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GERMINATION AND GROWTH INHIBITORY SESQUITERPENES FROM *IVA AXILLARIS* SEEDS

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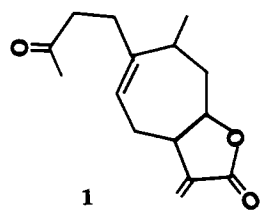
In our search for natural products with weed control potential (1), extracts of *Iva axillaris* Pursh. seeds showed good activity against velvetleaf (*Abutilon theophrasti* Medic). The active compounds proved to be sesquiterpenes. Study of germination and growth regulatory behavior of this compound class has begun in recent years (2-7), but only occasionally has quantitative information about specific compounds in specific bioassays been published (4-7). Identification of the active compounds was greatly facilitated by the work of Herz and co-workers, who have characterized the sesquiterpenes of many *Iva* species, including *I. axillaris* (8).

RESULTS AND DISCUSSION

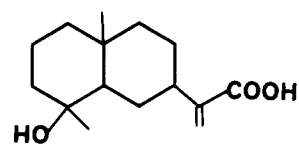
STRUCTURES.—The most potent germination inhibitor found in *I. axillaris* seed is compound **1** (Table 1), which was first isolated from *Parthenium tomentosum* (9) and later identified in *Inula viscosa* (10). Our identification is based on its ei mass and its nmr spectrum, which is identical to that published by Bohlmann *et al.* (10). Effective concentrations are near 10^{-3} M and, therefore, this compound is not as toxic to velvetleaf as benzyl isothiocyanate (1).

Compound **2**, ilicic acid, is slightly less active than **1** (Table 1) and also has not been recognized heretofore as an *Iva* constituent. It was characterized by Herz *et al.* from *Ambrosia ilicifolia* (11) and more recently was discovered in *Inula graveolens* (12). Again, mass and nmr spectra were consistent with the assigned structure. Treatments with compounds **1** and **2** combined showed that they were about as active upon combination as **1** was by itself.

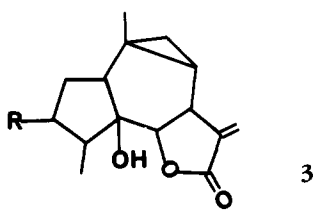
Axivalin (**3a**, and its congeners **3b**



1

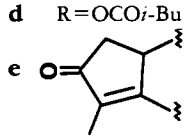


2



3

a R=OCOCH$_3$
b R=OH
c R=O
d R=OCO*i*-Bu



and **3c**, the major sesquiterpenes known in *I. axillaris*, were not inhibitory to germination, but they did have a profound effect on growth (Figure 1). Radicals of treated seeds were about one-fourth as

[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.

TABLE 1. Effect of Sesquiterpenes on Germination of Velvetleaf

Compound	Molarity[a]	Percent germination[a] (relative to controls)
1	4×10^{-3} each	0[b]
	2×10^{-3}	27[b]
	10^{-3}	42[b]
	6×10^{-4}	62[b]
2	4×10^{-3}	27[b]
	2×10^{-3}	87
	10^{-3}	100
1+2	2×10^{-3} each	13[b]
	10^{-3} each	57[b]
3a	10^{-3}	103
3b	10^{-3}	92
3c	10^{-3}	92
3e	8×10^{-4}	69[b]

[a]For bioassay details, see Wolf *et al.* (1).
[b]Significantly different from controls at 0.05 level or better.

long as the controls (2-3 cm vs. 9-10 cm) and were tightly coiled. Axivalin and ivaxillarin (**3c**) have been isolated from the above-ground parts of *I. axillaris* (8). In that study, deacetylaxivalin (**3b**) was not found in the extract. Because **3b** could easily be produced by hydrolysis of **3a**, we are not sure that it exists naturally in the seeds. Anhydroivaxillarin (**3e**) showed germination inhibition together with the abnormal growth associated with **3a-c**. All of these compounds were identified by their mass and nmr spectra (8).

During isolation of the sesquiterpene lactones, a small amount (ca. 1 mg) of another compound (**3d**) was detected. This compound migrated slightly further than **3a** on tlc and produced a blue-violet spot similar to that generated by **3a** after chromic acid treatment and charring. Distinctive tlc color reactions of sesquiterpene lactones have been extensively studied by Picman (13).

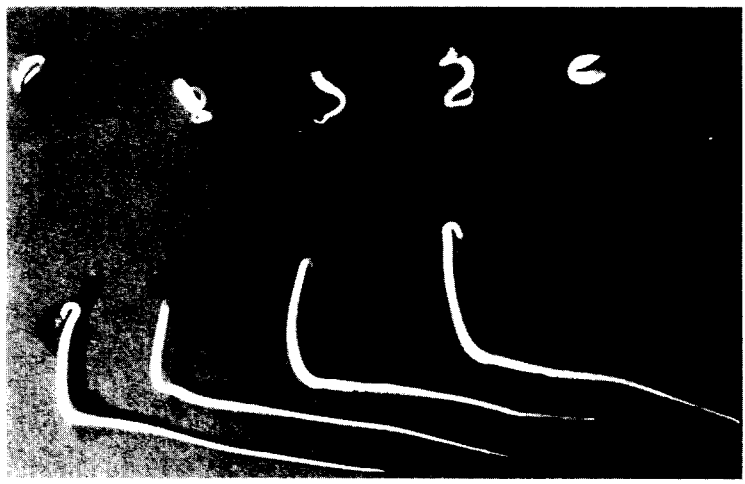


FIGURE 1. Velvetleaf seeds treated with 10^{-3}M axivalin (**3a**) (above) and controls (below). Control radicals are about 10 cm long. For bioassay details, see Wolf *et al.* (1).

Both the cims and eims were similar to those of **3a**, except that the molecular ions appeared to be 42 amu greater. The nmr spectrum was also similar to that of **3a**, except that no signal for an acetate group was forthcoming. These data indicated that the compound was the valerate ester of **3b**. Hydrolysis of **3d** with BF_3-MeOH and capillary gc/ms gave a peak identical in retention and spectrum to methyl isovalerate. No peak for *n*-valerate was detected. Tlc and gc/ms of the hydrolysis product confirmed the identity of the alcohol moiety as **3b**. Unfortunately, not enough of this compound could be isolated to perform a reliable bioassay.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded in $CDCl_3$ by a Bruker WM 300 spectrometer. Eims were obtained with a Kratos MS-30 instrument and cims with a Finnigan 4535/TSQ®. The column for gc/ms was 3% OV-1. For capillary gc/ms, a SE-54 WCOT column (30 m) was used. A 9.4×250 mm Zorbax C8 (Dupont) column and an Me_2CO-H_2O (4:6) solvent system provided hplc separations. Silica tlc plates (0.25 mm, Brinkmann) were developed in C_6H_6-EtOH (85:15), and spots were made visible by charring after the plate had been sprayed with chromic acid. Methanolysis was performed in 10% BF_3-MeOH (w/v) for 1 h with occasional shaking.

ISOLATION OF COMPOUNDS AND BIOASSAY.—Finely ground seed (450 g) was extracted in a Soxhlet apparatus for 24 h with hexane, followed by 24 h with Me_2CO. The concentrated Me_2CO extract was eluted through silica with C_6H_6, 5% (v/v) MeOH in C_6H_6, and 10% MeOH in C_6H_6. Each MeOH-C_6H_6 eluate was further partitioned on a reverse-phase column (RSil Prep C_{18}, Altech Assoc.) with Me_2CO-H_2O (50:50). The column was pressurized at 0.5 atm with an Omnifit® Liquid Handling System Ltd., (Omnifit, Atlantic Beach, NY) and 5-ml fractions were collected. Progress of this chromatography was monitored by tlc. Isolation of the sesquiterpenoids was completed by hplc.

The bioassay was carried out only on velvetleaf seeds essentially as described previously (1) and at the concentrations given in Table 1.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. R.B. Wolf, G.F. Spencer, and W.F. Kwolek, *J. Weed Sci.* (in press).
2. E. Rodriguez, G.H.N. Towers, and J.C. Mitchell, *Phytochemistry*, **15**, 1573 (1976).
3. D. Gross, *Phytochemistry*, **14**, 2105 (1975).
4. P.S. Kalsi, V.K. Vij, O.S. Sing, and M.S. Wadia, *Phytochemistry*, **16**, 784 (1977).
5. S. Del Amo and H.L. Anaya, *J. Chem. Ecol.*, **4**, 305 (1978).
6. P.S. Kalsi, V.B. Sood, A.B. Masih, D. Gupta, and K.K. Talwar, *Phytochemistry*, **22**, 1387 (1983).
7. S.D. Kanchan and Jayarchandra, *Plant and Soil*, **55**, 67 (1980).
8. W. Herz, V. Sudarsanam, and J.J. Schmid, *J. Org. Chem.*, **31**, 3232 (1966).
9. E. Rodriguez, H. Yoshioku, and T.J. Mabry, *Phytochemistry*, **10**, 1145 (1971).
10. F. Bohlmann, H. Czerson, and S. Schoneweiss, *Chem. Ber.*, **100**, 1330 (1977).
11. W. Herz, H. Chikamatsu, and L.R. Tether, *J. Org. Chem.*, **31**, 1632 (1966).
12. G. Fardella, *Fitoterapia*, **50**, 3 (1979); *Chem. Abstr.*, **91**, 154332a (1979).
13. A.K. Picman, "Analytical and Biological Studies of Sesquiterpene Lactones," Ph.d. Thesis, University of British Columbia, Vancouver, 1981.

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