Identification of Hymenoxon in *Baileya multiradiata* and *Helenium hoopsii*

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Extracts of *Baileya multiradiata* and *Helenium hoopsii* were analyzed by GLC and GC–MS for the presence of a toxic sesquiterpene lactone, hymenoxon. A compound was found in both plants that cochromatographed with hymenoxon on 3% OV-17 and 3% QF-1 GLC columns and gave a mass spectrum statistically identical with the mass spectrum of hymenoxon. Extracts of both plants were treated with base to convert hymenoxon to psilotropin. Hymenoxon could not be detected in the base-treated extracts; however, a compound was detected that cochromatographed with psilotropin on 3% OV-17 and 3% QF-1 columns and gave a mass spectrum statistically identical with the mass spectrum of psilotropin.

Hymenoxys odorata DC (bitterweed) is a major poisonous range plant in Texas. A toxic, sesquiterpene α-methylene lactone has been isolated and characterized from *H. odorata* by two groups (Kim et al., 1975; Ivie et al., 1975). The isolated compounds were shown to produce clinical signs characteristic of bitterweed intoxication. Several plants taxonomically related to *H. odorata* (Composite family) are also toxic to livestock and produce a syndrome similar to that observed in *H. odorata* intoxication (Kingsbury, 1964; Sperry et al., 1964). Three sesquiterpene lactones (fastigilin C, baileyolin, and baileyin) have been isolated from *Baileya multiradiata* (Waddell and Geissman, 1961). Sesquiterpene lactones have not been reported in *Helenium hoopsii*. The stereochemistry of hymenoxon (Figure 3) has been established by x-ray crystallography (Petterson and Kim, 1976). In this study, *B. multiradiata* and *H. hoopsii* were analyzed for the presence of hymenoxon and related lactones.

EXPERIMENTAL SECTION

Standards. Hymenoxon (mp 135–136 °C; lit. 135–142 °C; Kim et al., 1975) was extracted from *H. odorata* and repeatedly recrystallized by the procedure of Kim et al. (1975). Psilotropin was prepared from hymenoxon by dissolving approximately 1 g of hymenoxon in 1 N NaOH and allowing the solution to stand at room temperature for 15 min. The solution was adjusted to pH <1 with 2 N HCl, and then the solution was extracted with $CHCl_3$. The $CHCl_3$ was evaporated in vacuo, and the residue was dissolved in acetone. Analysis of the acetone solution by GLC (conditions described below) and comparison of the data with chromatograms of known standards obtained from Professor W. Herz showed that the solution contained a mixture of the two isomeric dilactones, psilotropin and greenein (Herz et al., 1970; Figure 3). Psilotropin was fractionally crystallized from the acetone by adding increasing amounts of petroleum ether to the solution. After each crystallization the psilotropin was checked for purity by GLC. Using a Fisher-Johns melting point apparatus the psilotropin crystals (ca. 300 mg) were observed to melt at 146–147 °C (lit. 144–145 °C; DeSilva and Geissman, 1970). The identity of hymenoxon and psilotropin was confirmed by NMR.

Collection of Plant Material. *B. multiradiata* was collected by Dr. Murl Bailey, Oct 1975, 50 miles north of Van Horn, Tex., on State Highway 54. Mature, flowering *H. hoopsii* was collected in 1970 by Dr. Wayne Binns (Poisonous Plant Research Lab., Agriculture Research Service, USDA, Logan, Utah). The aerial part from both plants was air-dried and ground.

Extraction of Plant Samples. The aerial part (800 g) of *B. multiradiata* was extracted three times by stirring with acetone for 3 h. The acetone was filtered and evaporated in vacuo, and the resultant residue was extracted with several volumes of $CHCl_3$. The $CHCl_3$ extracts were combined and evaporated in vacuo to yield a gummy, yellow residue. A few milligrams of the residue was dissolved in 1 mL of ethyl acetate for analysis on GLC.

The aerial part (200 mg) of *H. hoopsii* was extracted with 6 mL of acetone. The acetone was evaporated to dryness in a stream of air and yielded a yellow residue that was dissolved in 1 mL of acetone.

To convert hymenoxon to psilotropin, a few milligrams of the extract from each plant were dissolved in 0.5 mL of acetone, mixed with 2 mL of 0.5 N NaOH, and allowed to stand at room temperature for 15 min. The solutions were acidified with 2 mL of 2 N HCl and then each solution was extracted with 6 mL of ethyl acetate. The ethyl acetate extracts were evaporated to dryness under a stream of air, and the residues were dissolved in 0.5 mL of ethyl acetate.

Gas Chromatography (GC). The plant extracts containing hymenoxon and the base-treated extracts containing psilotropin were analyzed on a Varian 2100 GC or on a Barber Colman 5000 GC. The Varian GC contained a 0.125 in. × 4.8 ft glass column packed with 3% OV-17 on Gas-Chrom Q (100/120 mesh) with the column temperature at 205 °C, injector temperature at 218 °C, and FID temperature at 255 °C. Helium at a flow rate of 40 mL/min was used as the carrier gas. The Barber Colman GC was equipped with a 0.166 in. × 6 ft glass column containing 3% QF-1 on Gas-Chrom Q (100/120 mesh) at 202 °C with the injector temperature at 236 °C and the FID temperature at 250 °C. The carrier gas was nitrogen at a flow rate of 45 mL/min.

The retention time of hymenoxon and psilotropin was determined on both the 3% OV-17 column and the 3% QF-1 column. Each plant extract and base-treated extract was chromatographed on both columns to determine the presence of hymenoxon or psilotropin, and then each extract was cochromatographed with the appropriate standard.

Gas Chromatographic–Mass Spectrometric Analysis (GC–MS). The plant extracts and the base-treated extracts were chromatographed on a Dupont 21-491 (lot 4) GC–MS containing a 0.125 in. × 3 ft stainless steel microbore column packed with 6% Dexsil 300 on Supelcoport. The injector temperature was 260 °C while the column temperature was held isothermal at 190 °C for 150–200 s and then was programmed to 350 °C at 10 °C/min. One-fourth of the effluent was monitored by a

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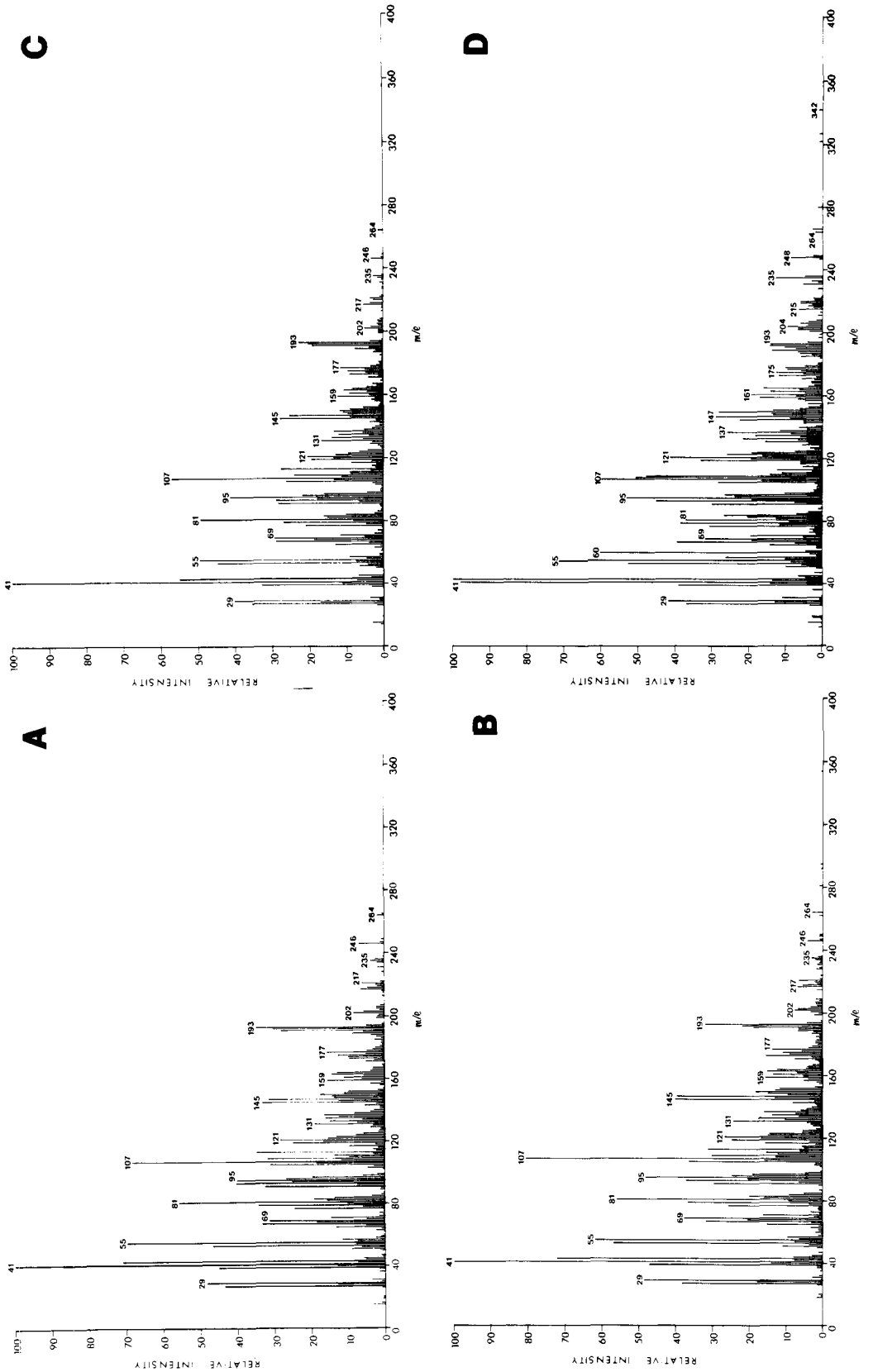

Figure 1. Mass spectra of (A) hymenoxon, (B) compound eluted from extract of *B. multiradiata*, (C) compound eluted from extract of *H. hoopsii*, (D) compound eluted from extract of *B. multiradiata* that was chromatographically separated from hymenoxon.

flame ionization detector (280 °C) and three-fourths of the effluent was monitored by the mass spectrometer. Mass spectra were statistically compared to known standards by a Dupont 21-094 data processing system (Hertz et al., 1971). The comparisons were made from a computer library containing the mass spectra of approximately 24 000 compounds. The spectra of hymenoxon and psilotropin were added to the library for this study.

RESULTS AND DISCUSSION

The presence of hymenoxon in *B. multiradiata* and *H. hoopsii* was indicated by the presence of a compound in

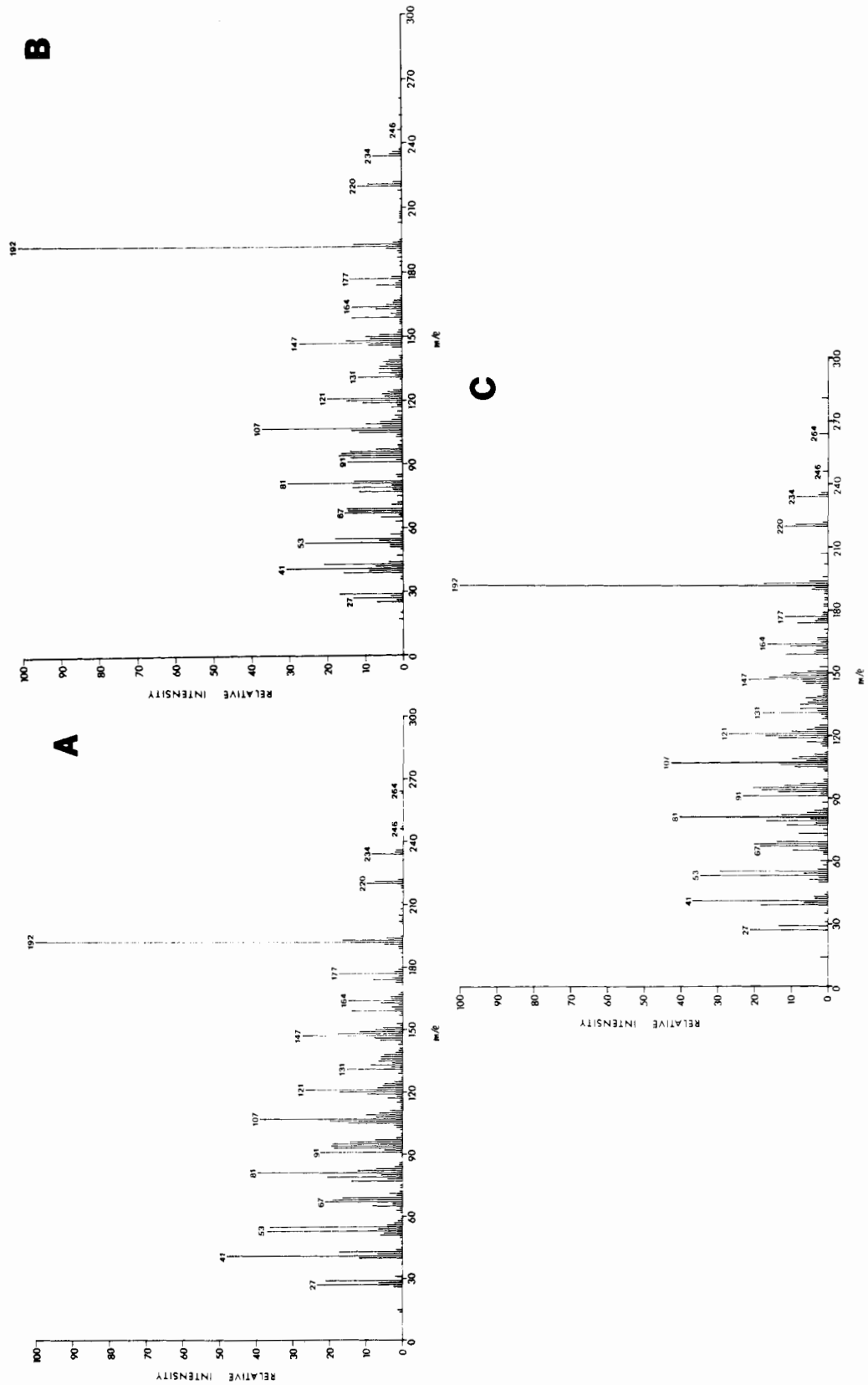

Figure 2. Mass spectra of (A) psilotropin, (B) compound eluted from the base-treated extract of *B. multiradiata*, (C) compound eluted from the base-treated extract of *H. hoopsii*.

the extract of these two plants that cochromatographed with hymenoxon on 3% OV-17 and on 3% QF-1 GLC columns (Table I). Treatment of the plant extracts with NaOH followed by acidification resulted in the disappearance of the peak corresponding to hymenoxon and the appearance of a new peak that cochromatographed with psilotropin (Table II). The basic conversion of hymenoxon to psilotropin has been reported by Kim et al. (1974, 1975). A manuscript describing the basic conversion of hymenoxon to psilotropin and greenein is in preparation.

For the identification of a mixture of hymenoxon, greenein, and psilotropin by retention time on GC, flavone

Table I. Retention Time of Hymenoxon and the Extracts of *B. multiradiata* and *H. hoopsii* on 3% OV-17 and 3% QF-1 GLC Columns

Sample	Retention time, min	
	3% OV-17	3% QF-1
Hymenoxon	8.3	15.5
B. multiradiata	8.4	15.4
B. multiradiata + hymenoxon	8.3	15.5
H. hoopsii	8.2	15.2
H. hoopsii + hymenoxon	8.3	15.5

Table II. Retention Time of Psilotropin and the Base-Treated Extracts of *B. multiradiata* and *H. hoopsii* on 3% OV-17 and 3% QF-1 GLC Columns

Sample	Retention time, min	
	3% OV-17	3% QF-1
Psilotropin	24.6	59.8
B. multiradiata	24.4	59.5
B. multiradiata + psilotropin	24.1	59.5
H. hoopsii	24.8	59.5
H. hoopsii + psilotropin	24.8	59.3

Table III. Retention Time of Hymenoxon, Greenein, Psilotropin, and Internal Standard (Flavone) on 3% OV-17

Sample	Retention time, min
Flavone	5.72
Hymenoxon	8.12
Greenein	15.16
Psilotropin	23.68

was selected as the internal standard. The retention time for a mixture of these compounds applied to a 0.125 in. × 4.8 ft glass column packed with 3% OV-17 on Gas-Chrom Q (100/120 mesh) with the column temperature at 205 °C is presented in Table III.

When the extracts from *B. multiradiata* and *H. hoopsii* were subjected to GC–MS analysis, a compound was eluted that gave a mass spectrum statistically identical with the mass spectrum of hymenoxon (Figure 1). Psilotropin was not detected in the original plant extracts. However, a compound with a mass spectrum statistically identical with psilotropin was detected in the base-treated plant extracts (Figure 2). The base-treated extracts did not contain hymenoxon.

The GC–MS of the extract from *B. multiradiata* also contained a compound that was chromatographically separated from hymenoxon and gave a mass spectrum (Figure 1) that was similar to hymenoxon but statistically different from hymenoxon. The compound was present in both the plant extract and the base-treated extract.

These data indicate that two other members of the composite family, *B. multiradiata* and *H. hoopsii*, contain the toxic sesquiterpene lactone hymenoxon. Other plants in this family may also contain hymenoxon since several plants have been reported to contain psilotropin (DeSilva and Geissman, 1970; Herz et al., 1970). Because of the ease with which hymenoxon is converted into psilotropin, hymenoxon may be present in these plants as a precursor of the dilactone. The methods described in this study should be useful in screening plants for hymenoxon and other sesquiterpene lactones.

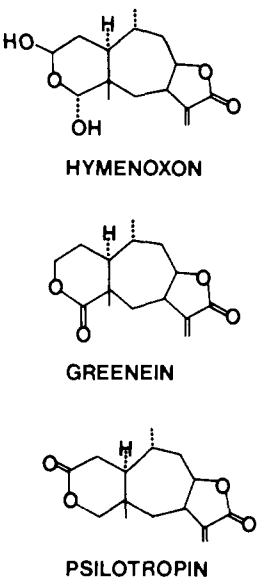

Figure 3. The stereochemical structure of three sesquiterpene lactones (hymenoxon, greenein, and psilotropin).

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