sponding to VI. Both diphenyldiazenes and diphenyldiazene oxides have recently been reported to be formed in the soil degradation of trifluralin (Golab et al., 1979).

ACKNOWLEDGMENT

We thank Eli Lilly Co. for a gift of 2,6-dinitro-4-(trifluoromethyl)[*ring*-^{14}C]benzenamine, and Edward Barnett for assistance with, and identification of, the microbiological cultures.

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Received for review August 27, 1979. Accepted December 14, 1979. Mention of a proprietary item does not constitute an endorsement by the USDA.

Growth Inhibitors from Spikerush

K. L. Stevens* and Glory B. Merrill

A phytotoxic compound, dihydroactinidiolide (I), has been isolated from spikerush (*Eleocharis* spp.), synthesized, and shown to be a potent inhibitor of root length elongation and seed germination.

The management of aquatic vegetation in ponds, canals, and drainage ditches has been the subject of considerable investigation which has led to a number of mechanical, chemical, and biological methods of control. In some instances, animals or competitive plants (biological control agents) can be introduced into the aquatic environment to remove selectively or greatly diminish the unwanted vegetation while not becoming pests themselves.

One such plant, spikerush (*Eleocharis* spp.), has been observed to "displace" other unwanted aquatic weeds. Spikerush species are short, rapidly growing perennial sedges, which form a fairly thick mat from a profuse and dense system of rhizomes. Propagation takes place by the growth of the rhizome system as well as the spreading of seed and tubercules.

The competitive nature of spikerush was reported by Oborn et al. (1954), who showed that it can eliminate pondweeds (*Potamogenton* spp.). Likewise, Yeo and Fisher (1970), observing the movement of spikerush in several aquatic sites in California, found that it had eliminated or greatly reduced infestations of pondweed and elodea. The mechanism by which spikerush successfully competes with larger and more vigorous plants was not determined. The rather diminutive nature of spikerush and its bottom-dwelling habit suggest that it could not displace larger species of aquatic plants by mere competition for light, space, and nutrients. After several studies demonstrated that spikerush (*Eleocharis coloradoensis*) successfully inhibited the growth and spreading of American and Sago pondweed, Frank (1975) ascribed the activity to allelopathy.

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MATERIALS AND METHODS

Collection and Extraction of Spikerush. Spikerush which had been grown for seed production in Davis, CA, was mowed, dried, and threshed to remove seeds. The intact plant material (1.1 kg) was then steeped for 1 week at room temperature in 40% aqueous ethanol (6 gal) (Keen, 1978) to extract plant growth regulators. Removal of ethanol in vacuo at 50 °C and liquid–liquid extraction of the aqueous residue for 1 day with ethyl acetate afforded 1.77 g of dark-brown oil.

Bioassay. Fractions or purified samples (0.1–6 mg) were dissolved in 0.5 mL of acetone or methanol and diluted with 60 mL of hot 0.35% agar solution. After thorough mixing, the agar–sample solution was poured into 7-cm petri dishes, allowed to cool, and seeded with approximately 20 watercress seeds (*Nasturtium officinale*). After 7 days in a growth chamber fluctuating between 18 °C (6-h night) and 28 °C (18-h day), root lengths were measured to the nearest 0.5 mm and compared to those of the controls. In each case, replicates were prepared and fractions which showed root-length reduction of 20% or greater at 100 ppm or less were considered active.

Separation of Active Components. The ethyl acetate extract was chromatographed on silica gel (500 g) and eluted with chloroform (2 L) to give material showing phytotoxic activity. The fraction crystallized to give the flavone tricin, which shows no toxicity. Preparative TLC of the mother liquor on silica plates (2% acetic acid/ether) gave an active band at R_f 0.77–0.85. Rechromatography of the band on silica plates (10% methanol/chloroform) gave an active band at R_f 0.68–0.72, which crystallized from methanol to give "γ-sitosterol", i.e., a mixture of sterols. The sterols were shown to be inactive. The mother liquor was vacuum transferred at 1 mm pressure and 50 °C to give, by GC, a compound of about 73% purity. Mass spectral analysis suggested the butenolide I (Figure 1), i.e., dihydroactinidiolide (Chen et al., 1970, which was con-

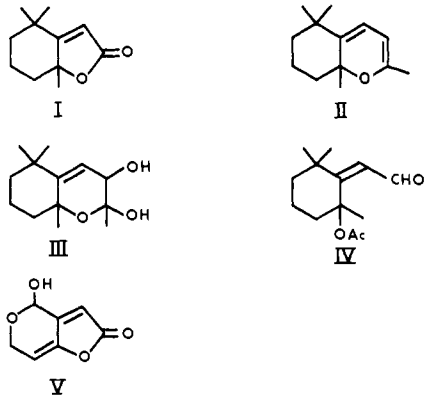

Figure 1. Structures of compounds mentioned in the text.

firmed by its NMR spectrum ($CDCl_3$): δ 1.19 (3 H, s), 1.22 (3 H, s), 1.50 (3 H, s), 5.59 (1 H, s); IR spectrum (neat): 1752 cm^{-1}; UV spectrum: λ_{max}^{EtOH} 212 (18000).

Synthesis of Butenolide (I). *Method A.* β-Ionone (19.2 g, 0.1 mol) was dissolved in 20 mL of ethanol, cooled in an ice bath, and reduced with 3.7 g (0.1 mol) of sodium borohydride dissolved in 30 mL of ethanol. The mixture was stirred for 5 min, then warmed to room temperature. TLC showed no β-ionone. Water, then dilute HCl, was added to the mixture which was then extracted with ether (6×). The ether extract was washed with water and saturated sodium chloride solution and dried over anhydrous magnesium sulfate. Removal of the ether in vacuo gave *trans*-β-ionol (19 g, 98%) as an oil: NMR spectrum ($CDCl_3$): δ0.97 (6 H, s), 1.30 (3 H, d, J = 7 Hz), 1.66 (3 H, s), 4.33 (1 H, dq, J = 7.7 Hz), 5.46 (1 H, dd, J = 7.16 Hz), 6.03 (1 H, d, J = 16 Hz).

trans-β-Ionol (1-g batches) and 2-acetonaphthone (10 mg) were dissolved in 1 L of cyclohexane, purged with helium, and irradiated in a quartz flask using 16 low-pressure, 350-nm lamps. The course of the reaction was followed by GC and stopped at 95% conversion to *cis*-β-ionol (approximately 2.5 h): NMR spectrum ($CDCl_3$) δ 0.97 (3 H, s), 1.19 (3 H, d, J = 6 Hz), 1.39 (3 H, s), 4.32 (1 H, m), 5.49 (1 H, dd, J = 7, 12 Hz), 5.88 (1 H, d, J = 12 Hz).

cis-β-Ionol (10 g) was dissolved in acetone and Jones reagent (Bowden, 1946) was added dropwise at room temperature until the orange color persisted. The mixture was then heated to reflux and more Jones reagent added until the characteristic color remained for 20 min. The mixture was cooled, water was added (150 mL), and the mixture was extracted with ether (3×). The ether was washed with water (2×), 10% sodium bicarbonate solution (2×), and water (3×) and dried over anhydrous magnesium sulfate. After filtration, the ether was evaporated, leaving an oil which was chromatographed on silica gel (500 g). The desired butenolide was eluted with 100% ether, rechromatographed on silica gel (300 g) using 50% ether/Skelly F to give pure (>99%) dihydroactinidiolide (73%) identical with the natural material.

Method B. β-Ionone (1 g) was dissolved in 1.2 L of cyclohexane, purged with helium, and irradiated through Pyrex for 1 h with a 450-W, medium-pressure Hanovia lamp. Evaporation of the solvent left a mixture which, by GC and TLC, showed the main component to be the pyran II. The pyran was separated by chromatography on silica gel (10% ether/Skelly F) and oxidized with Jones reagent in acetone to give a mixture of components. Upon standing, crystals formed which were separated and recrystallized from Skelly F/ether to give the diol III (Kurata et al., 1973). Preparative TLC (20% ether/Skelly F) of the mother liquor on silica gel afforded, in addition to the desired butenolide I (R_f 0.14) and the pyran II (R_f 0.64),

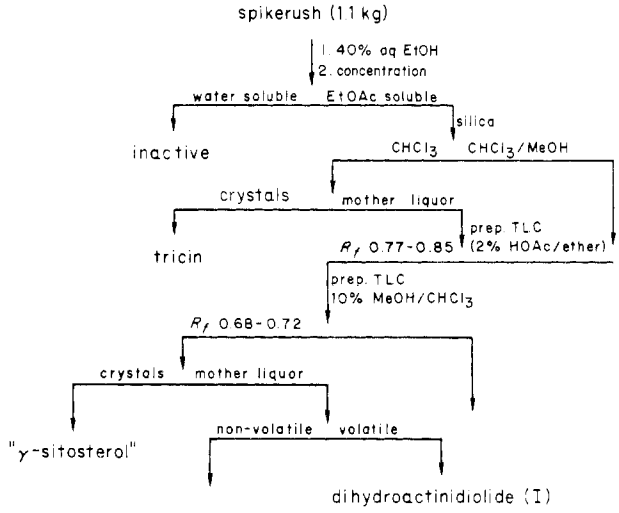

Figure 2. Schematic diagram of isolation of dihydroactinidiolide from spikerush.

the aldehyde IV (R_f 0.16): ^1H NMR ($CDCl_3$) δ 1.16 (3 H, s), 1.27 (3 H, s), 1.94 (3 H, s), 5.92 (1 H, d, J = 7 Hz), 10.36 (1 H, d, J = 7 Hz); IR (neat) 1665, 1745 cm^{-1}.

Quantitative Determination of Butenolide I and Aldehyde IV in Spikerush. Whole air-dried spikerush (50 g) was extracted continuously in a Soxhlet extractor for 24 h with Skelly F. Removal of the solvent left a dark-brown oil which was analyzed by GC using a 50 m × 0.25 mm i.d. glass column coated with SP2100 silicone oil. Comparison of integrated areas, using an HP5830A chromatograph, of the peak at 23.41 min (I) from the extract and from the extract plus a weighed amount of I gave 0.253 mg (I) in 50 g of sample of dried spikerush, which calculates to 5 ppm.

The aldehyde was not amenable to GC analysis; hence the extract was placed on preparative TLC plates and developed, and material was collected corresponding to the R_f value of the aldehyde. NMR analysis of the band showed no detectable amount of the aldehyde (<5 ppm).

RESULTS AND DISCUSSION

The search for phytotoxic substances in spikerush (*Eleocharis coloradoensis*) has been guided at every stage by biological assay which allows the separation of active compounds (often missed by the more classical approach) from the more abundant components or those easily separated. Figure 2 summarizes the isolation of an active fraction which was 73% of a single component tentatively identified as dihydroactinidiolide (I) by mass spectral analyses (Chen et al., 1970). Because adequate amounts were unavailable for biological testing and its similarity to patulin (V), a known phytotoxic compound (Jefferys, 1952), dihydroactinidiolide was synthesized to confirm its identity and determine its biological activity.

Reduction of *trans*-β-ionone with subsequent photolytic isomerization to *cis*-β-ionol followed by Jones oxidation gave good yields of the desired butenolide (I) (von Wartburg and Wolf, 1974), which was identical (by GC, MS, and TLC) with the natural material.

Biological testing of butenolide I shows a reduction in root length approximately 30% between 10 and 20 ppm. Due to its insolubility, difficulty was encountered in obtaining meaningful data at higher concentrations. Often the developing root became thickened and discolored, quite distinct from the thin, almost white roots, of the control. The level of activity compares favorably with other root growth inhibitors, e.g., Oyama et al. (1978) reported the root growth inhibition of Chinese cabbage, lettuce, and rice

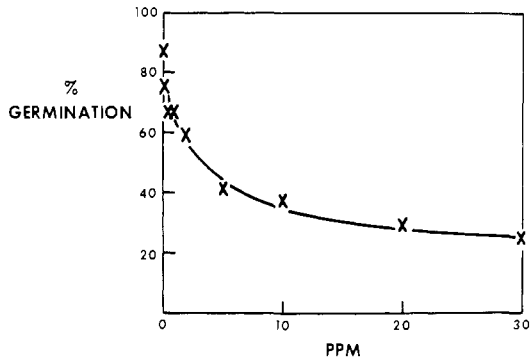

Figure 3. Percent germination of radish seeds (scarlet globe) vs. dihydroactinidiolide. The largest standard deviation between replicates was 5.9.

seedlings at a concentration of 100 ppm for *trans*-resorcylide, a macrocyclic fungal metabolite.

Dihydroactinidiolide (I) also affects the germination of radish seeds as shown in Figure 3. After 48 h, a 50% reduction in germination occurs at 5 ppm. At >50 ppm, no germination occurs even after 5 days.

Dihydroactinidiolide has been isolated from a number of plants, e.g., *Actinidia polygama* (Sakan et al., 1967), tea leaves (Ina et al., 1968), tobacco (Bailey et al., 1968), and cassia (Demole et al., 1969) and shown to be an important aroma constituent in tea and also an effective attractant for *Felidae* animals in lower order (Sakan et al., 1965). The phytotoxic nature of dihydroactinidiolide has not been previously observed and may be significant in acting as an allelopathic agent in spikerush. Quantitative analysis of whole, dried spikerush has revealed the presence of the butenolide on the order of 5 ppm which may be sufficient to have an appreciable effect on adjacent plants. In addition, its release and buildup in the environment (soil and water) may indeed reach levels where it could have an effect in the control of other plants. This hypothesis will be the subject of further testing.

Speculations concerning the origin of dihydroactinidiolide in spikerush led us to another synthesis of the butenolide (Kurata et al., 1973) which provides a number of intermediates which can be tested. Photolysis of β-ionone (inactive in root growth inhibition) with a medium pressure Hg lamp provided the unstable pyran II (also inactive) which readily formed the diol III (inactive) upon exposure to air. Jones oxidation of the pyran II provided not only the desired butenolide I but also an intermediate aldehyde IV.

Aldehyde IV is also active as a root growth inhibitor, giving a 21% reduction at 10 ppm in 0.35% agar gels. No root growth was observed >45 ppm even though germination occurs. Quantitative analysis of an extract of spikerush determined that, if the aldehyde is present, it is present at <5 ppm.

Other fractions from spikerush showing phytotoxic activity are currently being investigated and will be reported at a later time.

ACKNOWLEDGMENT

The cooperation of Peter Frank (SEA, Davis) and Floyd Ashton (University of California, Davis) is gratefully acknowledged.

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Received for review October 1, 1979. Accepted December 26, 1979. This work was done at Western Regional Research Center, Berkeley, California, in collaboration with the University of California, Davis California, under Cooperative Agreement No. 12-14-5001-325. Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.