PSII Inhibitory Activity of Resorcinolic Lipids from *Sorghum bicolor*

Agnes M. Rimando,*,† Franck E. Dayan,† and Jens C. Streibig‡

USDA-ARS, Natural Products Utilization Research Unit, P.O. Box 8048, University, Mississippi 38677, and Department of Agricultural Sciences, Section Weed Science, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Received August 28, 2002

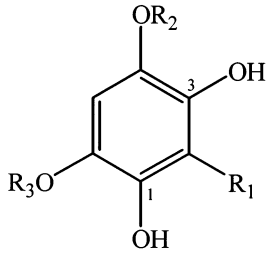
Resorcinolic lipids were isolated from the root extracts of *Sorghum bicolor* and identified as 4,6-dimethoxy-2-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]resorcinol (**4**), 4-methoxy-6-ethoxy-2-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]resorcinol (**5**), and 4-hydroxy-6-ethoxy-2-[(10′*Z*,13′*Z*)-10′,13′,16′-heptadecatriene]resorcinol (**6**). Compounds **4** and **5** inhibited photosynthetic oxygen evolution (IC_{50} 0.09 and 0.20 μM, respectively). Compound **4** could not be enzymatically converted to a quinone, suggesting that the quinone moiety is not required for its photosystem II inhibitory activity. Compounds **5** and **6** are reported for the first time.

Sorghum species are used in the southern United States as cover crops due to their ability to inhibit growth of weeds. Grain sorghum (*Sorghum bicolor*) has been demonstrated to be allelopathic.[1−3] Sorgoleone (**1**) is known to be the major phytotoxic constituent from the root exudates of grain sorghum.[4] In an earlier study, we isolated 5-ethoxysorgoleone (**2**) and prepared 2,5-dimethoxysorgoleone (**3**).[5] Both compounds were less phytotoxic than **1** when tested against lettuce seed germination.

Compound **1** inhibits photosynthetic electron transport by competing for the Q_B binding site of the natural substrate plastoquinone on photosystem II (PSII).[6−8] Compounds **2** and **3** were also found to inhibit photosynthetic electron transport.[5] The quinone moiety is believed to be the reactive group in these compounds.[9] In our further studies, however, three non-quinones were isolated from the root extracts of *S. bicolor* and were identified spectroscopically as resorcinolic lipids. Two of these compounds inhibited PSII electron transport. The structures and activities of these resorcinols are discussed.

Results and Discussion

In our continuing studies to identify phytotoxic constituents from root exudates of grain sorghum, three compounds were isolated and their structures were determined using various spectroscopic methods (UV, IR, MS, NMR). One of these was identified as a 4,6-dimethoxy-2-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]resorcinol (**4**), which was previously reported in *S. bicolor*.[10] The ^{1}H and ^{13}C NMR spectra of the two other compounds were very similar to **4** and proved to be analogues of **4**. Both compounds had resonances corresponding to the three methylene interrupted, skipped double bonds of the lipid side chain of the molecule (the ^{1}H and ^{13}C NMR resonances of the lipid side chain were previously determined[5]). One analogue differed from **4** in that the ^{1}H NMR methoxyl signal integrated for only three protons, and additional signals were observed at δ 4.03 (2H, q, J = 7.0 Hz) and 1.40 (3H, t, J = 7.0 Hz) (Table 1). These protons were coupled to each other (seen from the COSY spectrum), which indicated an ethoxy substituent. The position of the ethoxy group was determined from a HMBC experiment. Long-range correlation was observed between the methylene protons of the ethoxy moiety and C-6 (δ 137.8). Its HMBC spectrum also showed a correlation peak between the methoxy protons and the carbon resonance at δ 138.9, assigned as C-4. Long-range correlation was also observed between the protons at δ 2.67 and the carbon resonances at δ 138.5 and 138.7, assigned interchangeably as C-1 and C-3. The high-resolution electrospray ionization mass spectrum (HRESIMS) of this analogue gave a $[M + H]^+$ peak of m/z 389.2638 ($C_{24}H_{36}O_4$). On the basis of corroborating spectral data[5,10] this analogue was identified as 4-methoxy-6-ethoxy-2-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]resorcinol (**5**). The other analogue (**6**) was



	R_1	R_2	R_3
4	$-(CH_2)_7-(CH{=}CH-CH_2)_2-CH{=}CH_2$	CH_3	CH_3
5	$-(CH_2)_7-(CH{=}CH-CH_2)_2-CH{=}CH_2$	CH_3	CH_2CH_3
6	$-(CH_2)_9-(CH{=}CH-CH_2)_2-CH{=}CH_2$	H	CH_2CH_3

very similar to **5**, also bearing an ethoxy substituent as shown from the ^{1}H NMR and COSY spectra. The methylene protons of the ethoxy moiety showed long-range correlation with C-6 (δ 137.7) from its HMBC spectrum. Unlike **4** and **5**, this analogue did not have a methoxyl proton signal. Furthermore, from its ^{13}C NMR spectrum, in the upfield region between δ 29.0 and 29.5, six peaks instead of four peaks were observed, which indicated two additional methylene carbons in the aliphatic side chain. The presence of a C_{17} instead of a C_{15} side chain, and a hydroxyl instead of a methoxyl substituent at C-4, was substantiated by its HRESIMS, which gave a $[M + H]^+$ peak of m/z 403.2831 ($C_{25}H_{38}O_4$). This analogue was identified as 4-hydroxy-6-ethoxy-2-[(10′*Z*,13′*Z*)-10′,13′,16′-heptadecatriene]resorcinol (**6**). Compounds **5** and **6** are new to the literature.

Compound **4** was identified as a likely biosynthetic product from methylation of sorghum xenognosin for *Striga*

* Corresponding author. Tel: 662-915-1037. Fax: 662-915-1035. E-mail: arimando@ars.usda.gov.
† USDA-ARS, University, MS.
‡ Royal Veterinary and Agricultural University.

10.1021/np0203842 CCC: $25.00
Published on Web 12/06/2002

Table 1. NMR Data for Compounds **4**, **5**, and **6**[a]

	4		**5**		**6**	
position	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)	δ_C
1		138.5[b]		138.5[b]		138.9
2		116.4		116.2		116.1
3		138.8[b]		138.7[b]		138.9
4		138.5		138.9		138.9
5	6.42 (s)	95.2	6.41 (s)	96.4	6.40 (s)	97.6
6		138.8		137.8		137.7
1′	2.68 (dd, 7.7, 7.6)	23.6	2.67 (dd, 7.7, 7.5)	23.5	2.67 (dd, 7.6, 7.5)	22.5
2′	1.54−1.60 (m)	28.9	1.54−1.59 (m)	28.8	1.54−1.60 (m)	28.9
3′	1.25−1.39 (m)	29.3	1.25−1.33 (m)	29.2	1.25−1.30 (m)	29.0
4′	1.25−1.39 (m)	29.4	1.25−1.33 (m)	29.4	1.25−1.30 (m)	29.1
5′	1.25−1.39 (m)	29.5	1.25−1.33 (m)	29.5	1.25−1.30 (m)	29.2
6′	1.25−1.39 (m)	29.6	1.25−1.33 (m)	29.5	1.25−1.30 (m)	29.3
7′	2.01−2.05 (m)	27.2	2.00−2.04 (m)	27.1	1.25−1.30 (m)	29.5
8′	5.30−5.44 (m)	130.5	5.33−5.44 (m)	130.4	1.25−1.30 (m)	29.5
9′	5.30−5.44 (m)	127.4	5.33−5.44 (m)	127.3	2.00−2.04 (m)	27.1
10′	2.76−2.84 (m)	25.5	2.76−2.84 (m)	25.4	5.33−5.44 (m)	130.4
11′	5.30−5.44 (m)	129.3	5.33−5.44 (m)	129.2	5.33−5.44 (m)	127.3
12′	5.30−5.44 (m)	126.7	5.33−5.44 (m)	126.6	2.76−2.84 (m)	25.4
13′	2.76−2.84 (m)	31.5	2.76−2.84 (m)	31.4	5.33−5.44 (m)	129.2
14′	5.78−5.86 (m)	136.8	5.74−5.89 (m)	136.7	5.33−5.44 (m)	126.8
15′	a, 5.05 (ddt, 17.1, 1.7, 1.5) b, 4.98 (ddt, 11.7, 1.7, 1.8)	114.6	a, 5.03 (ddt, 17.1, 1.7, 1.4) b, 4.98 (ddt, 9.4, 1.7, 1.4)	114.5	2.76−2.84 (m)	31.4
16′					5.73−5.88 (m)	136.7
17′					a, 5.03 (ddt, 17.2, 1.7, 1.2) b, 4.98 (ddt, 10.1, 1.7, 1.2)	114.5
O*CH*$_3$	3.83 (s) ×2	57.0 × 2	3.81 (s)	56.9		
O*CH*$_2$CH$_3$			4.03 (q, 7.0)	65.8	4.01 (q, 6.9	65.7
OCH$_2$*CH*$_3$			1.40 (t, 7.0)	14.9	1.39 (t, 6.9)	14.9

[a] Recorded in $CDCl_3$ (^1H, 500 MHz; ^{13}C 125 MHz); δ in ppm. [b] Exchangeable.

Table 2. Effects of **1** and **4** on Development of Lettuce Seedlings[a]

concentration (mM)	**1**	**4**
3.33	5	5
1.00	4	5
0.30	3	4
0.10	1	3
control	0	0
control + solvent	0	0

[a] Activity based on visual rating of lettuce seedlings growth 4 days after treatment: 0 = no effect, 5 = death.

germination (SXSg), the chemical signal from the sorghum host initiating germination of the *Striga* parasitic plant.[11,12] It was demonstrated that **4** stabilizes and enhances the activity of SXSg by preventing its autoxidation.[10] SXSg is oxidized to **1**, and while **1** is inactive as *Striga* seed germination stimulant,[4] it is a potent phytotoxin[13,14] and is the major component in *S. bicolor* roots. The activity of **4** against lettuce seed germination was tested and was found to be more phytotoxic than **1** (Table 2). This may be due to a greater stability of the resorcinol ring of **4**, relative to the highly reactive benzoquinone ring of **1**. The minor components **5** and **6** were isolated in very small quantity, and we were not able to test the in vivo phytotoxicity of these compounds against lettuce seed germination.

The ability of **4** and **5** to inhibit PSII electron transport was investigated and compared with the activity of **1** and diuron, a commercial PSII inhibitor. Benzoquinone **1** and both resorcinol analogues **4** and **5** inhibited photosynthetic oxygen evolution from spinach thylakoid membranes with IC_{50} values of 0.09, 0.09, and 0.20 μM for **1**, **4**, and **5**, respectively (Figure 1 and Table 3). These I_{50} values are either lower than or equivalent to that obtained with the commercial herbicide diuron, which is 0.23 μM.

Benzoquinones (such as **1**) are known to be potent inhibitors of PSII by competing for the binding site of the

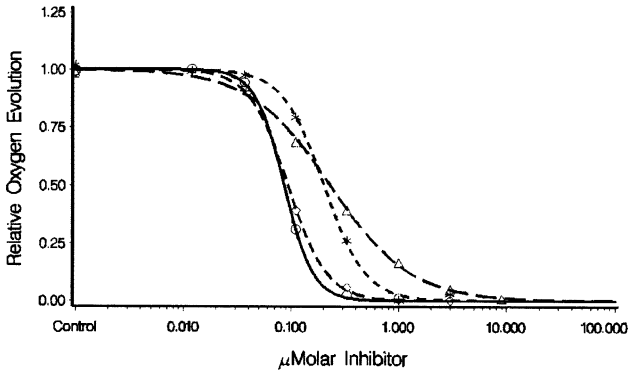


Figure 1. Effect of **1** (○) **4** (□), **5** (*), and diuron (△) on photosynthetic oxygen evolution from isolated thylakoid membranes.

Table 3. Summary of Regressions of Compounds **1**, **4**, and **5**[a]

	O_2 evolution, μmol mL^{-1} s^{-1}			IC_{50}	
compound	D	C	*b*	μM	confidence interval
1	1.02 (0.02)	0.12 (0.01)	3.20 (0.56)	0.09	0.08−0.10
4	1.28 (0.03)	0.20 (0.01)	2.39 (0.28)	0.09	0.08−0.10
5	1.05 (0.04)	0.10 (0.03)	2.25 (0.48)	0.20	0.15−0.26
diuron	1.16 (0.05)	0.12 (0.01)	1.16 (0.07)	0.23	0.20−0.26

[a] Standard deviations in parentheses.

natural electron acceptor plastoquinone at the Q_B binding site. The resorcinol analogues **4** and **5** were not expected to inhibit PSII as efficiently as **1**, because of the absence of a quinone moiety to act as the electron acceptor. Nonetheless, the aromatic ring of **4** and **5** was still able to interact with the binding pocket of the Q_B binding site. The lipid side chain may provide sufficient lipophilicity to these molecules to partition in the lipophilic environment of the thylakoid membranes where the binding site is located.

One concern was that the resorcinol ring could be converted to a quinone via either chemical or enzymatic activity. Compound **4** was incubated in the presence of active polyphenol oxidase at room temperature for up to 2 h without showing any sign of degradation (data not shown), as monitored by HPLC. Since the time of incubation for the PSII assay was 30 min on ice, the integrity of the resorcinol ring was not likely to have been altered during the experiments.

Statistical treatments of the dose response curves by four-parameter logistic analysis enabled the determination of the slope of these curves. As can be seen from Figure 1, the slopes of the curves of **1**, **4**, and **5** are similar to each other, whereas that of diuron is shallower. Difference in the slope of the curves is sometimes associated with different binding mechanisms. Diuron and natural products structurally related to **1**, **4**, and **5** bind to the same binding site,[7] and these compounds were demonstrated in this study to bind to the same site. As has been observed in previous studies,[15,16] the difference in the slope of diuron from those of **1**, **4**, and **5** suggests that these compounds interact with different regions of the same binding site on PSII.

Thus, one known (**4**) and two new (**5** and **6**) resorcinolic lipids were isolated from the root exudates of *Sorghum bicolor*. Compounds **4** and **5** inhibited photosynthetic oxygen evolution with IC_{50} values similar to that of **1**, the major sorghum phytotoxin. It was shown that **4** was not enzymatically converted into a quinone, suggesting that its activity does not require the presence of the quinone functionality and that it may exert inhibition by some other mechanisms. Compound **4** also inhibited lettuce seed germination, with activity greater than **1** at lower concentrations. Various biological activities for resorcinolic lipids have been reported,[17] but this is the first report on phytotoxic and PSII inhibitory activities for this class of compounds.

Experimental Section

General Experimental Procedures. UV spectra were obtained on a Shimadzu UV-3101 PC spectrophotometer. IR spectra were recorded with an ATI Mattson Genesis FT-IR instrument. HRESIMS was obtained from a Bruker FT-MS BioApex 3.0T spectrometer. NMR experiments were carried out on a Bruker Avance DRX (500 MHz) instrument. PTLC employed Merck Si gel F_{254} 20 × 20 cm, 0.5 mm thick plate (VWR Scientific, Atlanta, GA). All solvents used were HPLC grade (Fisher Scientific, Suwanee, GA). The HPLC system used for the quinone conversion experiment was composed of Waters Associates components (Milford, MA 01757), which included a Model 717 plus autosampler, a Model 600 controller, and Models 474 fluorescence and 996 photodiode array detectors.

Plant Material. *Sorghum bicolor* (L.) Moench (Poaceae), DeKalb Hybrid SX17 (Dekalb, IL), was grown as previously described.[8]

Isolation of 4, 5, and 6. Grain sorghum roots were dipped in acidified (0.25% HOAc) $CHCl_3$. The extract was evaporated under vacuum, and preparative layer chromatography was carried out on Si gel plates using hexane−*i*PrOH (90:10) as developing solvent to isolate the compounds, which were visualized under UV at 254 nm. The R_f values of **4**, **5**, and **6** were 0.52, 0.62, and 0.76, respectively.

4,6-Dimethoxy-2-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]-resorcinol (4): oily, UV (CH_2Cl_2) λ_{max} (log ϵ) 226 (3.92) 293 (3.75) nm; IR (film) ν_{max} 3508, 3008, 2927, 2848, 1487, 1435, 1354, 1252, 1201, 1148 cm^{-1}; ^1H and ^{13}C NMR (see Table 1).

4-Methoxy-6-ethoxy-2-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]resorcinol (5): oily, UV (CH_2Cl_2) λ_{max} (log ϵ) 2226 (3.76) 289 (3.53) nm; IR (film) ν_{max} 3525, 3006, 2922, 2851, 1489, 1355, 1205, 1150 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HRESIMS MH^+ 389.2638 (calcd for $C_{24}H_{36}O_4 + H^+$ 389.2686).

4-Hydroxy-6-ethoxy-2-[(10′*Z*,13′*Z*)-10′,13′,16′-heptadecatriene]resorcinol (6): oily, UV (CH_2Cl_2) λ_{max} (log ϵ) 226 (3.85) 282 (3.45) nm; IR (film) ν_{max} 3340, 2953, 2927, 2855, 1461, 1285, 1204, 1149 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HRESIMS MH^+ 403.2831 (calcd for $C_{25}H_{38}O_4 + H^+$ 403.2843).

Bioassays. Phytotoxicity and oxygen evolution assays were performed as in an earlier study.[5] In the oxygen evolution assay, dose response curves consisted of a half-log dilution series ranging from 0.01 to 100 uM, using a dilution factor of 3.0, plus an untreated control. The response, O_2 evolution (U), on concentrations of inhibitor (z), was described by the logistic model:[15]

$$U_j = C + \frac{D - C}{1 + \exp[b(\log(z_j) - \log(I_{50}))]}$$

where U_j denotes O_2 evolution at the jth concentration; D and C denote the upper and lower limits of O_2 evolution at zero and at infinite concentrations of inhibitor. IC_{50} denotes the concentration required to reduce O_2 evolution by half between the upper and lower limits, D and C; b is proportional to the slope of the curve around IC_{50}.

An experiment consisted of several dose response curves. As the O_2 evolution was measured consecutively for each dose response curve, the response curves were analyzed separately because we could consider them to have the same variance.[18] Stabilization of the variance of response by transforming both sides of the nonlinear regression and the tests for lack of fit of the models have been described elsewhere.[19]

Quinone Conversion of Compound 4. The possible enzymatic conversion of **4** into a quinone was studied. Polyphenol oxidase (PPO) was extracted from leaves of mungbean [*Vigna radiata* (L.) Wilczek] in 0.1 M sodium acetate buffer, pH 5.5. Twenty microliters of the cell-free extracts obtained (2.5 mg protein/mL) were added to 180 μL of oxygen-saturated sodium acetate buffer containing either 25 mM l-DOPA or **4** as substrate. PPO activity was determined spectrophotometrically at λ 490 nm for l-DOPA and by HPLC for the conversion of **4** to **1**, monitored at λ 280 nm using a photodiode array detector. The column used was a 3.9 × 300 mm (i.d.) μBondapak C18 reversed-phase column preceded by a Bio-Rad ODS-5S guard column. The solvent system consisted of a gradient beginning at 70% HPLC-grade MeOH and 30% double-distilled H_2O, reaching 100% MeOH within 20 min in a linear mode, holding 100% MeOH 20 min to clean the column, and reequilibrating to the original settings. Flow rate was 1 mL/min. Injection volume was 50 μL. The elution of the benzoquinone was monitored at 287 nm with the photodiode array detector scanning from 250 to 300 nm.

Acknowledgment. We acknowledge the technical assistance of Mr. Stacy Allen, Mr. Frank Wiggers, and Dr. Daniel C. Dunbar. We wish to thank the Danish Agricultural and Veterinary Research Council (Grant 9601215) for partially financing this work. We also thank Dr. Leslie Weston and Dr. Mark A. Czarnota for providing *Sorghum* root extract.

References and Notes

(1) Putnam, A. R.; DeFranck, J.; Barnes, J. P. *J. Chem. Ecol.* **1983**, *18*, 197−207.
(2) Einhellig, F. A.; Rasmussen, J. A. *J. Chem. Ecol.* **1989**, *15*, 951−960.
(3) Panasiuk, O.; Bills, D. D.; Leather, G. R. *J. Chem. Ecol.* **1986**, *12*, 1533−1543.
(4) Netzley, D. H.; Riopel, J.; Ejeta, G.; Butler, L. G. *Weed Sci.* **1988**, *36*, 441−446.
(5) Rimando, A. M.; Dayan, F. E.; Czarnota, M. A.; Weston, L. A.; Duke, S. O. *J. Nat. Prod.* **1998**, *61*, 927−930.
(6) Einhellig, F. A.; Souza, I. F. *J. Chem. Ecol.* **1992**, *18*, 1−11.
(7) Nimbal, C. I.; Yerkes, C. N.; Weston, L. A.; Weller, S. C. *Pestic. Biochem. Physiol.* **1996**, *54*, 73−83.
(8) Gonzalez, V. M.; Kazimir, J.; Nimbal, C. I.; Weston, L. A.; Cheniae, G. M. *J. Agric. Food Chem.* **1997**, *45*, 1415−1421.
(9) Kless, H.; Oren-Shamir, M.; Malkin, S.; McIntosh, L.; Edelman, M. *Biochem.* **1994**, *33*, 10501−10507.
(10) Fate, G. D.; Lynn, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 11369−11376.

(11) Chang, M.; Netzly, D. H.; Butler, L. G.; Lynn, D. G. *J. Am. Chem. Soc.* **1986**, *108*, 7858−7860.
(12) Netzly, D. H.; Riopel, J. L.; Ejeta, G.; Butler, L. G. *Weed Sci.* **1988**, *36*, 441−446.
(13) Einhellig, F. A.; Souza, I. T. *J. Chem. Ecol.* **1992**, *18*, 1−11.
(14) Nimbal, C. I.; Pedersen, J. F.; Yerkes, C. N.; Weston, L. A.; Weller, S. C. *J. Agric. Food Chem.* **1996**, *44*, 1343−1347.
(15) Rimando, A. M.; Dayan, F. E.; Streibig, J. C.; Weston, L. A.; Duke, S. O. Book of Abstracts, 216th ACS National Meeting, Boston, August 23−27; 1998, AGFD-067.
(16) Streibig, J. C.; Dayan, F. E.; Rimando, A. M.; Duke, S. O. *Pestic. Sci.* **1999**, *55*, 137−146.
(17) Kozubek, A.; Tyman, J. H. P. *Chem. Rev.* **1999**, *99*, 1−25.
(18) Madsen, K. H.; Jensen, J. E. *Weed Res.* **1995**, *35*, 105−111.
(19) Streibig, J. C.; Rudemo, M.; Jensen, J. E. In *Herbicide Bioassays*; Streibig, J. C., Kudsk, P., Eds.; CRC Press: Boca Raton, FL, 1993; Chapter 3, pp 29−55.

NP0203842