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Chalconoid and stilbenoid glycosides from *Guibourtia tessmanii*

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Abstract

Phytochemical studies on the stem bark of *Guibourtia tessmanii* yielded a dihydrochalcone glucoside, 2′,4-dihydroxy-4′-methoxy-6′-*O*-β-glucopyranoside dihydrochalcone and a new stilbene glycoside, 3,5-dimethoxy-4′-*O*-(β-rhamnopyranosyl-(1→6)-β- glucopyranoside) stilbene besides the known pterostilbene. Their structures were established on the basis of one and two dimensional NMR spectroscopic techniques, FABMS and chemical evidence.

Keywords: *Guibourtia tessmanii*; Leguminosae (Caesalpiniaceae); Stem bark; Stilbenoids; Chalconoids; Glycosides

1. Introduction

The plant *Guibourtia tessmanii* Leguminosae (subfamily Caesalpiniaceae) was identified in Cameroon at the Centre Province (Aubréville, 1970). It is well known in folk medicine in the Central Africa (Adjanohoun, 1984). Previous chemical studies on this species yielded stilbenoid compounds (Nyemba et al., 1995). Interest in this class of compounds have been stimulated by their biological activity and strong antimicrobial, antifungic and helmintic effects (Rimando et al., 1994). As a continuation of the chemical studies of the same species, we now report in this paper the isolation and structural determination of a new dihydrochalcone glucoside **1** and a new stilbene glycoside **2** along with the known pterostilbene **3**.

2. Results and discussion

The stem bark of *G. tessmanii* was extracted with chloroform and ethylacetate successively. The combined extracts were repeatedly subjected to column chromatography on silica gel using *n*-hexane, ethylacetate and methanol in increasing polarity. Compounds **1**–**3** were isolated.

The elemental composition of compound **1** was shown to be $C_{22}H_{26}O_{10}$ by HR–EIMS (*m*/*z* 450.1524 $[M^+]$) and CI/NH_3 MS (*m*/*z* 451 $[M+H]^+$, 468 $[M+NH_4]^+$), and its fast atom bombardment mass spectrum (FABMS) 451 $[M+H]^+$. The peaks in the EIMS at *m*/*z* 107 (C_7H_7O) and 342 resulting from a benzylic cleavage (Lien et al., 2000; Greenaway et al., 1989) revealed the existence of a dihydrochalcone skeleton with an hydroxyl substituting ring B and the ring A substituted with one hydroxyl, one methoxyl and one *O*-glucosyl groups. The ^1H NMR spectrum of **1** showed two methylene triplets at δ 3.12 and 2.80 with a coupling constant of 7.5 Hz in agreement with H_2-α and H_2-β respectively. The two doublets at δ 7.05 and 6.60 with a coupling constant of 8.7 Hz were assigned to the four aromatic protons of ring B, H-2/H-6 and H-3/H-5 respectively, thus the hydroxyl group was located at the C-4 position. On the other hand the presence of the glucosyl unit in **1** was confirmed by its MS which showed two dominant peaks at *m*/*z* 306 and 288. In addition, its ^1H NMR spectrum exhibited resonance for

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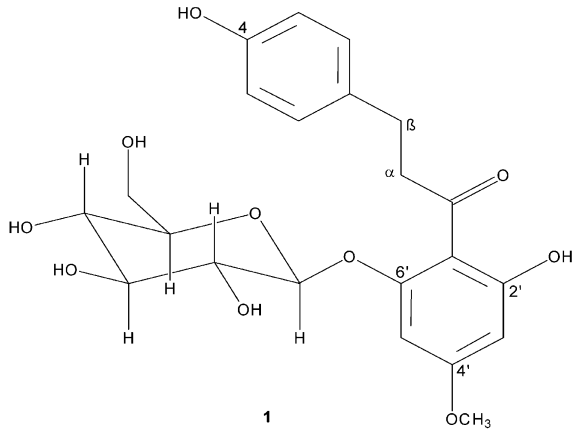

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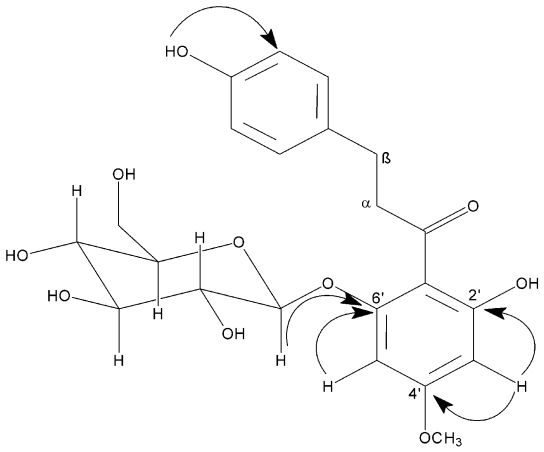
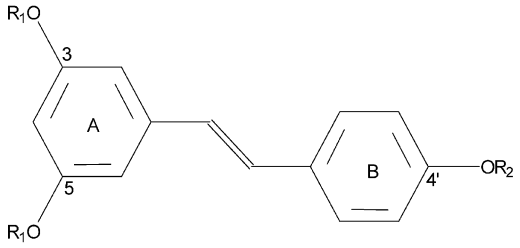


Fig. 1. COLOC correlations in compound **1**.



	R_1	R_2
2	Me	rutinosyl
3	Me	H

the anomeric proton of the β-glucose moiety at δ 5.05 (*d*, $J=7.0$ Hz). Furthermore, the hydroxyl signal at δ 13.36 indicated a chelat bonding; thus this group was linked to C-2′ of ring A. Finally, the two doublets at δ 6.15 and 6.30 with a coupling constant of 2.0 Hz were consistent with the two protons H-3′and H-5′ in ring A of the reported phlorizin (Proksa et al., 1988). Thus the methoxyl and *O*-glucopyranosyl groups were located at C-4′ and C-6′ with the respective positions to be determined. Assignments for all proton and carbon resonances (see Table 1) were achieved by COSY, HETCOR and COLOC experiments. The COLOC spectrum exhibited correlations (Fig. 1) particularly between the carbon at δ 166.3 and the protons at δ 3.80 (OCH_3), 6.15 (H-3′) and 6.30 (H-5′) on one hand and the carbon at δ 161.3 and the protons at δ 5.05 (anomeric proton H-1″) and 6.30 (H-5′) on the other hand. The carbon C-2′ at δ 166.1 correlated with the proton at δ 6.15 (H-3′). All these results clearly confirmed that, on ring A, the methoxyl group was linked to the C-4′ and the *O*-β-glucopyranosyl unit was linked to the C-6′ position. Thus the structure of compound **1** was established as 2′,4-dihydroxy-4′-methoxy-6′-*O*-β-glucopyranoside dihydrochalcone. It was shown to be the new 4′-*O*-methylderivative of the known phlorizin (Proksa et al., 1988).

Table 1
^{1}H and ^{13}C NMR chemical shifts for compound **1**

Attribution	^{13}C (DMSO)	^{1}H (DMSO) *J* (Hz)
α	46.3	3.12, *t*, $J=7.5$
β	30.0	2.80, *t*, $J=7.5$
1	132.5	–
2	130.3	7.05, *d*, $J=8.7$
3	116.0	6.60, *d*, $J=8.7$
4	156.3	–
5	116.0	6.60, *d*, $J=8.7$
6	130.3	7.05, *d*, $J=8.7$
1′	107.3	–
2′	166.1	–
3′	96.3	6.15, *d*, $J=2.0$
4′	166.3	–
5′	94.6	6.30, *d*, $J=2.0$
6′	161.3	–
CO	205.4	–
OCH_3	56.7	3.80, *s*
OH-4	–	9.10, *s*
OH-2′	–	13.36, *s*
1″	101.8	5.02, *d*, $J=7.0$
2″	74.3	
3″	78.4	3.00–3.24, *m*
4″	70.7	
5″	77.8	
6″	61.8	

Compound **2** was obtained as colourless crystals. Its elemental composition was shown to be $C_{28}H_{36}O_{12}$ by HR–EIMS (*m*/*z* 564.2205 $[M]^+$). The ^{13}C NMR spectrum showed 14 aryl/vinyl signals and no C=O resonances. Detailed analysis of the spectrum suggested that this compound was a stilbeneglycoside. The ^{1}H NMR spectrum of **2** showed the presence of nine aryl/vinyl proton resonances. Two of them formed an AB system at δ 6.82 and 7.15 (*d*, $J=16.0$ Hz), the large coupling constant indicating a *trans* geometry. The ^{13}C resonances at δ 125.9 and 128.7 assigned to the carbons C-α and C-β respectively confirmed that compound **2** had a

Table 2
^1H and ^{13}C assignments for compounds **2** and **3**

Attribution	**2** (DMSO)		**3** (DMSO)	
	^{13}C	^1H, *J* (Hz)	^{13}C	^1H, *J* (Hz)
α	125.9	7.14, *d*, *J*=16.0	125.2	6.82, *d*, *J*=16.0
β	128.7	6.95, *d*, *J*=16.0	128.8	7.15, *d*, *J*=16.0
1	139.3	–	139.6	–
2	107.1	6.77, *d*, *J*=2.2	104.0	6.77, *d*, *J*=2.1
3	159.0	–	159.0	–
4	100.5	6.47, *d*, *J*=2.2	100.4	6.46, *d*, *J*=2.1
5	158.8	–	159.7	–
6	107.1	6.77, *d*, *J*=2.2	107.0	6.77, *d*, *J*=2.1
1′	129.5	–	128.3	–
2′	127.9	7.50, *d*, *J*=8.8	127.8	7.46, *d*, *J*=8.8
3′	114.2	6.91, *d*, *J*=8.8	115.5	6.75, *d*, *J*=8.8
4′	160.5	–	158.0	–
5′	114.2	6.91, *d*, *J*=8.8	115.5	6.75, *d*, *J*=8.8
6′	127.9	7.50, *d*, *J*=8.8	127.9	7.46, *d*, *J*=8.8
OCH_3	55.2	3.76, *s*	55.3	3.76, *s*
OCH_3	55.1	3.76, *s*	55.2	3.76, *s*
1″	105.1	4.84, *d*, *J*=7.3	–	–
2″	73.2		–	–
3″	76.5[a]		–	–
4″	69.8	3.00–3.85, *m*	–	–
5″	75.4[a]		–	–
6″	66.3		–	–
1‴	101.5	4.43, *d*, *J*=6.2	–	–
2‴	70.8		–	–
3‴	70.3		–	–
4‴	72.1	3.00–3.85, *m*	–	–
5‴	68.3		–	–
6‴	17.9	1.18, *d*, *J*=6.2	–	–

[a] Values could be interchanged.

(*E*)-stilbene skeleton. A set of three proton resonances at δ 6.47 (1H) and 6.77 (2H) as doublets (*J*=2.2 Hz) were assigned to a 1,3,5-trisubstituted phenyl group. The 3,5-substituents were established to be two methoxyl groups with the six proton signals shown as a singlet at δ 3.76 and the ^{13}C signals at δ 55.2 and 55.1. Another set of four proton resonances at δ 6.90 (2H, H-2′/H-6′) and 7.42 (2H, H-3′/H-5′) with the coupling constant *J*=8.8 Hz were consistent with an AA′BB′ system of a *para*-substituted second phenyl group. Compound **2** gave a positive Molish test (α-naphtol 1%/H_2SO_4 cc) suggesting the presence of a glycosyl moiety. Acid hydrolysis of **2** yielded an aglycone that was identified to pterostilbene **3** (Rimando et al., 1994) and two sugars identified as glucose (Banks and Cameron, 1971) and rhamnose (Uniyal et al., 1990). Furthermore the ^1H NMR spectrum of **2** showed two doublets at δ 4.84 (*J*=7.3 Hz) and 4.43 (*J*=6.2 Hz) assigned to the anomeric protons of the glucosyl and rhamnosyl patterns respectively. The ^{13}C NMR spectrum indicated the respective anomeric carbons at δ 105.1 and 101.5. The EIMS of **2** showed ion fragments at *m*/*z* 418 $[M-146]^+$ and 256 $[M-308]^-$ suggesting the successive eliminations of the terminal rhamnosyl and the inner glucosyl fragments respectively. The linkages of the two sugars were established from the ^{13}C NMR/DEPT, HETCOR and COLOC spectral measurements. The COLOC spectrum of **2** showed an important correlation between the glucosyl anomeric proton H-1″ at δ 4.84 and the aglycone carbon signal at δ 160.5 (C-4′). The interconnectivity between the two sugars was deduced from the important correlation observed between the rhamnosyl anomeric proton H-1‴ at δ 4.43 and the glucosyl methylene carbon signal at δ 66.3 (C-6″). Thus, the structure of compound **2** was established as 3,5-dimethoxy-4′-*O*-(β-rhamnopyranosyl-(1→6)-β-glucopyranoside) stilbene.

The ^1H NMR spectrum of compound **3** was characterized by the presence of two vinyl proton signals with a *trans*-geometry. The ^{13}C NMR spectra showed 12 aryl signals, two vinyl signals and no C=O resonances. The ^1H NMR of **3** showed the presence of two methoxyl proton resonances. Furthermore, all NMR data of **3** (Table 2) were found to be in full agreement with the structure of the reported pterostilbene (Rimando et al., 1994).

3. Experimental

3.1. General

MPs were determined using a Kofler microhot stage apparatus. IR spectroscopy was performed on a Perkin-Elmer 257 spectrometer. $[\alpha]_D$ Were read on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Beckman 25 spectrometer. FABMS were recorded using ZAB Spec MS instrument. CIMS and EIMS were recorded on Nermag R10–10C spectrometer. NMR experiments were performed on a Varian Gemini 400 MHz instrument and a Bruker AC 300 spectrometer. Si gel 60H (5–40 μm), 60C (20–40 μm) and 60 (240–400 mesh) were used for CC under compressed air (300 mbar) while precoated Si gel $60F_{254}$ plates were used for TLC. The solvent used for spectra determination was DMSO-d_6.

3.2. Plant material

Stem bark of *G. tessmanii* was collected in Eseka locality (Centre Province of Cameroon) in June 1998. The plant was identified and collected by Mr Nole Tsabang, IMPM Yaoundé, in Cameroon. The herbarium specimen documenting the collection has been deposited in the National Herbarium, Yaoundé (HNC No. 5670).

3.3. Extraction and isolation

The plant material (1.5 kg) was air-dried, ground and extracted successively with $CHCl_3$ and EtOAc at room temperature. The solvents were evaporated using

vacumm pump and both crude extracts were combined under TLC control to yield a viscous material (100 g). Part of the crude extract (60 g) was chromatographed over Si gel with *n*-hexane, EtOAc and MeOH in increasing polarity to result three main series: **A** (800 mg) [*n*-hexane/EtOAc (7:3)], **B** (900 mg) [EtOAc/MeOH (9:1)] and **C** (350 mg) [EtOAc/MeOH (17:5)]. Further CC of three series yielded compounds **1**–**3**.

*3.3.1. 2′,4-Dihydroxy-4′-methoxy-6′-O-(β-glucopyranoside) dihydrochalcone **1***

Compound **1** (75 mg) was purified by CC of the series **B** [Si gel, EtOAc/MeOH (9:1); colourless crystals from CH_2Cl_2]. Mp 135–136 °C; R_f 0.27 [Si gel, $CHCl_3$/MeOH (9:1)]. IR ν_{max}^{KBr} $cm_{:}^{-1}$ 3530 (*br*. OH), 2930, 2850, 1650 (conj. C=O), 1600, 1570, 1470, 800. UV λ_{max}^{EtOH} nm (logϵ): 290 (4.19), 236 (*sh*), 204 (*sh*). Positive FABMS *m*/*z* 451 $[M+H]^+$. CI/NH_3 MS *m*/*z* (rel. int.): 468 $[M+NH_4]^+$ (20), 451 $[M+H]^+$ (18), 433 $[M+H-H_2O]^+$ (15), 419 $[M\text{-}OCH_3]^+$ (5), 342(10), 306 [M + NH_4-Glc] (100), 289 $[M+H-Glc]^+$(90), 272 [M-OGlc]$^+$ (10), 107 (5). EIMS (70 eV) *m*/*z* 450 $[M]^+$ (10), 342 (8), 288 $(M-Glc)^+$(80), 272 $(M-OGlc)^+$ (20), 328 (100), 107 (6). HR–EIMS (*m*/*z* 450.1524 $[M]^+$), $C_{22}H_{26}O_{10}$ required 450.1526. ^{1}H NMR (300 MHz, DMSO) and ^{13}C NMR (75 MHz, DMSO), see Table 1.

*3.3.2. 3,5-Dimethoxy-4′-O-(β-rhamnopyranosyl-(1→6)-β-glucopyranoside)stilbene **2***

Compound **2** (86 mg) was purified by CC of the series **C** [Si gel, EtOAc/MeOH (17:5); colourless crystals from CH_2Cl_2]. Mp 242–243 °C; R_f 0.25 [Si gel, $CHCl_3$/MeOH (4:1)]. $[\alpha]_D^{22}$ $-50\pm2^\circ$ (*c* 0.2, aq acetone). IR ν_{max} ^{KBr} cm^{-1}: 3350 (*br*. OH), 2950, 1615, 1592, 1300, 1260, 1253, 965. HR–EIMS (*m*/*z* 564.2205 $[M]^+$), $C_{28}H_{36}O_{12}$ required 564.2207. EIMS (70 eV) *m*/*z* (rel. int.): 564, $[M]^+$(10), 418 $[M-146]^+$ (10), 403 $[M-Glc-CH_3]$ (15), 385 [M−OGlc] (45), 369 $[M-OGlc-CH_3]$ (70), 256 $[M-rutinosyl]^+$(100). ^{1}H NMR (300 MHz, DMSO) and ^{13}C NMR (75 MHz, DMSO) see Table 2.

Acid hydrolysis of compound **2**: 30 mg of **2** were dissolved in 0.6 M HCl (4 ml)/MeOH (100 ml) and refluxed at room temperature for 10 h. The solvent was evaporated in vacuo and work up of the crude yielded two different sugars and one aglycone. The sugars were identified by TLC comparaison to glucose and rhamnose while the aglycone was identical to pterostilbene **3** also isolated from the same plant.

*3.3.3. 3,5-Dimethoxy-4′-hydroxystilbene (pterostilbene) **3***

Compound **3** (60 mg) was purified by CC of the series **A** [Si gel, *n*-hexane/EtOAc (3:1)]; oil; R_f 0.32 [Si gel, $CHCl_3$/MeOH (19:1)]. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390 (OH), 1620 (C=C), 1600, 1590. ^{1}H and ^{13}C NMR data (Table 2) were identical to the authentic compound (Rimando et al., 1994).

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