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ALLELOPATHIC POLYACETYLENES FROM *Centaurea repens* (RUSSIAN KNAPWEED)

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Abstract—The allelopathic weed Russian knapweed (*Centaurea repens*) was found to contain polyacetylenes VIII–XIV in the roots. Dose vs. response of the root length elongation against lettuce, alfalfa, barnyard grass, and red millet showed IX to be active. Closely related isomers were not active. Examination of the soil surrounding the Russian knapweed roots revealed the presence of IX in sufficient concentration to have an appreciable effect on the surrounding plant community.

Key Words—Allelopathy, polyacetylenes, Russian knapweed, *Centaurea repens, Compositae*

INTRODUCTION

It has been recognized for some time (Fletcher, 1963) that Russian knapweed (*Centaurea repens* L., Compositae), a rapidly spreading noxious weed, exhibits many of the characteristics of an allelopathic plant species. Earlier work (Stevens, 1982) showed the presence of a number of sesquiterpene lactones, many of which were shown to have some phytotoxic activity (Stevens and Merrill, 1985). However, both the concentrations of these sesquiterpene lactones in the plant and their toxicity to test species left some doubt as to whether these were responsible for the total phytotoxicity of Russian knapweed to the surrounding plant community.

Many polyacetylenic compounds have been isolated from composites (Bohlmann et al., 1973) but relatively few have been shown to be phytotoxic. Specifically, matricaria ester (I, ME), dehydromatricaria ester (II, DME), lachnophyllum ester (III, LE), and the two matricaria ester derivatives IV and V have been shown to have activity against rice seedlings (Kawazu et al., 1969; Kobayashi et al., 1974, 1980; Numata et al., 1973; Ichihara et al., 1976, 1978) (Figure 1). Since some of these compounds were found in the soil, it was concluded that the C_{10} polyacetylenes are probably allelopathic substances of ecological importance (Kobayashi et al., 1980). With the exception of the two

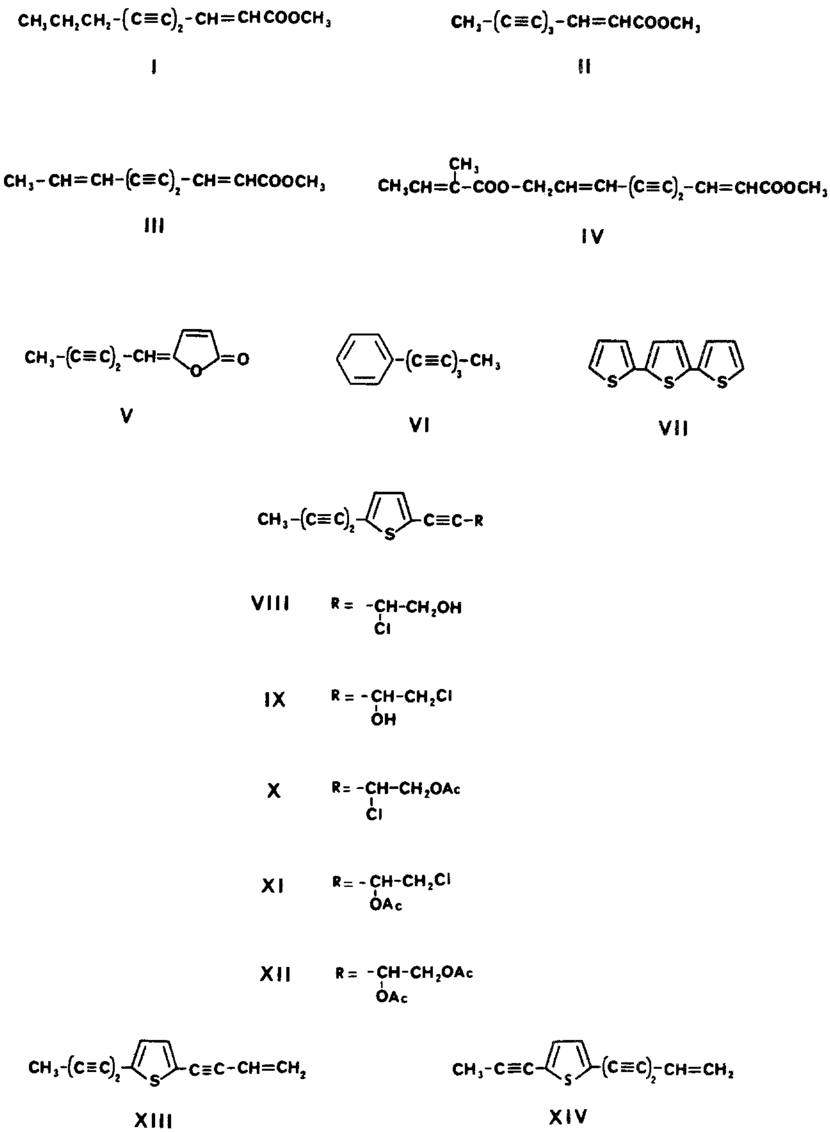

FIG. 1. Structures of Polyacetylenes

polyacetylenes phenyheptatriyne (VI, PHT) and α-terthienyl (VII), which have been found in the leaves of the tropical weed *Bidens pilosa* and the roots of common marigold *Tagetes erecta*, respectively (Campbell et al., 1982), there appear to be no data which would suggest that other polyacetylenes are phytotoxic. It must be recognized, however, that the vast majority of polyacetylenes described in the literature have never been tested for biological activity, particularly phytotoxicity.

METHODS AND MATERIALS

Extraction and Isolation. Roots of Russian knapweed were collected throughout its growing season near Discovery Bay (Hwy. 4 between Brentwood and Stockton), California. The severed roots were immediately placed on Dry Ice then ground in a rotating plate mill with Dry Ice to approximately $\frac{1}{8}$-in. particle size. The Dry Ice–root mixture was then slurried with ether–Skelly-F (1 : 2) in a larger beaker and allowed to warm to room temperature. Light was excluded to prevent any photodecomposition of the polyacetylenes. After warming to room temperature, the mixture was filtered and the solvent reduced in volume on a rotary evaporator at approximately 25°C.

Final separation of the polyacetylenes was accomplished by a combination of column chromatography on silica gel with benzene and preparative TLC (silica gel) using a variety of solvents, i.e., ether–Skelly-F (1 : 9), benzene, and cyclohexane–acetone (4 : 1). Polyacetylenes were collected from the preparative plates by scraping the bands from the plate and extracting the silica gel with ether. In all instances, care was taken to exclude light and keep the temperature of the solvents below 30°C.

Isolation of Polyacetylenes from Soil. Soil (375 g wet) from around the roots of Russian knapweed was sieved to remove organic material then placed in a chromatography column. Ether–Skelly-F (1 : 2) was percolated through the column and collected (2 : 1). The solvent was evaporated under reduced pressure and the residue chromatographed on preparative plates (20 × 20 cm. × 0.5 mm). Bands were scraped off and extracted with ether.

Identification of Compounds. Isolated compounds were analyzed by UV and [^{1}H]NMR spectroscopy and compared with reported values.

Bioassay. The seedling inhibition assays were carried out as follows: Seeds of lettuce (*Lactuca sativa*, black-seeded Simpson), barnyard grass (*Echinochloa crusgalli*), alfalfa (*Medicago sativa*), and red millet (*Panicum miliaceum*) were germinated on 0.5% agar (Bacto-Agar, Difco Laboratories, Detroit, Michigan) in a growth chamber set at 16-hr, 68°F days and 8-hr, 58°F nights. Twelve seedlings of each species were placed on 0.5% gels (20 ml agar/19 cm Petri dish) which had previously been slurried with the prescribed amount of polyacetylene in ether. In our tests, the concentrations were: 0, 5, 10, 20, 40, and

80 ppm. The polyacetylenes were added to the still-fluid gel at approximately 30°C to avoid thermal decomposition. Controls had only solvent incorporated into the gels. The seedlings were then placed in the dark at 20°C for 24 hr and root length measured to the nearest millimeter. Root growth studies with ME were carried out for 48 hr. Of the 12 seedlings whose root length were measured, the longest and shortest were discarded and the remaining 10 were used in the statistical analyses. In each case two replicates were run.

Data Analysis. The results of the seedling growth assays were analyzed separately using the Washington, D.C., Computing Center facilities and Statistical Analysis System (SAS Institute, Inc., Cary, North Carolina, 1982). The transformed data were subjected to Cochran's test for homogeneity of variances of all treatments and the least significant difference (LSD) test for differences between all treatment means.

RESULTS AND DISCUSSION

Five polyacetylenes, VIII–XII, obtained in pure form from Russian knapweed have been identified. Table 1 shows their respective UV and NMR spectra along with the literature reference. In addition to those listed in the table, com-

TABLE 1. UV AND NMR DATA OF POLYACETYLENES VIII–XII

Compound	UV(ether), λ_{max}[a]	NMR($CDCl_3$), 90 MHz, δ values(TMS)	Reference
VIII	344, 324, 270, 250, 206	2.04(s,3H,Me), 3.92(d,1H,J = 6), 3.97(d,1H,J = 5), 4.88(dd,1H, J = 5,6), 7.12(s,2H)	Bohlmann, 1965
IX	342, 322, 250, 236, 210	2.00(s,3H,Me), 3.70(d,1H,J = 6) 3.71(d,1H,J = 4), 4.79(dd,1H, J = 4,6), 7.02(d,1H,J = 3.5)	Bohlmann, 1970
X	342, 324, 270, 250, 236, 210	2.00(s,3H,Me), 2.08(s,3H,COMe), 4.37(d,1H,J = 6), 4.38(d,1H, J = 5), 4.91(dd,1H,J = 5,6), 7.08(s,2H)	Bohlmann, 1965
XI	342, 322, 250, 238	2.00(s,3H,Me), 2.10(s,3H,COMe), 3.73(d,2H,J = 6), 5.78(t,1H, J = 6), 7.06(s,2H)	Bohlmann, 1970
XII	342, 322, 248, 210	2.00(s,3H,Me), 2.07(s,3H,COMe), 2.09(s,3H,COMe), 4.22(dd,1H, J = 5,12), 4.34(dd,1H,J = 7,12), 5.80(dd,1H,J = 5,7), 7.02(s,2H)	Bohlmann, 1965

[a] Intensities were similar for all compounds [i.e., 342(2×10^4), 322(2.2×10^4), 250(8×10^3), 210(1.7×10^4)].

pounds XIII and XIV were isolated as a mixture. Lack of material, coupled with their instability, precluded separating the two isomers; however, they were readily identified by comparing the UV and NMR spectra of the mixture with those reported (Bohlmann et al., 1965).

Each of the isolated polyacetylenes is a C_{13} derivative, containing one thienyl group which can formally be envisioned (Bohlmann et al., 1973) as the addition of hydrogen sulfide across two triple bonds. These substances have not previously been found in other Composites. Compound VIII has been found in a number of *Echinops* spp. (Bohlmann et al. 1965) and *Pluchea dioscorides* (Bohlmann et al., 1973). Likewise, IX has been found in the roots of *Eclipta prostata* L. (Bohlmann et al., 1973) while X has been isolated from both *Echinops* spp. and *Centaurea cristata* (Bohlmann et al., 1966). Although a number of *Centaurea* species have been investigated for polyacetylenes (Bohlmann et al., 1973), VII, IX, and X represent new compounds to this genus. Bohlmann (Bohlmann et al., 1965) reported the presence of the diacetate XII in *Echinops sphaerocephalus* L.; however, it was only inferred to be present and was not isolated, nor were any data given. Its presence in Russian knapweed thus confirms it as a natural product.

The presumed intermediate oxidation products between the unsaturated compounds XIII, XIV, and the chlorohydrins and chlorohydrin acetates, viz., the epoxides, have not been found in spite of their presence in other *Centaurea* species (Bohlmann et al., 1973).

In all cases, with the exception of XIII and XIV, the isolated polyacetylenes are optically active, which rules out the possibility of their being hydrolysis products of XIII. As a typical example, IX showed $[\alpha]_{589}$ ($CHCl_3$) = $+17.5°$; $[\alpha]_{578} = +18.9°$; $[\alpha]_{546} = +20.6°$; $[\alpha]_{436} = +37.9°$.

Root length assay of VIII–XII has shown activity for only IX, with the results of lettuce, alfalfa, barnyard grass, and red millet tabulated in Table 2. The mean root length of the control for lettuce seedlings was 10.6 mm (starting length was approximately 3 mm), while at 10 ppm the root length was 6.9 mm. Fifty percent reduction in root length occurs at 12 ppm. The results of alfalfa, barnyard grass, and red millet were all similar, indicating that IX effectively inhibits root length elongation of grasses as well as broadleaf plants.

The possibility of synergism among the isolated polyacetylenes was tested by comparing the activity of the crude extract with that of IX. Allowing for concentration differences, it was found that IX accounted for all of the activity with no synergistic effect from other compounds.

Matricaria ester (ME, I), the first reported phytotoxic polyacetylene, was likewise tested under the same conditions against lettuce seedlings. Matricaria ester closely approximates the activity of IX with a 50% reduction in root length elongation occurring at 10 ppm. Little is known of the mode of action of the matricaria ester polyacetylenes. In a review (Numata et al., 1973) it was stated that addition of 5 ppm of either indoleacetic acid (IAA) or gibberelin (GA_3)

TABLE 2. EFFECT OF IX ON ROOT GROWTH OF WEED AND CROP SEEDLINGS

Seedling	Conc. of IX (ppm)	Mean root length (mm)[a]	
Lettuce	0	10.6	a
	10	6.9	b
	20	4.8	c
	40	3.7	d
	80	3.2	d
Barnyard grass	0	20.7	a
	5	16.3	b
	10	8.7	c
	20	4.7	d
	40	6.0	d
	80	5.0	d
Alfalfa	0	17.8	a
	5	14.6	a
	10	14.4	a
	20	10.1	b
	40	5.0	c
	80	5.1	c
Red millet	0	15.4	a
	5	19.1	a
	10	13.3	a
	20	6.1	b
	40	5.2	b
	80	2.0	c

[a] Means associated with a given test seedling with different letters are significantly different at the $\alpha = 0.05$ level according to the Least Significant Difference (LSD) test performed on transformed values. Original values are given.

failed to nullify the effects of 20 ppm of an ME derivative. It was concluded that the inhibitory effects were not based on a hormonal mechanism. The mechanism of plant growth inhibition by both matricaria esters and the thienyl compounds must await further studies.

To lend credence to the hypothesis that IX participates in the allelopathic action of Russian knapweed, the soil immediately surrounding the plant was extracted and found to contain 4–5 ppm of this particular compound. The analysis was based on actual material isolated, and hence represents a minimum amount because of the instability of the compound. The actual concentration in the soil may be considerably higher than 4–5 ppm. Extrapolation of the data from the bioassay to field conditions, gives a 30% reduction in root growth at 4 ppm. This is certainly sufficient to have an appreciable effect on the surrounding plant community (Kobayashi et al., 1974).

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