PERGAMON

Phytochemistry 54 (2000) 281–287

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Two antifeedant lignans from the freshwater macrophyte *Saururus cernuus*

Julia Kubanek[a,*], William Fenical[a], Mark E. Hay[b], Pam J. Brown[c], Niels Lindquist[c]

[a]*Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California — San Diego, La Jolla, CA 92093-0204, USA*

[b]*School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230, USA*

[c]*University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, NC 28557, USA*

Received 23 November 1999; received in revised form 12 January 2000

Abstract

Two diarylbutane derivatives of dihydroguaiaretic acid have been isolated from emergent portions of the southeastern United States freshwater angiosperm *Saururus cernuus* L. (Saururaceae). Bioassay-guided fractionation of organic extracts of *S. cernuus* led to the compounds, sauriols A and B, in addition to five previously known lignoids. These metabolites deter feeding by the omnivorous crayfish *Procambarus clarkii*. The two lignans were identified by analysis of nuclear magnetic resonance and mass spectral data, and by comparison with spectral data of dihydroguaiaretic acid.

Keywords: *Saururus cernuus*; Saururaceae; Lignans; Antifeedants; Sauriol A; Sauriol B; Chemical defense; Plant–herbivore interactions; *Procambarus clarkii*

1. Introduction

Few freshwater plants have been shown to possess chemical defenses against herbivores; only a small number of studies have reported the isolation of discrete molecules proven to play a defensive role (Newman et al., 1990, 1992, 1996; Bolser et al., 1998; Kubanek et al., 1999). However, freshwater herbivores are numerous, important in affecting macrophyte abundance and distribution (Lodge, 1991; Newman, 1991; Cyr and Pace, 1993), and they show distinct feeding preferences that are affected by secondary metabolites in freshwater plants (Lodge, 1991; Bolser et al. 1998, Cronin, 1998, Hay et al., unpublished).

Saururus cernuus (Saururaceae) is an emergent freshwater angiosperm commonly found throughout the southeastern United States. Also known as lizard's tail and breast weed, it has long been used as a folk remedy for inflammation of breasts, kidneys, and bladder (Phares, 1867), as a poultice for tumors (Hartwell, 1971), and as a sedative (Phares, 1867). The chemical constituents responsible for these properties have never been identified, although natural products with some notable biological activities have been isolated from this plant. To date, more than 20 lignans and neolignans, derivatives of cinnamic acid dimers, have been identified from *S. cernuus* (Rao and Alvarez, 1982, 1983; Rao and Chattopadhyay, 1990; Rao and Reddy, 1990; Rao and Rao, 1990; Rao and Oruganty, 1997). The most significant pharmacologic property reported from these compounds has been the murine neuroleptic activity of the dilignans, manassantins A and B (Rao et al., 1987). No ecological functions of the metabolites in *S. cernuus* have been postulated or tested.

In a survey of relative palatabilities of freshwater plants to the generalist omnivorous crayfish *Procambarus clarkii*, *S. cernuus* from North Carolina ranked

* Corresponding author. Tel.: +1-858-534-3953; fax: +1-858-558-3702.

E-mail address: jkubanek@ucsd.edu (J. Kubanek).

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00076-5

low relative to other local freshwater macrophytes in overall plant palatability (Bolser et al., 1998, Hay et al., unpublished). Its chemical extracts, both lipophilic and water-soluble, were found to significantly deter crayfish feeding as compared with controls. We recently conducted a study in which we used bioassay-guided fractionation to search for antifeedant constituents from lipophilic extracts of this freshwater macrophyte (Kubanek et al., 1999). In the course of this work, we identified seven lignoids that, at natural or close to natural plant concentrations, each significantly deterred feeding by the omnivorous crayfish *P. clarkii*. In this paper, we describe the isolation and structural elucidation of two of these compounds, the previously undescribed lignans sauriols A (**1**) and B (**2**). Extracts of *S. cernuus* collected in North Carolina contained five other deterrent lignoids (Kubanek et al., 2000) that have been previously identified from this plant: (−)-dihydroguaiaretic acid (**3**), (−)-licarin A (**4**), (+)-saucernetin (**5**), (−)-saucerneol (**6**), and (−)-saucerneol methyl ether (**7**) (Rao and Alvarez, 1982, 1983; Rao and Chattopadhyay, 1990; Rao and Oruganty, 1997).

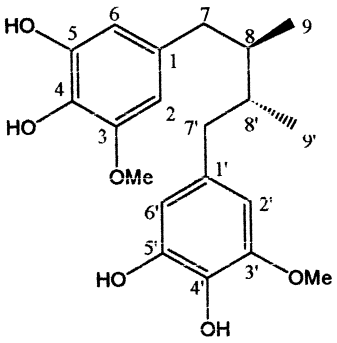
2. Results and discussion

Saururus cernuus was collected from a pond at the entrance to White Sands subdivision in Newport, NC, frozen at −70°C, and then freeze-dried and ground. Because emergent and submerged leaves of Saururus are morphologically similar, and the water level in local lakes varies as a function of recent weather conditions, it is difficult to distinguish between tissues that have grown mostly submerged and those that have grown mostly emergent. Plant material collected was estimated as 70% emergent at the time of collection. Plant dry weights were recorded before repetitive extraction with methanol/dichloromethane (1:1). Separation of extract constituents was followed by an aquarium crayfish choice feeding assay described in Kubanek et al. (2000) and Bolser et al. (1998). Chromatographic separation of deterrent fractions by size-exclusion chromatography, silica gel flash column chromatography, and then reversed phase HPLC led to the isolation of sauriol A (**1**) (0.010% by dry weight), sauriol B (**2**) (0.038%), (−)-dihydroguaiaretic acid (**3**) (0.11%), (−)-licarin A (**4**) (0.55%), (+)-saucernetin (**5**) (0.84%), (−)-saucerneol (**6**) (0.020%), and (−)-saucerneol methyl ether (**7**) (0.01%). Each of these compounds significantly deterred crayfish feeding at approximated natural concentrations by plant dry weight (Kubanek et al., 2000). The structures of compounds **3**–**7** were elucidated by comparison of spectral data (^{1}H- and ^{13}C-NMR, mass spectra, and optical rotation) with published values for these compounds (Aiba et al., 1973; Rao and Alvarez, 1982, 1983; Rao and Chattopadhyay, 1990; Rao and Oruganty, 1997).

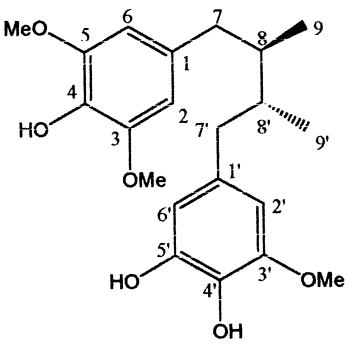
Sauriol A (**1**) had ^{13}C- and ^{1}H-NMR spectral characteristics very similar to those of **3**, indicating a close derivative that shared the C_2 symmetry of their planar structures. The molecular formula supported by mass spectral data,$C_{20}H_{26}O_6$, suggested that **1** contained two more hydroxyl groups than **3**. Differences in the NMR spectra were apparent only in the aromatic portions, indicating that the 1,4-(2,3-dimethyl)-butyl bridge between aromatic groups remained intact. The ^{1}H-NMR spectrum of **1** differed from that of **3** by the presence of two phenolic hydrogen signals (δ 5.17 and 5.20) instead of one; one fewer phenyl hydrogen resonance; and the collapse of a doublet of doublets aromatic resonance to a doublet (1.5 Hz) at δ 6.32. Two aromatic methine signals in the ^{13}C-NMR spectrum of **1** were shifted slightly upfield relative to those in **3**. Additionally, one methine carbon signal was missing and instead an additional quaternary phenolic carbon signal was observed at 130.0 ppm, its relatively upfield chemical shift indicating that it was flanked by two other phenolic carbons. These data suggested that compound **1** possessed the structure as shown for sauriol A. HMBC correlations confirmed this structure: correlations were observed between the methylene protons at δ 2.32 and δ 2.46 (2H-7) and three aromatic carbons at δ 103.7 (CH, C-2), 109.1 (CH, C-6), and 133.5 (quaternary C, C-1), establishing that the aromatic methines were *ortho* to the 2,3-dimethylbutane linkage. This forced the three oxygenated aromatic carbons (C-3,4,5) to the *meta* and *para* positions relative to the 2,3-dimethylbutane group, as in pyrogallol. The position of the methoxy functionality on each aromatic ring was established as being adjacent to one aromatic CH (^{1}H δ 6.15; ^{13}C δ 103.7; C-2) based on observed HMBC correlations (methoxy and phenyl H-2 each to phenolic C-3 at 146.5 ppm). Other HMBC correlations and the small couplings (1.5 Hz) of both aromatic methines (H-2,6) supported this substitution pattern in **1**. Although the absolute stereochemistry of **1** was not conclusively established, the optical activity of **1** predicted a non-meso form for **1**. The negative optical rotation for **1**, being the same as that of **3**, hinted that **1** probably shares the [*R*, *R*] configuration of **3**, and therefore, we propose the structure of **1** as shown.

With the structure of **1** established, the structural elucidation of sauriol B (**2**) was relatively straightforward. Mass spectral analysis supported a molecular formula of $C_{21}H_{28}O_6$, representing the addition of one CH_2 group relative to **1**. No major differences existed in the aliphatic portion of the NMR spectrum (neither between **2** and **3**, nor between **2** and **1**), except that **2** possessed two methoxy signals instead of one, and that one of these signals (δ 3.81) was of twice the

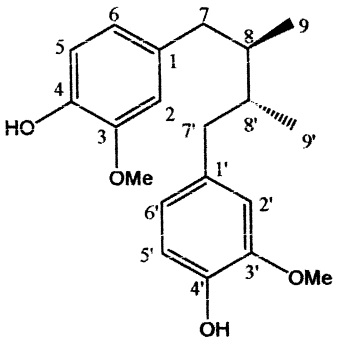
intensity of the other (δ 3.77). Close examination of splitting patterns of the ^{1}H resonances at δ 2.3–2.6 and δ 1.71 revealed some signal overlap not observed with **1** or **3**. The ^{13}C-NMR spectrum of **2** was considerably more complex than that of **1** or **3**, suggesting that the element of symmetry of the two latter compounds was not present in **2**. Differences between the resonance frequencies of carbons with similar shifts were more intense in the aromatic than aliphatic portion of the ^{13}C spectrum, and in the ^{1}H-NMR spectrum, the aromatic region was crowded with signals integrating to either two or one hydrogens. These two facts indicated that the difference between the two sides of the molecule lay in the aromatic rings.



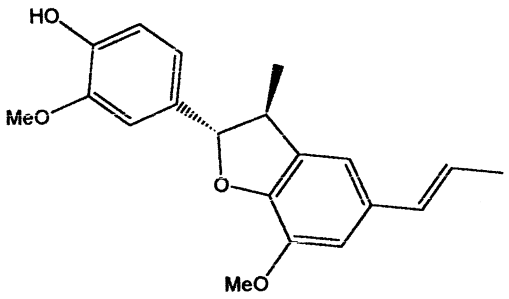
1



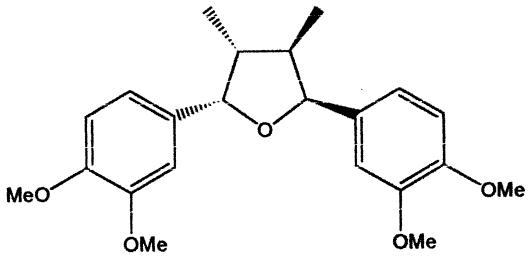
2



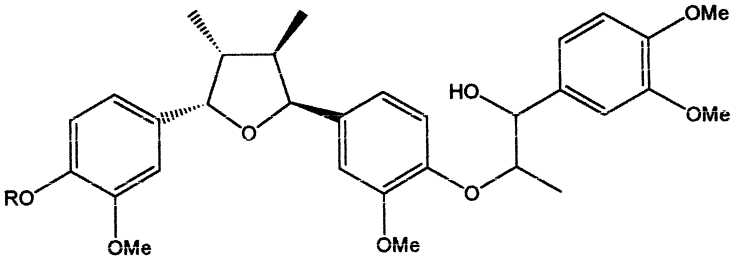
3



4



5



6 R = H
7 R = Me

One aromatic ring of **2** had identical spectral ^{1}H- and ^{13}C-NMR spectral data to that of **1**, confirming the 3-*O*-methylgallol substitution pattern for one side of **2**. The remaining ^{13}C and ^{1}H aromatic signals were apportioned to the other ring, which was concluded to possess mirror plane symmetry, because of the double intensity of its signals in the ^{1}H-NMR spectrum, and because of the low number of carbon resonances. Thus, we tentatively assigned the molecule as structure **2**, and turned to the two-dimensional NMR data for further proof. The methylene hydrogens at δ 2.37 and 2.51 (2H-7) and the strong methoxy signal at δ 3.81 (6H) all showed HMBC correlations to an intense aromatic methine carbon signal at δ 105.4 (C-2,6). In turn, this methine resonance (^{1}H δ 6.26, H-2, 6) was correlated to a phenolic carbon at δ 132.8 (C-4) and 147.0 (C-3, 5) and to its mirror image methine carbon at δ 105.4 (C-2, 6). A phenolic hydrogen singlet at δ 5.33 (OH on C-4) was also correlated to the phenolic carbons at δ 132.8 (C-4) and 147.0 (C-3, 5), but not to the methine carbon at δ 105.4 (C-2, 6). This placed the hydroxyl group *para* to the 2,3-dimethylbutane linkage, and confirmed the structure of **2** as that shown. We possess no firm evidence for absolute stereochemistry. However, the clear structural similarities between **1** and **2** isolated from the same plant material suggested that these compounds share the biosynthetic pathways leading to them, and therefore there was no compelling reason to suggest that the stereochemistry of **2** might be different from that of **1**. Indeed, the negative sign of the optical rotation supported the hesitant statement that a [*R*, *R*] stereochemistry, shared with that of **1** and **3**, was likely for **2**.

These natural products were isolated in the pursuit of ecologically important metabolites from a chemically defended freshwater plant, *S. cernuus* (Kubanek et al., 2000). Feeding deterrence in the crude lipophilic extract was followed through several chromatographic steps, yielding seven identified deterrent molecules and a number of deterrent unidentified complex mixtures. All identified antifeedant compounds fall into the lignoid family of natural products, although they represent several structural types within that category.

Sauriols A (**1**), B (**2**), dihydroguaiaretic acid (**3**), and saucernetin (**5**) are classical lignans, being oxygenated products of the β,β-coupling of two cinnamic acid residues (Ayres and Loike, 1990; Lewis and Davin, 1999). Compounds **1** and **2** were isolated from *S. cernuus* in minor quantities (0.010 and 0.038%, respectively, by plant dry weight) compared to **3** and **5** (0.11 and 0.84%), and were therefore assayed at close to these concentrations in order to approximate natural plant concentrations. When tested separately for feeding deterrence effects, **1** and **2** (at three and one times natural concentration, respectively) exerted similar effects on crayfish feeding at 0.030 and 0.038% to the effects of **3** and **5** at 0.11 and 0.84% (Kubanek et al., 2000). Thus, when comparing these four lignans to each other, it can be concluded that the order of antifeedant potency is approximately **1** $\sim$ **2** > **3** > **5**. Unfortunately, the small quantities of isolated metabolites prevented the determination of MIC (minimum inhibitory concentration) values for each compound. Amongst this small group of structurally related metabolites, antifeedant potency appears to be correlated with degree of aromatic oxidation and/or number of free phenolic hydroxyl groups. Saucernetin (**5**), which was the least potent lignan, possesses no free hydroxyls, while dihydroguaiaretic acid (**3**) possesses two, and its highly potent analogs, **2** and **1**, possess three and four, respectively. Because the only structural differences among **1**, **2**, and **3** are the degrees of hydroxylation and subsequent methylation, it seems likely that feeding deterrence activity amongst the *Saururus* lignans is related to the presence of phenolic hydroxyl groups.

The three other deterrent metabolites isolated from *S. cernuus* were the neolignan licarin A (**4**) and the sesquineolignans saucerneol (**6**) and saucerneol methyl ether (**7**). Compounds **4** and **6** also possess at least one free phenolic OH, in addition to several methylated ones. It is interesting to note that none of the deterrent compounds identified from this collection of *S. cernuus* possess the methylenedioxy functional group, seen in many lignoids of *S. cernuus* and terrestrial plants (Rao and Rao, 1990; Rao and Oruganty, 1997; Ward, 1999 and references cited therein). Because we pursued deterrent fractions only, and did not characterize non-active components of *S. cernuus*, it is uncertain whether the collected specimens did not contain other lignoid metabolites, whether they were in fact present but failed to deter crayfish feeding, or whether they were present and deterrent, but present in such small quantities that they were not detected. In an analytical study of the constituents of *S. cernuus*, Rao and Oruganty (1997) report the relative yields of metabolites and found that the major compound was austrobailignan-5, a methylenedioxy containing metabolite, present at 5–100 times the concentration of other lignoids. If this compound was present in our collections in similar quantities and deterred crayfish feeding, we would have detected it. For this reason, and because we accounted for the bulk of the feeding deterrence with seven non-methylenedioxy containing lignoids, it appears unlikely that the methylenedioxy functional group plays an important role in the antiherbivory properties of this plant. This is interesting because the methylenedioxy functional group has been identified as crucial to the biological activities of many lignoids, particularly as insecticide synergists. This is due to the action of methylenedioxy bearing molecules as blockers of insects' detoxicifying enzymes (polysubstrate monooxy-

genases) (Casida, 1970; MacRae and Towers, 1984; Bernard et al., 1989).

This work represents the first report of the existence of sauriols A (**1**) and B (**2**), perhydroxylated derivatives of dihydroguaiaretic acid (**3**) which together with several other lignoids protect *S. cernuus* from herbivory. It is expected that future efforts to understand the chemical ecology of freshwater plant–herbivore interactions will also reveal novel chemistry. Certainly, ecological studies bring to our attention compounds that may not be otherwise discovered by pharmacologically or agriculturally motivated research.

3. Experimental

3.1. General chemical methods

Solvents for extractions and open column chromatography were reagent grade and used without further purification. Solvents used for HPLC were analytical grade. HPLC was performed on Waters systems with refractive index and ultraviolet absorption detection. ^{1}H, ^{13}C, DEPT, and two-dimensional inverse-detected NMR experiments (COSY, HMQC, HMBC) were performed on Varian Inova 300 and Gemini 400 MHz spectrometers. NMR spectra were run in $CDCl_3$ and referenced to $CHCl_3$ (7.24 ppm). Infrared spectra were run as thin films on a Perkin-Elmer 1600 Series FTIR system. UV measurements were made on a Perkin-Elmer Lambda 3B UV/Vis spectrophotometer in chloroform. Low resolution electron impact mass spectra were run on a Hewlett-Packard 5988A mass spectrometer. Compounds were also submitted to The Scripps Research Institute for low and high resolution positive ion FAB, ESI, or MALDI mass spectral analysis. Optical rotations were measured on a Rudolph Research Autopol III polarimeter in chloroform at 20°C with a path length of 10 cm recorded on the sodium D line.

3.2. Feeding assays

Bioassay-guided fractionation was used to identify compounds of *S. cernuus* that deterred feeding by the crayfish *P. clarkii*. Details of bioassay design and data analysis are found in Bolser et al. (1998) and Kubanek et al. (2000). Artificial foods used in assays were prepared according to the methods in Bolser et al. (1998) and generalized in Hay et al. (1994, 1998). The treatment assay food consisted of chemical extracts or purified fractions of *S. cernuus* impregnated in agar-based food (agar plus freeze-dried broccoli and lettuce) with concentrations of both chemical and food materials matching those found in fresh *Saururus*. Control foods were made in the same manner as treatment foods but without the addition of extracts or purified compounds. For each assay, each of 30 individual adult crayfish was housed separately and offered a window screening strip embedded with a control and a treatment food square. Feeding was evaluated every 30 min and assays were terminated when approximately 50% of one choice had been eaten, or three hours had passed, whichever was sooner. Replicates in which greater than 90% or less than 10% of total food was consumed were discarded. Consumption was measured by counting the exposed grids of window screening, and feeding differences on control and treatment foods were analyzed using two-tailed paired *t*-tests.

3.3. Isolation of plant antifeedants

Emergent portions of *S. cernuus* were collected from a pond at the entrance to White Sands subdivision in Newport, North Carolina. Plants were returned to the laboratory at the Institute of Marine Sciences, UNC-CH and frozen at −70°C until ready for processing. Frozen plants were freeze-dried, ground to a powder, weighed, and then extracted with methanol/dichloromethane (1:2) six times (600 ml extraction solvent for 40 g freeze-dried plant). Extracts were filtered, concentrated in vacuo, and then suspended in deionized water and repeatedly extracted with dichloromethane. The dichloromethane extract (7.544 g green oil from 40 g dry plant) was fractionated using Sephadex LH-20 with methanol/dichloromethane (1:1) as eluent. Fractions with similar normal phase TLC characteristics were combined and assayed, leading to two deterrent fractions that were subjected to further fractionation. This was achieved using silica gel flash column chromatography employing a gradient eluent system from hexanes to diethyl ether and then to diethyl ether/methanol (1:1). Again, TLC properties were used to pool fractions, and all fractions were assayed. Deterrent fractions were purified by HPLC using a C-18 semi-preparative column with eluent systems methanol/water (9:1) or acetonitrile/water (7:3). Fractions approaching purity and pure compounds were protected from oxidation by the addition of 0.1–1.0 mg of ascorbic acid (vitamin C). Five known compounds, (−)-dihydroguaiaretic acid (**3**), (−)-licarin A (**4**), (+)-saucernetin (**5**), (−)-saucerneol (**6**), and (−)-saucerneol methyl ether (**7**) were isolated and identified by spectroscopic analysis and by comparison with literature data (Aiba et al., 1973; Rao and Alvarez 1982, 1983; Rao and Chattopadhyay, 1990; Rao and Oruganty, 1997). The lignans, sauriols A (**1**) and B (**2**), were purified and identified by spectroscopic analysis.

3.4. Characterization of novel plant antifeedants

Sauriol A (**1**). Isolated as a white amorphous solid

(0.5 mg from 5.0 g dry plant [0.01% by dry weight]); UV λ_{max} (log ϵ): 242 (3.7), 275 (3.4); IR ν_{max} cm^{-1}: 3378 (*br*), 2919, 2849, 1607, 1514, 1455, 1202, 1091; $[\alpha]_D$ (c = 0.03, $CHCl_3$) −240°; ^{1}H-NMR spectral data (300 MHz, $CDCl_3$): δ 0.79 (*d*, 6H-9/9′, J = 6.6), 1.72 (*br m*, 2H-8/8′), 2.32 (*dd*, 2H-7/7′, J = 14.0, 7.7), 2.46 (*dd*, 2H-7/7′, J = 13.7, 7.1), 3.79 (*s*, 6H-OMe on C-3/3′), 5.17 (*s*, 2H-OH on C-4/4′), 5.20 (*s*, 2H-OH on C-5/5′), 6.15 (*d*, 2H-2/2′, J = 1.5), 6.32 (δ, 2H-6/6′, J = 1.5); ^{13}C-NMR spectral data(100 MHz, $CDCl_3$) δ 13.9 (C-9/9′), 37.2 C-8/8′), 41.3 (C-7/7′), 56.0 (OMe on C-3/3′), 103.7 (C-2/2′), 109.1 (C-6/6′), 130.0 (C-4/4′), 133.5 (C-1/1′), 143.5 (C-5/5′), 146.5 (C-3/3′); HR-FABMS (m/z): [M + Na^+] calcd. for $C_{20}H_{26}O_6Na$, 385.1627; found 385.1640.

Sauriol B (**2**). Isolated as a white amorphous solid (1.9 mg from 5.0 g dry plant [0.038 % by dry weight]); UV λ_{max} (log ϵ): 241 (3.8), 272 (3.4); IR ν_{max} cm^{-1}: 3401 (*br*), 2931, 2849, 1614, 1519, 1455, 1326, 1214, 1108; $[\alpha]_D$ (c = 0.13, $CHCl_3$) −92°C; ^{1}H-NMR spectral data (300 MHz, $CDCl_3$), δ 0.80 (*d*, 6H-9/9′, J = 6.6), 1.71 (*br m*, 2H-8/8′), 2.34 (*dd*, 1H-7′), 2.37 (*dd*, 1H-7), 2.48 (*dd*, 1H-7′), 2.51 (*dd*, 1H-7′), 3.77 (*s*, 3H-OMe on C-3′), 3.81 (*s*, 3H-OMe on C-3/5), 5.19 (*s*, 1H-OH on C-4′ or 5′), 5.21 (*s*, 1H-OH on C-4′ or 5′), 5.33 (*s*, 1H-OH on C-4), 6.12 (*d*, 1H-2′, J = 1.7), 6.26 (*s*, 2H-2/6), 6.33 (*d*, 1H-6, J = 1.8); ^{13}C-NMR spectral data (100 MHz, $CDCl_3$): δ 13.9 (C-9/9′), 37.2 (C-8/8′), 41.3 (C-7′), 41.5 (C-7), 56.0 (OMe on C-3′), 56.2 (OMe's on C-3/5), 103.6 (C-2′), 105.4 (C-2/6), 109.1 (C-6′), 130.0 (C-4′), 132.5 (C-1), 132.8 (C-4), 133.5 (C-1′), 143.5 (C-5′), 146.5 (C-3′), 147.0 (C-3/5); HR-FABMS (m/z): [M + Na^+] calcd. for $C_{21}H_{28}O_6Na$, 399.1784; found 399.1796.

Acknowledgements

This work was supported by NSF grants CHE-9807098 to WF and DEB-9410336 to MEH, with additional support from the Harry and Linda Teasley endowment to Georgia Institute of Technology, and by an NSERC (Canada) postdoctoral fellowship to JK. We thank the SIO analytical facility for the use of spectroscopic instrumentation, The Scripps Research Institute for FAB, ESI, and MALDI mass spectral measurements, Peter Brueggeman at the SIO library for database searches, and Elizabeth Blackman and Sarah Lester for additional feeding assays. The manuscript was improved by the thoughtful suggestions of three anonymous reviewers.

References

Aiba, C.J., Correa, R.G.C., Gottlieb, O.R., 1973. Natural occurrence of Erdtman's dehydrodiisoeugenol. Phytochemistry 12, 1163–1164.

Ayres, D.C., Loike, J.D., 1990. Lignans: Chemical, Biological and Clinical Properties. Cambridge University Press, Cambridge.

Bernard, C.-B., Arnason, J.T., Philogene, B.J.R., Lam, J., Waddell, T., 1989. Effect of lignans and other secondary metabolites of the Asteraceae on the mono-oxygenase activity of the European corn borer. Phytochemistry 28, 1373–1377.

Bolser, R.C., Hay, M.E., Lindquist, N., Fenical, W., Wilson, D., 1998. Chemical defenses of freshwater macrophytes against crayfish herbivory. J. Chem. Ecol. 24, 1639–1658.

Casida, J.E., 1970. Mixed-function oxydase involvement in the biochemistry of insecticide synergists. J. Agric. Food Chem. 18, 753–772.

Cyr, H., Pace, M.L., 1993. Magnitude and patterns of herbivory in aquatic and terrestrial systems. Nature 361, 148–150.

Gronin, G., 1998. Influence of macrophyte structure, nutritive value, and chemistry on the feeding choices of a generalist crayfish. In: Jeppesen, E., Søndergaard, M., Christoffersen, K. (Eds.), The Structuring Role of Submerged Macrophytes in Lakes. Springer-Verlag, New York, pp. 307–317.

Hartwell, J.L., 1971. Plants used against cancer. A survey. Lloydia 34, 204–255.

Hay, M.E., Kappel, Q.E., Fenical, W., 1994. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant quality. Ecology 75, 1714–1726.

Hay, M.E., Stachowicz, J.J., Cruz-Rivera, E., Bullard, S., Deal, M.S., Lindquist, N., 1998. Bioassays with marine and freshwater macroorganisms. In: Haynes, K.F., Millar, J.G. (Eds.), Methods in Chemical Ecology (Bioassay Methods), vol. 2. Kluwer Academic Publishers, Norwell, MA, pp. 39–141.

Kubanek, J., Hay, M.E., Brown, P., Lindquist, N., Fenical, W., 2000. Herbivory on freshwater macrophytes mediated by lignoid chemistry: crayfish feeding deterrence in *Saururus cernuus* (submitted).

Lewis, N.G., Davin, C.B., 1999. Lignans, biosynthesis and function. In: Barton, D.H.R., Sir, Nakanishi, K., Meth-Cohn, O. (Eds.), Comprehensive Natural Products Chemistry. Vol. 1: Polyretites and other Secondary Metabolites. Elsevier, Amsterdam, pp. 639–712.

Lodge, D.M., 1991. Herbivory on freshwater macrophytes. Aquatic Bot. 41, 195–224.

MacRae, D.W., Towers, G.H.N., 1984. Biological activities of lignans. Phytochemistry 23, 1207–1220.

Newman, R.M., 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. J. N. Am. Benthol. Soc. 10, 89–114.

Newman, R.M., Hanscom, Z., Kerfoot, W.C., 1992. The watercress glucosinolate-myrosinase system: a feeding deterrent to caddisflies, snails and amphipods. Oecologia 92, 1–7.

Newman, R.M., Hanscom, Z., Kerfoot, W.C., 1996. Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. Ecology 77, 2312–2323.

Newman, R.M., Kerfoot, W.C., Hanscom, Z., 1990. Watercress and amphipods. Potential chemical defense in a spring stream macrophyte. J. Chem. Ecol. 16, 245–259.

Phares, D.L., 1867. Saururus cernuus. Am. J. Pharm. 39, 468.

Rao, K.V., Alvarez, F.M., 1982. Chemistry of *Saururus cernuus*. Part I: Saucernetin, a new neolignan. J. Nat. Prod. 45, 393–397.

Rao, K.V., Alvarez, F.M., 1983. Manassantins A/B and saucerneol: novel biologically active lignoids from *Saururus cernuus*. Tetrahedron Lett. 24, 4947–4950.

Rao, K.V., Chattopadhyay, S.K., 1990. Regioselective cleavage of the methylenedioxy group: conversion of (−)-austrobailignan-5 to (−)-dihydroguaiaretic acid. J. Org. Chem. 55, 1427–1429.

Rao, K.V., Oruganty, R.S., 1997. An improved method for the iso-

lation of the lignan constituents of *Saururus cernuus* by reverse phase column chromatography. J. Liq. Chrom. & Rel. Technol. 0, 3121–3134.

Rao, K.V., Puri, V.N., Diwan, P.K., Alvarez, F.M., 1987. Preliminary evaluation of manassantin A, a potential neuroleptic agent from Saururus cernuus. Pharmacol. Res. Commun. 19, 629–638.

Rao, K.V., Rao, N.S.P., 1990. Chemistry of *Saururus cernuus*. Part VI: Three new neolignans. J. Nat. Prod. 53, 212–215.

Rao, K.V., Reddy, G.C.S., 1990. Chemistry of *Saururus cernuus*. Part V: Sauristolactam and other nitrogenous constituents. J. Nat. Prod. 53, 309–312.

Ward, R.S., 1999. Lignans, neolignans and related compounds. Natural Product Reports 16, 75–96.