Volatile Components of Yellow Starthistle

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The volatile compounds of yellow starthistle flower buds (and leaves and stems) were isolated by Tenax trapping and analyzed by capillary GLC–MS. The major volatile component was identified as germacrene D, which formed 1 ppm of the floral buds. Other major components identified in much smaller relative amounts include bicyclogermacrene (tentative), caryophyllene, and 1-pentadecene.

INTRODUCTION

Yellow starthistle (*Centaurea* Solstitialis L.) is an introduced weed that has become naturalized to the United States and is responsible for considerable economic loss in California where it has invaded grainfields, orchards and vineyards, other cultivated crops, pastures and rangelands, and roadsides and wastelands (Maddox, 1981). This plant is widespread, especially in the Western United States, occurring in 208 counties in 23 states (Maddox, 1985b). In California the gross acreage of the plant has increased 6.4 times since 1958, the current gross acreage being estimated at nearly 3×10^6 ha (Maddox, 1985a). Two insect herbivores, a fly [*Urophora sirunaseva* (Hering)] and a weevil (*Bangasternus orientalis* Capiomont), have been introduced as biological control agents against yellow starthistle in the western United States, and other host plant specific insects are also being studied for possible use. An understanding of how these insects find their host plant could be useful in delineating plant–herbivore dynamics and in the more efficient management of these insects. It is thought that a volatile compound(s) associated with the bud stages of the plant might be involved in the orientation of the insect to the plant. The present work was begun to determine the nature of the volatile compounds available in starthistle flower buds that the insect might use. A literature search showed no previous reports of analysis of the volatile components of yellow starthistle, although some studies have been previously reported on the nonvolatile constituents (cf. Merril and Stevens, 1985; Stevens and Merril, 1985).

EXPERIMENTAL SECTION

Materials. The yellow starthistle buds were harvested in fields in Moraga, CA during the months of May to Aug 1985. Buds were obtained from a large number of different plants and combined to give a representative sample. The degree of maturity of the thistle flower buds has been described in terms of first to fourth bud stages (cf. Maddox, 1981). Third and fourth bud stage samples and leaves and stems were picked on different occasions. During transport the samples were stored in glass containers (covered with aluminum foil) in an ice-cooled insulated chest. The isolation was generally begun within 5 h after harvesting.

Isolation of Volatiles Using Tenax Traps. The method used was similar to that previously described by some of us for other plants (cf. Buttery et al., 1986). The buds (180–200 g) were enclosed in a 12-L flask. Air, drawn from outside the laboratory and purified by passage through activated charcoal, was led into the flask via a Teflon tube. The air passed over the buds and left through a Tenax trap (a Pyrex tube containing 10 g of Tenax, 14-cm length by 2.2-cm diameter). A reduced pressure (ca. 730 mm from an aspirator) was used at the end of the Tenax trap to draw the air through the system. The flow of air was 1 L/min and was continued for 24 h. The volatiles were later eluted from the Tenax trap with diethyl ether (freshly distilled containing a trace of Ethyl antioxidant 330), and the ether extract was concentrated (on a warm-water bath and low-hold-up Vigreux type distillation columns) to a small volume (5 μL).

Capillary Gas Chromatography–Mass Spectrometry (GLC–MS). The volatile concentrate from above was analyzed by using splitless injection with a 150-m length by 0.66-mm i.d. Pyrex glass capillary column coated with Carbowax 20-M. The GLC conditions were to hold the column at 60 °C for 40 min and then to program from 60 to 170 °C at 1°/min and to hold at the upper limit. The inlet pressure was 15 psi and flow velocity 28 cm/s He. The mass spectrometer conditions used were as described previously by Buttery et al. (1986). Authentic samples of sesquiterpenes were obtained from ylang-ylang, hop, and orange essential oils (cf. Buttery et al., 1986). Other compounds were obtained from reliable commercial sources. All authentic samples were purified by GLC separation and their identities verified by mass or infrared spectral means.

RESULTS AND DISCUSSION

The main study was carried out on the starthistle buds in their "third bud stage", as this stage was considered the most attractive to both the seed fly and the weevil (cf. Maddox, 1981). The volatile oil obtained by Tenax trapping from the third-stage buds was of the order of 2 ppm of the buds. Capillary GLC–MS analysis of this volatile oil showed the compounds listed in Table I. Figure 1 shows the GLC analysis. The peak numbers in Figure 1 are listed in Table I alongside the component's name.

By far the major component, peak 50, was identified as germacrene D by direct comparison of its mass spectrum and capillary GLC retention properties with that of an authentic sample isolated from ylang-ylang oil. Enough of peak 50 was isolated by packed-column GLC for measurement of its infrared absorption (IR) spectrum, which is shown in Figure 2. This IR spectrum was also consistent with that of the authentic sample of germacrene D. An optical rotation measurement was also carried out on peak 50. This showed $[\alpha]^{25}_D$ −196° (hexane), which compares fairly well with the literature figure of $[\alpha]^{27}_D$ −240° (chloroform) for authentic germacrene D (cf. Glasby 1982). Some of us had recently found germacrene D as a major component of walnut and fig leaves (Buttery et al., 1986).

The component second highest in relative concentration, peak 52, had a mass spectrum and GLC retention properties consistent with that of the sesquiterpene hydrocarbon bicyclogermacrene, which has a related structure to germacrene D. The mass spectrum of peak 52 showed the following (two most intense ions each 14 mass units above m/e 34, intensities in parentheses): 41 (45), 43 (21);

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Table I. Volatile Components of Starthistle Flower Buds

peak no.[a]	compd[b]	major MS ions (one ea 14 mass units)[c]	Kovat's GLC index[d]	rel %
		Aliphatic Alcohols, Aldehydes, and Esters		
12	(*E*)-2-hexenal	41, 55, 69, 83, *98*	1190	0.7
21	hexanol	56, 43, 31, 69, 84	1340	0.2
23	(*Z*)-3-hexenol	41, 67, 55, 31, 82	1370	0.4
29	(*Z*)-3-hexenyl propionate	57, 67, 82, 41, 105	1410	0.1
		Terpenoids		
8	myrcene	93, 41, 69, 79, 53, 121	1160	0.7
13	limonene	68, 93, 41, *136*, 53, 79	1180	0.2
14	(*E*)-β-ocimene	93, 41, 79, 53, 121, 67	1250	0.2
15	*p*-cymene	119, *134*, 91, 41, 77, 65	1280	0.1
17	perillene[e] (tentative)	69, 41, 81, 53, 107 *150*	1310	1.0
30	α-copaene	105, 119, 161, 93, 41, 81	1460	0.3
41	caryophyllene	41, 69, 93, 79, 133, 55	1570	4.3
48	(*E*)-β-farnesene	69, 41, 93, 79, 55, 133	1650	0.6
50	germacrene D	161, 105, 91, 119, 81, 133	1680	61
52	bicyclogermacrene[e] (tentative)	121, 93, 107, 41, 79, 136	1690	7.2
		Other Compounds		
24	2-methoxytoluene	*122*, 107, 91, 77, 65, 39	1390	0.2
37	1-pentadecene	43, 55, 69, 83, 97, 111	1540	4.9

[a] Peak numbers in Figure 1. [b] Complete mass spectrum and Kovat's GLC index are consistent with those of authentic samples except for peaks 17 and 52 (see footnote *e*). [c] The most intense ion each 14 mass units above m/z 34. Ions in descending order of intensity with molecular ion (if listed) in italic type. [d] Kovat's GLC index for the Pyrex Carbowax 20-M capillary column. [e] No authentic sample available but mass spectrum consistent with published spectra.

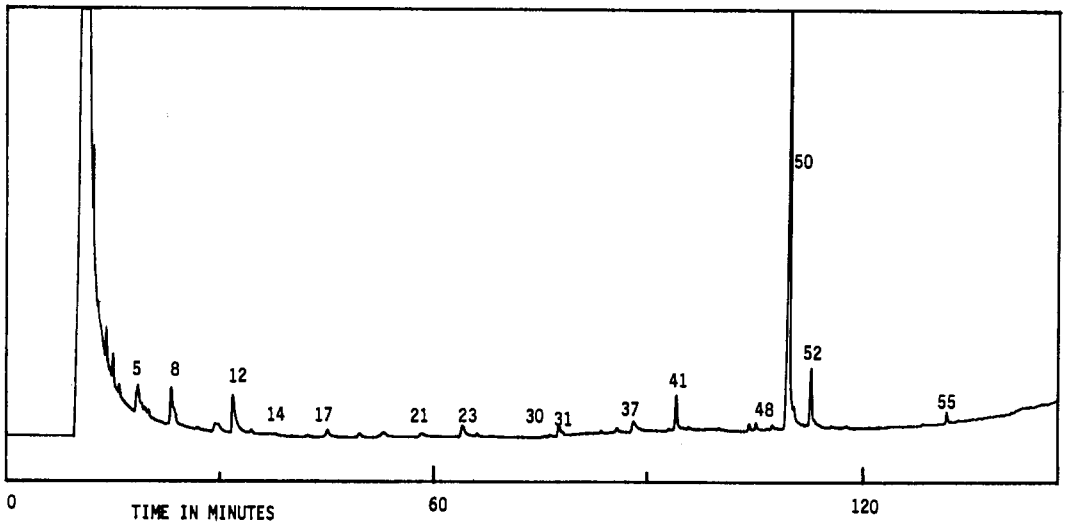

Figure 1. Capillary GLC analysis (fid detection) of the Tenax-trapped volatiles from third-stage yellow starthistle buds using the Carbowax 20-M pyrex capillary described in the Experimental Section.

53 (15), 55 (24); 67 (21), 69 (13); 79 (32), 81 (27); 91 (31), 93 (77); 105 (29), 107 (55); 119 (24), 121 (100), 133 (10), 136 (29); 147 (8), 148 (5); 161 (27); 189 (8); 204 (13). This is consistent with that reported by Tressel et al. (1983) for authentic bicyclogermacrene. The Kovat's index (KI) value on the 150-m length × 0.64-mm i.d. Carbowax 20-M column was 1690 (compared to that of 1570 for caryophyllene on the same column). The KI value on a DB-1 (silicone) fused silica capillary was 1488 (compared to 1415 for caryophyllene on the same column). The KI value for Carbowax 20 M is consistent with that reported by Maarse and Van Oss (1973) for authentic bicyclogermacrene using caryophyllene as an internal marker. Several nonvolatile sesquiterpene lactones, which have allelopathic activity, were recently identified in yellow starthistle by Stevens and Merrill (1985) and Merrill and Stevens (1985). These lactones, however, possess the guaiane-type sesquiterpene skeleton and do not appear to be related to the sesquiterpene hydrocarbons identified in the present work. Such multioxygenated sesquiterpenes could not be involved in attraction of the insect to the plant because they are nonvolatile. They could be involved in the insect's identification of the plant after the insect alights on its surface.

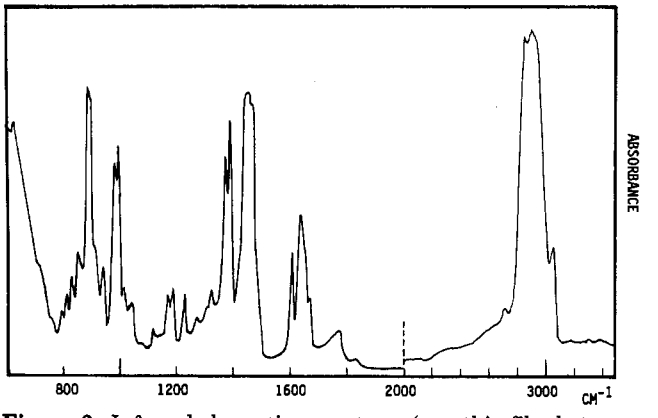

Figure 2. Infrared absorption spectrum (as a thin film between micro salt plates) of the major volatile component (peak 50, germacrene D) of yellow starthistle buds.

Minor Components. The C6 compounds identified such as (*Z*)-3-hexenol and (*E*)-2-hexenal are commonly found in green plants.

Perillene [2-(4-methyl-3-pentenyl)furan] is somewhat unusual. It was identified by comparison of its mass spectrum with that published by Thomas and Ozainne (1970). It could be considered an oxidation product of (*E*)-β-ocimene (or of myrcene). In reviewing their previous GLC–MS data on volatiles of other plant parts such as corn, wheat, barley, and oats where (*E*)-β-ocimene had been found, we also detected a compound with the spectral and GLC retention properties of perillene but had not had the reference spectrum at that earlier date for its identification with those studies (cf. Buttery et al., 1986).

Fourth-Stage Buds and Leaves and Stems. The volatile oil from fourth-stage yellow starthistle buds was qualitatively similar to that of the third-stage buds. The relative quantitative amounts of components were also similar. The total amount of volatile oil found in the fourth-stage buds was slightly less than that of the third bud stage, but the method used was not accurate enough to be sure of the small difference.

The volatiles were also isolated from the stems and the leaves of the starthistle plant by using the same procedure as for the buds. The leaves are very small on the yellow starthistle and were not separated from the stems. The qualitative analysis of the stems and leaves identified the same compounds that were found in the buds. Quantitatively the total amount of volatile oil was only 0.5 ppm, considerably less than that found in the buds. There was also larger concentrations of the C6 compounds relative to germacrene D. (*Z*)-3-Hexenol was one-tenth the concentration of germacrene D in the stems and leaves whereas this alcohol was less than $^1/_{100}$th the concentration of germacrene D in the buds. This is not unexpected because (*Z*)-3-hexenol has long been well-known to be associated with leaves and is sometimes known as "leaf alcohol".

Biological Tests. The starthistle seed fly (*U. sirunaseva*) and the weevil (*B. orientalis*) are only available for a few weeks during the summer months, and there was not sufficient time in the present study to test the volatiles identified against these insects. Both field and laboratory bioassays to test these compounds with the insects are planned for the 1986 season.

Registry No. (*E*)-CHOCH=CHPr, 6728-26-3; $HO(CH_2)_3Pr$, 111-27-3; (*Z*)-$HO(CH_2)_2CH$=CHEt, 928-96-1; (*Z*)-$EtCO_2$-$(CH_2)_2CH$=CHEt, 33467-74-2; *o*-MeC_6H_4OMe, 578-58-5; CH_2=$CH(CH_2)_{12}Me$, 13360-61-7; myrcene, 123-35-3; limonene, 138-86-3; (*E*)-β-ocimene, 3779-61-1; *p*-cymene, 99-87-6; perillene, 539-52-6; α-copaene, 3856-25-5; caryophyllene, 87-44-5; (*E*)-β-farnesene, 18794-84-8; germacrene D, 23986-74-5; bicyclogermacrene, 24703-35-3.

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GLC–MS Analysis of the Volatile Constituents of *Panicum sp.*

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The volatiles from the leaf and stem fractions of three selections of three species of *Panicum* were analyzed by GLC–MS. Major components were 6-methyl-5-hepten-2-ol, *cis*-3-hexenol, 1-octen-3-ol, 2-nonanol, linalool, and borneol. Lavandulol, an irregular monoterpene not generally found in grasses, was isolated in significant amounts from both leaf and stem fractions.

INTRODUCTION

Grazing preference exhibited by animals for plant material has been known for sometime (Johnson-Wallace, 1937) and is thought to depend on palatability, associated plant species, climate, soil and topographical conditions, kinds of animals, and animal physiology (Heardy, 1964) as well as the olfactory cues received from a given plant species. Scehovic (1985) has recently shown that the grazing preference for tall fescue (*Festuca arundinacea* Schreb.) and ryegrass (*Lelium perenne* L.) can be reversed by spraying the juices pressed from one plant on to the other and vice versa. Further, compounds were separated by classes and the total amounts in each class determined. The classes determined most important as attractants were the volatile esters, aldehydes, ketones, and volatile phenols. Although individual compounds were not indentified, the study illustrates the importance of olfactory cues in forage preference. Switchgrass (*Panicum virgatum* L.), a subtropical, perennial grass has shown potential to provide high daily gains for steers when grazed during early and middle summer (Burns et al., 1984). Preliminary studies to establish the grazing preferences of cattle among cultivars of several *Panicum* species have been conducted. In

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