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TWO NEW ISOFLAVANOIDS FROM *BOLUSANTHUS SPECIOSUS*

Gomotsang Bojase, Cornelius C.W. Wanjala and Runner R.T. Majinda*

Department of Chemistry, University of Botswana, Private Bag 00704, Gaborone, Botswana

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ABSTRACT. The structures of two new isoflavonoids from the combined ethyl acetate/methanolic extracts of the stem bark of Bolusanthus speciosus have been established as 4,7,2′-trihydroxy-4′-methoxyisoflavanol (**1**) and 5,7,3′,4′-tetrahydroxy-5′-(2-epoxy-3-methylbutyl)isoflavanone (**2**). Five other known isoflavonoids, 5,7,3′-trihydroxy-4′-methoxy-5′-γ,γ-dimethylallylisoflavanone, 5,7,2′-trihydroxy-4′-methoxy-6,5′-di(γ,γ-dimethylallyl)isoflavanone, 5,7,2′,4′-tetrahydroxy-8,5′-di(γ,γ-dimethylallyl)flavanone, 5,7,2′,4′-tetrahydroxy-8,3′-di(γ,γ-dimethylallyl)-isoflavanone, and derrone were also isolated. Compound **2** showed moderate activity against gram positive bacteria and weak activity against gram negative bacteria, while compound **1** was weakly active against both organisms in a TLC bioautography assay.

KEY WORDS: Bolusanthus speciosus, 4,7,2′-Trihydroxy-4′-methoxyisoflavanol, 5,7,3′,4′-Tetrahydroxy-5′-(2-epoxy-3-methylbutyl)isoflavanone, Antibacterial activity

INTRODUCTION

Bolusanthus speciosus Harms (Fabaceae), otherwise called tree wisteria, is a monotypic and endemic tree in subtropical Southern Africa [1, 2]. It occurs in open areas on granite basaltic or limestone soils [1]. *B. speciosus* is normally used as an ornamental tree in gardens and parks, because of its beauty [3]. The root infusion of this plant is used by some communities as an emetic while the dried inner bark is used to relieve abdominal pains [4]. Previous work on *B. speciosus* revealed the presence of alkaloids [5] and isoflavonoids which were identified as genestein, orobol, 3′-methoxyorobol, pratensein, 3′-O-methylpratensein and 3,5,7,3′-tetrahydroxy-4′-methoxyisoflavone (bolusanthin) [6].

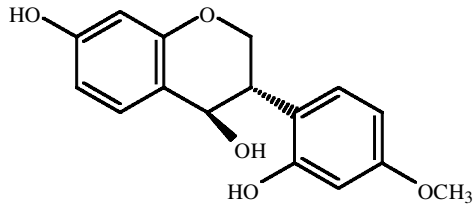
RESULTS AND DISCUSSION

The ethyl acetate and methanolic extracts from the stem bark of *B. speciosus* were worked up as described in the experimental section to give two new isoflavonoids which were identified using UV, IR, 1D and 2D NMR, EIMS and HRTOFMS spectroscopic methods.

The HRTOFEIMS of **1** showed the ion peak at 270.0892 corresponding to molecular formula $C_{16}H_{14}O_4$ [M-H_2O]. The low resolution EIMS also gave the highest molecular ion at *m/z* 270 which formed the base peak. This loss of 18 mass units from the expected molecular ion, $[C_{16}H_{16}O_5]^{+\cdot}$, is due to loss of water molecule, a common phenomenon in 4-hydroxyisoflavanols [7]. The EIMS gave other major mass fragments at *m/z* 137, 161 due to retro-Diels-Alder fragmentation. The fragment at *m/z* 137 confirms a flavonoid moiety with only one hydroxyl substituent on ring A [8]. The ^{1}H NMR spectra showed two proton *dd's* (*J* = 9.8, 10.2 Hz) at δ_H 3.59 (H-2a) and (*J* = 5.9, 9.8 Hz) at δ_H 4.28 (H-2b), one proton *ddd* (*J* = 5.7, 6.1, 10.2 Hz) at δ_H 3.61 (H-3) and one proton *d* (*J* = 5.7 Hz) at δ_H 5.52 (H-4). The DEPT spectrum also showed methine carbons at δ_C 39.9 and 79.1, a strong indication that compound **1** was an isoflavan-4-ol.

*Corresponding author. E-mail: majindar@mopipi.ub.bw

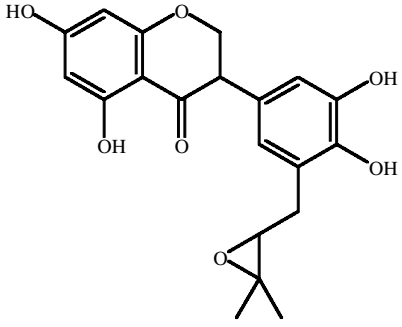
The ^{13}C NMR spectra showed sixteen carbon signals (Table 1) and the HMQC together with DEPT spectra confirmed that ten of these were protonated. The DEPT spectrum showed the presence of one methylene carbon, eight methine carbons and one methoxyl. The nature and identity of the isoflavan-4-ol was evident from the ^{1}H NMR which showed the following salient features: two 1,3-dioxyphenyl groups at δ_H = 6.57 (*dd*, *J* = 2.4, 8.4 Hz, 1H); 7.34, (*d*, *J* = 8.4 Hz, 1H); 6.36, (*d*, *J* = 2.4 Hz, 1H) and δ_H = 6.46 (*dd*, *J* = 2.3, 8.4 Hz, 1H); 7.24, (*d*, *J* = 8.1 Hz, 1H); 6.39, (*d*, *J* =2.3 Hz, 1H). The methoxyl group could be placed at either C-2′ or C-4′ of the isoflavanol moiety since both these carbons have resonances which showed they were oxygen bearing. The connectivity of the methoxyl (δ_H 3.76) group to this moiety was evident from the HMBC correlation with carbon at δ_C 161.7 (C-4′). The proton *ddd* at δ_H 3.61 (H-3) showed HMBC correlations with carbon signals δ_C 161.3 (C-2′) and δ_C 125.4 (C-6′) further confirming that the carbon atom resonating at δ_C 161.7 is indeed C-4′. The data available thus enable the identification of compound **1** as 4,7,2′-trihydroxy-4′-methoxyisoflavanol, named bolusanthol **D**, and it is being reported for the first time. The problem that remains unresolved is the absolute stereochemistry at C-3 and C-4. We were able to determine, from our data, the *trans* relationship between the two H-3 and H-4 and hence structure **1** as drawn shows only the relative stereochemistry at C-3 and C-4.



1

The HRTOFEIMS of **2** gave the ion peak at 372.1203 consistent with molecular formula $C_{20}H_{20}O_7$, with the low resolution EIMS also giving the molecular ion peak at *m/z* 372. The mass fragments shown at *m/z* 220 and 153 were consistent with the retro-Diels–Alder fragmentation, with former ion indicating substituent on ring B of the flavonoid moiety [8]. The ^{1}H NMR data (Table 1) shows the presence of the 2-epoxy-3-methybutyl substituent from the following features: two methyl singlets at δ_H 1.34 (3H) and 1.27 (3H); an aliphatic proton *dd* (*J* = 5.1, 7.3 Hz) at δ_H 3.70 and the non-equivalent methylene protons at δ_H 2.69 (*dd*, *J* = 7.3, 16.8 Hz) and 2.98 (*dd*, *J* = 5.1, 16.8 Hz). The presence of the 2-epoxy-3-methylbutyl substituent was also evident from ^{13}C NMR data which showed two methyl carbons at δ_C 20.0 and 24.7, a quaternary carbon at δ_C 77.6, a methine carbon at δ_C 69.6 and a methylene carbon at δ_C 31.1. The presence of three one-proton *dd's* at δ_H 3.8 (H-3) (*J* = 5.6, 9.0 Hz), δ_H 4.50 (H-2a) (*J* = 5.6, 11.4 Hz) and δ_H 4.52 (H-2b) (*J* = 9.0, 11.4 Hz) in the proton NMR spectrum together with a methine carbon at δ_C 50.7 and a methylene carbon at δ_C 71.6, in the ^{13}C NMR, indicated that compound **2** was an isoflavanone. The nature of the isoflavanone was evident from the ^{1}H NMR data (Table 1) which showed the following salient features: two shielded equivalent *meta*-coupled protons at δ_H 5.92 (*d*, *J* = 2.1 Hz, 2H) and another set of *meta*-coupled protons at δ_H 6.60 (1H, *d*, *J* = 2.1 Hz) and δ_H 6.53 (1H, *d*, *J* = 2.1 Hz). The ^{13}C NMR showed 20 carbon signals (Table 1), and the HMQC and DEPT confirmed that ten of these were protonated. The DEPT spectrum further showed the presence of six methines, two methylenes and two methyl carbons. The mass fragment at *m/z* 220 showed that the 2-epoxy-3-methylbutyl group was in the B-ring of the isoflavonoid moiety. The connectivity of the 2-epoxy-3-methylbutyl group to the isoflavanone

moiety was evident from the following HMBC correlations: the methylene protons at δ_H 2.69 and 2.98 showed correlations with carbons at δ_C 120.5 (C-6'), 121.1 (C-5'), 140.8 (C-4'), 69.5 (C- 2") and 77.6 (C-3"), while the *dd* at δ_H 3.70 showed correlations with carbons at δ_C 121.1 (C-5'), 77.6 (C- 3"), 20.0 (C- 4") and 24.7 (C-5"), thus establishing the position of attachment of the 2-epoxy-3-methylbutyl group to the isoflavonoid moiety as C-5′. Consequently compound **2** was identified as 5,7,3',4'-tetrahydroxy-5'-(2-epoxy-3-methylbutyl)isoflavanone, named bolusanthol **E**, and is also reported for the first time. Reported work [9,10] showed (+)-isoflavanones to have 3R configuration and (-)-isoflavanones to have 3S configuration. Since **2** is an (+)-isoflavanone, it probably has 3R configuration at C-3.



2

Table 1. ^{1}H and ^{13}C NMR data for Compounds **1** and **2** in acetone-d_6.

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
2a	3.59, *dd,* (9.8, 10.2)	66.5 (*t*)	4.50, *dd*, (5.6, 11.4)	71.6 (*t*)
b	4.28, *dd*, (6.1, 9.8)		4.52, *dd* (9.0, 11.4)	
3	3.61*ddd*,(5.7, 6.1, 10.2)	39.9 (*d*)	3.8, *dd*, (5.6, 9.0)	50.7 (*d*)
4	5.52, *d*, (5.7)	79.1 (*d*)		197.0 (*s*)
5	7.34, *d*, (8.4)	132.2 (*d*)		164.9 (*s*)
6	6.57, *dd*, (2.4, 8.4)	110.0 (*d*)	5.92, *d*, (2.1)	95.0 (*d*)
7		159.1 (*s*)		167.5 (*s*)
8	6.36, *d*, (2.4)	103.4 (*d*)	5.92, *d*, (2.1)	96.2 (*d*)
9		157.0 (*s*)		163.8 (*s*)
10		111.9 (*s*)		102.4 (*s*)
1'		119.8 (*s*)		127.8 (*s*)
2'		161.3 (*s*)	6.60, *d*, (2.1)	113.4 (*d*)
3'	6.39, *d*, (2.3)	96.7 (*d*)		146.0 (*s*)
4'		161.7 (*s*)		140.8 (*s*)
5'	6.46, *dd*, (2.3, 8.4)	106.4 (*d*)		121.1 (*s*)
6'	7.24, *d*, (8.4)	125.4 (*d*)	6.53, *d*, (2.1)	120.5 (*d*)
1"			2.98, *dd*, (5.1, 16.8) 2.69, *dd*, (7.3, 16.8)	31.1 (*t*)
2"			3.70, *dd*, (5.1, 7.3)	69.5 (*d*)
3"				77.6 (*s*)
4"			1.27, *s*	20.0 (*q*)
5"			1,34, *s*	24.7 (*q*)
-OCH_3	3.76, *s*	54.9 (*q*)		

Assignments were confirmed by COSY, HMQC, HMBC and DEPT.

The structures of the known compounds 5,7,3′-trihydroxy-4′-methoxy-5′-γ,γ-dimethylallylisoflavanone [11], 5,7,2′-trihydroxy-4′-methoxy-6,5′-di(γ,γ-dimethylallyl)-isoflavanone [12], 5,7,2′,4′-tetrahydroxy-8,3′-di(γ,γ-dimethylallyl)-isoflavanone [13] and 5,7,2′,4′-tetrahydroxy-8,5′-di(γ,γ-dimethylallyl)- flavanone [10] and derrone [14] were determined from spectroscopic data and by comparison of our spectral data with those reported in literature.

Compounds **1** and **2** were tested for antibacterial activity using the TLC bioautography technique [15, 16]. The results are shown in Table 2. Compound **2** seems to be consistently more active than **1** against the organism tested. The results seem to support the traditional use of the plant in treatment of abdominal pains, which mostly are due to bacterial infections.

Table 2. Antibacterial activity of compounds **1** and **2**.

Microorganism	Loading (μg)		
	1	**2**	Chloramphenicol
Bacillus subtilis (NCTC 3610)	100	10	0.001
Staphylococcus aureus	10	1	0.001
Escherichia coli (NCTC 09001)	100	10	0.01

EXPERIMENTAL

General experimental procedures. Melting point: Stuart Scientific (SMP1) melting point apparatus; specific rotation $[\alpha]_D$: Polatronic-D (Schimdt + Haensch) polarimeter; UV: Shimadzu UV-2101PC spectrophotometer; IR: Perkin Elmer 2000 FT-IR spectrometer. The 1D {^{1}H (300 MHz), ^{13}C (75.5 MHz), DEPT} and 2D (COSY, HMQC, HMBC) spectra acquired on Bruker Avance DPX 300 spectrometer and referenced to residual solvent signal. MS: HREIMS was done on autospec TOF spectrometer. EI on Finnigan MAT SSQ 700 single quadrupole instrument. Column chromatography-silica gel 60 particle size 0.040-0.063 mm for column chromatography (Merck); vacuum liquid chromatography: silica gel HF_{254} 5-15 μm mesh (Merck); Sephadex LH-20 (Sigma); preparative TLC: silica gel 60 $PF_{254+366}$ for preparative layer chromatography (Merck); analytical TLC: silica gel 60-F_{254} precoated alumina sheets (Merck), and visualized using UV (254 and 366 nm) and vanillin-sulfuric acid spray.

Plant material. The stem bark of *B. speciosus* was collected from Mapoka, North East District, Botswana in August 1997, identified by Dr. L. M. Turton and a voucher specimen (B 0897) was deposited at the University of Botswana Herbarium.

Extraction and isolation. The air dried stem bark was ground to fine powder (1.5 kg) and was sequentially extracted in MeOH-$CHCl_3$ and aqueous MeOH. The solvents were then removed to give 150 g of brown residue. The residue was then subjected to vacuum liquid chromatography by elution with hexane, ethyl acetate and methanol in order of increasing polarity. The ethyl acetate-methanol fractions were combined and applied to Sephadex LH-20 column and the elution was carried out with $CHCl_3$-MeOH (1:1), to give fractions A and B. Fraction A was then subjected to column chromatography silica gel 60 and eluted with toluene-EtOAc-HOAc (45:4:1) to obtain fractions A1 and A2. Concentrated fraction A1 was separated by multiple development on preparative TLC using solvent systems toluene-EtOAc-HOAc (35:14:1) and $CHCl_3$-EtOAc (6:1) to give 5,7,3′-trihydroxy-4′-methoxy-5′-γ,γ-dimethylallylisoflavanone (10

mg) [11], 5,7,2′-trihydroxy-4′-methoxy-6,5′-di(γ,γ-dimethylallyl)isoflavanone (60 mg) [12], 5,7,2′,4′-tetrahydroxy-8,5′-di(γ,γ-dimethylallyl)flavanone (15 mg) [13] and 5,7,2′,4′-tetrahydroxy-8,3′-di(γ,γ-dimethylallyl)-isoflavanone (15 mg) [10]. Fraction A2 was also subjected to multiple development preparative TLC with toluene-EtOAC-HOAc (45:4:1) to obtain 4,7,2′-trihydroxy-4′-methoxyisoflavanol (**1**) (30 mg) and derrone [14] (18 mg). Fraction B was applied to column chromatography silica gel 60 and eluted with $CHCl_3$-MeOH (14:1), followed by preparative TLC in the same solvent system to give 5,7,3′,4′-tetrahydroxy-5′-(2-epoxy-3-methylbutyl)isoflavanone (**2**) (75 mg).

4,7,2′-Trihydroxy-4′-methoxyisoflavanol (**1**) was obtained as brown syrup: $[\alpha]^{25}_D$ 21.6 (c 0.019, MeOH); UV (MeOH) λ_{max} (log ε) 290 (3.9), + $AlCl_3$ 376, 312, +$AlCl_3$/HCl 376, 310, +NaOAc 326, 248, +NaOAc/H_3BO_3 291, 248 nm; IR (KBr) ν_{max} 3401, 2979, 2928, 1638, 1497, 1378 cm^{-1}; ^1H (300 MHz) and ^{13}C (75.5 MHz) NMR (in CD_3COCD_3), (see Table 1); EIMS *m/z* 270 $[M-H_2O]^+$ (100), 255 (42), 161 (30) 137 (15); HRTOFEIMS *m/z* 270.0890 (calcd for $C_{16}H_{14}O_4$, 270.0892).

5,7,3′,4′-Tetrahydroxy-5′-(2-epoxy-3-methylbutyl)isoflavanone (**2**) was obtained as yellow crystals ($CHCl_3$/MeOH 1:1): m.p. 102-104 °C; $[\alpha]^{25}_D$ 6.0 (c 0.025, MeOH); UV (MeOH) λ_{max} (log ε) 286 nm (4.2), +NaOMe 290, 248, +$AlCl_3$ 286, 248, +$AlCl_3$/HCl 286,248, +NaOAc 286, +NaOAc/H_3BO_3 286 nm; IR (KBr) ν_{max} 3393. 1621, 1499, 944, 839 cm^{-1}; ^1H (300 MHz) and ^{13}C (75.5 MHz) NMR (in CD_3COCD_3), (see Table 1); EIMS *m/z* 153, $[M]^+$ (100), 372 (70), 220 (65), 149 (25); HRTOFEIMS *m/z* 372.1203 (calcd for $C_{20}H_{20}O_7$, 372.1209).

The isolated compounds **1** and **2** were tested against *Bacillus subtilis* (NCTC 3610), *Staphylococcus aureus* (NCTC 8532) and *Escherichia coli* (NCTC 09001) using a standard procedure [15, 16].

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REFERENCES

1. Allen, O.N.; Allen, E.K. *The Leguminoseae*, Macmillan Publishers: London; **1981**; p 101.
2. Fabian, A.; Germishhuizen, G. *Wild Flowers of Northern South Africa*, Fernwood Press: Vlaeberg; **1997**; pp 12, 166.
3. Van-Wyk, B.; Van-Wyk, P. *Field Guide to Trees of Southern Africa*, Struik Publishers: Cape Town; **1998**; pp 25, 452.
4. Venter, F.; Julye, A. *Making most of Indigenous Trees*, Briza Publications: Pretoria; **1996**; p 238.
5. Asres, K.; Phillipson, D.J.; Mascagani, P. *Phytochemistry* **1986**, 25, 1449.
6. Asres, K.; Mascagini, P.; O'neil, M.J.; Philipson, J.D. *Z. Naturforsch* **1985**, 40C, 617.
7. Porter, Q.N.; Baldas, J. *Mass Spectroscopy of Heterocyclic Compounds*, Taylor, E.C.; Weissberger, A. (Eds.); John Wiley: New York; **1985**; p 108.

8. Mabry, T.J.; Markham, K.R. *The Flavanoids*, Part 1, Harborne, J.B.; Mabry, T.J.; Mabry, H. (Eds); Academic Press: London; **1975**; p 81.
9. Osawa, K.; Yasuda, H.; Maruyama, T.; Morita, H.; Takeya, K.; Itokawa, H. *Chem. Pharm. Bull*. **1992**, 40, 2970.
10. Fukai, T.; Zeng, L.; Nishizawa, J.; Wang, Y.; Nomura, T. *Phytochemistry* **1994**, 36, 233.
11. Maillard, M.; Hamburger, M.; Gupta, M.P.; Hostettmann, K. *Planta Medica* **1989**, 55, 281.
12. Komatsu, M.; Yokoe, I.; Shirataki, Y. *Chem Pharm. Bull*. **1981**, 29, 532.
13. O'Neil, M.J.; Adesanya, S.A.; Roberts, M.F.; Pantry, I.R. *Phytochemistry* **1986**, 25, 315.
14. Chibber, S.S. *Phytochemistry* **1980**, 19, 1857.
15. Holmans, A.L.; Fuchs, J. *J. Chromatogr*. **1970**, 51, 327.
16. Rahalison, L.; Hamburger, M.; Hostettmann, K.; Monod, M.; Frenk, E. *Phytochem. Anal*. **1991**, 2, 199.