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Antimicrobial Flavonoids from *Bolusanthus speciosus*

Abstract

A new isoflavanone namely 3,5,7,2′,4′-pentahydroxy-8,3′-di(γ,γ-dimethylallyl)isoflavanone (bolusanthin II) and four new pterocarpans identified as 3-hydroxy-6′,6′-dimethylpyrano[2′,3′:1,2][6aR,11aR]-8,9-methylenedioxypterocarpan (bolucarpan A), 3-hydroxy-6′,6′-dimethyl-4′,5′-dihydropyrano[2′,3′:1,2][6aR,11aR]-8,9-methylenedioxypterocarpan (bolucarpan B), 3-hydroxy-9-methoxy-6′,6′-dimethylpyrano-[2′,3′:1,2][6aR,11aR]-pterocarpan (bolucarpan C) and 3-hydroxy-9-methoxy-6′,6′-dimethyl-4′,5′-dihydropyrano[2′,3′:1,2][6aR,11aR]-pterocarpan (bolucarpan D) and three known isoflavonoids were isolated from the methanolic extracts of the root bark, while eight known isoflavonoids were isolated from the stem bark of *Bolusanthus speciosus*. These compounds showed antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida mycoderma* using the agar overlay technique.

Key words

Bolusanthus speciosus · Fabaceae · Isoflavanones · Pterocarpans · antimicrobial activity

Introduction

Bolusanthus speciosus (Fabaceae), commonly called Tree Wisteria, is a monotypic and endemic tree in subtropical Southern Africa, growing up to ten meters or more in height [1], [2]. The root infusion is used as an emetic while the dried inner bark is used to treat tuberculosis and relieve abdominal pains [3], [4]. Previous phytochemical investigations of *B. speciosus* by Asres et al. afforded alkaloids [5] and isoflavonoids [6], while that by us gave isoflavonoids [7]. In the preliminary antibiotic screening in our laboratory, the methanolic extracts *B. speciosus* showed moderate activity against some Gram-positive and Gram-negative bacteria and yeast spores.

Materials and Methods

General methods

Melting point, specific rotation, UV, IR, NMR, MS, CC, vacuum liquid chromatography, analytical and preparative TLC, and visualization were determined as described [8]. Selective NOESY experiments were run on an Avance DRX 600 spectrometer (600 MHz) operating in Button Selective NMR mode and using the pulse programme Selnogp.3.

Plant material

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

Extraction and isolation

Root bark: The powdered root bark (1.6 kg) was extracted sequentially with EtOAc (5 L), MeOH (2.5 L) and 8:2 MeOH/H_2O mixture (2.5 L). Removal of solvent from the combined EtOAc/MeOH extracts gave 280 g of crude residue. Part of this (72 g) was adsorbed on silica gel PF 60 (120 g) and applied to column chromatography (5 × 100 cm) eluting with toluene/EtOAc/AcOH (TEA) and then EtOAc according to the following scheme: TEA

45:4:1 (frs 1–20, 200 ml each), 40:9:1 (frs 21–32, 200 ml each), 35:14:1 (frs 33–50, 200 ml each), and EtOAc (frs 51–65, 150 ml each). The fractions were monitored using TLC and were combined to give three major combined fractions R_1 (frs 1–26, 15 g), R_2 (frs 27–48, 23 g) and R_3 (frs 49–65, 30 g). Fractions R_1 and R_2 were each applied to Sephadex LH-20 [5 × 48 cm] separately, eluting with CHCl₃/MeOH (80:20) followed by preparative TLC (hexane/acetone 4:1) [× 3 developments, the R_f's of all isolated compounds were determined using the solvent systems that were used in preparative TLC to purify them] to give **3** (12 mg, R_f 0.45) and **5** (10 mg, R_f 0.40), and **2** (20 mg, R_f 0.55) and **4** (18 mg, R_f 0.55), respectively. C C (3 × 100 cm) of R_3 on silica gel (450 g) eluting with 6:1 CHCl₃/EtOAc (1.5 L) followed by gel filtration on Sephadex LH-20 [2 × 30 cm] with 1:1 MeOH/CHCl₃ (1 L) and preparative TLC (CHCl₃/EtOAc 6:1) yielded **1** (25 mg, R_f 0.30), 5,7,3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone, **6** (265 mg, R_f 0.40) [9], 5,7,2′-trihydroxy-4′-methoxy-6,5′-diprenylisoflavanone **7** (176 mg, R_f 0.36) [10] and 5,7,2′,4′-tetrahydroxy-8,3′-diprenylisoflavanone **8** (245 mg, R_f 0.33) [11].

Stem bark: The powdered stem bark (1.5 kg) was extracted sequentially with CHCl₃/MeOH (8:2) (3 L) and MeOH (4 L). The extracts were combined and solvents were removed to give a combined weight of 150 g of brown residue. Vacuum liquid chromatography (silica gel 600 g) of this residue was done eluting with *n*-hexane, dichloromethane and methanol in increasing polarity. The fractions obtained were combined into four groups: G-1 [3.5 g, C₆H₁₄ 100%, 1 L], G-2 [8.1 g, C₆H₁₄-CH₂Cl₂ 1:1 to 1:9, 1.5 L], G-3 [75 g, CH₂Cl₂-MeOH 100:0 to 70:30, 4 L], and G-4 [30 g, CH₂Cl₂-MeOH 60:40 to 00:100, 2.5 L]. Gel filtration on Sephadex LH-20 column [5 × 48 cm] of fraction G-3 (75 g), in three portions, eluted with CHCl₃/MeOH (1:1) gave three fractions S_1 (1.9–2.0 L, 12 g), S_2 (2.5–3.0 L, 18 g) and S_3 (3.2–3.5 L, 28 g). The concentrated fraction S_1 (12 g) was subjected to silica gel CC (2 × 40 cm) using TEA 45:4:1 to give fractions S_{11} [600–750 mL, 4 g], S_{111} [550–650 mL, 3.5 g] and S_{iv} [800 mL, 4.2 g]. The bulk of fraction S_{11} crystallized out to give lupeol (3 g). Fractions S_{111} and S_{iv} separately on preparative TLC (TEA 35:14:1, × 3 developments) yielded 4,2′,3′,4′-tetrahydroxy-6,7-methylenedioxyisoflavonol **9** (30 mg, R_f 0.25) [7], 5,7,2′,4′-tetrahydroxy-8,3′- di(γ,γ-dimethylallyl)isoflavanone **8** (15 mg, R_f 0.27) [12]. S_2 was separated by CC on silica gel [5 × 90 cm] with CHCl₃-MeOH 19:1 to give combined fractions I (950 mL, 7 g) and II (450 mL, 3 g). Fraction I was further separated by silica gel CC (5 × 48 cm, TEA 45:4:1, × 2 developments) to give subfractions III (500 mL, 3 g) and IV (450 mL, 3 g) which on preparative TLC (TEA 45:4:1) gave 4,7,2′-trihydroxy-4′-methoxyisoflavanol **10** (30 mg, R_f 0.45), derrone **11** (18 mg, R_f 0.42), and 5,7,3′-trihydroxy-4′-methoxy-5′-γ,γ-dimethylallylisoflavanone **6** (350 mg, R_f 0.33) [9]. Preparative TLC (× 2 developments, CHCl₃/EtOAc 6:1) of II gave 5,7,3′,4′-tetrahydroxy-5′-γ,γ-dimethylallyl-isoflavanone **12** (400 mg, R_f 0.25) [7], 5,7,4′-trihydroxy-6,3′-di(γ,γ-dimethylallyl)isoflavanone **13** (100 mg, R_f 0.40) [7], 5,7,2′-trihydroxy-4′-methoxy-6,5′-di(γ,γ-dimethylallyl)isoflavanone **7** (60 mg, R_f 0.33) [10]. S_3 gave one major spot on TLC and CC [3 × 30 cm] of part of this [5 g, CHCl₃/MeOH 14:1, 2 L] and subsequent purification by preparative TLC (CHCl₃/MeOH 14:1) gave 5,7, 3′, 4′-tetrahydroxy-5′-(2,3-epoxy-3-methylbutyl)isoflavanone **14** (40 mg, R_f 0.23).

3,5,7,2′,4′-Pentahydroxy-8,3′-bis(3,3-dimethylallyl)isoflavanone (bolusanthin II) *1*

Brown crystals, m. p. 112–114 °C; $[\alpha]_D^{25}$: –26.0° (MeOH, c 0.005); EI-MS: *m/z* (%) = 440 (100) [M]⁺, 422 (20) [M – H₂O]⁺, 262 (55), 178 (45), 123 (35), 221 (25); HREI-MS *m/z* = 440.1839 (calc for C₂₅H₂₈O₇, 440.1839); IR: ν_{max}^{MeOH} = 3424, 2922, 1635, 1448, 1176 cm⁻¹; UV: λ_{max}^{MeOH} (log ε) = 208 (4.56), 298 (4.02), 334, +NaOMe 206, 336, +AlCl₃ 205, 220, 305, +AlCl₃/HCl 204, 220, 311, +NaOAc 196, 340, +NaOAc/H₃BO₃ 220, 299 nm; ¹H- and ¹³C-NMR (CD₃COCD₃) (see Table **1**).

3-Hydroxy-6′,6′-dimethylpyrano[2′,3′:1,2][6aR,11aR]-8,9-methylenedioxypterocarpan (bolucarpan A) 2

Brown crystals (in acetone); m. p. 119–121 °C; $[\alpha]_D^{25}$: –170° (MeOH, c 0.005); EI-MS: *m/z* (%) = 368 (100) [M]⁺, 369 (20) [M + H]⁺; HREI-MS: *m/z* = 368.1250 (calc for C₂₁H₂₀O₆, 368.1250); IR: ν_{max}^{KBr} = 3455, 2973, 2928, 1732, 1614 cm⁻¹; UV: λ_{max}^{MeOH} (log ε) = 210 (4.43), 305 (3.83), +AlCl₃ 305, 210, +AlCl₃/HCl 305, 208, +NaOMe 310, 251, 209, +NaOAc 305, 220, 211, 193, + NaOAc/H₃BO₃ 305, 208 nm; ¹H- and ¹³C-NMR (CD₃COCD₃) (see Table **2**).

3-Hydroxy-6′,6′-dimethyl-4′,5′-dihydropyrano[2′,3′:1,2]-[6aR,11aR]-8,9-methylenedioxy-pterocarpan (bolucarpan B) 3

Brown semisolid; m. p. 68–70 °C; $[\alpha]_D^{25}$: –300° (MeOH, c 0.004); EI-MS: *m/z* (%) = 366 (100) [M]⁺, 367 (40) [M + H]⁺, 351 (25) [M – CH₃]⁺; HREI-MS: *m/z* = 366.1104 (calc for C₂₁H₁₈O₆, 366.1104); IR: ν_{max}^{KBr} = 3454, 2971, 2925, 1704, 1598, 1486, 1135, 1032 cm⁻¹; UV: λ_{max}^{MeOH} (log ε) = 207 (4.25), 221 (4.06), 230 (4.40), 272

Table 1 ¹H- (300 MHz) and ¹³C- (75.5 MHz) NMR and DEPT data for compound 1 (δ, ppm, CD₃COCD₃)

Position	δ_H	δ_C
2	4.84, d, (11.5) 4.44, d, (11.5)	73.8 (t)
3		75.0 (s)
4		196.6 (s)
5	11.77, s	163.9 (s)
6	6.07, s	96.5 (d)
7		166.7 (s)
8		109.1 (s)
9		160.7 (s)
10		101.7 (s)
1′		116.1 (s)
2′		156.7 (s)
3′		117.3 (s)
4′		157.8 (s)
5′	6.34, d, (8.1)	106.8 (d)
6′	7.03, d, (8.1)	123.5 (d)
1″	3.22, d, (7.0)	21.4 (t)
2″	5.21, m	124.0 (d)
3″		131.4 (s)
4″	1.75, s	17.3 (q)
5″	1.63, s	25.4 (q)
1‴	3.35, d, (7.0)	22.5 (t)
2‴	5.21, m	123.1 (d)
3‴		131.8 (s)
4‴	1.75, s	17.3 (q)
5‴	1.63, s	25.4 (q)

Assignments confirmed by HMQC, HMBC and COSY. *J* values (in Hz) given in parentheses.

(3.64), 280, 312, +AlCl₃ 192, 207, 221, 230, 272, 280, 312, +AlCl₃/ HCl 205, 221, 230, 262, 272, 280, 311, +NaOMe 197, 238, 288, 298, 353, + NaOAc 207, 223, 272, 280, 312, +NaOAc/H₃BO₃ 223, 272, 279, 311 nm; ¹H- and ¹³C-NMR (CD₃COCD₃) (see Table **2**).

3-Hydroxy-9-methoxy-6′,6′-dimethylpyrano[2′,3′:1,2]-[6aR,11aR]pterocarpan (bolucarpan C) 4

White amorphous powder; mp 78 – 80 °C; $[\alpha]_D^{25}$: –175° (MeOH, *c* 0.008); EI-MS: *m/z* (%) = 354 (100) [M]⁺ , 355 (25) [M + H]⁺; HREI-MS: *m/z* = 354.1466 (calc for $C_{21}H_{22}O_5$, 354.1466); IR: ν_{max}^{KBr} = 3454, 2930, 1618, 1489, 1142, 1091, 1026 cm⁻¹; UV: λ_{max}^{MeOH} (log ε) = 292 (3.16), +AlCl₃ 292, +AlCl₃/HCl 292, +NaOMe 294, +NaOAc 220, 292, +NaOAc/H₃BO₃ 292 nm; ¹H- and ¹³C-NMR (CD₃COCD₃) (see Table **2**).

3-Hydroxy-9-methoxy-6′,6′-dimethyl-4′,5′-dehydropyrano-[2′,3′:1,2][6aR,11aR]pterocarpan (bolucarpan D) 5

White amorphous powder; m. p. 83 – 85 °C; $[\alpha]_D^{25}$: –240° (MeOH, *c* 0.004); EI-MS: *m/z* (%) = 352 (100) [M]⁺, 353 (40) [M + H]⁺, 337 (50) [M – CH₃]⁺; HREI-MS *m/z* = 352.1310 (calc for $C_{21}H_{20}O_5$, 352.1310); IR: ν_{max}^{KBr} = 3451, 2926, 1606, 1460, 1141, 1093, 1026 cm⁻¹; UV: λ_{max}^{MeOH} (log ε) = 273, 282, 339, +AlCl₃ 227, 273, 282, 339, +AlCl₃/HCl 273, 281, +NaOMe 286, 353, +NaOAc 274, 282, 339, +NaOAc/H₃BO₃ 273, 281, 339 nm; ¹H- and ¹³C-NMR (CD₃COCD₃) (see Table **2**).

Activity tests

The antimicrobial activity tests were done using the TLC bioautographic technique using the standard procedures by Rahalison et

al. [13], against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida mycoderma*. The standards used for comparison were, chloramphenicol for bacteria and miconazole for fungi.

Results and Discussion

Compound **1** was isolated from the methanolic extract of the root bark as a brown amorphous powder and the HREI-MS gave the molecular ion peak at *m/z* 440.1839 consistent with the molecular formula $C_{25}H_{28}O_7$. The ¹H-NMR spectrum of **1** (Table **1**) showed two *ortho*-coupled aromatic protons at δ 7.03 (d, *J* = 8.1 Hz) and 6.34 (d, *J* = 8.1 Hz), a singlet at δ 6.07, two γ,γ-dimethylallyl units [δ 3.22 (2H, d, *J* = 7.0 Hz), 3.35 (2H, d, *J* = 7.0 Hz), 5.21 (2H, m), 1.75 (6H, s, CH₃×2) and 1.63 (6H, s, CH₃×2)] and two one-proton germinal doublets at δ 4.84 (d, *J* = 11.5 Hz) and 4.44 (d, *J* = 11.5 Hz) assignable to H-2 protons of an isoflavanone nucleus. The quaternary carbon at δ 75.0 suggested the presence of a 3-hydroxy group while the chelated hydroxy at δ 11.77 indicated the presence of 5-OH. The EIMS exhibited mass fragment at *m/z* 221 [A + H]⁺, which resulted from the *retro*-Diels-Alder fragmentation [14], suggesting that one of the γ,γ-dimethylallyl units was in ring A. The chemical shift of the hydroxy at C-5 position (δ 11.77) and of C-8 (δ 109.1) [which normally resonates around δ 100 when C-8 is unsubstituted] suggested the attachment of the γ,γ-dimethylallyl unit at position C-8 [11], [12], [15]. The observed HMBC correlations between the methylene protons of the γ,γ-dimethylallyl unit (H-1″, δ 3.22) and C-8 (δ 109.1), con-

Table 2 ¹H- (300 MHz), ¹³C- (75.5 MHz) NMR and DEPT data for compounds **2 – 5** (δ, ppm, CD₃COCD₃)

No.	2 δ_H	δ_C	3 δ_H	δ_C	4 δ_H	δ_C	5 δ_H	δ_C
1		149.7 (s)		150.3 (s)		149.8 (s)		150.4 (s)
2		121.9 (s)		121.7 (s)		122.2 (s)		121.8 (s)
3	7.45, s , OH	136.9 (s)	7. 55, s, OH	135. 4 (s)	7.59, s , OH	136.3 (s)	7.56, s, OH	135.4 (s)
4	6.12, s	102.5 (d)	6.17, s	103.8 (d)	6.27, s	101.6 (d)	6.17, s	103.7 (d)
4a		148.0 (s)		147.5 (s)		147.2 (s)		147.5 (s)
6	4.17, dd (4.9, 10.8) 3.54, dd (10.8, 11.3)	66.2 (t)	4.10, dd (4.8, 10.7) 3.32, dd (10.7, 11.4)	66.3 (t)	4.19, dd (4.0, 10.2) 3.54, dd (10.2, 11.5)	66.2 (t)	4.09, dd (4.4, 10.2) 3.41, dd (10.2, 11.4)	66.4 (t)
6a	3.38, ddd (4.9, 6.6, 11.3)	40.6 (d)	3.47, ddd (4.8, 5.7, 11.4)	40.5 (d)	3.47, ddd 4.0, 6.2, 11.5)	39.9 (d)	3.38, ddd (4.4, 6.4, 11.4)	39.8 (d)
7a		119.0 (s)		118.9 (s)		119.6 (s)		119.8 (s)
7	6.75, s	105.4 (d)	6.77, s	105.4 (d)	7.16, d (8.8)	125.3 (d)	7.11, d (8.3)	125.4 (d)
8		141.9 (s)		142.0 (s)	6.46, dd (2.2, 8.8)	106.4 (d)	6.33, dd (2.3, 8.3)	106.6 (d)
9		148.4 (s)		148.4 (s)		161.0 (s)		161.0 (s)
10	6.28, s	93.5 (d)	6.30, s	93.5 (d)	6.41, d (2.2)	96.7 (d)	6.28, d (2.3)	96.7 (d)
10a		154.6 (s)		154.7 (s)		161.5 (s)		161.6 (s)
11a	5.47, d (6.6)	77.7 (d)	5.46, d (5.7)	76.9 (d)	5.50, d (6.2)	77.7 (d)	5.48, d (6.4)	77.0 (d)
1a		109.1 (s)		107.1 (s)		109.1 (s)		107.0 (s)
4′	2.80, m 3.09, m	19.9 (t)	6.62, d (10.0)	119.9 (d)	2.80, m	20.1 (t)	6.65, d (10.0)	119.9 (d)
5′	1.92, m	32.9 (t)	5.72, d (10.0)	132.4 (d)	3.09 m, 1.92, m	32.9 (t)	5.73, d (10.0)	132.4 (d)
6′		74.1 (s)		75.9 (s)		74.1 (s)		75.9 (s)
6′,6′-(CH₃)₂	1.39, s	26.7 (q) 25.8 (q)	1.43, s	26.9 (q)	1.33, s	26.7 (q) 25.7 (q)	1.29, s	26.9 (q)
OCH₃					3.80, s	55.2 (q)	3.63, s	55.2 (q)
OCH₂O	5.92, d (10.0)	102.5 (t)	5.79, d (10.0)	101.6 (t)				

Assignments were confirmed by HMQC, HMBC, COSY and selective NOESY experiments. *J* values (in Hz) given in parentheses for ¹H-NMR data.

firmed a 5,7-dihydroxy-8-γ,γ-dimethylallyl substitution in ring A. The presence of aromatic *ortho*-coupled protons at δ 6.34 and 7.03 indicated that ring B moiety had substituents at 2′, 3′ and 4′ positions. The substitution could not be 2′,3′-dihydroxy or 3′,4′-dihydroxy since in *ortho*-dihydroxy substitution this would lead to mutual shielding of those positions in the ^{13}C-NMR spectrum, an effect which makes both hydroxylated aromatic carbons to resonate below δ 150 [16]. In compound **1**, however, these two oxygenated carbons were observed at δ 156.7 and 157.8 (Table **1**) suggesting a 2′,4′-dihydroxy substitution thus placing the γ,γ-dimethylallyl group at C-3′. Further confirmation of this was by observed HMBC correlation between the methylene protons of the γ,γ-dimethylallyl unit and C-2′ (δ 156.7), C-3′ (δ 117.3) and C-4′ (δ 157.8). Structure **1** was thus determined as 3,5,7,2′,4′-pentahydroxy-8,3′-di(γ,γ-dimethylallyl)isoflavanone (Fig. **1**), named bolusanthin II, because of its structural resemblance to bolusanthin [6].

Compound **2** was obtained as a brown amorphous powder with a melting point of 119 – 121 °C. The HREI-MS of **2** gave a base peak molecular ion at *m/z* 368.1250, consistent with the molecular formula $C_{21}H_{20}O_6$. The ^{1}H-NMR spectrum (Table **2**) showed four proton signals at δ 4.17 (1H, dd, *J* = 4.9, 10.8 Hz), 3.54 (1H, dd, *J* = 10.8, 11.3 Hz), 3.38 (1H, ddd, *J* = 5.0, 6.8, 11.3 Hz) and 5.47 (1H, d, *J* = 6.8 Hz) characteristic of the O-CH$_2$-CH-CH-O unit forming the B and C rings of a pterocarpan nucleus. The ^{1}H-NMR spectrum further revealed three one-proton singlets in the aromatic region at δ 6.12, 6.28 and 6.75, a hydroxy group proton at δ 7.45, a methylenedioxy group (δ 5.92, 2H, d, *J* = 10.0 Hz), and a dimethyldihydropyran unit [δ 1.39 (6H, s), 1.92 (CH$_2$, m), 2.80, 3.09 (1H each, m, CH$_2$)]. The placement of the dimethyldihydropyran substituent was deduced by the use of HMBC which showed correlations between H-4′ methylenic protons (δ 2.80, 3.09) of this substituent and C-1 (δ 149.7), C-2 (δ 121.9), C-3 (136.9), C-5′ (δ 32.9) and C-6′ (δ 74.1), suggesting a C-1/C-2 fusion of the pterocarpan nucleus to the dimethyldihydropyran unit. Other HMBC cross peaks were observed between the H-4 (δ 6.12) and C-1a (δ 109.1), C-2, C-3 and C-4a (δ 148.0), and between the hydroxy group proton (δ 7.45) and C-2, C-3, C-4 (δ$_C$ 102.5). The presence of a hydroxy proton (exchanged with D$_2$O) in each of the structures **2 – 5** (Fig. **2**) was helpful in their structure elucidation. Strong NOE interactions between the hydroxy proton, H-4 proton and the methyl groups of the cyclised prenyl unit, which were evident in all structures, clearly indicated that these protons are close together, a situation that is unlikely for neorautenanol **15**, edulenanol **16**, edulenane **17**, edulane **18**, neorautinin **19** type structures (Fig. **3**) [17], [18] where there is a hydroxy/methoxy at C-1 and the C-2 prenyl unit cyclised over C-3 oxygen atom. The methylenedioxy unit was placed at C-8/C-9 by way of
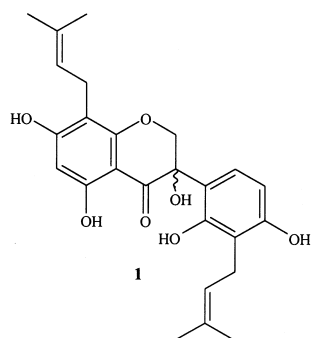
observed two-proton singlets (δ 6.28 (H-10) and 6.75 (H-7)) and the HMBC cross peaks between each of these singlets and two *ortho* oxygen bearing quaternary carbons resonating at 141.9 (C-8) and δ 148.4 (C-9). The methylenedioxy protons also showed a strong NOE with methyl protons of cyclised prenyl, with H-7 and with H-10 protons. The former observation also supports the placement of the dimethyldihydropyran unit, showing cyclisation of the C-2 prenyl unit has taken place over the C-1 oxygen atom. The assignment of the H-7 proton was confirmed also by observed NOE interaction between this proton and the H-6 and H-6a protons. Similar NOE interactions were also observed for compounds **3 – 5**. Compounds **2 – 5** all each show two carbon resonances at δ$_C$ ~136 for C-3 and δ$_C$ ~122 for C-2, which are rather unusual chemical shifts for carbons in those environments. Normally an oxygenated sp^2 cabon atom resonating at δ$_C$ 136 is associated with the middle carbon of pyrogallol type system where it experiences mutual shielding from adjacent *ortho* dioxygenated sp^2 carbon atoms. The carbon atom at δ$_C$ 122 also seems too deshielded for a carbon *ortho* to two oxygen bearing sp^2 carbons. Construction of molecular models for these compounds showed that C-3 lies in the shielding zone of aromatic D ring while C-2 lies in the deshielding zone of the same. The rest of the proton and carbon assignments were confirmed by HMBC data. The absolute configuration at C-6a and C-11a was determined to be 6a*R*, 11a*R* since **2** is a levorotatory (α_D^{25}: = −204°) pterocarpan [15],

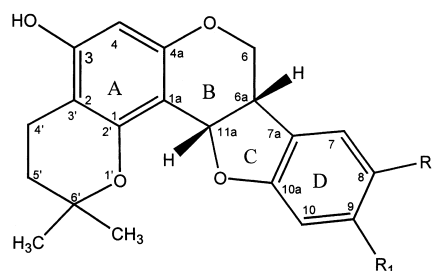Fig. **2** Structure of compound **2 – 5**.

	R^1	R^2	Δ$^{4',5'}$
2	-OCH$_2$O-		
3	-OCH$_2$O-		double bond
4	OCH$_3$	H	
5	OCH$_3$	H	double bond
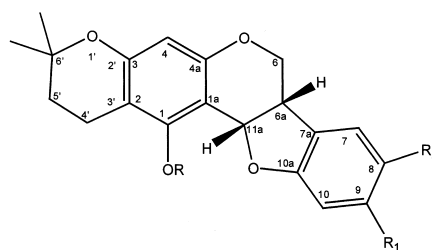
Fig. **3** Structure of compound **15 – 19**.

	R	R^1	R^2	Δ$^{4',5'}$
15	H	-OCH$_2$O-		double bond
16	CH$_3$	OH	H	double bond
17	CH$_3$	OCH$_3$	H	double bond
18	CH$_3$	OCH$_3$	H	
19	CH$_3$	-OCH$_2$O-		

Fig. **1** Structure of compound **1**.

[16]. Compound **2** was determined to be 3-hydroxy-6′,6′-dimethyl-4′,5′-dihydropyrano-[2′,3′:1,2][6aR,11aR]-8,9-methylenedioxypterocarpan, named bolucarpan A.

Compound **3** was obtained as brown paste and the HREI-MS showed the molecular ion, which formed the base peak, at *m/z* 366.1104 consistent with molecular formula $C_{21}H_{18}O_6$. A careful analysis of the ^{1}H-, ^{13}C-NMR (Table **2**) and HMBC data for **2** and **3** showed that the only difference between them is that the two sets of methylene protons due to H-4′ (δ 2.80, 1H; 3.09, 1H) and H-5′(δ 1.92, 2H) in **2** are replaced by a set of vinylic proton doublets (δ 5.72, 1H, d, *J* = 10 Hz and δ 6.62, 1H, d, *J* = 10 Hz) in the ^{1}H-NMR spectrum of **3**. The pyran ring in **3** seems to be unsaturated with a 4′,5′ double bond, making **3** a 4′,5′-dehydro derivative of **2**. Further support for the 4′/5′ unsaturation was provided by HMBC experiment which gave the expected correlations between H-4′ (δ 6.62) and C-1 (δ 149.7), C-2 (δ 121.9), C-3 (δ 135.4), C-6′ (δ 75.9) and between H-5′(δ 5.72) proton and C-2, C-6′(Fig. **4**). The rest of the spectral data were similar to those of **2** (Table **2**). Compound **3**, being also levorotatory (α_D^{25}: = –300°), was deduced to have 6aR, 11aR [19], [20], [21] absolute configuration with the structure determined as 3-hydroxy-6′,6′-dimethyl-4′,5′-dehydropyrano-[2′,3′:1,2][6aR,11aR]-8,9-methylenedioxypterocarpan, named bolucarpan B. The literature ^{1}H-NMR (^{13}C-NMR data not given) data for neorautenanol [17] shows very close resemblance to bolucarpin B, in fact these may be identical. In that case the structure of neorautenanol **15** may need to be revised to **3**. It also appears, judging from the similarity of the proton data of these compounds to ours and that these compounds were obtained from the same plant species as neorautenanol, that more experimental evidence maybe required to establish unambiguously the published structures of edulenanol, edulenane [17], edulane and neorautinin [18] which may also need revision to the basic substitution pattern of compounds **2 – 5** in the A ring.

Compound **4** was obtained as a white amorphous powder and the HREI-MS gave a molecular ion peak at *m/z* 354.1466 consistent with molecular formula $C_{21}H_{22}O_5$. The ^{1}H-NMR spectrum showed an ABD system of protons in the aromatic region at δ 7.16 (1H, d, *J* = 8.8 Hz), 6.46 (1H, dd, *J* = 2.2, 8.8 Hz) and 6.41 (1H, d, *J* = 2.2 Hz), a one proton aromatic singlet at δ 6.27 and a methoxyl group at δ 3.80 (3H, s). Comparison of the spectral data of **2** with those of **4**, shows that they only differ in ring D substitution. The ^{1}H-NMR spectrum (Table **2**) further shows the presence of a O-CH$_2$-CH-CH-O- [δ 4.19 (1H, dd, *J* = 4.0, 10.2 Hz), 3.54 (1H, dd, *J* = 10.2, 11.5 Hz), 3.47 (1H, ddd, *J* = 4.0, 6.2, 11.5 Hz), 5.50 (1H, d, *J* = 6.2 Hz)] and a dimethyldihydropyran substituent [δ 1.33 (6H, s, CH$_3$×2), 1.92 (CH$_2$, m), 2.80 (1H, m) and 3.09 (1H, m)]. The substitution pattern in **4** differs from **2** and **3** in that it
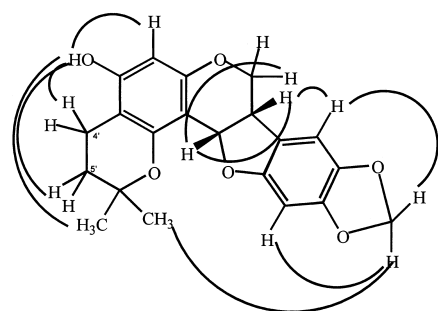
showed the presence of a methoxy group instead of a methylenedioxy unit, accounting for the observed ABD system. The placement of the methoxy group at C-9 was by an observed HMBC cross peak between the methoxyl group (δ 3.80) and C-9 (δ 161.0). Confirmation of this was by use of selective NOESY experiments (Fig. **5**) in which irradiation of the methoxy group enhanced H-8 and H-10, while that of H-10 only enhanced the methoxy protons. Furthermore, irradiation of H-8 enhanced H-7 and the methoxy group proving unambiguously the placement of the methoxy at C-9. The absolute configuration at C-6a and C-11a was determined to be *R*, *R* [19], [20], [21] from the negative (-175⁰) specific optical rotation. The data above enabled the structure of **4** to be determined as 3-hydroxy-9-methoxy-6′,6′-dimethyl-4′,5′-dihydropyrano[2′,3′:1,2][6aR,11aR]pterocarpan, named bolucarpan C.

Compound **5** was obtained as a white amorphous powder and its HREIMS showed the molecular ion peak at *m/z* 352.1310, consistent with molecular formula $C_{21}H_{20}O_6$. The spectral data of **5** (Table **2**), resembled that of **4** except that the proton multiplet signals representing two H-4′ and two H-5′ protons are replaced by two one-proton doublets at δ 6.79 (*J* = 10.0 Hz) and 5.88 (*J* = 10.0 Hz) assigned to H-4′ and H-5′ protons respectively, of the 2,2-dimethyl-3,4-dihydro-2*H*-pyrano unit. The placement of the methoxy at C-9 was similarly confirmed by NOE experiments. Compound **5** is a $\Delta^{4',5'}$ analogue of **4** and is assigned 6aR, 11aR configuration from the large negative (–240°) specific rotation [19], [20], [21]. Compound **5** was determined as 3-hydroxy-9-methoxy-6′,6′-dimethyl-4′,5′-dihydropyrano[2′,3′:1,2][6aR,11-aR]-pterocarpan, named bolucarpan D. In all the pterocarpans **2 – 5** prominent ^4J HMBC correlations were observed in the pterocarpan nucleus between H-4′ and C-1a, H-4′ and C-4, H-10 and C-6a. These couplings are normally not observed but their prominence here may be due to the structures possibly showing zig-zag or W couplings which are observed in H-H correlations.

Antimicrobial activity

The isoflavanones **1**, **7**, **8** and **13** showed highest activity against Gram-positive bacteria (Table **3**). It seems activity here is enhanced by the presence of prenyl groups at positions 6 or 8 in the A ring and 3′ or 5′ in B ring. It seems also that if there is only one prenyl unit and it is in in B ring, activity is somewhat reduced. The activity of isoflavanones against Gram-negative bacteria requires a free hydroxy at position 4′(and or 3′) and a prenyl group at either position 3′ or 5′, while that against fungi require a prenyl at position 6 or 8 in A ring and a prenyl at position 3′ or 5′ in B ring. The isoflavanone **6** has been previously reported to inhibit the growth of *Cladosporium cucumerinum* in a TLC bioassay with a loading of 2 µg of sample [9]. The pterocarpans bolucar-
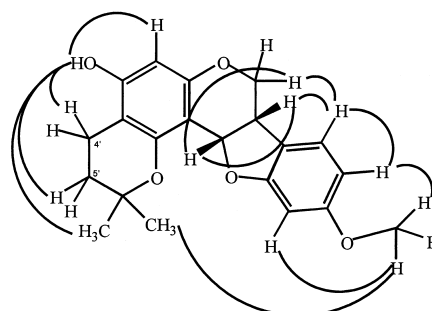
Fig. **4** Selective NOE interactions for **2** and **3** ($\Delta^{4',5'}$).

Fig. **5** Selective NOE interactions for **4** and **5** ($\Delta^{4',5'}$).

Table **3** The antibacterial and antifungal activity of the compounds using the agar overlay bioautography technique

| Compound | Microorganisms and the Minimum Inhibiting Amount in µg | | | | |
	E.coli	B.subtilis	S.aureus	C. mycoderma	S. cerevisiae
1	100	0.01	0.01	0.1	0.5
2	–	10	100	0.5	0.5
3	–	1	1	0.5	0.5
4	–	–	–	0.2	0.1
5	–	1	1	0.2	0.1
6	10.0	0.5	0.5	0.2	0.2
7	10	0.01	0.01	0.01	0.01
8	100	0.01	0.01	0.5	0.5
9	10.0	10.0	1.0	2.5	1.0
10	100	100	10	0.5	0.5
11	nt	nt	nt	nt	nt
12	5.0	0.1	0.1	0.1	0.1
13	1	0.01	0.01	0.01	0.01
14	10	10	1	10	10
Chloramphenicol	0.001	0.0001	0.0001		
Miconazole				0.0001	0.0001

Nt = not tested.

pan A-D (**2–5**), only showed moderate to weak antifungal activity, weak antbacterial activity against Gram-positive bacteria and no activity against Gram-negative bacteria.

Acknowledgements

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