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Six diprenylisoflavones, derrisisoflavones A–F, from *Derris scandens*

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Abstract

Chromatographic separation of EtOH extracts of the stems of *Derris scandens* has yielded six new diprenylisoflavones, named derrisisoflavones A–F, together with six known isoflavones. Their structures were elucidated by spectroscopic analyses. The known compounds were lupalbigenin, scandinone, erysenegalensein E, lupinisol A, lupinisoflavone G and 5, 7,4′trihydroxy-6, 8-diprenylisoflavone. Anti-dermatophyte activity of the isolated isoflavones from *D. scandens* against *Trichophyton mentagrophytes* was also examined.

Keywords: *Derris scandens*; Leguminosae; Derrisisoflavones A–F; Diprenylisoflavone; Dihydrohydroxypyranoisoflavone; Anti-dermatophyte activity; *Trichophyton mentagrophytes*

1. Introduction

In the course of screening for biologically active and chemically novel constituents of Asian medicinal plants and folk medicines, several interesting compounds such as anti-oxytocic alkaloids, asparagamine A–C from *Asparagus racemosus* and an acutely toxic amino acid, L-methionine S(*S*)-sulfoximine from *Cnestis palala* were isolated and their pharmacological activities have been examined (Ikegami et al., 1989; Sekine et al., 1991, 1993, 1995; Murakoshi et al., 1993). *Derris scandens* Benth. is a leguminous woody climber growing throughout southeast Asia, and in Thailand its stem is used for antidysenteric, diuretic and for relief of muscular pain. In this paper, we describe the isolation and structural elucidation of six new isoflavones (**1**–**6**) as well as the anti-dermatophyte activity of these and six previously described isoflavones (Tahara, Orihara, Ingham, & Mizutani, 1989; Rao, Krupadanam, & Srimannarayana, 1994; Wandji, Fomum, Tillequin, Seguin, & Koch, 1994) against *Trichophyton mentagrophytes*.

2. Results and discussion

The 75% EtOH extracts of the stems were partitioned with *n*-hexane, benzene, EtOAc and *n*-BuOH successively. The benzene extract was concentrated and subjected to silica gel, Chromatorex NH column chromatographies and prep. HPLC to yield **1**–**12** [Fig. 1]. Compounds **7**–**12** were identified as 5,7,4′-trihydroxy-6,8-diprenylisoflavone (**7**) (Rao et al., 1994), lupalbigenin (**8**) (Rao et al., 1994), scandinone (**9**) (Rao et al., 1994), erysenegalensein E (**10**) (Wandji et al., 1994), lupinisol A (**11**) (Tahara et al., 1989) and lupinisoflavone G (**12**) (Tahara et al., 1989), respectively, by comparison of their spectral data with those in the literature. Compounds **7**–**9** were estimated as major constituents of the benzene-soluble fraction

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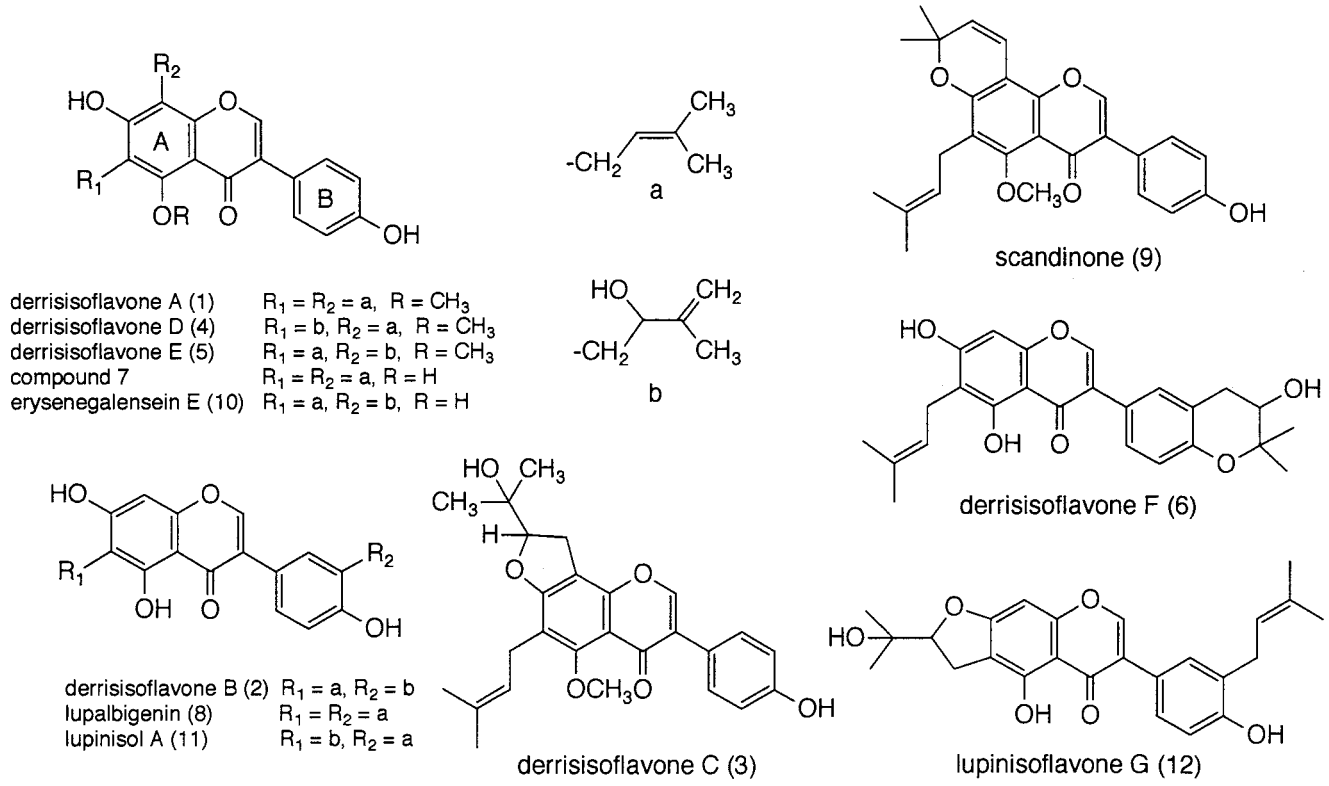

Fig. 1. Summary of compounds.

from the yields and TLC profiles, while **1**–**6** and **10**–**12** were minor components.

Compound **1** is a pale yellow amorphous solid with yellow fluorescence under irradiation of UV (365 nm). The molecular formula was determined as $C_{26}H_{28}O_5$ from HR-FABMS (m/z 421.2015 $[M+H]^+$, Δ 0 mmu), in accordance with thirteen degrees of unsaturation. The UV (λ_{max} 260 nm) and IR (ν_{max} 3369, 1631, 1588, 1229, 835 cm^{-1}) spectral data indicated the presence of an isoflavone nucleus. The ^{13}C NMR spectrum also showed 13 signals (δ 115.3–157.8) due to the isoflavone skeleton including two intense signals at δ 115.3 and 130.3 assigned to two equivalent aromatic carbons of the symmetrical B-ring and 10 signals due to two prenyl units (Table 1). The remaining signal at δ 62.2 was considered as a methoxyl group from its chemical shift and DEPT spectrum. The ^1H NMR spectrum showed the characteristic signal of H-2 of an isoflavone at δ 7.77 (1H, s) and AA′BB′ type signals at δ 7.24 (2H, d, J = 8.7 Hz), 6.77 (2H, d, J = 8.7 Hz) assignable to H-2′ 6′ and H-3′ 5′ of the B-ring, respectively, together with typical signals due to two sets of 3,3-dimethylallyl groups. Therefore, **1** was considered as a methoxyisoflavone having two prenyl groups at the C-6 and C-8 positions. This deduction was supported by correlations between H-1″ of one prenyl unit and C-5, C-6 and C-7, and also between H-1‴ of the other prenyl unit and C-7, C-8 and C-9 in the pulsed field gradient (PFG) HMBC spectrum (8 Hz)(Fig. 2). Moreover, the methoxyl hydrogen at δ 3.71 correlated with C-5 carbon at δ 156.0 in the PFG-HMBC spectrum. NOE studies displayed enhancements of the H-1″ and H-2″ signals upon irradiation of the methoxyl hydrogen. These findings enabled us to deduce the location of the methoxyl group to be at the C-5 position. On the basis of the above data, the structure of **1** was concluded to be 7,4′-dihydroxy-5-methoxy-6,8-diprenylisoflavone. Further confirmation came from comparing the observed ^{13}C and ^1H shifts to those of the known compound **7** also isolated in this study.

Compound **2** is a pale brown amorphous solid and its molecular formula was determined to be $C_{25}H_{26}O_6$ from HR-FABMS (m/z 423.1825 $[M+H]^+$, Δ+1.8 mmu) indicating thirteen degrees of unsaturation. The UV (λ_{max} 264 nm) and IR (ν_{max} 3421, 1647, 1278 cm^{-1}) spectral data indicated the presence of an isoflavone. The general feature of NMR spectra of **2** were similar to those of **8**, except for the signals due to one prenyl part. Thus, the ^{13}C NMR spectrum also showed 15 signals due to the isoflavone skeleton and 10 signals due to two prenyl units (Table 1). The ^1H NMR spectrum showed typical signals due to a 3,3-dimethylallyl group and ABX-type signals at δ 2.75 (1H, dd, J = 13.8 and 8.2 Hz, H-1‴), 2.89 (1H, dd, J = 13.8 and 4.5 Hz, H-1‴) and 4.34 (1H, dd, J = 8.2 and 4.5

Table 1
^{13}C NMR data of diprenylisoflavones (**1**–**12**) (125 MHz, ppm from TMS)

Carbon	1	2	3	4	5	6	7	8	9	10	11	12
C-2	150.5	154.5	152.4	152.8	152.5	154.6	152.6	152.2	150.3	154.4	148.6	154.1
C-3	125.3	124.7	126.8	126.5	126.4	124.3	121.2	123.6[a]	125.8	124.3	124.9[a]	124.1
C-4	176.0	182.3	177.3	177.7	177.7	182.1	181.3	180.8	175.8	182.7	182.3	181.8
C-5	156.0	160.5	159.6	157.5	157.8	160.5	157.4	159.1	158.0	159.0	161.2	157.9
C-6	119.4	113.0	117.6	120.5	123.1	113.1	110.1	111.5	121.8	113.8	110.3	110.2
C-7	157.8	163.7	165.1	161.1	161.7	163.7	156.9	161.5	155.9	162.7	164.5	167.5
C-8	111.5	93.8	110.5	114.5	111.2	93.8	105.3	93.0	105.8	105.3	94.3	89.2
C-9	154.9	157.5	154.1	157.0	156.5	157.5	153.3	155.9	152.1	154.9	157.8	158.9
C-10	112.7	106.0	113.4	112.9	112.9	106.0	105.3	105.3	112.6	106.2	105.9	106.8
C-1′	123.0	123.4	124.4	124.4	124.4	124.3	121.1	122.2[a]	123.6	123.5	123.2[a]	123.1
C-2′	130.3	133.2	131.6	131.5	131.5	131.6	130.3	130.0	130.4	131.4	131.3	131.2
C-3′	115.3	127.0	116.0	116.0	116.0	121.3	115.6	127.9	115.7	126.2	129.3	128.5
C-4′	156.6	157.0	158.5	158.5	158.5	154.5	155.8	154.7	156.1	158.7	156.2	155.9
C-5′	115.3	116.2	116.0	116.0	116.0	117.9	115.6	115.0	115.7	116.2	115.9	115.5
C-6′	130.3	129.5	131.6	131.5	131.5	129.3	130.3	127.7	130.4	131.4	128.6	128.5
C-1″	22.5	22.2	23.6	31.3	23.6	22.3	21.6	21.3	22.2	22.6	29.6	26.9
C-2″	121.5	123.4	123.1	78.1	124.0	123.4	121.2	121.8	122.5	123.0	76.2	92.7
C-3″	134.9	132.0	132.8	148.5	132.1	132.0	135.6	132.5	131.4	132.5	148.6	71.3
C-4″	17.8	17.9	18.0	110.9	18.0	17.9	17.9	17.6	17.9	17.9	111.0	25.8[a]
C-5″	25.6	25.9	25.8	18.2	25.8	25.9	25.8	25.6	25.7	25.9	17.6	25.4[a]
C-1‴	22.1	38.4	28.3	23.1	30.6	32.1	21.6	28.6	115.1	30.3	29.7	29.1
C-2‴	121.0	76.7	92.4	123.0	77.3	70.4	121.4	121.9	128.8	77.4	123.8	123.6
C-3‴	134.0	148.7	72.3	132.7	148.1	78.3	134.1	133.2	77.8	148.2	133.0	132.4
C-4‴	17.7	18.1	25.9[a]	17.9	18.4	25.9	17.9	17.6	28.1	18.4	17.8	17.8
C-5‴	25.6	111.1	24.6[a]	25.8	110.9	21.1	25.7	25.6	28.1	110.9	25.8	25.9
C_5-OMe	62.2		62.8	62.8	62.5				62.8			
Solvent	$CDCl_3$[b]	CD_3OD	CD_3OD	CD_3OD	CD_3OD	CD_3OD	$CDC1_3$	$CDC1_3$[b]	$CDC1_3$	CD_3OD	CD_3OD	acetone-d_6

[a] Assignments in the same column may be reversed.
[b] Small amount of CD_3OD was added.

Hz, H-2‴) in addition to three singlet signals at δ 1.76 (3H, H-4‴), 4.73 (1H, H-5‴) and 4.86 (1H, H-5‴) derived from a 2-hydroxy-3-methyl-3-butenyl group, together with characteristic signals of H-2 and H-8 of the isoflavone derivative at δ 7.95 (1H, s) and 6.32 (1H, s), respectively. The locations of the two prenyl groups were elucidated on the basis of the HMBC data. The PFG-HMBC spectrum of **2** showed cross peaks between H-1″ (δ 3.26) of the dimethylallyl group and C-5, C-6 and C-7 and between H-1‴ (δ 2.89) of the 2-hydroxy-3-methyl-3-butenyl group and C-2′, C-3′ and C-4′ (Fig. 2). These results confirmed the positions of the two prenyl units to be C-6 and C-3′ and the structure of **2** was finally elucidated as shown.

Compound **3**, isolated as a colourless amorphous solid, was formulated as $C_{26}H_{28}O_5$ from HR-FABMS (m/z 437.1957 $[M+H]^+$, Δ −0.7 mmu), in accordance with the same degrees of unsaturation as that of **1** and **2**, and showed UV and IR spectra closely approximating those of **1** and **2** indicating the presence of the same chromophores and functionalities. The presence of a 3,3-dimethylallyl group, a methoxyl group and an isoflavone skeleton possessing a symmetrical B-ring in **3** was easily deduced from its NMR spectral data (Table 1), which were closely related to those of scandinone (**9**). The locations of these two substituents were determined to be C-5 and C-6 positions from the HMBC and NOE correlations as shown in Fig. 2. The remaining partial structure, $-CH_2-CH(O-)C(CH_3)_2(OH)$, was deduced from the HMBC data and their chemical shifts. The HMBC correlations between H-1‴ (CH_2) and C-7, C-8 and C-10 confirmed the connection of this methylene group and C-8 of the isoflavone skeleton (Fig. 2). No bathochromic shift of band II (λ_{max} 260 nm) with NaOAc (Markham, 1982) was observed in the UV spectrum of **3**, indicating the absence of a free 7-hydroxyl group. This evidence, coupled with the molecular formula and degree of unsaturation, resulted in the formation of a dihydrobenzofuran ring composed of A-ring and the partial structure in an angular disposition. Therefore, the structure of **3** was concluded to be as shown in Fig. 1. Dihydrofurano-type isoflavones such as **3** have been previously found in *Lupinus* species (Tahara et al., 1989).

Compound **4** is a colourless amorphous solid showing $[\alpha]_D$ −10.8°. HR-FABMS (m/z 437.1936 $[M+H]^+$, Δ −2.8 mmu) of **4** showed the molecular formula to be $C_{26}H_{28}O_6$, with one oxygen more than **1**. The IR spectrum of **4** showed absorption bands for free

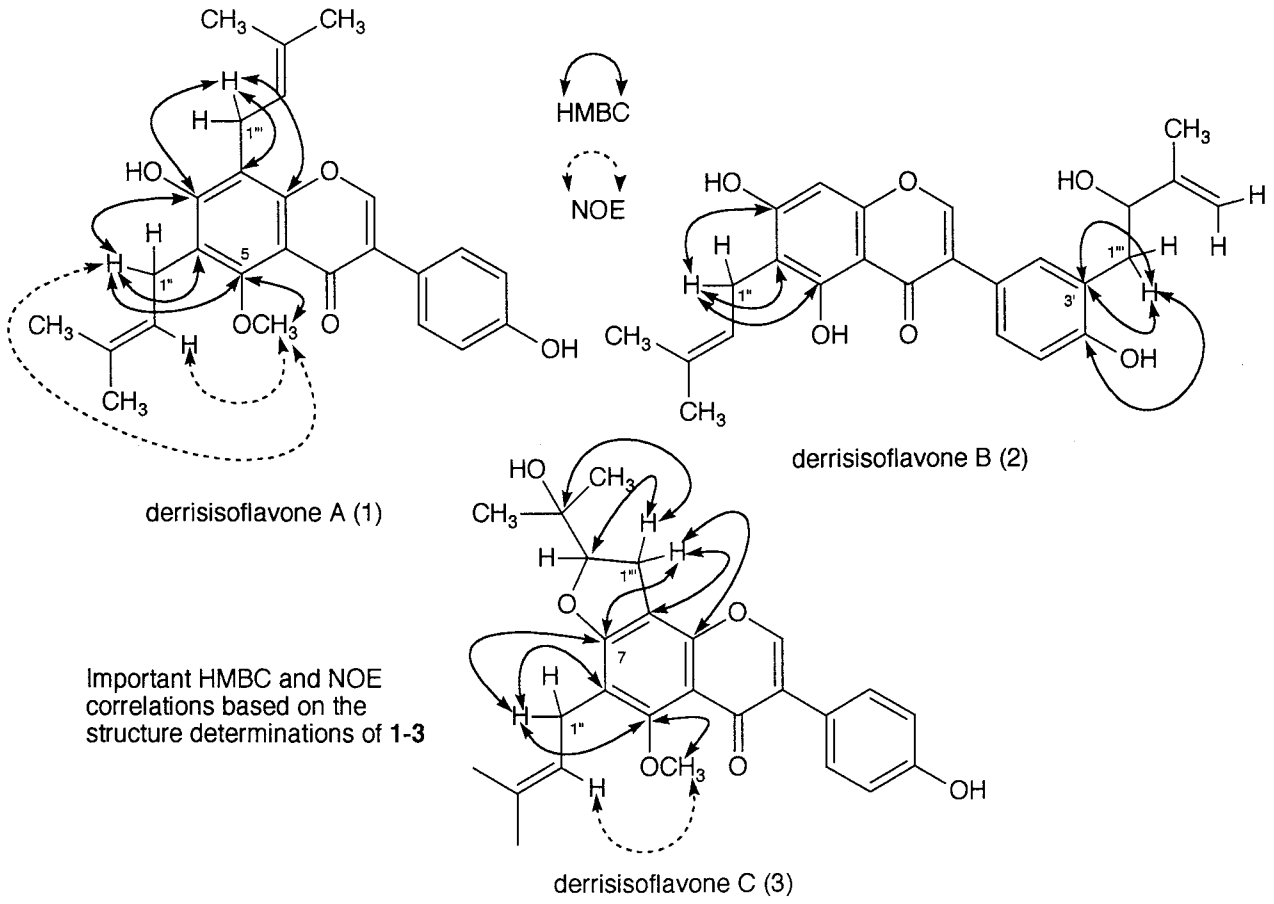

Fig. 2. Important HMBC and NOE correlations based on the structure determinations of **1**–**3**.

hydroxy (3392), conjugated carbonyl (1634), olefin (1588) and ether (1227 cm^{-1}) functionalities. An isoflavone nucleus was elucidated from the UV (λ_{max} 260 nm) data with various shift reagents (Markham, 1982). The ^{13}C NMR spectrum also showed 13 signals (δ 112.9–158.5) due to the isoflavone skeleton including two intense signals at δ 116.0 (C-3′ and C-5′) and 131.5 (C-2′ and C-6′) assigned to two equivalent aromatic carbon of a symmetrical B-ring, and 10 signals arising from two prenyl side chains (Table 1). The remaining signal was judged to be a signal of a methoxyl group from its chemical shift value (δ 62.8) and DEPT spectrum. The ^1H NMR spectrum revealed a characteristic signal of an isoflavone derivative at δ 7.98 (1H, s, H-2) and AA′BB′ type signals at δ 7.23 (2H, d, J = 8.5 Hz), 6.73 (2H, d, J = 8.5 Hz) assignable to H-2′, 6′, and H-3′, 5′ of the B-ring, respectively, together with typical signals due to a 3,3-dimethylallyl group (δ 3.43 (2H, d, J = 7.0 Hz, H-1‴), 5.10 (1H, t, J = 7.0 Hz, H-2‴), 1.72 (3H, s, H-4‴) and 1.56 (3H, s, H-5‴)). In addition, the ^1H NMR spectrum showed ABX-type signals at δ 2.71 (1H, dd, J = 14.5 and 8.6 Hz, H-1″), 3.10 (1H, d, J = 14.5 Hz, H-1″) and 4.19 (1H, d, J = 8.6 Hz, H-2″) along with three singlet signals at δ 1.74 (3H, H-4″), 4.74 (1H, H-5″) and 4.91 (1H, H-5″) derived from a 2-hydroxy-3-methyl-3-butenyl group. As the spectrum did not show a signal at δ 5.9-6.1 characteristic of H-6 and H-8, **4** was considered to be a 6,8-disubstituted-methoxyisoflavone. This deduction was supported by the correlations between H-1″ of the 2-hydroxy-3-methyl-3-butenyl group and C-5, C-6 and C-7, and also between H-1‴ of the other prenyl unit and C-7, C-8 and C-9 in the PFG-HMBC spectrum (Fig. 3). Therefore, the 3,3-dimethylallyl and butenyl side chains must be located at C-8 and C-6 position, respectively. The presence of a 5-methoxyl group is probable from the ^{13}C chemical shift value of C-4 at δ 176.0 (Agrawal and Bansal, 1989). Moreover, the methoxyl hydrogen at δ 3.67 correlated with C-5 carbon at δ 157.5 in the PFG-HMBC spectrum (Fig. 3). From these results, the structure of **4** was concluded as 7,4′-dihydroxy-5-methoxy-6-prenyl-8-(2′-hydroxy-3′-methyl-3′-butenyl) isoflavone. This was reinforced by the comparison of the observed ^{13}C and ^1H chemical shifts to those of previously isolated **1** (Table 1).

Compound **5**, a pale brown amorphous solid, was found to have an isomeric molecular formula,

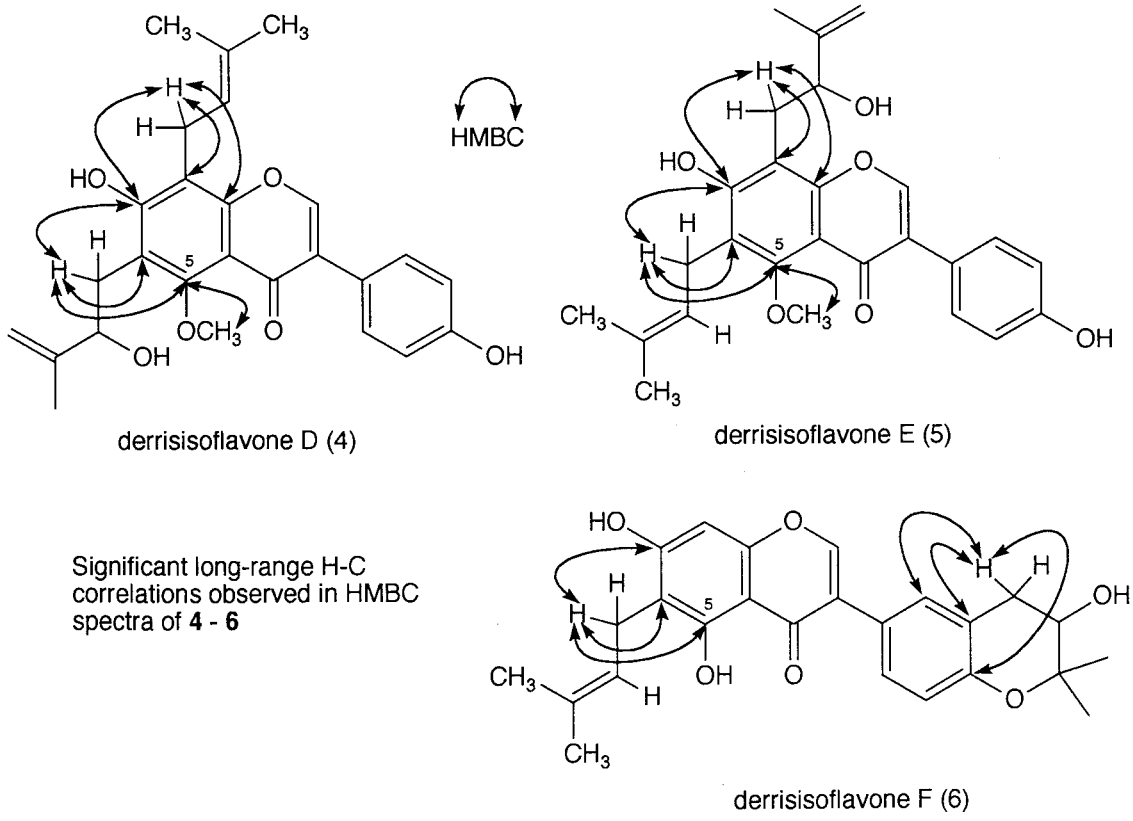

Fig. 3. Significant long-range H-C correlations observed in HMBC spectra of **4**–**6**.

$C_{26}H_{28}O_6$, from HR-FABMS (*m/z* 437.1977 $[M+H]^+$, Δ −1.3 mmu) and very similar UV (λ_{max} 260 nm) and IR (ν_{max} 3392, 1634, 1236 cm^{-1}) spectra to that of compound **4**, indicating an identical carbon skeleton. The close resemblance of ^{13}C and ^{1}H chemical shifts of **5** with those of **4** also strongly indicates that **5** is an isomer of **4** (Table 1). The validity of this deduction was demonstrated by the careful investigation of the HMBC spectrum. The PFG-HMBC spectrum of **5** showed cross peaks between H-1″ of a dimethylallyl group and C-5, C-6 and C-7, and also between H-1‴ of a 2-hydroxy-3-methyl-3-butenyl group and C-7, C-8 and C-9 (Fig. 2). Based on these spectroscopic data, **5** was reasonably concluded to be a positional isomer of **4** and represented by the structure formula in Fig. 3.

Compound **6**, isolated as a colourless amorphous solid, was formulated as $C_{26}H_{28}O_5$ from HR-FABMS (*m/z* 437.1957 $[M+H]^+$, Δ −0.7 mmu), in accordance with thirteen degrees of unsaturation. The UV (λ_{max} 264 nm), IR (ν_{max} 3421, 1647, 1266, 824 cm^{-1}) and NMR spectra (Table 1) are very similar to those of **4** and **5**, therefore **6** is an analogue of **4** and **5**, the main difference being in the ^{1}H and ^{13}C NMR spectra the presence of the singlet signal for H-8 at δ 6.35 and the absence of a set of the signals due to symmetrical B-ring. The 3′-,4′-disubstitution pattern of the B-ring, as in lupalbigenin (**8**) (Rao et al., 1994) was readily deduced from the chemical shift values and multiplicities for H-2′ (δ 7.24, d, J = 2.0 Hz), H-5′ (δ 6.78, d, J = 8.4 Hz) and H-6′ (δ 7.21, dd, J = 8.4 and 2.0 Hz) in the ^{1}H NMR spectrum. The location of a prenyl side chain was determined from HMBC correlations as shown in Fig. 3. The remaining partial structure, –$CH_2CH(OH)$– C(O–)$(CH_3)_2$, was also deduced from HMQC and HMBC data. The connection of this methylene group (C-1‴) and C-3′ of the isoflavone skeleton was confirmed by the HMBC correlations between H-1‴ (CH_2) and C-2′, C-3′ and C-4′ .Taking into account the degree of unsaturation and chemical shift values of the ^{13}C NMR spectrum, C-4′ (δ 154.5) of B-ring and C-5‴ (δ 78.3) of the partial structure were concluded to be linked through an oxygen atom to form a six-membered ring. Therefore the structure of **6** was unambiguously formulated as shown in Fig. 3.

To the best of our knowledge, while more than a thousand isoflavone derivatives have been reported (Barron & Ibrahim, 1996), this is the first isolation of **1**–**6**. Compounds **10**–**12** have been isolated from this plant for the first time (Rao et al., 1994).

The anti dermatophyte activity of several fractions and **1**–**12** obtained from *D. scandens* against *Tricophyton mentagrophytes* was examined by the microdilution method (Fukasawa et al., 1991), as our

screening test on anti-fungal compounds from Asian medicinal plants. The benzene-soluble fraction obtained from the EtOH extracts exhibited a weak activity against *T. mentagrophytes* at minimum inhibitory concentration (MIC) 500 μg/ml. The MIC of the standard agent lanoconazole (LNCZ) was 6 ng/ml. Among the isolated compounds, **3**, **7** and **8** showed a relatively high activity at 250 μg/ml, respectively, while other compounds **1**, **2**, **4**–**6** and **9**–**12** showed lower activity at 500–1000 μg/ml. Compounds **3**, **7** and **8**, at least, may be an anti-dermatophyte principle of *D. scandens* from the majority in the chromatograms and the magnitude of the activity.

3. Experimental

3.1. General

$[\alpha]_D$ values were measured with a JASCO DIP 181 polarimeter. The NMR spectra were obtained on a JEOL JNM-ALPHA 500 spectrometer, with chemical shifts reported in δ (ppm) using TMS as int. standard. FABMS were recorded on a JEOL HX-110A instrument in *m*-nitrobenzyl alcohol (NBA) matrix in the positive ion mode. TLC: Kieselgel 60 F254 0.25 mm (Merck), CC: Kieselgel 60 230–400 mesh (Merck).

3.2. Plant material

Stems of *D. scandens* were collected in Nakornpathom Province, Thailand. A voucher specimen is deposited at the herbarium of the Chulalongkorn University.

3.3. Isolation of isoflavones (*1*–*12*) from the stems of *D. scandens*

The dried stems (600 g) were mechanically pulverized and extracted with 75% EtOH. Half of the EtOH extracts (ca. 41 g) was suspended in H_2O and extracted with *n*-hexane, benzene, AcOEt and *n*-BuOH, successively. A portion (5 g) of the benzene extract with the highest anti-fungal activity was subjected to silica gel CC with elution of benzene EtOAc system to afford ten fractions (fr. 1–10). Among them, two fractions (fr. 3 and 5), eluting at approximately 10 and 12% AcOEt, were purified as **7** (49 mg) and **8** (326 mg), respectively. Fr. 7 (a complex mixture, 570 mg, elution at approximately 25% AcOEt) was chromatographed repeatedly on silica gel CC eluted with *n*-hexane–acetone, $CHCl_3$–Me0H systems to afford **1** (14 mg), **2** (7 mg) and **9** (34 mg). Fr. 6 (390 mg) was chromatographed repeatedly on silica gel CC eluted with *n*-hexane–acetone, and submitted to prep. HPLC ($CHCl_3$–MeOH–$NH_4$0H system) to afford **10** (5 mg) and **11** (6 mg). Fr. 8 (a complex mixture, 1100 mg, eluting at approximately 30% AcOEt) was chromatographed repeatedly on silica gel CC eluted with *n*-hexane–acetone, $CHCl_3$–MeOH systems, and submitted to prep. HPLC (LiChrosorb Si60, 5 μm, 4.0 × 250 mm)(*n*-hexane–EtOH) to afford **3** (4 mg), and also submitted to prep. HPLC ($CHCl_3$–EtOH system) to afford **4** (4 mg), **5** (8 mg), **6** (11 mg), **12** (17 mg) and **9** (178 mg).

3.4. Derrisisoflavone A (*1*)

Pale yellow amorphous, fluorescence (yellow), UV λ_{max} (MeOH) nm [log ε]: 260 [4.56], 202 [4.64]; +NaOMe 323^{sh} [4.54], 274 [4.81]; +$AlCl_3$ 260 [4.55]; $AlCl_3$/HCl 260 [4.55]; +NaOAc 343 [3.96], 273 [4.51], 220 [5.31]; +NaOAc/H_3BO_3 260 [4.55], IR ν_{max} (KBr) cm^{-1}: 3369, 2924, 1631, 1588, 1514, 1421, 1375, 1267, 1229, 1173, 1075, 988, 835, HR-FABMS [NBA] *m/z* 421.2015 $[M+H]^+$ (calcd. for $C_{26}H_{29}O_5$, 421.2015), ^1H NMR ($CDCl_3$+CD_3OD) δ 7.77 (1H, s, H-2), 3.71 (3H, s, C_5–OMe), 7.24 (2H, d, J = 8.7 Hz, H-2′, -6′), 6.77 (2H, d, J = 8.7 Hz, H-3′, 5′), 3.40 (2H, d, J = 7.0 Hz, H-1″), 5.10 (1H, t-like, J = 7.0 Hz, H-2″), 1.74 (3H, s, H-4″), 1.65 (3H, s, H-5″), 3.43 (2H, d, J = 7.0 Hz, H-1‴), 5.13 (1H, t-like, J = 7.0 Hz, H-2‴), 1.74 (3H, s, H-4‴), 1.63 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.5. Derrisisoflavone B (*2*)

Pale brown amorphous, $[\alpha]_D$: +3.0° (EtOH, c = 0.054), UV λ_{max} (MeOH) nm [log ε]: 264 [4.51], 210 [4.54]; +NaOMe 274 [4.94]; +$AlCl_3$ 264 [4.52], 211 [4.55]; +$AlCl_3$/HCl 264 [4.51], 211 [4.55]; +NaOAc 335 [3.89], 269 [4.47], 217 [5.23]; +NaOAc/H_3BO_3 264 [4.48], 217 [5.23], 209 [5.24], IR ν_{max} (KBr) cm^{-1}: 3421, 2825, 1648, 1570, 1499, 1458, 1279, 1252, 1120, 1057, 902, 817, HR-FABMS [NBA] *m/z* 423.1825 $[M+H]^+$ (calcd. for $C_{25}H_{27}O_6$, 423.1807), ^1H NMR (CD_3OD) δ 7.95 (1H, s, H-2), 6.32 (1H, s, H-8), 7.20 (1H, d, J = 2.2 Hz, H-2′), 6.78 (1H, d, J = 8.3 Hz, H-5′), 7.18 (H, dd, J = 8.3 and 2.2 Hz, H-6′), 3.26 (2H, d, J = 7.3 Hz, H-1″), 5.18 (1H, t, J = 7.3 Hz, H-2″), 1.73 (3H, s, H-4″), 1.61 (3H, s, H-5″), 2.89 (1H, dd, J = 13.8 and 4.5 Hz, H-1‴), 2.75 (1H, dd, J = 13.8 and 8.2 Hz, H-1‴), 4.34 (1H, dd, J = 8.2 and 4.5 Hz, H-2‴), 1.76 (3H, s, H-4‴), 4.86 (1H, s, H-5‴), 4.73 (1H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.6. Derrisisoflavone C (*3*)

Colourless amorphous, $[\alpha]_D$: −63.8° (EtOH, c = 0.043), UV λ_{max} (MeOH) nm [log ε]: 303^{sh} [4.05], 259 [4.59], +NaOMe 278 [4.78], 256 [4.76], 248 [4.76]; +$AlCl_3$ 302^{sh} [4.04], 259 [4.58]; +$AlC1_3$/HCl 302^{sh}

[4.04], 260 [4.58]; +NaOAc 305^{sh} [4.06], 259 [4.57], 223 [5.36], 218 [5.36]; +NaOAc/H_3BO_3 302^{sh} [4.04], 259 [4.57], 224 [5.39], IR ν_{max} (KBr) cm^{-1}: 3398, 2928, 1632, 1600, 1516, 1426, 1382, 1271, 1215, 1174, 1071, 835, HR-FABMS [NBA] m/z 437.1957 $[M+H]^+$ (calcd. for $C_{26}H_{29}O_6$, 437.1964), ^1H NMR (CD_3OD) δ 8.00 (1H, s, H-2), 3.78 (3H, s, C_5–OMe), 7.31 (2H, d, J = 8.7 Hz, H-2′, 6′), 6.82 (2H, d, J = 8.7 Hz, H-3′, 5′), 3.37 (2H, d, J = 7.0 Hz, H-1″), 5.21 (1H, t-like, J = 7.0 Hz, H-2″), 1.78 (3H, s, H-4″), 1.67 (3H, s, H-5″), 3.34 (2H, d, J = 8.5 Hz, H-1‴), 4.83 (1H, t, J = 8.5 Hz, H-2‴), 1.27 (3H, s, H-4‴), 1.26 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.7. Derrisisoflavone D (**4**)

Colourless amorphous, $[\alpha]_D$: −10.8° (EtOH, c = 0.088), UV λ_{max} (MeOH) nm [log ε]: 302^{sh} [3.94, 260 [4.57]; +NaOMe 272 [4.82], 209 [5.23]; +$AlC1_3$ 307^{sh} [3.93], 260 [4.57]; +$AlC1_3$/HCl 302^{sh} [3.94], 260 [4.56]; +NaOAc 334 [4.02], 271 [4.53], 221 [5.26], 218 [5.26], 212 [5.25]; +NaOAc/H_3BO_3 304^{sh} [3.95], 260 [4.55], 220 [5.28]; IR ν_{max} (KBr) cm^{-1}: 3392, 2923, 1634, 1588, 1514, 1420, 1269, 1227, 1080, 835, HR-FABMS [NBA] m/z 437.1936 $[M+H]^+$ (calcd. for $C_{26}H_{29}O_6$, 437.1964), ^1H NMR (CD_3OD) δ 7.98 (1H, s, H-2), 3.67 (3H, s, C_5–OMe), 7.23 (2H, d, J = 8.5 Hz, H-2′, 6′), 6.73 (2H, d, J = 8.5 Hz, H-3′, 5′), 3.10 (2H, d, J = 14.5 Hz, H-1″), 2.71 (1H, dd, J = 14.5 and 8.6 Hz, H-1″), 4.19 (1H, t, J = 8.6 Hz, H-2″), 1.74 (3H, s, H-4″), 4.91 (1H, s, H-5″), 4.74 (1H, s, H-5″), 3.43 (2H, d, J = 7.0 Hz, H-1‴), 5.10 (1H, t, J = 7.0 Hz, H-2‴), 1.72 (3H, s, H-4‴), 1.56 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.8. Derrisisoflavone E (**5**)

Pale brown amorphous, $[\alpha]_D$: +2.0° (EtOH, c = 0.089), UV λ_{max} (MeOH) nm [log ε] 301^{sh} [4.02], 260 [4.60]; +NaOMe 329 [4.50], 271 [4.79], 209 [5.23]; +$AlC1_3$ 303^{sh} [4.02], 260 [4.59]; +$AlC1_3$/HCl 260 [4.58]; +NaOAc 336 [4.13], 270 [4.61], 216 [5.28]; +NaOAc/H_3BO_3 302^{sh} [4.05], 261 [4.60], 218 [5.30], IR ν_{max} (KBr) cm^{-1}: 3392, 2922, 1634, 1610, 1586, 1514, 1374, 1236, 1075, 835, HR-FABMS [NBA] m/z 437.1977 $[M+H]^+$ (calcd. for $C_{26}H_{29}O_6$, 437.1964), ^1H NMR (CD_3OD) δ 8.01 (1H, s, H-2), 3.72 (3H, s, C_5–OMe), 7.29 (2H, d, J = 8.3 Hz, H-2′, -6′), 6.78 (2H, d, J = 8.3 Hz, H-3′, 5′), 3.39 (2H, d, J = 6.9 Hz, H-1″), 5.14 (1H, t, J = 6.9 Hz, H-2″), 1.75 (3H, s, H-4″), 1.61 (1H, s, H-5″), 3.18 (1H, dd, J = 14.6 and 2.6 Hz, H-1‴), 2.98 (1H, dd, J = 14.6 and 8.3 Hz, H-1‴), 4.31 (1H, dd, J = 8.3 and 2.6 Hz, H-2‴), 1.80 (3H, s, H-4‴), 4.91 (1H, s, H-5‴), 4.77 (1H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.9. Derrisisoflavone F (**6**)

Colourless amorphous, $[\alpha]_D$: +20.6° (EtOH, c = 0.12), UV λ_{max} (MeOH) nm [log ε]: 264 [4.54], 211 [4.54]; +NaOMe 329 [4.62], 271 [4.86]; +$AlC1_3$ 264 [4.53], 210 [4.54]; +$AlCl_3$/HCl 264 [4.52], 211 [4.52]; + NaOAc 336 [3.92], 269 [4.50], 220 [5.32], 212 [5.25]; +NaOAc/H_3BO_3 265 [4.51], 215 [5.25], IR ν_{max} (KBr) cm^{-1}: 3421, 2977, 2925, 1647, 1599, 1498, 1299, 1266, 1065, 824, HR-FABMS [NBA] m/z 423.1801 $[M+H]^+$ (calcd. for $C_{25}H_{27}O_6$, 423.1807), ^1H NMR (CD_3OD) δ 8.00 (1H, s, H-2), 6.35 (1H, s, H-8), 7.24 (1H, d, J = 2.0 Hz, H-2′), 6.78 (1H, d, J = 8.4 Hz, H-5′), 7.21 (1H, dd, J = 8.4 and 2.0 Hz, H-6′), 3.30 (2H, d, J = 7.3 Hz, H-1″), 5.21 (1H, t-like, J = 7.3 Hz, H-2″), 1.76 (3H, s, H-4″), 1.65 (3H, s, H-5″), 2.75 (1H, dd, J = 16.6 and 7.4 Hz, H-1‴), 3.04 (1H, dd,.J = 16.6 and 5.2 Hz, H-1‴), 3.77 (1H, dd, J = 7.4 and 5.2 Hz, H-2‴), 1.71 (3H, s, H-4‴), 1.69 (3H, s, H-5‴);^{13}C NMR spectrum (see Table 1).

3.10. 5,7,4′-Trihydroxy-6,8-diprenylisoflavone (**7**)

Yellow amorphous, UV λ_{max} (MeOH) nm [log ε]: 270 [4.49], IR ν_{max} (KBr) cm^{-1}: 3390, 2923, 1647, 1514, 1437, 1220, 836, FABMS m/z 407 $[M+H]^+$, ^1H NMR ($CDCl_3$) δ 7.82 (1H, s, H-2), 13.04 (1H, s, C_5-OH), 6.28 (1H, s, C_7-OH), 7.29 (2H, d, J = 8.6 Hz, H-2′, 6′), 6.78 (2H, d, J = 8.6 Hz, H-3′, 5′), 3.39 (2H, d, J = 5.4 Hz, H-1″), 5.19 (1H, t-like, J = 5.4 Hz, H-2″), 1.77 (3H, s, H-4″), 1.69 (3H, s, H-5″), 3.41 (2H, d, J = 5.4 Hz, H-1‴), 5.15 (1H, t-like, J = 5.4 Hz, H-2‴), 1.76 (3H, s, H-4‴), 1.67 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.11. Lupalbigettirt (**8**)

Pale yellow amorphous, UV ν_{max} (MeOH) nm [log ε]: 267 [4.53], 214 [4.59], IR ν_{max} (KBr) cm^{-1}: 3283, 2924, 1649, 1615, 1503, 1457, 1270, 1223, 824, FABMS m/z 407 $[M+H]^+$, ^1H NMR ($CDCl_3+CD_3OD$) δ 7.70 (1H s, H-2), 6.27 (1H, s, H-8), 7.09 (1H, d, J = 2.2 Hz, H-2′), 6.76 (1H, d, J = 8.2 Hz, H-5′), 7.11 (1H, dd, J = 8.2 and 2.2 Hz, H-6′), 3.30 (2H, d, J = 7.0 Hz, H-1″), 5.18 (1H, t-like, J = 7.0 Hz, H-2″), 1.72 (3H, s, H-4″), 1.62 (3H, s, H-5″), 3.29 (2H, d, J = 7.0 Hz, H-1‴), 5.27 (1H, t-like, J = 7.0 Hz, H-2‴), 1.66 (3H, s, H-4‴), 1.67 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.12. Scandinone (**9**)

Yellow amorphous, UV λ_{max} (EtOH) nm [log ε]: 267 [4.62], IR ν_{max} (KBr) cm^{-1}: 3380, 2922, 1630, 1610, 1250, 1220, 835, FABMS m/z 419 $[M+H]^+$, ^1H NMR ($CDCl_3$) δ 7.77(1H, s, H-2), 3.82 (3H, s, C_5–OMe),

7.24 (2H, d, J = 8.4 Hz, H-2′, 6′), 6.77 (2H, d, J = 8.4 Hz, H-3′, 5′), 6.44 (1H, brs, $C_{4'}$-OH), 3.34 (2H, d, J = 7.0 Hz, H-1″), 5.12 (1H, t-like, J = 7.0 Hz, H-2″), 1.76 (3H, s, H-4″), 1.63 (3H, s, H-5″), 6.72 (1H, d, J = 9.8 Hz, H-1‴), 5.60 (1H, d, J = 9.8 Hz, H-2‴), 1.44 (6H, s, H-4‴, -5‴ ^{13}C NMR spectrum (see Table 1).

3.13. Erysenegalensein E (*10*)

Colourless amorphous, $[\alpha]_D$: +4.8° (EtOH, c = 0.056), UV λ_{max} (MeOH) nm [log ε]: 271 [4.47], 213^{sh} [4.44]; IR ν_{max} (KBr) cm^{-1}: 3368, 2920, 1647, 1514, 1436, 1237, 836, FABMS m/z 423 $[M+H]^+$, ^{1}H NMR (CD_3OD) δ 8.08 (1H, s, H-2), 7.36 (2H, d, J = 8.7 Hz, H-2′, 6′), 6.83 (2H, d, J = 8.7 Hz, H-3′, 5′), 3.35 (2H, d, J = 7.0 Hz, H-1″), 5.22 (1H, t-like, J = 7.0 Hz, H-2″), 1.77 (3H, s, H-4″), 1.64 (3H, s, H-5″), 3.14 (1H, dd, J = 14.8 and 2.9 Hz, H-2‴), 2.94 (1H, dd, J = 14.8 and 8.3 Hz, H-1‴), 4.31 (1H, dd, J = 8.3 and 2.9 Hz, H-2‴), 4.94 (1H, s, H-4‴), 4.80 (1H, s, H-4‴), 1.82 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.14. Lupinisol A (*11*)

Pale yellow amorphous, $[\alpha]_D$: +36.1° (EtOH, c = 0.056), UV λ_{max} (MeOH) nm [log ε]: 267 [4.54], 212^{sh} [4.57], IR ν_{max} (KBr) cm^{-1}: 3394, 2923, 1647, 1558, 1271, 824, FABMS m/z 423 $[M+H]^+$, ^{1}H NMR (CD_3OD) δ 7.97 (1H, s, H-2), 6.36 (1H, s, H-8), 7.19 (1H, d, J = 2.2 Hz, H-2′), 6.79 (1H, d, J = 8.0 Hz, H-5′), 7.15 (1H, dd, J = 8.0 and 2.2 Hz, H-6′), 3.01 (1H, dd, J = 13.5 and 5.9 Hz, H-1″), 2.88 (1H, dd, J = 13.5 and 7.2 Hz, H-1″), 4.40 (1H, dd, J = 7.2 and 5.9 Hz, H-2″), 4.47 (1H, s, H-4″), 4.70 (1H, s, H-4″), 1.81 (3H, s, H-5″), 3.31 (2H, d, J = 7.3 Hz, H-1‴), 5.33 (1H, t-like, J = 7.3 Hz, H-2‴), 1.72 (3H, s, H-4‴), 1.72 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.15. Lupinisoflavone G (*12*)

Pale yellow amorphous, $[\alpha]_D$: −8.2° (EtOH, c = 0.14), UV λ_{max} (MeOH) nm [log ε]: 262 [4.54], 210 [4.57], IR ν_{max} (KBr) cm^{-1}: 3447, 2973, 2930, 1670, 1621, 1457, 1276, FABMS m/z 423 $[M+H]^+$, ^{1}H NMR (acetone-d_6) δ 8.12 (1H, s, H-2), 6.35 (1H, s, H-8), 7.33 (1H, d, J = 2.1 Hz, H-2′), 6.88 (1H, d, J = 8.2 Hz, H-5′), 7.26 (1H, dd, J = 8.2 and 2.1 Hz, H-6′), 3.18 (1H, dd, J = 15.6 and 7.7 Hz, H-1‴), 3.12 (1H, dd, J = 15.6 and 9.4 Hz, H-1‴), 4.82 (1H, dd, J = 9.4 and 7.7 Hz, H-2‴), 1.23 (3H, s, H-4‴), 1.27 (3H, s, H-5‴), 3.35 (2H, d, J = 7.3 Hz, H-1‴), 5.36 (1H, t-like, J = 7.3 Hz, H-2‴), 1.72 (3H, s, H-4‴), 1.72 (3H, s, H-5‴); ^{13}C NMR spectrum (see Table 1).

3.16. In vitro anti-fungal susceptibility test

The susceptibility test was performed according to the microdilution method using Sabouraud glucose broth (SGB) (Fukasawa et al., 1991). *Trichophyton mentagrophytes* TIMM1189 (supplied by Teikyo Institute for Medical Mycology) was cultivated and diluted with SGB to adjust the final inoculum concentration of 10^4 cells/ml. Test samples obtained from the plants were dissolved in DMSO or saline (10, 1, 0.1 and 0.01 mg/ml) and assayed in various concentrations at 27°C for 5 days. MICs were defined as the lowest concentration showing the complete inhibition of growth. Lanoconazole (LNCZ) was used as a reference standard (1, 0.1, 0.01 and 0.001 mg/ml).

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