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A New Isoflavone Glycoside from *Dalbergia vacciniifolia* (Fabaceae)

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Abstract

5,5'-Dihydroxy-2',4'-dimethoxy-7-[(6-*O*-β-D-apiofuranosyl-β-D-glucopyranosyl)-oxy]isoflavone (**1**) was isolated as the major constituent of *Dalbergia vacciniifolia* root bark ethanol extract together with the four known compounds 5,7-dihydroxy-2',4',5'-trimethoxyisoflavone (**3**), 5,7-dihydroxy-2',4'-dimethoxy-isoflavone (**4**), 5-hydroxy-2',4',7-trimethoxyisoflavone (**5**) and 7-hydroxy-2',4',5'-trimethoxyisoflavone (**6**). Identification of compounds was achieved through extensive analysis of 1D and 2D NMR and MS spectroscopy.

Keywords

Fabaceae • *Dalbergia vacciniifolia* • Apioglucoside • Isoflavone

Introduction

Dalbergia vacciniifolia Vatke (Fabaceae) is a shrub or small tree of *ca*. 1.3–10 m tall. The plant species grows in coastal bushland and thicket of Tanzania and Kenya where the decoction of the roots is used as a purgative [1, 2]. In the course of our continuing studies on the chemical constituents of this plant species, a new isoflavone glycoside, 5,5'-dihydroxy-2',4'-dimethoxy-7-[(6-*O*-β-D-apiofuranosyl-β-D-glucopyranosyl)oxy]isoflavone (**1**), from the ethanol extract of the root barks along with other four known compounds 5,7-dihydroxy-2',4',5'-trimethoxyisoflavone (**3**) [3–5], 5,7-dihydroxy-2',4'-dimethoxy-isoflavone (**4**) [3, 6], 5,7-dihydroxy-2',4',7-trimethoxyisoflavone (**5**) [7] and 7-hydroxy-2',4',5'-trimethoxyisoflavone (**6**) [4] (Fig. 1) was isolated. Recently, another isoflavone glycoside 2',4',5',6-tetramethoxy-7-[(6-*O*-β-D-apiofuranosyl-β-D-glucopyranosyl)oxy]-isoflavone (**2**) was isolated from the stem part of *Dalbergia vacciniifolia* [8]. This is the

second report of the occurrence of apioglucoside isoflavones in *D. vacciniifolia.* However, other isoflavone apioglucosides such as biochanin A 7-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] [9], prunetin 4'-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] [10], 7-methyl-tectorigenin 4'-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] [11], biochanin A 7-*O*-[β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] and tectorigenin 7-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] [12] have been previously isolated from *Dalbergia* species.

Results and Discussion

Isolation of compounds from the ethanolic extract of the root barks of *Dalbergia vacciniifolia* by column chromatography on silica gel eluting with 1:4 v/v methanol and dichloromethane yielded compound **1** as a major constituent in the extract. The compound was isolated as amorphous having absorption maxima at 261 and 342 nm. The ESI-MS showed a fragment peak at *m/z* 625 due to $[M^+ + H]$, 647 due to $[M^+ + Na]$, and *m/z* 659 due to $[M^+ + Cl]$ hence confirming the molecular weight of *m/z* 624 which corresponded to the formula $C_{28}H_{34}O_{16}$ of compound **1**. Both ^1H and ^{13}C NMR spectra data for compound **1** exhibit characteristic feature of isoflavone skeleton whose ring B is disubstituted. Identification attempts of the aromatic protons in ring B using HSQC, suggested that they were attached to C-6 (δ_H 6.46, δ_C 100.5) and C-8 (δ_H 6.69, δ_C 95.5). Furthermore, the ^1H NMR spectra for these protons showed meta coupling pattern (H-6, *d, J*=2Hz and H-8*, d, J*=2Hz). These chemical shifts and coupling patterns are typical for 5,7 di-*O*- isoflavones [13, 14], because the 6,7-di-*O*-isoflavone carbon signals would resonate at *ca*. δ 104 and 102 for C-5 and C-8, respectively [8, 15]. Presence of the hydroxyl group at C-5 suggests a chelation system with carbonyl group at C-4 (δ, 181.17) due to an intra-molecular hydrogen bonding [8, 13–15]. The 2D H/C correlation using HMBC showed C-6 and C-8 as having many common neighbors such as carbon signals at δ 162.10 (C-6), 163.46 (C-7), 158.01 (C-9), and 109.81 (C-10). In additional, HMBC showed H-8 as having a strong correlation with anomeric carbon of the glucose moiety (δ_C 100.42, δ_H 4.98) suggesting that the glycosidic linkage was at C-7. The first hydroxyl group in the structure was deduced to be attached to C-5 of the isoflavone because none of the methoxyl protons showed any correlation with carbon signal at δ 162.10 and δ 163.46 in the HMBC plot.

The rest of the three aromatic singlets in the ^1H NMR spectrum were due to aromatic methine protons at δ 8.13 (H-2, *s*), 6.82 (H-6', *s*) and 6.66 (H-3', *s*) as established both by H/C correlations such as HSQC and HMBC for compound **1**. The former proton signal is much deshielded due to α-inductive effect of the oxygen and the mesomeric electron withdrawing effect of the β-carbonyl group characteristic for isoflavones [15]. Assignment of the positions of the protons for structure **1**, were unambiguously reached because in the high field region, the ^1H NMR spectra showed aromatic methoxy proton singlets at δ 3.57 (H-4') and 3.70 (H-2'), whose corresponding ^{13}C NMR signals appeared at δ 57.31 and 56.80, respectively. The HMBC correlation also indicated that these methoxyl groups were attached to C-2' and C-4' at δ 152.74 and δ 141.89, respectively, while the signal at δ 148.47 which did not appear in the plot was attributed to the second hydroxyl group in structure **1**. These observations are consistent with other findings which reported the occurrence of isoflavonoids having 2',4',5',7-substitution pattern in *Dalbergia* species [13, 14] and particularly 2',4'-dimethoxyl substitution in *Dalbergia vacciniifolia* [8].

In the sugar region of the ^{13}C NMR spectrum, nine signals were observed which corresponded to two sugar units, one glucopyranosyl and one apiofuranosyl moiety in which three of these signals (δ 74.03, 68.08 and 64.57) were due to methylene (Table 1). The β-configuration of the glycosidic linkage was evident from the ^{1}H NMR spectrum due to signals at δ 4.98 (*J* = 7.5 Hz) and the observed ^{13}C NMR chemical shifts for the anomeric carbons of glucose (δ 100.42, C-1'') and apiose (δ 109.81, C-1''')[10,11]. The downfield shifts of C-2'' (δ 73.68), C-6'' (64.57) and C-5''' (δ 68.08) of the sugar moieties suggested an interglycosidic linkage for apiofuranosyl (1'''→6'') glucopyranosyl [10]. Complete assignments of the structures by using both 1D and 2D NMR spectra unambiguously established 5,5'-dihydroxy-2',4'-dimethoxy-7-[(6-*O*-β-D-apiofuranosyl-β-D-glucopyranosyl)oxy]isoflavone (**1**) as a new compound.

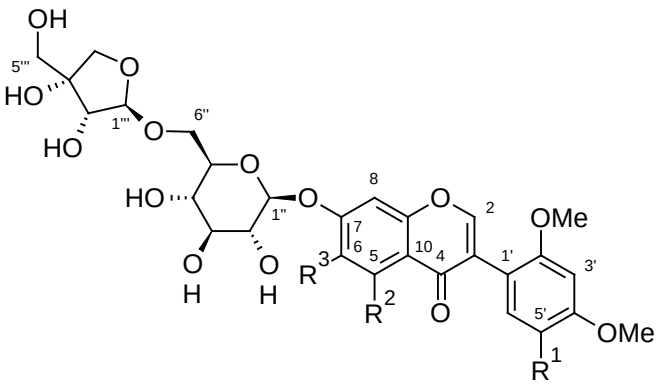
Tab. 1. NMR spectral data (500 MHz) for compound **1** from *Dalbergia vacciniifolia*

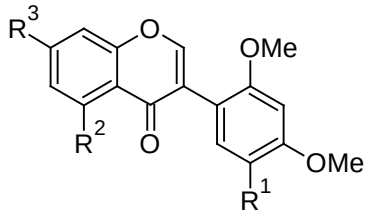
Position	δ_C*	δ_H* (*m*)	H/H COSY	HMBC
2	156.58, CH	8.13 (*s*)	–	3, 4, 9, 1'
3	121.36, qC	–	–	–
4	181.17, qC	–	–	–
5	162.10, qC	–	–	–
6	100.53, CH	6.46 (*d,* 2 *Hz*)	–	10, 6, 7, 8
7	163.46, qC	-	–	-
8	95.46, CH	6.69 (*d,* 2 *Hz*)	–	1', 6, 7, 9
9	158.01, qC	–	–	–
10	109.81, qC	–	–	–
1'	110.01, qC	–	–	–
2'	152.74, qC	–	–	2'-OMe
3'	101.53, CH	6.66 (*s*)	–	2' 4', 1'
4'	141.89, qC	–	–	4'-OMe
5'	148.47, qC	–	–	–
6'	116.95, CH	6.82 (*s*)	–	3, 1', 4', 5'
1''	100.42, CH	4.98 (*d*, *J* = 7.5 Hz)	2''	7
2''	73.68, CH	3.33 (*m*)	1''	1''
3''	76.86, CH	3.45 (*m*)	4'', 5''	2''
4''	70.53, CH	3.18 (*m*)	5'', 3''	5'', 6''
5''	76.82, CH	3.83 (*m*)	3''	2'', 4''
6''	64.57, CH_2	3.48, 3.57 (*m*)	–	–
1'''	109.81, CH	4.82 (*d*, *J* = 3.5 Hz)	2'''	2''', 3'''
2'''	76.22, CH	3.62 (*m*)	–	4''', 5'''
3'''	79.47, qC	–	–	–
4'''	74.03, CH_2	3.67, 3.85 (*m*)	–	1''', 3'''
5'''	68.08, CH_2	3.47, 3.88 (*m*)	–	1'''
2'-OMe	57.32, OCH3	3.57 (*s*)	–	2'
4'-OMe	56.80, OCH3	3.70 (*s*)	–	4'

* Samples were run in DMSO (TMS δ = 0).

The aglycone for compound **1** was not isolated in this study, but it has been previously reported to occur in *D. parviflora* [16]. However, its methyl derivative 5,7-dihydroxy-2',4',5'-

trimethoxyisoflavone (**3**) together with known compounds 5,7-dihydroxy-2',4'-dimethoxy-isoflavone (**4**), 5-hydroxy-2',4',7-trimethoxyisoflavone (**5**) and 7-hydroxy-2',4',5'-trimethoxy-isoflavone (**6**) were isolated [3–7]. Isolation of isoflavone apioglucoside from *D. vaccinifolia* which seems to have been reported from other *Dalbergia* species provides for a strong chemotaxonomic relationship with great value in plant biochemistry.





1: $R^1 = R^2 = OH$; $R^3 = H$

2: $R^1 = R^3 = OMe$; $R^2 = H$

3: $R^1 = OMe$; $R^2 = R^3 = OH$

4: $R^1 = H$; $R^2 = R^3 = OH$

5: $R^1 = H$; $R^2 = OH$; $R^3 = OMe$

6: $R^1 = OMe$; $R^2 = H$; $R^3 = OH$

Fig. 1. Chemical structure of compounds isolated from *D. vacciniifolia* root barks

Experimental

General experimental procedures

CC: silica gel (Merck, 230–400 Mesh, petroleum ether/dichloromethane/methanol); TLC: silica gel (60 F_{254}, Merck) precoated on plastic or aluminium plates; visualization: UV/VIS or anisaldehyde reagent [17]; FT-IR: Shimadzu 8400; UV-VIS: 168 diode array detector; 1D and 2D NMR: either Bruker Avance DRX 500 NMR spectrometers, operating at 500 MHz for ^{1}H NMR, and 150 MHz for ^{13}C NMR (δ= 0; TMS internal standard); MS: ESI mass spectrometer operating at 70 eV.

Plant materials

Dalbergia vacciniifolia root barks (voucher specimen reference No. 1682) were collected from Changanyikeni village in Kinondoni District, Dar es Salaam, Tanzania. The plant specimen was authenticated by Mr. Frank M. Mbago from the Department of Botany, University of Dare s Salaam. The voucher specimen is deposited at the Herbarium at the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences

Extraction and isolation

Air-dried pulverized root barks were soaked sequentially in dichloromethane and then in Ethanol, each two times for 72 h. Repeated column chromatograph of the ethanol extract (17 g) yielded seven fractions; Compounds **3** and **4** were obtained after repeated CC of the 3rd fraction on silica gel eluting with 3:2 v/v ethyl acetate and Petroleum ether while

further CC of each of the 2nd and 5th fractions on Sephadex® LH-20 eluting with 1:1 v/v MeOH and $CHCl_3$ gave compounds **5** and **6**, respectively. Repeated CC on silica gel eluting with 4:1 v/v CH_2Cl_2 and MeOH of the 6th fraction yielded compound **1**.

5,5'-Dihydroxy-2',4'-dimethoxy-7-[(6-O-β-D-apiofuranosyl-β-D-glucopyranosyl)oxy]-isoflavone
*(5-Hydroxy-3-(5-hydroxy-2,4-dimethoxyphenyl)-4-oxo-4H-chromen-7-yl 6-O-[(2R,3R,4R)-3,4-dihydroxy-4-(hydroxymethyl)tetrahydrofuran-2-yl]-β-D-glucopyranoside, **1**)*

Brown gum; yield, 695 mg; Anisaldehyde: pink, then black; UV, λ_{max} nm, UV, λ_{max} nm, 262 and 342; IR v_{max} cm^{-1}, 1710, 1025 and 949; MS, *m/z* (% rel. int.) 659 $[M^+ + Cl]^+$, 647 $[M^+ + Na]^+$, 625 $[M^+ + H]^+$, calc. for $C_{28}H_{32}O_{16}$: 624.16896); ^1H and ^{13}C NMR (see Tables 1).

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Author's Statement

Competing Interests

The author declares no conflict of interest.

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