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Phenylpropanoid glycosides of *Gnidia polycephala*

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Abstract

Two phenylpropanoid glucosides, 2-*O*- β -D-glucosyloxy-4-methoxybenzenepropanoic acid and its methyl ester, together with syringin and adicardin were isolated from the stem of *Gnidia polycephala* and characterized by physical and spectroscopic data.

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Keywords: *Gnidia polycephala*; Thymeleaceae; Phenylpropanoid glycosides; Acetate derivatives

1. Introduction

Gnidia polycephala (Thymeleaceae family) is a small plant widespread in the arid regions of Botswana, Zimbabwe and South Africa (Riley, 1963; Hutchinson, 1967; Barnes et al., 1994). It is called *makgonasotlhe* in Setswana and it grows well where other plants are difficult to propagate. Other *Gnidia* species found in Botswana are *G. burchellii*, *G. capitata*, *G. krausiana* and *G. sericocephala*.

In traditional medicine, *G. polycephala* has several preparations which are taken orally for stabilising heart conditions, treatment of tuberculosis and tonsillitis, and ashes are applied onto wounds (Hedberg and Staugard, 1989). Despite all these uses, the plant is very lethal when taken by livestock (Kellerman et al., 1988). Livestock often feed on it because of its prevalence and freshness in arid areas. Earlier phytochemical work led to the isolation of umbelliferone, and two glycosides (Rindl, 1917, 1933; Vermuelen and Pieterse, 1968), one of which was characterized as yuankanin (Ragot et al., 1988). We now report two new phenylpropanoid glycosides along with syringin and adicardin.

2. Results and discussion

The CH₂Cl₂ and MeOH extract of either the stem or roots was subjected to vacuum liquid chromatography

(VLC) using solvent gradients from hexane to EtOAc to CH₃COCH₃ to MeOH. Fractions that showed red spots under vanillin spray on heating were further subjected to flash chromatography and Sephadex LH-20. Following this treatment, fractions 5–7 gave phenylpropanoid **2** while fractions 8–12 gave acid **1**, syringin (**3**) (Sutarjadi et al., 1978) and adicardin (**4**) (Asheervadam et al., 1986). The latter two compounds were established by comparing to reported data and further confirmed by the NMR data of their acetate derivatives.

The molecular formula of compound **1** was established as C₁₆H₂₂O₉ from NMR and MS. The EI-MS showed a molecular mass at *m/z* of 358 while its API-MS showed four peaks at *m/z* 375.8 for [M + H₂O], 196.9 as the base peak for the aglycone moiety, 179.1 and 137.2. The last two peaks were fragments of the aglycone moiety via initial loss of water followed by ketene loss on the propanoic acid group.

From its ¹H NMR in CD₃OD, compound **1** showed three aromatic protons at δ 7.05 (*J* = 8.4 Hz), 6.66 (2.2 Hz), and 6.55 (8.4 and 2.2 Hz) as a doublet of doublets assignable to a 1, 2, 4-trisubstituted phenyl moiety. The β -glucosyl protons were at δ 4.95 for the anomeric proton, 3.64 and 3.82 for the C-6'' protons, each appearing as a doublet of doublets, and between δ 3.3 to 3.6 as multiplets for the other sugar protons. The two broad triplets at δ 2.29 and 2.77 were attributable to the propanoyl group protons at C-2 and C-3, respectively, and it had one methoxy group at δ 3.68.

The connectivity in compound **1** was determined using NOESY, NOEDIFF, HMQC and HMBC. NOEDIFF experiments on the methoxy group showed

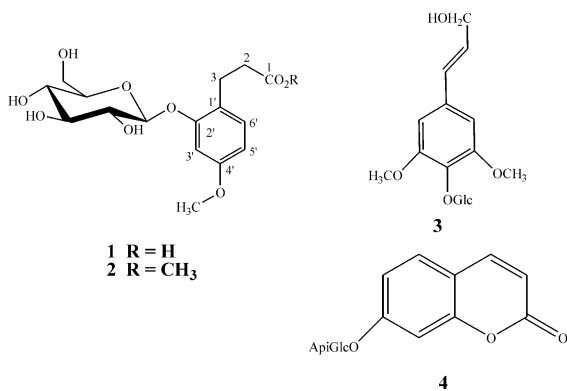
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greatest responses to protons at δ 6.55 and 6.66, and hence the methoxy group was between them. A similar experiment on the anomeric proton showed responses at δ 6.66 and 2.77, thus the glucosyl moiety and the propanoyl group were *ortho* to each other on the phenyl ring. HMBC and HMQC experiments confirmed these findings.

On acetylation with acetic anhydride and pyridine, compound **1** gave a crystalline tetraacetate **1a** with an EI-MS at m/z 526. The ^1H NMR of **1a** showed that the sugar protons were well resolved displaying large coupling constants in agreement with a glucopyranosyl group. All the acetyl groups (δ 1.99, 2.04, 2.05, and 2.07 in deuterated acetone) were fixed onto glucose as was confirmed from the EIMS fragmentation pattern which gave a base peak at m/z 331, consistent with a tetraacetate of glucosyl fragment. Thus compound **1** is 2-*O*- β -D-glucosyloxy-4-methoxybenzenepropanoic acid as shown in the figure below. Although compound **1** is new, its methylated aglycone moiety, methyl 2-hydroxy-4-methoxybenzenepropanoate, was isolated from the trunkwood of *Machaerium kuhlmannii* before (Ollis et al., 1978).

Compound **2** was only obtained in smaller amount. The only difference between NMRs of **2** and **1** was the presence of an extra methoxy group in the former and small variations in chemical shifts. Thus compound **2** was by analogy identified as methyl 2-*O*- β -D-glucosyloxy-4-methoxybenzenepropanoate. Large quantities of **2** were easily prepared from **1** on treatment with diazomethane. It was also observed that when compound **1** was left in methanol solution, traces of compound **2** were observed. The acetates of both compounds provided supporting evidence. In fact, isolation of compounds **1** and **2**, via acetylation, proved to be more efficient.



3. Experimental

3.1. General procedures

NMR spectra were measured on a Bruker Avance 300 Spectrometer at 300 MHz for protons and 75 MHz for carbons. All δ values are expressed in ppm. Low resolution mass spectra (LRMS CI and API) were obtained from a Finnigan Mat SSQ 7000 Single Quadrupole

Mass Spectrometer coupled to a Varian Gas Chromatograph and a Waters 600-MS, HPLC apparatus.

UV-vis data were run on Shimadzu UV-2401PC UV-VIS Scanning Spectrometer in MeOH. For IR spectra, a Perkin-Elmer System 2000 FT-IR spectrometer and KBr pellets were used. Optical rotations were measured in MeOH using a Polatron D Instrument (Schmidt and Haensch) and melting points (uncorr.) obtained from a Griffin Melting Point Instrument.

The gels used were silica gel 60 (60–120 mesh) for column chromatography, silica gel 60 PF₂₅₄ (prep. TLC) for flash chromatography and VLC. Sephadex LH-20 was also used. All solvents were general purpose reagents and redistilled before use.

3.2. Plant material

Whole plants were harvested on the road being constructed between Molepolole and Letlhakeng in Botswana. Dr. B. Hargreaves, the curator of National Herbarium, identified the plant, and a voucher specimen is kept in the National Herbarium, Gaborone, Botswana.

3.3. Extraction and separations

Dried and ground stem (300 g) was successively extracted with mixture of MeOH and CH₂Cl₂ (1:1) for 24 h and MeOH for 1 h. The combined extracts were evaporated and subjected to VLC using silica gel (120 g) and eluting with gradients of hexane to EtOAc to Me₂CO to MeOH, collecting 200 ml fractions. Three fractions (F1–F3) were collected using a mixture of hexane and EtOAc (1:1); two fractions (F4 and F5) with EtOAc only; two fractions (F6 and F7) with a mixture of EtOAc and Me₂CO (1:1); two fractions (F8 and F9) with Me₂CO only; and three fractions (F10–F12) with mixture Me₂CO and MeOH (4:1). TLC plates were performed in CHCl₃ and MeOH mixtures saturated with water.

Fractions 5–7 were further resolved using combination of flash chromatography (eluting with mixtures of CHCl₃ and Me₂CO and H₂O) and Sephadex LH-20 (2:1 CHCl₃ and MeOH mixture), and gave compound **2** detected as a red spot on TLC plate under vanillin spray on heating. Similar treatment of fractions 8–12 gave compound **1** (also detected as a red spot on TLC with vanillin spray), syringin (brown spot at 254 nm) and adicardin (blue spot at 366 nm). By the same procedure these compounds were obtained from the roots.

3.4. 2-*O*- β -D-glucosyloxy-4-methoxybenzenepropanoic acid (**1**)

Yield: 350 mg; mp 168–169 °C (from Me₂CO); burning through red to black under vanillin spray; $[\alpha]_D^{22}$ -80° , (MeOH; c 1.610); $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 224 (3.15), 278 (2.75) and 321 (2.08); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1716 (CO of acid),

1610 and 1510 (C=C of aromatic moiety); EIMS (probe) 70 eV, m/z (rel. int.): 328 $[M-CH_2O]^+$ (100), 314 $[M-CO_2]^+$ (63), 281 $[M-77]^+$ (59); NMR Tables 1 and 2.

3.4.1. Tetraacetate of compound 1 (1a):

Mp 115–116 °C (from EtOH and H₂O), burning through red to black under vanillin spray. $[\alpha]_D^{22}$ -40° , (CHCl₃; c 1.730). λ_{max}^{MeOH} nm (log ϵ): 217 (3.28) 276 (2.76) and 320 (2.32); IR ν_{max}^{KBr} cm⁻¹: 1747 (CO of acetyl groups), 1715 (CO of ester), 1616 and 1510 (C=C of the phenyl group). EIMS (probe) 70 eV, m/z (rel. int.): 526 $[M]^+$ (10) 331 $[M-195]^+$ (100) and 271 $[M-255]^+$ (20). ¹H NMR (CDCl₃): 7.15 (*d*, 8.3 Hz, H-6'), 6.80 (*d*, 2.5 Hz, H-3'), 6.60 (*dd*, 8.3 and 2.5 Hz, H-5'), 5.43 (*d*, 7.9 Hz, H-1''), 5.42 (*t*, 9.7 Hz, H-3''), 5.26 (*dd*, 9.6 and 7.9 Hz, H-2''), 5.13 (*t*, 9.6 Hz, H-4''), 4.1–4.3 (*m*, H-5''/6''), 3.80 (*s*, MeO-), 2.70–2.85 (*m*, H-2), 2.40–2.60 (*m*, H-3), 2.07, 2.05, 2.04, and 1.99 (*s*, AcO-). ¹³C NMR Table 2.

3.5. Methyl 2-O-β-D-glucosyloxy-4-methoxybenzenepropanoate (2)

Yield 30 mg; syrup; $[\alpha]_D^{22}$ -85° , (MeOH; c 0.9380). λ_{max}^{MeOH} nm (log ϵ): 223 (3.21), 277 (2.68) and 315 (2.04). IR ν_{max}^{KBr} cm⁻¹: 1716 (CO) 1624, 1512 (C=C of aromatic ring). ESIMS (probe) 70 eV, m/z (rel. int.): +ve 395 $[M+Na]^+$ (19), 390 $[M+H_2O]^+$ (100), -ve 371 $[M-1]^-$ (23), and 209 $[M-163]^-$ (100). This compound was also prepared from 1 and CH₂N₂. NMR Tables 1 and 2.

3.5.1. Tetraacetate of 2 (2a):

Mp 82–84 °C from Et₂O and hexane. $[\alpha]_D^{22}$ -80° , (CHCl₃; c 0.8660). λ_{max}^{MeOH} nm (log ϵ): 221 (3.29), and 277 (2.80). IR ν_{max}^{KBr} cm⁻¹: 1751 (CO) 1624, 1512 (C=C of aromatic ring). ESIMS (probe) 70 eV, m/z (rel. int.): +ve 577 $[M+K]^+$ (25), 563 $[M+Na]^+$ (100), 558 $[M+H_2O]^+$ (59), -ve 431 $[M-109]^-$ (21), 417 $[M-123]^-$ (23) and 209 $[M-331]^-$ (100). ¹H NMR (CDCl₃): 7.12 (*d*, 8.1 Hz, H-6'), 6.80 (*d*, 2.5 Hz, H-3'), 6.60 (*dd*, 8.1 and 2.5 Hz), 5.43 (*d*, 8.1 Hz, H-1''), 5.42 (*t*,

Table 1
¹H NMR for compounds 1 and 2 in CD₃OD

Compound	1		2	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
H-2	2.29	<i>t</i> , 7.5	2.55–2.65	<i>m</i>
H-3	2.77	<i>t</i> , 7.5	2.80–2.95	<i>m</i>
H-3'	6.66	<i>d</i> , 2.2	6.79	<i>d</i> , 2.5
H-5'	6.55	<i>dd</i> , 2.2, 8.4	6.52	<i>dd</i> , 2.5, 8.4
H-6'	7.05	<i>d</i> , 8.4	7.04	<i>d</i> , 8.4
4'-OMe	3.68	<i>s</i>	3.76	<i>s</i>
CO ₂ -Me			3.64	<i>s</i>
H-1''	4.95	<i>d</i> , 6.7	4.90	<i>d</i> , 6.7
H-2''–H-5''	3.3–3.6	<i>m</i>	3.3–3.6	<i>m</i>
H-6a''	3.82	<i>dd</i> , 12.4, 1.8	3.92	<i>dd</i> , 12.1, 1.9
H-6b''	3.64	<i>dd</i> , 12.4, 5.9	3.71	<i>dd</i> , 12.1, 4.9

9.6 Hz, H-3''), 5.25 (*dd*, 9.6 and 8.1 Hz, H-2''), 5.13 (*t*, 9.6 Hz, H-4''), 4.2–4.3 (*m*, H-5''/6''), 3.76 (*s*, MeO-), 3.80 (*s*, MeO-), 2.70–2.85 (*m*, H-2), 2.40–2.60 (*m*, H-3), 2.06, 2.05, 2.03, and 1.99 (*s*, AcO-). ¹³C NMR Table 2.

3.6. Syringin (3)

Yield 89 mg from EtOAc and MeOH data identical to that reported by Sutarjadi et al. (1978). Syringin acetate (3a): mp 109–111 °C from EtOAc and hexane. λ_{max}^{MeOH} nm (log ϵ): 222 (3.15) and 265 (2.65). IR ν_{max}^{KBr} cm⁻¹: 1743 (CO), 1587 and 1507 (aromatic C=C). ESIMS (probe) 70 eV, m/z (rel. int.): 582 $[M]^+$ (1), 331 $[M-251]^+$ (78), 252 $[M-330]^+$ (28), 169 $[M-413]^+$ (100) and 109 $[M-473]^+$ (48). ¹H NMR (CDCl₃): Aglycone; 6.58 (*s*, 2H, H-2'/6'), 6.55 (*dt*, 1.1, 1.1 and 16.2 Hz, H-3), 6.20 (*dt*, 6.5, 6.5 and 15.8 Hz, H-2), 4.70 (*dd*, 1.1 and 6.5 Hz, 2H, H-1), 3.82 (*s*, 6H, MeO): Glucose moiety; 5.29 (*bt*, 9.3 and 9.3 Hz H-3'') 5.27 (*bt*, 9.9 and 10.1 Hz, H-4'') 5.25 (*dd*, 6.6 and 8.6 Hz, H-2''), 5.05 (*d*, 7.0 Hz, H-1''), 4.24 (*dd*, 5.0 and 12.2 Hz, H-6''), 4.10 (*dd*, 2.6 and 12.2 Hz, H-6''), 3.68 (*ddd*, 2.3, 4.8, and 9.6 Hz, H-5'').

3.7. Adicardin (4)

The mixture containing of adicardin (blue spot at 366 nm) was acetylated, purified by chromatography to

Table 2
¹³C NMR for compounds 1, 1a, 2, and 2a

Compound	1	1a	1a	2	2a
Solvent	CD ₃ OD	CD ₃ Cl	(CD ₃) ₂ CO	CD ₃ OD	CD ₃ Cl
Carbon					
Propanoyl					
1	177.7	178.6	173.5	176.4	173.5
2	35.2	34.4	34.0	36.0	34.1
3	26.4	25.2	25.1	26.8	25.1
Phenyl					
1'	123.5	122.5	122.5	123.6	122.4
2'	157.7	155.8	156.0	158.1	155.5
3'	102.7	103.4	103.1	103.6	103.1
4'	160.9	159.8	159.8	161.3	159.4
5'	108.57	107.3	107.7	108.9	106.7
6'	131.3	131.1	130.8	131.8	130.7
Methoxy					
4'-OMe	55.8	55.8	55.2	56.2	55.4
CO ₂ -Me				52.5	51.5
Glucosyl					
1''	103.2	99.3	98.9	100.8	99.0
2''–5''	78.3*2	72.8	72.9	78.9*2	72.8
	75.0	72.1	72.2	75.4	72.1
	71.5	71.1	71.4	71.9	71.1
		68.5	69.0		68.5
6''	62.6	62.1	62.5	63.0	62.1
CH ₃ CO		171.1	170.2		170.6
		170.6	169.8		170.2
		169.9	169.6		169.4
		169.8	169.3		169.2
CH ₃ CO		21.0	20.1, 20.0		20.6

afford adicardin acetate 57 mg: ^1H NMR (CDCl_3): *Umbelliferone moiety*; 7.64 (d, 9.6 Hz, H-4), 7.42 (d, 9.3 Hz, H-5), 6.92 (d, 2.4 Hz, H-8), 6.91 (d, 2.4 and 9.3 Hz, H-6) and 6.30 (d, 9.6 Hz, H-3): *Glucose moiety*; 5.28 (dd, 7.8 and 9.1 Hz, H-2'), 5.26 (t, 9.1 and 9.1 Hz, H-3'), 5.17 (d, 7.7 Hz, H-1') 5.07 (bt, 8.4 and 9.1 Hz, H-4'), 3.87 (ddd, 3.2, 6.2 and 8.4 Hz, H-5'), 3.78 (dd, 2.4 and 11.6 Hz, H-6') and 3.59 (dd, 6.6 and 11.6 Hz, H-6'): *Apiose moiety*; 5.31 (bs, H-2''), 4.94 (bs, H-1''), 4.76 (d, 12.4 Hz, H-5''), 4.54 (d, 12.4 Hz, H-5''), 4.19 (d, 10.5 Hz, H-4'') and 4.11 (d, 10.5 Hz, H-4''): *Acetyls*; 2.11, 2.06, 2.05, 2.03, 2.01*2.

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