Isolation of Hymenovin from *Hymenoxys richardsonii* (Pingue) and *Dugaldia hoopesii* (Orange Sneezeweed)

Hymenovin, a sesquiterpene lactone previously reported as the major toxic constituent of *Hymenoxys odorata*, was isolated from extracts of *Hymenoxys richardsonii* and *Dugaldia hoopesii* and was shown to be the major constituent accounting for the toxicity of these plants as well. The finding that these three poisonous range plants contain an identical toxicant is consistent with prior observations of very similar toxicological responses in livestock poisoned with the whole plants.

Several species of the family Compositae, tribe Helenieae, are known to be poisonous when eaten by livestock. Three of these, *Hymenoxys odorata* DC. (western bitterweed), *Hymenoxys richardsonii* (Hook), Ckll. var. *floribunda* (pingue), and *Dugaldia hoopesii* (Gray) Rydb. (orange sneezeweed), cause heavy losses of sheep in several areas of the United States Southwest (Kingsbury, 1964). *H. odorata* is apparently most serious on overgrazed sheep ranges in Texas, whereas *H. richardsonii* has caused extensive losses of sheep in Colorado, New Mexico, and Arizona. *D. hoopesii*, also known as *Helenium hoopesii* (see Bierner, 1974, for recent taxonomic revision), is a major obstacle to sheep production on high altitude summer ranges in most of the Rocky Mountain states (Doran and Cassady, 1944; Kingsbury, 1964; Marsh et al., 1921).

Until recently, little was known concerning the nature of the poisonous principles of these plants, although Marsh et al. (1921) reported that the active constituent of *D. hoopesii* was a poorly defined glycoside to which the name dugaldin was given. Herz et al. (1970) identified several sesquiterpene lactones from *H. odorata* and *H. richardsonii* and speculated that these compounds might be responsible for the toxicity of the plants because related sesquiterpene lactones of plant origin are known to be biologically active (Kupchan, 1970). Recent studies have now shown that the major poisonous component of *H. odorata* is indeed a sesquiterpene lactone (Figure 1), which has been designated hymenovin by us (Ivie et al., 1975) and hymenoxon by others (Kim et al., 1975).

Because *H. richardsonii* and *D. hoopesii* are closely related to *H. odorata* taxonomically, it seemed likely that sesquiterpene lactones might account for the toxicity of these plants as well. This hypothesis was supported by the fact that toxicological symptoms and lesions in animals poisoned by the three plants are remarkably similar (Aanes, 1961; Marsh et al., 1921; Rowe et al., 1973).

MATERIALS AND METHODS

Mature *Hymenoxys richardsonii* var. *floribunda* and *Dugaldia hoopesii* were collected in early July, 1974, near Salina, Utah, at an elevation of approximately 8000 ft. The collection locality was within a few miles of the old Salina Experiment Station, which was established in 1915 by the U.S. Department of Agriculture primarily to study *D. hoopesii* poisoning in sheep. From this locality came Marsh's definitive study of the poisonous nature of this species (Marsh et al., 1921). The above-ground parts of the plants were air-dried for several days and then finely ground and stored in plastic bags. Both *H. richardsonii* and *D. hoopesii* retain their toxicity upon drying (Aanes, 1961; Marsh et al., 1921). Samples of *H. odorata* used in comparative studies were as previously described (Ivie et al., 1975).

Because we suspected that the poisonous principles of *H. richardsonii* and *D. hoopesii* might be related to hymenovin, the dried plant samples were extracted and fractionated in a manner similar to that used to isolate hymenovin from *H. odorata*. This procedure (Ivie et al., 1975) consisted of acetone extraction of the dried plant, precipitation of plant pigments, and, ultimately, analysis of the toxic extract by thin-layer chromatography (TLC). In the current studies, high-performance liquid chromatography (HPLC) was also used to analyze the plant fractions. Separations were made with 2.4 m of a 1-cm diameter column containing Porasil A, a normal phase silica packing (37–75 μm particle size). The instrument was a Waters Model ALC-401 liquid chromatograph with a refractive index detector. Sample injections in acetonitrile solution were made with a high-pressure septumless injector, and flow rate of the acetonitrile eluting solvent was maintained at 3.0 ml/min. Resolved components were collected as they eluted from the column for subsequent analysis or studies of their toxicity to hamsters (Ivie et al., 1975).

Table I. Toxicity of *Hymenoxys richardsonii* and *Dugaldia hoopesii* Fractions to Male Hamsters

Plant fraction[a]	Mortality at indicated treatment level (g equiv/hamster)[b] 2.5	5	10	20	40
Hymenoxys richardsonii					
1					
2			0/5	0/5	0/2
3[c]			0/5	3/5	2/2
4	0/5	4/5	5/5	5/5	
5			0/5	0/5	0/2
6			0/5	0/5	0/2
Dugaldia hoopesii					
1					
2[d]			0/2	0/2	0/2
3			0/2	0/2	0/2
4	0/5	1/5	5/5	3/3	
5			0/2	0/2	0/2
6			0/2	0/2	0/2

[a] Isolated by high-performance liquid chromatography or preparative thin-layer chromatography. Indicated fractions represent components as isolated by high-performance liquid chromatography (Figure 1). [b] Dosage in gram equivalents of the plant (dry weight) per 100-g hamster. Mortality figures indicate the number of animals killed out of the number treated. [c] This fraction contained appreciable hymenovin due to incomplete resolution from fraction 4 and this probably accounts for, at least in part, the observed toxicity of fraction 3. [d] Includes fraction 1 (see Figure 1).

RESULTS AND DISCUSSION

Examination of the extracts from *H. richardsonii* and *D. hoopesii* by TLC or HPLC indicated that each of the plants contained a component identical in chromatographic behavior with hymenovin from *H. odorata* (Figure 1). Subsequent toxicity studies with hamsters indicated that the suspected hymenovin (fraction 4, Figure 1) was the only toxic component in the *D. hoopesii* extract and

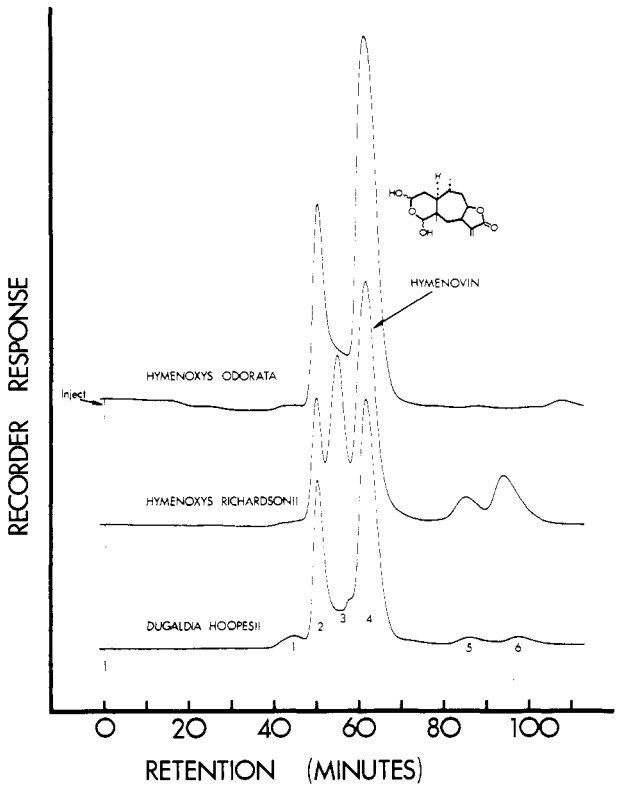

Figure 1.

was the major toxicant in the extract from *H. richardsonii* (Table I). Although fraction 3 from *H. richardsonii* exhibited some toxicity (Table I), this was likely due at least in part to hymenovin contamination as a result of incomplete HPLC resolution from the hymenovin fraction.

Hymenovin was subsequently isolated and recrystallized from both *H. richardsonii* and *D. hoopesii* by procedures reported for hymenovin isolation from *H. odorata* (Ivie et al., 1975). The yields from each plant averaged 0.5% by weight of the dry plant (corresponding yields of hymenovin from *H. odorata* were about 0.7%). Infrared, nuclear magnetic resonance, and mass spectral data obtained from each of the three hymenovin samples were identical. In addition, conversion of hymenovin from *H. richardsonii* and *D. hoopesii* to the known monoethylated derivative, hymenolide, and to bis(trimethylsilyl) ethers gave compounds that exhibited identical spectral data as reported previously for the same derivatives of hymenovin from *H. odorata* (Ivie et al., 1975).

The current studies thus indicate that hymenovin is the major if not the only toxic principle in *H. richardsonii* and *D. hoopesii*, as well as in *H. odorata*. It is not clear whether dugaldin, a toxic component of *D. hoopesii* reported by Marsh et al. (1921), contributes appreciably to the toxicity of *D. hoopesii*. Although dugaldin was reported to be a glycoside, the published data are inconclusive; thus, the chemical nature of dugaldin remains unclear. The reported isolation procedures and solubility characteristics of dugaldin are, however, consistent with the compound being similar to or identical with hymenovin.

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