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# BIOACTIVE STILBENES OF *SCIRPUS MARITIMUS*[1]

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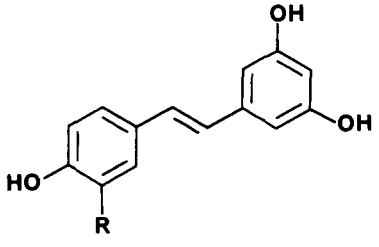
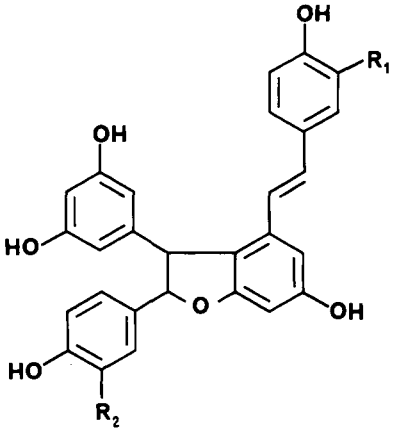
*Scirpus maritimus* L. is a perennial Cyperaceae species of wide occurrence in Europe, Asia, Africa, and North America. Despite the broad distribution of *S. maritimus* and its importance to the ecology of intertidal marsh communities and prairie saline wetlands (1,2), little is known of the chemistry of this plant. The root has been used in China as an astringent and as a diuretic (3). In addition, *S. maritimus* is a major weed pest of tropical lowland rice in the Philippines (4).

In our continuing effort to discover and evaluate bioactive compounds from plants, we observed that an alcoholic extract of *S. maritimus* seeds was active in vivo against P-388 lymphocytic (3 PS) murine leukemia (135% T/C at 200 mg/kg) (5). Fractionation of the extract, monitored by brine shrimp toxicity, yielded **1** (resveratrol or 3′,4,5′-trihydroxystilbene), **2** (piceatannol or 3,3′,4,5′-tetrahydroxystilbene), **3** (ε-viniferin), **4** (scirpusin A, a mixed dehydrodimer of **1** and **2**), and **5** (scirpusin B, a dehydrodimer of **2**). Structure/activity relationships for these compounds were investigated in a variety of systems; included were inhibition of 3 PS leukemia in mice, inhibition of crown gall tumors on discs of potato tubers (*Solanum tuberosum*) (6), brine shrimp (*Artemia salina*) toxicity (7), fall army worm (*Spodoptera frugiperda*) antifeedant activity (8), and growth inhibition of duckweed (*Lemna minor*) (9).

Structures of stilbenes **1-5** and their peracetylated derivatives were determined by comparison of their $^1$H nmr and ms with literature values and, for **2**, **4**, and **5**, by direct tlc, $^1$H nmr, and ms




**1** R=H
**2** R=OH




**3** $R_1$=$R_2$=H
**4** $R_1$=H, $R_2$=OH
**5** $R_1$=$R_2$=OH

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

comparison with known reference compounds. Results of the various bioassay procedures are summarized in Table 1. From the data presented in Table 1 it is evident that **2** and **5** are generally more toxic or inhibitory at lower levels in these bioassays than **1** and **4**.

oxidation product obtained by treatment of **1** with horseradish peroxidase (20). Scirpusins A and B [**4** and **5**] were previously known to occur only in rhizoma of *Scirpus fluviatilis* (21).

Growth inhibitory and allelopathic effects of stilbenes **1-5** towards a variety

TABLE 1. Bioassay Results for Resveratrol [**1**], piceatannol [**2**], Scirpusin A [**4**], and Scirpusin B [**5**][a]

| Compound | 3PS[b] | PD[c] | BS[d]$LC_{50}$ (μg/ml) | FAW[e] | *Lemna minor*[f] growth rate ($\times 10^{-3}$) |
|---|---|---|---|---|---|
| **1** | 100/37 | +39 | — | — | 129/142 |
| **2** | 131/35 | −19/−17 | 278 | 0.38 | 53/83 |
| **4** | 103/30 | +17 | 488 | 1.00 | 124/130 |
| **5** | 99/18 | −57/−40 | 389 | 0.23 | 90/59 |

[a]ϵ-Viniferin was not evaluated due to insufficient material.

[b]3PS activity is assumed when life span of leukemic mice is increased by 25% or more (T/C>125%), e.g., 131/25 indicates 131% T/C at 25 mg/kg (5). Insufficient material was available to test **4** and **5** at higher levels.

[c]Potato disc (PD) activity is assumed when the No. of crown gall tumors inhibited, vs. controls, is ca. 20% or more in two successive determinations (6), e.g., PD −19/−17 indicates 19% and 17% tumor inhibition in two successive determinations.

[d]Brine shrimp (BS) activity is assumed when the $LC_{50}$ is <1000 μg/ml (7).

[e]Fall army worm antifeedant activity (feeding ratio) expressed as: area of treated disc consumed/area of control disc consumed; treated leaf discs were dipped in 5% solutions of test compounds for 5 sec (8).

[f]Growth rate determined by the following formula (9):

$$\frac{\text{Log}_{10}\text{ (final frond No.)} - \text{Log}_{10}\text{ (initial frond No.)}}{\text{number of days}}$$

Values 129/142 are results of two successive determinations; control growth rates ranged from 131-152. All compounds were tested at 333 ppm.

Stilbenes **1** and **2** are phytoalexins or phytoalexin precursors that occur widely in plants, frequently but not invariably, under conditions of stress (10); both are photosynthesis inhibitors (11). Sources of **1** and various glycosides, oligomers, and/or partially methylated derivatives of **1**, include the groundnut (*Arachis hypogoea*) (12), rhubarb (*Rhei rhizoma*) (13), the grapevine (*Vitis vinifera*) (14) and *Gnetum* species (15). There is evidence to suggest that a glycoside of **1** (piceid) offers some protection from liver injury in rats fed peroxidized oils (16). Piceatannol is known to have antifungal, phytogrowth inhibitory, ichthyotoxic (17), and antileukemic (18) properties.

ϵ-Viniferin [**3**] is an antifungal compound previously isolated only from *V. vinifera* (14) and from *Vatica affinis* (19). Two other dehydrodimers of **1** are known; these are gnectin C (15) and an

of organisms undoubtedly contribute to the ability of *S. maritimus* to survive and to often dominate in various wetland plant communities.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Tlc was performed on silica gel 60 F-254 plates (E. Merck) developed with $CH_2Cl_2$-MeOH (9:1) for **1-5**, and (99:1) for their peracetylated derivatives. Silica gel (60-200 mesh, Baker) was used for column chromatography. For hplc we used a Waters ALC/PC-201 instrument, an RI detector, and a Whatman Partisil 10, M-9 (50 cm) column. $^1$H-nmr spectra were determined with a Bruker WM-300 instrument in acetone-$d_6$ [**1-5**] or $CDCl_3$ (peracetylated derivatives) solutions with TMS as an internal standard. Mass spectra were obtained with a Finnegan MAT 4535/TSQ instrument equipped with a DEP probe. Bioassays closely followed those described in the literature (5-9). Acetylations were carried out in $Ac_2O$-$C_5H_5N$ (1:1), 25°, 24 h.

PLANT MATERIAL.—Seeds of *S. maritimus*, collection No. NU 43769, were obtained in

Yugoslavia and identified by botanists at the Beltsville Agricultural Research Center, Beltsville, Maryland, where voucher specimens are maintained.

EXTRACTION AND FRACTIONATION.—*S. maritimus* seed (300 g) was finely ground and defatted with hexane in a Soxhlet apparatus. Further extraction with 95% EtOH provided 25 g of EtOH-soluble material. The EtOH solubles were divided into two equal portions, and each was chromatographed on columns packed with 300 g of silica; solvent consisted of a step-wise gradient of increasing MeOH in $CH_2Cl_2$. Similar fractions were combined on the basis on tlc analysis, and materials of interest were further concentrated by hplc on silica with $CH_2Cl_2$-MeOH (19:1, 9:1, or 17:3). Final purification of individual stilbenes by preparative tlc in $CH_2Cl_2$-MeOH-HOAc (85:15:1) gave, respectively, **1** (150 mg), **2** (470 mg), **3** (13 mg), **4** (99 mg), and **5** (35 mg). Separations were not optimized, and samples at each step of the fractionation process were consumed in biological testing.

RESVERATROL [**1**] AND TRIACETATE.—Resveratrol [**1**], $5.0\times10^{-2}$% yield, gave pale yellow crystals; mp 256-258°; eims *m/z* (rel. int.) 228 ($M^+$, 100), 211 (34), 181 (36). Triacetate; eims *m/z* (rel. int.) 354 ($M^+$, 12), 312 (38), 270 (39), 228 (72), 43 (100). $^1$H nmr values for **1** and the triacetate were identical to values quoted in the literature (21).

PICEATANNOL [**2**] AND TETRAACETATE.—Piceatannol [**2**], $1.6\times10^{-1}$% yield gave pale yellow crystals; mp 226-228°; eims *m/z* (rel. int.) 244 ($M^+$, 100), 197 (30). Tetraacetate; eims *m/z* (rel. int.) 412. ($M^+$, 12) 370 (31), 328 (79), 286 (71), 244 (76), 43 (100). $^1$H-nmr spectra of **2** and its tetraacetate were identical to spectra of known reference materials (21).

ε-VINIFERIN [**3**] AND PENTAACETATE.—ε-Viniferin [**3**], $4.0\times10^{-3}$%, pale brown powder, ca. 95% pure by tlc. Pentaacetate; eims *m/z* (rel. int.) 664 ($M^+$, 2), 622 (3), 580 (4), 538 (3), 496 (2), 454 (1), 43 (100). $^1$H nmr of **3** was identical to values quoted in the literature (14).

SCIRPUSIN A [**4**] AND HEXAACETATE.—Scirpusin A [**4**], $3.7\times10^{-2}$% yield, pale yellow powder; eims *m/z* (rel. int.) 470 ($M^+$, 20), 244 (82), 94 (100). Hexaacetate; eims *m/z* (rel. int.) 722 ($M^+$, 1), 680 (4), 638 (4), 596 (5), 554 (4), 512 (1), 43 (100). $^1$H-nmr spectra of **4** and its hexaacetate were identical to spectra of known reference materials (21).

SCIRPUSIN B [**5**] AND HEPTAACETATE.—Scirpusin B [**5**], $1.2\times10^{-2}$% yield, pale brown powder; eims *m/z* (rel. int.) 486 ($M^+$, 1), 110 (100). Heptaacetate; eims *m/z* (rel. int.) 780 ($M^+$, 0.1), 738 (0.5), 696 (1.8), 654 (2.0), 612 (2.0), 570 (1.3), 528 (0.6), 43 (100). $^1$H-nmr spectra of **5** and its heptaacetate were identical to spectra of known reference materials (21).

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