A New Diels−Alder-Type Adduct Flavonoid from *Dorstenia barteri*

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A new Diels−Alder-type adduct, dorstenone (**1**), was isolated from *Dorstenia barteri* together with three known flavonoids, 4,2′,4′-trihydoxy-3′-prenylchalcone; 4,2′,4′-trihydoxy-3,3′-diprenylchalcone; and 5,7,4′-trihydoxy-8-prenylflavone. The structure of **1** was elucidated using a combination of highfield NMR techniques, particularly, gradient-enhanced HMQC and HMBC.

The genus *Dorstenia* L. (Moraceae) is represented in Cameroon by 23 species made up largely of herbaceous perennials with succulent scrambling rhizomes.[1] Many of them have local traditional uses. The whole plants of *D. poinsettifolia* var. *angusta* Engl. and *D. barteri* Bureau, for example, are used in folk medicine for the treatment of yaws and infected wounds.[2] We have recently examined the constituents of *D. poinsettifolia* and reported the isolation of the novel geranylated flavonoids, poinsettifolins A and B, along with licoflavone C, isobavachromene, isobavachalcone, and the terpenoids, butyrospemol and lutein.[3] In continuation of our work on this important genus, we have investigated the whole plant extracts *of D. barteri* and report herein the isolation of a new Diels−Alder flavonoid adduct to which we have given the trivial name dorstenone (**1**), in addition to a known flavone, and two known chalcones.

A MeOH−CH_2Cl_2 (1:1) extract of the whole plants of *Dorstenia barteri* was subjected to sequential partition extraction with *n*-hexane and CH_2Cl_2. Vacuum liquid chromatography of the combined *n*-hexane and CH_2Cl_2 extracts followed by further purification by MPLC and Sephadex LH-20 gel filtration resulted in the isolation of four compounds, including the new Diels−Alder flavonoid adduct, dorstenone (**1**).

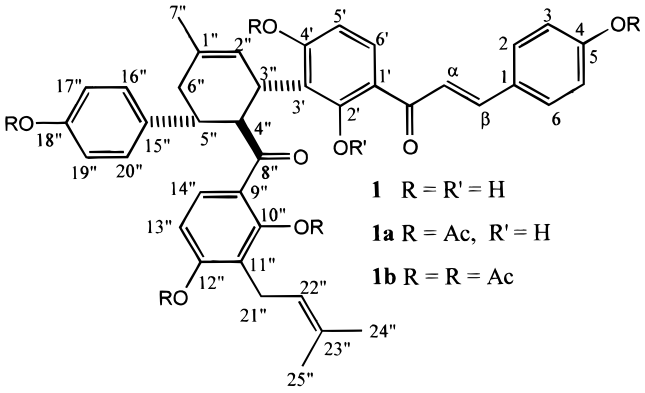
Compound **1** was obtained as a yellow amorphous powder, $[\alpha]_D$ −371°. The FABMS of **1** showed a protonated molecular ion at $[M + 1]^+$ 647, and the ^{13}C NMR spectrum (Table 1) indicated the presence of 40 carbon atoms. These observations are consistent with the molecular formula $C_{40}H_{38}O_8$. Treatment of **1** with a mixture pyridine−Ac_2O (1:1) gave a mixture of pentaacetylated (**1a**) and hexaacetylated (**1b**) derivatives. The ^1H NMR spectrum of **1a** showed a chemical shift at δ 12.70 and the IR spectrum an absorption at ν_{max} 3350 cm^{-1}, indicating that one chelated hydroxyl group was not acetylated.

The UV spectrum of dorstenone (**1**) exhibited absorption maxima at λ 225, 292, and 370 nm. The addition of $AlCl_3$ showed no red shift as reported for related compounds with no free *ortho* position to a chelated hydroxyl group.[4−6] Its IR spectrum showed strong absorption bands for hydroxyl, carbonyl, and benzene rings at ν_{max} 3350, 1600, and 1500 cm^{-1}, respectively. The ^1H NMR spectrum of **1** was well resolved and showed signals for a 3,3-dimethylallyl group at δ 1.51 and 1.59 (3H each, s), 3.05 (2H, br d, J = 6.4 Hz), and 4.95 (1H, m).[7] The ^1H NMR spectrum also indicated the presence of two 1,4-disubstituted and two 1,2,3,4-tetrasubstituted benzene rings along with a trans double bond at δ 7.61 and 7.17 (1H each, d, J = 15.3 Hz) characteristic of a chalcone.[7] In the ^{13}C NMR spectrum, the two carbonyl adsorptions at δ 208.8 and 192.9 were assigned to benzophenone and chalcone moieties, respectively. The 2D experiments, COSY, HMQC, and HMBC enabled us to complete the elucidation of the gross structure of dorstenone (**1**), although its stereochemistry still requires clarification.

Table 1. ^{13}C NMR Data (125 MHz) of Compounds **1**,[a] **1a**,[b] and **1b**[b]

carbon	**1**	**1a**	**1b**	carbon	**1**	**1a**	**1b**
1	128.3	128.6	128.6	6″	39.3	38.6	38.7
2	129.7	128.9	129.7	7″	23.0	23.1	23.1
3	114.7	121.4	122.1	8″	208.8	208.3	n.o.[c]
4	159.4	151.4	152.0	9″	113.3	118.4	121.5
5	114.7	121.4	122.1	10″	161.7	152.4	152.3
6	129.7	128.9	129.7	11″	115.8	121.9	121.5
α	115.7	124.9	125.0	12″	161.3	152.3	151.6
β	144.0	144.4	143.7	13″	106.4	112.6	119.6
C=O	192.9	190.2	189.3	14″	130.4	127.7	130.6
1′	113.9	122.1	121.4	15″	126.3	132.8	132.3
2′	164.8	162.0	152.0	16″	131.7	129.6	129.7
3′	117.2	122.1	120.9	17″	117.1	122.0	122.1
4′	161.8	154.3	152.0	18″	154.3	152.2	149.3
5′	107.2	112.7	119.4	19″	117.1	122.0	122.1
6′	128.4	128.1	129.7	20″	131.7	129.6	129.7
1″	135.1	132.8	132.3	21″	21.2	22.4	23.7
2″	123.9	122.9	123.1	22″	121.8	120.9	120.8
3″	37.9	39.9	38.7	23″	131.8	131.8	131.1
4″	47.3	49.1	52.5	24″	25.3	25.3	25.4
5″	44.1	42.9	38.7	25″	17.8	17.7	17.7

[a] In $CDCl_3$−CD_3OD (9:1). [b] In $CDCl_3$. [c] Not observed.



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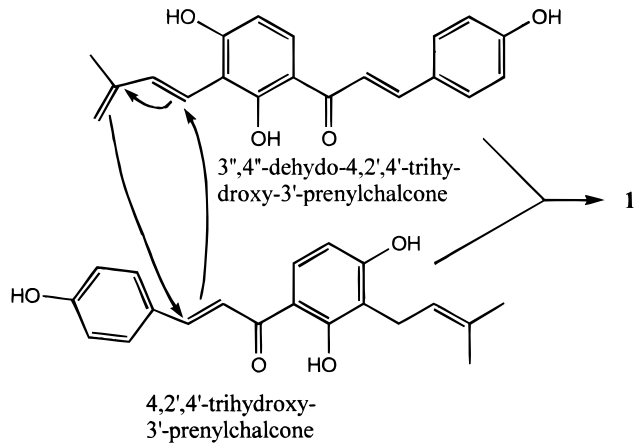

Figure 1. Formation of dorstenone (**1**).

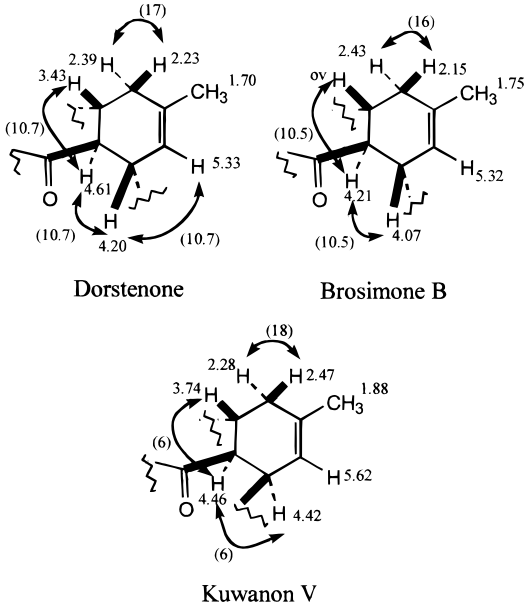

Figure 2. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) of the substituted cyclohexene ring dorstenone [run in $CDCl_3-CD_3OD$ (9:1)], brosimone B [run in $(CD_3)_2CO$)], and kuwanon V [run in $(CD_3)_2CO$]; ov = overlapped.

Dorstenone (**1**) is a Diels−Alder-type adduct (Figure 1) formed between the chalcone, 4,2′,4′-trihydoxy-3′-prenylchalcone,[8] also obtained in this study, and its dehydro derivative with three chiral centers in the methylcyclohexene ring. The relative configuration of dorstenone (**1**) has been established by comparison of the ^{1}H and ^{13}C NMR data of its methylcyclohexene ring with those of the corresponding ring in brosimone B[9] and kuwanon V,[7] all of known stereochemistry (Figure 2). The coupling constants $J_{3'',4''}$ and $J_{4'',5''}$ were found to have almost the same magnitude in **1** (10.7 Hz) and in brosimone B (10.5 Hz). In kuwanone V in which the two pairs of protons H-3″,H-4″ and H-4″,H-5″ are both cis oriented, $J_{3'',4''}$ and $J_{4'',5''}$ have the smaller compatible coupling constant (6 Hz). These data led to the conclusion that H-3″ and H-4″ as well as H-4″ and H-5″ are trans oriented, thus confirming the relative stereochemistry proposed in **1**. The configuration of **1** was further confirmed by the NOESY experiment. In this experiment, correlations were observed between H-4″ and H-6″α, and between H-5″,H-3″, and H-6″β. Dorstenone is thus the 3″-epimer of kuwanone V.[10] In addition, comparison of the optical rotations of **1** ($[\alpha]_D$ −371°) with that of kuwanone V ($[\alpha]_D$ +145°) confirmed the diastereoisomeric relationship of the two compounds and further supported structure **1** for dorstenone. Finally, it was observed from the ^{1}H NMR spectrum that **1** undergoes epimerization in solution. This behavior coupled with the high negative optical rotation is typical of these complex flavonoid type adducts.[5,6,9−11]

The others flavonoids were identified as 4,2′,4′-trihydroxy-3′-prenylchalcone;[8] 4,2′,4′-trihydroxy-3,3′-diprenylchalcone;[12] and 5,7,4′-trihydroxy-8-prenylflavone,[3,13] by comparison of their spectroscopic and physical data with those reported in the literature.

Experimental Section

General Experimental Procedures. All melting points were recorded with a Reichter microscope and are uncorrected. The optical rotations were measured with a Perkin−Elmer 141 polarimeter at 22 °C, while the UV and the IR spectra were recorded with a Varian Cary 2290 and a Perkin−Elmer 298 spectrometer, respectively. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ or in $CDCl_3-CD_3OD$ (9:1) using a Bruker ARX500 spectrometer with an inverse multinuclear 5-mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in parts per million with the solvent signals δ_H 7.26 and δ_C 77.0 as reference, while the coupling constants (J) are given in Hertz. COSY, HMQC, and HMBC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. MS were recorded with a JEOL SX102 spectrometer at 70 eV. Elemental analysis was performed at Lund Institute of Technology. Column chromatography was run on Merck Si gel 60 and gel permeation chromatography on Sephadex LH-20, while TLC was carried out on Si gel GF_{254} precoated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C or by visualizing with a UV lamp at 254 and 366 nm.

Plant Material. The whole plants of *Dorstenia barteri* Bureau were collected in the Korup Forest Reserve, Southwest Province, Cameroon, in January 1995. Mr. Paul Mezili, a retired botanist of the Cameroon National Herbarium, authenticated the voucher specimens (BUD 0265) that have been deposited at the Herbarium of the Botany Department of the University of Dschang.

Extraction and Isolation. The air-dried powdered plant material (2 kg) was extracted by percolation with a mixture of $MeOH-CH_2Cl_2$ (1:1) (5 L) to yield a crude extract (150 g) on evaporation in vacuo. The crude organic extract was suspended in 80% aqueous MeOH (2 L) and extracted three times with *n*-hexane (1 L). The resulting aqueous MeOH was diluted with H_2O to 70% MeOH (2 L) and extracted three times with CH_2Cl_2 (500 mL). The CH_2Cl_2 (40 g) and *n*-hexane (22 g) extracts were qualitatively very similar after TLC analysis. They were thus combined, and a portion (50 g) was subjected to vacuum liquid chromatography on Si gel (70−200 mesh) eluting with mixtures of hexane−Me_2CO (from 9:1, via 4:1, to 3:2) and finally with pure Me_2CO. Four successive fractions were obtained: I [5.0 g, *n*-hexane−Me_2CO (9:1)], II [3.3 g *n*-hexane−Me_2CO (4:1)], III [8.9 g *n*-hexane−Me_2CO (3:2)], and IV (3.6 g, Me_2CO). Further purification of these fractions was achieved by MPLC using a Baeckström Separo AB column (i.d., 15 mm) with a continuous gradient of *n*-hexane−Me_2CO. Fraction I yielded compounds 4,2′,4′-trihydroxy-3′-prenylchalcone (150 mg) and 4,2′,4′-trihydroxy-3,3′-diprenylchalcone (200 mg). Fraction II afforded 5,7,4′-trihydroxy-8-prenylflavone (220 mg), while a mixture

containing mainly **1** (6.9 g) was obtained from fraction III. Fraction IV also gave a mixture of three compounds that could not be resolved. For each of the compounds, an additional purification by Sephadex LH-20 gel permeation chromatography using *n*-hexane−CH_2Cl_2 (1:4) for 4,2′,4′-trihydroxy-3′-prenylchalcone and 4,2′,4′-trihydroxy-3,3′-diprenylchalcone and CH_2Cl_2−MeOH (1:1) for **1** and 5,7,4′-trihydroxy-8-prenylflavone was required to obtain pure analytical samples.

Dorstenone (1): yellow amorphous powder; $[\alpha]^{22}_D$ −371° (*c* 0.62, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (4.81), 241 (4.49), 292 (4.48), 370 nm (4.72); UV (MeOH + $AlCl_3$) λ_{max} (log ϵ) 225 (4.88), 241 (4.79), 283 (4.79), 390 (sh, 4.71), 425 nm (4.71); IR (KBr) ν_{max} 3350 (OH), 1600, 1550, 1500, 1480, 1360, 1305, 1285, 1220, 1160, 1095, 1020, 825, 790 cm^{-1}; ^{1}H NMR ($CDCl_3$−CD_3OD, 500 MHz) δ 1.51 (3H, s, H-25″), 1.59 (3H, s, H-24″), 1.70 (3H, s, H-7″), 2.23 (1H, m, H-6″α), 2.39 (1H, m, H-6″*β*), 3.05 (2H, br d, *J* = 6.4 Hz, H-21″), 3.34 (1H, m, H-5″), 4.20 (1H, br t, *J* = 10.7 Hz, H-3″), 4.61 (1H, br t, *J* = 10.7 Hz, H-4″), 4.95 (1H, m, H-22″), 5.98 (1H, d, *J* = 8.9 Hz, H-5′), 6.09 (1H, d, *J* = 8.8 Hz, H-13″), 6.53 (2H, d, *J* = 8.5 Hz, H-17″, H-19″), 6.76 (2H, d, *J* = 8,5 Hz, H-3, H-5), 7.01 (2H, d, *J* = 8.5 Hz, H-16″, H-20″), 7.17 (1H, d, *J* = 15.3 Hz, H-α), 7.30 (1H, d, *J* = 8.8 Hz, H-14″), 7.32 (1H, d, *J* = 8.9 Hz, H-6′), 7.61 (1H, d, *J* = 15.3 Hz, H-*β*) (when the NMR spectrum was run 4 h after dissolution of sample, a doubling of peaks was observed); ^{13}C NMR ($CDCl_3$−CD_3OD, 125.8 MHz) see Table 1; FABMS *m*/*z* $[M + 1]^+$ 647 (60), 591 (2), 527 (3), 469 (11), 441 (18), 325 (17), 323 (17), 307 (31), 289 (16) 269 (8), 205 (62), 203 (22), 154 (100), 149 (95), 136 (76), 110 (14), 107 (31), 89 (20), 77 (20), 39 (8); *anal*. C 74.26%, H 5.93%, calcd for $C_{40}H_{48}O_8$, C 74.28%, H 5.92%.

Acetylation of 1. A solution of dorstenone (**1**) (75 mg) was treated with pyridine (2 mL) and Ac_2O (4 mL). The reaction mixture was left at room temperature overnight and then concentrated with addition of toluene (3 × 10 mL) to give a residue that displayed two spots on TLC. Purification on a Si gel column with pure CH_2Cl_2 yielded compounds **1a** (79 mg) and **1b** (13 mg).

Pentaacetyldorstenone (1a): pale yellow amorphous powder; $[\alpha]^{22}_D$ −195° (*c* 1.3, $CHCl_3$); IR (KBr) ν_{max} 3400 (OH), 1760 (C═O), 1600, 1410, 1190, 1080, 1020, 905, 800 cm^{-1}; ^{13}C NMR ($CDCl_3$−CD_3OD, 125.8 MHz) see Table 1; EIMS (70 eV) *m*/*z* $[M]^+$ 856 (5), 814 (29), 772 (11), 737 (14), 567 (18), 525 (15), 483 (5), 407 (19), 365 (19), 363 (19), 349 (9) 323 (10), 289 (11), 247 (91), 205 (100), 189 (7), 187 (8), 149 (62), 147 (14), 107 (9), 43 (13).

Heaxaacetyldorstenone (1b): pale yellow amorphous powder; $[\alpha]^{22}_D$ −134° (*c* 0.92, $CDCl_3$); IR (KBr) ν_{max} 1760 (C═O), 1600, 1500, 1420, 1370, 1190, 1165, 1020, 905 cm^{-1}; ^{13}C NMR ($CDCl_3$−CD_3OD, 125.8 MHz) see Table 1; FABMS *m*/*z* $[M + 1]^+$ 899 (5), 857 (16), 839 (14), 797 (10), 737 (7), 595 (5), 567 (8), 525 (5), 409 (41), 311 (22), 289 (28) 247 (41), 205 (100), 149 (90), 147 (33), 107 (17), 43 (30).

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