Three flavonoids from *Cycas revoluta*

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Abstract

Three flavonoids compounds, 5,6,7,8,3',4'-hexamethoxyflavone (**1**)、5,6,7,8,4'-pentamethoxyflavon (**2**)、7,3'-dihydroisoflavones (**3**) were isolated from the Traditional Chinese medicine *Cycas revoluta* for the first time, and their structures were established on the basis of spectroscopic methods. The presences of these compounds (**1-3**) might be employed as the characteristic constituents of *Cycas revoluta* family.

Keywords: *Cycas revoluta*, flavonids, characteristic constituents

1. Introduction

Cycas revoluta belongs to the family *Cycas revoluta* and was widely distributed in Guangxi province, Yunnan province, Sichuan province, Guizhou province, Hainan province and Taiwan province in China^[1]. Previous phytochemical investigations resulted in the isolation of many compounds including biflavone, cycasin, amino acid, phenol glycosides, saccharide compounds, steroids, long chain compounds and pigment, etc^[2-6]. In this study compounds (**1-3**) were isolated for the first time. The structures of these compounds were shown in Fig. 1.

Fig. 1. Structure of compounds **1-3**

2. The Experiments And Materials

2.1 Separation And Extraction

The air-dried leaves was exhaustively extracted with 95% ethanol after grounding into powder (20.0 kg). The solvent was removed under reduced pressure to afford an extract (1.5 kg). Then the extract was successively partitioned with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The chloroform extract (110 g) was subjected to a silica gel chromatography, eluting with a gradient of CHCl₃/MeOH (100:1-1:1, v/v), to give six fractions (A-F). Fraction C (9.6 g) was applied to silica gel column chromatography, eluting with CHCl₃/MeOH (20:1 to 1:1, v/v), to furnish five fractions (1-5). Fraction 4 (5.3 g) was separated by a column of Sephadex LH-20 eluted with CHCl₃/MeOH 100:3, 100:7, 100:10 and 100:20 to gave compounds **1** (3.2 mg) and **2** (16.6 mg). Compounds **3** (6.4 mg) were obtained by recrystallization from CHCl₃.

2.2 Compounds Structural Analysis

Compound **1** was obtained as yellow needles. Its molecular formula was determined as C₂₂H₂₁O₈ by ESI-MS at m/z 403.1315[M+H]⁺. The ¹H-NMR and ¹³C-NMR spectra indicated six sp³ hybridization of high intensity of hydrogen proton signals; δ_H 4.02 (3H, s), 3.96 (3H, s), 3.88 (3H, s), 3.88 (3H, s), 3.85 (3H, s) and 3.79 (3H, s), ¹³C-NMR (75 MHz, DMSO- d₆) spectrum shown six carbon signals δ_C 62.0, 61.9, 61.6, 61.5 $\times$ 2 and 55.6, indicated the existence of 6 methoxy signals. δ_H 7.65 (1H, d, *J* = 8.7 Hz), 7.55 (1H, d, *J* = 1.8 Hz) and 7.16 (1H, d, *J* = 1.8, 8.7 Hz) revealed the structure with a benzene ring with ABX coupling system, benzene ring of carbon signal corresponding to the δ_C 127.9, 114.8 and 123.1. The ¹³C-NMR spectra given 21 carbon signals, remove 6 methoxyl matrix signal, the basic with the parent nucleus 15 carbon, speculated that the structure may be flavonoids compounds. δ_H 6.72 (1H, s) speculated that flavonoids three hydrogen proton signals. The above data, combined

with the literature [7], identification the compound as 5,6,7,8,3',4'-hexamethoxyflavone, the NMR spectrum data were shown in Fig. 2,3 and Table 1.

Fig. 2. The ^1H -NMR spectrum of compound **1**

Fig. 3. The ^{13}C -NMR spectrum of compound **1**

Table 1. ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) spectral data of compound **1**

Positio	δ_{C}	δ_{H} (J in Hz)
2	162.1	-
3	106.1	6.72 (1H, s)
4	175.9	-
5	147.6	-
6	143.6	-
7	151.0	-
8	137.8	-
9	147.2	-
10	123.1	-
1'	162.1	-
2'	127.9	7.16 (1H, dd, $J = 2.7, 8.7$ Hz)
3'	160.5	-
4'	154.3	-
5'	114.8	7.55 (1H, d, $J = 1.8$ Hz)
6'	127.9	7.65 (1H, d, $J = 8.7$ Hz)

Compound **2** was obtained as yellow needles. Its molecular formula was determined as $\text{C}_{20}\text{H}_{20}\text{O}_7$ by ESI-MS at m/z 374.1209 $[\text{M}+\text{H}]^+$. ^1H -NMR (300 MHz, DMSO- d_6) spectrum included five sp^3 hydrogen proton

signals at high intensity, δ_{H} 4.02 (3H, s), δ_{H} 4.02 (3H, s) $\times 2$, 3.85 (3H, s) and 3.78 (3H, s). ^{13}C -NMR (75 MHz, DMSO- d_6) given five carbon signals δ_{C} 62.0, 61.9, 61.6, 61.5 and 55.8, indicated the existence of 5 methoxy signals. δ_{H} 8.0 (1H, d, $J = 8.7$ Hz) $\times 2$ and 7.14 (1H, d, $J = 8.7$ Hz), suggested there is a counterpoint to replace benzene ring system, benzene ring of carbon signal corresponding to the δ_{C} 123.2, 119.4 and 114.6 $\times 2$. The ^{13}C -NMR spectra given 20 carbon signals, remove 5 methoxyl matrix signal, the basic with the parent nucleus 15 carbon, speculated that the structure may be flavonoids compounds. δ_{H} 6.77 (1H, s) speculated tha position three hydrogen proton signals in flavonoids. The above data, combined with the literature [7], identification the compound as 5,6,7,8,4'-pentamethoxyflavone, the NMR spectrum data were shown in shown in Fig. 4,5 and Table 2.

Fig.4. The ^1H -NMR spectrum of compound **2**

Fig. 5. The ^{13}C -NMR spectrum of compound **2**

Table 2. ¹H-NMR (300 MHz) and ¹³C-NMR (75 MHz) spectral data of compound 2

Positio	δ _C	δ _H (J in Hz)
2	160.4	-
3	106.4	6.77 (1H, s)
4	176.0	-
5	149.1	-
6	137.7	-
7	151.1	-
8	143.6	-
9	149.1	-
10	112.0	-
1'	123.2	-
2'	123.2	8.01 (1H, d, J = 8.7 Hz)
3'	114.6	7.14 (1H, d, J = 8.7 Hz)
4'	151.8	-
5'	114.3	7.14 (1H, d, J = 8.7 Hz)
6'	119.4	8.01 (1H, d, J = 8.7 Hz)

Compound 3 was obtained as colorless needles. Its molecular formula was determined as C₁₅H₁₂O₄ by ESI-MS at m/z 257.0736[M+H]⁺. ¹³C-NMR (75 MHz, DMSO- d₆) given fifteen carbon signals, speculated that the structure may be flavonoids compounds. δ_H 8.74 (1H, s) indicated the structure as isoflavone stem nucleus. δ_H 8.30 (1H, d, J = 2.7 Hz), 8.10 (1H, d, J = 9.0 Hz) and 7.52 (1H, dd, J = 2.7, 9.0 Hz), revealed the structure with a benzene ring with ABX coupling system, benzene ring of carbon signal corresponding to the δ_C 128.7, 116.2 and 112.6. The above data, combined with the literature^[8], identification the compound as 7,3'- dihydroisoflavones, NMR spectrum data were shown in Fig. 6,7 and Table 3.

Fig.6. The ¹H-NMR spectrum of compound 3

Fig. 7.The ¹³C-NMR spectrum of compound 3

Table 3. ¹H-NMR (300 MHz) and ¹³C-NMR (75 MHz) spectral data of compound 3

Positio	δ _C	δ _H (J in Hz)
2	156.0	8.74 (1H, s)
3	124.0	-
4	174.0	-
5	128.7	8.30 (1H, d, J = 2.7 Hz)
6	116.2	8.010 (1H, d, J = 9.0 Hz)
7	160.2	-
8	112.6	7.52 (1H, dd, J = 2.7, 9.0 Hz)
9	152.0	-
10	119.3	-
1'	130.1	-
2'	119.3	7.78 (1H, s)
3'	156.4	-
4'	134.1	7.72 (1H, m)
5'	129.9	7.65 (1H, m)

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4. Conclusions

The *Cycas revojuta* was an important genus in *Cycas* revojuta family, which has approximately 70 species in China. Three flavonoids compounds, 5,6,7,8,3',4'-hexamethoxyflavone (1)、5,6,7,8,4'-pentamethoxyflavon (2)、7,3'- dihydroisoflavones (3) were isolated from the fruits of *Cycas revojuta* for the first time, and their structures were established on the basis of spectroscopic

methods. Compounds **1-3** were isolated for the first time from the family *Cycas revoluta*, whose appears rich the compounds diversity to family *Cycas revoluta*.

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References

- (1) James A Ingham, PauII. Forster and Michael D.Crisp : "Ancient relicts or recent dispersal: how long have cycads been in central Australia", *Diversity and Distributions*, Vol. 19, No.3, pp. 307-316, 2013
- (2) Tapan Kumar Mohanta, Andrea Occipinti and Simon Atsbaha Zebelo : "Ginkgo biloba responds to herbivory by activating early signaling and direct defenses", *PLoS One* , Vol. 7, No.3, pp. e32822, 2012
- (3) Tomokazu Haraguchi, Keiichi Nomura and Fumio yagi : "Cloning and expression of a mannose-binding jacalin-related lectin from leaves of Japanese cycad (*Cycas revoluta* Thunb.)", *Bioscience, biotechnology, and biochemistry*, Vol. 70, No.9, pp. 2222-2229, 2006
- (4) Donald G Barceloux : "Cycad seeds and chronic neurologic disease (*Cycas* species)", *Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Plants, and Venomous Animals*, pp. 54-58, 2009
- (5) Thomas Krüger, Bettina Möncha and Steven Oppenhäusera : "LC-MS/MS determination of the isomeric neurotoxins BMAA methylamino-l-alanine) and DAB (2, 4-diaminobutyric acid) in cyanobacteria and seeds of *Cycas revoluta* and *Lathyrus latifolius*", *Toxicon* , Vol. 55, No.2, pp. 547-557, 2010
- (6) Moawad A, Hetta M, Zjawiony J K : "Phytochemical investigation of *Cycas circinalis* and *Cycas revoluta* leaflets: moderately active antibacterial biflavonoids", *Planta medica*, Vol.76, No.8, pp. 796-802, 2010
- (7) Saem Han, Hye Min Kim and Jeong Min Lee : "Isolation and identification of polymethoxyflavones from the hybrid *Citrus*, hallabong", *Journal of agricultural and food chemistry*, Vol.58, No.17, pp. 9488-9491, 2010
- (8) Terezinha de J. Faria, Luiz G. Fonseca e Silva and José D. de Souza Filho : "Synthesis and trypanocidal activity of 7, 2'-dioxxygenated isoflavones and oxypropanolamine derivatives", *Journal of the Brazilian Chemical Society*, Vol.16, No.16B, pp. 1415-1419, 2005