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Diprenylated chalcones and other constituents from the twigs of *Dorstenia barteri* var. *subtriangularis*

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Abstract

The twigs of *Dorstenia barteri* var. *subtriangularis* yielded three diprenylated chalcones: (−)-3-(3,3-dimethylallyl)-5′-(2-hydroxy-3-methylbut-3-enyl)-4,2′,4′-trihydroxychalcone, (+)-3-(3,3-dimethylallyl)-4′,5′-[2‴-(1-hydroxy-1-methylethyl)-dihydrofurano]-4,2′-dihydroxychalcone and 3,4-(6″,6″-dimethyldihydropyrano)-4′,5′-[2‴,-(1-hydroxy-1-methylethyl)-dihydrofurano]-2′-hydroxychalcone for which the names bartericins A, B and C, respectively, are proposed. Stipulin, β-sitosterol and its 3-β-D-glucopyranosyl derivative were also isolated. The structures of these secondary metabolites were determined on the basis of spectroscopic analysis, especially, NMR spectra in conjunction with 2D experiments, COSY, HMQC and HMBC. The structural relationship of bartericins B and C was further established by the chemical cyclization of one to the other.

Keywords: *Dorstenia barteri* var. *subtriangularis*; Moraceae; Twigs; Isolation; Prenylated chalcones; Bartericins A, B and C

1. Introduction

The genus *Dorstenia* Linne (Moraceae) is made up largely of herbaceous perennials with succulent scrambling rhizomes (Berg et al., 1989). Many of them are used as anti-snakebite, anti-infection and anti-rheumatic remedies in the medicinal plant therapy in Africa, Central and South America (Abegaz et al., 2000). The leaves of *Dorstenia poinsettifolia* Engl. and *D. barteri* Bureau, for example, are used for the treatment of yaws and infected wounds (Thomas et al., 1989); while the rhizomes of *D. psilurus* Welw. are used by the Bamileke tribe in Cameroon as spices for the ethnodietary preparation called *na'a poh* (Thomas et al., 1989). The methanol extracts of *D. barteri* var. *multiradiata* were found to be active against the amastigote stages of *Leishmania*. The antileishmanial activity was traced to chalcones (Ayafor et al., 1997). Previous study of *D. barteri* var. *multiradiata* resulted in the isolation of four compounds: 5,7,4′-trihydroxy-8-prenylflavone, 4,2′,4′-trihydroxy - 3′ - prenylchalcone,4,2′,4′ - trihydroxy - 3,3′-diprenylchalcone and the bichalcone dorstenone (**1**) (Tsopmo et al., 1999). In our research program to study all species of the genus *Dorstenia* available in our subregion, we have carried out the investigation of *D. barteri* var. *subtriangularis* in order to compare the chemical constitution of these two varieties: *D. barteri*. var. *subtriangularis* and *D. barteri* var. *multiradiata*.

2. Results and discussion

The polar fraction of the twigs extract containing flavonoids was passed through Sephadex LH-20 column followed by repeated silica gel column chromatography and preparative TLC separations to give compounds **2**–**5** (Table 1). Of these, **2** was identified as stipulin by direct comparison with an authentic specimen from an earlier study (Abegaz et al., 1998).

Compound **3**, $[\alpha]_D^{25}$ −107° (MeOH; *c* 0.015), was obtained as orange powder, mp 138–140 °C and its

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Table 1
^{1}H NMR assignments of compounds **2**–**6**[a]

H	**2** $CDCl_3$	**3** CD_3COCD_3	**4** CD_3COCD_3	**4** $CDCl_3$	**5** $CDCl_3$	**6** CD_3OD
2	7.42 (*brs*)	7.58 (*d*, 2.0)	7.62 (*d*, 1.9)	7.42 (*d*, 2.0)	7.39 (*d*, 1.8)	7.18 (*d*, 2.1)
5	6.87 (*d*, 8.3)	6.94 (*d*, 8.2)	6.95 (*d*, 8.3)	6.87 (*d*, 8.3)	6.84 (*d*, 8.5)	6.74 (*d*, 8.2)
6	7.44 (*d*, 8.3)	7.55 (*dd*, 2.0, 8.3)	7.59 (*dd*, 2.0, 8.2)	7.48 (*dd*, 2.0, 8.3)	7.47 (*dd*, 1.8, 8.5)	7.20 (*dd*, 2.1, 8.5)
3′	6.43 (*brs*)	6.37 (*s*)	6.25 (*brs*)	6.43 (*s*)	6.43 (*brs*)	6.37 (*s*)
6′	7.65 (*s*)	7.99 (*s*)	8.02 (*s*)	7.72 (*s*)	7.72 (*s*)	7.67 (*s*)
α	7.46 (*d*, 15.4)	7.74 (*d*, 15.3)	7.75 (*d*, 15.3)	7.43 (*d*, 15.4)	7.43 (*d*, 15.3)	3.05 (*ddd*, 1.4, 13.1, 14.4) 2.71 (ddd, 1.6, 3.0, 14.5)
β	7.84 (*d*, 15.4)	7.82 (*d*, 15.3)	7.83 (*d*, 15.3)	7.84 (*d*, 15.3)	7.85 (*d*, 15.3)	5.36 (*dt*, 2.4, 12.9)
1″	3.42 (*d*, 7.1)	3.38 (*d*, 7.3)	3.38 (*d*, 7.1)	3.42 (*d*, 7.2)	–	–
2″	5.35 (*t*, 7.1)	5.39 (*brt*, 7.4)	5.39 (*brt*, 7.2)	5.35 (*dt*, 1.4, 7.2)	–	–
4″	1.82 (*s*)	1.77 (*s*)	1.77 (*s*)	1.83 (*s*)	2.85 (*t*, 6.7)	2.82 (*t*, 6.7)
5″	1.82 (*s*)	1.76 (*s*)	1.75 (*s*)	1.83 (*s*)	1.87 (*t*, 6.7)	1.84 (*t*, 6.7)
1‴a	3.37 (*d*, 7.1)	2.94 (*dd*, 3.4, 14.4)	3.25 (*dd*, 7.6, 15.4)	3.16 (*d*, 8.5)	3.16 (*d*, 8.7)	3.15 (*d*, 8.5)
1‴b	3.37 (*d*, 7.1)	2.86 (*dd*, 8.3, 14.4)	3.18 (*dd*, 9.7, 15.3)	3.16 (*d*, 8.5)	3.16 (*d*, 8.7)	3.15 (*d*, 8.5)
2‴	5.35 (*t*, 7.1)	4.43 (*dd*, 3.2, 8.2)	4.77 (*brt*, 7.7)	4.74 (*t*, 8.6)	4.74 (*t*, 8.7)	4.72 (*t*, 8.6)
4‴	1.82 (*s*)	5.00 / 4.82 (*brs*)	1.29 (*s*)	1.37 (*s*)	1.28 (*s*)	1.28 (*s*)
5‴	1.82 (*s*)	1.84 (*s*)	1.24 (*s*)	1.26 (*s*)	1.26 (*s*)	1.23 (*s*)
2′-OH	13.34 (*s*)	13.48 (*s*)	14.02 (*s*)	13.81 (*s*)	13.86 (*s*)	–
Me					1.39,1.38(*s*)	1.33 (*s*)

[a] Chemical shifts are given in ppm; multiplicities and coupling constant *J* (parentheses) in Hz.

molecular formula was determined as $C_{25}H_{28}O_5$ from the CIMS and ^{13}C NMR spectra. The UV–visible absorption bands at λ_{max} 204, 242 and 380 nm were suggestive of a chalcone skeleton. The ^{1}H and ^{13}C NMR data, especially, the aluminium chloride induced bathochromic shift (Mabry et al., 1970) and the IR absorption at 1629 cm^{-1} indicated that compound **3** was a 2′-hydroxychalcone. The chemical shift of the carbonyl function at δ 192.3 and the highly deshielded signal at δ_H 13.48 ppm were noted as further evidence for the conjugated carbonyl and chelated hydroxyl moieties, respectively. The ^{1}H NMR of this compound showed the presence of 10 vinyl/aryl proton resonances. Two of them form an AB system at δ 7.74 and 7.82 (*d*, $J = 15.3$ Hz); the large coupling constant indicating the *trans* geometry; three of the proton signals form an ABX-system, a doublet at δ 6.94 ($J = 8.2$ Hz) an *ortho*- and *meta*-coupled double doublet at δ 7.55 ($J = 8.3$, 2.0 Hz), and a *meta*-coupled signal at δ 7.58 ($J = 2.0$ Hz). This ABX-system could be located on ring B because of the values of the chemical shifts. Two aryl proton signals appeared as sharp singlets at δ 6.37 and 7.99 and were assigned to the *para* oriented H-3′ and H-6′, respectively, taking into consideration the downfield chemical shift of the latter signal. This ^{1}H NMR displayed a prenyl group characterized by the following chemical shift values [3.38 (*d*, $J = 7.3$ Hz, methylene), 5.39 (*t*, $J = 7.3$ Hz, olefinic proton) and 1.77, 1.76 (3H each, two olefinic methyls)]. The presence of 2-hydroxy-3-methyl-3-butenyl group in **3** was deduced from its NMR spectra which showed signals for an sp^3 oxymethine [δ_C 76.1 (*d*)], a terminal methylene [δ_H 5.00 (*s*), 4.82 (*s*), δ_C 110.3 (*t*)], an sp^2 quaternary carbon [δ 147.9 (*s*)], a vinyl methyl [δ_H, 1.84 (*s*), δ_C 17.8 (*q*)] and an ABX-system [δ_H 2.86 (*dd*, $J = 14.4$, 8.3 Hz), 2.94 (*dd*, $J = 14.4$, 3.4 Hz) and 4.43 (*dd*, 3.2, 8.2 Hz)]. This hydroxymethylbutenyl group and the dimethylallyl moieties cannot be located in the same ring since the ring B contains an ABX system and should be *o*-disubstituted at positions 3 and 4; one of the substituents is assumed, also on biogenetic grounds, to be an hydroxyl group at C-4. The hydroxymethylbutenyl group was unambiguously fixed at C-5′ from HMBC and HMQC (Table 3) studies. The downfield proton signal at δ 7.99 (H-6′) was correlated from HMQC experiments to the aromatic carbon at δ 133.8, the latter, in turn, was also correlated from HMBC to two methylene proton signals of C-1‴ (δ 2.86 and 2.94). These two sp^3 proton resonances were also correlated to C-5′ (δ 118.6) and to one oxygenated sp^2 carbon signal at C-4′ (δ 164.0). Furthermore the downfield proton signal H-6′ (δ 7.99) also showed interaction in HMBC with C-β′, C-2′ and C-4′. The methylene proton signal (δ 3.38) of the second prenyl group displayed also correlations with C-4 (δ 158.2), C-2 (δ 131.3) and C-3 (δ 129.2). Compound **3**, tentatively named bartericin A, was then characterized as (−) 3-(3,3-dimethylallyl)-5′-(2-hydroxy-3-methylbut-3-enyl)-4,2′,4′-trihydroxychalcone. Paratocarpin D (**3a**), an isomer of **3** had been isolated from *Paratocarpus venenosa*, a Moraceae plant (Hano et al., 1995). The two prenyl groups in paratocarpin D (**3a**) are located at the 3 and 3′ positions and its ^{1}H NMR differs from **3** in having an AX system of two *ortho*-coupled proton signals in ring A. The ^{13}C NMR signals (Table 2) were fully assigned using DEPT spectra and by comparison of measured values with those reported for **3a** and for

Table 2
^{13}C NMR assignments of compounds **2–6**[a]

C	**2** $CDCl_3$	**3** CD_3COCD_3	**4** CD_3COCD_3	**4** $CDCl_3$	**5** $CDCl_3$	**6** CD_3OD
1	127.9 (*s*)	127.2 (*s*)	127.2 (*s*)	127.9 (*s*)	127.0 (*s*)	129.3 (*s*)
2	131.4 (*d*)	131.3 (*d*)	131.4 (*d*)	131.4 (*d*)	131.2 (*d*)	129.4 (*d*)
3	128.1 (*s*)	129.2 (*s*)	129.2 (*s*)	128.1 (*s*)	121.8 (*s*)	124.1 (*s*)
4	157.4 (*s*)	158.2 (*s*)	158.2 (*s*)	157.3 (*s*)	157.2 (*s*)	156.1 (*s*)
5	116.8 (*d*)	115.9 (*d*)	115.9 (*d*)	116.8 (*d*)	117.6 (*d*)	118.6 (*d*)
6	128.7 (*d*)	128.5 (*d*)	128.6 (*d*)	128.6 (*d*)	128.2 (*d*)	127.0 (*d*)
α	118.2 (*d*)	117.8 (*d*)	117.7 (*d*)	118.2 (*d*)	118.4 (*d*)	45.2 (*t*)
β	144.9 (*d*)	144.8 (*d*)	144.9 (*d*)	144.9 (*d*)	145.0 (*d*)	81.6 (*d*)
β′	192.4 (*s*)	192.3 (*s*)	192.1 (*s*)	192.0 (*s*)	192.0 (*s*)	193.8 (*s*)
1′	114.8 (*s*)	113.9 (*s*)	114.1 (*s*)	114.6 (*s*)	114.6 (*s*)	116.0 (*s*)
2′	165.3 (*s*)	165.8 (*s*)	167.5 (*s*)	167.4 (*s*)	167.4 (*s*)	169.1 (*s*)
3′	104.5 (*d*)	103.9 (*d*)	97.7 (*d*)	98.8 (*d*)	98.8 (*d*)	99.0 (*d*)
4′	161.9 (*s*)	164.0 (*s*)	167.4 (*s*)	166.7 (*s*)	166.7 (*s*)	165.9 (*s*)
5′	119.1 (*s*)	118.6 (*s*)	120.2 (*s*)	119.4 (*s*)	119.3 (*s*)	118.6 (*s*)
6′	131.5 (*d*)	133.8 (*d*)	126.5 (*d*)	126.0 (*d*)	125.9 (*d*)	125.6 (*d*)
1″	30.1 (*t*)	28.5 (*t*)	28.6 (*t*)	29.7 (*t*)	–	–
2″	121.5 (*d*)	122.8 (*d*)	122.9 (*d*)	121.5 (*d*)	–	–
3″	135.8 (*s*)	132.5 (*s*)	132.3 (*s*)	136.1 (*s*)	–	–
4″	18.4 (*q*)	17.4 (*q*)	17.4 (*q*)	18.4 (*q*)	22.8 (*t*)	23.8 (*t*)
5″	26.2 (*q*)	25.4 (*q*)	25.4 (*q*)	26.2 (*q*)	33.0 (*t*)	34.1 (*t*)
6″	–	–	–	–	75.7 (*s*)	75.9 (*s*)
1‴	29.6 (*t*)	37.6 (*t*)	29.1 (*t*)	30.2 (*t*)	29.7 (*t*)	30.3 (*t*)
2‴	122.1 (*d*)	76.1 (*d*)	91.9 (*d*)	91.6 (*d*)	91.6 (*d*)	93.0 (*d*)
3‴	136.1 (*s*)	147.9 (*s*)	70.9 (*s*)	72.2 (*s*)	72.2 (*s*)	72.7 (*s*)
4‴	18.4 (*q*)	110.3 (*t*)	25.0 (*q*)	24.6 (*q*)	24.6 (*q*)	25.6 (*q*)
5‴	26.2 (*q*)	17.8 (*q*)	25.5 (*q*)	26.4 (*q*)	26.4 (*q*)	25.7 (*q*)
Me	–	–	–	–	27.3 (*q*)	27.5 (*q*)

[a] Chemical shifts are given in ppm and multiplicities in parentheses.

Table 3
^1J (from HMQC), ^2J and ^3J gradient HMBC correlations for bartericin A (**3**)

Proton	Position	^1J-correlated carbon	^2J- and ^3J-correlated carbons
13.48	2′-OH	–	C-1′, C-2′, C-3′,
7.99	6′	133.8	C-2′, C-4′, C-β′, C-1‴
7.82	β	144.8	C-1, C-2, C-α, C-β′
7.74	α	117.8	C-1, C-β, C-β′
7.58	2	131.3	C-1, C-4, C-1″, C-β
7.55	6	128.5	C-2, C-4, C-β
6.94	5	115.9	C-1, C-3, C-4
6.37	3′	103.9	C-1′, C-2′, C-4′, C-5′
5.39	2″	122.8	C-1″, C-4″, C-5″
5.00	4‴	110.3	C-2‴, C-3‴, C-5‴
4.82	4‴	110.3	C-2‴, C-5‴
4.43	2‴	76.1	
3.38	1″	28.5	C-3, C-4, C-2″, C-3″,
2.94	1‴	37.6	C-4′, C-5′, C-6′, C-2‴, C-3‴,
2.86	1‴	37.6	C-4′, C-5′, C-6′, C-2‴, C-3‴
1.84	5‴	17.8	C-2‴, C-3‴, C-4‴
1.77	5″	25.4	C-2″, C-3″, C-4″
1.76	4″	17.4	

similar compounds (Agrawal, 1989). Important HMQC and HMBC correlations of **3** are showed in Table 3.

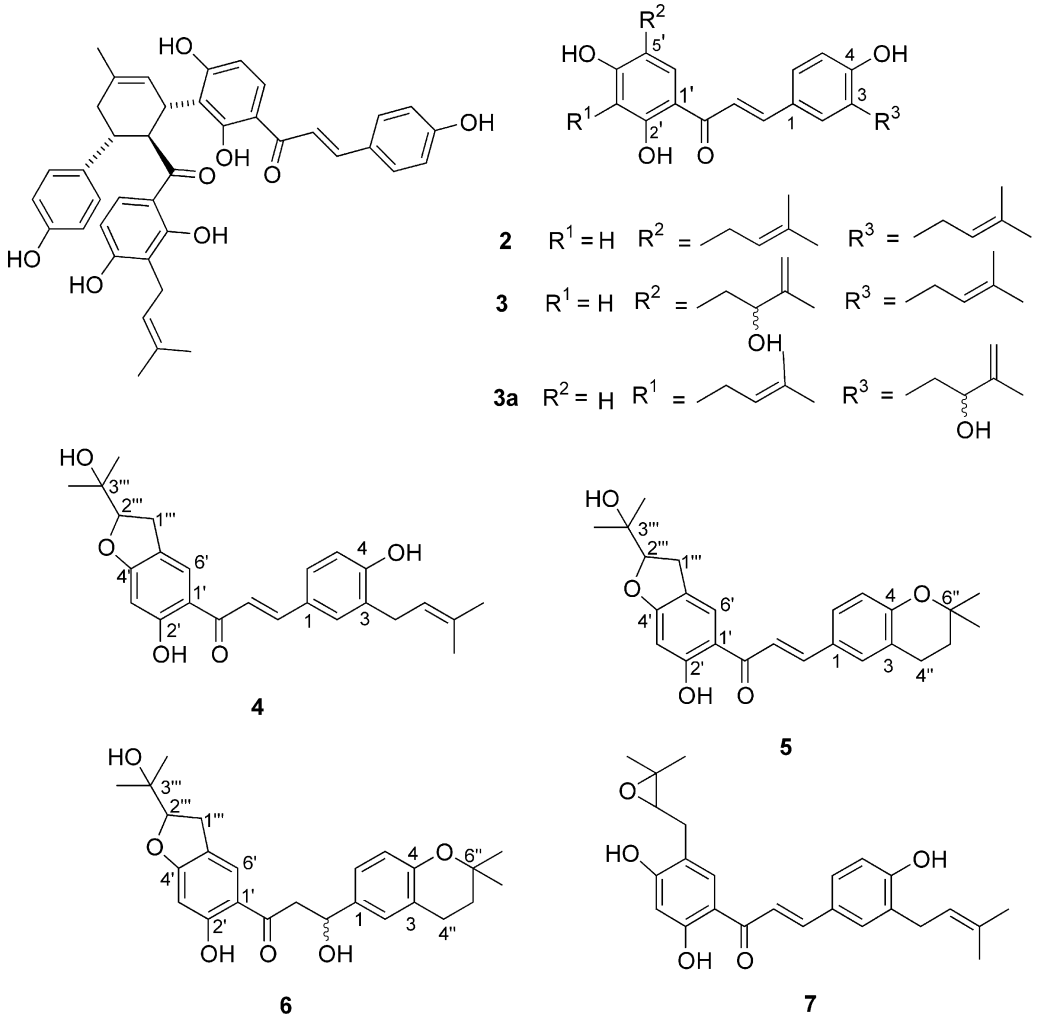
Bartericin B (**4**), $[\alpha]_D^{25}$ +125° (MeOH; *c* 0.015), was isolated as yellow plates. The IR spectrum displayed absorption bands due to hydroxyl, conjugated carbonyl and benzene ring at ν_{max} 3450, 1644 and 1570 cm^{-1}, respectively. Its molecular formula was determined as $C_{25}H_{28}O_5$ from the NMR and CIMS data $[M]^+$ at *m*/*z* 408. The NMR and UV spectra using shift reagents revealed the 2′-hydroxychalcone nature of **4**. Thus the UV-visible spectrum showed absorption bands at λ_{max} 245 and 383 nm as well as a bathochromic shift upon addition of aluminium chloride. The chelated 2′-hydroxyl proton at δ13.81 and the chemical shift of the carbonyl group at δ 192.0 were consistent with 2′-hydroxychalcone. Its ^1H NMR displayed eight aryl/vinyl proton signals; two of them form an AB system at δ 7.75 and 7.83 (*d*, *J* = 15.3 Hz), the large coupling constant is indicative of the *trans* geometry; a set of three proton signals at δ 6.95 (*d*, *J* = 8.3 Hz), 7.59 (*dd*, *J* = 8.3 and 2.0 Hz) and 7.62 (*d*, *J* = 1.9 Hz) which could be located in ring B. The two proton singlet signals at 6.25 and 8.02 ppm were assigned to H-3′ and H-6′, respectively, because of the downfield chemical shift of the latter as in the compound **2**. The ^1H NMR data (Table 1) also showed a prenyl group [δ 5.39 (*t*, *J* = 7.2 Hz, olefinic proton), 3.38 (2H, *d*, *J* = 7.1 Hz, methylene protons) and 1.77, 1.75 (3H each, olefinic methyl protons] and a 1-hydroxy-1-methylethyldihydrofuran ring [δ 1.29, 1.24 (3H each, *s*, gem-dimethyl protons), 3.25 (*dd*, *J* = 15.4, 7.6 Hz, methylene proton), 3.18 (*dd*, *J* = 15.4, 9.7 Hz, methylene proton) and 4.47 (*brt*, *J* = 7.7

Hz, oxymethine proton)]. The structure of bartericin B was determined to be (+)-3-(3,3-dimethylallyl)-4′,5′-[2‴-(1-hydroxy-1-methylethyl)-dihydrofurano]-4,2′-dihydroxychalcone (**4**). Convincing proof for structure **4** was obtained from HMBC spectra analysis (Table 4) which clearly showed correlations between H-1″, C-3, C-4 and C-3″ signals and also between H-1‴, C-4′, C-5′ and C-6′. The ^{13}C NMR (Table 2) signals were fully assigned using the DEPT spectra and by comparison of measured values with those reported (Agrawal, 1989) for similar compounds. The CIMS showed mass fragments at m/z 349 and 59 $[Me_2C{=}OH]^+$ typical for flavonoids possessing a 1-hydroxy-1-methylethyldi hydrofurano group (Tahara et al., 1984).

Bartericin C (**5**), $[\alpha]_D^{25}$ +301° (MeOH; c 0.033), was assigned $C_{25}H_{28}O_5$ as molecular formula from HREIMS measurements. Its ^1H NMR spectrum showed the signals of the following protons: ABX type aromatic protons: δ 6.84 (d, J = 8.5 Hz), 7.39 (d, J = 1.8 Hz), 7.47 (dd, J = 1.8 and 8.5 Hz); two aromatic proton

Table 4
^1J (from HMQC), ^2J and ^3J gradient HMBC correlations for bartericin B (**4**)

Proton	Position	^1J-correlated carbon	^2J and ^3J-correlated carbons
14.02	2′-OH	–	C-1′, C-2′, C-3′
8.02	6′	126.5	C-2′, C-β′, C-1‴
7.83	β	144.9	C-2, C-6, C-α, C-β′
7.75	α	117.7	C-1′, C-β, C-β′
7.62	2	131.4	C-6, C-1″
7.59	6	128.6	C-2, C-4, C-β
6.95	5	115.7	C-6, C-1′
6.25	3′	97.7	C-2′, C-5′
5.39	2″	122.9	C-4″, C-5″
4.77	2‴	91.7	
3.38	1″	28.6	C-3, C-4, C-3″
3.25	1‴a	29.1	C-4′, C-5′, C-2‴, C-3‴
3.18	1‴b	29.1	C-4′, C-5′, C-2‴, C-3‴
1.77	4″	17.4	C-2″, C-3″, C-5″
1.75	5″	25.4	C-2″, C-3″, C-4″
1.29	4‴	25.0	C-2‴, C-3‴, C-5‴
1.24	5‴	25.5	C-2‴, C-3‴, C-4‴



singlets δ 6.43 and 7.72; two olefinic *trans* protons at δ 7.43 and 7.85 (each 1H, *d*, $J=15.3$ Hz); protons in a 2,2-dimethyldihydropyran ring: δ 1.38, 1.39 (each 3H, *s*), 1.87, 2.85 (each 2H, *t*, $J=6.7$ Hz); proton in a hydrogen-bonded hydroxyl group, δ 13.86 (1H, *s*); protons in a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofurano ring δ 1.18 and 1.26 (each 3H, *s*), 3.16 (*d*, 8.7, 2H), 4.74 (*t*, 8.7, 1H) (Hano et al., 1995). The ^{13}C NMR spectrum of **5** was analysed by comparing with that of **4** (Table 2). In the spectrum, the chemical shifts of all the carbon atoms except those of ring B and isoprenoid moiety, were in good agreement with those of the relevant carbon atoms of **4**. From the foregoing data, the structure of compound **5** was determined as (+)-3,4-(6″,6″-dimethyldihydropyrano)-4′,5′-[2‴-(1-hydroxy-1-methylethyl) dihydrofurano]-2′-hydroxychalcone for which the name bartericin C is proposed. Bartericin C (**5**) was also obtained by its hemisynthesis from **4**. Acccordingly cyclization of **4** following the procedure of Hano et al. (1995) yielded bartericin C (**5**) and compound **6**.

It is possible to speculate the biosynthesis of these chalcones (**3**–**5**) from stipulin (**2**) which is in fact present in the plant. Accordingly, the epoxide intermediate (**7**) arising from **2**, could undergo ring opening reaction by loss of a proton from one of the methyl groups (4‴ or 5‴) to yield **3**; or by involvement of the 4′-OH group to form **4**. Further cyclization of this later compound at the other ring involving the 4-OH functionality can lead to compound **5**.

3. Experimental

3.1. General

M.p.s uncorr.; UV: MeOH solution; IR: KBr disk; CI and HREIMS: direct inlet 70 ev; ^{1}H and ^{13}C NMR ($CDCl_3$, CD_3COCD_3 and CD_3OD) 600 or 300 and 150 or 75 MHz, respectively, with the residual solvent peaks as internal references. COSY, HMQC and HMBC experiments were performed with gradient enhancements.

3.2. Plant material

The twigs of *D. barteri* var. *subtriangularis* were collected from Tombel in the South West Province of Cameroon in November 2001. Mr Victor Nana, of the National Herbarium in Yaounde identified the plant. Voucher specimen (19534/SRFCam) is deposited at the National Herbarium, Yaounde, Cameroon.

3.3. Extraction, isolation and characterization

The air-dried and powdered twigs of *D. barteri* var. *subtriangularis* (450 g) were macerated in the mixture of CH_2Cl_2/MeOH (1:1) and MeOH for 24 and 2 h, respectively, at room temp. These two extracts were combined. Removal of the solvent from the combined extracts under reduced pressure yielded 70 g of a dark green residue. A mass of 65 g of this organic extract was subjected to column chromatography on silica gel 60 (200 g) and eluted with petroleum ether (60/80) followed by pet. Ether–EtOAc (3:1, 1:1, 1:3) mixtures and then EtOAc to give five fractions, A–E of 500 ml each. The fr. A (5 g) eluted with pet. ether, contained mainly mixture of oils and was not investigated further. Fr. B (30.2 g) was passed through Sephadex LH-20 column and eluted with $CHCl_3$–MeOH (2:1). The post chlorophyll fr (10.5 g) was subjected to silica gel 60 (150 g) CC separations and eluted with hexane followed by hexane–EtOAc gradient. A total of 25 frs, 250 ml each, were collected and combined on the basis of TLC comparisons. Frs 4–12 obtained with hexane–EtOAc (9:1) yielded β-sitosterol (50 mg). Frs 13–25 eluted with hexane–EtOAc (7:3) were combined to give 3 g of a mixture of three compounds as shown by TLC; part (1 g) of this mixture was purified by PTLC using $CHCl_3$/MeOH (97:3) to give stipulin (**2**, 40 mg), bartericins A (**3**, 35 mg) and B (**4**, 30 mg). Combined frs C and D (4.5 g) were also passed through Sephadex LH-20 and eluted with $CHCl_3$/MeOH (2:1); the post chlorophyll frs (2 g) was subjected CC (Silica gel, 50 g) using hexane and hexane–EtOAc. Twelve frs each of 100 ml were collected. Frs 6–12, eluted with hexane–EtOAc (5:3) gave a mixture, which was purified on PTLC using $CHCl_3$–MeOH 95:5 to yield bartericin A (**3**, 5 mg) and bartericin C (**5**, 8 mg). Fraction E (10 g) gave a ppt (75 mg) in a mixture of hexane–EtOAc (1:1) which was identified as β-sitosterol glucoside by comparison with authentic specimen available in our Laboratory.

3.4. (−) 3-(3,3-Dimethylallyl)-5′-(2-hydroxy-3-methyl-but-3-enyl)-4,2′,4′-trihydroxychalcone: bartericin A (*3*)

Yellow amorphous powder from pet. ether–EtOAc mp 138–140 °C; $[\alpha]_D^{25}$ −107° (MeOH; *c* 0.015); UV λ_{max}^{MeOH} nm (log ϵ): 204 (4.69), 242 (4.16), 380 (4.56); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ϵ): 204 (4.73), 243 sh (4.18), 336 (4.09), 439 (4.60); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ϵ): no change; $\lambda_{max}^{MeOH+NaOAc}$ nm (log ϵ): 217 (5.20), 284 (4.20), 404 (4.60); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ϵ): 208 (5.07), 258 (4.16), 448 (4.73). IR ν_{max}^{KBr} cm^{-1}: 3410–3400 (OH), 1630 (C=O), 1553, 1429, 1374, 1255, 1181, 1138, 1018. ^{1}H NMR spectral data (600 MHz, $CDCl_3$): Table 1; ^{13}C-NMR spectral data (150 MHz, $CDCl_3$): Table 2. HMQC and HMBC: Table 3. CIMS (isobutane, probe) 200 ev, *m*/*z* (rel. int.): 409 $[M+H]^+$ (100), 337 $[M-C_4H_7O]^+$ (15), 219 (10), 187 (20). HREIMS: found: 408.1931; calc. for $C_{25}H_{28}O_5$: 408.1937.

3.5. (+)-3-(3,3-Dimethylallyl)-(4′,5′)-[2‴-(1-hydroxy-1-methylethyl)-dihydrofurano]-4,2′-dihydroxychalcone, bartericin B (4)

Yellow powder from pet. ether–EtOAc mp. 184–185 °C; $[\alpha]_D^{25}$ +125° (MeOH; *c* 0.015); UV λ_{max}^{MeOH} nm (log ϵ): 210 (4.42), 245 (4.10), 384 (4.47); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ϵ): 212 (4.43), 247 (4.05), 278 (3.88), 336 (4.00), 389 (4.22), 442 (4.52); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ϵ): no change; $\lambda_{max}^{MeOH+NaOAc}$ nm (log ϵ): 220 (4.50), 246 (4.10), 298 (3.92), 386 (4.40); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ϵ): 213 (4.49), 259 (4.09), 281 (4.03), 450 (4.48); IR ν_{max}^{KBr} cm^{-1}: 3415-3400 (OH), 1644 (C=O), 1568, 1483, 1406, 1245, 1153. ^{1}H NMR spectral data (600 MHz, CD_3COCD_3): Table 1; ^{13}CNMR spectral data (150 MHz, CD_3COCD_3): Table 2. CIMS (isobutane, probe) 200 ev, *m*/*z* (rel. int.): 409 $[M+H]^+$ (100), 354 (10), 349 $[M-C_3H_7O]^+$ (30), 294 (15), 270 (5), 221 $[C_{12}H_{13}O_4]^+$ (40), 188 $[C_{13}H_{16}O]^+$ (45), 175 $[C_{12}H_{15}O]^+$ (24), 59 $[C_3H_7O]^+$ (15). HREIMS: found 408.1932; calc. for $C_{25}H_{28}O_5$: 408.1937.

3.6. (+) 3,4-(6″,6″-Dimethyldihydropyrano)-4′,5′-[2‴-(1-hydroxy-1-methylethyl)-dihydrofurano]-2′-hydroxychalcone, bartericin C (5)

Yellow oil, $[\alpha]_D^{25}$ +301° (MeOH; *c* 0.033); UV λ_{max}^{MeOH} nm (log ϵ): 209 (4.36), 298 (3.80), 384 (4.30); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ϵ): 212 (4.33), 277 sh (3.79), 337 (3.84), 388 (4.48), 442 (4.35); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ϵ): no change; $\lambda_{max}^{MeOH+NaOAc}$ nm (log ϵ): 216 (4.48), 298 (3.81), 384 (4.30); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ϵ): 212 (4.44), 298 (3.84), 353 (4.69). IR ν_{max}^{KBr} cm^{-1}: 3420–3400 (OH), 1642 (C=O), 1542, 1420, 1240, 1130, 1050; ^{1}H NMR spectral data (600 MHz, $CDCl_3$): Table 1; ^{13}CNMR spectral data (150 MHz, $CDCl_3$): Table 2. CIMS (isobutane, probe) 200 ev, *m*/*z* (rel. int.): 409 $[M+H]^+$ (100), 393 $[M-CH_3]^+$ (15), 303 (10), 295 (15), 221 (10), 211 $[C_{14}H_{15}O_2]^+$ (15), 188 $[C_{13}H_{16}O]^+$ (30), 175 $[C_{12}H_{15}O]^+$ (20). HREIMS: found: 408.1940; calc. for $C_{25}H_{28}O_5$: 408.1937

3.7. Cyclisation of bartericin B (4)

A mixture of **4** (11.1 mg), 32% HCl (12 ml) and MeOH (40 ml) solution was refluxed for 6 h at 70 °C. The reaction mixture was poured into water (100 ml) and extracted with $CHCl_3$ (4x50 ml). The $CHCl_3$ extract was washed with water, dried on Na_2SO_4 and evaporated under reduced pressure. The residue 8 mg was separated and purified by PTLC to give compound **5** (3.7 mg, 33.3%) and **6** (1.1 mg, 9.5%).

3.8. (+)-3,4-(6″,6″-Dimethyldihydropyrano)-4′,5′-[2‴-(1-hydroxy-1-methylethyl)-dihydrofurano]-2′,3‴-dihydroxydihydrochalcone (6)

Yellow powder, mp 114–116 °C. $[\alpha]_D^{25}$ +414° (MeOH; *c* 0.013); UV λ_{max}^{MeOH} nm (log ϵ): 206 (4.82), 239 (4.52), 285 (4.37), 324 (4.07); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ϵ): 207 (4.91), 239 (4.66), 287 (4.51), 321 (4.28); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ϵ): no change; $\lambda_{max}^{MeOH+NaOMe}$ nm (log ϵ): 210 (5.21), 239 (4.54), 288 (4.38), 325 (4.17). IR ν_{max}^{KBr} cm^{-1}: 3440-3415 (OH),1620 (C=O), 1540, 1475, 1385, 1255, 1140. ^{1}H NMR spectral data (300 MHz, CD_3OD): Table 1; ^{13}C-NMR spectral data (75 MHz, CD_3OD): Table 2. CIMS (isobutane, probe) 200 ev, *m*/*z* (rel. int.): 409 $[MH_2O+H]^+$ (100), 393 $[M-H_2O-CH_3]^+$ (10), 357 (10), 221 (15), 188 $[M-C_{12}H_{12}O_4-H_2O]^+$ (80), 161 (35), 133 (45).

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