



ISOLATION AND STRUCTURAL DETERMINATION OF ISOFLAVANONES FROM DALBERGIA TONKINENSIS PRAIN COLLECTED IN DAK LAK

Dam Thi Bich Hanh¹, Nguyen Phu Khanh², Dang Thi Thuy My¹, Le Thi Thuy Loan¹, Nguyen Thi Van Dung¹,
Le Hong Thuy¹, Phan Hoang Thai Bao^{1*}

¹Faculty of Science and Technology, Tay Nguyen University, Buon Ma Thuot, Daklak, Vietnam

²Military Hospital 175, Ho Chi Minh City, Vietnam

Email address: phtbao@ttn.edu.vn

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Article info	Abstract
<p>Received: 5/4/2023</p> <p>Revised: 18/6/2023</p> <p>Accepted: 8/8/2023</p>	<p>From the ethyl acetate extract of the wood of <i>Dalbergia tonkinensis</i> Prain, was collected in Buon Ma Thuot city, DakLak province, two isoflavanones sativanone (1), and 3'-O-methylviolanonone (2) were isolated. The chemical structure of these compounds were determined by the interpretation of NMR spectral data and comparison with published data. This is the first time they are reported from wood of <i>Dalbergia tonkinensis</i>.</p>
<hr/> <p>Keywords</p> <p><i>Dalbergia tonkinensis</i>, <i>Fabaceae</i>, flavonoids, wood.</p> <hr/>	



PHÂN LẬP VÀ XÁC ĐỊNH CẤU TRÚC DALBERGIN TỪ CÂY SƯA (DALBERGIA TONKINENSIS PRAIN) Ở ĐẮC LẮC

Đàm Thị Bích Hạnh¹, Nguyễn Phú Khanh², Đặng Thị Thuỳ My¹, Lê Thị Thuý Loan¹, Nguyễn Thị Vân Dung¹,
Lê Hồng Thuý¹, Phan Hoàng Thái Bảo^{1*}

¹Trường Đại học Tây Nguyên, Việt Nam

²Bệnh viện Quân Y 175, Thành phố Hồ Chí Minh, Việt Nam

Địa chỉ email: phtbao@ttn.edu.vn

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Thông tin bài viết	Tóm tắt
Ngày nhận bài: 5/4/2023	Từ cao ethyl axetat của cây Sưa (<i>Dalbergia tonkinensis</i> Prain) thu được tại thành phố Buôn Ma Thuột, tỉnh Đắk Lắk, đã phân lập được hai hợp chất isoflavanone: sativanone (1) và 3'-O-methylviolanone (2). Cấu trúc hóa học của các hợp chất này được xác định bằng phổ cộng hưởng từ hạt nhân (NMR) đồng thời đối chiếu với các tài liệu tham khảo đã công bố. Đây là lần đầu tiên chúng được phân lập từ loài <i>Dalbergia tonkinensis</i> .
Ngày sửa bài: 18/6/2023	
Ngày duyệt đăng: 8/8/2023	
Từ khóa <i>Dalbergia tonkinensis</i> , <i>Fabaceae</i> , flavonoid, cây Sưa	

1. Introduction

There are over 310 recognized species in the *Dalbergia* genus, belonging to the family Fabaceae, are found widely distributed in tropical and subtropical regions [1]. In Vietnam the *Dalbergia* genus approximately 30 species. Most species in the *Dalbergia* genus have been widely used since in the long time in many countries to treat conditions such as nosebleeds, diarrhea, headache, syphilis, coughs, infections, fractures, fever scabies, worms, rheumatism, anti-inflammatory, antibacterial, absent-mindedness, blood pressure, etc [2, 3, 4].

Various biological activities of the *Dalbergia* species have reported, including antimicrobial, antidiarrheal,

anti-cancer, spermicidal, anti-inflammatory, antipyretic, analgesic, and cardiovascular activity [5]. In addition. Phytochemical studies of the genus *Dalbergia* have indicated that its chemical constituents are various flavonoids as flavones, isoflavones, neoflavones, flavanones, isoflavanones, flavanes, isoflavanes, neoflavans, 4-aryl-coumarins, phenols, sesquiterpenes, phytosterols, together coumaronochromone derivatives [6, 7, 8]

The *Dalbergia tonkinensis* Prain species (commonly called 'Sua Do'), is a medium-size floral species 5-13 meters, is widely distributed scattered from North to South in Vietnam and is classified as precious tree species listed in the Red Book. This is rare spices with high valuable economic [9, 10]

Previous studies have reported on the chemical constituent from the heartwood of the *Dalbergia tonkinensis* have carried by N.M.Cuong et al, two new carboxyethylflavanones: daltonkin (A), daltonkin (B), and neoflavonoid were isolated [5, 6]. In this paper, we describe the isolate and structural elucidation of two compounds, sativanone (**1**), and 3'-*O*-methylviolanone (**2**) from the wood of this species.

2. Experimental

2.1. Plant material

The plant and wood of *D. tonkinensis* Prain was collected in Buon Ma Thuot city, Daklak province, Vietnam in 2018. Its scientific name was identified by botanist Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST, Hanoi, Vietnam. A voucher specimen (Sua Do-SD-Go) is deposited with the Natural Product Chemistry Lab, Tay Nguyen University.

2.2. General Experimental Procedures

The $^1\text{H-NMR}$ (500 MHz) and $^{13}\text{C-NMR}$ (125 MHz) were measured on a Bruker Avance 500 MHz spectrometer. 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 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% H₂SO₄ followed by heating with a heat gun.

2.3. Extraction and Isolation

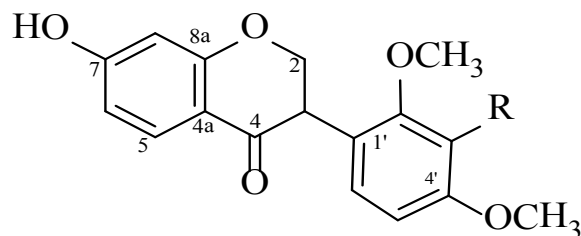
Dried powdered woods (3.7 kg) of *D. tonkinensis* were extracted with hot methanol (5 x 7.0 l) under reflux, filtered, and then concentrated under decreased pressure giving a black crude methanol residue (120 g). The suspension of the methanol residue in hot methanol-water (1:1, v/v) was successively partitioned with *n*-hexane, dichloromethane and ethyl acetate to give *n*-hexane (29.0 g, MH), dichloromethane (45.1 g, MD), ethyl acetate (11.1 g, ME) and water (12.0 g, MW) fractions, respectively.

The fraction ME (11.1 g) was chromatographed on a normal silica gel (40-63 mesh) chromatography column (CC) using a gradient of chloromethane and acetone as eluent to afford 12 fractions (ME1-ME12). Fraction ME1 (500 mg) was rechromatographed on a normal silica gel CC (chloroform-acetone 99-1 as eluent, v/v) to produce 8 sub-fractions (ME1.1- ME1.8). The

sub-fraction ME1.4 (240 mg) was further separated on a normal silica gel CC, eluting with *n*-hexane-ethyl acetate (8:1, v/v) to obtain compound **1** (6 mg). Fraction ME5 (310 mg) was rechromatographed on a normal silica gel CC (chloroform- ethylacetate 4-1 as eluent, v/v) to produce 15 sub-fractions (ME4.1- ME4.15). Compound **2** (12.0 mg) was obtained from the sub-fraction ME4.10.

Sativanone (1): white amorphous power (C₁₇H₁₆O₅, M = 300). $^1\text{H-NMR}$ (500 MHz, CD₃OD): $^1\text{H-NMR}$ (500 MHz, CD₃OD): 7.78 (1H, d, 8.5, H-5), 7.00 (1H, d, 8.5, H-6'), 6.58 (1H, d, 2.5, H-8), 6.52 (1H, dd, 8.5, 2.5, H-6), 6.49 (1H, dd, 8.5, 2.5, H-5'), 6.35 (1H, d, 2.5, H-3'), 4.56 (1H, t, 11.5, H_a-2), 4.40 (1H, dd, 11.5, 5.5, H_b-2), 4.17 (1H, dd, 11.5, 5.5, H-3), 3.80 (3H, s, 2'-OCH₃), 3.78 (3H, s, 4'-OCH₃). $^{13}\text{C-NMR}$ (125 MHz, CD₃OD): 194.4 (s, C-4), 166.4 (s, C-7), 165.8 (s, C-8a), 162.2 (s, C-4'), 159.9 (s, C-2'), 132.0 (d, C-5), 130.4 (d, C-6'), 117.4 (s, C-1'), 111.7 (d, C-3'), 106.0 (d, C-5'), 103.6 (d, C-6), 115.7 (s, C-4a), 99.9 (d, C-8), 70.0 (t, C-2), 56.6 (q, 4'-OCH₃), 55.8 (q, 2'-OCH₃), 49.7 (d, C-3).

3'-*O*-methylviolanone (2): white amorphous power (C₁₈H₁₈O₆, M = 330). $^1\text{H-NMR}$ (500 MHz, CD₃OD): 6.54 (1H, d, 8.5, H-5), 6.86 (1H, d, 8.5, H-6'), 6.36 (1H, d, 2.5, H-8), 6.54 (1H, dd, 8.5, 2.5, H-6), 6.75 (1H, dd, 8.5, H-5'), 4.55 (1H, t, 11.5, H_a-2), 4.15 (1H, dd, 11.5, 5.5, H_b-2), 4.44 (1H, dd, 11.5, 5.5, H-3), 3.82 (3H, s, 4'-OCH₃), 3.83 (3H, s, 2'-OCH₃), 3.85 (3H, s, 4'-OCH₃). $^{13}\text{C-NMR}$ (125 MHz, CD₃OD): 194.2 (s, C-4), 166.5 (s, C-7), 165.8 (s, C-8a), 155.1 (s, C-4'), 153.2 (s, C-2'), 130.4 (d, C-5), 126.0 (d, C-6'), 123.1 (s, C-1'), 143.6 (d, C-3'), 106.8 (d, C-5'), 111.8 (d, C-6), 115.6 (s, C-4a), 103.7 (d, C-8), 72.3 (t, C-2), 61.2 (q, 3'-OCH₃), 61.0 (q, 2'-OCH₃), 56.0 (q, 4'-OCH₃), 49.7 (d, C-3).



- 1** R = H
2 R = OCH₃

Figure 1. Chemical structure of isolated compounds **1-2** from the wood of *D. tonkinensis*.

3. Results and discussion

Compound **1** was obtained as white amorphous powder. The ^1H -NMR of **1** showed three protons in AMX spin system at δ_{H} 4.40 (1H, dd, $J = 11.5, 5.5$ Hz), 4.56 (1H, t, $J=11.5$ Hz) and 4.17 (1H, dd, $J = 11.5, 5.5$ Hz), typically assignable to H-2 and H-3 of isoflavanone skeleton.

Besides, the ^1H -NMR spectrum also supported the presence of two methoxy group at δ_{H} 3.80 (3H, s, 2'-OCH₃) and 3.78 (3H, s, 4'-OCH₃).

The ^{13}C -NMR and DEPT spectrum showed signals for seventeen carbons: a carbonyl carbons at δ_{C} 194.4 (C-4), six aromatic quaternary carbons at δ_{C} 164.4

(s, C-7), 165.8 (s, C-8a), 162.2 (s, C-4'), 159.9 (s, C-2'), 117.4 (s, C-1'), 115.7 (s, C-4a) and six aromatic methine carbons at δ_{C} 132.0 (d, C-5), 103.6 (d, C-6), 130.4 (d, C-6'), 99.9 (d, C-8), 111.7 (d, C-3') and 106.0 (d, C-5') together with an signal oxy methylen carbon at δ_{C} 70.0 (t, C-2), a methine carbon δ_{C} 49.7 (d, C-3), two methoxy group δ_{C} 56.6 (q, 4'-OCH₃) and 55.8 (q, 2'-OCH₃). In the ^{13}C -NMR spectrum suggested the presence of a isoflavanone skeleton. On the basis of above discussion and comparison with literature data [11], has identified **1** as sativanone. This compound was previously isolated from heartwood of *Dalbergia odorifera* [3].

Table 1. ^1H NMR and ^{13}C NMR spectroscopic data for **1** and reference compound.

Position	1 (*)		Sativanone [11]	
	δ_{H} (ppm) (J in Hz)	δ_{C} (ppm)	δ_{C}	δ_{H}
1	-	-	-	-
2	4.40, dd, 11.5, 5.5 4.56, t, 11.5	72.1	70.2	4.40, dd, 11.0, 5.5 4.54, d, 11.0
3	4.17, dd, 11.5, 5.5	49.7	47.5	4.16, dd, 11.0, 5.5
4	-	194.4	192.4	-
4a	-	115.7	113.7	-
5	7.78, d, 8.5	132.0	129.9	7.76, d, 8.7
6	6.52, dd, 8.5, 2.5	103.6	101.8	6.49, d, 8.7
7	-	166.4	164.2	-
8	6.58, d, 2.5	99.9	98.0	6.51, s
8a	-	165.8	163.6	-
1'	-	117.4	115.3	-
2'	-	159.9	157.7	-
2'-OCH ₃	3.80, s	55.8	54.0	3.77, s
3'	6.35, d, 2.0	111.7	109.8	6.33, d, 2.3
4'	-	162.2	160.0	-
4'-OCH ₃	3.78	56.0	54.2	3.74, s
5'	6.49, dd, 8.5, 2.5	106.0	104.0	6.46, dd, 8.4, 2.3
6'	7.00, d, 8.5	130.4	128.5	6.97, d, 8.4

(*) ^1H (500 MHz), ^{13}C (125 MHz) recorded in MeOH with TMS as an internal standard.

Compound **2** was isolated as white amorphous power. The ^1H -NMR spectrum exhibited similar signals of ring A of Compound **1**. The difference was the presence of two ortho-coupled protons of ring B at δ_{H} 6.86 (1H, d, $J = 8.5$ Hz, H-6') and 6.75 (1H, dd, $J=8.5$ Hz, H-5'). Furthermore, The ^1H -NMR spectrum also supported the presence of a methoxy group with a singlet signal at δ_{H} 3.82 (q, 3'-OCH₃) and 6.37 (1H, d, $J = 2.5$ Hz, H-8).

The ^{13}C -NMR and DEPT spectra of compound **1** are similar of compound **1**. The difference was the presence of a signal methoxy group at δ_{C} 61.2 (q, 3'-OCH₃) due to replacement of the aromatic proton H-3' in Compound **2** by a methoxy group, so showed signals an aromatic quaternary carbons at δ_{C} 143.6 (s, C-3'). Combined analysis of (^1H -, ^{13}C -NMR) and comparison with published data verified the compound **2** was established 3'-O-methylviolanonone [12].

Table 2. ¹H NMR and ¹³C NMR spectroscopic data for 2 and reference compound.

Position	2 (*)		3'-O-methylviolanonone [12]	
	δ_H (ppm) (J in Hz)	δ_C (ppm)	δ_C	δ_H
1	-	-	-	-
2	4.55, t, 11.5 4.15, dd, 11.5, 5.5	72.3	4.52, dd, 11.2, 5.2 4.15, dd, 11.2, 5.2	71.1
3	4.44, dd, 11.5, 5.5	49.7	4.45, dd, 11.2, 5.2	47.8
4	-	194.2	-	190.9
4a	-	115.6	-	114.3
5	7.79, d, 8.5	130.4	7.69, d, 8.8	129.4
6	6.54, dd, 8.5, 2.5	111.8	6.54, dd, 8.8, 2.4	111.1
7	-	166.5	-	164.8
8	6.36, d, 2.5	103.7	6.36, d, 2.4	102.8
8a	-	165.8	-	163.7
1'	-	123.1	-	122.1
2'	-	153.2	-	151.9
2'-OCH ₃	3.83, s	61.0	3.74, s	60.6
3'	-	143.6	-	142.2
3'-OCH ₃	3.82, s	61.2	3.71, s	60.9
4'	-	155.1	-	153.5
4'-OCH ₃	3.85, s	56.6	3.78, s	56.3
5'	6.75, d, 8.5	108.8	6.76, d, 8.8	108.2
6'	6.86, d, 8.5	126.0	6.85, d, 8.8	124.9

(*)¹H (500 MHz), ¹³C (125 MHz) recorded in MeOH with TMS as an internal standard.

4. Conclusions

By various chromatographic, two flavonoids, sativanone (1), and 3'-O-methylviolanonone (2) were isolated from the ethyl acetate extract of wood of *Dalbergia tonkinensis* was collected in Buon Ma Thuot city, DakLak province. Their chemical structures were determined by the interpretation of NMR spectral data and comparison with published data. Although these compounds were already reported in many different species, but this is the first time they are confirmed from wood of *Dalbergia tonkinensis*.

REFERENCES

[1] The Plant List. Available: <http://www.theplantlist.org/tpl1.1/search?q=dalbergia>, (Accessed, July 29, 2017)

[2] Chi V.V.- Dictionary of Vietnamese Medical plants, Medical Publishing House, 2(2012) 1047-1054.

[3] Sanjib S., Jamil A. S., Himangsu M., Faroque H., Md. Anisuzzaman, Md. Mahadhi H., Geoffrey A. C. (2013) *Ethnomedicinal, phytochemical, and pharmacological profile of the genus Dalbergia L. (Fabaceae)*. *Phytopharmacology* **4**, 291-346.

[4] Nhan, N.T., Nguyen, P.H., Hanh, D.T.B., My, D.T.T., Nguyen, N.P.D., Phong, T.B., Huong, N.T.M., Hung, T.M., Tram, N.T.T., & To, D.C. (2022) Anti-inflammatory activity of ingredients from the heartwood of Vietnamese *Dalbergia oliveri* Gamble ex, *European Journal of Inflammation*, 20, 1-8.

[5] Cuong, N.M., Son, N.T., Nhan, N.T., Yoshiyasu Fukuyama, Amer Ahmed, Simona Saponara, Alfonso Trezza, Beatrice Gianibbi, Ginevra Vigni, Ottavia Spiga and Fabio Fusi, (2022) Vietnamese *Dalbergia tonkinensis*: A Promising Source of Mono-and Bifunctional Vasodilators, *Molecules*, **27**(14), 4505.

[6] Cuong N.M., Nhan N.T., Son N.T., Nghi D.H., Cuong T.D. (2017). DaltonkinA and B, two new carboxyethylflavanone from the heartwood

of *Dalbergia tonkinensis*. Bull.Korean.Chem.Soc. 38(12),P.1511-1514.

[7] Ho P.H- An Illustrated Flora of Vietnam, 2nd ed, Youth Publisher, Ho Chi Minh City, Vietnam 1(1999).

[8] Mai N.T.H., Cuong N.M., Nhan N.T., Nguyen N.P.D., Trang N.T.P (2017). Molecular characteristics and classification ability of rpoC region for *Dalbergia cochinchinensis* Pierre and *Dalbergia tonkinensis* Prain species in Viet Nam. Proceedings of the 7th national scientific conference on ecology and biological resources. Journal of Science and Technology, Ha Noi, 814-817.

[9] Sorachai K., Saisunee L., Aphiwat T., Stephen G., Mary J., Boonsom L. -The isolation of bioactive

flavonoids from *Jacaranda obtusifolia* H. B. K. ssp. *rhombofolia* (G. F. W. Meijer) Gentry. Acta Pharm. (2012), 62, 181-190.

[10] Xiao Z., Wenli M., Mingfu G., Wenjian Z., Hongjin B., Haofu D. (2011). Antibacterial activity of the flavonoids from *Dalbergia odorifera* on *Ralstonia solanacearum*. Molecule. 16, 9775-9782.

[11] Jin W.L., Chul L., Qing H.J., Moon S.L., Young S.K., Jin T.H., Mi K.L., Bang Y.H. (2014). Chemical constituents from *Belamcanda chinensis* and their inhibitory effects on nitric oxide production in RAW 264.7 macrophage cells. Arch. Pharm. Res. 38(6), 991-997.