Volatile Components of Canada Thistle

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Volatile components of Canada thistle [(*Cirsium arvense* (L.) Scop.] roots were identified by gas chromatography–mass spectrometry. Among 24 compounds identified, there were seven C_{13} polyacetylenes, seven unsaturated C_{15}–C_{17} straight-chain hydrocarbons, and five epoxides derived from the C_{16} and C_{17} hydrocarbons. The major components are *cis*-8,9-epoxyheptadeca-1,11,14-triene and 8,9-dihydroxyheptadeca-1,11,14-triene. Sesquiterpenes γ-humulene and β-selinene are present. Compounds obtained from germinating thistle seed were inspected for C_{13} polyacetylene content and ability to stimulate germination of teliospores of the Canada thistle rust organism *Puccinia punctiformis*. As development of C_{13} polyacetylenes in the seedlings increased, so also did spore germination activity, and these compounds seem responsible for this activity.

Keywords: *Canada thistle; Cirsium arvense; volatiles; polyacetylenes; spore germination stimulants*

INTRODUCTION

Canada thistle [*Cirsium arvense* (L.) Scop.] is a noxious weed that is widely dispersed in the northern United States and Canada. Biological control agents could be helpful for control. One potential control agent is Canada thistle rust [*Puccinia punctiformis* (Strauss) Roehl]. Infection of the thistle is particularly devastating in the rust's aecial stage and is initiated from basidiospores produced by germinating teliospores.

To use teliospores effectively, a reliable way to break their dormancy and to stimulate their germination is necessary. Turner et al. (1982, 1986) found that Canada thistle root extracts when flooded over teliospores placed on water agar stimulated their germination. Subsequent studies by French et al. (1984, 1988) indicated that the stimulator was volatile and was present in the steam distillate obtained from Canada thistle roots. Compounds identified in a hexane extract of the steam distillate were 1-pentadecene, 1-heptadecene, a C_{17} diene, 1,8,11-heptadecatriene, and 1,8,11,14-heptadecatetraene (aplotaxene). When these compounds were separated by TLC of the hexane extract, most of the stimulating activity came from material recovered at the R_f of aplotaxene. However, tests of synthetic aplotaxene showed it to be inactive as a stimulatory agent (French et al., 1988). The other compounds are also inactive.

A similar situation was noted for safflower (*Carthamus tinctorius*). Safflower and Canada thistle are members of the Cynareae tribe of the Compositae family. Volatile components of safflower seedlings stimulated germination of teliospores of the safflower rust *Puccinia carthami* (Klisiewicz, 1973). Aplotaxene was tested and found inactive. Identified as active compounds were C_{13} polyacetylenic hydrocarbons (Binder et al., 1977).

We now report a qualitative and quantitative analysis of Canada thistle root volatiles and relationship of C_{13} polyacetylenes in seedlings to rust teliospore germination activity.

EXPERIMENTAL PROCEDURES

Materials. Roots of greenhouse-grown Canada thistle plants (30–40 cm tall) were washed free of soil and the small soft and fragile roots were discarded. Kilogram quantities of chopped roots (2–5 cm long) in 2.5 L of distilled water were steam distilled and volatiles were extracted with hexane. The sample discussed in detail herein was from roots collected in January 1991. Comparison of volatiles from this sample with volatiles from roots collected in September 1990, March 1991, and April 1991 showed substantial quantitative differences in the major components.

Seven 3-g batches of thistle seed were soaked in 0.003 M gibberellic acid solution to promote uniform germination and allowed to germinate as previously described (French et al., 1994). After each day of germination, for 7 days, the germinating seed in one of the batches was ground in acetone and extracted with hexane. Extracts were partitioned between hexane and water and the hexane extract was then concentrated to 1 mL. After these concentrates were tested for ability to stimulate spore germination, they were saponified and nonsaponifiables were extracted. An aliquot of the nonsaponifiables in hexane was hydrogenated with platinum oxide as catalyst.

Gas Chromatography. Chromatographic separations were carried out with Hewlett-Packard 5830 gas chromatographs fitted with flame ionization detectors. DB-1 and DB-Wax 60 m × 0.32 mm fused silica columns (J&W Scientific) were employed. Operating conditions for the DB-1 column were as follows: head pressure, 24 psi; temperature program, 50–230 °C at 4 °C/min and then 230 °C for 10 min. Operating conditions for the DB-Wax column were the same except head pressure was 21.5 psi. A measured amount of hexadecane was added to an aliquot of a volatiles concentrate to calculate yields. Correction for detector response was not attempted. The amount of tridecane in hydrogenated seedling extracts was determined by comparison to a measured amount of tetradecane.

Component Identification. Identifications were based on mass spectral data obtained with a Finnigan MAT 4500 gas chromatograph/mass spectrometer/data system and were verified by Kovats index comparisons on the DB-1 or DB-Wax column. ^{1}H NMR (200 MHz) spectra were obtained for $CDCl_3$ or benzene solutions with TMS as internal standard. Identification of some oxygenated compounds was aided by GC–EIMS (70 eV) and GC–CIMS (isobutane, source 140 °C) with

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a VG 70/70 magnetic mass spectrometer. Polyacetylenes in seedling extracts were identifiable by their UV spectrum obtained during a HPLC run.

Liquid Chromatography. Columns of silica gel 60 H in hexane along with hexane–ether mixtures as eluting solvents were used for separation of hydrocarbons, epoxides and hydroxy compounds. After removal of most of the aplotaxene and other linear compounds via their urea inclusion compounds from a mixture with a sesquiterpene, the sesquiterpene was separated from aplotaxene by chromatography on 15% silver nitrate on silica gel. A similar silver nitrate on silica column was used to resolve epoxide compounds. These were loaded onto the column in hexane and eluted with 5–20% ether in hexane. HPLC of polyacetylenes from seedling extracts was done on a silica column with hexane as eluting solvent and a diode array detector accumulated spectra.

Spectral Data for γ-Humulene. MS, *m/z* (relative intensity) 204 (51) (M^+), 189 (35), 161 (68), 147 (39), 133 (89), 119 (57), 105 (73), 93 (100), 91 (87), 79 (59); ^1H NMR ($CDCl_3$) δ 0.97 (s, 6H, CH_3), 1.43 (s, CH_3), 1.92 (m, 2H, H-11), 2.17 (m, 2H, H-3), 2.32 (m, 2H, H-4), 4.84 (dd, 2-H, $C{=}CH_2$), 5.28 (t, 1H, H-2), 5.56 (d, 1H, H-7), 5.87 (d, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 16.8, 21.3, 26.8, 29.7, 30.4, 31.5, 35.8, 42.2, 45.6, 113.0, 125.1, 126.2, 136.2, 143.4, 148.9.

***cis*-8,9-Epoxyheptadeca-1,11,14-triene.** GC–MS DB-Wax; EIMS, *m/z* (relative intensity) 133 (3), 122 (11), 111 (5), 108 (50), 95 (12), 93 (51), 79 (100), 67 (36), 55 (31), 41 (38); CIMS, *m/z* 249 $[M + H]^+$.

11,12-Epoxyheptadeca-1,8,14-triene. GC–MS DB-Wax; EIMS, *m/z* (relative intensity) 133 (9), 121 (11), 111 (25), 95 (55), 93 (38), 81 (68), 79 (46), 67 (100), 55 (68), 41 (80); CIMS, *m/z* 249 $[M + H]^+$.

***trans*-8,9-Epoxyheptadeca-1,11,14-triene.** GC–MS DB-Wax; EIMS, *m/z* (relative intensity) 133 (9), 122 (12), 111 (4), 108 (48), 95 (23), 93 (52), 79 (100), 67 (37), 55 (35), 41 (41); CIMS, *m/z* 249 $[M + H]^+$.

8,9-Epoxyheptadeca-1,11-diene. GC–MS DB-Wax; EIMS, *m/z* (relative intensity) 153 (5), 135 (6), 124 (13), 110 (45), 95 (77), 81 (99), 69 (73), 67 (100), 55 (97), 41 (100); CIMS, *m/z* 251 $[M + H]^+$.

7,8-Epoxyhexadeca-1,10,13-triene (Tentative). GC–MS DB-Wax; EIMS, *m/z* (relative intensity) 133 (1), 119 (3), 108 (42), 95 (17), 93 (60), 80 (24), 79 (100), 67 (38), 55 (31), 41 (40).

RESULTS AND DISCUSSION

Table 1 lists the volatile compounds identified in Canada thistle roots. Each compound listed was identified by the correlation of its mass spectrum obtained during a GC–MS run and the correspondence of experimental and reference retention indices or by the NMR spectrum of the isolated compound. Volatiles listed total 31.55 μg/g of roots.

Hydrocarbons constitute the major class of compounds and include two sesquiterpenes, six 1-alkenes, and seven polyacetylenes. Not listed in the table, but found to be present in trace amount, were C_{20}–C_{30} straight-chain alkanes. The compound with DB-1 retention index 1476 showed a mass spectrum similar to that of caryophyllene. After isolation, as described in the experimental section, its NMR spectrum was very similar to the spectrum (CCl_4 solution) published for γ-humulene (Yano and Nishijima, 1974) and our COSY confirmed this structure. The 1-alkenes make up about 15% of the root volatiles. In addition to the compounds previously identified in Canada thistle, there was a very small amount of 1-hexadecene and some 1,7,10,13-hexadecatetraene. The latter compound was previously found only in roots of *Senecio isatideus* (Boland and Jaenicke, 1982). Trideca-1,3(*E*),5(*Z*),11(*E*)-tetraene-7,9-diyne and trideca-1,3(*E*),5(*E*),11(*E*)-tetraene-7,9-diyne are reported here for the first time in a *Cirsium* species. The other polyacetylenes have been reported in *Cirsium* species although not with specification of double bond configuration (Bohlmann et al., 1966; Christensen, 1992). All of these and more were found in safflower and mass spectral data for them were given (Binder et al., 1990).

Table 1. Volatile Components of Canada Thistle Roots

compound	μg/g	Kovats index DB-1	Kovats index DB-Wax
methyl salicylate	0.01	1168	
γ-humulene	0.30	1476	1713
β-selinene	0.02	1480	1711
1-pentadecene	0.48	1489	1544
1,7,10,13-hexadecatetraene	0.03	1561	1769
1-hexadecene	0.01	1590	
trideca-1,3(*E*),5(*Z*),11(*E*)-tetraene-7,9-diyne	0.02	1598	
sesquiterpene alcohol	0.08	1633	
trideca-1,3(*E*),5(*E*),11(*E*)-tetraene-7,9-diyne	0.03	1648	
trideca-1,3(*Z*),11(*E*)-triene-5,7,9-triyne	0.30	1656	2394
1,8,11,14-heptadecatetraene (aplotaxene)	3.55	1658	1863
1,8,11-heptadecatriene	0.35		1801
1,8-heptadecadiene	0.18	1665	1763
trideca-1,11(*Z*)-diene-3,5,7,9-tetrayne	0.23	1672	2393
trideca-1,3(*E*),11(*E*)-5,7,9-triyne	0.09	1685	
1-heptadecene	0.06	1690	1745
trideca-1,11(*E*)-diene-3,5,7,9-tetrayne	0.22	1712	2489
7,8-epoxyhexadeca-1,10,13-triene (tentative)	0.09	1721	2128
unknown	0.09	1753	
1-tridecene-3,5,7,9,11-pentayne	0.05	1780	
trans-8,9-epoxyheptadeca-1,11,14-triene	0.49	1809	2208
11,12-epoxyheptadeca-1,8,14-triene	0.24	1815	2215
8,9-epoxyheptadeca-1,11-diene	0.35	1820	2180
cis-8,9-epoxyheptadeca-1,11,14-triene	10.9	1823	2235
unknown	0.47	1849	
unknown	0.17	1896	
hexadecanoic acid	0.67	1941	
8,9-dihydroxyheptadeca-1,11,14-triene	10.6	1987	2899
unknown	0.21	2020	
unknown	1.18	2059	
unknown	1.05	2063	2813
unknown	1.25	2107	
unknown	0.49	2111	
unknown	0.39	2116	

Two oxygenated derivatives of aplotaxene, (*cis*)-8,9-epoxyheptadeca-1,11,14-triene (Binder et al., 1992), also found in *Cirsium helenioides* roots (Christensen, 1992), and 8,9-dihydroxyheptadeca-1,11,14-triene (Binder et al., 1992), also found in *C. helenioides* (Christensen, 1992) and *C. nipponicum* roots (Takaishi et al., 1991), account for 69% of the volatiles.

One previously characterized aplotaxene-derived epoxide and three novel epoxides were present. Epoxides isolated by chromatography on silica gel were further resolved on $AgNO_3$ on a silica gel column. The compound with DB-1 RI 1815, isolated in greater than 90% purity, showed a ^1H NMR spectrum that matched the spectrum of the 11,12-epoxyheptadeca-1,8,14-triene found in *Cirsium hypoleucam* roots (Bohlmann and Abraham, 1981) and had the same molecular weight (248 for $C_{17}H_{28}O$). Another epoxide (DB-1 RI 1809) also had a 248 molecular weight and had a mass spectrum essentially indistinguishable from that of *cis*-8,9-epoxyheptadeca-1,11,14-triene. With these considerations, it seems most likely that this compound is the *trans* isomer of the 8,9-epoxy aplotaxene derivative. Their relative GC retention indices are consistent with this formulation. On both nonpolar and polar GC columns, the *trans* isomer of methyl 8,9-epoxyoctadecanoate elutes before the *cis* isomer (Gunstone and Jacobsberg, 1972). An epoxide concentrate was freed of *cis*-8,9-

Table 2. Amounts of C_{13} Polyacetylenic Hydrocarbons in Thistle Seed Germinated for 1–7 Days

germination time (days)	nmol of polyacetylenes/g of seed	germination time (days)	nmol of polyacetylenes/g of seed
1	none detected by GLC[a]	5	187[b]
2	1.5	6	388
3	32	7	565
4	120		

[a] A trace amount was probably present. See text. [b] A small indefinite amount of this sample was lost in a spill.

epoxyheptadeca-1,11,14-triene by repeated chromatography on silica gel. Then most of the 11,12-epoxide was eliminated by chromatography on 15% $AgNO_3$ on silica gel and a fraction of a milligram of the putative *trans*-8,9-epoxyheptadeca-1,11,14-triene was obtained in about 85% isomer purity. Its ^1H NMR spectrum was equivalent to that of the *cis*-8,9-epoxide in the δ 5.0–6.0 region (Binder et al., 1992; H-1,2,11,12,14,15). Of particular interest in the spectrum are peaks at δ 2.54 and 2.78 (C_6D_6 solution) with equal integrals. The peak at δ 2.78 can be attributed to H-13s and in this compound the epoxide protons may have their resonance shifted from the δ 2.7–2.8 position for the *cis* epoxide to δ 2.54 for the *trans* isomer. There is a report that unsaturated *cis* epoxides of fatty acids display signals at δ 2.69–2.75 whereas *trans* epoxides have signals at δ 2.49–2.50 for CCl_4 solutions (Gunstone and Schuler, 1975). An epoxide hidden in the peak for *cis*-8,9-epoxyheptadeca-1,11,14-triene when chromatographed on a DB-1 column was revealed at a significantly lower RI when a DB-Wax column was used. CIMS showed the molecular weight to be 250, indicating two olefinic bonds. Since the compound seemed most likely to be either 8,9-epoxyheptadeca-1,11-diene or 11,12-epoxyheptadeca-1,8-diene, a mixture of these compounds was synthesized from 1,8,11-heptadecatriene (Boland and Jaenicke, 1981) and one of the compounds matched the characteristics of the unknown. Authentic 11,12-epoxyheptadeca-1,8-diene was synthesized from vernolic acid (*cis*-12,13-epoxyoctadec-9-enoic acid) according to the procedure of Boland and Jaenicke (1981) and was found not to be the compound from the thistle roots. A trace amount of another thistle root epoxide had RIs on nonpolar and polar columns that were one C unit less than for *cis*-8,9-epoxyheptadeca-1,11,14-triene and so seemed likely to be a derivative of the 1,7,10,13-hexadecatetraene also found. The similarity of the mass spectra of the C_{16} and C_{17} compounds is so great that the structure 7,8-epoxyhexadeca-1,10,13-triene is indicated.

Ratios and amounts of the three major volatiles from root samples collected at various times during the year varied greatly. The average amount of aplotaxene was 10 μg/g of roots (range 4–21 μg/g), that of *cis*-8,9-epoxyheptadeca-1,11,14-triene was 37 μg/g of roots (range 11–66 μg/g), and that of 8,9-dihydroxyheptadeca-1,11,14-triene was 16 μg/g of roots (range 11–25 μg/g). The sample chosen for the extensive analysis given in Table 1 contains what seems to be less than usual amounts of these compounds.

The development of C_{13} polyacetylenes in germinating seeds was determined by inspection of UV spectra obtained during HPLC of nonsaponifiables and by measuring tridecane in samples of hydrogenated nonsaponifiables by use of GLC. Calculated values for C_{13} polyacetylenic hydrocarbons in seeds germinated for 1–7 days are given in Table 2. As indicated by UV spectra, the polyacetylene first to be produced is 1-tridecene-3,5,7,9,11-pentayne. A detectable amount occurred in the sample germinated for 1 day, and it was the major polyacetylene in all subsequent samples. The 3-day germination sample contained trideca-1,3-diene-5,7,9,11-tetrayne also. After 14 days of germination, in addition to the still predominant polyacetylene 1-tridecene-3,5,7,9,11-pentayne, GC–MS shows the presence of trideca-1-,11(*Z*)-diene-3,5,7,9-tetrayne, trideca-1,3(*E*),-11(*E*)-triene-5,7,9-triyne, trideca-1,11(*E*)-diene-3,5,7,9-tetrayne, trideca-1,3(*Z*)-diene-5,7,9,11-tetrayne, trideca-1,3(*E*),5(*E*)-triene-7,9,11-triyne, and trideca-1,3(*E*)-diene-5,7,9,11-tetrayne.

Table 3. Percent Germination on Test Plates of Teliospores of *P. punctiformis* Exposed to Extracts from Germinating Canada Thistle Seeds[a]

germination time (days)	0.01 μL	0.05 μL	0.1 μL	0.5 μL	1 μL	water control
1	0.1	0.4	0	1.5	1.1	0
2	3.0	7.3	8.3	17.3	28.4	0
3	14.5	30.5	25.0	38.5	30.9	0
4	30.0	41.4	38.8	43.4	44.8	0
5	29.8	38.1	44.5	43.0	36.5	0
6	47.5	54.6	54.9	56.3	44.9	0
7	44.1	45.5	53.8	55.0	48.5	0

[a] Amounts tested are aliquots of hexane extracts, concentrated to 1 mL, of 3 g of seed germinated for 1–7 days.

Compounds present in extracts from germinating seed strongly stimulated *P. punctiformis* teliospore germination (Table 3). Comparison of Tables 2 and 3 shows that increase in percent germination is correlated with development of polyacetylenes in the seedlings. The major increase in both is at 3 days. At 4 days, 30% spore germination was attained by extract representing 0.03 mg of seed. Data from Table 2 indicate that polyacetylene content would be 3.6 pmol and, if calculated as 1-tridecene-3,5,7,9,11-pentayne, would amount to 0.58 ng. In a previous study, Binder et al. (1977) found similar activity for polyacetylenes. For example, 1-tridecene-3,5,7,9,11-pentayne at 0.15 ng/cm^3 in a small chamber with teliospores of *Puccinia carthami* stimulated 15% germination.

These results and results of testing individual compounds (French et al., 1994) indicate that the germination stimulus is adequately accounted for by the C_{13} polyacetylene hydrocarbons.

ACKNOWLEDGMENT

We thank Mabry Benson for obtaining NMR spectra and Robert Flath and William Haddon for mass spectral data.

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Received for review June 16, 1994. Revised manuscript received September 26, 1994. Accepted October 4, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1994.