Limonoids from Nigerian *Harrisonia abyssinica* and Their Stimulatory Activity against *Striga hermonthica* Seeds

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Deoxyobacunone (**1**), a new limonoid with a double bond in ring D, has been isolated from the root bark of *Harrisonia abyssinica* collected in Nigeria. Also, the known limonoids obacunone (**2**), harrisonin (**3**), 12β-acetoxyharrisonin (**4**), and pedonin (**5**) have been isolated. The structure of **1** was assigned unambiguously by spectral data analysis. Under laboratory conditions, 10^{-3}−10^{-5} M concentrations of compounds **1**−**5** exhibited significant stimulatory activity (12−98%) against conditioned *Striga hermonthica* seeds. This study provided useful insight regarding the functionalities required for activity of limonoids against *Striga* seeds. The variation in activity was rationalized through quantitative structure−activity relationship (QSAR) models based on several molecular descriptors including van der Waals volume (VDW_v), molecular polarizability (α), dipole moment (μ), log *P*, and the differences between the highest occupied molecular orbital and lowest unoccupied molecular orbital (HOMO−LUMO gap).

The Nigerian shrub *Harrisonia abyssinica* Oliv. (Simaroubaceae) is used as a remedy for the treatment of fever, bubonic plague, tuberculosis, hemorrhoids, and snakebite.[1] Several limonoids have been isolated from its root bark, including obacunone (**2**), harrisonin (**3**), 12β-acetoxyharrisonin (**4**), and pedonin (**5**).[2,3] Crude extracts of the root bark of this plant have been found to exhibit antimicrobial, cytotoxic, insect antifeedant, and plant growth inhibitory activities.[4] The nature of the chemical constituents of the Nigerian *H. abyssinica*, together with our ongoing program on the search for potent *Striga*[5] seed germination stimulants and antimycobacterial and antimicrobial agents, prompted us to further investigate the root bark of *H. abyssinica*.

Striga (common name "witchweed") (Scrophulariaceae) is an obligate root-parasitic flowering plant genus that causes considerable yield reductions of cereals and legumes in sub-Saharan Africa and Asia.[6] There are four economically important *Striga* species, namely, *S. asiatica* (L.) Kuntze, *S. aspera* (Willd.) Benth., *S. gesnerioides* (Willd.) Vatke, and *Striga hermonthica* (Del.) Benth. Mature *Striga* plants produce large quantities (50 000 to 500 000) of microscopic seeds (0.3 mm long × 0.15 mm wide), which remain dormant in the soil for as long as 20 years. The dormant seeds will only germinate if exposed to exudates from roots of host crops. Due to limited endosperm nutrients, the developing *Striga* seedlings will die if they do not attach to the roots of a host crop within 7 days. Controlling *Striga* species is a very challenging task. This is because their life cycle is closely associated with those of their hosts. Current *Striga* control methods in African countries include hand-weeding, use of resistant crops, use of biological pests, and chemical methods.[5] Most of these methods are tedious, expensive, and too hazardous for human health. In the United States, *Striga* seeds are eliminated or reduced by injecting ethylene gas into infected soil. This is not an economically viable approach for resource-poor African farmers. Recently, it has been shown that the density of *Striga* seeds in the soil can be significantly reduced by direct application of extracts from native African plants.[7] In this endeavor, crude extracts from roots, stems, and leaves of the Nigerian *H. abyssinica* shrub have exhibited high *Striga* seed germination.[5] The isolation of a new limonoid, deoxyobacunone (**1**), and its structural elucidation through ^{1}H−^{1}H COSY,[8] 2D NOESY,[9] HMQC,[10] and HMBC[11] NMR experiments is described herein. Also, *Striga* germination data for limonoids **1**−**5** and their molecular properties calculated using Alchemy 2000 and QSARIS molecular modeling programs are reported.

Several types of African shrubs and trees of the family Simaroubaceae are rarely attacked by insects. Extracts from the leaves or fruits of these plants can be used as pesticides for the protection of other plant varieties.[3] Previous phytochemical studies of *Harrisonia* species have resulted in the isolation of several biologically active limonoids.[2−5] The insect antifeedant and antimicrobial activities of these limonoids have been reported.[2] In the present study, bioassay-guided fractionation of the methanolic extract from the root bark of the Nigerian *H. abyssinica* led to the isolation of a new limonoid, **1**, and four known limonoids (**2**−**5**). The IR spectrum of **1** exhibited bands that are typical of a limonoid skeleton (α,β-substituted furan at 1502 and 877 cm^{-1}, three CO bands at 1734, 1710, and 1644 cm^{-1}; the last band being due to an α,β-unsaturated C=O). The major fragments in the mass spectrum of **1** were similar to those for obacunone (**2**).[3] All proton and carbon NMR signals were unambiguously assigned by concerted application of 1D and 2D NMR techniques using literature pulse sequences.[8−11] Because we had worked before with obacunone (**2**), it was very easy to assign all spectral data of **1**. Compound **1** showed a characteristic Ehrlich-positive reaction, indicating the presence of a furan ring.[12] Moreover, the NMR spectra of **1** showed chemical shift signals that are characteristic of a limonoid: singlets due to the protons at C-17, C-15, and C-methyl groups, an AB-type system from the protons at C-1 and C-2, and multiplets due to the protons at C-11 and C-12. Detailed analyses of 2D NMR spectra revealed the presence of six diagnostic segments, A−F (Figure 1). The

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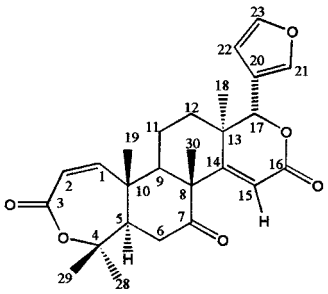

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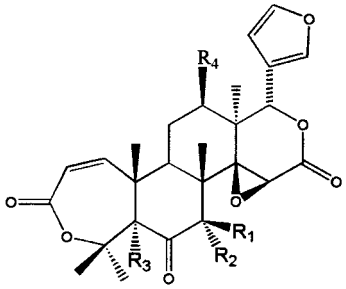
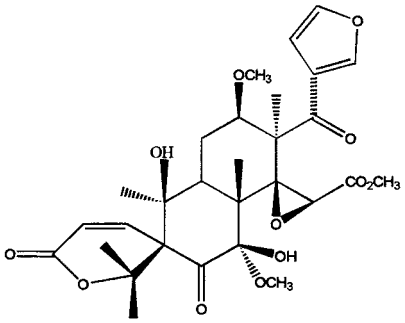


2 $R_1 = R_2 = =O$; $R_3 = R_4 = H$

3 $R_1 = R_3 = OH$; $R_2 = OCH_3$; $R_4 = H$

4 $R_1 = R_3 = OH$; $R_2 = OCH_3$; $R_4 = OAc$



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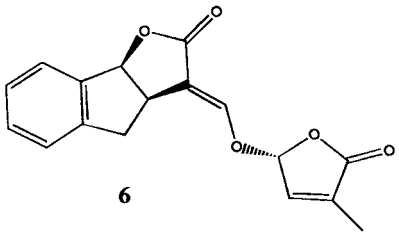

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chemical shifts for A−C rings were closely similar to those of **2**−**4**. [2,3] However, the chemical shifts of H_3-18, H-17, and furan protons (H-21, H-22, and H-23) differed by δ 0.03−0.45 compared to those of obacunone (**2**), suggesting different structural features in ring D. A pair of doublets of a conjugated double bond at δ 5.99 and 6.35 (1H each, J = 12.2 Hz) indicated that ring A in **1** is the same as that found in obacunone (**2**).[3] Clearly, the characteristic signals for an epoxy moiety at C-14−C-15 were absent in the NMR spectra of **1**. Instead, the ^{1}H NMR spectrum of **1** showed a sharp olefinic singlet at δ 6.47 that was ascribed to H-15 based on proton−carbon correlations discernible from HMQC[10] and HMBC[11] spectra. In comparing the ^{13}C NMR spectrum of **1** with that of **2**, the chemical shifts for carbons

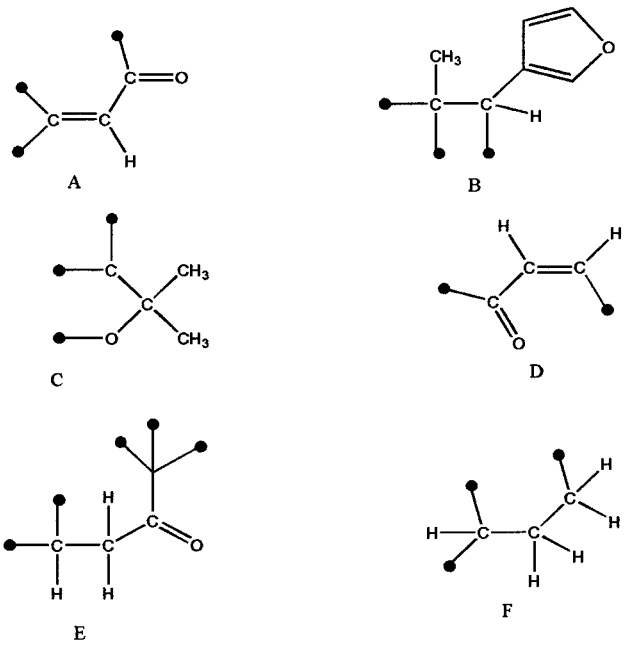

Figure 1. Partial fragments deduced from 2D NMR spectra of **1**.

C-13, C-17, and C-20 differed by less than δ 2.0. Large chemical shift differences were observed for C-14 and C-15 carbons, again indicating changes in the D ring area of the molecule. All of the oxygen functional groups were accounted for in the ^{13}C NMR spectrum. The multiplicity of all 26 carbon atoms observed in the ^{13}C NMR spectrum was determined by DEPT experiment.[13] The DEPT broadband spectrum exhibited five methyl, three methylene, nine methine, and three carbonyl signals, while the remaining six signals in the spectrum were due to the quaternary carbon atoms. The chemical shifts due to five methyl groups (at δ 1.18, 1.50, 1.55, and two overlapping at δ 1.43) and nine quaternary carbon atoms were unambiguously assigned through NOESY[9] and HMBC[11] techniques. The methyl singlet at δ 1.55 was assigned to H_3-30 because it showed $^3J_{H-C}$ coupling with C-14 in the HMBC spectrum and strong spatial interactions with H_β-6 and one or both of the methyl groups at δ 1.43. The quaternary carbon signal at δ 52.4 showed three-bond coupling to the olefinic H-15 signal in the HMBC spectrum and was assigned to C-8. The methyl proton signal at δ 1.50 was assigned to H_3-28 because it showed very strong spatial correlation with H_α-5 and H_α-6 in the NOESY spectrum. The remaining methyl singlets at δ 1.43 and 1.50 showed strong NOE correlations with H_3-30 and H_β-6 and were assigned to H_3-19 and H_3-29, respectively. The quaternary carbon signal at δ 84.4 was assigned to C-4 because it showed three-bond proton−carbon correlation with H_α-6 and H_β-6 in HMBC spectrum. The geminal methyl groups (H_3-28 and H_3-29) showed three- and four-bond proton−carbon correlations to C-5 and C-3, respectively. This shows that the seven-membered ring A in **1**, as in the known limonoids **2**−**4**, is intact. In fact, very few limonoids with seco-A rings have been reported in the West African *H. abyssinica*.[4] On the basis of spectral data (see Experimental Section), the structure of **1** was assigned to deoxyobacunone. A search of the literature, including the reviews of limonoids,[14−17] revealed several deoxyobacunone-type compounds that have double bonds in the D ring. To obtain the lowest energy conformer of deoxyobacunone (**1**), the three-dimensional (3D) X-ray structure of perforatin[18] was modified using the NMR data of **1** and analyzed by Alchemy

Table 1. Percentage Germination of Conditioned *Striga hermonthica* Seeds[a] and Molecular Descriptors of Deoxyobacunone (**1**), Obacunone (**2**), Harrisonin (**3**), 12β-Acetoxyharrisonin (**4**), Pedonin (**5**), and GR 24 (**6**)

compd	% germination[b]	energy[c]	VDW_v[d]	VDW_A[e]	μ[f]	log *P*	ovality	α[g]	LUMO[h]	HOMO[i]	HOMO−LUMO	spcP[j]
1	98, 80, 26	113.5	346	399	5.99	3.58	1.68	11.61	−11.0764	−11.1692	−0.093	0.039
2	85, 90, 22	181.1	304	361	5.47	3.14	1.65	11.61	−11.5580	−11.708	−0.150	0.038
3	28, 30, 12	256.2	337	383	9.67	2.83	1.64	11.22	−11.4475	−11.6367	−0.189	0.033
4	86, 89, 22	199.0	293	341	2.87	4.00	1.60	13.16	−11.9001	−12.0625	−0.162	0.045
5	70, 80, 18	137.8	343	387	7.80	5.27	1.63	14.71	−11.1861	−11.4561	−0.270	0.042
6[k]	98, 93, 85	38.3	253	317	8.85	2.57	1.64	5.42	−11.1135	−11.4048	−0.291	0.021

[a] Seeds were treated with aqueous solutions of **1**−**5**. [b] % germination at 10^{-3}, 10^{-4}, and 10^{-5} M, respectively. [c] Total steric energy (kcal/mol). [d] van der Waals molecular volume (Å^3). [e] van der Waals molecular area (Å^2). [f] Diple moment. [g] Molecular polarizabilty. [h] Lowest unoccupied molecular orbital. [i] Highest occupied molecular orbital. [j] spc polarizability. [k] Positive control.

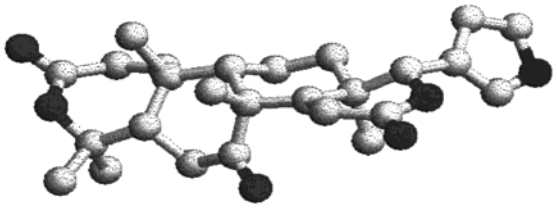

Figure 2. Perspective view of a molecule of **1**.

2000. A computer-generated perspective drawing of deoxyobacunone (**1**) is shown in Figure 2.

Recent structure−activity relationship studies on a wide range of compounds have revealed the complexities involved in interpreting *Striga* germination data.[5,19] The percentage germination data for limonoids **1**−**5** are shown in Table 1. In each test series, aqueous solutions of DMSO (0.1% v/v) and the strigol analogue 2-methyl-4-(2-oxo-2,3,-3a,8b-tetrahydro-4*H*-indeno[1,2*b*]furan-3-ylidenemethoxy)-but-2-en-4-olide (commonly referred to as "GR 24") were used as negative and positive controls, respectively. There was no consistent relationship between decreasing the concentration with stimulation of germination. Compounds **1**−**5** induced significant stimulation of germination at 10^{-3}−10^{-5} M concentration ranges. The maximum stimulatory activities were at 10^{-4} M, while 10^{-5} M was the optimum concentration. This is encouraging because not only are many limonoids available in very large quantities[2] but also there are a number of practical sources. GR 24 (**6**), at any given concentration, showed significantly higher activity than all the limonoids tested. This was anticipated because **6** contains several structural features that are found in strigol,[20] the most potent *Striga* seed germination stimulant known. As expected, the conditioned *Striga* seeds did not germinate in 0.1% DMSO. It is worth noting that deoxyobacunone (**1**), having one less oxygen atom on the D ring, was almost as active as the parent compound, obacunone (**2**). Limonoids **1**−**4** are similar in that they are tetranortriterpenoids with α,β-unsaturated lactone and α,β-epoxy δ-lactone moieties in rings A and D, respectively. The presence of an acetoxy group in 12β-acetoxyharrisonin (**4**) made it more active than harrisonin (**3**). Not surprisingly, the presence of an OH functionality (inhibitor[5] of *Striga* seed germination) in **3** resulted in a dramatic loss of activity. Pedonin (**5**) was less active compared to **3** and **4** probably due to the differences in rings A and D. The differences in the stimulatory activities of limonoids **1**−**5** and GR 24 (**6**) are best rationalized by invoking a stimulant−*Striga* receptor interaction model.[19] Although detailed quantitative structure−activity relationship (QSAR) studies involving several structurally similar limonoids are required in order to deduce their specific reactive site(s), molecular descriptors[21] offer some useful insights (Table 1).

The mechanism of stimulation of germination involves sequential steps similar to those described.[5,19] It is plausible to suggest that the stimulation of germination of *Striga* seeds by compounds **1**−**6** occurs either through chiral recognition processes or through the formation of ethylene. The specific structural requirements for a chiral recognition mechanism in aqueous media was recently formulated through NMR spectroscopy.[22,23] In chiral recognition, a minimum of three simultaneous interactions is required; at least one of the interactions in the transient "compound−*Striga* seed rececptor" adduct must be stereochemically dependent. Hydrogen bond association is the major factor governing chiral recognition in that it fixes the interacting molecules in proper orientations. Limonoids **1**−**5** have several chiral centers. Chiral recognition studies [22−24] suggest that host−guest noncovalent interactions involve a complex set of events. Therefore in the present study, the interactions between **1**−**6** ("guests") and *Striga* seed receptor ("host") involve (1) hydrogen bonds between carbonyl groups of **1**−**6** and hydrogen donating sites in receptors, (2) van der Waals ($\pi-\pi$) interactions between the aromatic (furan or benzene) double bonds of **1**−**6** and chiral protons of receptors, and (3) multiple CH−π interactions of **1**−**5** and receptors. Limonoids **1**−**5** have four functional groups (epoxide, α,β-unsaturated carbonyl, furan ring, and an alkene in ring D) that may be involved (directly or indirectly) in the stimulation of germination of *S. hermonthica* seeds. For example, the epoxide group may react with biological nucleophiles, in particular, thiol-containing enzymes, phosphofructokinase, and glycogen synthetase.[25] *Striga* seeds contain methionine, which is also found in several seeds.[26] The enzyme-mediated reaction of limonoids **1**−**5** with methionine results in the formation of ethylene, the compound that induces germination of *Striga* seeds.[27]

Irreproducibility of germination data is a major problem in *Striga* bioassays. Several factors need to be taken into consideration. These include the type of *Striga* isolate, bioassay conditions, and the stability of test compounds. Our bioassays have shown that limonoids undergo degradation[5] especially in polar solvents (e.g., methanol, acetone, and ethanol) to form crystalline seco-A, B, C, and D ring artifacts. During bioassay tests, conditioned *Striga* seeds were treated with aqueous solutions of test compounds. The compounds must penetrate the outer seed coat in order to induce germination. Repulsive interactions of a compound with the aqueous phase in *Striga* seed would drive the compound away from water to a nonpolar organic phase (receptor sites). Therefore, understanding the mechanisms of stimulation of germination and designing QSAR models[21] that predict and explain the differences in activities is a sensible way to solve the dilemma highlighted by the irreproducible *Striga* bioassays. The differences between HOMO and LUMO (HOMO−LUMO gap) of the structurally similar limonoids (**1** and **2**; **3** and **4**) appeared to be one of the molecular descriptors that best explain the effect of functional groups on bioactivity (Table 1). The HOMO

represents the electron-donating power of the molecule and relates to hydrogen bond acceptor basicity. The LUMO is the electron-accepting power of the molecule and relates to hydrogen bond donor acidity. The present study reveals that limonoids **1**−**5** adopt specific conformations. The effect of substituents on C-5, C-6, C-7, C-12, and C-14−C-15 positions on the HOMO−LUMO gap energies of the structurally similar **1**−**4** is dramatic. VDW_v, μ, and α represent molecular bulk, molecular polarity, and molecular hydrophobicity, respectively. The hydrophobic chiral cavities of compounds **1**−**5** differ in size (ranging from 293 to 346 Å^3), and it is possible that they adopt different geometrical orientations (e.g., Figure 2) when interacting with *Striga* seed receptors.

Experimental Section

General Experimental Procedures. The melting point was measured on a Thomas-Hoover apparatus (uncorrected). IR spectra were recorded on a Perkin-Elmer 1760X spectrometer. 1D and 2D NMR spectra were recorded on a Bruker ARX 300 MHz spectrometer in deuterated chloroform ($CDCl_3$). The solvent chemical shift signals at δ_H 7.24 and δ_C 77.0 were used to reference ^{1}H and ^{13}C NMR spectra, respectively. Carbon multiplicities were deduced from DEPT[13] and HMQC[10] spectra. NOESY[9] spectra were recorded with 800 ms mixing time (τ_m). The acquisition parameters used in HMBC experiments were identical to those described in the literature.[11] Mass spectra were recorded on a Hewlett-Packard 5971A GC−MS or a TSQ70 FAB mass spectrometer. Vacuum-liquid chromatographic (VLC) separations[5] were performed on silica gel (MN Kieselgel). Conformational analysis was performed using Alchemy 2000 software (MOPAC 1993 PM3; SciVision, Burlington, MA). The structures of **2**−**6**, whose stereochemistry have been determined,[2−4,24] were used as a starting points for energy minimization. The 3D structures were built using the standard atoms, functional groups, and fragments available in the molecule library of the Alchemy 2000 software. The structures were subjected to geometry optimization through MOPAC using PM3 parametrization. The lowest energy conformers were obtained after running 10^5 iterations at various temperature regimes. To explain the *Striga* bioactivites, molecular descriptors of the lowest energy conformers were deduced using QSARIS software (SciVision, Burlington, MA). The molecular properties used in the QSAR model include van der Waals molecular volume (VDW_v); van der Waals molecular area (VDW_A); dipole moment (μ); spc polarizability; highest occupied molecular orbital (HOMO); lowest unoccupied molecular orbital (LUMO); ovality, log *P*; steric energy; and the HOMO−LUMO gap (Table 1). *Striga* bioassays and structure elucidation experiments were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and Louisiana State University, Baton Rouge, LA, respectively.

Plant Material. The root bark of *Harrisonia abyssinica* was collected in Moniya, Nigeria, in August 1994. A voucher specimen (Rugutt No. 61), identified by Mr. C. W. Agyakwa, is deposited in the Herbarium of the Weed Science Department, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Extraction and Isolation. Air-dried root bark of *H. abyssinica* (1.4 kg) was extracted with methanol (4 × 250 mL) at room temperature. Evaporation of the solvent in vacuo yielded 85.3 g of crude extract. An aliquot (30.1 g) of the crude extract was adsorbed on 25 g of Si gel (MN Kieselgel G) and placed on a VLC column (3.5 cm in diameter and 31 cm long) containing 65 g of Si gel. Compounds **2**−**5** were isolated and characterized as described in the literature.[5] The spectroscopic data (IR and NMR) of the known obacunone (**2**, 70 mg), harrisonin (**3**, 20 mg), 12β-acetoxyharrisonin (**4**, 18 mg), and pedonin (**5**, 66 mg) were in agreement with the literature values.[2−5,28]

Deoxyobacunone (1, 6 mg): amorphous powder, mp 229−233 °C; $[\alpha]^{20}_D$ −51° (*c* 0.99, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 215 (2.44) nm; IR (KBr) ν_{max} 1734, 1711 (carbonyls), 1645 (α,β-unsaturated carbonyl), 1507, 834 (β-substituted furan ring), 1755 cm^{-1} (δ-lactone); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.35 (1H, d, J = 12.2 Hz, H-1), 5.99 (1H, d, J = 12.2, H-2), 2.58 (1H, dd, J = 9.7, 4.7 Hz, H-5), 2.83 (1H, dd, J = 14.7 Hz, H_β-6), 2.78 (1H, dd, J = 14.7, H_α-6), 2.24 (1H, dd, J = 7.6, 9.3, H-9), 1.93−2.05 (2H, m, H-11), 1.60−1.95 (2H, m, H-12), 6.47 (1H, s, H-15), 5.00 (1H, s, H-17), 1.18 (3H, s, H_3-18), 1.43 (3H, s, H_3-19), 7.49 (1H, s, H-21), 6.42 (1H, d, H-21), 7.43 (1H, d, H-23), 1.43 (3H, s, H_3-28), 1.50 (3H, s, H_3-29), 1.55 (3H, s, H_3-30); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.5 (d, C-1), 120.9 (d, C-2), 166.3 (s, C-3), 84.4 (s, C-4), 54.3 (d, C-5), 39.6 (t, C-6), 206.8 (s, C-7), 52.4 (s, C-8), 46.1 (d, C-9), 44.5 (s, C-10), 17.4 (t, C-11), 26.2 (t, C-12), 38.2 (s, C-13), 166.6 (s, C-14), 116.9 (d, C-15), 164.9 (s, C-16), 82.3 (d, C-17), 20.0 (q, C-18), 16.0 (q, C-19), 119.9 (s, C-20), 143.1 (d, C-21), 109.9 (d, C-22), 141.3 (d, C-23), 31.2 (q, C-28), 25.0 (q, C-29), 26.8 (q, C-30); EIMS *m/z* $[M]^+$ 438 (40), 331 (100), 273 (35), 174 (15), 68 (9); 54 (25); HRFABMS $[M + H]^+$ *m/z* 438.5264 (calcd for $C_{26}H_{30}O_6$, 438.5254); TLC, R_f 0.20 (Si gel, EtOAc−hexane, 4:9 v/v).

***Striga* Biological Assays.** Stock solutions (10^{-3} M) of pure samples (>99% by ^{1}H NMR quantification) of limonoids **1**−**5** and GR 24 (**6**) (a positive control)[19] were prepared in dimethyl sulfoxide (DMSO). The solutions were refrigerated at 4 °C until tested. Fresh solutions of lower concentrations (10^{-4} and 10^{-5} M) were prepared by serial dilution of the stock solution with sterile deionized water. The solutions were tested for stimulation of germination of conditioned *Striga hermonthica* seeds collected during the 1993 sorghum-harvesting season in Bida (Nigeria). Each test solution (600 μL) was applied to 12 5-mm diameter glass fiber disks (in a 9 cm diameter Petri dish) containing approximately 360 conditioned *Striga* seeds. The tests were performed in triplicate and the average percentage germination values determined. For each treatment, GR 24 (**6**) and DMSO (0.1% v/v) served as positive and negative controls, respectively. The Petri dishes were sealed with Parafilm and incubated at 28 °C for 24 h. The nongerminated and germinated seeds were counted under a binocular dissecting microscope at 20× magnification. Seeds were considered to have germinated when the radicle had protruded from the conditioned *Striga* seed coat.

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