

Isolation and identification of compounds from *Bauhinia championii* (Benth.) Benth

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Abstract

Phytochemical investigation of *Bauhinia championii* (Benth.) Benth. rattan ethanol extract allowed to isolate compounds characterized as β -sitosterol, triacontane, hexacontane, quercetin, myricitrin, 2,4,6-trimethoxyphenol-1-O- β -D-(6'-O-galloyl)-glucopyranoside, 5,7,3',4',5'-hexamethoxyflavone and oblongixanthone A. Oblongixanthone A was isolated for the first time and its structural characterization was obtained on the basis of extensive NMR and MS spectral studies.

Keywords: *Bauhinia championii* (Benth.) Benth.; Spectral analysis; oblongixanthone A; quercetin; myricitrin

Introduction

Bauhinia championii (Benth.) Benth., a perennial lianas, belongs to *Bauhinia leguminosae* family. It is mainly distributed in Zhejiang, Jiangxi, Fujian, Guangdong, Guangxi, Hunan, Hubei, Guizhou et al. provinces [1]. *Bauhinia championii* (Benth.) Benth. is a characteristic minority medicine. It is bitter and acerbic in taste, mild in nature. It has the properties of expelling wind to eliminate dampness, promoting blood circulation to stop pain, invigorating the spleen and regulating qi [2]. In folk medicine, it is mainly used to treat epigastric pain [3], rheumatism arthritis [4], acute and chronic pain of lumbar and leg [5,6].

Previous phytochemical studies on *Bauhinia championii* (Benth.) Benth. had resulted in the isolation of flavonoids, sterol, aromatic acid, terpenoids and so on [7,8]. To our knowledge, there are no more published data documenting research on isolation of it. The present chemical investigations of *Bauhinia championii* (Benth.) Benth have led to the isolation and identification of a new oblongixanthone A together with several known compounds.

Experimental

Reagents and General Materials

MDS-3 type melting point apparatus was employed to determined melting point, it was bought from YANAGIMOTO MFG, CO. MS spectra were measured with Agilent 1100 series LC/MSD mass spectrometer from Agilent. The IR spectrum was run on FTIR spectrometer (BRUKER-VECTOR22) that is from BRUKER Company, Germany, recorded as KBr patches; Bruker ac f200 type NMR was used to measure the NMR spectrum and it came from BRUKER Company, Germany. Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), Silica gel (300-400 mesh) and

silica gel GF254 sheets (0.20-0.25 mm) that both were from Qingdao Haiyang Chemical Group Co., Shandong Province, China and were used for column chromatography and TLC, respectively.

Plant material

The rattans of *Bauhinia championii* (Benth.) Benth. were collected in the mountain woods of Minhou (E119.22, N25.88, Fuzhou, China) in April 2011, and confirmed by Professor Lu Wei, Fujian University of Traditional Chinese Medicine. A voucher specimen (BC201104) was deposited in Pharmacy college of Fujian University of Traditional Chinese Medicine, Fujian, P. R. China. All samples were dried, milled, passed through a stainless steel sieve and stored until use. The same batch of sample was used through this study.

Extraction and isolation

Samples of *Bauhinia championii* (Benth.) Benth. (8.0 kg) were extracted with 80% aq. ethylalcohol for 2 times/each 2 h at room temperature and then concentrated in vacuo to yield a crude extract. The extract was suspended in water and then successively partitioned by EtOAc and n-butyl alcohol.

The EtOAc extract (100 g) was subjected to gel column chromatography (160 ~ 200 mesh) and eluted with a gradient system of Methylene chloride-methanol, and volumetric eluent was collected. Eluent was merged after TLC inspection. Affording fractions were further separated and purified by silica gel, Sephadex LH-20 column chromatography, polyamide column chromatography and ODS column chromatography. Finally compounds I, II, III, IV, V, VI, VII, VIII, IX were obtained.

The n-butyl alcohol extract (100 g) was done as the above process, and compounds V, X were obtained.

Identification of chemical structure

The structures of the isolated compounds were established by comparing their physical, chemical and spectroscopic data with those previously reported. Further confirmation has been done by co-chromatography (TLC) with authentic samples.

Results and discussion

β -sitosterol (I): whitish needles (acetone), mp:141-142 °C. IR (KBr) cm^{-1} : 3432, 2936, 2860, 1461, 1381, 1054, 959, 800. Its matching degree with β -sitosterol was more than 90% which was searched in Sigma Biological Sample Library. Then it was detected by TLC, and the R_f value was consistent with sitosterol at the different conditions of petroleum ether-ethyl acetate, petroleum ether-acetone, methylene chloride-acetone. Mixed melting point don't fall. Its properties was also in agreement with references [9]. Herein it was identified as β -sitosterol, whose structure was shown in Fig.1.

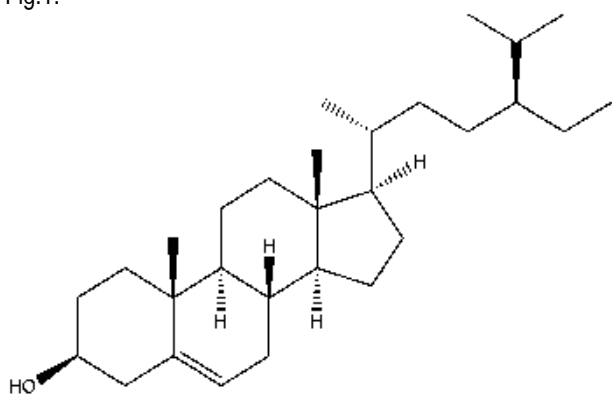


Figure 1 Chemical structures of β -sitosterol (I)

Daucosterol (II): whitish needles (acetone), mp: 288~289 °C. IR (KBr) cm^{-1} : 3397, 2959, 2933, 2869, 1463, 1379, 1073, 102. Matching degree with daucosterol of it was more than 90% which was searched in Aldrich Condensed Phase Sample Library. When it was detected by TLC, the R_f value was consistent with daucosterol at the different conditions of methylene chloride-acetone, methylene chloride-methanol, methylene chloride-methanol-water. And mixed melting point showed don't fall. Its $^1\text{H-NMR}$ properties was in agreement with references [10]. So it was identified as daucosterol, whose structure was shown in Fig.2.

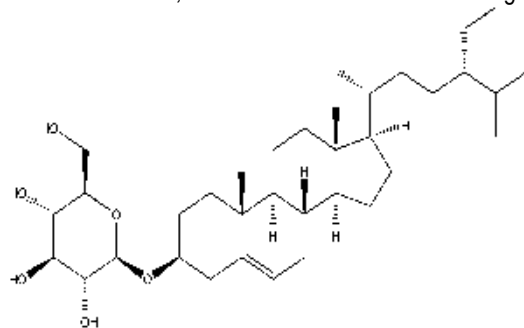


Figure 2 Chemical structures of daucosterol (II)

Triacontane (III): whitish needles (Petroleum ether-ethyl acetate), mp: 65~66 °C. IR (KBr) cm^{-1} : 2917, 2848, 1472, 1061, 719. Matching degree with triacontane was more than 90% which was searched in Aldrich Condensed Phase Sample Library. And it was showed single spot on a silica G plate at the different conditions, the R_f value was consistent with triacontane when detected by TLC. Hence it was identified as triacontane.

Hexacontane (IV): White flake or solid (Petroleum ether-ethyl acetate), mp: 97~98 °C. IR(KBr) cm^{-1} : 2917, 2849, 1742, 1472, 1363, 1239, 1179. Matching degree with hexacontane was more than 90% which was searched in Aldrich Condensed Phase Sample Library. And detected by TLC it was showed single-point on a silica G plate at the different conditions, the R_f value was consistent with hexacontane. Therefore it was identified as hexacontane. Quercetin (V): yellowish needles (Chloroform-methanol), mp: >300°C. IR (KBr) cm^{-1} : 2918, 2849, 1664, 1610, 1521, 1450, 1381, 1318, 1261, 1198, 1167, 1013. Matching degree with quercetin searching in HR Aldrich Aldehydes and Ketones was more than 90%. $^1\text{H-NMR}$ (CD_3OD , 400 MHz): 7.70 (1H, d, $J = 2$ Hz, H-2''), 7.62 (1H, dd, $J = 2, 2.5$ Hz, H-6), 6.85 (1H, d, $J = 2$ Hz, H-5'), 6.34 (1H, d, $J = 2$ Hz, H-8), 6.08 (1H, d, $J = 1.5$ Hz, H-6). $^{13}\text{C-NMR}$ (CD_3OD , 100 MHz): 175.9 (C-4), 164.1 (C-7), 161.1 (C-5), 156.7 (C-9), 147.4 (C-2), 146.6 (C-3'), 144.5 (C-4'), 122.8 (C-1'), 120.3 (C-6'), 114.7 (C-5''), 114.6 (C-2'), 103.0 (C-10), 97.8 (C-6), 93.1 (C-8). $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ datas was the same as literature reported [11,12]. Moreover it was showed single-point on a silica G plate at the different conditions, the R_f value was consistent with quercetin. So it was identified as quercetin, the structure was in Fig.3.

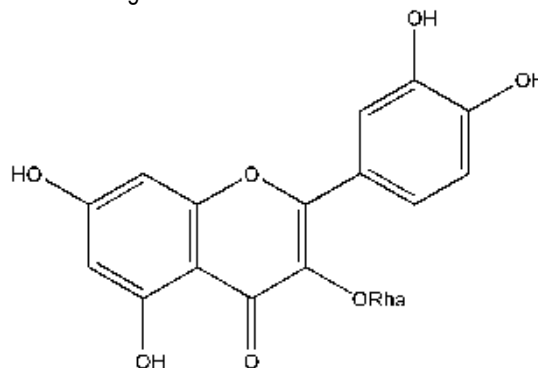


Figure 3 Chemical structures of quercetin (V)

2,4,6-trimethoxyphenol-1- β -D-(6'-*O*-galloyl)-glucopyranoside (VI): whitish needles. $^1\text{H-NMR}$ (DMSO- d_6 , 400MHz): 3.80 (6H,s, -OMe 2), 3.73 (3H,s, -OMe), 4.20-4.30 (4H, m, H-2', 3', 4', 5'), 4.95 (1H, dd, $J = 11.9, 5.9$ Hz, H-6'), 5.21 (1H, dd, $J = 11.9, 1.2$ Hz, H-6'), 5.58 (1H, dd, $J = 7.2$ Hz, H-1'), 6.54 (2H,s, H-3, 5), 7.80 (2H,s, H-2'', 6''); $^{13}\text{C-NMR}$ (CD_3OD , 400MHz): 56.2 (q, -OMe 2), 60.6 (q, -OMe), 64.6 (t, C-6'), 71.0 (d, C-4'), 74.4 (d, C-2'), 75.5 (d, C-5'), 77.3 (d, C-3'), 95.6 (d, C-3, 5), 102.2 (d, C-1'), 109.6 (d, C-2'', 6''), 121.1 (s, C-1''), 134.1 (s, C-1), 138.8 (s, C-4''), 145.8 (s, C-3'', 5''), 154.1 (s, C-2,6), 154.9 (s, C-4), 166.9 (s, C-7''). $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$

NMR datas were consistent with reported 2,4,6-trimethoxyphenol-1-*O*- β -D-(6'-*O*-galloyl)-glucopyranoside in literature [10]. Consequently, it was identified as 2,4,6-trimethoxyphenol-1-*O*- β -D-(6'-*O*-galloyl)-glucopyranoside, the structure was in Fig.4.

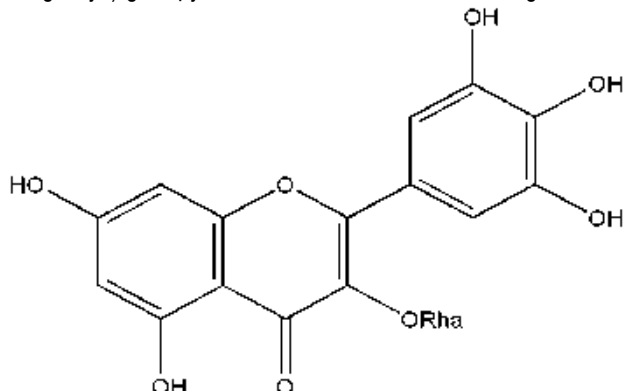


Figure 4 Chemical structures of 2,4,6-trimethoxyphenol 1-*O*- β -D-(6'-*O*-galloyl)-glucopyranoside (VI)

Myricitrin (VII) : yellow powder. $^1\text{H-NMR}$ (DMSO- d_6 , 400MHz): 0.90 (3H,d, $J=6.2$ Hz, H-6'), 3.35 (1H, t, $J=3, 1$ Hz, H-3''), 3.51 (1H, m, H-5''), 3.78 (1H, dd, $J=3.3, 9.5$ Hz, H-4''), 4.22 (1H, dd, $J=1.5, 3.1$ Hz, H-2''), 5.35 (1H, d, $J=1.1$ Hz, H-1''), 6.20 (1H, d, $J=2.2$ Hz, H-6), 6.35 (1H, d, $J=2.1$ Hz, H-8), 6.95 (2H, s, H-2', 6'). Matching degree with myricitrin searching in HR Aldrich Aldehydes and Ketones was more than 90%. $^1\text{H-NMR}$ datas was the same as literature reported [7]. Moreover it was showed a single-point on a silica G plate at the different conditions, the R_f value was consistent with myricitrin. Thus it was identified as myricitrin, the structure was in Fig.5.

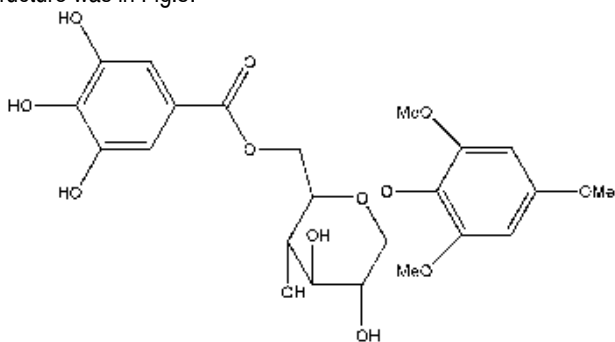


Figure 5 Chemical structures of myricitrin (VII)

5,7,3',4',5'-hexamethoxyflavone (VIII): whitish needles (acetone). $^1\text{H-NMR}$ (DMSO- d_6 , 400MHz): 3.90 (3H,s, OMe), 3.95(6H,s, OMe 2), 3.98(3H,s, OMe), 4.00(3H,s,OMe), 6.42 (1H,br s,3-H), 6.60 (1H, br s, 6-H), 6.71 (1H, s, 8-H), 7.10 (2H, s, 2', 6'-H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 56.2 (q, -OMe 3), 55.8 (q, -OMe), 61.2 (q, -OMe), 103.5 (C-6'),153.6 (C-5'), 140.9 (C-4'), 153.6 (C-3'), 103.5 (C-2'), 126.8 (C-1'), 160.5 (C-2), 108.6 (C-3), 177.5 (C-4), 161.1 (C-5), 96.8 (C-6), 164.8 (s, C-7), 92.1 (C-8), 159.9 (C-9), 109.9 (C-10). The datas of $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ were agreed with 5,7,3',4',5'-hexamethoxy flavone (Fig.6) in literature [13].

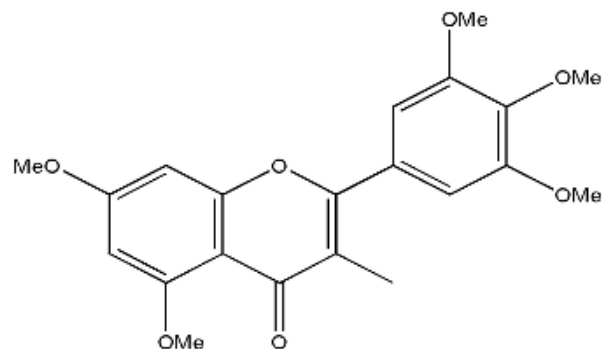


Figure 6 Chemical structures of 5,7,3',4',5'-hexamethoxyflavone (VIII)

Oblongixanthone A (IX): Yellow granular crystals, mp: $>280^\circ\text{C}$. Its IR (KBr) cm^{-1} was 3395, 2926, 1648, 1613, 1601, 1464, 1377, 1305, 1264, 1165, 1125. $^1\text{H-NMR}$ (DMSO- d_6 , 400MHz) 7.877(1H, s), 6.804(1H, d), 6.402(1H, d), 6.129(1H, d), 5.935(1H, d), 1.468(6H, s). The molecular formula was deduced as $\text{C}_{18}\text{H}_{14}\text{O}_6$ by TOF-MS-ESI at m/z 327.2[M+H]. The $^1\text{H-NMR}$ spectrum was very similar to the known structure xanthone, which had characteristic peaks of a 1,2,3,5-tetrasubstituted benzene ring B [H 6.169 (1H, d, $J=1.9$ Hz) and 6.402 (1H, d, $J=1.9$ Hz)], a chelated hydroxy proton [H 13.339 (OH-1, s)], an aromatic proton singlet at H 7.877 and a dimethyl chromene ring [H 6.804, 5.935 (1H each, d, $J=10.0$ Hz) and 1.468 (6H, s)]. The highest field aromatic protons at H 6.129 and 6.402 coupled to each other and both were attributed to H-1 and H-3 in the 1,2,3,5-tetrasubstituted benzene ring B, respectively. The other aromatic proton at H 7.877 was assigned to ring A. It was confirmed via comparing the NMR datas with these of xanthone and the correlation peaks observed in the HMBC spectrum (Fig.7). The HMBC indicated that this proton may be attributed to H-8 that was correlations of the ring A aromatic proton with a ketone carbon (C 178.9 s) and three oxygenated aromatic carbons at C 147.3, 145.3 and 143.4 (C-6, C-7 and C-10a). The dimethyl chromene ring unit was located at C-5 and C-6 on the basis of the HMBC correlations of H-11 with C-5, C-6 and C-10a. Above $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ datas was the same as reported literature [14]. Accordingly, the structure of IX was established in Fig.8 and was assigned as 1,3,7-trihydroxy-13,13-dimethyl-2H-pyran [5,6-b] xanthone, named oblongixanthone A.

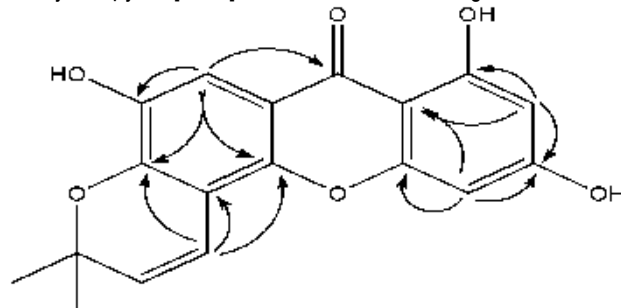


Figure 7 Key HMBC correlations of oblongixanthone A

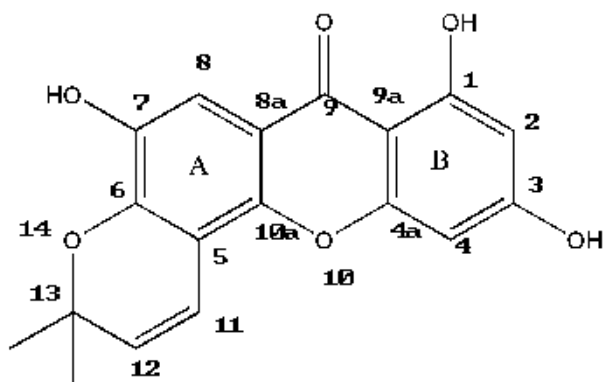


Figure 8 Chemical structures of oblongixanthone A(IX)

Conclusion

Phytochemical investigation of *Bauhinia championii* (Benth.) Benth. afforded, in addition to known β -sitosterol, triacontane, hexacontane, quercetin, 2,4,6-trimethoxyphenol-1- α - β -D-(6'- α -galloyl)-glucopyranoside, myricitrin, 5,7,3',4',5'-hexamethoxyflavone, a new oblongixanthone A. The availability of

the new oblongixanthone A will be useful for our ongoing studies to evaluate the potential effect as modulators of drug in RA therapy.

Authors' contributions

Wei Xu carried out extraction and isolation studies and drafted the manuscript. Kedan Chu carried out the identification of chemical structure. Huang Li participated in the isolation studies. Mei Sha and Yuqin Zhang participated in the identification of chemical structure. Mingqing Huang carried out the data analysis. Lidian Chen conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

Acknowledgment

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