COMPOUNDS FROM THE LEAVES OF STEREOSPERMUM BINHCHAUENSIS

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TÓM TẮT

MỘT SỐ HỢP CHẤT CÔ LẬP TỪ LÁ CÂY QUAO BÌNH CHÂU (STEREOSPERMUM BINHCHAUENSIS)

Từ cao n-hexane chiết xuất từ lá cây quao Bình Châu, chúng tôi đã cô lập được hai hợp chất flavonoid là 5,7-dimethoxyflavone (1), kaempferol (2), một phenolic 5,7-dihydroxychromone (3), và một hợp chất sterol là 3β-hydroxystigmast-5-en (4). Cấu trúc của các hợp chất được xác định bằng các phương pháp phổ hiện đại MS, NMR (1D & 2D) và so sánh với tài liệu tham khảo. Đây là lần đầu tiên các hợp chất (1-4) được cô lập từ chi Stereospermum

Từ khóa: *Stereospermum binhchauensis, Bignoniaceae, flavonoid, phenolic.*

1. INTRODUCTION

Stereospermum binhchauensis V.S. Dang (Bignoniaceae), a new variety was discovered and indentified in 2015[6]. As part of our continuing study on bioactive compounds of the family Bignoniaceae[3,4,8], this paper illustrated the isolation and structural elucidation of two flavonoids (**1-2**), one phenolic **(3)** and one sterol (**4**) from the *n*-henxane extract of the leaves *Stereospermum binhchauensis* collected in Binh Chau, Phuoc Buu District, Ba Ria Province, Vietnam.

2. EXPERIMENTAL

2.1. Plant material

The leaves of *S. binhchauensis* were collected in Binh, Phuoc Buu District, Ba Ria Province, Vietnam, in June 2016; and identified Chau by Dr. Van Son Dang, Institute of Tropical Biology. A voucher specimen (No.VH/PHAT-0616B) was deposited in Bioactive Compounds Laboratory, Institute of Chemical Technology, Vietnam Academy of Science and Technology.

2.2. Methods

The mass spectrometric analysis (MS) was performed on a UPLC-MSQ Plus spectrometer (Thermo, USA). The ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), DEPT, COSY, HSQC, HMBC spectra were recorded on a Bruker AM500 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard. Column chromatography was carried out using silica gel normal-phase (230-400 mesh). Analytical TLC was carried out on silica gel plates (Kieselgel 60 F_{254}, Merck). Compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3-5 min.

2.3. Extraction and isolation

Powdered leaves of *Stereospermum binhchauensis* (7000 g) were extracted with 96^o EtOH for three times (3 x 30 L, total amount 90 L) at room temperature, residue was filtered, solvents were removed under low pressure and the crude extract (1050 g) was obtained. The crude extract was added distilled water and applied to liquid-liquid extraction procedures and successively partitioned into *n*-hexane (340 g), EtOAc (150 g) and aqueous partition (495 g). The *n*-hexane extract was applied to silica gel column chromatography with mobile phase *n*-hexane-EtOAc (100-0, 75-25, 50-50, 25-75, 0-100, v/v, respectively) to give five fractions, H.I (45 g), H.II (75 g), H.III (74 g), H.IV (75 g) and H.V (65 g). Fraction H.III (74 g) was subjected on silica gel with eluted solvent *n*-hexane-EtOAc gradient (70-30 to 40-60, v/v) to give seven sub-fractions (H.III.1-H.III.7). Fraction H.III.2 (6 g) was eluted with solvent system $CHCl_3$–MeOH (100:0 to 98: 2 , v/v) to afford **3** (12 mg) and **4** (10 mg)

The same as fraction H.III, fraction H.IV (75g) was applied on column chromatography with mobile phase *n*-hexane-EtOAc (60–40 to 0–100, v/v) to obtain seven subfractions (H.IV.1-H.IV.7.). Subfraction H.IV.2 (4 g) was purified on silica gel with $CHCl_3$–MeOH (100:0 to 98:2, *v*/*v*) to give (**1)** (5 mg). Subfraction H.IV.5 (5 g) was purified onsilica gel column with $CHCl_3$–MeOH solvent system (98:2 to 96: 4, v/v) to afford (**2)** (9 mg).

3. RESULTS AND DISCUSSION

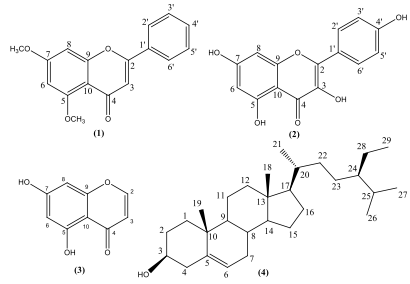

Figure 1. Chemical structures of compounds 1-4

Compound **1** was obtained as a yellow amorphous powder. ESI-MS: positive *m/z*: 283,28.^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 159.3 (C-2), 108.1 (C-3), 175.5 (C-4), 160.1 (C-5), 96.0 (C-6), 163.6 (C-7), 93.1 (C-8), 159.0 (C-9), 108.3 (C-10), 130.8 (C-1′), 125.7 (C-2′), 128.9 (C-3′), 131.1 (C-4′), 128.9 (C-5′), 125.7 (C-6′), 55.9 (5-OCH_3), 55.7 (7-OCH_3). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.68 (1H, *s*, H-3), 6.41 (1H, *d*, *J* = 2.0, H-6), 6.72 (1H, *d*, *J* = 2.5, H-8), 7.95 (1H, *dd*, *J* = 2.0, 8.0, H-2′), 7.49 (1H, *m*, H-3′), 7.49 (1H, *m*, H-4′) 7.49 (1H, *m*, H-5′), 7.95 (1H, *dd*, *J* = 2.0, 8.0, H-6′)**,** 3.79 (3H, *s*, 5-OCH_3), 3.85 (3H, *s*, 7-OCH_3).

Compound **2** was obtained as a yellow amorphous powder. ^{13}C-NMR (125 MHz, acetone-d_6, δ ppm): 147.0 (C-2), 136.7 (C-3), 176.6 (C-4), 157.8 (C-5), 99.1 (C-6), 165.0 (C-7), 94.4 (C-8), 160.1 (C-9), 104.1 (C-10), 123.3 (C-1′), 130.4 (C-2′), 116.3 (C-3′), 162.3 (C-4′), 116.3 (C-5′), 130.4 (C-6′). ^{1}H-NMR (500 MHz, acetone-d_6, δ ppm): 6.27 (1H, *d*, *J* = 2.0, H-6), 6.53 (1H, *d*, *J* = 2.0, H-8), 8.14 (1H, *d*, *J* = 8.5, H-2′), 7.01 (1H, *d*, *J* = 8.5, H-3′), 7.01 (1H, *d*, *J* = 8.5, H-5′)**,** 8.14 (1H, *d*, *J* = 8.5, H-6′), 12.96 (1H, *s*, OH-5).

Compound **3** was obtained as a white amorphous powder. ESI -MS: negative *m/z*: 177.68. ^{13}C-NMR (125 MHz, $CDCl_3$, δ ppm): 155.7 (C-2), 110.9 (C-3), 181.9 (C-4), 161.7 (C-5), 99.4 (C-6), 164.2 (C-7), 94.4 (C-8), 158.3 (C-9), 105.8 (C-10). ^{1}H-NMR (500

MHz, $CDCl_3$, δ ppm): 7.75 (1H, *d*, *J* = 6.0, H-2), 6.19 (1H, *d*, *J* = 6.0, H-3), 6.28 (1H, *brs*, H-6), 6.35 (1H, *brs*, H-8).

Compound **4** was obtained as a white amorphous powder. ^{13}C-NMR (125 MHz, acetone-d_6, δ ppm): 38.3 (C-1) , 32.5 (C-2), 71.7 (C-3), 43.3 (C-4), 142.4 (C-5), 121.5 (C-6), 32.6 (C-7), 32.8 (C-8), 51.2 (C-9), 37.3(C-10), 21.8 (C-11), 40.7 (C-12), 43.1 (C-13), 57.7 (C-14), 24.9 (C-15), 29.0 (C-16), 57.0 (C-17), 12.2 (C-18), 19.8 (C-19), 36.9 (C-20), 19.4 (C-21), 34.7 (C-22), 29.5 (C-23), 46.7 (C-24), 26.8 (C-25), 19.2 (C-26), 20.1 (C-27), 23.8 (C-28), 12.3 (C-29). ^{1}H-NMR (500 MHz, acetone-d_6, δ ppm): 3.89 (1H, *m*, H-3), 5.31 (1H, *brd*, *J* = 5.0, H-6), 0.73 (3H, *s*, H-18), 1.05 (3H, *s*, H-19), 0.85 (3H, *d*, *J* = 7.0, H-21), 0.97 (3H, *d*, *J* = 6.5, H-26), 0.86 (3H, *d*, *J* = 7.0, H-23), 0.87 (3H, *t*, *J* = 7.5, H-29).

Compound **1** (Figure 1) was obtained as a yellow amorphous powder. The molecular formula was established as $C_{17}H_{14}O_4$ by ESI-MS data ($[M+H]^+$ *m/z* 283.28). The ^{13}C-NMR data of **1** exhibited seventeen carbons, including one carbon carbonyl at δ_C 181.9 (C-4), four oxygenated aromatic carbons, ten aromatic carbons and two methoxy carbons. The aglycone of **1** was identified as the flavone, according signals: two *meta*-coupled aromatic protons at δ_H 6.41 (1H, *d*, *J* = 2.0 Hz, H-6) và 6.72 (1H, *d*, *J* = 2.5 Hz, H-8); five methine aromatic protons at δ_H 7.95 (2H, *dd*, *J* = 2.0, 8.0 Hz, H-2', H-6'), δ_H 7.49 (3H, *m*, H-3', H-4', H-5'), and one proton olefin at δ_H 6.68 (1H, *s*, H-3). In addition, six methoxy protons at 3.79 (3H, *s*, 5-OCH_3) and 3.85 (3H, *s*, 7-OCH_3) correlated with two carbons at δ_C 160.1 (C-5) và δ_C 163.6 (C-7), respectively, in HMBC. Based on the above analysis, the structure of **1** was indicated **5,7-dimethoxyflavone** [7].

Compound **2** (Figure 1) was obtained as a yellow amorphous powder. The ^{1}H-NMR data of **2** exhibited one intramolecular proton at δ_H 12.16 (1H, *s*, OH-5), four *ortho*-coupled protons at δ_H 8.14 (1H, *d*, *J* = 8.5, H-2', H- 6'), and 7.01 (1H, *d*, *J* = 8.5 , H-3', H-5'), two *meta*-coupled protons at δH 6.53 (1H, *d*, *J* = 2.0, H-8), and 6.27 (1H, *d*, *J* = 2.0, H-6). The ^{13}C-NMR spectra of **2** described fifteen carbons including one carbonyl carbon at δ_C 176.6 (C-4), six oxygenated aromatic carbons at δ_C 147.0 (C-2), 136.7 (C-3), 157.8 (C-5), 165.0 (C-7), 160.1 (C-9), and 162.3 (C-4'), two quaternary aromatic carbons at δ_C 104.1 (C-10), 123.3 (C-1′), and six sp^2 methine carbons at δ_C 99.1 (C-6), 94.4 (C-8), 130.4 (C-2′), 116.3 (C-3′), 162.3 (C-4′), 116.3 (C-5′), 130.4 (C-6′), which were confirmed a flavonoid bearing five hydroxyl groups. Thus, the structure of **2** evidenced as **kaempferol** [9], which was isolated from the Arremisia vulgaris leaves collected in Thai Nguyen, Viet Nam [2].

Compound **3** (Figure 1) was obtained as a white amorphous powder. The molecular formula was established as $C_9H_6O_4$ by APCI-MS data ($[M-H]^-$ *m/z* 177.68). The ^{13}C-NMR data of **3** exhibited nineteen carbons, including one carbon carbonyl at δ_C 181.8 (C-4), four oxygenated aromatic carbons, four aromatic carbons. The aglycone of **3** was identified as the chromone, according signals two *meta*-coupled aromatic protons at δ_H 6.28 (1H, *s*, H-6); δ_H 6.35 (1H, *s*, H-8), and two *cis*-coupled protons at δ_H 7.75 (1H, *d*, *J* = 6.0 Hz, H-2); δ_H 6.19 (1H, *d*, *J* = 6.0 Hz, H-3). Consequently, base one MS and NMR data of **3**, the structure of **3** was evidenced as **5,7-dihyroxychromone** [5].

Compound **4** (Figure 1) was obtained as a white amorphous powder. The ^{1}H-NMR data of 4 showed olefinic proton at δ_H 5.31 (1H, *brd*, *J* = 5.0, H-6); oxymethine proton at δ_H 3.89 (1H, *m*, H-3); tertinary methyl groups at δ_H 1,05 (3H, *s*, H-19), and 0.73 (3H, *s*, H-18); secondary methyl protons at δ_H 0.85 (3H, *d*, *J* = 6.5, H-21), 0.97 (3H, *d*, *J* = 6.5, H-26), and 0.86 (3H, *d*, *J* = 7.0, H-27); primary methyl protons at 0.87 (3H, *t*, *J* = 7.5, H-29). The ^{13}C-NMR spectra of **4** possessed tewnty nine carbons including two olefine carbon at δ_C 142.5 (C-5), 121.5 (C-6); one oxymethine carbon at δ_C 71.7 (C-3); two quaternary carbons at δ_C 37.3 (C-10), 43.1 (C-13), seven

methine carbons at δ_C 32.8 (C-8), 51.2 (C-9), 57.7 (C-14), 57.0 (C-17), 36.9 (C-20), 46.7 (C-24), 26.8 (C-25), eleven methylene carbons at δ_C 38.3 (C-1), 32.5 (C-2), 43.3 (C-4), 32.6 (C-7), 21.8 (C-11), 40.7 (C-12), 24.9 (C-15), 29.0 (C-16), 34.7 (C-22), 29.5 (C-23), 23.8 (C-28), and six methyl carbons at δ_C 12.2 (C-18), 19.8 (C-19), 19.4 (C-21), 19.2 (C-26), 20.1 (C-27), 12.3 (C-29), which concentrated stigmast-5-en. So, the structure of 4 was indicated as **3β-hydroxystigmast-5-en** [1]**.**

4. CONCLUSION

From the *n*-hexane extract of the leaves of *S. binhchauensis*, using column chromatography, two flavonids, one phenolic, and one sterol were isolated. Base on the analysis of mass spectroscopy and nuclear magnetic resonance as well as comparision with published data, the structure of four compounds were identified as 5,7-dimethoxyflavone **(1)**, kaempferol **(2)**, 5,7-dihydroxychromone **(3)**, and 3β-hydroxystigmast-5-en **(4)**. Compounds **1-4** were reported for the first time from the genus *Stereospermum.*

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