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INOSITOL, MEGASTIGMANES AND FLAVONOIDS FROM THE LEAVES OF *BAUHINIA VARIEGATA* IN VIETNAM

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Summary

Inositol, Megastigmanes and Flavonoids from the Leaves of *Bauhinia variegata* in Vietnam

The leaves of *Bauhinia variegata* (Ban trang) were collected in Dien Bien province and were used to study the chemical composition. From the *n*-BuOH fraction, five compounds were isolated including pinitol (**1**), (9*S*)-9-hydroxymegastigma-4,7-dien-3-one 9-*O*-*β*-D-glucopyranoside (**2**), (9*S*)-roseoside (**3**), rutin (**4**), and tamarixetin 3-robinobioside (**5**). Their structures were determined through analysis of NMR and MS spectral data in comparison with those published in the literatures. Notably, these substances were isolated for the first time from *Bauhinia variegata* in Vietnam.

Keywords: *Bauhinia variegata, Pinitol, (9S)-9-hydroxymegastigma-4,7-dien-3-one 9-O-β-D-glucopyranoside, (9S)-roseoside, Rutin, Tamarixetin 3-robinobioside.*

1. Introduction

Bauhinia variegata L. (common local names: Ban Trang, Mong bo soc, Hoa ban, Mong bo doi mau) belongs to the Caesalpiniaceae family [1],[2]. The plant distributed mainly in tropical and subtropical regions of South Asia and Southeast Asia such as India, Myanmar, Vietnam, Laos, China, Thailand... In Vietnam, it grows naturally in the mountains of the northwestern provinces such as Lai Chau, Son La, Dien Bien, Hoa Binh. Nowadays for the decorating purpose, *B. variegata* has been planted in many cities such as Hanoi, Ho Chi Minh, Da Nang, Hai Phong… [1]. According to traditional medicine, the flower buds of *B. variegata* are used to treat diarrhea and dysentery; flowers are used to relieve pain or to treat colic; roots are used to treat ureteral stones; bark has the effect of firming, and is used as a tonic to restore the body, to treat tuberculosis, skin diseases, boils, and sores, while the leaves are used to treat cough [1]. Following modern pharmacology, *Bauhinia variegata* leaf extracts showed anti-inflammation, anti-tumor activity, anti-bacterial activity, anti-diabetic activity, anti-carcinogenic activity…[3],[4],[5],[6]. Studies on the chemical composition of the leaves show the presence of various types of compounds, including flavonoids, proanthocyanidins, saponins, fatty acids, reducing sugars, anthraquinones, alkaloids, tannins, terpenoids... Among them, flavonoids and saponins were attributed as the main groups of compounds [7],[8],[9]. To the best of our knowledge, there is no report on the chemical constituents from this species in Vietnam. In this report, the isolation of several compounds from the *n*-butanol fraction of *B. variegata* leaves and their chemical structural characterizations are presented and discussed.

2. Experimental

2.1. Plant materials

The plant materials were collected in Dien Bien province, Vietnam in September 2020. The scientific name of the plant was identified as *Bauhinia variegata* L. by MSc. Nguyen Quynh Nga and MSc. Phan Van Truong. A voucher specimen, No. DL-19202, has been kept at the Department of Medicinal Plant Resources - Institute of Medicinal Materials.

Samples were prepared by chopping, firstly drying in the shade and then in an oven at 50°C. The sample was then ground to the raw powder and preserved in sealed plastic bags in a cool dry place and got rid of direct sunlight.

2.2. General experimental procedures

The NMR data were measured with Bruker AM 500 MHz and/ or 600 MHz FT-NMR spectrometer using TMS as an internal standard. The electrospray ionization mass spectrometry (ESI-MS) spectra were obtained on an Agilent 1260 Series Single Quadrupole LC/MS Systems (Agilent, USA). Optical rotations were measured on a Jasco P-2000 Polarimeter serial A060161232. Column chromatography was performed on *silica gel* (Merck, Darmstadt, Germany particle size 63 - 200 μm or 40 - 63 μm), reversed phase YMC ODS-AA12S75 (75 μm), and Sephadex® LH-20 (GE Healthcare). High Performance Liquid Chromatography (HPLC) was carried out on a preparative HPLC Shimadzu LC20-AP with a Supelco Analytical Discovery ® HS C_{18} (250 × 21,2 mm; 10 μm) column. Thin-layer chromatography (TLC) was performed on DC-

Alufolien 60 F_{254} pre-coated plates (Merck 1.05715). Spots were detected by UV radiation or by spraying with 10% aq. H_2SO_4 followed by heating or 5% $FeCl_3$/EtOH.

2.3. Extraction and isolation

The dried leaves of *B. variegata* (6.0 kg) were extracted with 75% aqueous ethanol three times (40L × 3 times, 4 days/time) at room temperature. After filtration, solvents were removed under reduced pressure to obtain an ethanol extract. This extract was suspended in water and successively fractionated with dichloromethane, ethyl acetate, and *n*-butanol. The solvents were evaporated in the vacuum condition to obtain the corresponding dichloromethane (81.21 g), ethyl acetate (36.54 g), and *n*-butanol (74.83 g) fractions.

The *n*-butanol fraction (60.0 g) was subjected to a *silica gel* column and eluted with a gradient solvent system of EtOAc-MeOH-H_2O (100:1:0.1 - 0:100:0.1, v/v/v) to give 13 fractions (BTB1-BTB13). The BTB5 (1.2 g) fraction was separated on a Sephadex LH-20 column eluting with MeOH to obtain four sub-fractions (BTB5.1-BTB5.4). Sub-fractions BTB5.1 (0.45 g) gave a precipitate which was separated by filtration and recrystallization in methanol to obtain compound **1** (70.0 mg). Sub-fraction BTB5.2 (0.38 g) was purified by Sephadex LH-20 column using 100% methanol to yield compound **4** (10.0 mg). Fraction BTB7 (1.8 g) was subjected to an LH-20 Sephadex column, and the system was eluted with MeOH to yield three sub-fractions (BTB7.1-BTB7.3). Sub-fraction BTB7.1 (0.4 g) was further purified by preparative HPLC with a solvent system of acetonitrile and 0.01% TFA in water (0-5 min: 20% ACN, 5-40 min: 20%-50%, ACN, 10 mL/min and UV 254 nm) to obtain compound **5** (12.0 mg, t_R = 18.9 min). Fraction BTB4 (1.05 g) was fractionated by column chromatography on Sephadex LH-20 using MeOH to yield four sub-fractions (BTB4.1-BTB4.4). Sub-fraction BTB4.2 (200.0 mg) was subjected to preparative HPLC with a solvent system of acetonitrile and 0,01% TFA in water (0-10' min: 15% ACN; 10-20' min: 15% - 30% ACN, 20 - 35' min: 30% - 20% ACN, 10 mL/min, UV 205 nm and 254 nm) to give compound **3** (17.0 mg, t_R = 20.5 min). Fraction BTB3 (1.1 g) was separated by reversed-phase *silica gel* CC using MeOH-H_2O (3:7-10:0, v/v) to yield five sub-fractions (BTB3.1-BTB3.5). Sub-fraction BTB3.2 (200.0 mg) was further purified by preparative HPLC with a solvent system of acetonitrile and 0,01% TFA in water (0-2' min: 20% ACN; 2-28' min: 20%-40% ACN, 28-35' min: 40%-20% ACN, 10 mL/ min, UV 205 nm and 254 nm) to obtain compound **2** (4.0 mg, t_R = 15.7 min).

Compound 1: White solid. $[\alpha]_D^{22}$ = +16.45° (c = 1, H_2O). ESI-MS: *m/z* 177 $[M-H_2O+H]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6), δ_H (ppm): 3.42 (1H, *m,* H-1), 3.35 (1H, m, H-2), 3.00 (1H, t, *J* = 9.6 Hz, H-3), 3.51 (1H, m, H-4), 3.63 (2H, brs, H-5, H-6), 3.44 (3H, s, OCH_3). ^{13}C-NMR (150 MHz, DMSO-d_6), δ_C (ppm): 70.9 (C-1), 72.6 (C-2), 83.8 (C-3), 70.0 (C-4), 72.4 (C-5), 71.9 (C-6), 59.6 (OCH_3).

Compound 2: Yellow oil. $[\alpha]_D^{22}$ = +29.2° (c = 0.5, CH_3OH). ^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 2.09 (1H, d, *J* = 16.2 Hz, Ha-2), 2.50 (1H, d, *J* = 16.2 Hz, Hb-2), 5.91 (1H, s, H-4), 2.72 (1H, d, *J* = 9.6 Hz, H-6), 5.78 (1H, dd, *J* = 9.6, 15.0 Hz, H-7), 5.61 (1H, dd, *J* = 7.2, 15.0 Hz, H-8), 4.51 (1H, m, H-9), 1.32 (3H, d, *J* = 6.6 Hz, H-10), 1.05 (3H, s, H-11), 1.01 (3H, s, H-12), 2.06 (3H, d, *J* = 1.2 Hz, H-13), *Glc*: 4.31 (1H, d, *J* = 7.8 Hz, H-1′), 3.20 (1H, m, H-2′), 3.28 (2H, m, H-3′, H-4′), 3.17 (1H, m, H-5′), 3.88 (1H, dd, *J* = 2.4, 12.0 Hz, Ha-6'), 3.66 (1H, dd, *J* = 6.0, 12.0 Hz, Hb-6'). ^{13}C-NMR (125 MHz, CD_3OD), δ_C (ppm): 37.2 (C-1), 48.5 (C-2), 202.0 (C-3), 126.2 (C-4), 165.7 (C-5), 56.9 (C-6), 131.2 (C-7), 137.1 (C-8), 74.8 (C-9), 22.2 (C-10), 27.4 (C-11), 28.0 (C-12), 23.9 (C-13), *Glc*: 101.2 (C-1′), 75.0 (C-2′), 78.4 (C-3′), 71.7 (C-4′), 78.2 (C-5′), 62.9 (C-6′).

Compound 3: Pale yellow oil. $[\alpha]_D^{22}$ = +38.3° (c = 0.5, CH_3OH). ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 2.19 (1H, d, *J* = 16.8 Hz, Ha-2), 2.62 (1H, d, *J* = 16.8 Hz, Hb -2), 5.89 (1H, brs, H-4), 6.00 (1H, d, *J* = 15.6 Hz, H-7), 5.75 (1H, dd, *J* = 7.2, 15.6 Hz, H-8), 4.55 (1H, t, *J* = 6.6 Hz, H-9), 1.31 (3H, d, *J* = 6.6 Hz, H-10), 1.06 (3H, s, H-11), 1.04 (3H, s, H-12), 1.96 (3H, d, *J* = 1.2 Hz, H-13), *Glc*: 4.29 (1H, d, *J* = 7.2 Hz, H-1′), 3.21 (1H, m, H-2′), 3.32 (2H, m, H-3′, H-4 ′), 3.18 (1H, m, H-5′), 3.65 (1H, dd, *J* = 6.0, 12.0 Hz, Ha-6′), 3.86 (1H, dd, *J* = 2.4, 12.0 Hz, Hb-6′). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 42.4 (C-1), 50.7 (C-2), 201.3 (C-3), 127.1 (C-4), 167.1 (C-5), 80.0 (C-6), 133.7 (C-7, C-8), 74.7 (C-9), 22.2 (C-10), 23.5 (C-11), 24.7 (C-12), 19.6 (C-13), *Glc*: 101.3 (C-1′), 75.0 (C-2′), 78.4 (C-3′), 71.7 (C-4′), 78.2 (C-5′), 62.8 (C-6′).

Compound 4: Pale yellow solid. ESI-MS: *m/z* 611.0 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 6.24 (1H, d, *J* = 2.4 Hz, H-6), 6.42 (1H, d, *J* = 2.4 Hz, H-8), 7.69 (1H, d, *J* = 1.8 Hz, H-2′), 6.90 (1H, d, *J* = 8.4 Hz, H-5′), 7.65 (1H, dd, *J* = 1.8, 8.4 Hz, H-6′), *Glc*: 5.12 (1H, d, *J* = 7.8 Hz, H-1"), 3.48

(1H, m, H-2"), 3.43 (1H, m, H-3"), 3.65 (1H, m, H-4"), 3.33 (1H, m, H-5"), 3.41 (1H, m, H-6"), 3.82 (1H, dd, J = 1.8, 10.8 Hz, H-6"), *Rham:* 4.54 (1H, d, J = 1.8 Hz, H-1‴), 3.28 (1H, m, H-2‴), 3.56 (1H, dd, J = 3.6, 9.6 Hz, H-3‴), 3.34 (1H, m, H-4‴), 3.46 (1H, m, H-5‴), 1.14 (3H, d, J = 6.6 Hz, H-6‴). ^{13}C-NMR (125 MHz, CD_3OD), δ_C (ppm): 158.6 (C-2), 135.7 (C-3), 179.5 (C-4), 163.1 (C-5), 100.0 (C-6), 166.1 (C-7), 94.9 (C-8), 159.4 (C-9), 105.7 (C-10), 123.2 (C-1′), 117.8 (C-2′), 145.9 (C-3′), 149.9 (C-4′), 116.1 (C-5′), 123.6 (C-6′), *Glc*: 104.8 (C-1"), 75.8 (C-2"), 78.3 (C-3"), 72.2 (C-4"), 77.3 (C-5"), 68.6 (C-6"), *Rham*: 102.5 (C-1‴), 71.5 (C-2‴), 72.3 (C-3‴), 74.0 (C-4‴), 69.8 (C-5‴), 17.9 (C-6‴).

Compound 5: Pale yellow solid. ^{1}H-NMR (500 MHz, DMSO-d_6), δ_H (ppm): 6.20 (1H, d, J = 2.0 Hz, H-6), 6.41 (1H, d, J = 2.0 Hz, H-8), 7.52 (1H, d, J = 2.5 Hz, H-2′), 7.00 (1H, d, J = 9.0 Hz, H-5′), 7.75 (1H, dd, J = 2.5, 8.5 Hz, H-6′), 12.52 (1H, s, 5-OH), 3.86 (4'-OCH_3), *Gal*: 5.32 (1H, d, J = 7.5 Hz, H-1"), 3.56 (1H, m, H-2"), 3.40 (1H, m, H-3"), 3.36 (1H, m, H-4"), 3.55 (1H, m, H-5"), 3.26 (1H, m, H-6"), 3.60 (1H, m, H-6"), *Rham*: 4.54 (1H, s, H-1‴), 3.39 (1H, m, H-2‴), 3.30 (1H, m, H-3‴), 3.10 (1H, m, H-4‴), 3.60 (m, H-5‴), 1.06 (3H, d, J = 6.0 Hz, H-6‴). ^{13}C-NMR (125 MHz, DMSO-d_6), δ_C (ppm): 156.4 (C-2), 133.8 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.2 (C-7), 93.6 (C-8), 156.1 (C-9), 103.9 (C-10), 122.5 (C-1′), 115.6 (C-2′), 145.9 (C-3′), 150.0 (C-4′), 111.3 (C-5′), 121.6 (C-6′), 55.6 (4'-OCH_3), *Gal*: 102.1 (C-1"), 71.0 (C-2"), 73.0 (C-3"), 68.2 (C-4"), 73.6 (C-5"), 65.3 (C-6"), *Rham*: 100.0 (C-1‴), 70.4 (C-2‴), 70.6 (C-3‴), 71.9 (C-4‴), 68.0 (C-5‴), 17.9 (C-6‴).

3. Results and discussions

Compound 1 was isolated as a white solid. The molecular formula of **1** was suggested as $C_7H_{14}O_6$ based on the positive *quasi*-molecular peak at *m/z* 177 $[M\text{-}H_2O+H]^+$. The ^{1}H-NMR spectrum showed signals of hydroxymethin protons (CH-OH) at δ_H 3.42 (1H, m, H-1), 3.35 (1H, m, H-2), 3.00 (1H, t, J = 9.6 Hz, H-3), 3.51 (1H, m, H-4), and 3.63 (2H, brs, H-5, H-6). The ^{13}C-NMR spectrum displayed 6 carbon signals at δ_C 70.9 (C-1), 72.6 (C-2), 83.8 (C-3), 70.0 (C-4), 72.4 (C-5), and 71.9 (C-6). In addition, the ^{1}H-NMR spectrum also showed signals of hydroxyl protons in the range of δ_H 4.3 to 4.7 ppm, demonstrating the structure of **1** containing a polyhydroxy cyclohexanol moiety. Moreover, **1** also exhibited the signal of one methoxy group at δ_H 3.44 (3H, s) and δ_C 59.6 and its position was determined at C-3 through an HMBC correlation between the proton δ_H 3.44 (OCH_3) and carbon δ_C 83.8 (C-3). Based on the obtained spectral data, combined with the comparison with published data [10], **1** was identified as **pinitol** (Fig. 1). This compound is known for many effects such as anti-inflammatory, anti-diabetic, appetite stimulant [11].

Compound 2 was isolated as a yellow oil. The ^{1}H-NMR spectrum showed a singlet signal at δ_H 5.91 (1H, s, H-4), a *trans* double bond at δ_H 5.78 (1H, dd, J = 9.6, 15.0 Hz, H-7) and 5.61 (1H, dd, J = 7.2,15.0 Hz, H-8), one oxymethin group at δ_H 4.51 (1H, m, H-9), and four methyl groups at δ_H 1.32 (3H, d, J = 6.6 Hz, H-10), 1.05 (3H, s, H-11), 1.01 (3H, s, H-12) and 2.06 (3H, d, J = 1.2 Hz, H-13), suggesting **2** is a megastigmane. Additionally, on the ^{1}H-NMR spectrum, signals of a β-D-glucopyranosyl unit appeared in the presence of an anomer proton at δ_H 4.31 (1H, d, J = 7.8 Hz, H-1′) together with CHOH and CH_2OH groups in the region of δ_H 3.17 - 3.88. The ^{13}C-NMR and DEPT spectra of **2** showed the presence of 19 carbons, including 3 non-hydrogenated carbons at δ_C 37.2 (C-1), 202.0 (C-3), and 165.7 (C-5); 4 methine groups at δ_C 56.9 (C-6), 131.2 (C-7), 137.1 (C-8), and 74.8 (C-9); 1 methylene group at δ_C 48.5 (C-2), 4 methyl groups at δ_C 22.2 (C-10), 27.4 (C-11), 28.0 (C-12), 23.9 (C-13), and 6 carbons belonging to the β-D-glucopyranosyl moiety at δ_C 101.2 (C-1′), 75.0 (C-2′), 78.4 (C-3′), 71.7 (C-4′), 78.2 (C-5′), and 62.9 (C-6′). The position of the sugar moiety at C-9 was determined through interactions in the HMBC spectrum between H-1′ (δ_H 4.29) and H-7 (δ_H 5.78) with C-9 (δ_C 74.6). The stereochemistry at C-9 was determined to be 9*S* based on the chemical shifts of carbons C-7 (δ_C 131.1), C-8 (δ_C 137.1), and C-9 (δ_C 74.7) [12]. The analysis of the spectral data in comparison with the previously published data [12] confirmed that **2** was **(9*S*)-9-hydroxymegastigma-4,7-dien-3-one-9-*O*-β-D-glucopyranoside** (Fig. 1).

Compound 3 was isolated as a yellow oil. The NMR data of **2** and **3** were very similar except for the absence of a signal at δ_H 2.72 (1H, d, J = 9.6 Hz) instead of the appearance of the >C-OH group at δ_H 80.0, indicating the hydroxylation at the C-6 position. The configuration C-9 was also determined to **2** based on the chemical shifts of C-7 (δ_C 133.74), C-8 (δ_C 133.81), and C-9 (δ_C 74.67) [13]. The structure of **3** was elucidated as **(9*S*)-roseoside** when comparing the spectra with that described in the literature [13] (Fig. 1).

Compound 4 was isolated as a yellow solid and its molecular formula ($C_{27}H_{30}O_{16}$) was determined

by the ESI-MS spectrum (*m/z* 611.0 $[M+H]^+$) and NMR data. The 1D-NMR spectra of **4** gave the characteristic signals of a flavonoid glycoside. The ^{1}H-NMR spectrum of **4** exhibited signals of 5 aromatic protons including 3 signals of an ABX system at δ_H 7.69 (1H, d, J = 1.8 Hz, H-2′), 6.90 (1H, d, J = 8.4 Hz, H-5′), and 7.65 (1H, dd, J = 1.8, 8.4 Hz, H-6′) in the B ring and two *meta*-interacting proton signals at δ_H 6.24 (1H, d, J = 2.4 Hz, H-6) and 6.42 (1H, d, J = 2.4 Hz, H-8) of the A ring. These signals were indicated for the quercetin skeleton. Furthermore, the ^{1}H-NMR spectrum showed the signal of two anomeric protons at δ_H 5.12 (1H, d, J = 7.8 Hz, H-1") and 4.54 (1H, d, J = 1.8 Hz, H-1‴). In addition, the hydroxymethine and hydroxymethylene proton signals were in the range of δ_H 3.28 - 3.82, together with a doublet methyl groups signal at δ_H 1.14 (3H, *d*, J = 6.6 Hz) suggested the presence of β-D-glucopyranosyl and α-L-rhamnopyranosyl moieties. The ^{13}C-NMR and DEPT spectra of **4** showed 27 carbon signals, of which 15 were assigned to quercetin aglycon and 12 belong to the 2 hexose moieties. The positions of the sugar moieties were determined through the observation of interactions in the HMBC and HSQC spectra. The interaction in the HMBC spectrum between H-1" (δ_H 5.12) with carbon signal at δ_C 135.7 confirmed the *O*-glycosidic linkage of the β-D-glucopyranosyl sugar moiety with the aglycon at C-3. The position of the rhamnose moiety at C-6" was determined through the HMBC interaction between H-1‴ (δ_H 4.54) and C-6" (δ_C 68.6) as well as the downfield shift of the hydroxymethylene carbon compared with the corresponding carbon signals of the β-glucopyranosyl residues in the structure of **2** (δ_C 62.9) and **3** (δ_C 62.8). Analysis of the spectral data in comparison with published data [14] concluded that **4** was quercetin 3-*O*-α-L-rhamnopyranosyl(1->6)-β-D-glucopyranoside or **rutin** (Fig. 1). This compound has been found in the leaves of *B. variegata* and showed potent antioxidant effects [15].

Compound 5 was isolated as a yellow solid. Spectral data of **5** and **4** showed similarities. However, **5** appeared an additional signal of 1 methoxy group (at δ_H 3.86 and δ_C 55.6) and its location at C-4' was elucidated by HMBC correlations between the proton signals at δ_H 3.86 (3H, s), 7.52 (1H, d, J = 2.5 Hz, H-2'), 7.00 (1H, d, J = 9.0 Hz, H-5'), and 7.75 (1H, dd, J = 2.5, 8.5 Hz, H-6') and carbon δ_C 150.0 (C-4'). In addition, the chemical shift of the sugar unit bonded to C-3 [δ_C102.1 (C-1"), 71.0 (C-2"), 73.0 (C-3"), 68.2 (C-4"), 73.6 (C-5"), and 65.3 (C-6")] was different from the corresponding sugar unit of **4** [δ_C 104.8 (C-1"), 75.8 (C-2"), 78.3 (C-3"), 72.2 (C-4"), 77.3 (C-5"), and 68.6 (C-6")]. It was determined to be β-galactopyranoside by NMR data and reported literature [16]. From the above data analysis combined with the comparison of published data [16], the structure of **5** was identified as **tamarixetin 3-robinobioside** (Fig. 1).

Table 1. ^{13}C-NMR data of compound **5** and related references

C	Compound 5	Tamarixetin 3-robinobioside [16]	Tamarixetin 3-rutinoside [17]
	[a,b]δ_C	[a,b]δ_C	[c,b]δ_C
2	156.4	156.7	156.4
3	133.8	133.3	133.6
4	177.4	177.6	177.5
5	161.2	161.4	161.2
6	98.7	98.9	98.8
7	164.2	164.4	164.3
8	93.6	94.0	93.7
9	156.1	156.6	156.5
10	103.9	104.2	104.0
4'-OCH_3	55.6	56.2	55.7
1'	122.5	121.3	121.5
2'	115.6	113.7	111.4
3'	145.9	147.2	150.1
4'	150.0	149.7	145.9
5'	111.3	115.4	115.7
6'	121.6	122.2	122.5
	3'-*O*-Gal		3'-*O*-Glc
1"	102.1	102.0	101.3
2"	71.0	71.4	74.1
3"	73.0	73.2	76.4

4"	68.2	68.5	69.8
5"	73.6	73.2	75.8
6"	65.3	65.5	66.9
6"-*O*-Rham			
1‴	100.0	100.3	100.8
2‴	70.4	70.6	70.4
3‴	70.6	70.8	70.6
4‴	71.9	72.1	71.9
5‴	68.0	68.2	68.3
6‴	17.9	18.1	17.8

[a] 125 MHz, [b] DMSO-d_6, [c] 100 MHz.

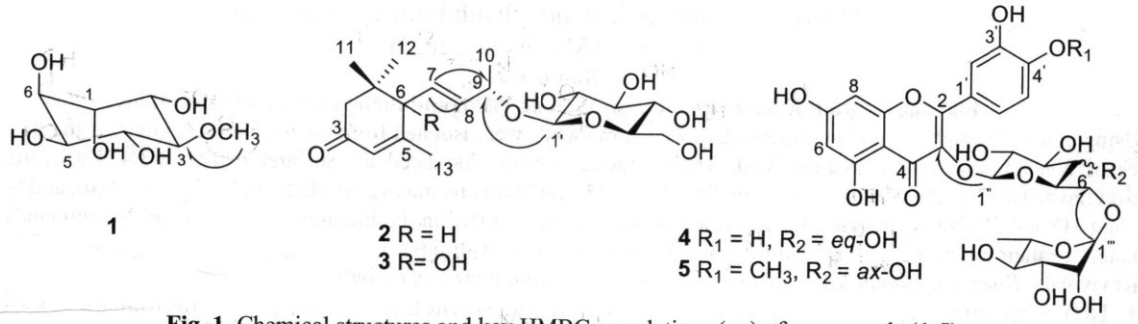

Fig. 1. Chemical structures and key HMBC correlations (→) of compounds (**1-5**)

5. Conclusions

By using various chromatographic techniques, 5 compounds were isolated from the *n*-butanol fraction of *B. variegata* leaves. The isolated substances were identified including one inositol: pinitol (**1**), 2 megastigmanes: (9*S*)-9-hydroxymegastigma-4,7-dien-3-one-9-*O*-*β*-D-glucopyranoside (**2**) and (9*S*)-roseoside (**3**), and 2 flavonoids: rutin (**4**) and tamarixetin 3-robinobioside (**5**). Among them, compounds **2**, **3**, and **5** were isolated for the first time from this species. The structures of the compounds were confirmed through analysis of their spectral data in comparison with previously published data.

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