

FLAVONOIDS ISOLATED FROM CAESALPINIA BONDOC

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TÓM TẮT

CÁC HỢP CHẤT FLAVONOID PHÂN LẬP TỪ LOÀI CAESALPINIA BONDOC

Năm hợp chất flavonoid kaempferol-3-O- α -L-rhamnopyranoside (**1**), kaempferol-3-O- β -D-glucopyranoside (**2**), kaempferol-3-O- β -D-galactopyranoside (**3**), dihydrokaempferol (**4**), và taxifolin (**5**) được phân lập từ phần chiết methanol của loài điệp mắt mèo *Caesalpinia bonduc* bằng các phương pháp sắc ký hiện đại. Cấu trúc của các chất này được xác định dựa trên phân tích các dữ kiện phổ khối lượng, phổ cộng hưởng từ hạt nhân một chiều và hai chiều, và kết hợp với các dữ liệu phổ đã công bố.

Từ khóa: *Caesalpinia bonduc*, điệp mắt mèo, flavonoid.

1. INTRODUCTION

Caesalpinia bonduc L. is a flowering plant belonging to *Caesalpinia* in the Caesalpinaceae family. “Bonducella” the name of the species is derived from the Arabic word “Bonduce” meaning a “little ball” which indicates the globular shape of the seed [1]. This plant is distributed up to an altitude of 1,000 m in Himalayas and wild throughout the plains on wastelands or coastal areas in India. It is also found in the deltaic region of western, eastern, and southern India. Found particularly in the seacoast throughout the hotter parts of India, Burma, Sri Lanka, and Vietnam [2]. It has been used as a herbal medicine for diseases such as diabetes, hypertension [3]. Besides, it also showed antioxidant, antitumor, antibacterial, and antifilarial activities [3-6]. In Vietnam, Dang et al reported the presence of ten compounds including three new cassane-type furanoditerpenes and seven known cassane diterpenoids from the seed of *C. bonduc* [1]. Our previous study indicated the appearance of lignans, phloroglucinols, flavonoids, and phenolics from the leaves of *C. bonduc*, which have rarely been explored to date [7]. In our going research on the phytochemistry of the

genus *Caesalpinia*, we reported herein the isolation and structure elucidation of five flavonoids in more detail from the methanol extract of the leaves of *C. bonduc*.

2. MATERIALS AND METHODS

2.1. Plants materials

The dried leaves of *Caesalpinia bonduc* were collected in Vinh Phuc, Viet Nam in November, 2019 and were identified by botanist Dr. Nguyen The Cuong at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (CB1-19) was deposited at Lab of Pharmaceutical Chemistry, VNU University of Science, Hanoi.

2.2. General experimental procedures

All NMR spectra, including ^1H -NMR (500 MHz), ^{13}C (125 MHz), HSQC and HMBC were recorded on a Bruker AM500 FT-NMR spectrometer and TMS was used as an internal standard. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (30-50 μm , Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) was done using

pre-coated silica gel 60 F₂₅₄ (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and separation

Dried *C. bonduc* seeds (4.8 kg) were grinded into fine powder and extracted with methanol (3 times x 15L) in ultrasonic extractor for 2 h each. After removal of the solvent under reduced pressure to get the residue was 550 g. Water was added to this extract and then successively partitioned by solvents with dichloromethane and EtOAc to obtain the dichloromethane (CBD, 120 g), EtOAc (CBE, 50.2 g) and aqueous layer (CBW) after evaporating solvents in *vacuo* under reduced pressure to dryness.

The CBE fraction was carried out by a silica gel column eluting with methanol: water (1:1.8, v/v) to yield fractions E1– E3. E2 fraction was then separated on a silica gel column and eluted with a gradient mobile phase to give three fractions, E2A – E2C. E2A fraction (215 mg) was chromatographed by a silica gel column eluting with dichloromethane-acetone (6:1, v/v) which afforded compound **4** (6.0 mg). E2C fraction (657 mg) was further separated on a silica gel column eluting with dichloromethane-ethanol (2.0:1, v/v) which afforded compound **5** (5.0 mg).

The CBW fraction was treated with Diaion HP-20 column chromatography to remove sugar with water and eluted with gradually increasing the concentration of MeOH solvent in water to yield fractions W1 – W4. W3 fraction (875 mg) was separated on a silica gel column eluting with dichloromethane-

methanol (10:1, v/v), to give two fractions W3A and W3B. W3A was then separated on a silica gel column to afford compound **1** (8.0 mg), **2** (6.0 mg) and **3** (7.0 mg).

Kaempferol-3-O- α -L-rhamnopyranoside (1): pale-yellow powder; ESI-MS m/z 433.46 $[M+H]^+$, C₂₁H₂₀O₁₀; C₂₇H₃₀O₁₅; ¹H- and ¹³C-NMR (CD₃OD): see Table 1.

Kaempferol-3-O- β -D-glucopyranoside (2): light-yellow amorphous powder; ESI-MS m/z 449.42 $[M-H]^-$, C₂₁H₂₀O₁₁; ¹H- and ¹³C-NMR (CD₃OD): see Table 1.

Kaempferol-3-O- β -D-galactopyranoside (3): pale-yellow powder; C₂₁H₂₀O₁₁; ¹H- and ¹³C-NMR (CD₃OD): see Table 1.

Dihydrokaempferol (4): pale-yellow needle; ¹H- and ¹³C-NMR (CD₃OD): see Table 2.

Taxifolin (5): pale-yellow needle; ¹H- and ¹³C-NMR (CD₃OD): see Table 2.

3. RESULTS AND DISCUSSIONS

Compound **1** was obtained as a pale-yellow powder. Its ESI-MS had a pseudomolecular ion peak at m/z 433.46 $[M+H]^+$, calculating a molecular formula of C₂₁H₂₀O₁₀. Its NMR features suggested a kaempferol glycoside containing signals of an AA'BB' coupling type aromatic ring at δ_H 7.78 (2H, d, J = 8.5 Hz) and 6.95 (2H, d, J = 8.5 Hz); two *meta*-coupled aromatic protons at δ_H 6.21 (1H, d, J = 2.0 Hz) and 6.38 (1H, d, J = 2.0 Hz). Moreover, typical signals due to a rhamnopyranosyl moiety were observed at δ_H 5.03 (1H, br s, H-1'') and 0.80 (3H, d, J = 5.5 Hz, H_{rham}-6''), of which the broad singlet signal of anomeric proton indicated the α -linkage with the aglycone. The ¹³C-NMR spectra of

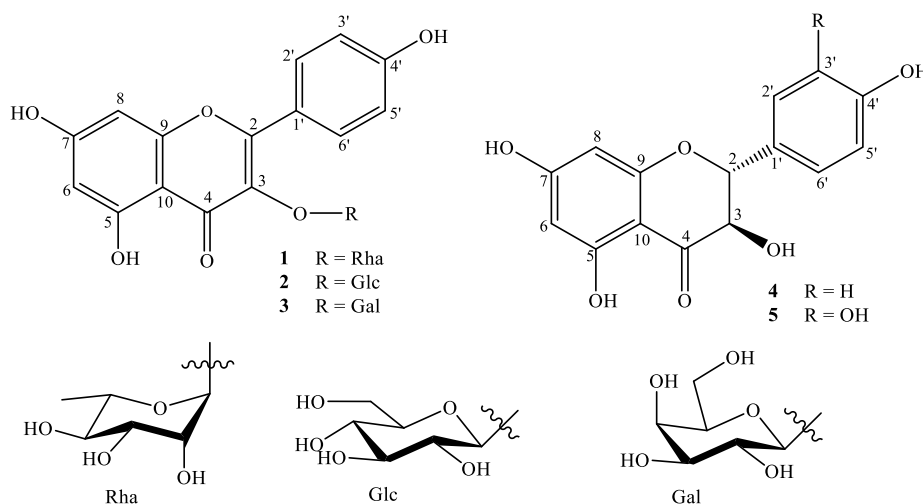


Figure 1. Chemical structure of compounds 1-5

Table 1. The ^1H - and ^{13}C -NMR data of compounds 1-3 and reference compounds

| | C | 1 | | | 2 | | | 3 | | |
|-----------|-----|---------------------|----------------------------------|--|---------------------|----------------------------------|--|---------------------|----------------------------------|--|
| | | δ_{C} | $\delta_{\text{C}}^{\text{a,b}}$ | $\delta_{\text{H}}^{\text{a,c}}$ (mult, J in Hz) | δ_{C} | $\delta_{\text{C}}^{\text{a,b}}$ | $\delta_{\text{H}}^{\text{a,c}}$ (mult, J in Hz) | δ_{C} | $\delta_{\text{C}}^{\text{a,b}}$ | $\delta_{\text{H}}^{\text{a,c}}$ (mult, J in Hz) |
| Aglycone | 2 | 157.1 | 157.1 | - | 158.5 | 158.5 | - | 158.0 | 159.1 | - |
| | 3 | 134.2 | 134.1 | - | 135.5 | 135.5 | - | 134.6 | 135.6 | - |
| | 4 | 177.6 | 177.6 | - | 179.5 | 179.5 | - | 179.8 | 179.7 | - |
| | 5 | 161.2 | 161.2 | - | 163.1 | 163.1 | - | 162.1 | 163.1 | - |
| | 6 | 98.7 | 98.9 | 6.21 (s) | 99.9 | 100.0 | 6.23 (br s) | 98.8 | 100.0 | 6.23 (br s) |
| | 7 | 164.2 | 164.9 | - | 166.0 | 166.1 | - | 165.0 | 166.2 | - |
| | 8 | 93.7 | 93.8 | 6.38 (s) | 94.7 | 94.8 | 6.42 (br s) | 93.5 | 94.8 | 6.43 (br s) |
| | 9 | 156.4 | 156.5 | - | 159.1 | 159.1 | - | 157.5 | 158.6 | - |
| | 10 | 104.1 | 103.9 | - | 105.7 | 105.7 | - | 106.2 | 105.6 | - |
| | 1' | 120.5 | 120.5 | - | 122.8 | 122.8 | - | 121.5 | 122.7 | - |
| | 2' | 132.3 | 132.3 | 7.78 (d, 8.5) | 132.3 | 132.3 | 8.07 (d, 8.5) | 131.1 | 132.4 | 8.11 (d, 8.5) |
| | 3' | 116.1 | 116.1 | 6.95 (d, 8.5) | 116.1 | 116.1 | 6.91 (d, 8.5) | 114.9 | 116.6 | 6.91 (d, 8.5) |
| | 4' | 161.6 | 161.6 | - | 161.6 | 161.6 | - | 105.3 | 105.2 | - |
| | 5' | 116.1 | 116.1 | 6.95 (d, 8.5) | 116.1 | 116.1 | 6.91 (d, 8.5) | 114.9 | 116.6 | 6.91 (d, 8.5) |
| | 6' | 132.3 | 132.3 | 7.78 (d, 8.5) | 132.3 | 132.3 | 8.07 (d, 8.5) | 131.1 | 132.4 | 8.11 (d, 8.5) |
| Glycoside | 1'' | 101.7 | 101.8 | 5.30 (br s) | 104.1 | 104.2 | 5.26 (d, 7.0) | 105.0 | 105.0 | 5.16 (d, 8.0) |
| | 2'' | 70.3 | 70.3 | 3.47 (m) | 75.7 | 75.8 | 3.46 (dd, 7.0, 9.0) | 73.1 | 73.0 | 3.81 (dd, 8.0, 9.0) |
| | 3'' | 70.5 | 70.6 | 3.12 (m) | 78.1 | 78.1 | 3.43 (dd, 9.0, 9.0) | 75.0 | 75.1 | 3.55 (dd, 9.0, 4.0) |
| | 4'' | 71.1 | 71.1 | 3.12 (m) | 71.4 | 71.4 | 3.35 9 (m) | 70.0 | 70.0 | 3.84 (br d, 4.0) |
| | 5'' | 70.0 | 70.1 | 3.98 (m) | 78.4 | 78.4 | 3.23 (m) | 77.1 | 77.1 | 3.46 (dd, 6.0, 11.0) |
| | | 17.4 | 17.4 | 0.80 (d, 5.5) | | | 3.71 (br d, 12.0) | 62.0 | 62.0 | 3.54 (dd, 6.0, 11.0) |
| | 6'' | | | | 62.6 | 62.7 | 3.55 (dd, 5.0, 12.0) | | | 3.64 (dd, 6.0, 11.0) |

Measured in $^a\text{CD}_3\text{OD}-d_4$, $^b125\text{ MHz}$, $^c500\text{ MHz}$

1 showed signals of 21 carbons comprising 15 carbons of an aglycone and 6 carbons of a rhamnopyranosyl unit at δ_{C} 101.7, 70.3, 70.5, 71.1, 70.0, and 17.4. The ^1H and ^{13}C -NMR data suggested compound **1** was a flavonol glycoside. The assignment of all carbons and protons and thereby the structure of the compound was resolved by 2D experiments, notably HSQC and HMBC experiments. The location of a rhamnopyranosyl unit was determined at C-3 of an aglycone by the HMBC correlations observed from H-1'' (δ_{H} 5.30 br s) to C-3 (δ_{C} 134.1) as shown in Figure 2.

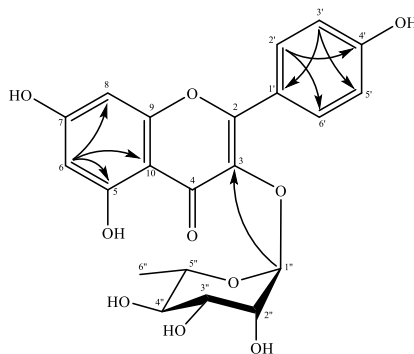


Fig. 2. The key HMBC correlations of compound **1** Its NMR data was found to match perfectly with those reported in the literature [8] (table 1). Thus, the structure of compound **1** was identified as kaempferol-3- O - α -L-rhamnopyranoside.

Compound **2** was isolated as a light-yellow amorphous powder. The ESI-MS of

compound **2** showed a molecular ion peak at m/z 449.42 $[M-H]^-$, calculating a molecular formula of $C_{21}H_{20}O_{11}$. The 1H - and ^{13}C -NMR spectra of compound **2** were similar to those of **1** except for the signals at sugar moiety. Typical signals due to a glucosyl moiety were observed at δ_H 5.26 (1H, d, $J = 7.0$ Hz, H-1''), 3.71 (1H, br d, $J = 12.0$ Hz, H_{glc} -6''a), and 3.55 (1H, dd, $J = 5.0, 12.0$ Hz, H_{glc} -6''b). These signals were determined to be β -D-glucopyranose due to the large coupling constant of anomeric proton $J = 7.0$ Hz. Thus, compound **2** was proposed as kaempferol-3- O - β -D-glucopyranoside. Its 1H and ^{13}C NMR data were identical to those reported in the literature [9]. Compound **3** was isolated as a pale-yellow powder. The molecular formula is determined to be $C_{21}H_{20}O_{11}$. The 1H - and ^{13}C -NMR spectra of compound **3** were similar to those of compounds **1** and **2** except for the slight differences in signals at sugar moiety suggested **3** was also a kaempferol glycoside. Detailed analysis of ^{13}C -NMR data and 1H -NMR spin coupling pattern (table 1) identified the sugar moiety as β -D- galactopyranose [10]. Consequently, compound **3** had been

determined as kaempferol-3- O - β -D-galactopyranoside or trifolin [11], a compound previously isolated from the flowers and leaves of *Trifolium repens* [10].

Compound **4** was obtained as a pale-yellow needle. The 1H -NMR spectra of **4** also showed characteristic of an AABB coupling system at [δ_H 6.85 (2H, d, $J = 7.5$ Hz) and 7.37 (2H, d, $J = 7.5$ Hz)]; two *meta*-coupled aromatic protons at δ_H 5.95 (d, $J = 2.0$ Hz), and 5.90 (d, $J = 2.0$ Hz). However, two additional oxymethine resonances at δ_H 4.56 and 5.01 ppm suggested that it might be a dihydroflavonol. The ^{13}C -NMR spectra of **4** revealed signals of 15 carbons including one carbonyl group at δ_C 198.4, six methines at δ_C 96.3, 97.4, 116.2 (2CH) and 130.3 (2 CH); two tertiary carbons at δ_C 103.9 and 129.3; four O-bearing C-atoms at δ_C 159.2, 164.5, 165.3, 168.8; and two oxymethine at δ_C 73.6 and 85.0. A comparison of the 1H - and ^{13}C -NMR data of **4** with reported data in the literature for dihydrokaempferol showed excellent agreement [12] (Table 2). Compound **4** was therefore characterized as dihydrokaempferol or aromadendrin.

Table 2. The 1H - and ^{13}C -NMR data of compounds **4-5** and reference compounds

| C | 4 | | | 5 | | |
|----|--------------|------------------|------------------------------------|------------|------------------|------------------------------------|
| | @ δ_C | $\delta_C^{a,b}$ | $\delta_H^{a,c}$ (mult, J in Hz) | δ_C | $\delta_C^{a,b}$ | $\delta_H^{a,c}$ (mult, J in Hz) |
| 2 | 85.0 | 85.0 | 4.56 (d, 11.5) | 85.1 | 85.11 | 4.52 (d, 11.5) |
| 3 | 73.6 | 73.6 | 5.01 (d, 11.5) | 73.7 | 73.7 | 4.93 (d, 11.5) |
| 4 | 198.4 | 198.4 | - | 198.4 | 198.4 | - |
| 5 | 169.3 | 168.8 | - | 168.9 | 168.8 | - |
| 6 | 97.5 | 97.4 | 5.95 (d, 2.0) | 97.3 | 97.3 | 5.94 (d, 2.0) |
| 7 | 165.4 | 165.3 | - | 165.3 | 165.3 | - |
| 8 | 96.4 | 96.3 | 5.90 (d, 2.0) | 96.3 | 96.3 | 5.91 (d, 2.0) |
| 9 | 164.6 | 164.5 | - | 164.5 | 164.5 | - |
| 10 | 102.9 | 102.0 | - | 101.8 | 101.8 | - |
| 1' | 129.3 | 129.3 | - | 129.9 | 129.9 | - |
| 2' | 130.4 | 130.3 | 7.37 (d, 7.5) | 115.9 | 115.9 | 6.99 (d, 1.5) |
| 3' | 116.1 | 116.2 | 6.85 (d, 7.5) | 147.1 | 147.1 | - |
| 4' | 159.2 | 159.2 | - | 146.3 | 146.3 | - |
| 5' | 116.1 | 116.2 | 6.85 (d, 7.5) | 116.1 | 116.1 | 6.82 (8.0) |
| 6' | 130.4 | 130.3 | 7.37 (d, 7.5) | 120.9 | 120.9 | 6.87 (dd, 1.5; 8.0) |

Measured in aCD_3OD - d_4 , b125 MHz, c500 MHz

Compound **5** was obtained as a pale-yellow needle. The ^1H - and ^{13}C -NMR spectra of compound **5** were similar to those of **4** except for the replacement of proton signals in ring B with signals of an AA'BB' coupling type aromatic ring in **4** instead of an ABX coupling system [δ_{H} 6.99 (1H, d, $J = 1.5$ Hz), 6.82 (1H, d, $J = 8.0$ Hz) and 6.87 (1H, dd, $J = 1.5$; 8.0 Hz)] in **5**. The above evidence implied that the structure of **5** was similar to **4** except for the addition of hydroxy group at the B ring in **5**. Moreover, a comparison of the NMR data of compound **5** with those of reference compound [12], led to the elucidation of the structure of compound **5** as taxifolin

4. CONCLUSION

Five flavonoids kaempferol-3-*O*- α -L-rhamnopyranoside (**1**), kaempferol-3-*O*- β -D-glucopyranoside (**2**), kaempferol-3-*O*- β -D-galactopyranoside (**3**), Dihydrokaempferol (**4**), and taxifolin (**5**) were isolated from the methanol extract of leaves of *C. bonduc*.

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