FURTHER STUDY ON CHEMICAL CONSTITUENTS FROM THE HEARTWOOD OF _DALBERGIA TONKINENSIS_ COLLECTED IN DAKLAK PROVINCE

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ABSTRACT

From the methanol extract of the heartwood of _Dalbergia tonkinensis_ Prain collected in DakLak province, Vietnam, five flavonoids including liquiritigenin (1), 7,3',5'-trihydroxyflavanone (2), sativanone (3), 3'-O-methylviolanone (4) and sulfurin (5) were isolated by several chromatography techniques. The chemical structures of isolated compounds were determined by the interpretation of NMR spectral data, mass spectra as well as comparison with data from the literature. Among them, 7,3',5'-trihydroxyflavanone (2) was isolated from the genus _Dalbergia_ for the first time.

Keywords: _Dalbergia tonkinensis_, Fabaceae, flavonoids, aurone, heartwood.

1. INTRODUCTION

There are over 300 recognized _Dalbergia_ species (Fabaceae) that are widely distributed in tropical and subtropical regions [1]. In Vietnam, the _Dalbergia_ genus consists of 27 species [2] which have been used to treat nosebleeds, diarrhea, coughs, infections, fever scabies, rheumatism and inflammation [3]. The heartwood of _Dalbergia odorifera_ species is used in traditional Chinese medicines for treating cardiovascular diseases, myocarditis, and coronary failure [4]. Phytochemical studies of _Dalbergia_ genus have indicated that major chemical constituents include phenolics, flavonoids, coumarins, sesquiterpenes, phytosterols and coumaronochromone derivatives [3, 5].

The _D. tonkinensis_ Prain, a medium to large tree of 10–25 meter height [6], is widely distributed from North to South in Vietnam. The colour of flowers of _Dalbergia tonkinensis_ is white, but this species is commonly called ‘Sưa Đỏ’ in Vietnam as the heartwood of perennial
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plant is red-brown to red-black. *D. tonkinensis* is also known as ‘Sua Trang’, ‘Trac Bac Bo’, ‘Trac thi’ [2], ‘Hue Moc vang’, ‘Huynh dan’ [6], and ‘Sua Bac Bo’ [7]. Recently our investigations on *D. tonkinensis* led to the isolation of new carboxyethylflavanones, neoflavonoids, isoflavonoids, and pterocarpan, together with their antibacterial [5, 8, 9] and antidiabetic [10] activities. Tuan Anh *et al.* reported the isolation of two isoflavones, genistein, lanceolarin and a phenanthrene from the methanol extract of *D. tonkinensis* [7], however the studied plant part was not described. In this paper, we describe the isolation and structural determination of five compounds (1–5) from ethyl acetate fraction of the methanol extract of *D. tonkinensis* heartwood.

2. MATERIALS AND METHODS

2.1. Plant material

The plant and heartwood of *Dalbergia tonkinensis* Prain (over ten-years old) were collected in Buon Ma Thuot city of Daklak province in Vietnam in 2016. The plant was identified by botanist Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST, in Hanoi, Vietnam. A voucher specimen (C-612) was deposited in Department of Bioactive Products, Institute of Natural Products Chemistry, VAST, in Ha Noi, Viet Nam.

2.2. General experimental procedures

$^1$H-NMR (500 MHz) and $^{13}$C-NMR (125 MHz) were measured on a Bruker Avance 500 MHz spectrometer. ESI-MS was obtained from a Varian FT-MS spectrometer and MicroQ-TOF III (Bruker Daltonics, Ettlingen, Germany). Column chromatography was carried out on silica gel (Si 60 F254, 40–63 mesh, Merck, St. Louis, MO, USA). All solvents were redistilled before use. Pre-coated thin layer chromatography (TLC) plates (Si 60 F254) were used for analytical purposes. Compounds were visualized under UV radiation (254, 365 nm) and by spraying plates with 10 % H$_2$SO$_4$ followed by heating with a heat gun.

2.3. Extraction and Isolation

Dried powdered heartwoods (1.2 kg) of *D. tonkinensis* were extracted with hot methanol (5 × 3.0 L) under reflux, filtered, and then concentrated under decreased pressure giving a black crude methanol residue (60.3 g). The suspension of the methanol residue in hot water was successively partitioned with hexane, dichloromethane and ethyl acetate to give hexane (1.6 g, HDT-1), dichloromethane (27.2 g, HDT-2), ethyl acetate (2.6 g, HDT-3), and water (3.7 g, HDT-4) fractions, respectively.

The fraction HDT-3 (2.6 g) was chromatographed on a normal silica gel (40-63 mesh) chromatography column (CC) using a gradient of hexane and acetone (99:1→0:1, v/v) as eluent to afford 12 fractions (HDT3.1-HDT3.12). Fraction HDT3.1 (500 mg) was rechromatographed on a normal silica gel CC (chloroform-acetone 30-2 as eluent, v/v) to produce 8 sub-fractions (HDT3.1.1-HDT3.1.8). The sub-fraction HDT3.1.2 (200 mg) was further separated on a normal silica gel CC and eluted with chloroform-ethyl acetate (11/3, v/v) to obtain compound 3 (10.0 mg). Compounds 1 (8.0 mg) and 4 (6.0 mg) were obtained from fraction HDT3.2 (120 mg) by a normal silica gel CC, eluting with hexane-acetone (3:1, v/v). Fraction HDT3.4 (420 mg) was rechromatographed on a normal silica gel CC (chloroform-ethylacetate 3-1 as eluent, v/v) to produce 15 sub-fractions (HDT3.4.1-HDT3.4.15). Compound 5 (10.0 mg) was obtained from the
sub-fraction HDT3.4.14 by a normal silica gel CC, eluting with chloroform-ethylacetate (4:1, v/v). The sub-fraction HDT3.4.3 was further separated on a reverse phase C18 column, eluting with a gradient of methanol-water (2:1, v/v) to afford compound 2 (4.0 mg).

**Liquiritigenin (1):** yellow amorphous powder; ESI-MS *m/z*: 257 [M + H]+ (C15H12O5); 1H-NMR (500 MHz, MeOD-d4) δH: 7.47 (1H, d, 8.5 Hz, H-5), 7.33 (2H, d, 8.5 Hz, H-2', H-6'), 6.83 (2H, d, 8.5 Hz, H-3', d-5'), 6.61 (1H, dd, 2.0, 8.5 Hz, H-6), 6.37 (1H, d, 2.0 Hz, H-8), 5.39 (1H, dd, 3.0, 13.0 Hz, H-2), 3.06 (1H, dd, 13.0, 17.0 Hz, H-3α), 2.71 (1H, dd, 3.0, 17.0 Hz, H-3β); 13C-NMR (125 MHz, MeOD-d4) δC: 193.5 (C-4), 169.9 (C-9), 165.6 (C-7), 158.9 (C-4'), 131.4 (C-1'), 129.8 (C-5), 129.0 (C-2', C-6'), 116.1 (C-3', C-5'), 114.9 (C-10), 111.8 (C-6), 103.8 (C-8), 81.0 (C-2), 44.9 (C-3).

**7,3',5'-trihydroxyflavanone (2):** colorless needles; ESI-MS *m/z*: 273 [M + H]+ (C15H11O5); 1H-NMR (500 MHz, DMSO-d6) δH: 7.62 (1H, d, 8.5 Hz, H-5), 6.88 (1H, s, H-4'), 6.74 (2H, s, H-2', H-6'), 6.48 (1H, dd, 2.5, 8.5 Hz, H-6), 6.31 (1H, d, 2.5 Hz, H-8), 5.36 (1H, dd, 3.0, 12.5 Hz, H-2), 3.02 (1H, dd, 12.5, 16.5 Hz, H-3α), 2.61 (1H, dd, 3.0, 16.5 Hz, H-3β); 13C-NMR (125 MHz, DMSO-d6) δC: 193.8 (C-4), 165.7 (C-9), 164.9 (C-7), 146.2 (C-3', C-5'), 131.8 (C-1'), 129.8 (C-5), 119.4 (C-4), 115.2 (C-2', C-6'), 114.8 (C-10), 111.8 (C-6), 103.8 (C-8), 81.2 (C-2), 44.8 (C-3).

**Sativanone (3):** white amorphous powder; ESI-MS *m/z*: 301 [M + H]+ (C15H11O5); 1H-NMR (500 MHz, Acetone-d6) δH: 9.40 (1H, br s, 7-OH), 7.77 (1H, d, 9.0 Hz, H-5), 7.01 (1H, d, 8.5 Hz, H-6'), 6.59 (1H, d, 2.5 Hz, H-8), 6.58 (1H, dd, 9.0, 2.5 Hz, H-6), 6.48 (1H, dd, 8.5, 2.5 Hz, H-5'), 6.40 (1H, d, 2.5 Hz, H-3'), 4.56 (1H, t, 11.0 Hz, H-2α), 4.45 (1H, dd, 11.0, 5.5 Hz, H-2β), 4.18 (1H, dd, 11.0, 5.5 Hz, H-3), 3.79 (3H, s, 2'-OCH3), 3.78 (3H, s, 3'-OCH3); 13C-NMR (125 MHz, Acetone-d6) δC: 190.7 (C-4), 164.5 (C-7), 164.3 (C-9), 161.1 (C-4'), 159.1 (C-2'), 131.2 (C-5), 129.7 (C-6'), 117.0 (C-1'), 115.6 (C-10), 110.8 (C-6), 105.3 (C-5'), 103.1 (C-3'), 99.2 (C-8), 71.4 (C-2), 55.6 (4'-OCH3), 55.2 (2'-OCH3), 47.6 (C-3).

**3'-O-methylviolanone (4):** white amorphous powder; ESI-MS *m/z*: 331 [M + H]+ (C15H11O6); 1H-NMR (500 MHz, MeOD-d4) δH: 7.78 (1H, d, 9.0 Hz, H-5), 6.86 (1H, d, 8.5 Hz, H-6), 6.75 (1H, dd, 8.5, H-5'), 6.54 (1H, dd, 9.0, 2.0 Hz, H-6), 6.37 (1H, d, 2.0 Hz, H-8), 4.57 (1H, t, 11.0, H-2α), 4.44 (1H, dd, 11.0, 5.5, H-2β), 4.15 (1H, dd, 11.0, 5.5, H-3), 3.85 (3H, s, 4'-OCH3), 3.83 (3H, s, 2'-OCH3), 3.82 (3H, s, 3'-OCH3); 13C-NMR (125 MHz, MeOD-d4) δC: 194.3 (C-4), 166.4 (C-7), 165.7 (C-9), 155.0 (C-4'), 153.1 (C-2'), 143.6 (C-3'), 130.3 (C-5), 126.0 (C-6'), 123.0 (s, C-1'), 115.6 (C-10), 111.8 (C-6), 106.8 (C-5'), 103.6 (C-8), 72.3 (C-2), 61.2 (3'-OCH3), 61.0 (2'-OCH3), 56.5 (4'-OCH3), 49.7 (C-3).

**Sulfuretin (5):** yellow amorphous powder; ESI-MS *m/z*: 271 [M + H]+ (C14H8O3); 1H-NMR (500 MHz, DMSO-d6) δH: 7.59 (1H, d, 8.5 Hz, H-5), 7.44 (1H, d, 2.0, 8.5 Hz, H-8), 7.23 (1H, d, 2.0, 8.5 Hz, H-6), 6.83 (1H, d, 8.5 Hz, H-5'), 6.74 (1H, d, 2.0 Hz, H-2'), 6.69 (1H, dd, 2.0, 8.5 Hz, H-6), 6.62 (1H, s, H-2); 13C-NMR (125 MHz, DMSO-d6) δC: 181.2 (C-4), 167.5 (C-7), 166.3 (C-9), 148.2 (C-4'), 145.7 (C-3), 145.6 (C-3'), 125.7 (C-5), 124.5 (C-1'), 123.4 (C-6'), 117.9 (C-2'), 116.0 (C-5'), 113.1 (C-10), 112.9 (C-6), 111.9 (C-2), 98.4 (C-8).

**3. RESULTS AND DISCUSSION**

The methanol extract of the heartwood of *D. tonkinensis* was partitioned into hexane, dichloromethane, ethyl acetate-soluble fractions and a water layer. Chromatographic purification of the ethyl acetate-soluble fraction led to the isolation of five compounds (1–5) (Figure 1).
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Figure 1. Chemical structure of isolated compounds 1–5 from *D. tonkinensis*.

Compound 1 was isolated as yellow amorphous powder. The molecular formula of 1 was $C_{15}H_{12}O_4$, due to the presence of an ion peak at $m/z$ 257 [M + H]$^+$ in the electrospray ionization mass spectrometry (ESI-MS). The presence of an oxymethine group $[\delta_H 5.39 \ (1H, \ dd, \ J = 3.0, 13.0 \ Hz, \ H-2)/\delta_c 81.0 \ (C-2)]$, a methylene group $[\delta_H 3.06 \ (1H, \ dd, \ J = 13.0, 17.0 \ Hz, \ H-3\text{a})$ and $2.71 \ (1H, \ dd, \ J = 3.0, 17.0 \ Hz, \ H-3\text{b})/\delta_c 43.1 \ (C-3)]$, and a carbonyl carbon at $\delta_c 193.5 \ (C-4)$ in the $^1H$ and $^{13}C$ NMR spectra indicated 1 to be a flavanone (Figure 1) [5]. The $^1H$ NMR spectrum of 1 showed signals of a 1,2,4-trisubstituted benzene ring (ring A) at $[\delta_H 7.74 \ (1H, \ d, \ J = 8.5 \ Hz, \ H-5), 6.50 \ (1H, \ dd, \ J = 2.0, 8.5 \ Hz, \ H-6)$ and $6.33 \ (1H, \ d, \ J = 2.0 \ Hz, \ H-5)]$, and a 1,4-disubstituted benzene ring (ring B) characterized with an $A_2B_2$ spin system $[\delta_H 7.33 \ (2H, \ d, \ J = 8.5 \ Hz, \ H-2', \ H-6')$ and $6.83 \ (2H, \ d, \ J = 8.5 \ Hz, \ H-3', \ H-5')]$ (Figure 1). The $^{13}C$ NMR spectrum which exhibiting fifteen signals due to twelve $sp^2$ carbons at $\delta_c 103.8–169.9$ indicate the presence of two benzene rings. Based on these evidences and in comparison with the published data compound 1 was identified as liquiritigenin [11]. This compound was isolated from the heartwood of *D. odorifera* [12]. Liquiritigenin possesses hepatoprotective [13] and neuroprotective activities [14].

Compound 2 obtained as colorless needles. The molecular formula of 2 was clarified as $C_{13}H_{12}O_4$ based on the ion at $m/z$ 273 [M + H]$^+$ in the ESI-MS spectrum. The $^1H$ and $^{13}C$ NMR spectra of 2 were similar to those of 1 except for the presence of 1,3,5-trisubstituted benzene ring at $[\delta_H 6.88 \ (1H, \ s, \ H-4')$ and $6.74 \ (2H, \ s, \ H-2', \ H-6')$ in 2 (Figure 1). Therefore, compound 2 was identified as 7,3',5'-trihemxyflavanone when compared with literature data [15]. This compound was isolated from the genus *Dalbergia* for the first time. It possesses hepatoprotective activity [16].

Compound 3 obtained as a white amorphous powder with a molecular formula was $C_{17}H_{16}O_5$, determined by the an ion at $m/z$ 301 [M + H]$^+$ in the ESI-MS spectrum. The presence of an oxymethylene group $[\delta_H 4.56 \ (1H, \ t, 11.0 \ Hz, \ H-2\text{a})$ and $4.45 \ (1H, \ dd, \ 11.0, 5.5 \ Hz, \ H-2\text{b})/\delta_c 71.4 \ (C-2)]$, a methine group $[\delta_H 4.18 \ (1H, \ dd, \ 11.0, 5.5 \ Hz, \ H-3)/\delta_c 47.6 \ (C-3)]$, and a carbonyl carbon at $\delta_c 190.7 \ (C-4)$ in the $^1H$ and $^{13}C$ NMR spectra indicated 3 to be a isoflavanone (Figure 1) [17]. The $^1H$-NMR of 3 showed signals of 1,2,4-trisubstituted benzene rings (ring A and B) characterized with two ABX systems $[\delta_H 7.77 \ (1H, \ d, \ J = 9.0 \ Hz, \ H-5), 6.58 \ (1H, \ dd, \ J = 9.0, 2.5 \ Hz, \ H-6)$ and $6.59 \ (1H, \ d, \ J = 2.5 \ Hz, \ H-8)]; [\delta_H 7.01 \ (1H, \ d, \ 8.5 \ Hz, \ H-6), 6.48 \ (1H, \ dd, \ 8.5, 2.5 \ Hz, \ H-5')$ and $6.40 \ (1H, \ d, \ 2.5 \ Hz, \ H-3')]$ (Figure 1). The signals of two methoxy groups at $\delta_H 3.79 \ (3H, \ s, \ 2'-OCH_3)$ and $3.78 \ (3H, \ s, \ 4'-OCH_3)$ were also observed in the $^1H$ NMR spectrum. The $^{13}C$-NMR and DEPT spectra showed signals of six aromatic quaternary carbons at $\delta_c 164.5 \ (C-7), 164.3 \ (C-9), 161.1 \ (C-4'), 159.1 \ (C-2'), 117.0 \ (C-1'), 115.5 \ (C-10)$, six aromatic methine carbons at $\delta_c 131.2 \ (C-5), 129.7 \ (C-6'), 110.8 \ (C-6), 105.3 \ (C-5'), 103.1 \ (C-3'), 99.2 \ (C-8)$, two methoxy groups at $\delta_c 55.6 \ (4'-OCH_3)$ and 55.2 (2'-OCH_3) (Figure 1). On the
basis of the above evidence and comparison with literature data [12], compound 3 was identified as sativanone. This isoflavanone was also isolated from the heartwood of D. parviflora [18] and D. odorifera [12]. Sativanone was found to show anti-bacterial [12] and anti-oxidant activities [19].

Compound 4 was isolated as a white amorphous powder. Its molecular formula was determined to be C_{18}H_{18}O_{6} based on the ion at m/z 331 [M + H]^+ in the ESI-MS spectrum. The ^1H and ^13C NMR spectra of 4 were similar to those of 3 except for the presence of a methoxy group [δ_H 3.82 (3H, s, 3'-OCH_3); δ_C 61.2 (3'-OCH_3)] in 4 (Figure 1). Therefore, compound 4 was identified as 3'-O-methylviolanone when compared with literature data [20]. This isoflavonane was isolated from the heartwood of D. odorifera. It was found to possess anti-inflammatory activity [21].

Compound 5 was obtained as yellow amorphous powder. The ESIMS spectrum of compound 5 showed the pseudo molecular ion at m/z 271 [M + H]^+, indicating the molecular formula C_{15}H_{16}O_{5}. The ^1H-NMR of 5 showed signals of 1,2,4-trisubstituted benzene rings (ring A and B) characterized with two ABX systems [δ_H 7.59 (1H, d, J = 8.5 Hz, H-5), 7.44 (1H, d, J = 2.0 Hz, H-8) and 7.23 (1H, dd, J = 2.0, 8.5 Hz, H-6); δ_H 6.83 (1H, d, 8.5 Hz, H-5'), 6.74 (1H, d, 2.0 Hz, H-2'), and 6.69 (1H, dd, 2.0, 8.5 Hz, H-6')], together with an olefinic proton at δ_H 6.62 (1H, s, H-2). The ^13C-NMR spectrum of 5 showed signals for six aromatic quaternary carbon at δ_C 167.5 (C-7), 166.3 (C-9), 148.2 (C-4'), 145.6 (s, C-3'), 124.5 (s, C-1'), 113.1 (C-10) and six aromatic methine carbons at δ_C 125.7 (C-5), 123.4 (C-6'), 117.9 (C-2'), 116.0 (C-5'), 112.9 (C-6) and 98.4 (C-8). Furthermore, a carbonyl carbon at δ_C 181.2 (C-4), together with an olefinic group [δ_C 145.7 (C-3), and 111.9 (C-2)] observed in the ^13C NMR spectrum indicated that 5 was aurone skeleton (Figure 1) [13]. Based on the above evidence and comparison with reported data [22], compound 5 was identified as 7,3',4'-trihydroxyaurone, named sulfuretin. This compound was also isolated from the heartwood of D. odorifera [11]. Sulfuretin possesses anti-inflammatory [23], anti-cancer [24] and neuroprotective activities [25].

4. CONCLUSIONS

The methanol extract of the heartwood of D. tonkinensis was partitioned into hexane, dichloromethane, ethyl acetate-soluble fractions and a water layer. Five compounds, liquiritigenin (1), 7,3',5'-trihydroxyflavanone (2), sativanone (3), 3'-O-methylviolanone (4) and sulfuretin (5) were isolated from the ethyl acetate fraction. Their chemical structures were determined by the interpretation of NMR spectral data and comparison with published data. 7,3',5'-trihydroxyflavanone (2) was isolated from the genus Dalbergia for the first time.

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