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Kwanzoquinones A–G and other constituents of *Hemerocallis fulva* 'Kwanzo' roots and their activity against the human pathogenic trematode *Schistosoma mansoni*

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Abstract—Schistosomiasis is a debilitating disease caused by parasitic trematodes of the genus *Schistosoma* that afflicts 200 million people worldwide. Daylilies (*Hemerocallis* spp.) have been used in Asia for the treatment of schistosomiasis; however, the active principles have not been fully characterized. In our studies of *Hemerocallis fulva* 'Kwanzo' Kaempfer roots, we have isolated seven new anthraquinones, kwanzoquinones A (**1**), B (**2**), C (**4**), D (**5**), E (**6**), F (**7**), and G (**9**), two known anthraquinones, 2-hydroxychrysophanol (**3**) and rhein (**8**), one new naphthalene glycoside, 5-hydroxydianellin (**11**), one known naphthalene glycoside, dianellin (**10**), one known flavone, 6-methylluteolin (**12**), and α-tocopherol. The structures of the compounds were elucidated by spectroscopic and chemical methods. Compounds **1**–**11** and the monoacetates of kwanzoquinones A and B, **1a** and **2a**, respectively, were tested for their activity against multiple life-stages of *Schistosoma mansoni*. Compound **3** immobilized all cercariae within 15 s at 3.1 μg/mL. However, upon removal of the compound, 20% of the immobilized cercariae recovered after 24 h. In contrast, compound **6** immobilized cercariae within 12–14 min at 25 μg/mL. Following removal of the compound, all cercariae died within 24 h. The adult worms were also immobilized within 16 h by compounds **3** and **6** at 50 μg/mL. None of the compounds had an effect on the schistosomula stage.

1. Introduction

Schistosomiasis is a disease caused by parasitic digenetic trematodes of the genus *Schistosoma*. The World Health Organization estimates that *Schistosoma* species currently infect 200 million people, while another 600 million are at risk.[1] A large number of schistosomes are known; however, only five appear to be primarily responsible for human infections. These include *Schistosoma haematobium*, *Schistosoma intercalatum*, *Schistosoma japonicum*, *Schistosoma mansoni*, and *Schistosoma mekongi*.[2,3] Schistosomes pass through a complex life-cycle in which free-swimming cercariae emerge from their intermediate freshwater snail hosts and infect humans by attaching to the skin via mucus secretions. Cercariae then penetrate the skin by releasing proteolytic enzymes.[4] Concurrently, the cercariae shed their tails and transform into schistosomula that enter the venous vascular system where they are carried to the heart and lungs, before reaching the systemic circulation. Ultimately, the schistosomula arrive at the liver where they grow into sexually mature adults. Male and female adults form copulating pairs in the portal venous system. Later, they migrate to the mesenteric or vesical veins depending on the specific species of schistosome, and begin laying eggs for a period of typically 3–5 years. The eggs evoke a host immune response that results in the formation of granulomas leading to fibrosis and the sequelae of clinical manifestations.[2,3,5,6] These clinical manifestations of schistosomiasis may include bloody diarrhea and hematuria, portal and pulmonary hypertension, hepatosplenomegaly, and death. There are limited options available for the chemotherapeutic treatment for *Schistosoma* infections with the drug-of-choice being the pyrazionoisoquinoline, praziquantel.[2] Unfortunately, the long-term, worldwide application of the drug coupled with the recent discovery of praziquantel-tolerant schistosomes has generated concern over the development of drug-resistant *Schistosoma* strains.[7–9] With few other options available for combating schistosomiasis, there is an urgent need to develop new methodologies for the treatment and prevention of *Schistosoma* infections.[7,8]

Daylily roots (*Hemerocallis* spp., Hemerocallidaceae) have been used in Asia to treat schistosomiasis.[10,11] However, this method of treatment has been disfavored due to a host of toxic side effects and deaths associated with the administration of *Hemerocallis* root extracts to humans.[12] Previous

Keywords: anthraquinones; *Hemerocallis fulva* 'Kwanzo'; natural products; schistosomiasis.

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efforts to identify the active constituent responsible for the therapeutic properties of *Hemerocallis* roots lead to the isolation of a neurotoxic binaphthalenetetrol known as stypandrol[13] which had been shown to cause paralysis, blindness and death in mammals.[14,15] In another report,[16] researchers obtained a yellow powdery isolate to which was ascribed both the biological activity against schistosomes, as well as, the toxic side effects associated with the use of *Hemerocallis* roots; however, its structure was never identified. While other studies have described additional compounds found in daylilies, none of these efforts have addressed the need to fully characterize the bioactive anti-schistosome chemical constituents from *Hemerocallis* roots.

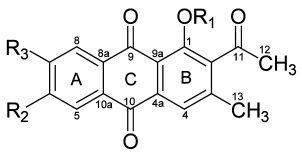
In our studies of the roots of *Hemerocallis fulva* 'Kwanzo' Kaempfer roots, we isolated a series of seven new and two known anthraquinones, one new and one known naphthalene glycosides, and one flavone. These compounds were tested for activity against the cercariae, schistosomula, and adult life-stages of the human-pathogenic trematode *S. mansoni*. The structure elucidation and biological activity of these compounds are reported.

2. Results and discussion

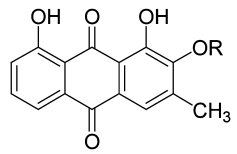
The roots of *H. fulva* 'Kwanzo' were successively extracted with hexane, EtOAc, and MeOH. The hexane and MeOH extracts were selected for further study and subsequently subjected to a combination of chromatographic procedures including Si gel MPLC and PTLC, ODS MPLC and preparative HPLC, and crystallization. This work led to the isolation of seven new anthraquinones, kwanzoquinones A–G (**1**, **2**, **4**–**7**, **9**), and a new naphthalene glycoside (**11**). The structures and complete ^{1}H and ^{13}C NMR spectral assignments for these new compounds, as well as those for compounds **3**, **10**, and **12**, were determined based on extensive 1D and 2D NMR studies and are reported here for the first time.

The hexane extract was subjected to a series of chromatographic procedures, leading to the isolation of 12 mg of fine yellow needles following crystallization from $CHCl_3$–hexane. Initial inspection of the ^{1}H and ^{13}C NMR spectra of this product indicated a doubling of most proton and carbon signals that suggested it was perhaps a large dimeric compound composed of more than 31 unique carbon nuclei. However, positive FABMS indicated a major signal at *m/z* 295 $[M+H]^+$ that suggested the product was a mixture of two structurally related isomers each with a formula of $C_{18}H_{14}O_4$. This was supported by the presence of a significant fragment ion at *m/z* 273 $[M+H-H_2O]^+$. Further evidence was also provided by HMBC experiment that showed two sets of contours representing the $^{2-3}J_{CH}$ connectivities for two compounds, each composed of 18 carbon and 14 proton spins. Extensive efforts to separate these two compounds by Si gel MPLC and TLC, ODS MPLC and preparative HPLC, and crystallization with a variety of solvent systems proved unsuccessful. Further attempts were made to separate the acetylated products (**1a** and **2a**) from one another, but this method also failed. Therefore, the structure elucidation and full ^{1}H and ^{13}C NMR assignments of compounds **1** and **2** (Table 1) were performed on the inseparable 1:1 mixture of these two constitutional isomers.

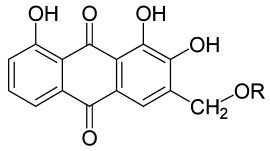
Compounds **1** and **2** were determined to each be composed of substituted 1-hydroxyanthraquinone moieties. Evidence for this came from a combination of HRFABMS with *m/z*



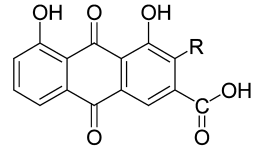
1 R_1=H, R_2=CH_3, R_3=H
1a R_1=Ac, R_2=CH_3, R_3=H
2 R_1=H, R_2=H, R_3=CH_3
2a R_1=Ac, R_2=H, R_3=CH_3



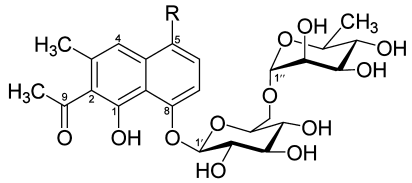
3 R=H
4 R=β-D-glucopyranoside
5 R=malonyl-(1→6)-β-D-glucopyranoside



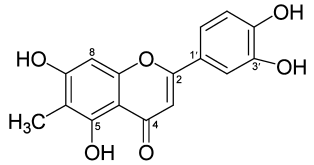
6 R=H
7 R=β-D-glucopyranoside



8 R=H
9 R=CH_3



10 R=H
11 R=OH



12

Table 1. NMR spectral data for kwanzoquinones A (**1**) and B (**2**) in $CDCl_3$

Position	**1**			**2**		
	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]	δ_H (J in Hz)[a]	δ_C[b]	HMBC[c]
1	–	159.6 (s)	1-OH	–	159.6 (s)	1-OH
2	–	114.4[d] (s)	1-OH, H-13, H-4	–	114.5[d] (s)	1-OH, H-13, H-4
3	–	144.7[e] (s)	H-4, H-13	–	144.9[e] (s)	H-4, H-13
4	7.61 (s)	121.5 (d)	H-13	7.61 (s)	121.5 (d)	H-13
4a	–	133.1 (s)	H-4	–	133.1 (s)	H-4
5	8.04 (s)	127.8 (d)	H-14	8.13 (d, 7.5)	127.7 (d)	H-6
6	–	146.2 (s)	H-8, H-14	7.58 (d, 7.5)	135.6 (d)	H-8, H-14
7	7.58 (d, 7.5)	135.1 (d)	H-5, H-14	–	145.6 (s)	H-5, H-14
8	8.15 (d, 7.5)	127.1 (d)	H-7	8.05 (s)	127.2 (d)	H-14
8a	–	133.4 (s)	H-8	–	131.2 (s)	H-8
9	–	188.1 (s)	H-8	–	188.5 (s)	H-8
9a	–	135.7[f] (s)	1-OH, H-4	–	135.8[f] (s)	1-OH, H-4
10	–	182.3 (s)	H-4, H-5	–	181.9 (s)	H-4, H-5
10a	–	130.9 (s)	H-5	–	133.0 (s)	H-5
11	–	203.0 (s)	H-12	–	203.0 (s)	H-12
12	2.59 (s)	31.9 (q)	–	2.59 (s)	31.9 (q)	–
13	2.37 (s)	20.2 (q)	H-4	2.37 (s)	20.2 (q)	H-4
14	2.51 (s)	21.9[g] (q)	H-5, H-7	2.51 (s)	22.0[g] (q)	H-6, H-8
1-OH	12.95 (s)	–	–	12.90 (s)	–	–

All spectra were recorded using 12 mg of a 1:1 mixture of compounds **1** and **2** dissolved in 1 mL of $CDCl_3$ with a 5 mm probe at 25°C.
[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz. Multiplicities were determined by DEPT experiment.
[c] HMBC data were recorded using a J_{CH}=8 Hz and are expressed as protons exhibiting $^{2-3}J_{CH}$ couplings to the carbons as indicated.
[d] Assignments bearing the same letter may be interchanged.
[e] Assignments bearing the same letter may be interchanged.
[f] Assignments bearing the same letter may be interchanged.
[g] Assignments bearing the same letter may be interchanged.

295.0971 $[M+H]^+$ (calcd 295.0970) and spectroscopic studies. The IR spectrum of compounds **1** and **2** exhibited a number of diagnostic absorption bands at 3438 (broad, O–H stretch), 1670 (C=O stretch, non-chelated), and 1633 cm^{-1} (C=O stretch, chelated). The UV spectrum showed a λ_{max} at 403 nm that suggested the presence of a single *peri*-hydroxyl functionality.[17] This was supported by the ^1H NMR spectrum that revealed two sharp singlets at δ_H 12.90 and 12.95 for compounds **2** and **1**, respectively, that were both exchanged upon addition of D_2O. Further evidence for the presence of a single hydroxyl functionality in compounds **1** and **2** came from their acetylation products **1a** and **2a**. Both of these acetyl derivatives exhibited the same molecular ion under HRFABMS at *m/z* 337.1068 $[M+H]^+$ (calcd for $C_{20}H_{17}O_5$, 337.1076) confirming the addition of a single acetate to **1** and **2**. The ^1H NMR spectrum of **1a** and **2a** no longer displayed downfield peaks between δ_H 12 and 13 while the ^{13}C NMR spectrum exhibited new signals at δ_C 19.6 ($-OCOCH_3$) and 169.0 ($-OCOCH_3$).

^1H NMR and DEPT experiments revealed the presence of two aromatic (δ_C 20.2 q×2, 21.9 q, and 22.0 q) and one acetyl (δ_C 31.9 q×2) methyl groups in both compounds **1** and **2**. Data from the HMBC experiment (Table 1) provided evidence for the assignment of these functionalities for compounds **1** and **2**. Further support in favor of this conclusion was obtained from long-range COSY and difference NOE experiments (Fig. 1). Both compounds **1** and **2** exhibited reciprocal NOE correlations upon irradiation of the methyl protons of C-12 (both δ_H 2.59) and 1-OHs (δ_H 12.95 and 12.90, respectively). In addition, NOE enhancements and long-range COSY correlations were noted between the methyl protons of C-13 (both δ_H 2.37) and the H-4 aromatic singlet (both δ_H 7.61). Together, these data confirmed the proposed ring B assignments for compounds **1** and **2**.

Compound **1** exhibited reciprocal NOE enhancements and COSY correlations amongst H-7 (δ_H 7.58 d, J=7.5 Hz) and H-8 (δ_H 8.15 d, J=7.5 Hz), as well as between the methyl protons of C-14 (δ_H 2.51 s) and protons at positions H-7 and H-5 (δ_H 8.04 s) (Fig. 1). This evidence confirmed that the aromatic methyl C-14 (δ_H 21.9) was attached at position 6 on ring A of compound **1**. Compound **2** differed from compound **1** by displaying reciprocal NOE enhancements and long-range COSY correlations between the methyl protons of C-14 (δ_H 2.51 s) and protons H-6 (δ_H 7.58 d, J=7.5 Hz) and H-8 (δ_H 8.05 s) (Fig. 1). Similar NOE and COSY correlations were noted between H-6 and H-5 (δ_H 8.13 d, J=7.5 Hz). Therefore, the assignment of the aromatic methyl C-14 (δ_C 22.0) was confirmed at position 7 on ring A of compound **2**. Compounds **1** and **2** have been named kwanzoquinones A and B, respectively, in recognition of their biogenic source.

The MeOH extract was subjected to repeated ODS and Sephadex LH-20 gel column chromatography yielding compounds **3**–**12**. Following purification, compound **3** was obtained from MeOH as orange needles. HREIMS (*m/z* 270.0532 $[M]^+$ (calcd for $C_{15}H_{10}O_5$, 270.0528)) and spectral evidence (IR, UV, 1D and 2D NMR) confirmed that compound **3** (1,2,8-trihydroxy 3-methylanthraquinone) had been previously isolated from *Myrsine africana* L. (Myrsinaceae) and was given the trivial name 2-hydroxychrysophanol.[18] Previous studies had only given partial ^1H and no ^{13}C NMR assignments for this compound; therefore, we undertook a thorough NMR investigation of **3** in order to confirm its proposed structure. This is the first report of

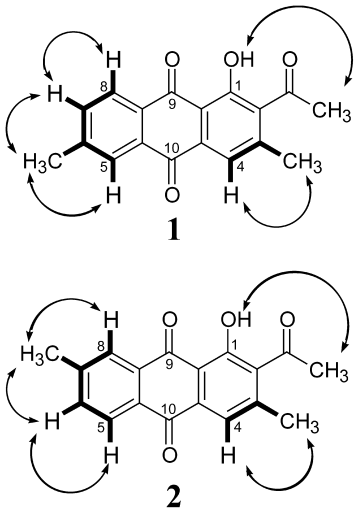


Figure 1. Difference NOE (→) and long-range COSY (▬) correlations used to establish the structures of kwanzoquinones A (**1**) and B (**2**).

compound **3** from daylilies and its complete ^{13}C NMR spectral data are presented in Table 2.

Compound **4** was obtained as yellow needles and exhibited many spectral characteristics similar to **3**. The IR spectrum of **4** revealed absorption bands at 3455 (broad, O–H stretch), 1671 (C=O stretch, non-chelated), and 1624 cm^{-1} (C=O stretch, chelated). The UV spectrum presented a λ_{max} at 429 nm that was in accordance with the presence of two *peri*-hydroxyl functionalities.[17,19,20] In addition, the ^1H NMR spectrum showed two downfield peaks (δ_H 12.00 and 12.04) that were exchanged with D_2O. Together this evidence supported the presence of a 1,8-dihydroxyanthraquinone moiety for compound **4**.

Table 2. ^{13}C NMR assignments for compounds **3**–**7** and **9**

Position	3	4	5	6	7	9
1	149.4 s	153.9 s	153.9 s	149.1 s	153.4 s	158.6 s
2	150.2 s	147.7 s	147.7 s	148.4 s	146.0 s	131.2 s
3	132.3 s	141.4 s	141.5 s	136.7 s	145.3 s	140.4 s
4	122.8 d	121.5 d	121.4 d	119.0 d	117.9 d	112.0 d
4a	123.1 s	128.0 s	128.0 s	123.3 s	128.2 s	136.2 s
5	119.0 d	119.0 d	119.0 d	119.1 d	119.1 d	118.1 d
6	137.3 d	137.1 d	137.1 d	137.4 d	137.2 d	135.9 d
7	123.7 d	124.1 d	124.0 d	123.7 d	124.0 d	124.2 d
8	161.2 s	161.2 s	161.2 s	161.3 s	161.2 s	161.3 s
8a	115.9 s	115.9 s	115.9 s	116.0 s	116.1 s	116.7 s
9	192.2 s	191.5 s	191.4 s	192.3 s	191.7 s	189.2 s
9a	114.3 s	115.2 s	115.2 s	114.6 s	115.7 s	122.4 s
10	180.1 s	180.8 s	180.6 s	180.2 s	180.8 s	181.8 s
10a	133.7 s	133.2 s	133.1 s	133.8 s	133.3 s	132.3 s
11	16.4 q	17.2 q	17.2 q	57.8 t	58.1 t	19.5 q
12	–	–	–	–	–	167.8 s
1′	–	102.9 d	102.8 d	–	102.7 d	–
2′	–	74.2 d	74.0 d	–	74.1 d	–
3′	–	76.3 d	76.0 d	–	76.2 d	–
4′	–	69.7 d	69.7 d	–	69.7 d	–
5′	–	77.3 d	73.8 d	–	77.2 d	–
6′	–	60.8 t	63.7 t	–	60.7 t	–
1″	–	–	166.4 s	–	–	–
2″	–	–	41.1 t	–	–	–
3″	–	–	167.4 s	–	–	–

Data recorded in DMSO-d_6 at 125 MHz at 25°C. Multiplicities were determined by DEPT experiment and confirmed by analysis of HMQC spectra.

FABMS gave *m/z* 433 $[M+H]^+$ that represented a molecular composition of $C_{21}H_{21}O_{10}$. ^1H NMR provided important evidence for the substitution pattern of rings B and A in compound **4**. Three protons representing an ABC spin system at δ_H 7.70 (dd, *J*=1.0, 7.5 Hz), 7.79 (dd, *J*=7.5, 8.0 Hz), and 7.36 (dd, *J*=1.0, 8.0 Hz) were assigned to C-5, C-6, and C-7, respectively, on ring A of compound **4**. ^{13}C NMR and DEPT experiments (Table 2) provided further evidence for the identity of the substituents attached to ring B of compound **4** with one methine (δ_C 121.5), one *C*-linked (δ_C 141.4) methyl (δ_C 17.2), and two quaternary carbon each linked with a hetero-atom (δ_C 147.7 and 153.9). These carbons were assigned positions in ring B of compound **4** based on their respective chemical shifts and the results from HMBC and HMQC experiments. Five additional methine (δ_C 69.7, 74.2, 76.3, 77.3, and 102.9) and one methylene (δ_C 60.8) spins were observed that exhibited chemical shift values that coincided with those for a glucopyranose moiety. Further evidence of the presence of a glucopyranose moiety in compound **4** was obtained by comparative TLC of the hydrolysate with authentic D-glucopyranose. The glucopyranose was assigned a β-configuration based on the coupling of H-1′ (δ_H 5.07, d, *J*=7.5 Hz). The complete structure of compound **4** was confirmed by HMBC experiment. Compound **4** is a new anthraquinone glycoside and has been given the name kwanzoquinone C.

The molecular formula of compound **5** was determined to be $C_{24}H_{22}O_{13}$ based on FABMS analysis that exhibited *m/z* 519 $[M+H]^+$. The spectral data of **5** were very similar to those obtained for compound **4**. The most significant difference was observed in the ^1H and ^{13}C (Table 2) NMR spectra with the addition of three new carbon signals at δ_C 41.1, 166.4, and 167.4 and a new proton resonance at δ_H 3.23 integrating for two hydrogens. These chemical shifts were characteristic of those expected for a malonyl moiety. The linkage of the malonyl group in compound **5** was established as malonyl-(1→6)-β-D-glucopyranoside based on the observed downfield shift of C-6′ to δ_C 63.7 versus that observed for compound **4** (Δ=+2.9 ppm). This was verified by HMBC analysis (Fig. 2) which exhibited weak $^4J_{CH}$ correlations from H-6a′ (δ_H 4.12) and H-6b′ (δ_H 4.27) to C-1″ (δ_C 41.1) and H-2″ (δ_H 3.23) to C-6′ (δ_C 63.7). This confirmed that compound **5** was a new anthraquinone malonyl-glucoside and was named kwanzoquinone D.

EIMS analysis of compound **6** gave a molecular ion of *m/z* 286 $[M]^+$ indicating a molecular formula of $C_{15}H_{10}O_6$. The UV (λ_{max} at 426 nm) and IR (absorption bands at 3469 (broad, O–H stretch), 1667 (C=O stretch, non-chelated), and 1620 cm^{-1} (C=O stretch, chelated)) spectra suggested a 1,8-dihydroxyanthraquinone chromaphore for compound **6**. The ^1H NMR spectrum provided evidence for four exchangeable protons at δ 12.06, 11.92, 10.47, and 5.40 representing three aromatic and one aliphatic hydroxyl functionalities. An ABC spin system was observed with protons at δ_H 7.70 (dd, *J*=0.5, 7.8 Hz), 7.78 (overlapping dd, *J*=7.8, 7.8 Hz), and 7.33 (dd, *J*=0.5, 7.8 Hz) which

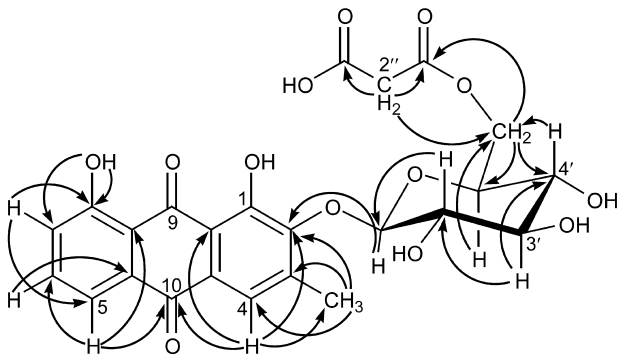


Figure 2. Selected HMBC correlations used to determine the structure of kwanzoquinone D (**5**).

occupied contiguous positions attached to C-5, C-6, and C-7, respectively, on ring A of compound **6**.

^{1}H and ^{13}C (Table 2) NMR and DEPT experiments of compound **6** gave evidence that ring B possessed quaternary carbons with *ortho*-hydroxyl functionalities (δ 149.1 s and 148.4 s), a hydroxy-methylene moiety (δ_H 4.59 s, 2H and δ_C 57.8 t) attached to a quaternary carbon (δ_C 136.7), and a methine (δ_C 119.0). An HMBC experiment was used to make full assignments of these proton and carbon spins as shown for compound **6** (Fig. 3). Compound **6** was identified as a new anthraquinone and has been named kwanzoquinone E.

Compound **7** exhibited spectral data similar to **6** with the addition of five methine (δ_C 69.7, 74.1, 76.2, 77.2, and 102.7) and one methylene (δ_C 60.7) spins that coincided with those for a glucopyranose moiety. The addition of a glucopyranose moiety in compound **7** was confirmed by HRFABMS which gave a molecular ion of *m/z* 449.1082 $[M+H]^+$ (calcd 449.1084 for $C_{21}H_{21}O_{11}$) that represented a molecular formula of $C_{21}H_{20}O_{11}$. The glucopyranose moiety was determined to be O-linked at position 11 due to the downfield shift of this carbon signal to δ_C 58.1 (Δ=+0.4) and the change in the splitting pattern of the attached protons. While the enantiotopic C-11 protons of compound **6** (δ_H 4.59, 2H) appeared as a singlet, the diastereotopic C-11 protons of compound **7** (δ_H 4.65, 1H and 4.73, 1H) were each a doublet (J=16.0 Hz) in achiral solvent (0.75 mL DMSO-d_6 with two drops of D_2O). Further evidence in support of the composition of compound **7** was obtained from acid hydrolysis that yielded kwanzoquinone E and D-glucopyranose based on co-TLC with authentic sugar samples. The assignments of all proton and carbon (Table 2) spins in compound **7** were confirmed by HMBC experiment. Compound **7** is a new conjugated anthraquinone glucoside and has been given the name kwanzoquinone F.

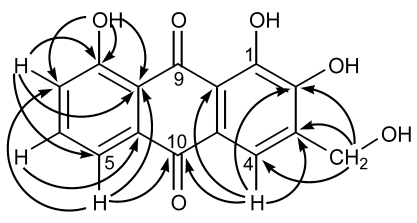


Figure 3. Selected HMBC correlations used to determine the structure of kwanzoquinone E (**6**).

Compound **8** was obtained as an amorphous yellow powder and its spectral data were found to match those reported for rhein, a known anthraquinone.[21] Compound **9** exhibited spectral data similar to **8** with the main differences in the ^{1}H NMR spectrum being the loss of an aromatic doublet (ca. δ_H 8.15, 1H, J=1.5 Hz) and the concomitant loss of splitting in the proton signal at δ_H 7.59 (s, 1H). This indicated that position 2 in ring B of compound **9** was substituted. These observations coincided with the appearance of an aromatic methyl (δ_H 2.67 s, 3H and δ_C 19.5 q) and the downfield shift of C-2 in compound **8** from δ_C 124.2 to 131.2 (Δ=+7.0 ppm) in compound **9** (Table 2). HMBC experiment was able to confirm that this methyl was a substituent of C-2 based on the long-range coupling of the C-11 methyl protons to C-2 (δ_C 131.2) and C-3 (δ_C 140.4). Compound **9** is a new anthraquinone and has been named kwanzoquinone G.

FABMS of compound **10** provided a molecular ion of 525 $[M+H]^+$ and the ^{13}C NMR spectrum exhibited 10 sp^2 carbon signals between 110 and 155 ppm along with 12 additional sp^3 carbon signals that were characteristic of a rutinose moiety. In light of the presence of three additional carbon signals that represented an aromatic methyl (δ_C 19.0) and an acetyl moiety (δ_C 31.9 and 204.4), it was determined that compound **10** was a substituted naphthalene diglycoside. Acid hydrolysis of compound **10** yielded D-glucopyranose and L-rhamnopyranose based on co-TLC with authentic sugar samples. HMQC and HMBC experiments established the aglycone portion of compound **10** as 2-acetyl-3-methyl-1,8-dihydroxynaphthalene, dianellidin. Further scrutiny of the HMBC data provided for the assignment of an 8-*O*-linkage to the rutinoside moiety based on a correlation from H-1′ (δ_H 5.04, d, J=7.5 Hz) to C-8 (δ_C 154.2 s). According to these data, compound **10** was identified as dianellin, previously isolated from *Dianella* spp. (Liliaceae).[22] This is the first account of compound **10** from daylilies and the first report detailing its ^{1}H and ^{13}C NMR spectral data.

The ^{1}H, ^{13}C and DEPT NMR data of compound **11** were very similar to those observed for **10**. The loss of one aromatic methine spin in compound **10** was replaced by a quaternary carbon (δ_C 148.3) that was linked to a heteroatom. The FABMS analysis of compound **11** yielded a molecular ion at *m/z* 541 $[M+H]^+$ indicating a molecular formula of $C_{25}H_{32}O_{13}$. A comparison of the ^{1}H and ^{13}C NMR spectral data for the aglycone portion of compound **11**, with the reported values for the naphthalene glycoside stelladerol,[23] demonstrated that both possessed the same aglycone moiety. However, these compounds differed with respect to their glycosidic moiety. Acid hydrolysis of the new naphthalene glycoside revealed the presence of D-glucopyranose and L-rhamnopyranose moieties in compound **11**. HMBC correlation data for compound **11** (Fig. 4) showed that it possessed an 8-*O*-β-D-rhamnopyranosyl-(1→6)-β-D-glucopyranoside moiety. Significant HMBC correlations that were used to deduce these connectivities included those observed for H-1′ (δ_H 4.87) to C-8 (δ_C 146.7), and H-6a′ (δ_H 3.92) and H-6b′ (δ_H 3.52) to C-1″ (δ_C

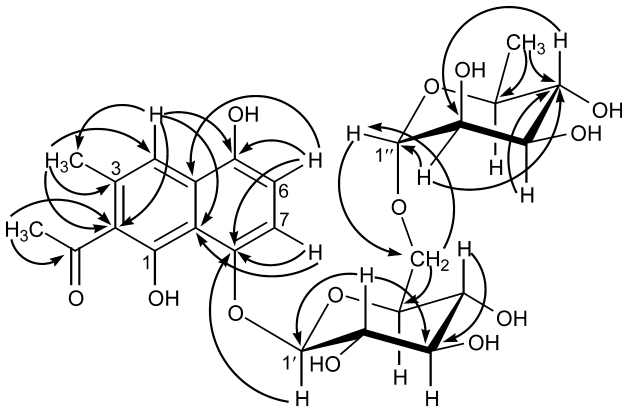


Figure 4. Selected HMBC correlations used to determine the structure of 5-hydroxydianellin (**11**).

100.7), as well as, from H-1″ (δ_H 4.61) to C-6′ (δ_C 66.6). Based on these data, compound **11**, 5-hydroxydianellin (1-(1,5,8-trihydroxy-3-methyl-naphthalen-2-yl)-ethanone-8-*O*-β-D-rhamnopyranosyl-(1→6)-β-D-glucopyranoside), was identified as a new naphthalene glycoside.

Compound **12** was obtained as a clear glass-like solid and identified as 5,7,3,4-tetrahydroxy-6-methylflavone(6-methylluteolin) that was previously reported from *Salvia nemorosa* L. (Lamiaceae).[24] The structure of compound **12** was confirmed based on detailed 1D and 2D NMR studies and by comparisons of its UV and IR spectral data with those reported. This is the first report of compound **12** from daylilies and the first detailed account of its ^{1}H and ^{13}C NMR spectral properties.

Compounds **1**–**11**, including **1a** and **2a**, were tested in vitro for their activity against multiple life-stages (cercariae, schistosomula, adult) of the human pathogenic trematode *S. mansoni*. At a concentration of 25 μg/mL, compounds **3** (2-hydroxychrysophanol) and **6** (kwanzoquinone E) exhibited significant activity by completely immobilizing all cercariae within 15 s and 14 min, respectively. The dose response effect of these compounds is shown in Fig. 5. The potency of

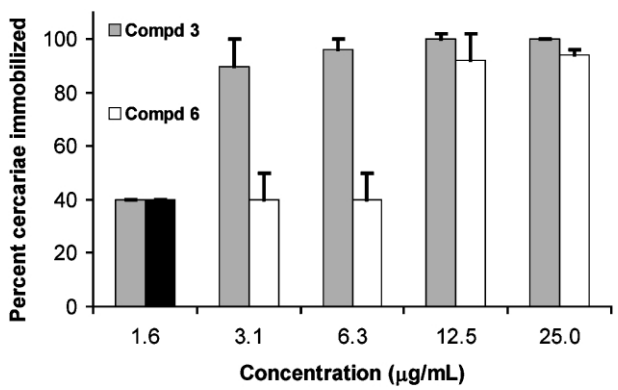


Figure 5. Dose response effect of 2-hydroxychrysophanol (**3**) and kwanzoquinone E (**6**) on *S. mansoni* cercariae mobility. Mobility was accessed based on the movement and swimming behavior of the invasive aquatic larval stage 10 min after the addition of test compound. There was no effect on the mobility of cercariae in the DMSO control. Data are represented as mean±one standard deviation (n=10).

compound **3** was not diminished even when diluted to a concentration of 3.1 μg/mL. After 30 min of exposure to test compounds, the test solution was removed and replaced with fresh medium. Cercariae treated with compound **3** exhibited 80% mortality after 24 h while those exposed to compound **6** were all dead. None of the other compounds isolated from *H. fulva* roots, including the glycosides of compounds **3** and **6**, compounds **4** and **7**, respectively, exhibited any activity at 25 μg/mL. The adult worms were also immobilized within 16 h by compounds **3** and **6** at 50 μg/mL. Following removal of the compounds, 35 and 55% of the adults exposed to compounds **3** and **6**, respectively, were dead. In contrast to the effects on the cercariae and adults, the intermediate schistosomula stage was refractory to all compounds at 25 μg/mL. Based on these promising results, compounds **3** and **6** are being investigated further in order to determine their mode of action and for potential development as topical anti-cercarial agents for the prevention of *Schistosoma* infection.

3. Experimental

3.1. General procedures

^{1}H NMR spectra were recorded at 500 and 600 MHz on Varian (Palo Alto, CA) VRX (500 MHz) and INOVA (600 MHz) instruments, respectively. ^{13}C NMR spectra were obtained at 125 MHz on a Varian VRX instrument. NMR spectra of compounds **1** and **2** were obtained in $CDCl_3$ while all other spectra were recorded in DMSO-d_6 (Cambridge Isotope Laboratories, Inc., Andover, MA). Standard pulse sequences were employed for all 1D (^{1}H, ^{13}C, DEPT, selective ^{1}H decoupling, and difference NOE) and 2D (DQF-COSY, long-range COSY, NOESY, HMQC, and HMBC) NMR experiments. Mass spectra were acquired at the Michigan State University Mass Spectrometry Facility using a JOEL AX-505H double-focusing mass spectrometer operating at 70 eV for EIMS analysis and a JEOL HX-110 double-focusing mass spectrometer (Peabody, MA) operating in the positive ion mode for FABMS experiments. The UV spectra were recorded in EtOH using a Shimadzu UV-260 recording spectrophotometer (Kyoto, Japan). IR spectra were obtained on a Mattson Galaxy Series FTIR 3000 using WinFIRST software (Thermo Nicolet, Madison, WI). Optical rotations were measured with a Perkin–Elmer Polarimeter 341 (Shelton, CT). Melting points were determined using a Thomas Model 40 Hot Stage (Philadelphia, PA). Sephadex LH-20 was purchased from Sigma-Aldrich (St Louis, MO). Si gel (particle size 40–63 μm) was obtained from Fischer Scientific (Pittsburgh, PA). Amberlite XAD-16 resin was purchased from Supelco (Bellefonte, PA). LC-SORB SP-A-ODS gel (particle size 25–40 μm) was obtained from Dychrom (Santa Clara, CA). Si gel PTLC plates (20×20 cm; 250, 500, and 1000 μm thick) were acquired from Analtech, Inc. (Newark, DE). Preparative HPLC was performed on a Japan Analytical Industry Co. model LC-20 recycling preparative HPLC with a JAIGEL-C_{18} column (10 μm, 20 mm×250 mm). Standards (α-tocopherol, D-glucopyranose and L-rhamnopyranose) were purchased from Sigma-Aldrich (St Louis, MO). All other solvents and chemicals were purchased from

Spectrum Laboratory Products, Inc. (New Brunswick, NJ) and were of ACS analytical grade.

3.2. Plant material

H. fulva 'Kwanzo' plants were purchased from Dr Linda Sue Barnes, Perennial Patch (Wade, North Carolina) in August 1999. The plants were grown on the Michigan State University campus before being harvested in April 2001. The leaves were removed and the roots and crowns of 124 plants (10 kg) were washed and frozen at −4°C. The frozen roots were lyophilized and ground in a Waring blender, yielding 2.2 kg of fine light-brown powder.

3.3. Extraction and isolation of compounds 1–12

The lyophilized, powdered roots (2.0 kg) were sequentially extracted with 3×8 L portions of hexane, EtOAc, and MeOH yielding 25, 23, and 130 g of extracts, respectively. The hexane extract was redissolved in 500 mL of hexane and partitioned with 3×500 mL portions of MeOH. The MeOH fractions were pooled, yielding 15 g of extract that was applied to Si gel VLC and eluted with 4 L hexane, 3 L hexane–acetone (9:1), and 3 L hexane–acetone (3:2). The hexane eluate (8.5 g) was subjected to Si gel MPLC under gradient conditions with 100% hexane to 100% acetone, and a total of 30 fractions, each 200 mL, were collected. All fractions were analyzed by TLC and pooled according to similarities in their profiles, yielding fractions A1–A4.

The hexane–acetone (9:1) eluate from the Si gel VLC (4.5 g) was subjected to Si gel MPLC under gradient conditions with 100% hexane to hexane–acetone (1:1) providing 900 mL fractions B1–B4. Fraction B2 (1.5 g) was rechromatographed by Si gel MPLC under gradient conditions with 100% hexane to 100% EtOAc and a total of 18 fractions, each with a volume of 200 mL, were collected and pooled based on TLC profiles giving fractions C1–C4. Fractions A3 (1 g), A4 (1 g), C2 (300 mg), and C3 (300 mg) were pooled based on further examination by TLC and applied to Si gel MPLC. Elution was carried-out under gradient conditions with 100% hexane to 100% $CHCl_3$ to $CHCl_3$–ethanol (1:1) and 18 mL fractions, D1–D90, were collected. Fractions D1–D10 were pooled (500 mg) and further subjected to Si gel MPLC under gradient conditions with 100% hexane to hexane–acetone (97:3) and 15 mL fractions E1–E40 were collected. Fractions E6–E20 (200 mg) were composed of primarily one major component and thus pooled and subjected to sequential Si gel PTLC with hexane–EtOAc (10:1) (72 mg), hexane– diethyl ether (6:1) (51 mg), and benzene–$CHCl_3$ (20:1), yielding 30 mg of α-tocopherol as a clear oil that exhibited spectral characteristics matching those reported in the literature,[25] and was found identical in all respects to an authentic standard.

Fraction D12–D45 (300 mg) were combined, applied to Si gel PTLC plates, and developed twice in benzene–$CHCl_3$ (10:1). A bright yellow band (44 mg) was obtained and following extraction from the Si gel, it was dissolved in a minimal volume of $CHCl_3$ and hexane added drop-wise until a slight degree of turbidity was noted. The solution was stored at −20°C, yielding an inseparable 1:1 mixture (based on ^{1}H NMR) of compounds **1** and **2** as fine yellow needles (12 mg). Compounds **1** and **2**, and their monoacetates **1a** and **2a**, were subjected to a variety of chromatographic techniques including further Si gel TLC and MPLC, as well as, ODS MPLC and ODS preparative HPLC, but failed to separate them as single entities.

The MeOH extract of the roots was dissolved in 800 mL MeOH–H_2O (3:1) and left at 4°C until a precipitate formed. The mixture was centrifuged (16,000×*g*, 15 min, 4°C) and the supernatant decanted to give 30 g of extract. The extract was applied to a column of XAD-16 resin and eluted with 10 L H_2O, 6 L 25% aqueous MeOH, and 8 L 100% MeOH. The MeOH eluate (18 g) was dissolved in 500 mL H_2O and partitioned with $CHCl_3$ (3×300 mL). The $CHCl_3$ fractions were pooled and evaporated under reduced pressure, yielding 2 g of extract that was applied to ODS MPLC, eluted with 50–100% MeOH, and 16 mL fractions F1–F166 were collected. Fractions F116–F125 were pooled giving 100 mg of residue that was dissolved in MeOH–acetone (3:1) and stored at −20°C, yielding 7 mg of compound **8** as a yellow powder. Compound **8** was identified as rhein based on comparisons of its physical and spectral data to those reported in the literature.[21]

The aqueous phase (16 g), from partitioning with $CHCl_3$, was dissolved in 50 mL of MeOH and 450 mL of acetone was slowly added while stirring and the mixture left at 4°C. The supernatant (14 g) was applied to ODS MPLC and eluted with 45–100% MeOH under gradient conditions, yielding 750 mL fractions G1–G6. Fraction G3 (1 g) was again applied to ODS MPLC and eluted with CH_3CN–MeOH–H_2O–TFA (25:25:50:0.1–30:30:40:0.1) under gradient conditions yielding fractions H1–H6. Fraction H5 (170 mg) was applied to Sephadex LH-20 with MeOH. The major component eluted as a yellow band (25 mg) and was further purified by ODS preparative HPLC with CH_3CN–MeOH–H_2O–TFA (50:20:30:0.1), yielding 16 mg of compound **9** as a yellow powder.

Fraction G1 (10 g) was applied to ODS MPLC with 10–50% CH_3CN under gradient conditions and 550 mL fractions (I1–I7) were collected. Fraction I3 (410 mg) was chromatographed on Sephadex LH-20 with MeOH, yielding 80 mg of yellow amorphous solid. This material was further purified by successive Si gel PTLC chromatography with EtOAc–$CHCl_3$–MeOH–H_2O–HCOOH (65:25:10:0.8:0.1) (75 mg) followed by $CHCl_3$–MeOH–H_2O (8:2:1) (70 mg). Final purification by ODS preparative HPLC with 60% MeOH gave 61 mg of compound **10** as a clear-yellow glass-like solid.

Fraction I4 (1.5 g) was applied to Sephadex LH-20 and eluted with MeOH giving 150 mL fractions J1–J6. Fractions J3–J6 (400 mg), I7 (300 mg), and H2–H4 (700 mg) were pooled and subjected to ODS MPLC with CH_3CN–MeOH–H_2O–TFA (20:20:60:0.1–40:40:20:0.1) under gradient conditions and 16 mL fractions K1–K105 were collected. Fractions K22–K38 (430 mg) were combined and chromatographed on Sephadex LH-20 with MeOH giving fractions L1–L2. Fraction L1 (300 mg) was applied to Si gel PTLC and developed twice with $CHCl_3$–MeOH–H_2O (8:2:0.1) giving a single band that was further purified

by ODS preparative HPLC with 60% MeOH to yield 31 mg of compound **11** as a clear glass-like solid.

Fractions L2 (130 mg) was applied to Sephadex LH-20 and eluted with MeOH to give 80 mg of a yellow amorphous solid. This material was dissolved in MeOH and placed at −20°C yielding 62 mg of precipitate. The precipitate was chromatographed twice by ODS preparative HPLC with CH_3CN–MeOH–H_2O–TFA (40:15:45:0.1) to give 30 mg of yellow amorphous solid. Further purification of it was achieved by using 60–100% MeOH as the solvent under gradient conditions, yielding a single fraction. It was reduced in vacuo and kept at −20°C yielding 1 mg of compound **7** as a yellow powder.

Fractions K50–K55 were combined (98 mg), subjected to Sephadex LH-20 chromatography using MeOH as the eluent, and 125 mL fractions (M1–M5) were collected. Fraction M5 (40 mg) was dissolved in MeOH and left at room temperature whereupon 25 mg of compound **4** was obtained as fine yellow needles.

Fractions K56–K62 were pooled (130 mg), applied to Sephadex LH-20 and eluted with MeOH to yield fractions N1–N3. Fraction N1 (50 mg) was subjected to further Sephadex LH-20 chromatography with MeOH giving a fraction (35 mg) that was chromatographed again on ODS preparative HPLC using CH_3CN–MeOH–H_2O–TFA (50:20:30:0.1). A single fraction was collected, reduced in vacuo, and placed at −20°C, yielding 6 mg of compound **5** as golden-yellow needles. Fraction N2 (7 mg) was further purified by ODS preparative HPLC using CH_3CN–MeOH–H_2O–TFA (50:20:30:0.1) as the mobile phase to yield 1 mg of compound **12** as a yellow glass-like solid.

Fractions K63–K77 were pooled and subjected to Sephadex LH-20 chromatography using MeOH as the mobile phase and 100 mL fractions (O1–O5) were collected. Fraction O3 (30 mg) was applied to ODS preparative HPLC using CH_3CN–MeOH–H_2O–TFA (50:20:30:0.1) as the mobile phase and yielded an amorphous yellow solid (6 mg). This material was further purified by ODS preparative HPLC under the same conditions and the resultant fraction reduced in vacuo and placed at −20°C to yield 4 mg of compound **6** as fine yellow needles.

Fractions K94–K100 were reduced in vacuo to dryness, yielding 13 mg of an orange amorphous solid. This material was dissolved in a minimal volume of MeOH and left at −20°C providing 7 mg of compound **3** as orange needles.

3.3.1. Kwanzoquinones A and B (1 and 2). Yellow needles; 165–167°C; UV λ_{max} (EtOH) 212, 262, 287, 403 nm; IR (KBr) ν_{max} 3438, 1700, 1696, 1691, 1685, 1670, 1652, 1630, 1595, 1559 cm^{-1}; ^1H NMR ^{13}C NMR data, see Table 1; HRFABMS *m/z* 295.0971 $[M+H]^+$ (calcd for $C_{18}H_{15}O_4$, 295.0970).

3.4. Acetylation of compounds 1 and 2

A portion (4 mg) of the 1:1 mixture of compounds **1** and **2** was dissolved in 1 mL of pyridine and 1 mL of Ac_2O was added and the solution was stirred at room temperature for 16 h. Deionized H_2O was added to the reaction mixture and it was subsequently partitioned with $CHCl_3$ (×3). The $CHCl_3$ fractions were reduced in vacuo and applied to a silica gel PTLC plate. The plate was repeatedly developed (×3) in hexane–diethylether–$CHCl_3$ (5:1:0.1) giving two UV active bands. Band 1 (R_f=0.4) (0.7 mg) was found to be identical to kwanzoquinones A and B (**1** and **2**) while band 2 (R_f=0.2) (3.6 mg) was crystallized from MeOH to give yellow needles that were an inseparable mixture of kwanzoquinone A and B monoacetates (**1a** and **2a**, respectively).

3.4.1. Kwanzoquinone A and B monoacetates (1a and 2a). Yellow needles; IR (KBr) ν_{max} 1773, 1706, 1675, 1592, 1457, 1438, 1368, 1328, 1251, 1187 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 8.10 (4H, m, H-5s and H-8s), 8.02 and 7.99 (2H, s, H-4′s), 7.55 (2H, m, H-6 and H-7 for compounds **2a** and **1a**, respectively), 2.49 (12H, brs, H-12s and −$OCOCH_3$s), 2.45 (6H, s, H-14s), 2.41 (6H, s, H-13s); HRFABMS at *m/z* 337.1068 $[M+H]^+$ (calcd for $C_{20}H_{17}O_5$, 337.1076).

3.4.2. 2-Hydroxychrysophanol (3). Orange needles; mp 239–240°C; UV λ_{max} (EtOH) (log ε) 208 (4.19), 235 (4.05), 258 (4.11), 426 (3.73) nm; IR (KBr) ν_{max} 3408, 1653, 1620, 1560, 1473, 1456, 1434, 1310, 1271, 1190, 1023 cm^{-1}; ^1H NMR (DMSO-d_6) δ_H 12.04 (1H, brs, 1-OH), 11.90 (1H, s, 8-OH), 10.34 (1H, brs, 2-OH), 7.76 (1H, dd, *J*=8.0, 7.5 Hz, H-6), 7.66 (1H, dd, *J*=7.5, 1.0 Hz, H-5), 7.55 (1H, s, H-4), 7.31 (1H, dd, *J*=8.0, 1.0 Hz, H-7), 2.26 (1H, s, 3-CH_3); ^{13}C NMR, see Table 2; EIMS *m/z* 270 $[M]^+$ (100), 253 (2), 242 (8), 213 (4), 196 (3), 185 (2), 168 (5), 139 (11); HREIMS *m/z* 270.0532 $[M]^+$ (calcd for $C_{15}H_{10}O_5$, 270.0528) (for literature values see Refs. 18 and 26).

3.4.3. Kwanzoquinone C (4). Fine yellow needles; mp 233–234°C; $[\alpha]_D^{20}=-46°$ (*c* 0.031, EtOH); UV λ_{max} (EtOH) (log ε) 206 (4.20), 227 (4.23), 260 (4.17), 429 (3.78) nm; IR (KBr) ν_{max} 3433, 1671, 1624, 1559, 1473, 1382, 1373, 1293, 1263, 1067 cm^{-1}; ^1H NMR (DMSO-d_6) δ_H 12.04 (1H, brs, 8-OH), 12.00 (1H, s, 1-OH), 7.79 (1H, dd, *J*=7.5, 8.0 Hz, H-6), 7.70 (1H, dd, *J*=1.0, 7.5 Hz, H-5), 7.61 (1H, s, H-4), 7.36 (1H, dd, *J*=1.0, 8.0 Hz), 5.07 (1H, d, *J*=7.5 Hz, H-1′), 3.60 (1H, ddd, *J*=2.0, 5.5, 12.0 Hz, H-6a′), 3.42 (1H, ddd, *J*=6.0, 11.5, 11.5 Hz, H-6b′), 3.31 (1H, m, H-2′), 3.25 (1H, m, H-3′), 3.16 (1H, m, H-4′), 3.13 (1H, m, H-5′), 2.42 (3H, s, H-11); ^{13}C NMR data, see Table 2; HRFABMS *m/z* 433.1139 $[M+H]^+$ (calcd for $C_{21}H_{21}O_{10}$, 433.1135).

3.4.4. Kwanzoquinone D (5). Golden-yellow needles; mp 174–175°C; $[\alpha]_D^{29}=-313°$ (*c* 0.008, EtOH); UV λ_{max} (EtOH) (log ε) 205 (4.28), 227 (4.35), 260 (4.31), 290 sh (3.91), 430 (3.96) nm; IR (KBr) ν_{max} 3430, 1734, 1717, 1699, 1670, 1653, 1559, 1457, 1268, 1066 cm^{-1}; ^1H NMR (DMSO-d_6) δ_H 12.57 (1H, brs, 1-OH), 11.96 (1H, s, 8-OH), 7.77 (1H, dd, *J*=7.5, 8.0 Hz, H-6), 7.67 (1H, dd, *J*=1.0, 7.5 Hz, H-5), 7.57 (1H, s, H-4), 7.33 (1H, dd, *J*=1.0, 8.0 Hz), 5.06 (1H, d, *J*=7.5 Hz, H-1′), 4.27 (1H, dd, *J*=2.5, 11.9 Hz, H-6a′), 4.12 (1H, dd, *J*=6.5, 11.9 Hz, H-6b′), 3.38 (1H, m, H-5′), 3.33 (1H, m, H-2′), 3.28 (1H, m, H-3′), 3.23 (2H, s, H-2″), 3.21 (1H, m, H-4′), 2.37 (3H, s, H-11); ^{13}C NMR data, see Table 2; HRFABMS *m/z* 519.1139 $[M+H]^+$ (calcd for $C_{24}H_{23}O_{13}$ 519.1151).

3.4.5. Kwanzoquinone E (6). Fine yellow needles; mp 196–197°C; UV λ_{max} (EtOH) (log ε) 209 (4.32), 235 (4.10), 258 (4.27), 354 (3.72), 426 (3.76) nm; IR (KBr) ν_{max} 3469, 1652, 1619, 1559, 1473, 1458, 1382, 1321, 1273, 1092 cm^{-1}; ^1H NMR (DMSO-d_6) δ_H 12.06 (1H, brs, 1-OH), 11.92 (1H, s, 8-OH), 10.47 (1H, brs, 2-OH), 7.87 (1H, d, J=0.5 Hz, H-4), 7.78 (1H, dd, J=7.8, 7.8 Hz, H-6), 7.70 (1H, dd, J=0.5, 7.8 Hz, H-5), 7.33 (1H, dd, J=0.5, 7.8 Hz, H-7), 5.40 (1H, brs, 11-OH), 4.59 (2H, s, H-11); ^{13}C NMR data, see Table 2; EIMS m/z 286 $[M]^+$ (62), 268 (89), 240 (56), 212 (100), 184 (50), 155 (14), 128 (19), 120 (19); HREIMS m/z 286.0479 $[M]^+$ (calcd for $C_{15}H_{10}O_5$, 286.0477).

3.4.6. Kwanzoquinone F (7). Yellow powder; mp 204–206°C; $[\alpha]_D^{29}$=−38° (c 0.01, EtOH); UV λ_{max} (EtOH) (log ε) 228 (4.04), 259 (4.03), 291 (3.57), 432 (3.68) nm; IR (KBr) ν_{max} 3450, 1698, 1684, 1652, 1635, 1559, 1540, 1457, 1262, 1027 cm^{-1}; ^1H NMR (0.75 mL DMSO-d_6/two drops D_2O) δ_H 7.88 (1H, s, H-4), 7.79 (1H, dd, J=7.5, 8.0 Hz, H-6), 7.71 (1H, dd, J=1.0, 7.5 Hz, H-5), 7.36 (1H, dd, J=1.0, 8.0 Hz, H-7), 5.07 (1H, d, J=7.5 Hz, H-1′), 4.37 (1H, d, J=16.0 Hz, H-11a), 4.65 (1H, d, J=16.0 Hz, H-11b), 3.60 (1H, d, J=3.0, 12.5 Hz, H-6a′), 3.40 (1H, dd, J=5.5, 12.0 Hz, H-6b′), 3.26 (1H, m, H-2′), 3.25 (1H, m, H-3′), 3.15 (1H, m, H-4′), 3.12 (1H, m, H-5′); ^{13}C NMR data, see Table 2; HRFABMS m/z 433.1132 $[M+H]^+$ (calcd for $C_{21}H_{21}O_{10}$, 433.1135).

3.4.7. Kwanzoquinone G (9). Yellow powder; mp 235–236°C; λ_{max} (EtOH) (log ε) 219 (4.25), 283 (4.19), 413 (3.63) nm; IR (KBr) ν_{max} 3420, 1717, 1700, 1670, 1634, 1577, 1365, 1320, 1261, 1223 cm^{-1}; ^1H NMR (DMSO-d_6) δ_H 12.82 (1H, s, 8-OH), 12.81 (2H, brs, 1-OH and 12-OH), 7.67 (1H, dd, J=8.1, 8.1 Hz, H-6), 7.57 (1H, dd, J=1.2, 8.1 Hz, H-5), 7.56 (1H, s, H-4), 7.28 (1H, dd, J=1.5, 8.1 Hz, H-7), 2.67 (3H, s, H-11); ^{13}C NMR data, see Table 2; HRFABMS m/z 299.0547 $[M+H]^+$ (calcd for $C_{16}H_{11}O_6$, 299.0556).

3.4.8. Dianellin (10). White needles; mp 156–157°C; $[\alpha]_D^{29}$=−137° (c 0.01, EtOH); UV λ_{max} (EtOH) (log ε) 225 (4.75), 301 (3.80), 334 (3.78) nm; IR (KBr) ν_{max} 3416, 2923, 1651, 1633, 1579, 1467, 1443, 1356, 1270, 1067 cm^{-1}; ^1H NMR (DMSO-d_6) δ_H 9.53 (1H, brs, 1-OH), 7.47 (1H, dd, J=1.0, 8.0 Hz, H-5), 7.40 (1H, dd, J=8.0, 8.0 Hz, H-6), 7.30 (1H, dd, J=1.0, 8.0 Hz, H-7), 7.21 (1H, s, H-4), 5.04 (1H, d, J=7.5 Hz, H-1′), 4.62 (1H, d, J=1.5 Hz, H-1″), 3.93 (1H, dd, J=1.5, 11.0 Hz, H-6a′), 3.68 (1H, m, H-2″), 3.59 (1H, m, H-5′), 3.50 (2H, m, H-6b′ and H-3″), 3.49 (1H, m, H-5″), 3.39 (1H, m, H-2′), 3.36 (1H, m, H-3′), 3.20 (1H, m, H-4″), 3.18 (1H, m, H-4′), 2.52 (3H, s, H-12), 2.25 (3H, s, H-13), 1.12 (3H, d, J=6 Hz, H-6″); ^{13}C NMR (DMSO-d_6) δ_C 204.4 (s, C-11), 154.2 (s, C-8), 150.2 (s, C-1), 135.7 (s, C-10), 132.8 (s, C-3), 127.3 (d, C-6), 125.2 (s, C-2), 122.3 (d, C-5), 119.4 (d, C-4), 113.2 (s, C-9), 110.7 (d, C-7), 102.6 (d, C-1′), 100.7 (d, C-1″), 76.2 (d, C-3′), 76.0 (d, C-5′), 73.3 (d, C-2′), 71.9 (d, C-4″), 70.7 (d, C-3″), 70.4 (d, C-2″), 70.1 (d, C-4′), 68.4 (d, C-5″), 66.6 (t, C-6′), 31.9 (q, C-12), 19.0 (q, C-13), 17.7 (q, C-6″); HRFABMS m/z 525.1970 $[M+H]^+$ (calcd for $C_{25}H_{33}O_{12}$, 525.1972).

3.4.9. 5-Hydroxydianellin (11). Yellow amorphous solid; mp 152–153°C; $[\alpha]_D^{29}$=−212° (c 0.01, EtOH); UV λ_{max} (EtOH) (log ε) 224 (4.92), 318 (4.13), 346 (4.15) nm; IR (KBr) ν_{max} 3420, 1698, 1684, 1653, 1635, 1559, 1457, 1364, 1257, 1059 cm^{-1}; ^1H NMR (DMSO-d_6) δ_H 9.71 (2H, brs, 1-OH and 5-OH), 7.43 (1H, s, H-4), 7.16 (1H, d, J=8.0 Hz, H-7), 6.76 (1H, d, J=8.0 Hz, H-6), 4.87 (1H, d, J=7.5 Hz, H-1′), 4.61 (1H, m, H-1″), 3.92 (1H, m, H-6a′), 3.69 (1H, brs, H-2″), 3.52 (1H, m, H-6b′), 3.51 (1H, m, H-5′), 3.50 (1H, m, H-3″), 3.48 (1H, H-5″), 3.34 (2H, m, H-2′ and H-3′), 3.21 (1H, m, H-4″), 3.18 (1H, m, H-4′), 2.51 (3H, s, H-12), 2.26 (3H, s, H-13), 1.14 (3H, d, J=6 Hz, H-6″); ^{13}C NMR (DMSO-d_6) δ_C 204.7 (s, C-11), 150.2 (s, C-1), 148.3 (s, C-5), 146.7 (s, C-8), 131.3 (s, C-3), 126.4 (s, C-10), 125.6 (s, C-2), 114.2 (s, C-9), 113.8 (d, C-4), 111.9 (d, C-7), 108.6 (d, C-6), 103.5 (d, C-1′), 100.7 (d, C-1″), 76.3 (d, C-3′), 75.9 (d, C-5′), 73.3 (d, C-2′), 72.0 (d, C-4″), 70.8 (d, C-3″), 70.5 (d, C-2″), 70.0 (d, C-4′), 68.4 (d, C-5″), 66.6 (t, C-6′), 31.9 (q, C-12), 19.3 (q, C-13), 17.7 (q, C-6″); HRFABMS m/z 541.1910 $[M+H]^+$ (calcd for $C_{25}H_{33}O_{13}$, 541.1921).

3.4.10. 6-Methylluteolin (12). Yellow glass-like solid; UV and IR data were identical to literature values;[24] ^1H NMR (DMSO-d_6) δ_H 10.92 (1H, s, 5-OH), 9.71 (1H, s, 7-OH), 9.55 (1H, s, 4′-OH), 9.23 (1H, s, 3′-OH), 7.40 (1H, d, J=2.0 Hz, H-2′), 7.16 (1H, dd, J=2.0, 8.5 Hz, H-6′), 6.80 (1H, d, J=8.5 Hz, H-5′), 6.47 (1H, s, H-3), 6.32 (1H, s, H-8), 1.92 (3H, s, $-CH_3$); ^{13}C NMR (DMSO-d_6) δ_C 180.1 (s, C-4), 165.0 (s, C-5), 164.2 (s, C-9), 154.5 (s, C-7), 147.4 (s, C-4′), 145.6 (s, C-3′), 145.3 (s, C-2), 123.9 (d, C-6′), 123.5 (s, C-1′), 117.5 (d, C-2′), 115.8 (d, C-5′), 109.8 (d, C-3), 105.8 (s, C-6), 102.8 (s, C-10), 90.2 (d, C-8), 7.5 (q, $-CH_3$); HRFABMS m/z 301.0709 $[M+H]^+$ (calcd for $C_{16}H_{13}O_6$, 301.0712).

3.5. Hydrolysis of compounds 4, 5, 7, 10, and 11

Approximately 0.5 mg of compounds **4**, **5**, **7**, **10**, and **11** were each combined with 2.5 mL of 15% aqueous HCl and left at 50°C for about 6 h with constant stirring. The mixtures were each neutralized with the drop-wise addition of 5% aqueous NaOH and partitioned with EtOAc. The EtOAc fractions were reduced in vacuo, the resultant residues were spotted on an analytical silica gel TLC plate along with compounds **3** and **6**, and the plate was developed in toluene–EtOAc–HOAc (4:2:0.1). After development, the plate was dried, lightly sprayed with 10% aqueous H_2SO_4, and charred with a heat-gun. The hydrolysate from compounds **4** and **5** exhibited a bright pink spot (R_f=0.8) that was identical to that observed for 2-hydroxychrysophanol (**3**). Similarly, the hydrolysate of compound **7** yielded a pink spot (R_f=0.4) that was identical to kwanzoquinone E (**6**). The aqueous portions from compounds **4**, **5**, **7**, **10**, and **11** were also reduced in vacuo and the residues spotted on analytical silica gel TLC plates along with D-glucopyranose and L-rhamnopyranose. The plate was developed in *n*-ButOH–HOAc–H_2O (3:1:1), air dried, sprayed with 10% aqueous H_2SO_4, and charred with a heat-gun. The hydrolysate from compounds **4**, **5**, **7**, **10**, and **11** each exhibited a black spot (R_f=0.6) that was identical with that observed for D-glucopyranose. In addition, compounds **10** and **11** also exhibited a second dark greenish-black spot (R_f=0.7) that matched L-rhamnopyranose.

3.6. *Schistosoma mansoni* cercariae assay

S. mansoni (Puerto Rican strain) cercariae were obtained from infected *Biomphalaria glabrata* snails by light induction. Details regarding the methods used for the maintenance of both *S. mansoni* and *B. glabrata* cultures have been previously reported.[27] A total of 50–100 cercariae in 100 μL distilled H_2O were collected and placed in Costar 96-well vinyl assay plates (Acton, MA). Stock solutions of compounds **1**–**11**, including **1a** and **2a**, were prepared by dissolving 1 mg of test compound in 100 μL of DMSO and 19.9 mL of distilled H_2O. This stock solution was further diluted as needed and 100 μL aliquots were added to each well. Cercariae mobility (i.e. tail movement and swimming behavior) was observed under a dissecting microscope. Viability of the cercariae was determined by removing the test compound after 10 h, and replacing it with fresh water. Recovery from exposure to the test compounds was assessed after 24 h.

3.7. *Schistosoma mansoni* schistosomula assay

Schistosomula were prepared from *S. mansoni* cercariae by shearing the tails and incubating the organisms for 2 days in RPMI-1640 media with pen/strep plus FBS in flat-bottomed Costar 96 well Cell Culture Cluster tissue culture plates. Test compounds were added to the media as described for the cercariae assay and the schistosomula were observed for changes in movement, feeding, and viability.

3.8. *Schistosoma mansoni* adult assay

Adult worms were perfused from Syrian Golden hamsters as described.[28] Twenty male and female adult worms were cultured in 24 well Falcon plates at 37°C in 1 mL of RPMI-1640 media supplemented with 2 g/L glucose, 0.3 g/L L-glutamate, and 2.0 g/L $NaHCO_3$, 15% fetal bovine serum (heat inactivated), pen/strep, and 15 μL of hamster red blood cells (washed with RPMI). Five microliter aliquots of test compounds in DMSO or DMSO control were added to each well. The movement, feeding, and viability of the adult worms were monitored for 24 h. The media were removed and replaced with fresh media to which the test compounds were added again and the adult worms observed for another 24 h. Finally, the media were again removed and replaced with fresh media without test compounds and the recovery of the adult worms was monitored for another 24 h.

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