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New Iridoid and phenethyl glycosides from Malagasy medicinal plant, *Phyllarthron madagascariense*

Harinantenaina Liva R. R.[a], Ryoji Kasai[a], Marcelle Rakotovao[b], Kazuo Yamasaki[a]*

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine , [a] 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan and Laboratoire de Chimie des Produits Naturels d'Origine Végétale , Faculté des Sciences, [b] BP: 906 Université d'Antananarivo, Madagascar.

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Two new iridoids, 6-*O* -caffeoyl ajugol and 6-*O* -isoferuloyl ajugol, and one new phenethyl glycoside, (3,4 dihydroxyphenyl) ethyl 5-*O* -isoferuloyl-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, were isolated from the dried leaves of *Phyllarthron madagascariense*, along with seven known compounds identified as minecoside, verminoside, methyl caffeate, salvigenin, apigenin 6,7-dimethyl ether, (4-hydroxyphenyl) ethyl apiofuranosyl-(1→6)-β-D- glucopyranoside, 7-*O* - acetyl loganic acid. The structures of these new compounds were determined on the basis of their spectral data.

Keywords: *Phyllarthron madagascariense*; leaves; Bignoniaceae; 6-*O* -caffeoyl ajugol; 6-*O*-isoferuloyl ajugol; (3,4-dihydroxyphenyl) ethyl 5-*O*- isoferuloyl-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

The genus *Phyllarthron* (Bignoniaceae), represented by 13 species, has its center of distribution in Madagascar and Comores. Some species of this genus are exotic in Mauritius and Reunion islands.[1,2]

Phyllarthron madagascariense is an endemic medicinal plant widely distributed in the Center and East of Madagascar. The leaf of this plant is commonly used in the traditional pharmacopoeia for its anti-inflamatory, anti-cataplasm and anti-syphilitic properties.[3] To the best of our knowledge, no phytochemical investigation has been appeared in the literature.

The present study deals with the isolation and structure elucidation of two new iridoid glucosides (**1**, **2**) and one new phenethyl glycoside (**3**) together with three known iridoids (**4**-**6**), one known phenethyl glycoside (**7**), two known flavonoids (**8**, **9**) and methyl caffeate (**10**) from the leaves of the plant.

RESULTS AND DISCUSSION

The methanolic extract of the leaves of *P. madagascariense* afforded ten compounds (**1**-**10**). Seven were identified as known compounds: Minecoside (**4**), verminoside (**5**),[4] 7-*O*-acetyl loganic acid (**6**),[5] 2-(4-hydroxyphenyl) ethyl apiofuranosyl-(1→6)-β-D-glucopyranoside (**7**),[6] salvigenin (**8**), apigenin 6,7-dimethyl ether (**9**)[7] and methyl caffeate (**10**) by comparison of their spectral data with the reported data.

Compound **1** was obtained as a pale yellow amorphous powder, whose molecular formula was determined as $C_{24}H_{30}O_{12}$ by HR FAB-Mass spectral analysis

The ^1H NMR of **1** showed signals, due to a 1,3,4 trisubstituted benzene (δ 6.76, d, J = 8.3 Hz; δ 6.93, dd,

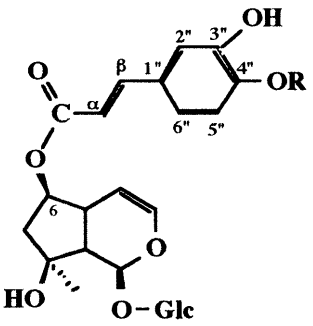
J = 8.3 and 1.7 Hz, and δ 7.03, d, J = 1.7 Hz), a *trans* olefin (δ 6.27 and 7.54, each 1H, d, J = 15.9 Hz) and a *cis* olefin (δ 4.95, dd, J = 6.2, 2.9 Hz, and δ 6.20, dd, J = 6.2, 2.2 Hz). A pair of large geminal coupling at δ 2.22 (1H, dd, J = 13.6, and 6.3 Hz) and δ 2.00 (1H, dd, J = 13.6, and 4.6 Hz) could be assigned to a methylene.

^{13}C NMR spectrum showed 24 signals, fifteen of them attributable to an ajugol and nine to a caffeoyl group. Alkaline hydrolysis of **1** in MeOH gave ajugol (**1a**)[8] and methyl caffeate. Acylation shift were observed for the signals of **1** due to C-6 (+2.1 ppm), C-5 (-2.0 ppm) and C-7 (-2.2 ppm), in comparing the ^{13}C NMR spectra to those of ajugol. Further, long range correlations, CO/H-6; C-1/H-1'; C-1'/H-1 were observed, in the HMBC spectrum, confirming that the caffeoyl moiety is attached at C-6 of ajugol and the glucosyl unit at C-1. Thus, the structure was concluded as 6-*O*-caffeoyl ajugol.

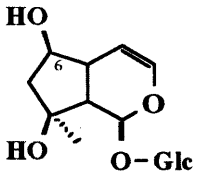
The molecular formula of Compound **2** was deduced as $C_{25}H_{32}O_{12}$ from HR FAB-MS. The ^1H (Table1) and ^{13}C NMR (Table2) signals of **2** were similar to those of **1** in all respects except for the presence of the signal at δ 56.4 (^1H NMR, δ 3.88, s) assigned to a methoxyl group in the acyl portion. The ^1H and ^{13}C resonances (Tables 1 and **2**) arising from the acyl moiety showed better agreement with those of isoferulate[9] than ferulate.[10] Morever, in the NOE difference experiment, irradiation at a methoxyl proton signal (δ 3.88) enhanched the signal at δ 6.95 (1H, d, J = 8.3 Hz). The fact that the ^{13}C chemical shifts due to the ajugol are very similar to those of **1** indicates that the acyl group is also attached at C-6. These were supported by the long range correlations, C-4"/OMe (δ 3.88) and H-1/C-1', H-1/C-9; CO/H-6 in the HMBC spectrum. On the basis of this evidence, the structure of **2** was elucidated as 6-*O* - isoferuloyl ajugol.

Compound **3** was obtained as an amorphous powder, whose molecular formula was determined as $C_{29}H_{36}O_{15}$ by HR FAB-Mass spectral analysis. The ^1H NMR showed signals, due to the presence of two ABX systems, a methylene proton signal at δ 2.78 (2H, t, J =7.0 Hz), oxomethylene proton signals at δ 3.70 (1H, m) and δ 3.97 (1H, m), a methoxyl proton signal at δ 3.89 (3H, *s*), two *trans* olefinic protons (δ 7.60, d, J = 15.8 Hz and δ 6.32, d, J = 15.8 Hz), as well as a glucosyl and apiosyl anomeric protons (δ 4.30, d, J = 8.0 Hz and δ 5.10, d, J = 2.2 Hz, respectively). These are characteristic of phenylethanoid glycosides.[11]

^{13}C NMR spectrum of **3** is very similar to 2-(4-hydroxyphenyl) ethyl 5-*O*-*trans*-feruloyl-β-D-apiosyl-(1→6)-β-D-glucopyranoside[6] except for the signals arising from the aromatic rings. Instead of feruloyl, the isoferuloyl group was observed in **3** and the other ABX system must be that of the aglycone. Since, the six signals arising from the aromatic ring (δ 117.1, 116.3, 121.3, each CH and 131.3, 144.5, 146.0 each C by DEPT experiment) and the methylenes (δ 72.1 and 36.6, each CH_2) were superimposable on those of the aglycone of sanangoside,[12] the structure of (3,4-dihydroxyphenyl) ethyl was present in **3**. Furthermore, the chemical shift of the glucosyl anomeric proton (δ 4.26) and the signal of the C-1' (δ 104.3) showed that the sugar linkage is not an ester-linkage glucosyl. Based on the above data, the attachment must be at position 8 of the aglycone. Therefore, the structure of **3** was identified as (3,4 dihydroxyphenyl) ethyl (5-*O*-isoferuloyl-*O*-β-D-apio-furanosyl-(1→6)-β-D-glucopyranoside).



1 R = H
2 R = CH_3



1a

Glc : β-D-Glucopyranoside

EXPERIMENTAL

NMR spectra (^1H, ^{13}C, HSQC, HMBC) were recorded in CD_3OD using a JEOL JNM A-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR). MS were recorded on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (150x20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector, flow rate 6ml/min. For CC, silica gel G 60 (Merck), RP-18 (50 mm, YMC) and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) Hexane-EtOAc (6:4), (II) EtOAc-MeOH (8:2), (III)15-100% MeOH, (IV) 45% MeOH, (V) 20-100% MeOH, (VI) 20-60% MeOH, 25%CH_3CN. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

Plant material. Plant material was collected in August, 1999 from Ambohimanga-Antananarivo, Madagascar. The identity of the plant was confirmed by Dr. Armand Rakotozafy from Institut malgache de recherches appliquées.

Extraction and isolation of constituents of P.madagascariense. The dried leaves (1.0 kg) of *P. madagascariense* were extracted with MeOH. After removal of the solvent by evaporation, the residue (200 g) was suspended in water and extracted with hexane, EtOAc and *n*-BuOH, successively. The EtOAc extract (90 g) was subjected to a column of silica gel (system I), to afford compounds **8** (52 mg), **9** (24 mg) and **10** (248 mg). The *n*-BuOH extract (45.0 g) was subjected to a column of highly porous copolymer of styrene and divinylbenzene, and eluted with H_2O, 35% MeOH, 70% MeOH, MeOH and Me_2CO, successively. The fraction eluted with 35% MeOH was chromatographed on a column of silica gel (systems II), affording eight fractions. Fractions 2 and 4 were subjected to RP-18 and ODS-HPLC using system III and system IV, respectively to afford compound **1** (257 mg), **4** (353 mg), **5** (1.6 g). Fractions 3 and 5 were further purified by RP-18 using system V to provide compounds **6** (154 mg) and **7** (32 mg).

Silica gel column chromatography of the fraction eluted with 70% MeOH, (system II), afforded five fractions. Fractions 3 and 4 were subjected to RP-18 and ODS-HPLC column using system V and system VII, respectively to afford compound **2** (30 mg) and **3** (20 mg).

Compound 1. Pale yellow amorphous powder. $[\alpha]_D^{30}$ -128° (*c* 1.2, MeOH); ^1H NMR: Table 1; ^{13}C NMR: Table 2; Negative HR-FAB-MS, *m/z* : 509.1601 $[M-H]^-$ ($C_{24}H_{29}O_{12}$ requires 509.1658)

Alkaline hydrolysis of compound 1. Compound **1** (20 mg) was dissolved in 1N NaOH-MeOH (2ml) and kept at room temperature for 30 min. The reaction mixture was neutralized with amberlite MB-3 to separate the mixture of the two compounds by precipitation in MeOH. The two compounds were identified as methyl caffeate (**10**) and ajugol (**1a**) by TLC and NMR spectra respectively.

Compound 2. Amorphous powder. $[\alpha]_D^{30}$ -132° (*c* 0.5, MeOH); ^1H NMR: Table 1; ^{13}C NMR: Table 2; Negative HR-FAB-MS, *m/z* : 523.1790 $[M-H]^-$ $C_{25}H_{31}O_{12}$ (requires 523.1815).

Compound 3. Amorphous powder. $[\alpha]_D^{30}$ -55° (*c* 0.9, MeOH); ^1H NMR and ^{13}C NMR: Table 3; Negative HR-FAB-MS, *m/z* : 623.1970 $[M-H]^-$ $C_{29}H_{35}O_{15}$ (requires 623.1975).

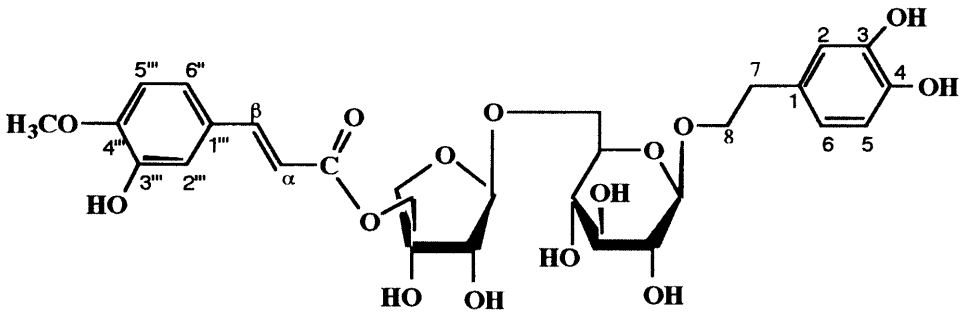


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Table 1

^{1}H NMR spectral data for compounds **1**, **1a** and **2** (400MHz, CD_3OD)

H	1*	1a	2*
1	5.49 d (2.4)	5.36 d (2.4)	5.48 d (2.4)
3	6.20 dd (6.2, 2.2)	6.10 dd (6.2, 2.2)	6.25 dd (6.2, 2.1)
4	4.95 dd (6.2, 2.9)	4.77 dd (6.2, 3.0)	4.92 dd (6.2, 2.9)
5	2.91 brd (9.3)	3.10 m	2.92 brd (9.0)
6	4.85 m	3.80 m	4.98 m
7a	2.22 dd (13.6, 6.3)	1.93 dd (13.4, 6.0)	2.23 dd (14.2, 6.3)
7b	2.00 dd (13.6, 4.6)	1.71 dd (13.4, 4.6)	2.00 dd (14.2, 4.1)
9	2.57 dd (9.3, 2.4)	2.46 dd (9.6, 2.4)	2.57 dd (9.0, 2.4)
10	1.37 s	1.36 s	1.37 s
Glucosyl moiety:			
1'	4.66 d (7.8)	4.54 d (7.8)	4.66 d (7.8)
2'	3.20-3.42 m	3.20 dd (9.2,7.8)	3.19-3.45 m
3'	3.20-3.42 m	3.36 dd (9.2,7.9)	3.19-3.45 m
4'	3.20-3.42 m	3.24 dd (9.5,7.9)	3.19-3.45 m
5'	3.20-3.42 m	3.25 m	3.19-3.45 m
6'a	3.65 dd (4.6,12)	3.58 dd (5.1,12)	3.67 dd (4.6,12)
6'b	3.90 dd (12, 2)	3.84 dd (1.9,12)	3.90 dd (12, 2)
Caffeoyl moiety:			Isoferuloyl moiety:
2"	7.03 d (1.7)		7.05 d (1.9)
5"	6.76 d (8.3)		6.95 d (8.3)
6"	6.93 dd (8.3,1.7)		7.02 dd (8.3,1.9)
α	6.27 d (15.9)		6.35 d (15.9)
β	7.54 d (15.9)		7.57 d (15.9)
OCH_3			3.88 s

*Assignments based on HSQC.
J (Hz) in parentheses

Table 2

^{13}C NMR spectral data for compounds **1**, **1a** and **2** (CD_3OD, 100MHz)

C	1	1a	2
1	93.4	93.7	93.5
3	141.0	140.4	141.1
4	104.6	105.9	104.6
5	39.3 (-2.0)*	41.3	39.4
6	80.3 (+2.1)*	78.2	80.4
7	47.8 (-2.2)*	50.0	47.9
8	77.9[a]	78.0	78.2[a]
9	51.6	51.8	51.7
10	26.0	25.2	26.1
Glucosyl moiety:			
1'	99.3	99.4	99.4
2'	74.7	74.8	74.8
3'	78.1[a]	77.8	78.0[a]
4'	71.6	71.7	71.7
5'	79.1	79.5	79.0
6'	62.8	62.8	62.9
Caffeoyl moiety:			Isoferuloyl moiety:
1"	127.7		128.9
2"	115.3		112.5
3"	146.9		151.5
4"	149.5		148.0
5"	116.6		116.4
6"	122.9		122.8
a	115.1		115.6
β	146.7		146.6
CO	169.0		168.8
OCH_3			56.4

*Δδ (1-1a)

[a]Assignment may be interchangeable in each column.

Table 3

^{1}H and ^{13}C NMR spectral data for compound **3** (CD_3OD, 400MHz and 100MHz, respectively)

C	δH	δC		δH	δC
			Apiosyl moiety:		
1		131.3	1"	5.10 d (2.2)	110.6
2	6.69 d (1.8)	117.1	2"	4.25 m	78.5
3		146.0	3"	*	79.0
4		144.5	4"	*	75.0
5	6.65 d (8.3)	116.3	5"	*	67.5
6	6.53 dd (1.8, 8.3)	121.3	Isoferuloyl moiety:		
7	2.78 t (7.0)	36.6	1'"		128.8
8a	3.97 m	72.1	2'"	7.07 d (1.9)	112.5
8b	3.70 m		3'"		151.5
Glucosyl moiety:			4'"		148.0
1'	4.30 d (8.0)	104.3	5'"	6.92 d (8.3)	115.7
2'	*	74.9	6'"	7.05 dd (8.3, 1.9)	123.0
3'	*	78.0	α	6.32 d (15.8)	114.8
4'	*	71.7	β	7.60 d (15.8)	147.8
5'	*	76.8	CO		168.7
6	*	68.5	OCH_3	3.89 s	56.3

* Overlapped, *J* (Hz) in parentheses

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