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The Research of Lygodium

Zhang Guo-Gang, He Ying-Cui,
Liu Hong-Xia, Zhu Lin-Xia and Chen Li-Juan
College of Traditional Chinese Materia Medica,
Shenyang Pharmaceutical University, Shengyang,
China

1. Introduction

Lygodium is the dry root and rhizome of *Lygodium japonicum* (Thunb.)Sw. which belongs to the family Lygodiaceae. *Lygodium* is the only genus of Lygodiaceae comprises 45 species throughout the world. In China, there are 10 species of *Lygodium* distributed in the southwest and south China, and five species of them had been used for Chinese herbs medicine to treat hepatitis and dysentery^[1]. They are named Lygodium, Lygodium of hainan, Crankshaft Lygodium, Angustifolia Lygodium, Pinnately lobed Lygodium, Willow-like leaves Lygodium, Reticulata Lygodium, yunnan Lygodium, Lobular Lygodium, Palm leaf Lygodium.

Fig. 1. Leaves and branches of *Lygodium japonicum* (Thunb.)Sw.

Fig. 2. Dried powder of *Lygodium japonicum* (Thunb.) Sw.

2. Chemical composition

Currently, the research on the active components in the *Lygodium* were done on the underground parts. We summarize the structure and classification of these compounds from *lygodium*.

2.1 The main components of the *Lygodium* root^[3]

2.1.1 Ecdysteroid

2.1.2 Triterpenes

Friedelin

2 α -hydroxyursolic acid

2.1.3 Flavonoids

Kaempferol-3-O- α -L-rhamnopyranoside-7-O- α -L-rhamnopyranoside

2.1.4 Phytosterols

22-hydroxyhopane

β -phytosterol

Daucosterol

2.1.5 Glycosides

3,4-dihydroxybenzoic acid 4-O- (4'-O-methyl)- β -D-glucopyranoside

2.1.6 Organic acids

Succinic acid

Methylmalic acid

2.1.7 Naphthoquinone

2-isopropyl-7-methyl-6-hydroxy- α -(1,4) naphthoquinone*

2.2 The main components of the root of *Lygodium* from n-butanol layer^{[2][3]}

2.2.1 flavonoids

Linaribn

Dosinin

Nicotflorin

Tilianine

Kaempferol-α

Kaempferol-1-rhamnopyranoside

2.2.2 Phenylpropanoids

Caffeic acid

P-coumaric acid

6-O-p-coumaroyl-D-glucopyranose

6-O-caffeoyl-D-glucopyranose

2.2.3 Phenolic acids^[4]

2.2.4 Sterols

A new steroidal saponins was Isolated from the root of *Lygodium*, which is (24- *R*)-stigmastan-3β, 5α,6β-triol-3-*O*-β-*D*-glucopyranoside, in addition to daucosterol and β-sitosterol.

2.2.5 Others

Hexadecanoic acid 2, 3-dihydroxy, propyl ester, hexacosanoic acid, 1-hentriacontanol, pentacosanoic acid, palmitic acid, linoleic acid, (6*S*, 9*R*) -6 - hydroxy -3- ketone - Violet alcohol-*D*-β-9-*O*-glucoside (roseoside) and so on.

2.2.6 Diterpenoids

3. New compounds from *Lygodium japonicum*

3.1 New naphthalene ketone from the root of *Lygodium japonicum*^[5]

3.1.1 Extraction and isolation

Air-dried roots of *L. japonicum* (Thunb.) Sw. (4 kg) were crushed and extracted twice under reflux with 70% EtOH. Evaporation of the solvent under reduced pressure delivered the 70% EtOH extract (around 280 g). The extract was partitioned successively with CHCl₃, AcOEt and n-BuOH. The n-BuOH-soluble fraction (50.0 g) was further eluted on a silica gel column using gradient elution with CHCl₃-MeOH (100:1-1:1) to give ten fractions. Fraction 2 (2.3 g) was subjected to another silica gel column chromatography eluted with petroleum ether (PE)-EtOAc (20:1-1:1) to afford a further five fractions (frs. 2-1 to 2-5). Fraction 2-2 was purified twice by Sephadex LH-20 eluted with MeOH to give the new compound 1 (9 mg).

3.1.2 Apparatus

Melting points were determined on an X4-A micro-melting point apparatus and were uncorrected. ESI-MS spectra were measured on an Agilent 1100 LC-MSD-Trap-SL, and HR-ESI-MS spectra were measured on a Bruker Daltonics MicroTOFQ. NMR spectra were measured on a Bruker ARX-600 and 300 NMR spectrometer with tetra-methylsilane (TMS) as the internal reference and chemical shifts are expressed with δ (ppm). UV spectra were recorded on a Shimadzu UV-2201 spectrometer. IR spectra were recorded on a Bruker IFS-55 spectrophotometer. TLC was performed on silica gel GF254 (10-40 μ m; Qingdao, China). Separations were performed by Semiprep-HPLC named Shimadzu SPD-10A apparatus equipped with UV detector under ODS column (i.d. 10 mm $\times$ 200 mm).

3.1.3 Physical data of the new compound 1

The new compound, yellow powder, melting point: 193-194°C. The molecular formula was determined as C₁₄H₁₄O₃ by HR-ESI-TOF-MS (m/z 231.1004 [M + H]⁺, calcd. 231.1016), along with ¹H-NMR and ¹³C-NMR data. The UV spectrum displayed absorption bands at 207, 267 and 347 nm, closely resembling that of 1,4-naphthoquinones. The ¹³C-NMR spectrum revealed 14 carbon resonances; in the low field area of it, two were assigned as carbonyl carbons, eight were assigned as aromatic carbons. However, in the high field area of ¹³C-NMR spectrum, there were four carbon resonances all that assigned as sp³ carbons. By observing these data of

¹³C-NMR spectrum, nucleus of naphthoquinone was revealed. All protonated carbons were assigned by analysis of the HSQC spectrum (Table 1).The ¹H-NMR spectrum showed signals of two aromatic protons at δ 7.30 (1H, s, H-5), 7.77 (1H, s, H-8) and one aromatic methyl proton at d 2.24 (3H, s, 7-CH₃) that were assigned by analyzing HMBC spectrum (Table 1; Fig. 1). Additionally, δ 1.12 (6H, d, J = 6.8 Hz,H-12, H-13) and 6.68 (1H, s, H-3) correlated, respectively, with d: δ 26.6 (C-11), δ 156.6 (C-2) in the HMBC spectrum and δ 3.09 (1H, m, H-11) correlated with δ 21.4(C-12 and 13), 156.6 (C-2), 131.6 (C-3), 183.4 (C-1) all that revealed the presence of isopropyl and it connected C-2 of quinone ring. Other detailed correlations in the HMBC spectrum see Table 1. All these spectroscopic data discussed above showed compound 1 as 6-hydroxy-2-isopropyl-7-methyl-1,4- naphthoquinone.

C No.	HSQC	HMBC	
	δ _C	δ _H , mult	
1	183.5		
2	156.6		
3	131.6	6.68 (1H, s)	C2/C11
4	185.2		
5	110.3	7. 30 (1H, s)	C6/C9/C4
6	160.9		
7	131.8		
8	129.5	7. 77 (1H, s)	C1/C10/C7 (CH ₃)/C6
9	124.1		
10	131.7		
CH ₃ -7	16.2	2.24 (3H, s)	C6/C8
11-CH-	26.6	3.09 (1H, m)	C12/ C13/C2
12-CH ₃	21.4	1.13 (6H, d, J=6.8 Hz)	C11/C2
13-CH ₃	21.4	1.11 (6H, d, J=6.8 Hz)	C11/C2

Table 1. ¹H and ¹³C data for New naphthalene ketone (300and 75MHz,in DMSO- d₆).

Fig. 3. The key HMBC correlations of the new compound 1.

3.2 The new compound 2 from the roots of *Lygodium japonicum*^[6]

lygodiumsteroside B

3.2.1 Extraction and isolation

The air-dried roots of *L. japonicum* (Thunb.) Sw were crushed and extracted twice using reflux with 70% ethanol; the solution was concentrated under reduced pressure to obtain the residue, and then the residue was extracted with MeOH. The MeOH-soluble fraction (100 g) was isolated by column chromatography on silica gel and gradient elution with CHCl_3 :MeOH (50 : 1 to 1 : 1) gave 14 fractions. Fraction 8 was isolated by semipreparative ODS column using MeOH:H₂O (65 : 35) as eluent to afford the new compound 2 (13mg).

3.2.2 Apparatus

Melting point: X-4 micro melting point determination apparatus (uncorrected). ESI-MS spectra: LC-MSD-Trap-SL. HR-ESI-MS spectra: Bruker MicroTOFQ. ¹H NMR (600MHz) and ¹³C NMR (150MHz): Bruker ARX-600. UV: Shimadzu UV-260 UV-Vis. Semiprep-HPLC: Shimadzu SPD-10A apparatus equipped with UV detector under ODS column (i.d. 10mm*200mm).

3.2.3 The spectrum of new compound

1. ¹H-NMR and ¹³C-NMR data for the new compound 2

Fig. 4. The key HMBC correlations of the new compound 2.

C	HMQC		HMBC
	δ_C (ppm)	δ_H (ppm)	
1	38.7	1.74(brd, $J=12.6\text{Hz}$), 2.02(m)	C-2,C-3,C-9,C-10,C-19
2	67.5	4.07(br.dt, $J=10.8\text{ Hz}$),	
3	77.7	4.28(brs)	
4	30.6	1.65, 2.20(each m)	
5	51.4	2.93(m)	C-4,C-9
6	203.0	—	
7	121.7	6.23(brs)	C-5,C-9,C-14
8	166.3	—	
9	34.3	3.53(t, $J=8.4\text{ Hz}$)	
10	39.1	—	
11	21.1	1.67, 1.80(each m)	C-8,C-9,C-12,C-13
12	32.0	2.02(m),2.58(dt, $J=4.2\text{ and }12.6\text{ Hz}$)	C-9,C-11,C-18
13	48.1	—	
14	84.2	—	
15	31.9	1.92, 2.17(each m)	C-13,C-16
16	21.4	2.08, 2.45(each m)	C-17
17	50.0	2.89(m)	C-13,C-15,C-16,C-18
18	18.0	1.20(s)	C-12,C-13,C-14,C-17
19	24.1	0.88(s)	C-1,C-2,C-5,C-10
20	76.9	—	
21	21.6	1.58(s)	C-17, C-20, C-22
22	74.2	3.90(m)	C-20
23	36.9	1.45, 1.63(each m)	C-21,C-24,C-28
24	35.6	1.93(m)	
25	33.7	1.46(m)	C-23,C-26,C-27
26	20.3	0.80(d, $J=6.6\text{ Hz}$)	C-24,C-25,C-27
27	18.7	0.76(d, $J=6.6\text{ Hz}$)	C-24,C-25,C-26
28	15.2	0.83(d, $J=6.6\text{ Hz}$)	C-23,C-24,C-25,C-27
C-1'	104.2	4.92(d, $J=7.8\text{ Hz}$)	C-3
C-2'	74.7	4.03(overlap)	C-3'
C-3'	78.7	4.20(overlap)	C-2', C-4'
C-4'	71.6	4.21(m)	C-5', C-6'
C-5'	78.5	3.92(m)	C-4', C-5'
C-6'	62.6	4.32(m),4.52(brd, $J=10.8\text{ Hz}$)	C-4', C-5'

Table 2. ¹H and ¹³C NMR data for lygodiumsteroside B (600 and 150 MHz, in C₅D₅N).

2. Other spectrum data for the new compound 2

The new compound 2(lygodiumsteroside B): white powder, m.p. 294–295°C,UV| max (MeOH): 243 nm; ESI-MS:m/z 675.5 [M+Cl]⁻, m/z 639.7 [M+H]⁺; HR-ESI-MS: m/z 639.3701 [M+H]⁺(Calcd forC₃₄H₅₅O₁₁ 639.3750);¹H-NMR (600MHz, in C₅D₅N) and ¹³C-NMR (150MHz, inC₅D₅N), see table 2 .

3. NMR Analysis of the new compound 2

Lygodiumsteroside B, white powder, m.p. 294–295°C, gave positive responses to Liebermann–Burchard and Molish reactions, which suggested a steroid glycoside structure. The sugar was identified as glucose by co-TLC with authentic sample after acid hydrolysis. The molecular formula was established to be $C_{34}H_{56}O_{11}$ based on HR-ESI-MS($[M+H]^+$, m/z 639.3701, Calcd for $C_{34}H_{56}O_{11}$ 639.3750). Additionally, the UV spectrum showed a maximum at 243 nm [Check this typing] for an α, β -unsaturated carbonyl group. The 1H NMR (600 MHz, C_5D_5N) spectrum showed an olefinic proton at δ 6.23 (1H, brs) and six methyl signals at δ 1.58 (3H, s), 1.20 (3H, s), 0.88 (3H, s), 0.83 (3H, d, $J=4.6$ Hz), 0.80 (3H, d, $J=4.6$ Hz), 0.76 (3H, d, $J=4.6$ Hz). The ^{13}C -NMR (150 MHz, C_5D_5N) spectrum showed six methyl signals and a typical α, β -unsaturated carbonyl group signals at δ 203.0 (C-6), 166.3 (C-8) and 121.7 (C-7). The HMBC spectrum, showed the long-range correlations between δ 6.23 (1H, brs, H-7) and δ 34.3 (C-9), 51.4 (C-5), 84.2 (C-14), the correlations between methyl proton signal at δ 0.88 (3H, s, H-19) and the carbon signals at δ 38.7 (C-1), 39.1 (C-10), 51.4 (C-5) and 67.5 (C-2) could also be observed. In addition, the correlations between methyl proton signal at δ 1.20 (3H, s, H-18) and the carbon signals at δ 32.0 (C-12), 48.1 (C-13), 50.0 (C-17) and 84.1 (C-14) could also be found. Thus, the ecdysteroid-type skeleton was identified. In the HSQC spectrum, δ 4.07 (1H, brd, H-2) had the correlation with δ 67.5 (C-2) and δ 4.28 (1H, brs, H-3) had the correlation with (1H, brs, H-3) had the correlation with (1H, brs, H-3) had the correlation with δ 77.7 (C-3). δ 1.58 (3H, s, H-21) showed the correlation with δ 50.0 (C-17), δ 74.2 (C-22) and δ 76.9 (C-20) in the HMBC. So the signals of the five hydroxyl carbons C-2, C-3, C-14, C-20, C-22 were evident. Additionally, δ 0.83 (3H, d, H-28) showed correlation with δ 1.93 (1H, m, H-24) in the 1H - 1H COSY, and the HMBC spectrum showed the correlations between δ 0.83 (3H, s, H-28) and δ 18.7 (C-27), 33.7 (C-25), 35.6 (C-24), 36.9 (C-23). These facts indicated that a methyl group (C-28) was attached to C-24.

Compared with polyporusterone A (Ishida et al., 1999; Ohsawa, Yukama, Takao, Murayama, & Bando, 1992), the chemical shifts of C5–C22 were very similar; this fact suggested that the positions of the substituents on the steroid rings and side-chain of the new compound were identical with polyporusterone A except for C24, and the configuration of hydroxyls were 14 α , 20R, 22R. Since the signals at 33.7 (C-25), 20.3 (C-26), 18.7 (C-27) were different from the corresponding values of polyporusterone A, the configuration of C-24 could be different. Compared with the compound schizaeasterone A (Fuchino et al., 1997), which has 24R configuration, the chemical shifts of C20–C28 were very similar to those of schizaeasterone A. Moreover, in the NOESY spectrum, a cross peak was observed between δ 3.91 (1H, overlap, H-22) and δ 0.83 (3H, d, H-28). So all these facts indicated that the configuration of C-24 of compound 1 was R (Figure 2). The NOESY spectrum also showed the correlation between the proton signal at δ 4.07 (1H, brd, H-2) and δ 4.28 (1H, brs, H-3), δ 1.65 (1H, m, H-4), so the relative configuration was confirmed to be 2 β , 3 β .

Since the signal at δ 77.7, which could assignable to C-3, was downfield shifted by 9 ppm, and the signal at δ 30.6 (C-4) was upfield shifted, glycosylation was present at C-3. The chemical shifts of the sugar moiety in ^{13}C -NMR (δ 104.2, 74.7, 78.7, 71.6, 78.5, 62.6) also

confirmed the presence of glucose. The HMBC correlation was observed between the anomeric proton signal at δ 4.92 (1H, d,H-10) and the carbon signal at δ 77.7 due to C-3 of the aglycone moiety. The anomeric configurations of glucose were determined to be on the basis of the JH-H values ($J^{1/4}$ 7.8Hz).Therefore, the structure of 2 was elucidated as 2 β ,3 β ,14 α , 20R, 22R - pentahydroxy-24R-methly-5-cholest-7-en-6-one-3-O- β -D-glucopyranoside, and named lygodiumsteroside B

C No.	HMQC		HMBC
	δ_c (ppm)	δ_H (ppm)	
1	38.7	1.76(m), 2.10(m)	C-2,C-3,C-9,C-10,C-19
2	67.5	4.10(br.dt, J =11.4 Hz),	
3	76.8	4.30(overlap)	
4	30.3	1.73, 2.20(each m)	C-1,C-5,C-9,C-19
5	51.4	2.93(m)	C-1,C-4,C-6,C-9
6	203.1	—	
7	121.7	6.23(d, J =1.8 Hz)	C-5,C-9,C-14
8	166.4	—	
9	34.3	3.55(t, J =8.4 Hz)	C-1,C-11,C-19
10	39.0	—	
11	21.1	1.65, 1.83(each m)	C-8,C-9,C-13
12	32.0	2.02(m),2.58(dt, J =4.2 and 12.6 Hz)	C-11,C-13,C-17,C-18
13	48.1	—	
14	84.2	—	
15	31.8	1.92, 2.15(each m)	C-13,C-16
16	21.5	2.08, 2.44(each m)	C-17
17	50.1	2.91(m)	C-11,C-13,C-15,C-18,C-22
18	17.9	1.19(s)	C-12,C-13,C-14,C-17
19	24.1	0.87(s)	C-1,C-2,C-5,C-9
20	77.7	—	
21	21.6	1.58(s)	C-17,C-22,C-23
22	76.8	3.81(brd, J =10.8 Hz)	C-21,C-24
23	28.2	1.47(m)	C-21,C-24,C-25,C-26,C-27
24	37.2	1.40, 1.70(each m)	C-23,C-26,C-27
25	30.6	1.54(m)	
26	23.4	0.81(d, J =6.0 Hz)	C-23,C-24,C-27
27	22.4	0.82(d, J =6.0 Hz)	C-23,C-24,C-26
C-1'	104.2	4.90(d, J =7.8 Hz)	C-3
C-2'	74.7	4.03(m)	C-3'
C-3'	78.7	4.20(m)	C-2', C-4'
C-4'	71.6	4.18(m)	C-5', C-6'
C-5'	78.5	3.93(m)	C-4', C-5'
C-6'	62.6	4.32(m),4.53(brd, J =10.2 Hz)	C-4', C-5'

Table 3. ¹H and ¹³C NMR data for polyporusterone A (600 and 150 MHz, in C₅D₅N).

4. Attached figure:

Fig. 5. Lygosteroside B (¹H-NMR).

Fig. 6. Lygosteroside B (¹³C-NMR).

Fig. 7. Lygosteroside B (HSQC).

Fig. 8. Lygosteroside B (HSQC).

Fig. 9. Lygosteroside B (HMBC).

Fig. 10. Lygosteroside B (HMBC).

Fig. 11. Lygosteroside B (HMBC).

Fig. 12. Lygosteroside B (¹H-¹H COSY).

Fig. 13. Lygosteroside B (NOESY).

Fig. 14. Lygosteroside B (NOESY).

Fig. 15. Lygosteroside B (ESI).

3.3 The new compound 3 from *Lygodium* [7]

3.3.1 Extraction and isolation

The air-dried roots of *L. japonicum* (Thunb.) Sw. (4 kg) were crushed and extracted twice under reflux with 70% EtOH. The solution was concentrated under reduced pressure to obtain the residue, and then the residue was extracted with MeOH. The MeOH-soluble fraction (100 g) was isolated by column chromatography on silica gel using gradient elution with CHCl_3 -MeOH (50:1 to 1:1), which gave 14 fractions. Fraction 9 (10 g) was subjected to silica gel column chromatography using CHCl_3 -MeOH (40:1 to 1:1) in gradient to give fractions 1-4. Fraction 4 (3.7 g) was chromatographed on an ODS column eluting with

MeOH-H₂O system, giving two fractions. Fraction 2 (1.3 g) was isolated by a semi-preparative ODS column using MeOH-H₂O (65:35) as the eluent to afford lygodium A (9 mg) and a kown compound ponastteroside A(30 mg), respectively.

3.3.2 Apparatus

Melting points were determined on an X4-A micro-melting point apparatus and were uncorrected. ESI-MS spectra were measured on an Agilent1100 LC-MSD-Trap-SL, and HR-ESI-MS spectra were measured on a Bruker Daltonics MicroTOFQ. NMR spectra were measured on a Bruker ARX-600 NMR spectrometer with tetramethylsilane (TMS) as the internal reference and chemical shifts are expressed with δ (ppm). UV spectra were recorded on a Shimadzu UV-2201 spectrometer. IR spectra were recorded on a Bruker IFS-55 spectrophotometer. TLC was performed on silica gel GF254 (10-40 l; Qingdao, China).Separation was performed by semiprep HPLC using Shimadzu SPD-10A apparatus equipped with a UV detector under an ODS column (i.d. 10 mm * 200 mm).

3.3.3 The spectrum of new compound

1. NMR data (nuclear magnetic resonance) of lygodiumsteroside A

C No.	HMQC		HMBC
	δ_C (ppm) DEPT	δ_H (ppm)	
1	38.0 (t)	1.72(t, $J=12.6$ Hz), 2.05(m)	C-2,C-3,C-9,C-10,C-19
2	66.8 (d)	4.06(br.dt, $J=11.4$ Hz),	
3	77.1 (d)	4.29(brs)	
4	29.9 (t)	1.66, 2.16(each m)	
5	50.7 (d)	2.90(m)	C-4,C-9
6	202.3 (s)	—	
7	121.1 (d)	6.18(brs)	C-5,C-9,C-14
8	165.4 (s)	—	
9	33.5 (d)	3.50(brt)	
10	38.4 (s)	—	
11	20.3 (t)	1.64, 1.77(each m)	C-8,C-9,C-10,C-12
12	31.2 (t)	1.86, 2.57(each m)	C-9,C-11,C-13,C-18
13	47.2 (d)	—	
14	83.4 (s)	—	
15	31.1 (t)	1.86, 2.12(each m)	C-14,C-16,C-17
16	20.7 (t)	2.05, 2.39(each m)	C-17
17	49.2 (d)	2.93(m)	C-13,C-16,C-18
18	17.3 (q)	1.09(s)	C-12,C-13,C-14,C-17
19	23.4 (q)	0.86(s)	C-2,C-5,C-9,C-10
20	75.1 (s)	—	
21	20.7 (q)	1.44(s)	C-17, C-20, C-22
22	85.3 (d)	4.44(dd, $J=11.5$ Hz and 2.5Hz)	C-23,C-24

C No.	HMQC		HMBC
	δ_C (ppm) DEPT	δ_H (ppm)	
23	29.0 (t)	1.41, 2.05(each m)	C-24,C-25,C-28
24	39.5 (d)	1.41(m)	
25	40.7 (d)	2.17(m)	C-24,C-27,C-28
26	173.9 (s)	—	
27	15.2 (q)	1.28(d, $J=7.2\text{Hz}$)	C-24,C-25,C-26
28	25.9 (t)	1.01,1.37(each m)	C-23,C-24,C-29
29	9.6 (q)	0.67(t, $J=7.2\text{Hz}$)	C-24,C-28
C-1'	103.5 (d)	4.89(d, $J=7.8\text{Hz}$)	C-3
C-2'	74.0 (d)	4.01(t-like)	C-3'
C-3'	78.0 (d)	4.18(overlap)	C-2', C-4'
C-4'	70.9 (d)	4.06(overlap)	C-5', C-6'
C-5'	77.8 (d)	3.90(t-like)	C-4', C-5'
C-6'	61.9 (t)	4.31(m), 4.49(brd, $J=11.5\text{Hz}$)	C-4', C-5'

Table 4. ¹H and ¹³C NMR data for lygodiumsteroside A (600 and 150 MHz, in C₅D₅N).

2. Correlative spectrum data:

Fig. 16. The key correlations of the new compound 3.

3. Analysis and conclusions of the new compound 3:

The new compound 3,white powder, mp.245–246°C, gave positive response to Liebermann–Burchard reaction and Molish reaction, suggesting a steroid glycoside structure. The sugar was identified as glucose by co-TLC with authentic sample after acid hydrolysis.

The molecular formula was determined as $C_{35}H_{54}O_{12}$ by HR-ESI-MS (m/z 701.3309 $[M+CL]^-$, calcd. 701.3309), along with 1H -NMR and ^{13}C -NMR data. Additionally, the UV spectrum of compound 1 showed a maximum at $\lambda = 243$ nm and the IR spectrum exhibited absorption at $1,730\text{ cm}^{-1}$ for an α, β -unsaturated carbonyl group. The 1H -NMR (600 MHz, C_5D_5N) spectrum showed an olefinic proton at δ 6.18 (1H, brs, H-7) and five methyl signals at δ 1.44 (3H, s, H-21), 1.28 (3H, d, $J = 7.2$ Hz, H-27), 1.09 (3H, s, H-18), 0.86 (3H, s, H-19), 0.67 (3H, t, $J = 7.2$ Hz, H-29). The ^{13}C -NMR (150 MHz, C_5D_5N) showed five methyl signals and a typical α, β -unsaturated carbonyl group signals at δ 202.3 (C-6), 165.4 (C-8), and 121.1 (C-7), which revealed an ecdysteroid-type nucleus. It was also confirmed by the HMBC correlations of the new compound 3. In the HSQC spectrum, δ 4.06 (1H, brd, $J = 11.4$ Hz, H-2) and δ 4.29 (1H, brs, H-3) had direct correlation with δ 66.8 (C-2) and δ 77.1 (C-3), respectively, which combined with the information of long correlation in the HMBC spectrum – δ 1.44 (3H, s, H-21) correlated with δ 49.2 (C-17), δ 85.2 (C-22), and δ 75.1 (C-20), respectively – all of these signals elucidated the presence of five hydroxyl carbons C-2, C-3, C-14, C-20, and C-22. Additionally, δ 1.09 (3H, s, H-18) and δ 1.44 (3H, s, H-21), δ 2.17 (1H, m, H-25) and δ 1.28 (3H, d, $J = 7.2$ Hz, H-27), δ 2.93 (1H, m, H-17) and δ 2.05, 2.39 (2H, each m, H-16) correlated mutually with each other in the 2D- COSY spectrum. The occurrence of α - β lactone ring in the side chain was evidenced by the carbonyl absorption at $1,730\text{ cm}^{-1}$ in the IR and the δ 173.9 ppm in the ^{13}C -NMR. The HMBC spectrum showed the long correlation between the methyl proton signal at δ 1.28 (3H, d, $J = 7.2$ Hz, H-27) and the carbon signals at δ 39.5 (C-24), 40.7 (C-25), and 173.9 (C-26). The correlation between the methyl proton signal at δ 0.67 (3H, t, $J = 7.2$ Hz, H-29) and the carbon signals at δ 25.9 (C-28), 39.5 (C-24), and the correlation between the methyl proton signal at δ 2.19 (1H, m, H-25) and the carbon signals at δ 15.2 (C-27), 25.9 (C-28), and 39.5 (C-24) could be observed, respectively. Moreover, δ 4.44 (1H, dd, $J = 11.5$ Hz and 2.5 Hz, H-22) also showed the correlation with δ 29.0 (C-23), 39.5 (C-24) in the HMBC. Taken together, the structure of the side chain was identified. The HMBC correlation was observed between the anomeric proton signal at δ 4.89 (1H, d, H-10) and the carbon signal at δ 77.1 due to C-3 of the aglycone moiety. This key long-range cross peak fixed the glycosidation position. The chemical shifts of the sugar moiety in ^{13}C -NMR (δ 103.5, 74.0, 78.0, 70.9, 77.8, 61.9) also confirmed the presence of β -glucopyranose. The anomeric configuration of glucose was determined to be on the basis of the J_{H-H} values ($J = 7.8$ Hz) and δ 4.09 (1H, brs, H-3) correlated with δ 4.85 (1H, d, anomeric proton) in the NOE spectrum. (The NOESY spectra mentioned in this text was recorded in CD_3OD because the key proton signals overlapped in C_5D_5N). The relative configuration of the new compound 3 was identified by the NOESY correlation between the proton signal at δ 4.09 (1H, brs, H-3) and δ 3.85 (1H, brd, H-2), δ 1.87 (1H, m, H-4). Careful comparison ^{13}C -NMR data of the new compound 3 and a known compound, named capitasterone^{[8][9]}, revealed that the B, C, and D rings and the side chain were very similar to that of capitasterone. Furthermore, the stereochemistry of C-20 and C-22 of capitasterone had established R, in the NOE spectrum (in CD_3OD) of the new compound 3, δ 4.25 (1H, dd, $J = 11.5$ Hz and 2.5 Hz, H-22) had correlated with δ 1.56 (1H, m, H-24); however, δ 1.56 (1H, m, H-24) never correlated with δ 2.17 (1H, m, H-25). All of these confirmed the stereochemistry of C24 and C25 as R and S, respectively. The 1H -NMR and ^{13}C -NMR signals of the aglycone moiety of the new compound 3 were found to be similar to that of capitasterone. Therefore, the structure of the new compound 3 was elucidated safely as Lygodiumsteroside A (Fig. 15).

Fig. 17. Lygosteroside A (¹H-NMR, in C₅D₅N).

Fig. 18. Lygosteroside A (¹³C-NMR, in C₅D₅N).

Fig. 19. Lygosteroside A (DEPT).

Fig. 20. Lygosteroside A (HSQC, in C₅D₅N).

Fig. 21. Lygosteroside A (HSQC, in C₅D₅N).

Fig. 22. Lygosteroside A (HMBC, in C₅D₅N).

Fig. 23. Lygosteroside A (HMBC, in C_5D_5N).

Fig. 24. Lygosteroside A (HMBC, in C_5D_5N).

Fig. 25. Lygosteroside A (HSQC, in MeOH).

Fig. 26. Lygosteroside A (HSQC, in MeOH).

Fig. 27. Lygosteroside A (NOESY, in MeOH).

Fig. 28. Lygosteroside A (ESI).

4. Pharmacological actions

4.1 Antibacterial action^[10]

Inhibiting *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *shigella Flexneri*.

4.2 Antivirus^[11]

Water extract and alcohol extract of *Lygodium* spores both can inhibit HIV-1 virus. The Concentration of water extract above 125ug/ml can wholly inhibit HIV-1 virus.

4.3 Resistance to male hormone and effect on hair growth^[12]

50% alcohol extract of *Lygodium* Spores can inhibit the activity of Testosterone 5- α reductase in vitro and activate hair follicle.

4.4 Normalizing functioning of the gallbladder and dissolving stone^[13]

4.5 Liver protection^[14]

The water extract of *Lygodium* (50ug/ml) can significantly reduce the GPT action of liver Cell cultures containing GaIN 5×10^{-3} mol/L, so it has effect on liver protection markably.

4.6 Cure urinary impassability, tumescent feeling below umbilicus,

4.7 Urgent pain: *Lygodium* powder drinking by licorice soup.

4.8 Have a good effect on urinary tract infection, urinary calculus, nephritis edema, cold with fever, urine content small and cardiac, enteritis diarrhea.

5. Indications^[10]

5.1 Stranguria marked by chyluria, stranguria caused by the passage of urinary stone, stranguria from urolithiasis and strangury due to heat. For dribbling and painful micturition, there is powder of climbing fern spore from *standards of diagnosis and treatment*: Climbing fern spore 6g, red poria 9g, umbellate pore-fungus 9g, white atractylodes rhizome 9g, peony root 9g, oriental water plantain rhizome 15g, talc 21g, pyrrosia leaf 3g. Grind these herbs into a fine powder. Take 9g each time. For stranguria marked by chyluria, there is powder of climbing fern spore: Climbing fern spore 30g, talc 30g, licorice root 7.5g. Grind these herbs into a fine powder. Take 6g each time.

5.2 Dampness in the spleen, general edema, distension in the abdomen can be treated with powder of fern spore from *Inventions of medicine*: Morning glory seed 45g, kansui root 15g, climbing fern spore 15g, grind these herbs into a fine powder. Take 6g each time before meals.

6. Conclusion

Lygodium japonicum (Thunb.)Sw., which belongs to the genus *Lygodium* of the family Lygodiaceae, is the dry root and rhizome of *Lygodium japonicum* (Thunb.)Sw. There are different constituents in different parts of it and distribution of content of these constituents

are different. The paper aims at making a systematical research for the root of *Lygodium japonicum* (Thunb.) Sw. that is one specie of medicinal *Lygodium*. According to the existing literature, the main constituents of *Lygodium* are steroidal, flavonoids, organic acids and other substances, but it is no clear whether all these chemical constituents are effective composition or not.

This paper summarize the research on chemical constituents and pharmacological actions of *Lygodium*. From our systematic research on *Lygodium*, we have isolated and identified many kinds of compounds. In this paper, we focus on introduction of three new compounds: lygodiumsteroside A and lygodiumsteroside B and 2-isopropyl-7-methyl-6-hydroxy- α -(1,4) naphthoquinone, as well as the data analysis of UV, IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, 2D-NMR and HR-MS. We also generalize pharmacological actions and clinical applications of *Lygodium*.

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Drug Discovery Research in Pharmacognosy

Edited by Prof. Omboon Vallisuta

ISBN 978-953-51-0213-7

Hard cover, 244 pages

Publisher InTech

Published online 16, March, 2012

Published in print edition March, 2012

This book, Drug Discovery Research in Pharmacognosy provides a full picture of research in the area of pharmacognosy with the goal of drug discovery from natural products based on the traditional knowledge or practices. Several plants that have been used as food show their potential as chemopreventive agents and the claims of many medicinal plants used in traditional medicine are now supported by scientific studies. Drug Discovery Research in Pharmacognosy is a promising road map which will help us find medicine for all!

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Zhang Guo-Gang, He Ying-Cui, Liu Hong-Xia, Zhu Lin-Xia and Chen Li-Juan (2012). The Research of Lygodium, Drug Discovery Research in Pharmacognosy, Prof. Omboon Vallisuta (Ed.), ISBN: 978-953-51-0213-7, InTech, Available from: <http://www.intechopen.com/books/drug-discovery-research-in-pharmacognosy/the-research-of-lygodium>

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InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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