Gaboroquinones A and B and 4′-*O*-Demethylknipholone-4′-*O*-*β*-D-glucopyranoside, Phenylanthraquinones from the Roots of *Bulbine frutescens*

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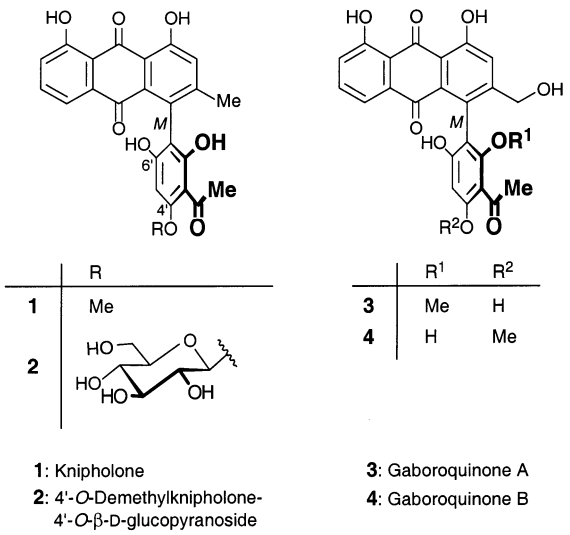
The novel phenylanthraquinones 4′-*O*-demethylknipholone-4′-*O*-*β*-D-glucopyranoside (**2**) and gaboroquinones A (**3**) and B (**4**) were isolated from the African medicinal plant *Bulbine frutescens*. Biaryl **2** represents the first phenylanthraquinone glucoside, while **3** and **4** are the first side-chain-hydroxylated phenylanthraquinones. Their constitutions were determined by spectroscopic analysis, in particular by HMBC, HMQC, and ROESY NMR investigations, and by chemical transformations. The axial configurations were elucidated chemically, by deglucosylation of **2** and by side-chain deoxygenation of **3** and **4** to give the known phenylanthraquinones 4′-*O*-demethylknipholone (**5**), isoknipholone (**6**), and knipholone (**1**), respectively, and chiroptically, by CD investigations. Compounds **2**, **3**, and **4** showed moderate to good antiplasmodial and antitrypanosomal activities in vitro.

Bulbine frutescens (L.) Wild (Asphodelaceae) is an ornamental herb that grows widely, e.g., in the city of Gaborone, Botswana. It is also used medicinally to enhance the healing of wounds.[1] As part of our continuing program to investigate secondary metabolites from marketed plants, we have reported the presence of anthraquinones, phenylanthraquinones, and isofuranonaphthoquinones in *B. capitata* and *B. abyssinica*.[2−5] Phenylanthraquinones are a new class of antiplasmodial substances. The few that have been tested so far show activities comparable to or only slightly weaker than the commercial drug chloroquine.[6] The cooccurring isofuranonaphthoquinones have been found to have antioxidant and also mild antiplasmodial properties.[5] The first phenylanthraquinone, knipholone (**1**), was reported from *Kniphofia foliosa*[7] in 1984 and has been found subsequently in other *Kniphofia*,[8] *Bulbinella*,[8] *Bulbine* (all Asphodelaceae),[9−11] and *Senna* species (Fabaceae).[12] Recent reports have indicated that the genus *Bulbine* produces a greater diversity of phenylanthraquinones: eight knipholone-type phenylanthraquinones have been described so far, differing in the *O*-methylation pattern of the acetylphloroglucinol part and/or the oxidation state of the anthraquinone moiety.[2−4,7,11,13,14] All of these constitutionally unsymmetric biaryls are optically active ($[\alpha]_D \neq 0°$) and thus stereochemically stable. The antiplasmodial activity of the knipholones appears to be associated intrinsically with the complete molecular array of a phenylanthraquinone (including the stereogenic axis), since neither chrysophanol nor phloroacetophenone possesses significant antiplasmodial activity.[6] Structurally related, but simplified synthetic substances have also been tested and have been found to be devoid of activity (IC_{50} values >10 μm).[6] The absolute configuration of **1** as *M* was determined by quantum chemical CD calculations.[15] Recently, a first, atropo-enantioselective total synthesis was developed for knipholone and 6′-*O*-methylknipholone, which has the same stereo-orientation at the axis (although, for formal reasons, being *P*-configured).[16,17] The present study on *B. frutescens* was undertaken in an attempt to search for more knipholone-type structures and to investigate their antiparasitic properties. Three such novel compounds have been isolated now (**2**−**4**), and this report describes their structural elucidation and antiprotozoal properties.



1: Knipholone
2: 4′-*O*-Demethylknipholone-4′-*O*-β-D-glucopyranoside
3: Gaboroquinone A
4: Gaboroquinone B

Results and Discussion

The roots of *B. frutescens* were successively extracted with $CHCl_3$ and MeOH. After evaporation of the solvent, the gummy organic extract was subjected to various fractionation and separation procedures to yield the known metabolite (*M*)-knipholone (**1**), along with the new compounds (*M*)-4′-*O*-demethylknipholone-4′-*O*-*β*-D-glucoside (**2**) and gaboroquinones A (**3**) and B (**4**).

Compound **2** was isolated as a red pigment. FABMS indicated a protonated molecular ion peak at *m*/*z* 583.1466

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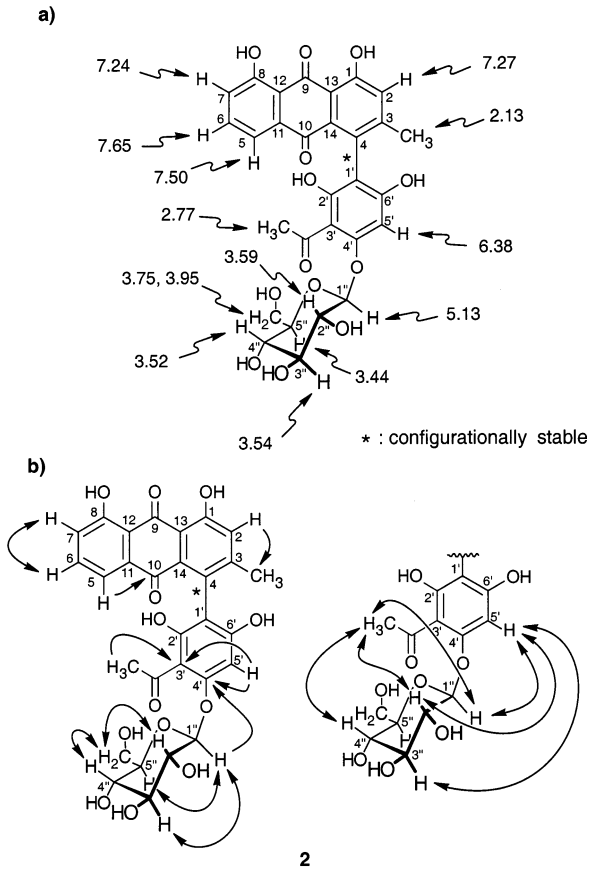


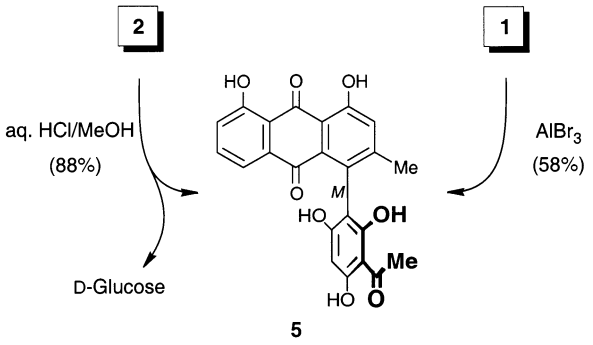
Figure 1. Selected ^{1}H NMR shifts (δ values in ppm) (a) as well as HMBC (single arrows) and ROESY interactions (double arrows) (b) of the glycoside **2**.

(calcd for $C_{29}H_{27}O_{13}$ 583.1452) $[MH]^+$. The IR spectrum showed a broad band at 3421 cm^{-1} for free OH groups and a sharp (C=O stretch) peak at 1627 cm^{-1}. As in the case of knipholone, the ^{1}H NMR spectrum of **2** (Figure 1) displayed an ABC pattern for the protons H-5, H-6, and H-7 at δ 7.50, 7.65, and 7.24, respectively. The singlet in the aromatic region at position 7.27 was attributed to H-2. Other singlets in the ^{1}H NMR spectrum at δ 6.38 (1H), 2.77 (3H), and 2.13 (3H) were assigned as the aromatic proton H-5′, an aryl acetyl (at C-3′), and an aryl methyl group (at C-3), respectively. The *O*-glycosidic nature of this compound became evident from a doublet at δ 5.13 (7.7 Hz), attributed to an anomeric proton (H-1″), and additional signals that were observed in the region from δ 3.00 to 4.00. These signals were well resolved in CD_3OD and were easily assigned to glucose. The ^{13}C NMR data displayed also the expected number of carbons and chemical shifts for glucose, which were confirmed by ROESY interactions and by hydrolysis and TLC comparison with an authentic sample of glucose (see below).

The position of the glucose unit was deduced unambiguously from ROESY experiments, which showed interactions of H-1″, H-2″, and H-3″ or H-4″ with both H-5′ and the $COCH_3$ protons. Therefore, the sugar unit is situated between C-3′ and C-5′, i.e., at position C-4′, and the new glycoside has the constitution shown in Figure 1.

An assignment of the absolute configuration of **2** to be identical to that of knipholone (**1**) was attained by their conversion (see Scheme 1) to configurationally authentic *O*-demethylknipholone (**5**). Thus, aglycon **5**, as prepared by deglucosylation of **2**, proved to be identical to the material obtained by *O*-demethylation of **1**, spectroscopically and with respect to its optical rotation and CD data.

Scheme 1. Stereochemical Identity of Glycoside **2** and (+)-Knipholone (**1**) by Their Joint Conversion to 4′-*O*-Demethylknipholone (**5**)



The new glycoside **2** is therefore stereochemically identical to **1** and **5** and is *M*-configured. As well as leading to the phenylanthraquinone **5**, the cleavage of **2** expectedly also gave D-glucose, identical to an authentic sample by ^{1}H NMR, $[\alpha]_D$, and co-chromatography on TLC. Phenylanthraquinone **2** represents the first D-glucoside of a phenylanthraquinone. During our ongoing work, Karuso's group reported on a related glycoside with D-gentobioside at *O*-8 and knipholone as the aglycon.[11]

Compound **3** was obtained as a red pigment; HRMS *m*/*z* 450.0954 (calcd for $C_{24}H_{18}H_9$, 450.0950). The aromatic region of the ^{1}H NMR spectrum (Figure 2) showed a pattern similar to that of knipholone (**1**), viz., an ABC pattern for the protons in the 5-, 6-, and 7-positions at δ 7.59, 7.78, and 7.33, respectively, and a singlet at δ 7.72. The spectrum also displayed a set of signals (Ar−CO−Me, −OMe, and ArC−H) typical of the acetylphloroglucinol unit observed in phenylanthraquinones such as knipholone (**1**).[7] The molecular ion peak was observed at *m*/*z* 450, which is 16 mass units higher than that of knipholone (MW = 434) and hinted at the presence of an additional oxygen atom. A noticeable difference in the proton NMR spectrum of **3** was the absence of the aromatic methyl signal at ca. δ 2.1 usually observed in phenylanthraquinones (e.g., the peak at δ 2.13 for **2**) and the appearance of two, one-proton doublets at δ 4.22 and 4.51 for **3**, instead. The above data suggest that **3** consists of an oxygenated, i.e., aloe-emodin-like, anthraquinone unit linked to an acetylphloroglucinol methyl ether.

From 2-D NMR experiments, e.g., by the HMBC and HMQC interactions of the chelated OH group and of H-2 with C-1, the site of attachment of the phloroglucinol portion to the anthraquinone was unambiguously located at C-4. The high-field shift of the methoxy signal in **3** (δ 3.43) is consistent with proximity to the biaryl axis and thus with the location of the methyl ether unit in the 2′-position of the acetylphloroglucinol part, as observed for isoknipholone.[2,14] Irradiation of these OMe protons showed signal enhancement of the $COCH_3$ protons and of the CH_2OH protons. Consequently, on the basis of further HMBC, HMQC, and ROESY results (see Figure 2), gaboroquinone A must have the structure shown, i.e., that of an isoknipholone additionally oxygenated on the side chain. Through extensive 1- and 2-D NMR experiments, including COSY, HMQC, HMBC, and NOE (see Figure 2) measurements, complete assignments of the proton and carbon signals were achieved. This is the first example of a natural phenylanthraquinone further oxygenated in the methyl group of the anthraquinone part, which is thus an aloe-emodin rather than a chrysophanol building block.

As to the axial configuration, the close similarity of the CD spectra of gaboroquinone A (**3**) with those of authentic

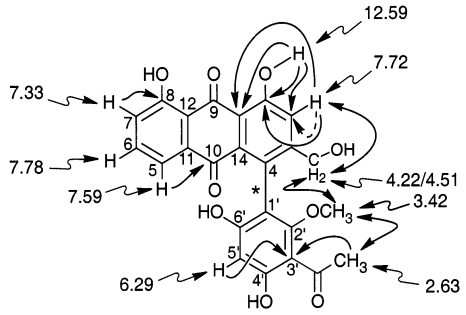


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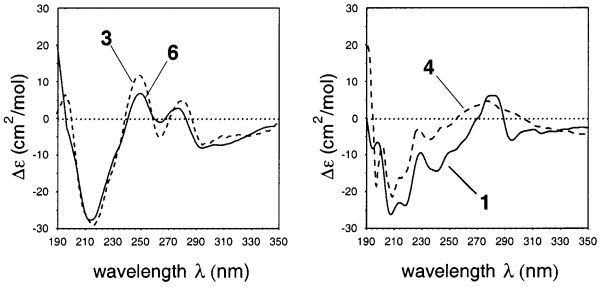
Figure 2. Selected ^{1}H NMR shifts (δ values in ppm) as well as HMBC (single arrows), HMQC (broken arrow), and ROESY interactions (double arrows) indicative of the structure of gaboroquinone A.

Scheme 2. Absolute Configurations of **3** and **4** by CD Comparison with Isoknipholone (**6**) and Knipholone (**1**) and by Reductive Side-Chain Deoxygenation of **3** and **4** to Give **6** and **1**, Respectively



	R^1	R^2
3	Me	H
4	H	Me

	R^1	R^2
6	Me	H
1	H	Me

knipholone (**1**) and naturally occurring isoknipholone (**6**) (see Scheme 2) suggests that these compounds are stereochemically identical, with the $COCH_3$ group above the anthraquinone plane, and thus *M*-configured. The stereostructure of **3** was confirmed by reductive deoxygenation of its side chain, using a two-step procedure according to a previously established protocol,[16−18] to give isoknipholone (**6**). Despite the low yield, the product was established to be identical with an authentic sample from *Bulbine capitata*,[2] by its spectral and chiroptical data and by cochromatography.

Compound **4** was obtained as a red pigment, mp 152−159 °C; $[\alpha]_D^{25}$ +134° (*c* 0.010, EtOH). The spectroscopic properties of this substance were very similar to those of gaboroquinone A (**3**), and further analysis of the MS and ^{13}C NMR data revealed clearly that the two compounds were isomeric. However, it was observed that the methoxy group of the acetylphloroglucinol unit in **4** resonated at lower field than that of **3**. This difference was accounted for by the structure shown in Figure 3, where the methoxy group is now located at C-4′ and thus is no longer subject to a high-field shift from the proximate anthraquinone portion at C-1′. A similar shift difference was observed previously for knipholone (**1**; 2′-OH, 4′-OMe) and isoknipholone (**6**; 2′-OMe, 4′-OH).[2,14] The proton and carbon resonances were completely assigned using extensive 1-D and 2-D NMR measurements. The constitution and configuration of **4** were also confirmed by reductive deoxygenation, furnishing knipholone (**1**). This reduction confirmed the stereochemical identity of these two compounds.

Table 1. Bioactivities of **1** and the New Compounds **2**−**4**

	IC_{50} (μg/mL)			
	1	**2**	**3**	**4**
P. falciparum (strain K1) standard: chloroquine 0.057	0.67	0.41	4.8	>5.0
P. falciparum (strain NF54) standard: chloroquine 0.0049	0.91	0.43	4.2	>5.0
T. cruzi standard: benznidazole 0.53	7.6	6.8	33.1	>90
T. b. rhodesiense standard: melarsoprol 0.0009	9.3	0.7	5.1	45.5
cytotoxicity L6 (MIC) standard: >90	33	90	>90	>90

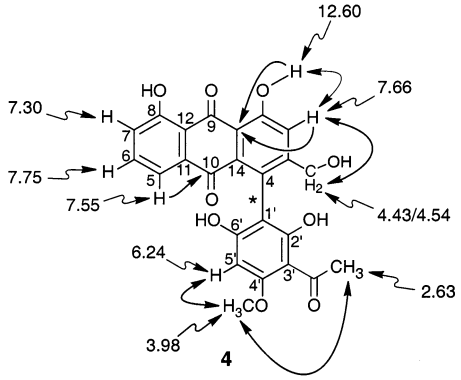


Figure 3. Constitution of gaboroquinone B (**4**) as deduced from selected ^{1}H NMR shifts (δ values in ppm) and from HMBC (single arrows) and ROESY interactions (double arrows).

Recently, knipholone (**1**) and related phenylanthraquinones showed activity against *Plasmodium falciparum*,[6] the pathogenic agent of malaria.[19] The new glycoside **2** displayed better activity against the chloroquine- and pyrimethamine-resistant K1 strain and against the sensitive strain NF54 (sensitive to all known drugs) as compared to **1**[6] and was less active than the standard only by a factor of 7 (K1) (Table 1). The phenylanthraquinone **3**, by contrast, was only weakly active, while its isomer **4** did not show any effect at 5.0 μg mL^{-1} (highest concentration tested). None of these compounds exhibited any cytotoxic effects on mammalian cells below 90 μg mL^{-1} (highest concentration tested).

The new natural products **2**−**4** were tested in vitro against two other protozoan parasites, *Trypanosoma cruzi*, which is the pathogen of the South American Chagas disease, and *T. brucei rhodesiense*, which causes African sleeping sickness (Table 1). Compounds **2** and **3** displayed weak activity against *T. cruzi*, and **2** was slightly active against *T. b. rhodesiense*, showing the antiplasmodial activity to be quite pathogen-specific. The in vitro activities against these pathogenic agents warrant further research into the activities of other phenylanthraquinones.

Experimental Section

General Experimental Procedures. Flash chromatography was carried out using columns packed with silica gel 60 (particle size 0.040−0.063 mm). For preparative TLC, plates

(0.5 mm thick) were prepared using either silica gel $60PF_{254+366}$ (Merck, Germany) or silica gel $60PF_{254}$ containing $CaSO_4$ (Merck, Germany) on 20 × 20 cm glass plates. Spots were detected under UV light (at 254 nm) and by spraying plates with vanillin−H_2SO_4 or 5% KOH in MeOH and heating. Optical rotations were taken on a Perkin-Elmer 241MC polarimeter (25 °C, 10 cm cell). UV−vis spectra were taken on a Shimadzu UV-250 recording spectrophotometer; IR spectra on a Perkin-Elmer 1429 spectrophotometer; and CD spectra (25°, EtOH, 0.1 cm cell) on a JASCO J-715 spectropolarimeter. ^{1}H NMR (600.13 MHz) and ^{13}C NMR (150.9 MHz) spectra were measured on a Bruker DMX 600 instrument using CD_3COCD_3 (δ 2.05 and 29.82) and CD_3OD (δ 3.30 and 49.01) as solvents and internal ^{1}H and ^{13}C standards. Proton-detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 7 Hz). Mass spectra were obtained on MAT-CH 7 (Varian), MAT 2000, and MAT SSQ 7000 single quadrupole mass spectrometers (both Finnigan). Positive electron impact (EI) or chemical ionization (CI) probes were used to produce ions and fragments of the samples. Isobutane was used as reagent gas in CI measurements.

Plant Material. The roots of *B. frutescens* were collected from the Experimental Garden at the Department of Chemistry, University of Botswana, in February 2001 and were identified by Mr. G. Pope (Royal Botanical Garden, Kew, UK). Voucher specimens are deposited at the University of Botswana Herbarium (code BA 205) and at the University of Würzburg (code 60).

Extraction and Isolation. Air-dried powdered roots (638 g) were extracted with $CHCl_3$ (3.6 L) and subsequently with MeOH (2.0 L). The two extracts were combined and freed of solvent to give a brown gummy solid (35 g). A portion (17 g) of this residue was purified by flash chromatography on silica gel (petroleum ether−EtOAc, 4:1 → petroleum ether−EtOAc, 1:1 → petroleum ether−EtOAc, 1:4 → EtOAc) to yield seven fractions. Further purification of the third fraction by Sephadex LH-20 ($CHCl_3$−MeOH, 2:1) and preparative TLC ($CHCl_3$−EtOAc, 4:1) yielded gaboroquinone A (**3**) (3.5 mg, 0.01%) and knipholone (**1**) (2.1 mg, 0.06%). In the same way, fraction 5 gave gaboroquinone B (**4**) (22.6 mg, 0.065%). (*M*)-4′-*O*-Demethylknipholone-4′-*O*-*β*-D-glucoside (**2**) (6.1 mg, 0.017%) was obtained by preparative TLC ($CHCl_3$−MeOH, 4:1) of the seventh fraction.

4′-*O*-Demethylknipholone-4′-*O*-*β*-D-glucoside (2): red solid; mp 178−180 °C; $[\alpha]_D^{25}$ −218° (*c* 0.020, MeOH); CD (EtOH) $\Delta\epsilon_{217}$ −9.1, $\Delta\epsilon_{231}$ −3.7, $\Delta\epsilon_{239}$ −4.6, $\Delta\epsilon_{279}$ 4.1, $\Delta\epsilon_{299}$ −1.5, $\Delta\epsilon_{347}$ 1.1; UV (MeOH) λ_{max} (log ϵ) 225 (3.81), 254 (3.57), 287 (3.54), 429 (3.05) nm; IR (KBr) ν_{max} 3421, 2923, 1627, 1281, 1201, 1080 cm^{-1}; ^{1}H NMR (CD_3OD) δ 2.13 (3H, s, $ArCH_3$), 2.77 (3H, s, $COCH_3$), 3.42−3.46 (1H, m, H-5″), 3.50−3.53 (2H, m, H-3″, H-4″), 3.58−3.60 (1H, m, H-2″), 3.75 (1H, dd, *J* = 12.0, 5.7 Hz, H-6″), 3.95 (1H, dd, *J* = 12.0, 1.9 Hz, H-6″), 5.13 (1H, d, *J* = 7.7 Hz, H-1″), 6.38 (1H, s, H-5′), 7.24 (1H, d, *J* = 8.3 Hz, H-7), 7.27 (1H, s, H-2), 7.50 (1H, d, *J* = 6.8 Hz, H-5), 7.65 (1H, dd, *J* = 8.0, 7.9 Hz, H-6); ^{13}C NMR (CD_3OD) δ 21.0 (CH_3-3), 33.6 ($COCH_3$), 62.6 (C-6″), 71.2 (C-5″), 74.8 (C-2″), 78.5, 78.7 (C-3″, C-4″), 95.4 (C-5′), 102.3 (C-1″), 106.9 (C-3′), 110.4 (C-1′), 116.1 (C-13), 116.9 (C-12), 120.3 (C-5), 124.4 (C-7), 125.7 (C-2), 129.6 (C-4), 133.2 (C-14), 136.1 (C-11), 138.1 (C-6), 153.1 (C-3), 161.9 (C-6′), 162.7 (C-4′), 163.0 (C-8), 163.7 (C-1), 164.0 (C-2′), 183.9 (C-10), 194.4 (C-9), 205.0 (*C*OCH_3); CIMS *m*/*z* 583 $[MH]^+$ (10), 163 (100); HRFABMS *m*/*z* 583.1466 $[MH]^+$ (calcd for $C_{29}H_{27}O_{13}$ 583.1452).

Gaboroquinone A (3): red solid; mp 145−150 °C; $[\alpha]_D^{23}$ +164° (*c* 0.020, MeOH); CD (EtOH) $\Delta\epsilon_{198}$ 0.2, $\Delta\epsilon_{216}$ −8.3, $\Delta\epsilon_{250}$ −4.5, $\Delta\epsilon_{264}$ 1.0, $\Delta\epsilon_{278}$ 2.75, $\Delta\epsilon_{296}$ −1.1, $\Delta\epsilon_{356}$ 1.0; UV (MeOH) λ_{max} (log ϵ) 226 (3.87), 254 (3.70), 285 (3.60), 332 (3.17) nm, 431 (3.23); IR (KBr) ν_{max} 3439, 2924, 2852, 1625, 1365, 1277, 1084 cm^{-1}; ^{1}H NMR (CD_3COCD_3) δ 2.63 (3H, s, $COCH_3$), 3.42 (3H, s, OCH_3), 4.22 (1H, d, *J* = 16.5 Hz, C*H*HOH), 4.51 (1H, d, *J* = 16.5 Hz, C*H*HOH), 6.29 (1H, s, H-5′), 7.33 (1H, dd, *J* = 8.5, 1.2 Hz, H-7), 7.59 (1H, dd, *J* = 7.6, 1.1 Hz, H-5), 7.72 (1H, s, H-2), 7.78 (1H, dd, *J* = 8.5, 7.6 Hz, H-6), 11.96 (1H, s, OH-8), 12.59 (1H, s, OH-1), 13.57 (1H, s, OH-4′), 14.20 (s, 1H, OH-6′); ^{13}C NMR (CD_3COCD_3) δ 31.59 (CO*C*H_3), 61.20 (OCH_3), 62.11 (CH_2OH), 100.45 (C-5′), 109.86 (C-3′), 113.33 (C-1′), 116.00 (C-13), 116.53 (C-12), 120.22 (C-5), 121.65 (C-2), 124.35 (C-7), 126.06 (C-4), 132.96 (C-14), 135.79 (C-11), 138.29 (C-6), 156.18 (C-3), 161.00 (C-2′), 162.30 (C-6′), 162.75 (C-8), 164.07 (C-1), 166.67 (C-4′), 183.39 (C-10), 194.18 (C-9), 204.08 (*C*OCH_3); EIMS *m*/*z* 450 $[M]^+$ (32), 435 $[M - CH_3]^+$ (7), 418 $[435 - OH]^+$ (32), 401 $[418 - OH]^+$ (100); HRMS *m*/*z* 450.0954 (calcd for $C_{24}H_{18}O_9$, 450.0950).

Gaboroquinone B (4): red solid; mp 152−159 °C; $[\alpha]_D^{23}$ +134° (*c* 0.010, EtOH); CD (EtOH) $\Delta\epsilon_{198}$ 0.2, $\Delta\epsilon_{197}$ −3.46, $\Delta\epsilon_{202}$ −0.76, $\Delta\epsilon_{210}$ −4.19, $\Delta\epsilon_{230}$ 0.48, $\Delta\epsilon_{237}$ −0.23, $\Delta\epsilon_{283}$ 2.39; UV (EtOH) λ_{max} (log ϵ) 224 (1.06), 254 (0.53), 288 (0.49), 431 (0.18); IR (KBr) ν_{max} 3435, 2925, 2855, 1625, 1384, 1279, 1085 cm^{-1}; ^{1}H NMR (CD_3COCD_3) δ 2.62 (3H, s, $COCH_3$), 3.98 (3H, s, OCH_3), 4.43 (1H, dd, *J* = 16.5, 1.2 Hz, C*H*HOH), 4.54 (1H, dd, *J* = 16.6, 1.1 Hz, C*H*HOH), 6.24 (1H, s, H-5′), 7.30 (1H, dd, *J* = 8.4, 1.1 Hz, H-7), 7.55 (dd, *J* = 7.6, 1.2 Hz, H-5), 7.66 (1H, s, H-2), 7.75 (dd, *J* = 8.2, 7.7 Hz, 1H, H-6), 12.01 (1H, s, OH-8), 12.60 (1H, s, OH-1), 14.20 (1H, s, OH-2′); ^{13}C NMR (CD_3COCD_3) δ 33.19 (CO*C*H_3), 56.10 (OCH_3), 61.92 (CH_2OH), 91.88 (C-5′), 106.18 (C-3′/C-1′), 106.18 (C-1′/C-3′), 115.91 (C-13), 116.46 (C-12), 120.10 (C-5), 121.15 (C-2), 124.09 (C-7), 126.04 (C-4), 132.52 (C-14), 135.70 (C-11), 138.18 (C-6), 156.62 (C-3), 161.00 (C-2′), 161.81 (C-6′), 162.74 (C-8), 163.83 (C-4′), 164.09 (C-1), 182.76 (C-10), 194.17 (C-9), 203.65 (*C*OCH_3); EIMS *m*/*z* 450 $[M]^+$ (27), 435 $[M - CH_3]^+$ (6), 418 $[435 - OH]^+$ (37), 401 $[418 - OH]^+$ (100); HRMS *m*/*z* 450.0952 (calcd for $C_{24}H_{18}O_9$, 450.0950).

***O*-Demethylation of Knipholone (1).** A solution of **1** (2.0 mg, 4.61 μmol, from previous isolation work[15]) in chlorobenzene (1 mL) was treated under argon with $AlBr_3$ (12.0 mg, 44.8 μmol) and stirred at 80 °C for 2 h. After cooling to room temperature, water (1 mL) and 2 M aqueous HCl (1 mL) were added, and the mixture was extracted thoroughly with EtOAc. The combined organic phases were dried ($MgSO_4$) and filtered, and the solvent was evaporated. Purification of the residue by preparative HPLC using an LC 25 mm module with two Nova-Pak C_{18} column segments (25 × 100 mm) from Waters (Eschborn, Germany) and a 510 pump (Waters, Eschborn, Germany) with a flow of 4.0 mL min^{-1} and UV detection at 254 nm (solvent: MeOH−water, 75:25, acidified with 0.1% trifluoroacetic acid) afforded **5** (1.1 mg, 2.67 μmol, 58%) as a red solid. The chromatographic (HPLC), spectroscopic (^{1}H NMR), and chiroptical properties, as well as the optical rotation, were identical to those of an authentic sample of **5** from *Bulbine capitata*[3] and to those of synthetic material as obtained by total synthesis.[16]

Hydrolysis of Glycoside 2. A sample (6.00 mg, 10.3 μmol) of **2** was stirred in 2 N HCl−MeOH (1:1) (5 mL) at 70 °C for 2.5 h and cooled to room temperature, and the solvent evaporated. The residue was dissolved in EtOAc (3 mL) and water (3 mL), and the phases were separated. Evaporation of the aqueous phase gave D-glucose. Identity to an authentic sample (Aldrich) was confirmed by TLC (silica gel, $CHCl_3$−MeOH−water, 9:6:1, using the vanillin−sulfuric acid spray reagent), ^{1}H NMR, and optical rotation. Evaporation of the organic phase and column chromatography on silica gel (CH_2Cl_2−EtOAc, 2:1) afforded 4′-*O*-demethylknipholone (**5**) (3.8 mg, 9.04 μmol, 88%). The chromatographic (TLC), spectroscopic (^{1}H NMR), and chiroptical properties were identical to those of the sample prepared above from knipholone (**1**) and to material obtained previously by isolation from *B. capitata*[3] and by total synthesis.[16]

Reductive Deoxygenation of Gaboroquinone A (3). A solution of **3** (4.00 mg, 8.88 μmol) in dry CH_2Cl_2 (1 mL) was treated with polymer-bound triphenylphosphine (12.0 mg, 35.3 μmol) at room temperature and stirred for 15 min, after which $(CBrCl_2)_2$[18] (5.55 mg, 16.9 μmol) was added and the suspension was stirred for another 10 min at room temperature. The mixture was filtered through a pad of Celite, which was then washed thoroughly with CH_2Cl_2. The solvent from the combined organic phases was evaporated. The residue was dis-

solved in dry MeOH (10 mL), and after addition of Pd/C (10%) (2.0 mg) and NaOAc (3.0 mg, 11.3 μmol), the mixture was hydrogenated for 30 min at 3.5 bar H_2. Filtration, evaporation of the solvent, and flash chromatography on silica gel (CH_2Cl_2−EtOAc, 10:2) afforded **6** (0.83 mg, 1.91 μmol, 22%), identical to an authentic sample from *B. capitata*.[2]

Reductive Deoxygenation of Gaboroquinone B (4). The deoxygenation of **4** (3.0 mg, 6.66 μmol) was performed as described above for **3** using polymer-bound triphenylphosphine (9.0 mg, 40.0 μmol) and $(CBrCl_2)_2$[18] (5.0 mg, 15.2 μmol) for the first step and Pd/C (10%) (2.0 mg) and NaOAc (3.0 mg, 11.3 μmol) for the second. Flash chromatography on silica gel (CH_2Cl_2−EtOAc, 10:2) afforded **1** (600 μg, 1.38 μmol, 21%), identical to an authentic sample from *B. capitata*.[2]

Biological Experiments. The biological experiments were performed as previously described.[20]

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References and Notes

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