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Algicide Constituents from *Swinglea glutinosa*

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Oscillatoria perornata, a cyanobacterium (blue-green alga), common in catfish production ponds in the southeastern United States, produces the monoterpene 2-methylisoborneol (MIB), which is absorbed into catfish flesh and imparts a "musty" taste, rendering them unpalatable and unmarketable. Algicides that are currently in the commercial market to control *O. perornata* have broad-spectrum toxicity toward other beneficial phytoplankton, such as the green alga *Selenastrum capricornutum*, as well as low biodegradability. As part of our continuing efforts to search for natural-product-based algicides, the ethyl acetate extract of the roots of *Swinglea glutinosa* was investigated. This report describes isolation and structure elucidation of one novel coumarin, two known coumarins, and nine acridone alkaloids from *S. glutinosa* root extracts and the evaluation of these compounds for algicidal activity against *O. perornata*.

KEYWORDS: Algicide; *Oscillatoria perornata*; *Selenastrum capricornutum*; *Swinglea glutinosa*; Rutaceae

INTRODUCTION

Commercial channel catfish (*Ictalurus punctatus* Rafinesque) production in the southeastern United States continues to experience problems with "off-flavor" caused by compounds produced by certain types of cyanobacteria (blue-green algae) that grow in catfish ponds. The most common off-flavor problem is "musty", which is due to the accumulation of 2-methylisoborneol (MIB) in the flesh of catfish, thereby rendering them unpalatable and unmarketable. The species of cyanobacteria attributed as the main source of musty off-flavor problems in Mississippi catfish ponds is the MIB-producing cyanobacterium *Oscillatoria perornata* Skuja (*1*, *2*). The most common management approach used by catfish farmers is the application of algicides to the catfish ponds to reduce the abundance of *O. perornata* and, subsequently, reduce the levels of MIB in pond water and eventually in catfish. The two types of algicides currently approved by the U.S. Environmental Protection Agency for use by catfish farmers to manage musty off-flavor episodes are copper-based products and diuron [*N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea]. However, these products have several negative attributes, including lengthy environmental persistence and broad-spectrum toxicity toward nontarget organisms. The discovery of environmentally safe natural compounds for selective phytoplankton control and economically effective alternatives to the currently used compounds would greatly benefit the U.S. channel catfish industry. Thus, plant extracts have been investigated to search for such compounds.

The small tropical tree *Swinglea glutinosa* (Blanco) Merr. is a member of the Rutaceae family. Originally brought to South America from southeast Asia, it is used as an ornamental plant in Colombia and as a natural barrier in rural areas and gardens. Extracts from this tree have been assessed for cytotoxic and antimalarial activity in previous studies (*3*, *4*). Acridone alkaloids have been isolated from this plant that exhibited *in vitro* antiplasmodial activity against chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* (*5*). However, *S. glutinosa* extracts have never been evaluated for selective algicide activity against odor-producing species of cyanobacteria. This report describes the following: (1) the isolation and structure elucidation of one novel coumarin, two known coumarins, and nine acridone alkaloids from *S. glutinosa* root extracts and (2) the evaluation of these compounds for algicidal activity against *O. perornata*.

MATERIALS AND METHODS

General Experimental Procedures. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Varian 400 Fourier transform NMR spectrometer. ^{13}C multiplicities were deduced from 135° distortionless enhancement by polarization transfer (DEPT) experiments. NMR assignments were made by correlation spectroscopy (COSY), gradient heteronuclear multiple-quantum coherence (HMQC), and gradient heteronuclear multiple-bond correlation (HMBC) experiments. High-resolution electrospray ionization mass

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spectra (HRESI−MS) were obtained using an Agilent 1100 high-performance liquid chromatograph (HPLC) coupled to a JEOL AccuTOF (JMS-T100LC). Column chromatography was performed using a Biotage, Inc., Horizon Pump equipped with a Horizon Flash Collector and fixed wavelength (254 nm) detector. Analytical thin-layer chromatography (TLC) was performed using 250 mm silica-gel TLC plates with a fluorescent indicator (Analtech, Newark, DE). Spots were visualized by ultraviolet (UV) light (254 and 365 nm) or by spraying with Dragendorff's and anisaldehyde TLC spray reagents.

Plant Material. Plant materials of *S. glutinosa* were collected and identified by Dr. Charles Burandtat in 2004 at the Montgomery Botanical Center, Coral Gables, FL. A voucher specimen BUR250903 is deposited at the University of Mississippi herbarium.

Extraction and Isolation. Dried roots, fruits, and stems of *S. glutinosa* were extracted separately at room temperature with EtOAc twice for 12 h. After evaporation of the solvent under reduced pressure at 40 °C, the root extract (4.0 g) was fractionated by silica-gel flash column chromatography eluting with hexane and EtOAc starting from 10% EtOAc up to 100% and finally with EtOAc in CH_3OH (19:1). Fractions with similar TLC profiles were combined to yield 16 fractions.

Fractions 1 (750 mg) and 2 (600 mg) were combined and purified by silica-gel column chromatography with hexane in EtOAc (19:1) to afford fractions A−F. Fraction B was determined to be pure compound **3** (25 mg). Fraction D was further purified by TLC using hexane in ethyl ether (4:1) to give compounds **1** (45 mg) and **2** (40 mg).

Fraction 4 was purified by crystallization from hexane and EtOAc to afford compound **11** (25 mg). Further purification of fraction 6 (400 mg), by silica-gel column chromatography using hexane in $CH_3)_2CO$ (9:1) and TLC plates using CH_2Cl_2 in ethyl ether (19:1), gave pure compounds **12** (14 mg), **6** (10 mg), **9** (6 mg), **8** (17 mg), and **5** (21 mg).

Fraction 10 (260 mg) was rechromatographed on a silica-gel column eluting with CH_2Cl_2 in ethyl ether (97:3) to afford compounds **7** (12 mg) and **4** (20 mg). Fraction 11 (130 mg) was rechromatographed on a silica-gel column eluting with CH_2Cl_2 in ethyl ether (19:1) to give 11 subfractions. The ninth subfraction was purified by TLC using CH_2Cl_2 in $(CH_3)_2CO$ (9:1) to yield pure compound **10** (3 mg).

6-(3,3-Dimethylallil)seselin (3). HRESI−MS: *m*/*z* 297.1491 $[M + H]^+$, calculated for $C_{19}H_{21}O_3$, 297.149070. ^1H NMR ($CDCl_3$) δ: 7.54 (1H, d, J = 9.6 Hz, H-4), 7.02 (1H, s, H-5), 6.84 (1H, d, J = 10.2 Hz, H-1″), 6.16 (1H, d, J = 9.6 Hz, H-3), 5.68 (1H, d, J = 10.2 Hz, H-2″), 5.22 (1H, t, J = 7.2 Hz, H-2′), 3.24 (2H, d, J = 7.2 Hz, H-1′), 1.72 (3H, s, H-5′), 1.70 (3H, s, H-4′), 1.44 (6H, s, H-4″ and H-5″). ^{13}C NMR ($CDCl_3$) δ: 161.5 (C-2), 154.2 (C-7), 148.9 (C-8a), 144.3 (C-4), 133.2 (C-3′), 130.6 (C-2″), 127.3 (C-5), 126.7 (C-6), 121.9 (C-2′), 115.6 (C-1″), 112.6 (C-3), 112.2 (C-4a), 109.1 (C-8), 78.0 (C-3″), 28.3 (C-4″ and C-5″), 28.0 (C-1′), 26.0 (C-5′), 18.1 (C-4′).

1,3,5-Trihydroxy-4-(γ,γ-dimethylallyl)-10-methylacridone (5). HRESI−MS: *m*/*z* 324.1264 $[M - H]^-$, calculated for $C_{19}H_{18}NO_4$, 324.1236. ^1H NMR (CD_3OD) δ: 7.63 (1H, dd, J = 1.6 and 7.6 Hz, H-8), 7.08 (1H, dd, J = 1.6 and 7.6 Hz, H-6), 7.04 (1H, t, J = 7.6 Hz, H-7), 6.17 (1H, s, H-2), 5.31 (1H, t, J = 7.8 Hz, H-2′), 3.56 (3H, s, N-Me), 3.41 (2H, d, J = 7.8 Hz, H-1′), 1.73 (3H, s, H-4′), 1.67 (3H, s, H-5′). ^{13}C NMR (CD_3OD) δ: 182.5 (C-9), 164.3 (C-3), 162.1 (C-1), 151.1 (C-4a), 148.8 (C-5), 138.2 (C-10a), 131.0 (C-3′), 124.6 (C-8a), 124.1 (C-2′), 122.7 (C-7), 119.2 (C-6), 115.7 (C-8), 108.0 (C-4), 106.7 (C-9a), 96.8 (C-2), 47.6 (N-Me), 25.9 (C-1′), 24.7 (C-5′), 17.0 (C-4′).

4,9-Dihydroxy-2-(2-hydroxypropan-2-yl)-11-methoxy-10-methyl-2,3-dihydrofuro[3,2-*b*]acridin-5(10*H*)-one (10). HRESI−MS: *m*/*z* 370.1318 $[M - H]^-$, calculated for $C_{20}H_{20}NO_6$, 370.1291. ^1H NMR (CD_6CO) δ: 7.79 (1H, dd, J = 1.6 and 8.0 Hz, H-8), 7.27 (1H, dd, J = 1.6 and 8.0 Hz, H-6), 7.15 (1H, t, J = 8.0 Hz, H-7), 4.87 (1H, dd, J = 8.0 and 9.2 Hz, H-2′), 3.85 (3H, s, 4-OMe), 3.82 (3H, s, N-Me), 3.20 (2H, m, H-1′a,b), 1.33 (3H, s, H-4′), 1.29 (3H, s, H-5′). ^{13}C NMR (CD_6CO) δ: 182.3 (C-9), 159.8 (C-3), 154.2 (C-1), 147.8 (C-5), 143.1 (C-4a), 137.3 (C-10a), 125.7 (C-4), 124.8 (C-8a), 122.9 (C-7), 119.7 (C-6), 116.8 (C-8), 107.5 (C-2), 107.3 (C-9a), 92.4 (C-2′), 72.0 (C-3′), 60.6 (4-OMe), 46.5 (N-Me), 27.5 (C-1′), 25.5 (C-5′), 24.2 (C-4′).

Algicide Bioassay. Cultures of the cyanobacterium *O. perornata* and the green alga *Selenastrum capricornutum* Printz were maintained separately in continuous, steady-state growth using the conditions indicated by Schrader et al. (*6*) to provide a source of cells growing at a fairly constant rate. *S. capricornutum* was used as a representative species for green algae to determine selective toxicity of the extracts, extract fractions, and pure isolated compounds. Methanol (100%) was used to dissolve crude extracts, extract fractions, and isolated compounds for the bioassay. Stock solutions of the crude extracts and extract fractions (20, 200, and 2000 ppm) and pure compounds (2, 20, 200, and 2000 μM) were added to the empty wells (10 μL/well) of a 96-well microplate. The solvent was allowed to evaporate completely before adding continuous culture material (200 μL/well). Final test concentrations were 1, 10, and 100 ppm for the crude extracts and extract fractions and 0.1, 1, 10, and 100 μM for each pure compound. Controls did not include any test material, only culture material. Three replications were used for each concentration of extracts, pure compounds, and the control. Microplates were placed in a growth chamber held at 29−30 °C and were illuminated continuously by fluorescent lights (40 W, cool white) at a photon flux density of 21−27 $\mu E\ m^{-2}\ s^{-1}$. Absorbance measurements of each well were measured at 650 nm at 24 h intervals for 4 days using a Packard model SpectraCount microplate photometer. Mean values and standard deviations of absorbance measurements were calculated and graphed to determine the lowest observed effect concentration (LOEC) (lowest concentration that inhibited growth) and the lowest complete inhibition concentration (LCIC) (lowest concentration that completely inhibited growth).

Table 1. Algicidal Activity of Crude Extracts of *S. glutinosa*

	O. perornata		*S. capricornutum*	
EtOAc extract	LOEC[a] (ppm)	LCIC[b] (ppm)	LOEC (ppm)	LCIC (ppm)
roots	10	100	100	>100
fruits	>100	>100	100	>100
stem	>100	>100	>100	>100

[a] Lowest observed effect concentration. [b] Lowest complete inhibition concentration.

RESULTS AND DISCUSSION

The ethyl acetate extracts of *S. glutinosa* roots, fruits, and stems were initially evaluated for antialgal activity toward *O. perornata*. The root extract was found to be selectively toxic toward *O. perornata* when compared to *S. capricornutum* (**Table 1**). Therefore, the root extract was chosen for further investigation via bioassay-guided fractionation.

The root extract was fractionated by silica-gel flash column chromatography eluting with hexane and EtOAc. Fractions with similar TLC profiles were combined to yield 16 fractions, and these were further purified by column chromatography and TLC, yielding one new compound (**3**), two known coumarins (**1** and **2**), and nine acridone alkaloids (**4**−**12**). Seselin (*7*) (**1**) and 5-methoxyseselin (*8*) (**2**) were identified by a comparison to spectroscopic data previously reported. The HRESI mass spectrum of compound **3** showed a molecular ion peak $[M + H]^+$ at *m*/*z* 297.1491 corresponding to the molecular formula $C_{19}H_{20}O_3$. The ^{13}C NMR and DEPT spectra indicated the presence of four methyl carbons, one methylene carbon, six methine carbons, and eight quaternary carbons. The ^1H NMR spectrum (**Table 2**) showed two doublets at δ 7.54 and 6.16 (J = 9.6 Hz) attributed to H-4 and H-3 of the coumarin skeleton, the characteristic signals of a dimethylchromene ring at δ 6.84 and δ 5.68 (d, J = 10.2 Hz), and two methyl groups at δ 1.44 (6H, s). Furthermore, the spectrum showed a singlet at δ 7.02 and a prenyl chain at δ 5.22 (1H, t, J = 7.2 Hz), 3.24 (2H, d, J = 7.2 Hz), 1.72, and 1.70 (each 3H). The protons were correlated to the corresponding carbons by the HMQC experiment. In a HMBC spectrum, H-1′ showed correlation with C-2′, C-3′, C-5, and C-7; H-1″ showed correlation with C-7; H-2″ showed correlation with C-3″ and C-8; and H-5 showed correlation with C-1′, C-4, C-7, and C-8a. These data established the structure of coumarin **3** as 6-(3,3-dimethylallyl)seselin.

Table 2. NMR ($CDCl_3$, 400 MHz) Data for Compound **3**

position	^{13}C (ppm)[a]	^{1}H δ [m, J (Hz)]	HMBC[a]
2	161.5		
3	112.6	6.16 (d, 9.6)	2, 4a
4	144.3	7.54 (d, 9.6)	2, 5, 8a
4a	112.2		
5	127.3	7.02 (s)	1′, 4, 7, 8a
6	126.7		
7	154.2		
8	109.1		
8a	148.9		
1′	28.0	3.24 (d, 7.2)	2′, 3′, 5, 6, 7
2′	121.9	5.22 (t, 7.2)	
3′	133.2		
4′	18.1	1.70 (s)	2′, 3′
5′	26.0	1.72 (s)	2′, 3′
1″	115.6	6.84 (d, 10.2)	7, 8, 8a
2″	130.6	5.68 (d, 10.2)	3″, 8
3″	78.0		
4″	28.3	1.44 (s)	2″, 3″
5″	28.3	1.44 (s)	2″, 3″

[a] HMBC correlations from H to C.

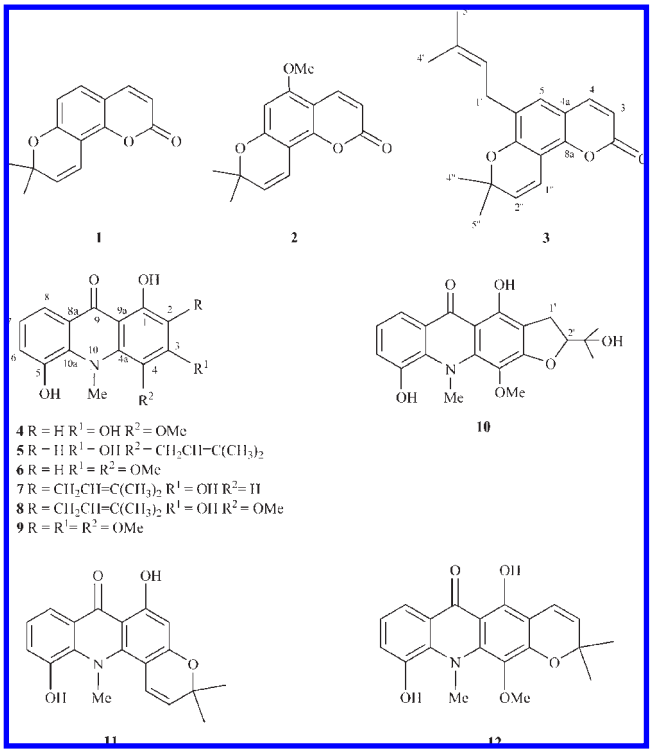

Figure 1. Structures of constituents isolated from the ethyl acetate extract of the roots of *S. glutinosa*.

The acridone alkaloids were identified as citrusinine II (*9*) (**4**), citrusinine I (*9*) (**6**), junosine (*10*) (**7**), glycocitrine IV (*11*) (**8**), citbrasine (*8*) (**9**), 4,9-dihydroxy-2-(2-hydroxypropan-2-yl)-11-methoxy-10-methyl-2,3-dihydrofuro[3,2-*b*]acridin-5(10*H*)-one (*3*) (**10**), atalaphyllidine (*12*) (**11**), and pyranofoline (*12*) (**12**) by analysis of their NMR spectra and comparison to spectroscopic data reported in the literature (**Figure 1**).

The molecular formula $C_{19}H_{19}NO_4$ of compound **5** was deduced from the molecular ion peak $[M - H]^-$ at m/z 324.1264 in the HRESI mass spectrum. The ^{13}C NMR and DEPT spectra indicated the presence of 3 methyl carbons, 1 methylene carbon, 5 methine carbons, and 10 quaternary carbons.

Table 3. NMR (CD_3OD, 400 MHz) Data for Compound **5**

position	^{13}C (ppm)	^{13}C (ppm)[a]	^{1}H δ [m, J (Hz)]	^{1}H δ [m, J (Hz)][a]	HMBC[b]
1	162.1	161.5			
2	96.8	95.8	6.17 (s)	6.20 (s)	1, 3, 4, 9a
3	164.3	161.1			
4	108.0	99.9			
4a	151.1	139.8			
5	148.8	144.7			
6	119.2	114.7	7.08 (dd, 1.6, 7.6)	7.20 (d, 2.5)	8, 10a
7	122.7	121.3	7.04 (t, 7.6)	7.11 (d, 8.3)	5, 8a
8	115.7	115.8	7.63 (dd, 1.6, 7.6)	7.60 (dd, 2.5, 8.3)	6, 9, 10a
8a	124.6	119.3			
9	182.5	180.5			
9a	106.7	103.5			
10a	138.2	133.3			
1′	25.9	21.4	3.41 (d, 7.8)	3.48 (d, 8.4)	2′, 3, 3′, 4, 4a
2′	124.1	121.7	5.31 (t, 7.8)	5.13 (t, 8.3)	
3′	131.0	130.6			
4′	17.0	17.9	1.73 (s)	1.79 (s)	2′, 3′, 5′
5′	24.7	25.9	1.67 (s)	1.98 (s)	2′, 3′, 4′
N-Me	47.6	44.3	3.57 (s)	3.63 (s)	4a, 10a

[a] Reported NMR data (500 MHz) (*12*). [b] HMBC correlations from H to C.

Table 4. Algicidal Activity of Pure Compounds from *S. glutinosa*

	O. perornata		*S. capricornutum*	
compound	LOEC[a] (μM)	LCIC[b] (μM)	LOEC (μM)	LCIC (μM)
1	10	100	10	100
2	10	100	0.1	>100
3	10	100	>100	>100
4	>100	>100	>100	>100
5	100	100	>100	>100
6	100	100	100	>100
7	>100	>100	>100	>100
8	100	>100	>100	>100
9	10	10	100	>100
11	>100	>100	>100	>100

[a] Lowest observed effect concentration. [b] Lowest complete inhibition concentration.

The ^{1}H NMR spectrum of **5** (**Table 3**) showed three correlated aromatic protons at δ 7.63 (1H, dd, J = 1.6 and 7.6 Hz), δ 7.08 (1H, dd, J = 1.6 and 7.6 Hz), and δ 7.04 (1H, t, J = 1.6 Hz), a singlet at δ 6.17, a prenyl chain at δ 5.31 (1H, t, J = 7.8 Hz), δ 3.41 (2H, d, J = 7.8 Hz), δ 1.73, and δ 1.67 (each 3H, s), and a N-Me group at δ 3.57. Detailed analysis of HMQC, HMBC, COSY, and DEPT spectra allowed the complete assignment of ^{1}H and ^{13}C signals and led us to identify the acridone alkaloid (**5**) as 1,3,5-trihydroxy-4-(γ,γ-dimethylallyl)-10-methylacridone. In the HMBC spectrum, H-1′ had a correlation with C-2′, C-3, C-3′, and C-4a; H-2 had a correlation with C-1, C-3, C-4, and C-9a; and H-10 (Me) had a correlation with C-4a and C-10a. Observed correlations and the signal of C-1′ at δ 25.9 ppm in the ^{13}C spectrum (*10*) supported the location of the prenyl chain at C-4. These NMR data were not in accordance with data previously reported for this structure (**Table 3**) (*12*).

Among the three ethyl acetate extracts of *S. glutinosa* (root, fruit, and stem) evaluated in the algicide bioassay, the root extract demonstrated the most toxicity toward *O. perornata* based on LOEC and LCIC results (**Table 1**). The lack of activity of the root extract against *S. capricornutum* indicated selective toxicity toward *O. perornata*. For these reasons, the root extract was chosen

to pursue bioassay-guided fractionation to isolate and identify the active compound(s).

Results of evaluation of the isolated compounds for antialgal activity toward *O. perornata* and *S. capricornutum* are provided in **Table 4**. The most toxic of the isolated compounds was found to be citbrasine **9**, with a LCIC of 10 μM. In addition, it was selectively toxic toward *O. perornata* when compared to LOEC and LCIC values for *S. capricornutum*. On the basis of LCIC results, the other 10 compounds were not very toxic toward *O. perornata*, while LOEC results indicated some growth inhibition of *O. perornata* by compounds **1**−**3** at 10 μM. None of the test compounds was very toxic toward *S. capricornutum* based on LCIC results; however, compound **1** partially inhibited growth at 10 μM (LOEC), while compound **2** was very active in terms of partial growth inhibition of *S. capricornutum*, with a LOEC of 0.1 μM, but growth was not completely inhibited even at 100 μM test concentration. Citbrasine **9** was previously examined for cytotoxic activity on several cancer cell lines showing only weak activity (*13*).

This research is the first to report on the selective algicidal activity of citbrasine toward *O. perornata*. Further evaluation of citbrasine for potential use as a selective algicide in catfish aquaculture will need to include assessment of toxicity toward other nontarget organisms (e.g., catfish), persistence in the environment, and efficacy of a water-soluble formulation in pond studies.

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LITERATURE CITED

(1) van der Ploeg, M.; Dennis, M. D.; de Regt, M. Q. Biology of *Oscillatoria* cf. *chalybea*, a 2-methylisoborneol producing blue-green alga of Mississippi catfish ponds. *Water Sci. Technol*. **1995**, *31*, 173–180.

(2) Schrader, K. K.; Dennis, M. E. Cyanobacteria and earthy/musty compounds found in commercial catfish (*Ictalurus punctatus*) ponds in the Mississippi Delta and Mississippi−Alabama Blackland Prairie. *Water Res*. **2005**, *39*, 2807–2814.

(3) Braga, P. A. C.; Dos Santos, D. A. P.; Da Silva, M. F. D. G. F.; Vieira, P. C.; Fernandes, J. B.; Houghton, P. J.; Fang, R. In vitro cytotoxicity activity on several cancer cell lines of acridone alkaloids and *N*-phenylethyl-benzamide derivatives from *Swinglea glutinosa* (Bl.) Merr. *Nat. Prod. Res*. **2007**, *21*, 47–55.

(4) Weniger, B.; Robledo, S.; Arango, G. J.; Deharo, E.; Argon, R.; Munoz, V.; Callapa, J.; Lobstein, A.; Anton, R. Antiprotozoal activities of Colombian plants. *J. Ethnopharmacol*. **2001**, *78*, 193–200.

(5) Weniger, B.; Um, B.-H.; Valentin, A.; Estrada, A.; Lobstein, A.; Anton, R.; Maille, M.; Sauvain, M. Bioactive acridone alkaloids from *Swinglea glutinosa*. *J. Nat. Prod*. **2001**, *64*, 1221–1223.

(6) Schrader, K. K.; de Regt, M. Q.; Tucker, C. S.; Duke, S. O. A rapid bioassay for selective algicides. *Weed Technol*. **1997**, *11*, 767–774.

(7) El-Shafae, A. M.; Ibrahim, M. A. Bioactive kaurane diterpenes and coumarins from *Fortunella margarita*. *Pharmazie* **2003**, *58*, 143–147.

(8) Wu, T. S.; Kuoh, C. S.; Furukawa, H. Acridone alkaloids and a coumarin from *Citrus grandis*. *Phytochemistry* **1983**, *22*, 1493–1497.

(9) Wu, T.-S.; Furukawa, H. Acridone alkaloids. VII. Constituents of *Citrus sinensis* Osbeck var. brasiliensis Tanaka. Isolation and characterization of three new acridone alkaloids, and a new coumarin. *Chem. Pharm. Bull*. **1983**, *31*, 901–906.

(10) Juichi, M.; Inoue, M.; Aoki, K.; Furukawa, H. Acridone alkaloids. XVI. Constituents of domestic Citrus plants. III. New acridone alkaloids from *Citrus junos* Tanaka. *Heterocycles* **1986**, *24*, 1595–1597.

(11) Ito, C.; Kondo, Y.; Wu, T.-S.; Furukawa, H. Chemical constituents of *Glycosmis citrifolia* (Willd.) Lindl. Structures of four new acridones and three new quinolone alkaloids. *Chem. Pharm. Bull*. **2000**, *48*, 65–70.

(12) Furukawa, H.; Yogo, M.; Wu, T. Acridone alkaloids. X. Carbon-13-nuclear magnetic resonance spectra of acridone alkaloids. *Chem. Pharm. Bull*. **1983**, *31*, 3084–3090.

(13) Wansi, J. D.; Wandji, J.; Mbaze, M. A. L.; Kamdem, W. A.F.; Ranjit, R.; Khan, S. N.; Asma, A.; Iqbal, C. M.; Lallemand, M.-C.; Tillequin, F.; Fomum, T. Z. α-Glucosidase inhibitory and antioxidant acridone alkaloids from the stem bark of *Oriciopsis glaberrima* Engl. (Rutaceae). *Chem. Pharm. Bull*. **2006**, *54*, 292–296.

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