2013

Parviflorals A-F, trinorcadalenes and bis-trinorcadalenes from the roots of Decaschistia parviflora

Nikhom Wongsa
Khon Kaen University, nw174@uowmail.edu.au

Somdej Kanokmedhakul
Khon Kaen University

Kwanjai Kanokmedhakul
Khon Kaen University

Palangpon Kongsaeree
Mahidol University

Samran Prabpai
Mahidol University

See next page for additional authors

Publication Details

Wongsa, N., Kanokmedhakul, S., Kanokmedhakul, K., Kongsaeree, P., Prabpai, S. & Pyne, S. G. (2013). Parviflorals A-F, trinorcadalenes and bis-trinorcadalenes from the roots of Decaschistia parviflora. Phytochemistry, 95 368-374.

Parviflorals A-F, trinorcadalenes and bis-trinorcadalenes from the roots of Decaschistia parviflora

Abstract
Trinorcadalenes, parviflorals A and B (1 and 2), and four bis-trinorcadalenes, parviflorals C–F (3–6), together with the known trinorcadalenes, syriacusins A (7) and C (8), scopoletin (9) and stigmasterol were isolated from roots of Decaschistia parviflora. Their structures were established by spectroscopic techniques. The CD spectra of the bis-trinorcadalenes (3–6) established their absolute configurations at the binaphthyl axis. Further, structure 6 was confirmed by a single-crystal X-ray crystallographic analysis. Compounds 2 and 6 showed antimalarial activity against Plasmodium falciparum with IC50 values of 11.45 and 6.85 μM, respectively. Compounds 1, 5, 7 and 8 also exhibited weak antifungal activity against Candida albicans, with IC50 values in the range of 37.03–197.68 μM. Compounds 1–3 and 5–8 showed weak antimycobacterial activity against Mycobacterium tuberculosis with MIC values in the range of 54.30–192.13 μM. In addition, several of these compounds possessed cytotoxicity towards the cancer cell lines, KB, MCF7 and NCI-H187 with IC50 values in the range of 2.20–90.09 μM.

Keywords
trinorcadalenes, bis, f, roots, parviflorals, decaschistia, parviflora, CMMB

Publication Details
Wongsa, N., Kanokmedhakul, S., Kanokmedhakul, K., Kongsaeree, P., Prabpai, S. & Pyne, S. G. (2013). Parviflorals A-F, trinorcadalenes and bis-trinorcadalenes from the roots of Decaschistia parviflora. Phytochemistry, 95 368-374.

Authors
Nikhom Wongsa, Somdej Kanokmedhakul, Kwanjai Kanokmedhakul, Palangpon Kongsaeree, Samran Prabpai, and Stephen G. Pyne

Parviflorals A-F, trinorcadalenes and bis-trinorcadalenes from the roots of *Decaschistia parviflora*

Nikhom Wongsa,[1] Somdej Kanokmedhakul,[1] Kwanjai Kanokmedhakul,[*1] Palangpon Kongsaeree,[2,3] Samran Prabpai,[2,3] and Stephen G. Pyne[4]

[1]Natural Products Research Unit, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

[2]Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

[3]Center for Excellence in Protein Structure and Function, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

[4]School of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia

[*]Corresponding author. Tel: +66-43-202222-41, ext. 12243. Fax: +66-43-202373.

E-mail: kwanjai@kku.ac.th.

ABSTRACT

Two new trinorcadalenes, parviflorals A and B (**1** and **2**), and four new bis-trinorcadalenes, parviflorals C−F (**3**−**6**), together with two known trinorcadalenes, syriacusins A (**7**) and C (**8**), and two known compounds scopoletin (**9**) and stigmasterol have been isolated from the roots of *Decaschistia parviflora*. Their structures were established by spectroscopic techniques. The CD spectra of the bis-trinorcadalenes (**3**−**6**) identified their absolute configurations at the binaphthyl axis. Further, the structure of **6** was confirmed by a single-crystal X-ray crystallographic analysis. Compounds **2** and **6** showed antimalarial activity against *Plasmodium falciparum* with IC_{50} values of 11.45 and 6.85 μM, respectively. Compounds **1**, **5**, **7** and **8** exhibited weak antifungal activity against *Candida albicans* with IC_{50} values in the range of 37.03−197.68 μM. Compounds **1**−**3** and **5**−**8** showed weak antimycobacterial activity against *Mycobacterium tuberculosis* with MIC values in the range of 54.30−192.13 μM. In addition, several of these compounds possessed cytotoxicity towards the cancer cell lines, KB, MCF7 and NCI-H187 with IC_{50} values in the range of 2.20−90.09 μM.

Keywords: Decaschistia parviflora, Malvaceae, trinorcadalenes, binaphthyl, parvifloral, cytotoxicity, antimalarial

1. Introduction

Decaschistia parviflora Kurz (Malvaceae) is a small shrub, up to 1 m in height, growing throughout Thailand. It is known as "Thong Phun Dun" or "Chaba Nu" in Thai and the fresh root is used by local people as an embrocation in animals (Pharmaceutical Science, 1996). The genus *Decaschistia* comprises ca. 16 species distributed from India through Southeast Asia to Australia. Five species, *Decaschistia crotonifolia, D. harmadii, D. intermedia, D. siamensis,* and *D. parviflora* have been found in Thailand (Smitinand, 2001). Only *D. crotonifolia* has been investigated phytochemically, resulting in the isolation of three flavonols; gossypitrin, quercimeritrin, and gossypin (Radhakrishniah, 1981). As part of our search for bioactive compounds from Thai plants we found that the crude EtOAc extract from the roots of *D. parviflora* had cytotoxicity against a KB cancer cell line with an IC_{50} value of 6.25 μg/mL. We report herein the isolation, characterization, and bioactivities of the new trinorcadalenes, **1** and **2**, the new binaphthyl derivatives, **3**–**6**, and four known compounds from the root extracts of *D. parviflora*.

2. Results and Discussion

Air-dried roots of *D. parviflora* were extracted sequentially with hexane and EtOAc. The crude extracts were separated by column chromatography and crystallization to yield six new trinorcadalenes, parviflorals A-F (**1**–**6**) and four known compounds, syriacusins A (**7**) and C (**8**) (Yoo et al., 1998), scopoletin (**9**) (Siddiqui et al., 2007), and stigmasterol (Fig. 1). The structures of the known compounds were identified by comparison of their physical and spectroscopic data with those reported in literature. While, the structures of the new compounds were determined on the basis of their spectroscopic data (IR, UV, CD, 1D and 2D NMR, MS) and, in the case of **6**, also by a single-crystal X-ray crystallographic analysis.

Compound **1** was obtained as yellow needles, and its molecular formula, $C_{13}H_{12}O_4$, was determined from HRESITOFMS (observed *m*/*z* 233.0814 $[M + H]^+$), indicating eight degrees of unsaturation. The UV spectrum showed absorption maxima at 223, 253, 281, and 350 nm which indicated a highly conjugated naphthalene system. The IR spectrum showed bands for the presence of a hydroxyl (3332 cm^{-1}), an aromatic carbonyl (1616 cm^{-1}), and an aromatic (1508 cm^{-1}) system. The ^1H NMR spectrum of **1** (Table 1) showed two doublet signals at δ 7.77 and 7.02 for two aromatic protons, both having a coupling constant of 9.2 Hz, and a singlet aromatic signal at δ 7.17. These signals indicated a pentasubstituted naphthalene. A signal at δ 11.30 (s, 1H) which correlated to a carbonyl carbon (δ 199.3) in the HSQC spectrum indicated an aldehyde substituent. The other substituents identified included a methyl group (δ 2.45, s, 3H), a methoxy group (δ 3.91, s, 3H), and two hydroxy groups (1H, singlets at δ 6.75 and 14.21). The HBMC spectrum showed correlations of the most downfield hydroxyl proton at δ 14.21 (2-OH) to C-1 (δ 113.1), C-2 (δ 166.0), and C-3 (δ 119.0); the aldehydic proton (H-9) to C-1, C-2, and C-3; 8-OH (δ 6.75) to C-7, C-8 and C-8a; the methoxy protons to C-7 (δ 145.2); and the methyl protons to C-5, C-6 and C-7. The last correlation indicated the location of the methyl group at C-6 on the naphthalene ring (Fig. 2). The NOESY spectrum of **1** showed correlations between H-3 and H-4, H-5 and H-10, and 7-OMe and H-10. We found that our proposed structure of **1** has been reported as hibicuslide C (colorless oil) from *Hibiscus taiwanensis* (Wu et al., 2005). However, its ^1H NMR spectroscopic data did not correlate well with those of **1** but were identical to those of **7** that we also isolated from this plant. Thus, the structure of hibicuslide C should be revised to that of **7**. On the basis of the above data, compound **1** was determined to be a new trinorcadalene; 2,8-dihydroxy-7-methoxy-6-methyl-1-naphthalenecarbaldehyde and it was named as parvifloral A.

Compound **2** was obtained as pale yellow needles, and its molecular formula, $C_{14}H_{12}O_5$, was determined from HRESITOFMS (observed *m/z* 283.0603 $[M + Na]^+$), indicating nine degrees of unsaturation. The UV spectrum showed absorption maxima at 224, 238, 259, and 346 nm indicated a conjugated naphthalene system. The IR spectrum showed the presence of a hydroxyl (3204 cm^{-1}), a lactone (1722 cm^{-1}), and an aromatic (1634 cm^{-1}) system. The ^1H and ^{13}C NMR spectra of **2** (Table 1) were similar to those of **1**, except for the absence of signals for an aldehydic proton at δ_H 11.30 and the downfield hydroxyl proton. The chemical shifts of the carbons at C-8 (δ 131.3) and C-9 (δ 166.3) were in good agreement with those of the naphthalenecarbolactone, syriacusin C (**8**) (Yoo et al., 1998), except for those at C-4 and C-2 which were substituted by a hydroxy and a methoxy group, respectively. The complete interpretation of the NMR spectroscopic data of **2** was established as a result of extensive DEPT, COSY, HSQC, HMBC, and NOESY experiments (Table 1). Thus, compound **2** was determined to be a new trinorcadalene lactone, and it was named as parvifloral B.

Compound **3** was obtained as a yellow solid, and its molecular formula, $C_{26}H_{22}O_8$, was determined from HRESITOFMS (observed *m/z* 463.1394 $[M + H]^+$), indicating sixteen degrees of unsaturation. The UV spectrum showed absorption maxima at 227, 255, 282, 353, and 391 nm. The IR spectrum showed bands for the presence of a hydroxyl group (3351 cm^{-1}) and an aromatic aldehyde (1624 cm^{-1}). The ^{13}C NMR and DEPT spectra of **3** contained only 13 signals which were attributable to a carbonyl group (δ 199.6), eight sp^2 quaternary (δ 165.8, 145.2, 143.9, 129.6, 127.4, 125.6, 120.8, and 113.4) and two sp^2 methine (δ 136.1 and 119.5) carbons, one methyl (δ 13.4), and one methoxy (δ 61.3) group. Since the ^1H and ^{13}C NMR spectra (Table 2) showed only half the number of resonances expected for a compound having 22 protons and 26 carbons, the structure must be that of a symmetrical dimer. The ^1H and ^{13}C NMR spectra of the two monomeric units were similar to those of **1**, except for the absence of an aromatic proton signal at C-5 which indicated that the two naphthyl units were

connected *via* C-5 and C-5′. Furthermore, the assignment of the absolute configuration of the binaphthyl dimer was concluded to be *R* from an analysis of its CD spectrum. The CD spectrum of **3** showed a positive Cotton effect at 223 nm ($\Delta\varepsilon$ +70.3) and a negative one at 237 nm ($\Delta\varepsilon$ -48.6), similar patterns have been reported for the CD spectra of other *R*-2,2′-homodisubstituted-1,1′-binaphthyls (Di Bari et al., 1999). Therefore, compound **3** was determined to be a new dimer of **1**, and it was named as parvifloral C.

Compound **4** was obtained as light brown needles, and its molecular formula, $C_{26}H_{18}O_8$, was determined from HRESITOFMS (observed *m*/*z* 481.0899 $[M + Na]^+$), indicating eighteen degrees of unsaturation. The UV spectrum showed absorption maxima at 225, 259, and 361 nm. The IR spectrum showed the presence of hydroxyl (3383 cm^{-1}), lactone (1737 cm^{-1}) and aromatic (1626 cm^{-1}) groups. Since the ^1H and ^{13}C NMR spectra (Table 2) showed only half the number of resonances expected for a compound having 18 protons and 26 carbons, the structure of **4** must also be that of a symmetrical dimer. The ^1H NMR resonance signals of **4** were similar to those of **8**, except for the absence of the aromatic signal for H-5, suggesting that the two naphthyl units in **4** were also connected *via* C-5 and C-5′. The absolute configuration of this dimer was deduced to be *S* from analysis of its CD spectrum, which revealed a positive Cotton effect at 230 nm ($\Delta\varepsilon$ +7.6) and a negative one at 225 nm ($\Delta\varepsilon$ -15.0) (Di Bari et al., 1999). Therefore, compound **6** was determined to be a new dimer of **8**, and it was named as parvifloral D.

Compound **5** was obtained as a yellow solid, and its molecular formula, $C_{26}H_{20}O_8$, was determined from HRESITOFMS (observed *m*/*z* 461.1236 $[M+H]^+$), indicating seventeen degrees of unsaturation. The UV spectrum showed absorption maxima at 227, 257, 282, and 361 nm for a naphthalene conjugated system. The IR spectrum showed the presence of hydroxyl (3389 cm^{-1}), lactone (1753 cm^{-1}) and aldehyde (1627 cm^{-1}) groups. An analysis of the ^1H and ^{13}C NMR spectroscopic data of **5** (Table 2), including those obtained using 2D

NMR techniques (COSY, HSQC, HMBC, and NOESY), indicated that the structure of **5** contained two different core structures, which were based on compounds **1** and **8**. The ^{13}C NMR spectrum of **5** showed resonances for 26 carbons, indicating an unsymmetrical dimer. The ^{1}H NMR spectrum of **5** displayed two sets of resonance patterns related to those of **1** and **8**, except for the absence of two aromatic proton signals at C-5 and C-5′. The HMBC spectrum of **5** showed a combination of the correlations found in **1** and **8** (Fig. 3). The absolute configuration of the binaphthyl system was deduced to be *R* from its CD spectrum, which showed a positive Cotton effect at 226 nm ($\Delta\varepsilon$ +9.3) and a negative one at 247 nm ($\Delta\varepsilon$ -3.6) (Di Bari et al., 1999). Therefore, compound **5** was determined to be a dimer of **1** and **8**, and it was named as parvifloral E.

Compound **6** was obtained as yellow needles, and its molecular formula, $C_{27}H_{22}O_9$, was determined from HRESITOFMS (observed *m/z* 513.1139 $[M+Na]^+$), indicating seventeen degrees of unsaturation. The UV spectrum showed absorption maxima at 201, 214, 233, 277, and 399 nm for a naphthalene conjugated system. The IR spectrum showed the presence of hydroxyl (3435 cm^{-1}), ketone (1655 cm^{-1}) and aldehyde (1643 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra indicated 27 carbons which were contributable to three carbonyl groups (δ 198.5, 190.6, and 181.1), fifteen sp^2 quaternary (δ 172.3, 169.2, 163.9, 150.0, 150.0, 148.2, 136.3, 132.5, 130.4, 128.0, 125.1, 121.7, 119.3, 117.1, and 111.1), one sp^3 quaternary (94.0) and three sp^2 methine (δ 134.8, 124.3, and 89.0) carbons and two methyl (δ 11.0 and 10.2), and three methoxy (δ 60.4, 60.0, and 57.2) groups. The ^{1}H NMR spectrum showed three aromatic protons at δ 6.50 (s, H-3′), 7.06 (d, J = 9.2 Hz, H-3), and 6.99 (d, J = 9.2 Hz, H-4). Two downfield singlet resonances at δ 10.22 and 11.06, which correlated to the carbons at δ 190.6 and 198.5, respectively in the HSQC spectrum, revealed the presence of the two aldehyde groups in the molecule. While the two downfield singlet signals at δ 13.02 and 12.65 were assigned to two chelated OH groups that were H-bonded to

the carbonyl oxygens of their nearby aldehyde groups. Three methoxy groups appeared as singlet signals at δ 4.06, 3.90, and 3.88. In addition, the two methyl groups resonated as two singlet signals at δ 1.82 and 1.64. The complete interpretation of the NMR data of **6** (Table 2) was established as a result of extensive DEPT, COSY, HSQC, HMBC and NOESY experiments. The HMBC technique confirmed the structure of **6** from the correlations of: (1) H-3′ to C-1′, C-2′, C-4′ and C-4a′; (2) H-4 to C-2, C-3 and C-8a; (3) H-3 to C-1, C-2 and C-4a; (4) the aldehydic proton (H-9′) to C-1′ and C-8a′; (5) the aldehydic proton (H-9) to C-1 and C-2 and C-3; (6) the methyl protons at δ 1.82 to C-5′, C-6′ and C-7′; (7) the methyl protons at δ 1.64 to C-5, C-6 and C-7; and (8) the methoxy protons at δ 4.06 to C-2′, δ 3.90 to C-7′, and those at δ 3.88 to C-7 (Fig. 4). The absolute configuration of the binaphthyl system was suggested to be *R* from its CD spectrum, which showed a positive Cotton effect at 220 nm ($\Delta\varepsilon$ +4.28) and a negative one at 241 nm ($\Delta\varepsilon$ -0.9) (Di Bari et al., 1999). Finally, the structure of **6** was confirmed by a single-crystal X-ray crystallographic analysis (Fig. 5). Thus, the structure of **6** was deduced as a new unsymmetrical bis-trinorcadalene, and it was named as parvifloral F.

We proposed that compound **6** is derived biosynthetically from an oxidative coupling reaction at C-5 between compound **1** and the hypothetical compound **10** to provide a bis-trinorcadalene in the first step. Subsequent oxidation of the hydroxy group at C-8 followed by a C-O coupling between C-5 and OH-C4′ could then give the dihydrofuran ring of **6** (Fig. 6). Although **10** has not been isolated, it is also proposed as a precursor of compound **2** by oxidation of the C-9 aldehyde to a carboxylic acid and then lactonization with the hydroxy group at C-8 (Fig. 6).

The biological activities of the isolated compounds are shown in Table 3. Compounds **1**−**9** showed cytotoxicity against the cancer cell lines, KB, MCF7 and NCI-H187 with IC_{50} values in the range of 2.20−90.09 μM. Among these, **6** was the most active

compound against the KB cell line with an IC_{50} value of 2.20 μM. Compounds **2** and **6** exhibited antimalarial activity against *Plasmodium falciparum* with IC_{50} values of 11.45 and 6.85 μM, respectively. Compounds **2**, **5**, **7** and **8** showed weak antimycobacterial activity against *Mycobacterium tuberculosis*. In addition, four compounds, **1**, **5**, **7** and **8**, showed weak antifungal activity against *Candida albicans*.

3. Conclusion

Chromatographic separation of the roots of *D. parviflora* led to the isolation of ten compounds. Their structures were determined using spectroscopic techniques as two new trinorcadalenes named as parviflorals A (**1**) and B (**2**), four new bis-trinorcadalenes named as parviflorals C−F (**3**−**6**), along with two known trinorcadalenes, syriacusins A (**7**) and C (**8**), a known coumarin, scopoletin (**9**), and a common phytosterol, stigmasterol. Although the structure of **1** has previously been reported, this is the first report of its structure with the corrected physical and spectroscopic data. In addition, the absolute configuration of the bis-trinorcadalenes (**3**−**6**) were deduced from an analysis of their CD spectra. In addition, the structure of **6** was confirmed by a single-crystal X-ray crystallographic analysis. Finally, the isolated compounds, **1**−**9** were evaluated for their biological activities.

4. Experimental

4.1. General Experimental Procedures

Optical rotations and CD spectra were measured on a JASCO DIP-1000 digital polarimeter and a JASCO J-815 apparatus, respectively. UV spectra were recorded using an Agilent 8453 UV-visible spectrophotometer. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ and CD_3OD on a Varian Mercury Plus 400 spectrometer, using residual solvent as an internal standard ($CHCl_3$;

δ_H 7.26, δ_C 77.0/ MeOH; δ_H 3.30, δ_C 48.7). HRESITOFMS spectra were recorded on Micromass Q-TOF-2 spectrometer. Column chromatography (CC) was carried out on Merck silica gel 60 (230-400 mesh). TLC and reverse phase TLC were performed with precoated Merck silica gel 60 PF_{254} and silica gel RP-18 F_{254s} aluminum sheets, respectively; the spots were visualized under UV light (254 nm and 366 nm) and further by spraying with anisaldehyde then heating until charred.

4.2. Plant Materials

The roots of *D. parviflora* were collected from Mueng District, Khon Kaen Province in November 2008. A plant specimen (voucher number S. Kanokmedhakul-14) was identified by Prof. Pranom Chantaranothai, and was deposited at the herbarium of the Department of Biology, Faculty of Science, Khon Kaen University, Thailand.

4.3. Extraction and isolation

Air-dried roots of *D. parviflora* (6.1 kg) were ground into a powder and then extracted sequentially with hexane and EtOAc (3 x 16 L, three days each) at room temperature. Removal of solvents from each extract under reduced pressure gave the crude hexane (133.1 g) and EtOAc (80.4 g) extracts, respectively.

The crude hexane extract was separated by flash column chromatography (FCC) over silica gel. Elution with a gradient system of EtOAc-hexane and EtOAc-MeOH afforded 5 fractions designated as HF_1−HF_5. Fraction HF_2 was filtered and crystallized from hexane to give colorless needles of stigmasterol (1.60 g). Fraction HF_3 was purified by FCC over silica gel FCC. Eution with EtOAc-hexane (1:4) gave 3 sub-fractions, $HF_{3.1}$-$HF_{3.3}$. The solid from sub-fraction $HF_{3.2}$ was crystallized from hexane to give an additional amount of stigmasterol (204.3 mg). Fraction HF_4 was purified by FCC over silica gel, elution with EtOAc-hexane

(3:7) gave 3 sub-fractions, $HF_{4.1}$−$HF_{4.3}$. The precipitate of sub-fraction $HF_{4.2}$ was crystallized from CH_2Cl_2 to give a yellow solid of **3** (804.2 mg). Sub-fraction $HF_{4.3}$ was purified by CC over silica gel, elution with EtOAc-CH_2Cl_2 (1:9) gave colorless crystals of **9** (9.1 mg).

The crude EtOAc extract was separated by FCC over silica gel. Elution with a gradient system of EtOAc-hexane and EtOAc-MeOH afforded 5 fractions designated as EF_1−EF_5. Fraction EF_2 was purified by FCC over silica gel, elution with CH_2Cl_2 gave 3 sub-fractions, $EF_{2.1}$−$EF_{2.3}$. The precipitate of sub-fraction $EF_{2.2}$ was purified on reverse phase precoated Merck silica gel RP-18 F_{254S} aluminum sheets, elution with MeOH-H_2O (1:1) gave two yellow compounds **1** (16.4 mg) and **7** (21.7 mg), both of which crystallize as yellow needles. The precipitate of sub-fraction $EF_{2.3}$ was purified by crystallization from hexane to give stigmasterol (3.42 g)**.** Fraction EF_3 was purified by FCC over silica gel, elution with EtOAc-hexane (1:4) gave 4 sub-fractions, $EF_{3.1}$−$EF_{3.4}$. Purification of sub-fraction $EF_{3.2}$ by FCC over silica gel and elution with EtOAc-hexane (1:4), gave yellow needles of **6** (15.2 mg) and an additional amount of **3** (3.99 g). Sub-fraction $EF_{3.3}$ was purified by FCC over silica gel, elution with EtOAc-hexane (1:4) gave a yellow solid of **8** (12.3 mg). Sub-fraction $EF_{3.4}$ was purified by FCC over silica gel, elution with EtOAc-CH_2Cl_2 (1:4) gave pale yellow needles of **2** (10.5 mg) and a yellow solid of **5** (72.6 mg). Fraction EF_4 was purified by FCC over silica gel, elution with EtOAc-CH_2Cl_2 (1:9) gave 3 sub-fractions, $EF_{4.1}$-$EF_{4.3}$. Sub-fraction $EF_{4.2}$ was crystallized from CH_2Cl_2-hexane to give an additional amount of **3** (30.1 mg). Sub-fraction $EF_{4.3}$ was crystallized from MeOH-CH_2Cl_2 to give an additional amount of **9** (22.4 mg). Fraction EF_5 was purified by FCC over silica gel FCC, elution with MeOH-CH_2Cl_2 (1:19), gave light brown needles of **4** (14.8 mg).

*4.3.1. Parvifloral A (**1**)*

Yellow needles; mp 158-160 ^{o}C; UV (MeOH) λ_{max} (log ε) 223 (5.08), 253 (4.61), 281 (4.51), 350 (4.20); IR (KBr) ν_{max} 3332, 3064, 2942, 2851, 1616, 1508, 1461, 1419, 1355, 1286, 1151, 1098, 1002, 829 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESITOFMS *m*/*z* 233.0814 $[M + H]^+$ (calcd for $C_{13}H_{12}O_4 + H^+$, 233.0814).

*4.3.2. Parvifloral B (**2**)*

Pale yellow needles; mp 248-250 ^{o}C; UV (MeOH) λ_{max} (log ε) 224 (4.83), 238 (4.77), 259 (5.03), 346 (4.60); IR (KBr) ν_{max} 3204, 3008, 1952, 2854, 1722, 1634, 1531, 1473, 1419, 1383, 1296, 1219, 1052, 958 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESITOFMS *m*/*z* 283.0603 $[M + Na]^+$ (calcd for $C_{14}H_{12}O_5$ + Na, 283.0582).

*4.3.3. Parvifloral C (**3**)*

Yellow solid; mp 181-183 ^{o}C; $[\alpha]^{22}_{D}$ +54.8 (*c* 0.20, EtOH); UV (MeOH) λ_{max} (log ε) 227 (4.78), 255 (4.39), 282 (4.25), 353 (3.93), 391 (3.96); CD (EtOH) nm 223 ($\Delta\varepsilon$ +70.3), 237 ($\Delta\varepsilon$ -48.6); IR (KBr) ν_{max} 3351, 2943, 2856, 1624, 1506, 1464, 1392, 1344, 1282, 1218, 1173, 1104, 999, 833 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESITOFMS *m*/*z* 463.1394 $[M + H]^+$ (calcd for $C_{26}H_{22}O_8 + H^+$, 463.1393).

*4.3.4. Parvifloral D (**4**)*

Light brown needles; decomposed at 295 ^{o}C; $[\alpha]^{22}_{D}$ -18.3 (*c* 0.20, EtOH); UV (MeOH) λ_{max} (log ε) 225 (5.30), 259 (5.18), 361 (4.86); CD (EtOH) 225 ($\Delta\varepsilon$ -15.0), 230 ($\Delta\varepsilon$ +7.6) nm; IR (KBr) ν_{max} 3383, 1992, 2952, 2856, 1737, 1626, 1486, 1386, 1272, 1245, 1149, 1123, 1036, 957 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESITOFMS *m*/*z* 481.0899 $[M + Na]^+$ (calcd for $C_{26}H_{18}O_8$ + Na, 481.0899).

*4.3.5. Parvifloral E (**5**)*

Yellow solid; decomposed at 280 ^{o}C; $[\alpha]^{22}_{D}$ -57.9 (*c* 0.20, EtOH); UV (MeOH) λ_{max} (log ε) 227 (5.07), 257 (4.84), 282 (4.37), 361 (4.48); CD (EtOH) nm 226 ($\Delta\varepsilon$ +9.3), 247 ($\Delta\varepsilon$ -3.6); IR (KBr) ν_{max} 3389, 2992, 2948, 2857, 1753, 1627, 1476, 1393, 1270, 1165, 1144, 1104, 1036, 998, 961, 832 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESITOFMS *m*/*z* 461.1236 $[M + H]^{+}$ (calcd for $C_{26}H_{20}O_8 + H^{+}$, 461.1236).

*4.3.6. Parvifloral F (**6**)*

Yellow needles; mp 240-242 ^{o}C; $[\alpha]^{25}_{D}$ -2.43 (*c* 0.014, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.64), 214 (3.65), 233 (3.60), 277 (3.37), 399 (2.97); CD (EtOH) nm 220 ($\Delta\varepsilon$ +4.9), 241 ($\Delta\varepsilon$ +0.9); IR (KBr) ν_{max} 3435, 2917, 2850, 1655, 1643, 1613, 1571, 1490, 1439, 1376, 1324, 1202, 1166, 1104, 1058, 898 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESITOFMS *m*/*z* 513.1139 $[M + Na]^{+}$ (calcd for $C_{27}H_{22}O_9$ + Na, 513.1162).

*4.3.7. Syriacusin A (**7**)*

Yellow needles; mp 180-182 ^{o}C; UV (MeOH) λ_{max} (log ε) 230 (4.75), 274 (3.75), 365 (4.02); IR (KBr) ν_{max} 3238, 2949, 2921, 2851, 1622, 1509, 1479, 1447, 1293, 1234, 1164, 1060, 924, 814 cm^{-1}; HRESITOFMS *m*/*z* 233.0814 $[M + H]^{+}$ (calcd for $C_{13}H_{12}O_4 + H^{+}$, 233.0814).

*4.3.8. Syriacusin C (**8**)*

Yellow solid; mp 229-231 ^{o}C; UV (MeOH) λ_{max} (log ε) 224 (4.68), 253 (4.61), 377 (4.09); IR (KBr) ν_{max} 3307, 3066, 2956, 2850, 1734, 1642, 1506, 1488, 1342, 1291, 1258, 961, 861 cm^{-1}; HRESITOFMS *m*/*z* 253.0476 $[M + Na]^{+}$ (calcd for $C_{13}H_{10}O_4$ + Na, 253.0477).

4.4. X-ray crystallographic analysis of 6

Orange prism-like crystals of **6** were obtained from a mixture of CH_2Cl_2/MeOH by slow evaporation. X-ray diffraction data were measured on a Bruker-Nonius kappaCCD diffractomer with graphite monochromated MoKα radiation (λ = 0.71073 Å) at 298(2) K. The structure was solved by direct methods by SIR97 (Altomare et al, 1999), and refined with full-matrix least-squares calculations on F^2 using SHELXL-97 (Sheldrick, 2008). All non-hydrogen atoms were refined anisotropically and the hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. Crystallographic data of parvifloral F (**6**) has been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 933196.

3.4.1. X-ray data of ***6****.* $C_{27}H_{22}O_9$, M = 490.46, Monoclinic, dimensions: 0.15 × 0.20 × 0.30 mm, D = 1.423 g/cm^3, space group $P2_1/c$, Z = 4, a = 7.4460 (2), b = 22.8870 (7), c = 14.0200(5) Å, β = 106.565 (1)° V = 2290.1 (1) Å^3, reflections collected/unique: 7263/2820, number of observation [$I > 2\sigma(I)$] 1982, final R indices [$I > 2\sigma(I)$]: R_1 = 0.0791, wR_2 = 0.2090.

4.5. Bioactivity Assays

4.5.1. Antimalarial Assay

Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrugresistant strain), using the method of Trager and Jensen (1967). Quantitative assessment of malarial activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al. (1979). The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in

parasite growth as indicated by the *in vitro* uptake of [3H]-hypoxanthine by *P. falciparum*. The positive control compound was dihydroartemisinin (Table 3).

4.5.2. Antifungal Assay

An antifungal assay was performed against a clinical isolate of *Candida albicans* using a method modified from the soluble formazan assay described by Scudiero et al. (1988). The number of living cells was determined by measuring the absorbance of XTT formazan at 450 nm. The reference substance was amphotericin B (Table 3).

4.5.3. Antimycobacterial assay

Antimycobacterial activity was assessed against *M. tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) (Collins, et al., 1976). The reference substance was streptomycin (Table 3).

4.5.4. Cytotoxicity Assay

Cytotoxicity assays against the human epidermoid carcinoma (KB), human breast cancer (MCF7), and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method as described by Skehan et al. (1990). The reference substances were ellipticine and doxorubicin (Table 3).

Acknowledgements

This research was supported by a grant under the program Strategic Scholarships for Frontier Research Network for the Joint Ph.D. Program Thai Doctoral Degree from the Office of the Higher Education Commission, Thailand. We thank the Center for Innovation in Chemistry (PERCH-CIC) and Udon Thani Rajabhat University for partial support. We are indebted to the Bioassay Research Facility of the National Center for Genetic Engineering

and Biotechnology via the Bioresource Research Network (BRN) for bioactivity tests. P.K. acknowledges the Thailand Research Fund and Mahidol University for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi…../j.phytochem.

References

Altomare, A., Burla, M. C., Camalli, M., Cascarano, G. L., Giacovazzo, C., Guagliardi, A., Moliterni, A. G. G.; Polidori, G.; Spagna, R. 1999. *SIR*97: a new tool for crystal structure determination and refinement. J. Appl. Cryst., 32, 115-119.

Collins, L., Franzblau, S. G., 1997. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob. Agents Chemother. 41, 1004-1009.

Desjardins, R.E., Canfield, C.J., Haynes, J.D., Chulay, J.D., 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. 16, 710-718.

Di Bari, L.; Pescittelli, G.; Salvadori, P. 1999. Conformational study of 2,2´-homosubstituted 1,1´-binaphthyl by mean of UV and CD spectroscopy. J. Am. Chem. Soc. 121, 7998-8004.

Pharmaceutical Sciences, Mahidol University. Saim-Phi-Chacha-Ya-Prug; Amarin Printing and Publishing: Bangkok, 1996; p 98.

Radhakrishniah, M., 1981. Yellow flavonols of *Decaschistia crotonifolia*. J. Indian Chem. Soc. 58, 101.

Scudiero, D.A., Shoemaker, R. H, Paull, K.D., Monks, A., Tierney, S., Nofziger, T.H.,

Currens, M. J., Seniff, D., Boyd, M. R. 1988. Evaluation of a soluble Tetrazolium/Formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines Cancer Res. 48, 4827-4833.

Sheldrick, G. M. *SHELX-97*: Program for X-ray crystal structure solution and refinement; University of Göttingen: Göttingen, Germany, 1997.

Siddiqui, B.S., Sattar, F.A., Ahmad, F., Begum, S., 2007. Isolation and structural elucidation of chemical constituents from the fruits of *Morinda citrilotia* Linn. Arch. Pharm. Res. 30, 919-923.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T, Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer. Inst. 82, 1107-1112.

Smitinand, T. Thai plant names, Revised Edition; Prachachon Co., Ltd.: Bangkok, 2001; pp 174.

Trager, W., Jensen, J.B., 1967. Human malaria parasites in continuous culture. Science 193, 673-675.

Wu, P. L., Wu, T. S., He, C. X., Su, C. H., Lee, K. H., 2005. Constituents from the stems of *Hibiscus taiwanensis*. Chem. Pharm. Bull. 53, 56-59.

Yoo, I.D., Yun, B.S., Lee, I.K., Ryoo, I.J., Choung, D.H., Han, K.H., 1998. Three naphthalenes from root bark of *Hibiscus syriacus*. Phytochemistry 47, 799-802.

List of Figures (1-6)

List of Tables (1-3)

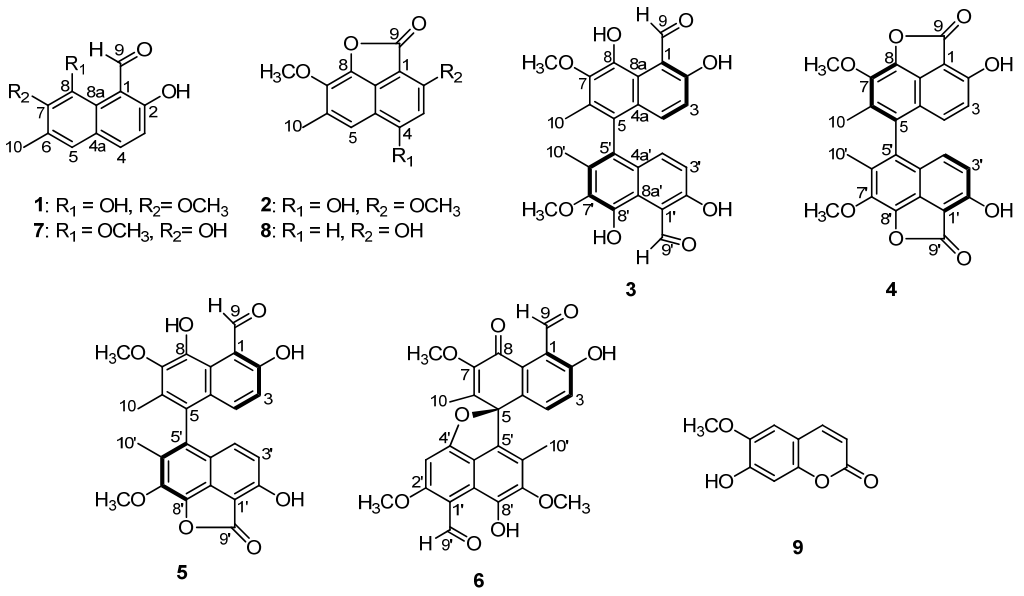

Fig. 1. Structures of the isolated compounds from *D. parviflora* (**1**-**9**).

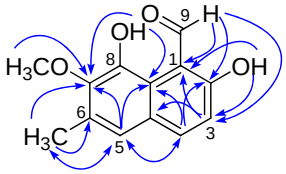

Fig. 2. Key HMBC correlations (H→C) for compound **1**.

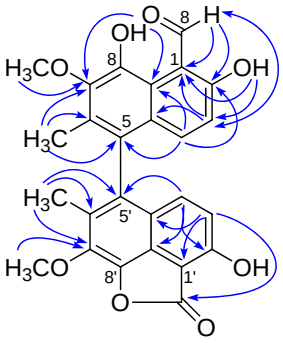

Fig. 3. Key HMBC correlations (H→C) for compound **5**.

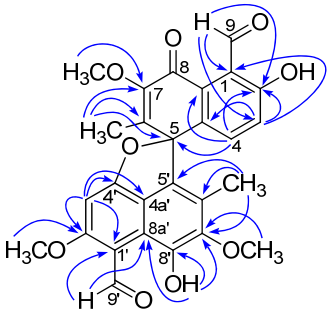

Fig. 4. Key HMBC correlations (H→C) for compound **6**.

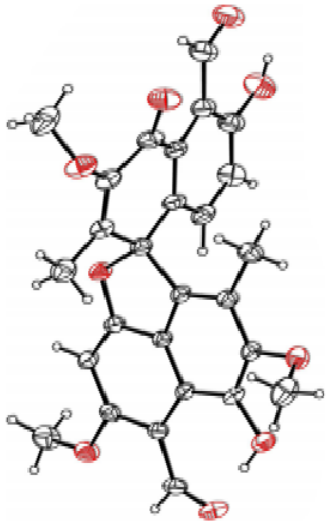

Fig. 5. ORTEP plot of compound **6.**

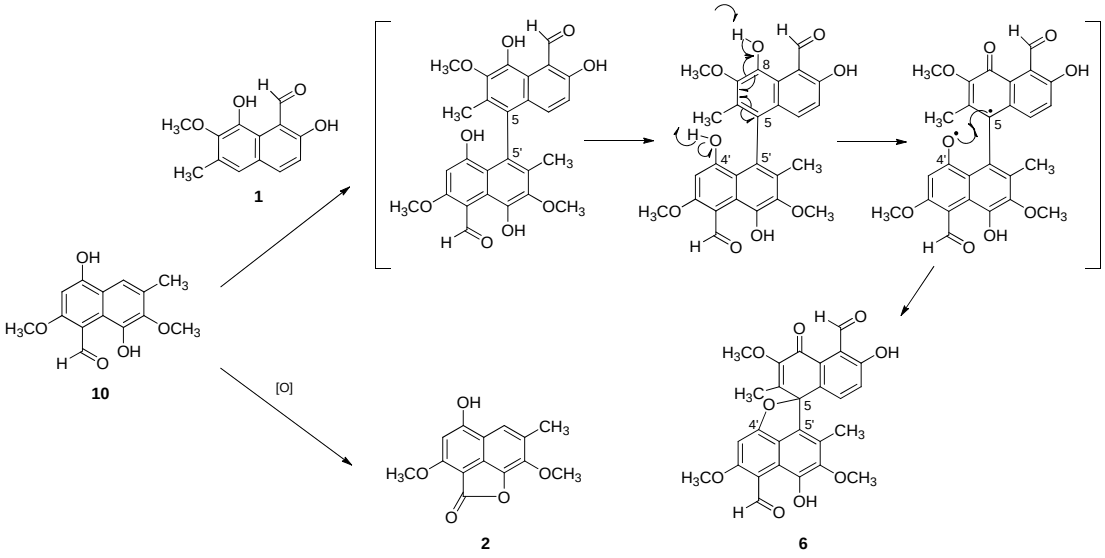

Fig. 6. Proposed the biosynthesis of **2** and **6**.

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2**

position	**1**[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
1		113.1		93.7
2		166.0		161.2
3	7.02, d (9.2)[c]	119.0	6.43, s	97.3
4	7.77, d (9.2)	138.3		163.0
4a		126.2		110.6
5	7.17, s	122.8	7.43, s	117.9
6		127.4		128.4
7		145.2		140.4
8		144.1		131.3
8a		120.1		134.0
9	11.30, s	199.3		166.3
10	2.45, s	16.1	2.28, s	17.6
2-OMe			4.02, s	56.6
7-OMe	3.91, s	61.0	4.23, s	59.5
8-OMe				
2-OH	14.21, s			
7-OH				
8-OH	6.75, s			

[a]In $CDCl_3$
[b]In CD_3OD
[c]Values in parentheses are coupling constants in Hz.

Table 2. ^{1}H and ^{13}C NMR spectral data of **3-6**

position	**3**[a]		**4**[b]		**5**[a]		**6**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		113.4		100.5		113.4		117.1
2		165.8		158.5		165.8		163.9
3	6.83, d (9.2)[c]	119.5	6.86, d (8.8)	118.6	6.82, d (9.2)	119.4	7.06, d (9.2)	124.3
4	7.13, d (9.2)	136.1	7.12, d (8.8)	134.2	7.19, d (9.2)	136.2	6.99, d (9.2)	134.8
4a		125.6		119.2		125.4		132.5
5		129.6		130.0		128.2		94.0
6		127.4		128.8		127.2		136.3
7		145.2		140.0		145.0		150.0[d]
8		143.9		167.3		144.0		181.1
8a		120.8		131.5		120.6		130.4
9	11.41, s	199.6		131.6	11.41, s	199.6	11.06, s	198.5
10	1.94, s	13.4	1.93, s	14.3	1.98, s	13.5	1.64, s	11.0
1′		113.4		100.5		100.7		111.1
2′		165.8		158.5		156.8		172.3
3′	6.83, d (9.2)	119.5	6.86, d (8.8)	118.6	6.92, d (8.8)	118.4	6.50, s	89.0
4′	7.13, d (9.2)	136.1	7.12, d (8.8)	134.2	7.12, d (8.8)	134.1		169.2
4a′		125.6		119.2		119.7		121.7
5′		129.6		130.0		131.2		125.1
6′		127.4		128.8		129.7		128.0
7′		145.2		140.0		140.3		150.0[d]
8′		143.9		167.3		131.4		148.2
8a′		120.8		131.5		130.8		119.3
9′	11.41, s	199.6		131.6		167.6	10.22, s	190.6
10′	1.94, s	13.4	1.93, s	14.3	1.94, s	14.3	1.82, s	10.2
OH-2	14.16				14.15, s		12.65, s	
OH-8	6.94				7.02, s			
7-OMe	3.96	61.3	4.36, s	59.9	3.96, s	61.3	3.88, s	60.0
OH-2′	14.16							
OH-8′	6.94						13.02, s	
7′-OMe	3.96	61.3	4.36, s	59.9	4.41, s	60.0	3.90, s	60.4
2′-OMe							4.06, s	57.2

[a] In $CDCl_3$
[b] In CD_3OD
[c] Values in parentheses are coupling constants in Hz.
[d] Overlapping of the signals.

Table 3. Biological activities of the isolated compounds

compound	antimalarial[a] (IC_{50}, μM)	antifungal[b] (IC_{50}, μM)	anti-TB[b] (MIC, μM)	cytotoxicity (IC_{50}, μM)[b]		
				KB[c]	MCF7[d]	NCI-H187[e]
1	inactive	64.50	inactive	3.88	16.66	36.64
2	11.45	inactive	192.13	37.43	nd	14.68
3	inactive	nd	nd	36.05	76.03	36.05
4	inactive	inactive	inactive	inactive	nd	inactive
5	inactive	92.93	54.30	5.56	20.50	29.06
6	6.85	inactive	nd	2.20	inactive	31.22
7	inactive	37.03	107.65	9.73	33.37	4.99
8	inactive	197.68	54.30	90.09	nd	inactive
9	inactive	inactive	inactive	66.24	inactive	inactive
dihydroartemisinin	0.004					
amphotericin B		0.06				
streptomycin			0.27-0.54			
ellipticine				0.78		5.64
doxorubicin				0.29	0.13	0.13

[a] Inactive at final concentration 10 μg/mL.
[b] Inactive at final concentration 50 μg/mL.
[c] Human oral epidermoid carcinoma.
[d] Human breast cancer cells.
[e] Human small cell lung cancer.
nd = not determined