-1″), 154.0 (C-4′), 210.1 (C-3); HMBC correlations: H-1 (C-2, C-3, C-1′, C-2′/C-6′), H-2 (C-1, C-3, C-4, C-1′), H-4 (C-2, C-3, C-5, C-6), H-5 (C-3, C-4, C-6, C-7), H-6 (C-4, C-5, C-7, C-1″), H-7 (C-5, C-1″, C-2″/C-6″), H-2′/H-6′ (C-1, C-3′/C-5′), H-3′/H-5′ (C-1′); ESMS (-ve): *m*/*z* 279 $[M-H]^-$; 559 $[2M-H]^-$; HR-TOFMS (ES^+): *m*/*z* 281.1525 $[M+H]^+$; calcd for $C_{19}H_{20}O_2$+H, 281.1536.

4.3.3. Compound 16

Prisms from EtOAc–hexane, mp 129–130 °C (lit.[25] 129.3–130.0 °C). IR (KBr) ν_{max}: 3380, 3026, 2928, 1673, 1638, 1618, 1595, 1513, 1445, 1368, 1219, 1175, 1100, 1009, 829, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.86 (br s, 4H, H-1, H-2), 6.26 (d, *J* = 15.6 Hz, 1H, H-4), 6.72 (d, *J* = 8.4 Hz, 2H, H-3′, H-5′), 6.84 (dd, *J* = 15.6, 10.1 Hz, 1H, H-6), 6.92 (d, *J* = 15.6 Hz, 1H, H-7), 7.06 (d, *J* = 8.4 Hz, 2H, H-2′, H-6′), 7.25–7.35 (m, 3H, H-3″-H-5″), 7.33 (partially overlapping signal, 1H, H-5), 7.45 (br d, *J* = 7.1 Hz, 2H, H-2″, H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.4 (C-1), 42.5 (C-2), 115.3 (C-3′, C-5′), 126.5 (C-6), 127.2 (C-2″, C-6″), 128.8 (C-3″, C-5″), 129.2, 129.3, 129.4 (C-4, C-2′, C-6′, C-4″), 133.0 (C-1′), 135.9 (C-1″), 141.6 (C-7), 143.0 (C-5), 154.0 (C-4′), 200.1 (C-3); HMBC correlations: H-1/H-2 (C-3, C-1′), H-4 (C-2, C-3, C-6), H-5 (C-3, C-7), H-6 (C-5, C-1″), H-7 (C-5, C-2″/C-6″), H-2′/H-6′ (C-1, C-4′), H-3′/H-5′ (C-1′, C-4′), H-2″/H-6″ (C-7); ESMS (+ve): *m*/*z* 279 $[M+H]^+$, 301 $[M-Na]^+$, 579 $[2M+Na]^+$; HR-TOFMS (ES^+): *m*/*z* 279.1367 $[M+H]^+$; calcd for $C_{19}H_{18}O_2$+H, 279.1380.

The MeOH extract (210.0 g) was chromatographed (Merck silica gel, 0.063–0.200 mm, see Scheme 4), using a gradient CH_2Cl_2, CH_2Cl_2–MeOH, and MeOH, to give six combined fractions (M1–M6). Fraction M1 contained a small quantity (8 mg) of compounds **11** and **12**, whereas fraction M2 constituted a small quantity (4 mg) of compounds **15** and **16** mixture.

A half portion (45.0 g) of fraction M5 was chromatographed, using ingredient elution with CH_2Cl_2-MeOH, to yield a mixture of two enantiomers **17a** and **17b** (14 mg), mp 94–95 °C (from CH_2Cl_2–hexane) (lit.[26] 94–95 °C), $[\alpha]_D^{26} + 1.73^\circ$ (MeOH, *c* 0.17), and the ^1H NMR data of which were identical to the reported values of compound **6**.[26] Further elution with increasing proportion of MeOH afforded compound **18** (21 mg), mp 100–101 °C (from CH_2Cl_2–hexane) (lit.[16] 99–100 °C), $[\alpha]_D^{28} - 5.2^\circ$ (EtOH,*c* 0.41), and the ^1H NMR data of this compound were identical to those reported for compound **7**.[26] A half portion (11.0 g) of fraction M6 was similarly subjected to column chromatography and, after repeated column chromatography, the glucoside **8**[16] (18 mg), mp 222–224 °C, was resulted.

4.4. Determination of C-3 absolute configuration of diarylheptanoids

To a solution of the diarylheptanoid **11** (2.1 mg, 0.0078 mmol) in dry pyridine (100 μL) was added (*R*)-(−)-MTPA chloride (15 μL) at 10 °C and the mixture was stirred for 5 min. Stirring continued at ambient temperature and the completion of reaction was monitored by TLC. Two milliliters of *n*-hexane was added to the reaction mixture and the hexane-soluble part was subjected to flash column chromatography using hexane and 3% EtOAc/hexane as eluting solvent to give the (*S*)-MTPA ester **11x** (3.1 mg). The procedure was repeated, but using (*S*)-(+)-MTPA chloride in place of (*R*)-(−)-MTPA chloride, to yield the (*R*)-MTPA ester **11y** (4.5 mg). The ^1H NMR spectra of **11x** and **11y** were recorded in $CDCl_3$; the chemical shift differences of the proton resonances between the (*S*)-MTPA ester **11x** and the (*R*)-MTPA ester **11y** were calculated and the results are summarized in Figure 1.

Following the above procedure, the absolute configurations of compounds **12**, **13a** and **13b**, **14**, **17a** and **17b**, and **18** were determined and the results are summarized in Figure 1.

4.5. Chemical modifications of diarylheptanoids

The procedures for chemical modifications of the diarylheptanoids **11**, **12**, **15** and **16** are summarized in Schemes 5 and 6.

4.5.1. (3*S*)-1,7-Diphenyl-3-methoxy-(6*E*)-6-heptene (19)

To a solution of compound **11** (100 mg, 0.376 mmol) in DMF (5 mL) were added methyl iodide (0.7 mL, d = 2.27 g/mL, 11.28 mmol) and silver oxide (784 mg, 3.38 mmol), and the mixture was stirred at ambient temperature for 24 h. Water (30 mL) was added and the mixture was extracted with EtOAc (3 × 40 mL). The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by column chromatography under isocratic condition using hexane/EtOAc (10:1) to afford compound **19** (61 mg, 58%) as colorless oil; $[\alpha]_D^{27} - 5.3^\circ$ (EtOH, *c* 0.29); IR (neat) ν_{max}: 3025, 2929, 2856, 1600, 1494, 1452, 1182, 1096, 965, 742,

696 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.60–1.76 (m, 2H, H-4), 1.76–1.92 (m, 2H, H-2), 2.22–2.33 (m, 2H, H-5), 2.60–2.80 (m, 2H, H-1), 3.27 (m, 1H, H-3), 3.38 (s, 3H, OMe), 6.23 (dt, J = 15.8, 6.8 Hz, 1H, H-6), 6.41 (d, J = 15.8 Hz, 1H, H-7), 7.15–7.25 (m, 4H, H-2′, H-4′, H-6′, H-4″), 7.25–7.38 (m, 6H, H-3′, H-5′, H-2″, H-3″, H-5″, H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.7 (C-5), 31.5 (C-1), 33.0 (C-4), 35.2 (C-2), 56.4 (OMe), 79.5 (C-3), 125.7 (C-4″), 125.9 (C-2″, C-6″), 126.8 (C-4′), 128.3 and 128.4 (C-2′, C-3′, C-5′, C-6′, C-3″, C-5″), 130.0 (C-7), 130.5 (C-6), 137.7 (C-1″), 142.3 (C-1′); ESMS (+ve): m/z 281 $[M+H]^+$; HR-TOFMS (ES^+): m/z 281.1886 $[M+H]^+$; calcd for $C_{20}H_{24}O$+H, 281.1900.

4.5.2. 1-(4-Methoxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-one (20)

Compound **15** (20 mg, 0.071 mmol) was subjected to methylation in the same manner to the preparation of **19** from **11**, except that the reaction time was only 2 h, to give, after usual column chromatographic purification, the product **20** (18 mg, 86%) as colorless needles (from $CHCl_3$–hexane), mp 69 °C; IR (KBr) ν_{max}: 3033, 2947, 2929, 2832, 1704, 1610, 1512, 1510, 1460, 1438, 1413, 1373, 1301, 1278, 1242, 1180, 1092, 1036, 969, 750 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.45 (dt, J = 7.1, 6.9 Hz, 2H, H-5), 2.54 (t, J = 6.9 Hz, 2H, H-4), 2.70 (t, J = 7.5 Hz, 2H, H-2), 2.83 (t, J = 7.5 Hz, 2H, H-1), 3.75 (s, 3H, OMe), 6.14 (dt, J = 15.8, 6.8 Hz, 1H, H-6), 6.36 (d, J = 15.8 Hz, 1H, H-7), 6.79 (br d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.07 (br d, J = 8.5 Hz, 2H, H-2′, H-6′), 7.19–7.32 (m, 5H, H-2″-H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.9 (C-5), 28.8 (C-1), 42.3 (C-4), 44.5 (C-2), 55.1 (OMe), 113.8 (C-3′, C-5′), 125.9 (C-2″, C-6″), 126.9 (C-4″), 128.3 (C-3″, C-5″), 128.7 (C-6), 129.1 (C-2′, C-6′), 130.6 (C-7), 132.9 (C-1′), 137.3 (C-1″), 157.8 (C-4′), 209.1 (C-3); ESMS (+ve): m/z 317 $[M+Na]^+$, 611 $[2M+Na]^+$; HR-TOFMS (ES^+): m/z 295.1691 $[M+H]^+$; calcd for $C_{20}H_{22}O_2$+H, 295.1693.

4.5.3. 1-(4-Methoxyphenyl)-7-phenyl-(4*E*,6*E*)-4,6-heptadien-3-one (21)

Compound **16** (25 mg, 0.09 mmol) was subjected to methylation in the same manner to the preparation of **20** from **15** to give, after usual column chromatographic purification, the product **21** (22 mg, 82%) as colorless oil; IR (neat) ν_{max}: 2928, 1707, 1612, 1512, 1450, 1247, 1178, 1034, 827, 752, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.84–2.94 (m, 4H, H-1, H-2), 3.77 (s, 3H, OMe), 6.26 (d, J = 15.4 Hz, 1H, H-4), 6.82 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 6.84 (dd, J = 15.5, 10.0 Hz, 1H, H-6), 6.92 (d, J = 15.5 Hz, 1H, H-7), 7.12 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 7.28 (dd, J = 15.4, 10.1 Hz,, 1H, H-5), 7.28–7.38 (m, 3H, H-3″-H-5″), 7.45 (br d, J = 7.1 Hz, 2H, H-2″, H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.3 (C-1), 42.6 (C-2), 55.2 (OMe), 113.9 (C-3′, C-5′),126.6 (C-6), 127.2 (C-2″, C-6″), 128.8 (C-3″, C-5″), 129.1 (C-4″), 129.2 (C-2′, C-6′), 129.5 (C-4), 133.3 (C-1′), 136.0 (C-1″), 141.3 (C-7), 142.6 (C-5), 157.9 (C-4′), 199.5 (C-3); ESMS (+ve): m/z 293 $[M+H]^+$, 607 $[2M+Na]^+$; HR-TOFMS (ES^+): m/z 293.1531 $[M+H]^+$; calcd for $C_{20}H_{20}O_2$+H, 293.1536.

4.5.4. (3*S*)-3-Acetoxy-1,7-diphenyl-(6*E*)-6-heptene (22)

To a solution of compound **11** (50 mg, 0.187 mmol) in pyridine (4 mL) was added acetic anhydride (0.3 mL, d = 1.081 g/mL, 3.15 mmol), and the mixture was stirred at ambient temperature for 3 h. Water (30 mL) was added and the mixture was extracted with EtOAc (3× 40 mL). The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by column chromatography (silica gel, 0.063–0.20 mm) under isocratic condition using hexane/EtOAc (10:1) to yield compound **22** (44 mg, 76%) as colorless oil; $[\alpha]_D^{28}$ − 7.3° (EtOH, c 0.26); The spectroscopic (IR, ^1H NMR and MS) data were consistent with the reported data of compound **1** isolated earlier from this plant species.[15]

4.5.5. (3*R*)-3-Acetoxy-1,7-diphenyl-(4*E*,6*E*)-4,6-heptadiene (23)

Compound **12** (25 mg, 0.0.094 mmol) was acetylated in the same manner described for the preparation of **22** from **11** to afford compound **23** (23 mg, 79%) as colorless oil; $[\alpha]_D^{27}$ + 12.3° (EtOH, c 0.30). The spectroscopic (IR, ^1H NMR, and MS) data were consistent with the reported data of the acetate derivative of compound **4** isolated from other plant species.[19]

4.5.6. 1-(4-Acetoxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-one (24)

Compound **15** (20 mg, 0.071 mmol) was acetylated in the same manner described for the preparation of **22** from **11** to afford compound **24** (20 mg, 87%) as colorless oil; IR (neat) ν_{max}: 3407, 3029, 2924, 2855, 1760, 1713, 1639, 1505, 1447, 1370, 1198, 1015 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.27 (s, 3H, OAc), 2.46 (dt, J = 7.2, 6.9 Hz, 2H, H-5), 2.55 (t, J = 7.2 Hz, 2H, H-4), 2.73 (t, J = 7.6 Hz, 2H, H-2), 2.88 (t, J = 7.6 Hz, 2H, H-1), 6.15 (dt, J = 15.7, 6.8 Hz, 1H, H-6), 6.37 (d, J = 15.8 Hz, 1H, H-7), 6.95 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.16 (br d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.16–7.32 (m, 5H, H-2″-H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.0 (OCO*Me*), 27.0 (C-5), 29.0 (C-1), 42.4 (C-4), 44.3 (C-2), 121.4 (C-3′, C-5′), 126.0 (C-2″, C-6″), 127.0 (C-4″), 128.4 (C-3″, C-5″), 128.7 (C-6), 129.2 (C-2′, C-6′), 130.8 (C-7), 137.3 (C-1″), 138.6 (C-1′), 148.9 (C-4′), 169.5 (*C*OCOMe), 208.8 (C-3); ESMS (+ve): m/z 345 $[M+Na]^+$, 667 $[2M+Na]^+$; HR-TOFMS (ES^+): m/z 323.1644 $[M+H]^+$; calcd for $C_{21}H_{22}O_3$+H, 323.1642.

4.5.7. 1-(4-Acetoxyphenyl)-7-phenyl-(4*E*,6*E*)-4,6-heptadien-3-one (25)

Compound **16** (15 mg, 0.054 mmol) was acetylated in the same manner described for the preparation of **22** from **11** to afford compound **25** (14 mg, 78%) as colorless oil; IR (KBr) ν_{max}: 3433, 3063, 3035, 2925, 2855, 1759, ca. 1700 (shoulder), 1636, 1610, 1508, 1450, 1370, 1218, 1197, 1167, 1017, 913 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.27 (s, 3H, OAc), 2.86–2.98 (m, 4H, H-1, H-2), 6.26 (d, J = 15.4 Hz, 1H, H-4), 6.85 (dd, J = 15.5, 10.3 Hz, 1H, H-6), 6.93 (d, J = 15.6 Hz, 1H, H-7), 6.98 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.20 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.26–7.37 (m, 3H, H-3″-H-5″), 7.33 (obscured signal, 1H, H-5), 7.45 (br d, J = 7.1 Hz, 2H, H-2″, H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.1 (CO*Me*), 29.4 (C-1), 42.2 (C-2), 121.4 (C-3′, C-5′), 126.6 (C-6), 127.2 (C-2″, C-6″), 128.8 (C-3″, C-5″), 129.2 (C-4″), 129.3 (C-2′, C-6′), 129.4 (C-4), 135.9 (C-1″), 138.8 (C-1′), 141.4 (C-7), 142.7 (C-5), 148.9 (C-4′), 169.6 (*C*OMe), 199.1 (C-3); ESMS (+ve): m/z 663 $[2M+Na]^+$; HR-TOFMS (ES^+): m/z 321.1493 $[M+H]^+$; calcd for $C_{21}H_{20}O_3$+H, 321.1485.

4.5.8. (3*S*)-1,7-Diphenylheptan-3-ol (26)

Compound **11** (50 mg, 0.187 mmol) in EtOH (5 mL) was hydrogenated at atmospheric pressure for 20 min, with 10% Pd-C (20 mg, 0.018 mmol) as a catalyst. The mixture was filtered through Celite; the solid was washed with EtOAc and the filtrate was concentrated in vacuo. The residue was purified by column chromatography under isocratic condition using hexane/EtOAc (80:20) to afford compound **26** (49 mg, 97%) as colorless needles (from EtOAc–hexane), mp 59–60 °C; $[\alpha]_D^{27}$ + 8.0° (EtOH, c 0.27); IR (KBr) ν_{max}: 3416, 2927, 2856, 1627, 1550, 1497, 1452, 1382, 1020, 749, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.30–1.54 (m, 4H, H-5, H-6), 1.61 (m, 2H, H-4), 1.74 (m, 2H, H-2), 2.62 (t, J = 7.7 Hz, 2H, H-7), 2.65 (m, 1H, H-1a), 2.77 (m, 1H, H-1b), 3.60 (m, 1H, H-3), 7.10–7.20 (m, 6H, H-2′, H-4′, H-6′, H-2″, H-4″, H-6″), 7.22–7.30 (m, 4H, H-3′, H-5′, H-3″, H-5″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.2 (C-5), 31.4 (C-4), 32.0 (C-1), 35.8 (C-7), 37.4 (C-6), 39.0 (C-2), 71.2 (C-3), 125.6 and 125.7 (C-4′, C-4″), 128.2 and 128.3 (C-2′, C-3′, C-5′, C-6′, C-2″, C-3″, C-5″, C-6″), 142.1 (C-1′), 142.5 (C-1″); ESMS: m/z 291 $[M+Na]^+$; HR-TOFMS (ES^+): m/z 291.1717 $[M+Na]^+$; calcd for $C_{19}H_{24}O$+Na, 291.1719.

4.5.9. 1,7-Diphenylheptan-3-one (27)

Compound **2** (10 mg, 0.039 mmol) was hydrogenated in the same manner to that of compound **11** to afford compound **27** (9 mg, 95%) as colorless oil; IR (neat) ν_{max}: 3027, 2930, 2858, 1712, 1603, 1495, 1453, 1408, 1371, 1099, 1026, 747, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.58 (m, 4H, H-5, H-6), 2.37 (t, J = 6.5 Hz, 2H, H-4), 2.58 (t, J = 6.9 Hz, 2H, H-7), 2.68 (t, J = 7.8 Hz, 2H, H-2), 2.87 (t, J = 7.8 Hz, 2H, H-1), 7.10–7.20 (m, 6H, H-2′, H-4′, H-6′, H-2″, H-4″, H-6″), 7.22–7.30 (m, 4H, H-3′, H-5′, H-3″, H-5″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.3 (C-5), 29.7 (C-1), 30.8 (C-6), 35.6 (C-7), 42.7 (C-4), 44.2 (C-2), 125.7 and 126.0 (C-4′, C-4″), 128.2, 128.3 and 128.4 (C-2′, C-3′, C-5′, C-6′, C-2″, C-3″, C-5″, C-6″), 141.0 (C-1′), 142.1 (C-1″), 209.9 (C-3); ESMS (+ve): m/z 267 $[M+H]^+$, 533 $[2M+H]^+$; HR-TOFMS (ES^+): m/z 267.1748 $[M+H]^+$; calcd for $C_{19}H_{22}O$+H, 267.1743.

4.5.10. (3*S*)-3-Acetoxy-1,7-diphenylheptane (28)

Compound **26** (20 mg, 0.075 mmol) was acetylated in the same manner described for the preparation of **23** from **12** to afford compound **28** (20 mg, 86%) as colorless oil; $[\alpha]_D^{27}$ − 4.2° (EtOH, *c* 0.48); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.30 (m, 2H) and 1.57 (m, 4H) (H-4, H-5, H-6), 1.80 (m, 2H, H-2), 2.00 (s, 3H, OAc), 2.60 (m, 4H, H-1, H-7), 4.90 (m, 1H, H-3), 7.12–7.19 (m, 6H, H-2′, H-4′, H-6′, H-2″, H-4″, H-6″), 7.23–7.29 (m, 4H, H-3′, H-5′, H-3″, H-5″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.1 (OCO*Me*), 24.8 (C-5), 31.2 (C-4), 31.7 (C-7), 33.9 (C-6), 35.7 (C-1, C-2), 73.8 (C-3), 125.6 and 125.8 (C-4′, C-4″), 128.2 and 128.3 (C-2′, C-3′, C-5′, C-6′, C-2″, C-3″, C-5″, C-6″), 141.6 (C-1′), 142.4 (C-1″), 170.8 (O*C*OMe); ESMS (+ve): m/z 333 $[M+Na]^+$; HR-TOFMS (ES^+): m/z 333.1825 $[M+Na]^+$; calcd for $C_{21}H_{26}O_2$+Na, 333.1825.

4.5.11. 1-(4-Hydroxyphenyl)-7-phenyl-(1*E*)-1-hepten-3-one (30)

Compound **15** (40 mg, 0.143 mmol) was hydrogenated as described for the preparation of compound **19** from **11** to afford compound **29** (38 mg, 94%). A portion (30 mg, 0.106 mmol) of compound **29** was heated with DDQ (120 mg, 0.528 mmol) in benzene (2 mL) at reflux for 3 h to give, after column chromatography (hexane/EtOAc, 3:1), compound **30** (14 mg, 47%).

4.5.11.1. Compound 29. Colorless viscous oil; IR (neat) ν_{max}: 3390, 3025, 2932, 2859, 1702, 1614, 1515, 1452, 1372, 1226, 1172, 1099, 1030, 828, 748, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.58 (m, 4H, H-5, H-6), 2.39 (t, J = 6.8 Hz, 2H, H-4), 2.58 (t, J = 7.0 Hz, 2H, H-7), 2.67 (t, J = 7.5 Hz, 2H, H-2), 2.81 (t, J = 7.5 Hz, 2H, H-1), 6.74 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.01 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.10–7.19 (m, 3H, H-3″, H-4″, H-5″), 7.20–7.27 (m, 2H, H-2″, H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.3 (C-5), 28.9 (C-1), 30.9 (C-6), 35.6 (C-7), 42.8 (C-4), 44.4 (C-2), 115.3 (C-3′, C-5′), 125.7 (C-4″), 128.2 (C-3″, C-5″), 128.3 (C-2″, C-6″), 129.3 (C-2′, C-6′,), 133.0 (C-1′), 142.1 (C-1″), 153.9 (C-4′), 210.6 (C-3); ESMS (−ve): m/z 281 $[M-H]^-$, 562 $[2M-H]^-$; HR-TOFMS (ES^+): m/z 283.1686 $[M+H]^+$; calcd for $C_{19}H_{22}O_2$+H, 283.1693.

4.5.11.2. Compound 30. Pale yellow amorphous solid; IR (KBr) ν_{max}: 3379, 3058, 3024, 2938, 2927, 2864, 1676, 1585, 1516, 1438, 1371, 1268, 1170, 1104, 1076 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.53–1.75 (m, 4H, H-5, H-6), 2.63 (t, J = 7.0 Hz, 2H, H-7), 2.68 (t, J = 6.8 Hz, 2H, H-4), 6.60 (d, J = 16.1 Hz, 1H, H-2), 6.88 (d, J = 8.6 Hz, 2H, H-3′, H-5′), 7.13–7.19 (m, 3H, H-3″-H-5″), 7.22–7.30 (m, 2H, H-2″, H-6″), 7.42 (d, J = 8.6 Hz, 2H, H-2′, H-6′), 7.70 (d, J = 16.1 Hz, 1H, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.3 (C-5), 31.0 (C-6), 35.7 (C-7), 40.5 (C-4), 116.2 (C-3′, C-5′), 123.3 (C-2), 125.8 (C-4″), 126.5 (C-1′), 128.3 and 128.4 (C-2″, C-3″, C-5″, C-6″), 130.5 (C-2′, C-6′), 142.1 (C-1″), 143.9 (C-1), 159.1 (C-4′), 202.3 (C-3); ESMS (−ve): m/z 279 $[M-H]^-$, 559 $[2M-H]^-$; HR-TOFMS (ES^+): m/z 281.1536 $[M+H]^+$; calcd for $C_{19}H_{20}O_2$+H, 281.1536.

4.5.12. 1-(4-Hydroxyphenyl)-7-phenyl-(4*E*)-4-hepten-3-one (33)

Compound **15** (60 mg, 0.143 mmol) and SeO_2 (60 mg, 0.541 mmol) in dioxane (3 mL) were refluxed for 4 h. The evaporated mixture was chromatographed (isocratic elution with hexane/EtOAc, 3:1) to give 1-(4-hydroxyphenyl)-7-phenyl-(4*E*)-4-hepten-5-ol-3-one (**31**) (27 mg), which was subsequently hydrogenated as described for the preparation of compound **26** from **11** to afford compound **32**, ESMS (+ve): m/z 299 $[M+H]^+$, 619 $[2M+Na]^+$; HR-TOFMS (ES^+): m/z 299.1638 $[M+H]^+$; calcd for $C_{19}H_{22}O_3$+H, 299.1642, which in turn was subjected to dehydration using *p*-TsOH (10 mg) in benzene (3 mL) at 50 °C for 1 h to give, after column chromatography under isocratic condition using hexane/EtOAc (3:1), compound **33** (12 mg) as colorless viscous oil. The overall yield of **33** from compound **15** was 20%.

4.5.12.1. 1-(4-Hydroxyphenyl)-7-phenyl-(4*E*)-4-hepten-5-ol-3-one (31). Amorphous solid; IR (KBr) ν_{max}: 3381, 2917, 1698, 1597, 1514, 1412, 1310, 1233, 1105, 1018, 816, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.68 (d, J = 5.9 Hz, 2H, H-4), 2.75 (t, J = 6.8 Hz, 2H, H-2), 2.84 (t, J = 6.8 Hz, 2H, H-1), 4.72 (dt, J = 6.1, 5.9 Hz, 1H, H-5), 6.15 (dd, J = 15.9, 6.1 Hz, 1H, H-6), 6.59 (d, J = 15.9 Hz, 1H, H-7), 6.72 (d, J = 8.3 Hz, 2H, H-3′, H-5′), 7.02 (d, J = 8.3 Hz, 2H, H-2′, H-6′), 7.20–7.35 (m, 5H, H-2″-H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.6 (C-1), 45.4 (C-2), 49.3 (C-4), 68.5 (C-5), 115.3 (C-3′, C-5′), 126.4 (C-2″, C-6″), 127.7 (C-4″), 128.5 (C-3″, C-5″), 129.4 (C-2′, C-6′), 130.0 (C-6), 130.4 (C-7), 132.7 (C-1′), 136.1 (C-1″), 153.9 (C-4′), 210.3 (C-3); ESMS (+ve): m/z 615 $[2M+Na]^+$; HR-TOFMS (ES^+): m/z 319.1297 $[M+Na]^+$; calcd for $C_{19}H_{20}O_3$+Na, 319.1305.

4.5.12.2. 1-(4-Hydroxyphenyl)-7-phenyl-(4*E*)-4-hepten-3-one (33). Amorphous solid; IR (KBr) ν_{max}: 3375, 3026, 2927, 2860, 1658, 1620, 1515, 1451, 1367, 1227, 1109, 975, 829, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.50 (dt, J = 7.3, 7.1 Hz, 2H, H-6), 2.74 (t, J = 7.3 Hz, 2H, H-7), 2.78 (d, J = 6.1 Hz, 2H, H-2), 2.82 (d, J = 6.1 Hz, 2H, H-1), 4.58 (br s, 1H, OH), 6.08 (d, J = 15.9 Hz, 1H, H-4), 6.72 (d, J = 8.2 Hz, 2H, H-3′, H-5′), 6.81 (dt, J = 15.9, 7.1 Hz, 1H, H-5), 7.02 (d, J = 8.2 Hz, 2H, H-2′, H-6′), 7.15 (d, J = 7.2 Hz, 2H, H-2″, H-6″), 7.19 (br t, J = 7.2 Hz, 1H, H-4″), 7.27 (br t, J = 7.2 Hz, 2H, H-3″, H-5″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.2 (C-1), 34.0 (C-6), 34.3 (C-7), 41.9 (C-2), 115.2 (C-3′, C-5′), 126.1 (C-4″), 128.2 (C-2″, C-6″), 128.4 (C-3″, C-5″), 129.4 (C-2′, C-6′), 130.6 (C-4), 133.3 (C-1′), 140.6 (C-1″), 146.2 (C-5), 153.8 (C-4′), 199.6 (C-3); ESMS (+ve): m/z 615 $[2M+Na]^+$; HR-TOFMS (ES^+): m/z 281.1530 $[M+H]^+$; calcd for $C_{19}H_{20}O_2$+H, 281.1536.

4.6. Assay for estrogenic activity in human cervical cancer HeLa cell line

The estrogenic activity of diarylheptanoids was investigated in a cervical carcinoma HeLa (ATCC) cell line using RT-PCR assay. HeLa cells were grown in Dulbecco minimal essential medium (DMEM) (GIBCO Invitrogen Corporation, USA) supplemented with 5% FBS, 100 U/mL of penicillin G, 100 μg/mL of streptomycin, and 2.5 μg/mL of amphotericin B in 5% CO_2 incubator at 37 °C for 24 h, and then subcultured (at 7.5×10^5 cells/plate) onto phenol red-free DMEM supplemented with 5% charcoal stripped FBS (GIBCO Invitrogen) for another 24 h to remove serum steroids. Cells were washed with PBS once and exposed to non-toxic final concentration of 10^{-8} M of pure compounds in the same phenol red-free medium for 24 h. Cultures were also treated with freshly prepared 17β-estradiol (solubilized with DMSO and diluted with the med-

ium) at the final concentration of 10^{-8} M or 0.01%DMSO alone which were then used as positive and negative controls, respectively. After 24 h treatment, cells were collected and total RNA was purified using TRIzol Reagent (Pacific Science Co., Ltd). cDNA was synthesized by Superscript™ II RNase H⁻ Reverse-Transcriptase kit (Invitrogen). The primers used included primers for Bcl-xL (sense 5′-CTGGTGGTTGACTTTCTCTC-3′ and anti-sense 5′-GAGTTCATTCACTACCTGTTC-3′), ERβ (sense 5′-CGCTAGAACA CACCTTACCTG-3′ and anti-sense 5′-TTCACCATTCCCACTTCGTA-3′) and their internal standard, HPRT (sense 5′-TGTGATGAAGGAGATGGGAGG-3′ and anti-sense 5′-AAGCTTGCGACCTTGACCATCT-3′). PCR amplification was performed using an automated PTC-200® Peltier Thermal Cycler (MJ Research, Massachusetts, USA) in 25 μL of PCR containing 2 mM $MgCl_2$, 0.2 mM each dNTPs, 10 mM Tris–HCl, pH 8.3 and 50 mM KCl, 0.2 pmol of each primer and 0.5 U of Taq polymerase (Invitrogen). The profiles consisted of 28 cycles at 94 °C for 30 s, 60 °C for 30 s (Bcl-xL and HPRT) or 32 cycles at 94°C for 30 s, 56 °C for 30 s (ERβ) and at 72°C for 30 s, except for 7 min in the last cycle. The RT-PCR products of Bcl-xL (368 bp), ERβ (320 bp), and HPRT (333 bp) were detected and analyzed using Gel-Doc 2000 (Bio-Rad, USA). After normalization, results were expressed as means of three independent experiments.

Acknowledgments

This work was supported by the research team strengthening grant of the National Center for Genetic Engineering and Biotechnology. Support from the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry is gratefully acknowledged. We are grateful to Dr. Thaya Jenjittikul, Department of Biology, Mahidol University, Bangkok, for identifying the *Curcuma* plant species from different parts of Thailand. We are indebted to Suttiporn Pikulthong, Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, for recording the high resolution mass spectra.

References and notes

1. Dixon, R. A.; Ferreira, D. *Phytochemistry* **2002**, *60*, 205.
2. Beck, V.; Rohr, U.; Jungbauer, A. *J. Steroid Biochem. Mol. Biol.* **2005**, *94*, 499.
3. Kuiper, G. G.; Lemmen, J. G.; Carisson, B.; Corton, J. C.; Safe, S. H.; van der Saag, P. T.; van der Burg, B.; Gustafsson, J. A. *Endocrinology* **1998**, *139*, 4252.
4. Kostelac, D. S.; Rechkemmer, G.; Briviba, K. *J. Agric. Food Chem.* **2003**, *51*, 7632.
5. Fritz, S. E.; Coward, L.; Wang, L.; Lamartiniere, C. A. *Carcinogenesis* **1998**, *19*, 2151.
6. Lamartiniere, C. A. *Am. J. Clin. Nutr.* **2000**, *71*, 1705.
7. Hwang, J. L.; Hodis, H. N.; Sevanian, A. *J. Agric. Food Chem.* **2001**, *49*, 308.
8. MerzDemlow, B. E.; Duncan, A. M.; Wangen, K. E.; Xu, X.; Carr, T. P.; Phipps, W. R.; Kurzer, M. S. *Am. J. Clin. Nutr.* **2000**, *71*, 1462.
9. Alekel, D. L.; StGermain, A.; Pererson, C. T.; Hanson, K. B.; Stewart, J. W.; Toda, T. *Am. J. Clin. Nutr.* **2000**, *72*, 844.
10. Cos, P.; De Bruyne, T.; Apers, S.; Vanden Berghe, D.; Pieters, L.; Vlietinck, A. J. *Planta Med.* **2003**, *69*, 589.
11. Ricketts, M. L.; Moore, D. D.; Banz, W. J.; Mezei, O.; Shay, N. F. *Nutr. Biochem.* **2005**, *16*, 321.
12. Piyachaturawat, P.; Ercharuporn, S.; Suksamrarn, A. *Int. J. Pharmacog.* **1995**, *33*, 334.
13. Piyachaturawat, P.; Ercharuporn, S.; Suksamrarn, A. *Asia Pacific J. Pharmacol.* **1995**, *10*, 121.
14. Piyachaturawat, P.; Gansar, R.; Suksamrarn, A. *Int. J. Pharmacog.* **1996**, *34*, 174.
15. Jurgens, T. M.; Frazier, E. G.; Schaeffer, J. M.; Jones, T. E.; Zink, D. L.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J. *J. Nat. Prod.* **1994**, *57*, 230.
16. Suksamrarn, A.; Eiamong, S.; Piyachaturawat, P.; Byrne, L. T. *Phytochemistry* **1997**, *45*, 103.
17. Tolbert, L. M.; Ogle, M. E. *J. Am. Chem. Soc.* **1990**, *112*, 9519.
18. Thiele, G.; Streitwieser, A. *J. Am. Chem. Soc.* **1994**, *116*, 446.
19. Claeson, P.; Panthong, A.; Tuchinda, P.; Reutrakul, V.; Kanjanapothi, D.; Taylor, W. C.; Santisuk, T. *Planta Med.* **1993**, *59*, 451.
20. Kuronayagi, M.; Noro, T.; Fukushima, S.; Aiyama, R.; Ikuta, A.; Itokawa, H.; Morita, M. *Chem. Pharm. Bull.* **1983**, *31*, 1544.
21. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
22. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
23. Ali, M. S.; Tezuka, Y.; Awale, S.; Banskota, A. H.; Kadota, S. *J. Nat. Prod.* **2001**, *64*, 289.
24. Sirat, H. M.; Rahman, A. A.; Itokawa, H.; Morita, H. *Planta Med.* **1996**, *62*, 188.
25. Baranovsky, A.; Schmitt, B.; Fowler, D. J.; Schneider, B. *Synth. Commun.* **2003**, *33*, 1019.
26. Suksamrarn, A.; Eiamong, S.; Piyachaturawat, P.; Charoenpiboonsin, J. *Phytochemistry* **1994**, *36*, 1505.
27. Itokawa, H.; Morita, H.; Midorikawa, I.; Aiyama, R.; Morita, M. *Chem. Pharm. Bull.* **1985**, *33*, 4889.
28. Perillo, B.; Sasso, A.; Abbondanza, C.; Palumbo, G. *Mol. Cell. Biol.* **2000**, *20*, 2890.
29. Li, L. C.; Yeh, C. C.; Nojima, D.; Dahiya, R. *Biochem. Biophy. Res. Commun.* **2000**, *275*, 682.
30. Sohrabji, F.; Miranda, R. C. G.; Toran-Allerand, C. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11110.
31. Amantea, D.; Russo, R.; Bagetta, G.; Corasaniti, M. T. *Pharmacol. Res.* **2005**, *52*, 119.
32. Lovekamp-Swan, T.; Glendenning, M. L.; Schreihofer, D. A. *Brain Res.* **2007**, *1159*, 54.
33. Pike, C. *J. Neurochem.* **1999**, *72*, 1552.
34. Nair, H. B.; Luthra, R.; Kirma, N.; Liu, Y. G.; Flowers, L.; Evans, D.; Tekmal, R. R. *Cancer Res.* **2005**, *65*, 11164.
35. Jun, S. S.; Chen, Z.; Pace, M. C.; Shaul, P. W. *J. Clin. Invest.* **1998**, 176.