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STILBENE GLYCOSIDES FROM *GUIBOURTIA TESSMANNII*

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Key Word Index—*Guibourtia tessmannii*; Cesalpiniaceae; stem bark; chemistry; stilbene glycoside.

Abstract—(*E*)-3,4′-Dimethoxy-5-rutinosyl stilbene, rhaponticin and piceid have been isolated from the stem bark of *Guibourtia tessmannii*.

INTRODUCTION

Guibourtia tessmannii (Harms Leonard), a plant which is used as a source of commercial timber (Bubinga), is also an important medicinal plant in the central and southern provinces of Cameroon. Its preparations are used for the treatment of hypertension, the prevention of abortion and as an anti-helmintic.

A preliminary pharmacological screening of an extract of this plant revealed the presence of an interesting anti-fungal activity [1].

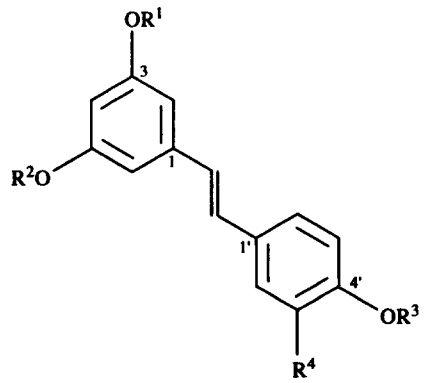
Earlier chemical work on this plant afforded two flavanols, leucofisetinidin and guibourtacacidin, tannins and sugars [2, 3]. Studies on other *Guibourtia* species have resulted in the isolation of many stilbene glycosides [4–6]. (*E*)-3,4′-Dimethoxy-5-rutinosyl stilbene was identified in *G. coleosperma* and was characterized as its acetate [6]. This report describes the isolation and characterization of (*E*)-3,4′-dimethoxyl-5-rutinosyl stilbene and other stilbenes from *G. tesmanii*.

RESULTS AND DISCUSSION

From the acetone extract of the stem bark, three compounds, **1–3**, were separated by a combination of different chromatographic techniques.

Compound **1**, a glycoside, gave a positive Molish test. Hydrolysis of **1** afforded an aglycone **1a** and two sugars characterized as D-glucose and L-rhamnose by chromatography.

The aglycone **1a** was shown to be a stilbene from the following information. The IR spectrum showed absorptions at 3390 (OH), 1620 (C=C), 1600 and 1520 (benzene ring) cm^{-1}. Its ^1H NMR spectrum (90 MHz, $CDCl_3$) showed two *trans* olefinic protons appearing as an AB quartet at δ6.83 (1H, $J = 16$ Hz) and 7.14 (1H, $J = 16$ Hz). The occurrence of two separate aromatic rings was established by the presence of signals for three protons at δ6.47 (1H, *t*, $J = 2.3$ Hz) and 6.76 (2H, *d*, $J = 8.7$ Hz) indicating a 1,3,5-trisubstituted benzene ring and an AA′BB′ type system at δ6.89 (2H, $J = 8.7$ Hz) and 7.43 (2H, $J = 8.7$ Hz) for a 1,4-disubstituted benzene ring. The spectrum also contained two methoxy signals at δ3.95 (3H, *s*, OMe) and 3.98 (3H, *s*, OMe). From this evidence, the partial structure **1a** was assumed to be a stilbene bearing two methoxy groups and a hydroxy function. The EI-mass spectrum (70 eV) showed positive ions at *m/z* 256 $[M]^+$ (100) and 195 $[M - 2 \times OMe]^+$ (12). The position of each of these groups in the stilbene structure was assigned by comparison of the ^{13}C NMR signals of **1a** with those of a similar compound [7]. Thus the chemical shifts at C-5 (δ161.1) and C-3 (δ156.9) established that the methoxy and hydroxy groups were on the same benzene ring, and that the second methoxy group was on the other ring. Consequently, the aglycone



	R^1	R^2	R^3	R^4
1	Me	rutinosyl	Me	H
1a	Me	H	Me	H
1b	Me	hexa-acetoxy rutinosyl	Me	H
2	H	glucosyl	Me	OH
2a	H	H	Me	OH
3	H	glucosyl	H	H
3a	Me	glucosyl	Me	H

1a was shown to be (*E*)-3,4'-dimethoxy-5-hydroxy stilbene. The EI-mass spectrum (70 eV) of **1** exhibited a molecular ion at *m/z* 564 $[M]^+$. This was followed by two significant ions at *m/z* 418 $[M - 148]^+$ (2.1) and 256 $[M - 308]^+$ (100) due to successive elimination from the molecular ion of a rhamnosyl and a glucosyl moiety. The link between the rhamnosyl and glucosyl portions was deduced from an examination of the ^{13}C NMR spectrum (25.15 MHz) which showed signal patterns similar to those of rutinose [8].

Thus **1** was found to be (*E*)-3,4'-dimethoxy-5-rutinosyl stilbene. The configuration at the anomeric carbons was found to be β for the glucosyl and α for the rhamnosyl moieties from the ^1H NMR spectrum of **1**.

Acetylation of **1** yielded a peracetate (**1b**) which was identical with the one reported in the literature [6].

Compound **2**, $C_{21}H_{24}O_9$, gave a positive Molish test for a glycoside. Acid hydrolysis of **2** afforded an aglycone (**2a**) which was identified as rhapontigenin [7]. Comparison of the physical and spectral data of **2** with those described in the literature [7] showed that **2** is rhaponticin.

Compound **3**, $C_{20}H_{22}O_8$, gave a positive Molish test for a glycoside. The ^1H NMR spectrum of **3** was similar to that of piceid [9]. An attempt to prepare the known derivative of piceid [4'-methoxy piceid (desoxyrhaponticin)] gave the dimethylated derivative 3,4'-dimethoxy piceid ($C_{22}H_{26}O_8$).

EXPERIMENTAL

Plant material. Stem bark of *Guibourtia tessmannii* was collected from Eseka (Centre Province of Cameroon). It was identified by the National Herbarium of Cameroon where a sample is deposited.

General. Mps: uncorr.; ^1H and ^{13}C NMR: CD_3OD or $CDCl_3$ or DMSO with TMS as int. standard; flash chromatography: silica gel 60 H, spots detected by UV fluorescence and by spraying with H_2SO_4 and heating at 100° for 5–10 min, or by I_2 vapour; TLC: $CHCl_3$–MeOH (9:1 and 4:1).

Extraction and isolation. Pulverized stem bark (1 kg) of *G. tessmannii* was defatted with hexane and then extracted successively with Me_2CO and MeOH. The Me_2CO extract, after removal of solvent, was subjected to flash chromatography over silica gel using $CHCl_3$ and MeOH as solvents.

Two main frs were obtained. The first contained a viscous residue which was discarded and the second contained the mixt. of stilbenes (7.2 g). Crystallization of this mixt. in MeOH afforded a crystalline mixt. of **1–3**. Three g of a mixt. of **1–3** was purified by prep. TLC ($CHCl_3$–MeOH, 4:1).

Compound **1**. Crystals (EtOH) (360 mg) mp 142–143°. Found C 59.57, H 6.38 required for $C_{28}H_{36}O_{12}$: C 59.5, H 6.4% IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 3050, 1620 (C=C), 1600, 1590, 1510 (benzene ring); ^1H NMR (CD_3OD, 300 MHz): δ1.2 (3H, *d*, J = 7.4 Hz, Me rhamnose), 3.76 (3H, *s*, OMe), 3.79 (3H, *s*, OMe), 3.29–3.41 (*m*, CH sugars), 4.7 (1H, *d*, J = 1.6 Hz anomeric proton of α-L rhamnose), 4.9 (1H, *d*, J = 7.5 Hz anomeric proton of β-D-glucose), 6.58 (1H, *t*, J = 2.6 Hz, H-4), 6.75 (1H, *t*, J = 1.6 Hz, H-2), 6.82 (1H, *t*, J = 1.6 Hz, H-6), 6.87 and 7.04 (2H, ABq, J = 16 Hz, *trans* olefinic proton), 6.88 and 7.42 (4H, AA'BB', J = 8.7 Hz, H-2', H-3', H-5', H-6'); ^{13}C NMR: Table 1.

Acid hydrolysis of **1**. Compound **1** (200 mg) was mixed with 50 ml 0.4 M HCl in MeOH and refluxed for 9 hr in a water bath. The solvent was reduced by evapn and upon cooling, crystals were obtained. Further crystallization from H_2O afforded the aglycone **1a** (32 mg). Compound **1a**: Found C 75, H 6.25, required for $C_{16}H_{16}O_3$: C 74.9, H 6.29%; crystals, mp 119–120°; IR ν_{max} (KBr) cm^{-1}: 3390 (OH), 1620 (C=C), 1600, 1580, 1520 (benzene ring); ^1H NMR (90 MHz, $CDCl_3$): δ3.95 (3H, *s*, OMe), 3.98 (3H, *s*, OMe), 6.47 (1H, *t*, J = 2.3 Hz, H-4), 6.76 (2H, *d*, J = 2.3 Hz, H-2, H-6), 6.83 and 7.14 (2H, ABq, J = 16 Hz, olefinic protons), 6.89 and 7.43 (4H, AA'BB', J = 8.0 Hz, H-2', H-3', H-5', H-6'); ^{13}C NMR: Table 1.

Acetylation of **1**. Compound **1** (100 mg) was left overnight in a mixt. of 5 ml pyridine and 5 ml Ac_2O and then worked up in the usual way. Purification by prep. TLC with $CHCl_3$ as solvent yielded a peracetate (**1b**) (88 mg).

Compound **1b** was identified as (*E*)-3,4'-dimethoxy-5 [2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-*o'*-L-rhamnosyl)-β-D-glucopyranosyloxy]stilbene by comparison of its ^1H NMR, ^{13}C NMR and MS data with those reported in the lit. [6].

Found C 58.82, H 5.88 calc. for $C_{40}H_{48}O_{18}$ C 58.8, H 5.92%. IR ν_{max} (KBr) cm^{-1}: 1760 (C=O), 1600, 1595 (benzene ring); ^1H NMR (90 MHz, $CDCl_3$): δ1.3 (3H, *d*, J = 6 Hz, Me rhamnose), 1.95 (3H, *s*, MeCOO), 2.02 (6H, *s*, 2 MeCOO), 2.2 (9H, *s*, MeCOO), 3.82 (3H, *s*, OMe); 4.73 (1H, anomeric proton of rhamnose), 5.05–5.4 (7H, *m*, anomeric proton of glucose, 6 CH-OAc), 6.44 (1H, *t*, J = 2 Hz, H-4), 6.74 (2H, *d*, J = 2 Hz, H-2 and H-6), 6.81 and 7.02 (2H, ABq, J = 16 Hz, *trans* olefinic proton), 6.88 and 7.44 (4H, AA'BB' system, J = 8 Hz, H-2', H-3', H-5', H-6'); ^{13}C NMR: Table 1.

Compound **2**. Rhaponticin (300 mg) crystals (EtOH) mp 241–242° (lit. [7] 246–248°). Found C 60, H 5.71 calc. for $C_{21}H_{24}O_9$: C 59.9, H 5.75%. IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 1620 (C=C-Ar), 1580, 1510 (benzene ring); ^1H NMR (DMSO-d_6, 90 MHz): δ3.25–3.35 (6H, *m*, 4H and $-CH_2$), 3.76 (3H, *s*, OMe), 4.85 (1H, *d*, J = 6 Hz, anomeric proton), 5–5.3 (4H, *br s*, OH glucose), 6.34 (1H, *br s*, H-4), 6.55 (1H, *br s*, H-2), 6.75 (1H, *br s*, H-6), 6.8–7.5 (5H, *m*, H-2', H-3', H-5', H-α and H-β), 8.9 (1H, *s*, OH phenolic), 9.35 (1H, *s*, OH phenolic).

Acid hydrolysis of **2**. Compound **2** (200 mg) was hydrolysed as previously described. The residue was purified over silica gel with $CHCl_3$–MeOH (19:1) and (9:1). Frs of 25 ml were collected. Frs 30–36 deposited, after evapn, a powder (40 mg) of rhapontigenin **2a** mp 196–197°. Found C 69.7, H 5.42 calc. for $C_{15}H_{14}O_4$: C 69.8, H 5.46%. ^1H NMR (300 MHz, CD_3OD): δ3.75 (3H, *s*, OMe), 6.15 (1H, *t*, J = 2.1 Hz, H-4), 6.42 (2H, *d*, J = 2.1 Hz, H-2, H-6), 6.71 (1H, *d*, J = 8 Hz, H-5'), 6.83

Table 1. ^{13}C NMR spectral data of **1b** [6], **1**, **1a**, **1b**, **2**, **2a**, **3** and **3a**

C	1	1a	1b	1b [6]	2	2a	3	3a
1	140.9 *s*	140.1	140.1	140.5	139.2	139.7	139.9 *s*	139.4 *s*
2	100.3 *d*	105.8	106.5	108.2	105	105.0	106.0 *d*	105.8 *d*
3	162.0 *s*	156.9	161.0	158.7	158.3	157.3	158.4 *s*	159.1
4	103.2 *d*	100.7	102.5	102.6	103	102.0	102.6 *d*	101.7 *d*
5	160.0 *s*	161.1	158.2	161.6	158.8	157.3	157.7 *s*	159.0 *s*
6	108.6 *d*	104.8	107.9	106.8	107.3	105.3	107.3 *d*	106.5
OMe	55.9 *q*	55.4	55.4	55.2	55.5	55.2		55.3
OMe	55.9 *q*	55.4	55.4	54.8		—		55.2
α	127.1 *d*	126.3	126.1	126.6	126.1	126.8	125.2	126.1
β	129.8 *d*	129.0	129.4	129.7	128.6	126.5	128.7	128.8 *d*
			170.3(1C)	169.9				
C=O			170.1(1C)					
			169.8(2C)					
			169.5(2C)					
1′	130.8 *s*	130.2	129.9	130.2	129.9	130.9	128.7	129.6 *s*
2′	126.1 *d*	127.9	128.0	128.3	112.3	112.2	127.6	128.0 *d*
3′	114.9 *d*	114.1	114.0	114.5	146.5	145.7	115.2	114.3 *d*
4′	160.9 *s*	158.4	159.0	160.0	147.7	147.0	156.5	160.5
5′	114.9 *d*	114.1	114.0	114.5	112.3	111.0	115.2	114.3 *d*
6′	126.1 *d*	127.8	128.0	128.3	118.6	119.0	127.6	128.0 *d*
Me C=0			20.7	20.3				
Glucose								
1	101.8 *d*		91.1	98.9	100.6		100.6 *d*	100.8 *d*
2	73.8 *d*		73.2	71.8	73.3		73.1 *d*	73.4 *d*
3	77.6 *d*		78.3	73.3	77.0		76.2 *d*	79.9 *d*
4	71.1 *d*		71.4	69.9	69.8		69.7 *d*	70.0 *d*
5	76.6 *d*		77.0	73.4	76.7		76.2 *d*	76.9 *d*
6	67.5 *t*		66.7	67.1	60.7		61.3 *t*	60.9 *t*
Rhamnose								
1	102.2 *d*		98.0	98.4				
2	72.1 *d*		72.8	69.9				
3	71.8 *d*		71.0	69.8				
4	74.7 *d*		75.8	71.2				
5	69.5 *d*		69.5	67.3				
6	17.8 *q*		17.3	17.7				

(1H, *dd*, J = 2 Hz, J = 8 Hz, H-6′), 6.96 (1H, *d*, J = 2 Hz, H-2′), 6.70 and 6.82 (1H each, *d*, J = 16 Hz *trans* olefinic protons); ^{13}C NMR: Table 1.

Compound **3**. Piceid (446 mg): Found C 61.53, H 5.64 calc. for $C_{20}H_{22}O_8$: C 61.53, H 5.68%. Pale yellow crystals, mp 220° (lit. 225° [9]). ^{1}H NMR (CD_3OD and $CDCl_3$, 200 MHz): δ3.17–3.6 (6H, *m*, 4CH and CH_2 glucose), 4.7 (1H, *d*, J = 5 Hz, anomeric proton), 6.27 (1H, *dd*, J = 2 Hz, J = 1.8 Hz, H-4), 6.44 (1H, *dd*, J = 2 Hz, J = 1.8 Hz, H-2), 6.5 (1H, *dd*, J = 2 Hz, J = 1.8 Hz, H-6), 6.54 and 6.64 (1H each, *d*, J = 18 Hz, *trans* olefinic protons), 6.58 and 7.13 (4H, AA′BB′ type signal, H-2′, H-3′, H-5′, H-6′).

Methylation of **3**. Compound **3** (70 mg) was dissolved in 50 ml dry Me_2CO. MeI (3 ml) was added together with 3 g K_2CO_3 then refluxed for 5 hr. The reaction mixt. was cooled and extracted with EtOAc. The residue (1 g) from the EtOAc extract after further purification by prep. TLC using $CHCl_3$–MeOH (4:1) as solvent gave **3a** (48.5 mg) as yellow crystals, mp 174–175°. Found C 63.15, H 6.22 required for $C_{22}H_{26}O_8$: C 63.1, H 6.26%. ^{1}H NMR (200 MHz, DMSO-d_6): δ3.1–3.6 (6H, *m*, 4H, CH_2), 3.75 (3H, *s*, OMe), 3.76 (3H, *s*, OMe), 4.7 (1H, *br s*, OH), 4.9 (1H, *d*, J = 7 Hz anomeric proton), 5.15 (1H, *br s*, OH), 5.17 (1H, *br s*, OH), 5.45 (1H, *br s*, OH), 6.51 (1H, *dd*, J = 1.7 Hz, J = 2 Hz, H-4), 6.8 (1H, *dd*, J = 1.7 Hz, J = 2 Hz, H-2), 6.9 (1H, *dd*, J = 1.7 Hz, J = 2 Hz, H-6), 6.93 and 7.52 (4H, AA′BB′ type signal, J = 8 Hz, H-2′, H-3′, H-5′, H-6′), 7.0 and 7.21 (2H, ABq, J = 18 Hz, H_α and H_β); ^{13}C NMR: Table 1.

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