Hymenovin. Major Toxic Constituent of Western Bitterweed (*Hymenoxys odorata* DC.)

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A toxic component of *Hymenoxys odorata* DC., an important livestock plant poison of the American Southwest, has been isolated and identified. This material, a sesquiterpene lactone which has been designated hymenovin, is a mixture of epimeric dihemiacetals and is lethal to sheep when given orally at levels as low as 100 mg/kg. Hymenovin probably exerts its toxic action, at least in part, by alkylating sulfhydryl groups of key enzymes and other body constituents. Because *H. odorata* and related livestock plant poisons exhibit similar toxicity syndromes, it is likely that the sesquiterpene lactone content of these plants is the major factor governing their toxicity.

Hymenoxys odorata DC. (Compositae), known as Western bitterweed, bitter rubberweed, or bitterweed, is an important livestock poison that primarily affects sheep. Bitterweed grows from Kansas south into Mexico and from central Texas west to California (Hardy et al., 1931). The plant is a significant obstacle to sheep production in some areas; as late as 1962, annual losses of sheep and goats to bitterweed poisoning in Texas were estimated at more than 3 million dollars (Jaggi, 1962). Bitterweed has been reported to cause occasional losses of cattle (Dollahite et al., 1973).

The nature of the toxic constituents of bitterweed is not known, although it has been suggested, without supportive data, that saponins (Couch, 1937) or sesquiterpene lactones (Herz et al., 1970) might be responsible. Isolation of a toxic, incompletely characterized lactone from *H. odorata* has recently been reported (Kim et al., 1974a). We now report the isolation of hymenovin (**2**, Figure 1), as the major toxic constituent of this important livestock poison.

MATERIALS AND METHODS

Extraction and Bioassay. *H. odorata* in full bloom was collected in early spring near Sonora, Tex., and the aerial parts were dried at 50°. Previous studies have shown that bitterweed retains full toxicity upon drying (Hardy et al., 1931), and thus the dried plant was used in all feeding and isolation studies. Attempts to isolate toxic constituents by solvent extraction of the dried plant and subsequent fractionation procedures were in all cases accompanied by animal bioassay studies to monitor the presence of toxic components. The animals used were female sheep (30–40 kg, mixed breed, taken from populations maintained at this laboratory), and male hamsters (100 g, Ela:Eng-Syr-Strain, Engle Laboratory, Farmersburg, Ind.). Hamsters were selected because of their appropriate size, and because they were susceptible to poisoning by bitterweed extract, exhibiting a toxicity syndrome similar to that observed in sheep. Ground bitterweed and certain of its fractions were administered orally to sheep in gelatin capsules via a balling gun; where water was an appropriate carrier, the animals were treated by stomach tube. Hamsters were treated with water solutions or suspensions via stomach tube to the lightly etherized animals. Toxicity studies were also conducted using male white mice (20 g, Camm Research Institute, Wayne, N.J.). Following all treatments, mortality was recorded 1 week later.

Studies with the dried bitterweed plant indicated that the poisonous principles were extractable into polar organic solvent. A 500-g portion of the finely ground bitterweed was extracted twice with 1000-ml portions of acetone by homogenization with a Willems Polytron Homogenizer (Brinkman Instruments, Westbury, N.Y.). The solvent was removed, and the residue was administered orally to a 40-kg ewe. The animal died within 48 hr while exhibiting typical symptoms of acute bitterweed poisoning (abdominal distress, CNS depression, labored breathing, loss of appetite). The extracted plant residue, when given to sheep at levels equivalent to 1 kg of dried bitterweed, was not poisonous.

The toxic acetone extracts of bitterweed were subjected to the following cleanup procedure. The extract from 1 kg of dried plant was taken to dryness, and the residue was dissolved in 400 ml of ethanol. This was diluted with 1 l. of water; then the ethanol was removed by concentrating the solution to about 800 ml at reduced pressure and at a temperature not exceeding 50°. The sample was subsequently cooled to 5° and centrifuged at 48,000*g* for 15 min. This procedure removed most of the plant pigments and gave a light amber liquid that was toxic to sheep and hamsters, whereas the precipitated pigment fraction was essentially nontoxic. The toxic water phase was subsequently concentrated to dryness at reduced pressure, and the crystalline residue subjected to preparative thin-layer chromatography (TLC).

Chromatography. Preparative TLC was accomplished using 20 × 20 cm glass plates coated with silica gel GF-254 (1.0 mm gel thickness, Merck, Darmstadt, Germany). Before use, the plates were activated by heating overnight at 100°. The toxic *H. odorata* fraction was dissolved in acetone for spotting, and the plates were developed in a solvent mixture containing chloroform–ether–acetone (3:3:1). The resolved components were visualized by acid charring or nondestructively by iodine vapors (Stahl, 1969). Hamster bioassay of the TLC resolved bitterweed components was conducted by scraping the appropriate gel regions from the plates, extracting the gel with acetone, and subsequently administering the compounds as water suspensions.

Analytical Procedures. Infrared (ir) spectra were recorded as 1% potassium bromide pellets on a Beckman IR-18A or Perkin-Elmer Model 257 spectrophotometer. Nuclear magnetic resonance (NMR) studies were conducted using a JEOL Model JNM-MH-100 or Bruker 90 MHz spectrometer using tetramethylsilane as an internal refer-

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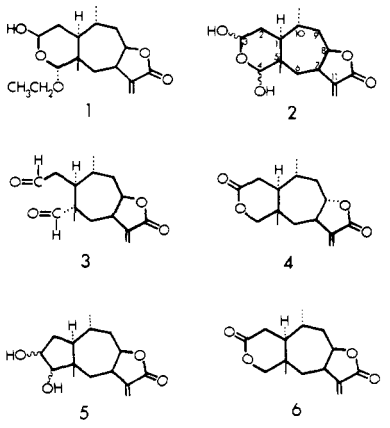


Figure 1. Structures of sesquiterpene lactones.

ence. Low resolution mass spectra (MS) were run on a Varian-Mat-CH-7 90° sector magnetic scan spectrometer, utilizing a direct insertion probe; high resolution MS studies were run on a MS-902 mass spectrometer.

Chemical Experimental. Hymenovin. A sample of crystalline material, mp 125–127°, exhibited NMR signals ($CDCl_3$) at 6.22 d (2) and 5.55 d (2, H-13, superimposed on slightly displaced signals of minor isomer), 5.2 c (H-3 of major and minor isomer), 4.71 s (H-4), 3.9 c (H-8), 3.5 c (H-7), 1.05 d br (C-10 methyl of major and minor isomer), 0.99 and 0.94 (C-5 methyl of major and minor isomer; DMSO-d_5), 6.26 br (OH), 6.01 d (2.5) and 5.51 d (2.5) superimposed on H-13 of minor isomer, 4.85 c (2 p, H-3 and H-8), 4.45 s (H-4), 0.99 d br and 0.88 s (C-10 and C-5 methyl, superimposed on signals of minor isomer).

Anal. Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85; mol wt, 282. Found: C, 63.81; H, 8.10; mol wt (MS), 282.

In the high resolution mass spectrum, the peak of highest mass number (390) was found at *m/e* 264.1387; calcd for $C_{15}H_{20}O_4$ ($M - H_2O$), 264.1361.

Hymenovin Bistrimethylsilyl Ether. A solution of 40 mg of hymenovin in 1.5 ml of pyridine was allowed to stand at room temperature for 3 hr with 0.5 ml each of trimethylchlorosilane and hexamethyldisilazane. Solvent and excess reagents were removed in vacuo; the residue was dissolved in $CDCl_3$ and filtered. The NMR spectrum was sharp except for the C-10 methyl signal whose presence suggested the presence of a mixture of epimers; signals at 6.18 d (2.5) and 5.48 d (2.5, H-13), 5.16 d of d (10, 2.5, H-3), 4.92 m (H-8), 4.8 s (H-4), 3.4 m (H-7), 1.08 d br (6, C-10 methyl, probably superimposed on corresponding signal of minor isomer), 1.00 s (C-5 methyl).

Diacetylhymenovin. A mixture of 50 mg of hymenovin, mp 125–127°, 2 ml of pyridine, and 2 ml of acetic anhydride was left at room temperature overnight. Decomposition with ice water followed by the usual work-up gave 62.5 mg of crude diacetate which was purified by TLC (silica gel $Pf_{254+366}$, benzene–ethyl acetate, 9:1); yield, 41 mg. The NMR spectrum indicated that the gummy product was a mixture of epimers; signals ($CDCl_3$) at 6.21 d and 5.38 d (2.5, H-13), 6.02 d of d (H-3), 5.70 s (H-4) with indications of the minor constituent's signal at 5.66), 4.8 c (H-8), 3.2 m (H-7), 2.18 s and 2.05 s (acetates, superimposed on two minor acetate singlets), 1.13 s (C-5 methyl), and 1.15 m (superimposed major and minor C-10 methyl doublets).

Anal. Calcd for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 61.87; H, 7.39.

Dibenzoylhymenovin. Benzoylation of 52 mg of hymenovin in 2 ml of pyridine with excess benzoyl chloride gave a gummy mixture of epimeric dibenzoates (NMR spectrum). Preparative TLC afforded a very low yield (6 mg) of a crystalline homogeneous dibenzoate: mp 221–223°; ir bands at 1740, 1725, 1660, 1600, and 1270 cm^{-1}; NMR signals at 6.42 d (10, 2.5, H-3), 6.09 s (H-4), 6.02 d (2.5), 5.06 d (2.5, H-13), 4.78 m (H-8), 3.31 m (H-7), 1.26 (C-5 Me), and 1.19 d (7, C-10 Me).

Anal. Calcd for $C_{29}H_{30}O_7$: C, 71.01; H, 6.16. Found: C, 70.81; H, 6.23.

Diethylhymenovin. A solution of 0.2 g of hymenovin, in 5 ml of ethanol and 1 ml of concentrated HCl, was allowed to stand at room temperature for 2 hr, diluted with water, and extracted with $CHCl_3$. The washed and dried organic solvent was evaporated and the residue (214 mg) purified by TLC over silica gel (hexane–ethyl acetate, 7:3). The NMR spectrum of the gummy product indicated that it was homogeneous; signals ($CDCl_3$) at 6.22 d and 5.50 d (2, H-3), 4.80 d of d (H-3, superimposed on H-8 multiplet), 4.27 s (H-4), 3.7 c (two CH_3CH_2O quartets partially superimposed on H-7 multiplet), 1.25 t, 1.23 t (7, two CH_3CH_2), 1.10 d (6, C-10 methyl), 1.04 s (C-5 methyl).

Anal. Calcd for $C_{19}H_{30}O_5$: C, 67.43; H, 8.93; O, 23.64; mol wt, 338. Found: C, 67.14; H, 8.74; O, 23.67; mol wt (MS), 338.

Other significant peaks in the low resolution mass spectrum were found at *m/e* 293 ($M^+ - C_2H_5O$) and *m/e* 264 ($M^+ - 2C_2H_5O$).

Ethylation of hymenolide in the same manner gave gummy material indistinguishable (TLC, ir, NMR, MS) from the above.

Conversion of Hymenovin to Hymenolide. A solution of 0.1 g of hymenovin in 2 ml of dioxane, 4 drops of ethanol, and 4 drops of concentrated HCl was allowed to stand at room temperature, the reaction being monitored by TLC. After 15 min, the mixture was diluted with water and extracted with $CHCl_3$. The washed and dried extract was evaporated; preparative TLC of the residue afforded 27 mg of crystalline material, mp 115–116°, identical in all respects (ir, NMR, mixture melting point) with authentic hymenolide.

Antitumor Studies. C3H strain mice, having high susceptibility to C3H mouse mammary carcinoma, were obtained from Texas Inbred Mice Co., Houston, Tex. Mature mice which had spontaneously developed tumors were used as a source of tumor cells. The tumors were harvested from freshly sacrificed animals, and a 20% (w:v) suspension of cells was prepared by homogenizing the tumor in Eagle's basal medium (Grand Island Biological Co., Buffalo, N.Y.) at low speed using a Polytron homogenizer. Juvenile mice (15–20 g) were inoculated subcutaneously with 0.25 ml of the cell suspension, which was equivalent to 50 mg of tumor.

Two experiments were conducted to evaluate the antitumor properties of hymenovin. In the first study, 10 mice were inoculated with 50 mg of tumor; then 7 days later, half of the animals were treated with hymenovin intraperitoneally at a level equivalent to 10 mg/kg per treatment. The hymenovin injections (in 0.25 ml of sterile water) were repeated at 48-hr intervals and were continued for 3 weeks (11 injections). The five control mice received injections of sterile water only. At the end of the treatment period, the animals were sacrificed and tumor weight:body weight comparisons were made. In the second experiment 30 mice were inoculated with tumor cells as above and then 15 immediately began receiving hymenovin injections (12.5 mg/kg) at 48-hr intervals. The 15 control animals were treated with sterile water at 48-hr intervals. Because hymenovin caused some mortality after seven treatments, the study was terminated at that point and tumor weights determined.

Hymenovin Antagonists. Cysteine was studied as a potential antidote for acute hymenovin poisoning in hamsters. L-Cysteine hydrochloride (Aldrich, Milwaukee, Wis.) was administered as a water solution to hamsters (75 mg/animal) which had received a minimum LD_{100} dose of hy-

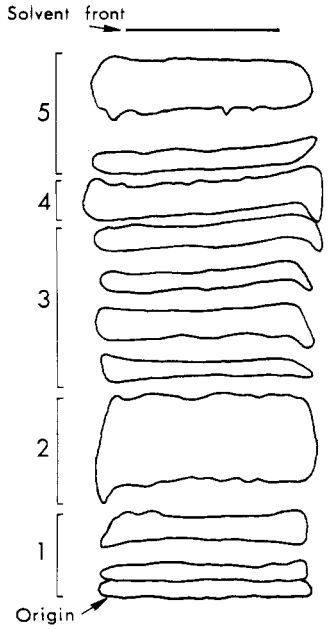


Figure 2. TLC resolution of components of a toxic extract from *Hymenoxys odorata* DC. Brackets indicate fractions which were subsequently assayed for toxicity to hamsters (Table I).

menovin just prior to the cysteine treatment. The animals were subsequently observed for toxicity symptoms and mortality.

Plant Part Analysis. Studies were made to determine the distribution of hymenovin in roots, leaves, flowers, and stems of *H. odorata*. A 100-g sample of the dried plant was separated into its respective parts, and these were extracted by the usual procedure. A semiquantitative determination of the hymenovin content of the plant organs was then made by TLC comparisons of the color intensity of known quantities of hymenovin with that in the plant extracts.

RESULTS

Isolation of Hymenovin. TLC analysis of the poisonous *H. odorata* fraction revealed the presence of at least 11 separate components (Figure 2). Because it was expected that many of these would not show appreciable biological activity, the TLC plates were conveniently divided into five gel regions for hamster bioassay (Figure 2). Extraction of the compounds from the gel and hamster treatment revealed that toxicity was associated almost entirely with gel region 2 (Table I). This component (R_f 0.30, Figure 2) was the major constituent of the toxic *H. odorata* fraction, and appeared to consist of a single compound. It was toxic to hamsters at levels equivalent to 5.0 g of the original dried *H. odorata*, while the remaining fractions showed little or no toxicity when tested at 10 times this level (Table I). Because it was apparent that fractions 1, 3, 4, and 5 contributed little to the toxicity of *H. odorata*, all subsequent efforts were directed toward isolation, characterization, and biological activity studies of the constituents of fraction 2.

The toxic component, which was designated hymenovin, was isolated in gram quantities by preparative TLC. The material crystallized with difficulty, but efforts at crystallization from ether–hexane were eventually successful. However, the melting point of hymenovin thus obtained varied among different preparations in the range of 115–137°; ir and NMR spectra of the various preparations did not differ significantly except as noted below. The average yield of hymenovin was approximately 0.7% by weight of the dry plant.

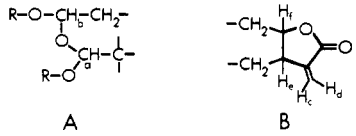
Formula 2, a mixture of C-2 or C-4 epimers, was deduced as the structure of hymenovin in the following manner. The

Table I. Toxicity of *Hymenoxys odorata* Fractions to Male Hamsters

	Mortality at indicated dose[b]				
Fraction[a]	2.5	5	7.5	15	50
1					0/3
2	0/4	2/4	3/3	3/3	
3					1/3
4					0/3
5					0/3

[a] Isolated by preparative thin-layer chromatography (Figure 1). [b] Dosage in gram equivalents *H. odorata* (dry weight) per 100 g of hamster. Mortality figures indicate the number of animals killed out of the number treated.

ir spectrum indicated the presence of hydroxyl (3600 cm^{-1}) and α,β-unsaturated γ-lactone (1755 and 1660 cm^{-1}) moieties. Mass spectroscopy and elemental analysis indicated a molecular formula of $C_{15}H_{22}O_5$. High resolution mass spectrometry did not permit observation of the molecular ion, but gave $C_{15}H_{20}O_4$ (M − H_2O) as the composition of the peak of highest mass number. Formation of a bistrimethylsilyl ether established the presence of two hydroxyl groups and permitted a more detailed analysis of the NMR spectrum which in conjunction with the empirical formula indicated the presence of partial structures A and B.



Partial structure B is characteristic of many naturally occurring α,β-unsaturated sesquiterpene lactones, H_c and H_d appearing as doublets centered at 6.18 and 5.48 ppm, respectively, each spin-coupled (verified by double resonance experiments) to H_e (broad signal centered at 3.4 ppm) which was in turn coupled to H_f (multiplet at 4.75 ppm).

Partial formula A was corroborated by formation of a gummy diacetate, dibenzoate, and diethyl acetal; although the two esters (R = Ac and Bz) were homogeneous on TLC, their NMR spectra which exhibited the expected chemical shifts for H_a and H_b suggested the presence in varying proportions (depending on the melting point of the starting material) of two closely related substances apparently epimeric at carbon a or b. Similarly, the NMR spectra of hymenovin itself suggested that the presence of somewhat different proportions of two epimers might be responsible for the variable melting point. One of the dibenzoates (R = Bz) was isolated in pure form, albeit in very poor yield, and the diethyl acetal (R = Et) appeared to be homogeneous, possibly as the result of equilibration under the acid conditions leading to its preparation.

Short-term exposure of hymenovin to ethanolic hydrogen chloride, elution of the monoethylated fraction, and rechromatography eventually permitted the isolation of crystalline material identical in all respects with hymenolide (**1**, Figure 1), a sesquiterpene lactone previously isolated from collections of *H. odorata* (Herz et al., 1970). Similarly, hymenovin diethyl acetal (R = Et) was indistinguishable (TLC, ir, NMR) from the monoethyl ether of hymenolide. Consequently, hymenovin can be formulated as **2**, an epimeric mixture of hydrated dialdehyde **3** (Figure 1), and is an obvious biological oxidation product of the sesquiterpene lactone hymenoratin (**5**), which has been isolated (Ortega et al., 1968; Romo et al., 1969) from a San Luis Potosi,

Table II. Antitumor Properties of Hymenovin Against C3H Mammary Carcinoma in Living Mice Inoculated with a Suspension of Tumor Cells

Parameter	Hymenovin treatment level, mg/kg per dose			
	10.0		12.5	
	Control	Treated	Control	Treated
Waiting period, days[a]	7	7	0	0
Number of treatments[b]	11[c]	11	7[c]	7
Mortality, %	0	0	0	20
% body wt as tumor	7.7	3.1	4.6	3.2
% tumor reduction		59.7		30.4

[a] Days between inoculation with tumor and first hymenovin treatment. [b] Treatments at 48-hr intervals. Animals sacrificed 48 hr after final treatment. [c] Control animals treated with sterile water only.

Mexico collection of *H. odorata*. (Since the hymenolide isolated by Herz et al. (1970) constituted only a small portion of the total extract of *H. odorata* and since the extraction procedure included a step utilizing ethyl alcohol, it is quite possible that hymenolide was an artifact.) At the present time we have no definitive evidence for deciding whether the two hymenovin components are epimeric at C-2 or C-4.

Distribution of Hymenovin in the Plant. TLC analysis of extracts from roots, leaves, flowers, and stems of *H. odorata* indicated that hymenovin is concentrated primarily in the leaves and flower heads of the growing plant. Roots contained only about 0.05% hymenovin (dry weight basis) and stems 0.2% while the leaves and flowers consisted of about 1.1–1.3% hymenovin.

Toxicology. Hymenovin is toxic to hamsters and white mice; the acute oral LD_{50} values are 250 mg/kg for the former and 150 mg/kg for the latter. The substance is more toxic to sheep. Two animals dosed orally with hymenovin at 200 and 150 mg/kg died within 24 hr, two treated at 100 mg/kg died between 48 and 96 hr later, while a single sheep treated with hymenovin at 50 mg/kg showed no visible signs of poisoning. Necropsy of hymenovin poisoned sheep revealed severe congestion and small areas of eroded mucosal surface on the rumen, reticulum, and abomasum. Microscopically, there was congestion, hemorrhage, and edema of the forestomachs, abomasum, and cecum. Severe toxic nephrosis with heavy proteinaceous casts and tubular degeneration was noted, primarily at the cortico-medullary junction. Toxic hepatosis with occasional areas of paracentral necrosis was also observed. These findings were consistent with those observed following *H. odorata* poisoning with the whole plant (Witzel, 1975).

Cysteine is an effective antagonist to the toxic effects of hymenovin. When hymenovin treatment was followed immediately by cysteine, hamsters survived normally lethal hymenovin doses, and exhibited no significant toxicity symptoms.

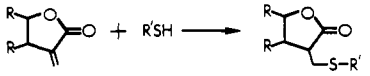
Cytotoxicity of Hymenovin. Intraperitoneal administration of hymenovin to tumor inoculated mice caused an inhibition of tumor growth (Table II). Animals receiving 11 doses of hymenovin at 10 mg/kg each exhibited tumors which averaged 60% less than those of control mice, when compared by tumor weight:body weight ratios. These treatments were apparently just below the level of acute toxicity, however, as mice receiving only 7 injections of hymenovin at 12.5 mg/kg experienced 20% mortality after the last treatment. At that time, tumor weights of treated animals were 30% lower than those of controls.

DISCUSSION

The current studies indicate that hymenovin is the major toxic constituent of *H. odorata*, at least in the samples studied here. However, other sesquiterpene lactones have been isolated from *H. odorata* (Herz et al., 1970; Ortega et al., 1968; Romo et al., 1969), and it is possible that these may contribute to the toxicity of this plant.

The finding that hymenovin is quite toxic to sheep has implications relative to the possible toxic agents in other livestock poisons of the family Compositae (Kingsbury, 1964). Because some of these elicit symptoms very similar to those of Western bitterweed, it seems likely that sesquiterpene lactones or their precursors also account for their toxicity. Rimington and coworkers (1936) reported that "vomiting disease" among sheep grazing on South Africa *Geigeria* species was caused by "vermeeric acid", a poorly defined compound which gradually transformed to another substance, vermeerin, on standing. Although vermeeric acid has not been isolated since Rimington's work, vermeerin has been studied extensively and was characterized as the sesquiterpene dilactone 4 (Figure 1) (Anderson et al., 1967; Herz et al., 1970). Vermeerin has been found (Herz et al., 1970) in *Hymenoxys richardsonii* (Hook.) Cockl., another stock poison of the American Southwest, which suggests that vermeerin or its precursors may contribute to the toxicity of *H. richardsonii* as well. Although Rimington implies that vermeeric acid, not vermeerin, is primarily responsible for the toxicity of *Geigeria spp.*, the nature of vermeeric acid remains obscure. The reported chemical properties are not compatible with the earlier deduction that it is simply the dicarboxylic acid of vermeerin, now that the structure of vermeerin is known. Based on the current studies, it seems possible that vermeeric acid may be a hymenovin isomer which is related to vermeerin as hymenovin is related to floribundin (**6**, Figure 1). Floribundin is known to occur in *H. richardsonii*, but has not been reported from *H. odorata* (Herz et al., 1970).

Previous studies have shown that some sesquiterpene lactones exhibit cytotoxic activity and are potential antitumor agents, and that the presence of an O=CC=CH_2 system is essential for significant antitumor activity (Hanson et al., 1970; Kupchan, 1970; Kupchan et al., 1970, 1971). Hymenovin, which contains this system, was shown to be an in vivo inhibitor of C3H mouse mammary carcinoma. The biological mechanism of poisoning by *H. odorata*, and probably by some related poisonous plants, can thus be considered in relation to the known cytotoxic properties of the sesquiterpene lactones. Current evidence supports the hypothesis that the cytotoxic activity of these compounds is due at least partly to their ability to alkylate sulfhydryl groups of key enzymes (Cavallito and Haskell, 1945; Hanson et al., 1970; Kupchan, 1970; Kupchan et al., 1970, 1971). The fact that the mercaptan cysteine is antidotal to hymenovin poisoning in hamsters suggests a similar mode of action for hymenovin. The alkylation reaction probably occurs through the exocyclic methylene as has been shown for similar compounds (Kupchan et al., 1970).



Mercaptans may be useful antidotes for poisoning by bitterweed and related plants. Although these compounds may inactivate the toxin within the digestive tract or bloodstream, they cannot be expected to reverse the biochemical lesion if alkylation is indeed a major mechanism of action. A brief report by Kim and coworkers (1974b), which appeared after completion of the studies reported here, also demonstrated the potential antidotal properties of mercaptans toward bitterweed poisoning. An additional consideration in livestock poisoning by bitterweed and related plants is the possibility that the sesquiterpene lac-

tone toxicants may alter the microbial composition of the rumen and thus affect its vital metabolic functions. Some sesquiterpene lactones from Compositae are potent antimicrobial agents (Vichkanova et al., 1971), and studies in these laboratories have shown that bitterweed extracts are highly toxic and possibly mutagenic to some Gram-positive bacteria (Norman, 1975). It therefore seems likely that rumen dysfunction may contribute to bitterweed toxicity.

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Toxicity and Milk Bittering Properties of Tenulin, the Major Sesquiterpene Lactone Constituent of *Helenium amarum* (Bitter Sneezeweed)

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Constituents of *Helenium amarum* (Rafin.) H. Rock. were isolated by high performance liquid chromatography and were studied for toxicity in hamsters and sheep. Tenulin, a sesquiterpene lactone that is known to account for the bitterness of this plant, was by far the major poisonous component observed. Although tenulin is of a low order of toxicity, it occurs in *H. amarum* to the extent of about 3% of the dried above ground plant material, and it is probably the primary toxicant involved in livestock poisoning by *H. amarum*. Oral administration of tenulin to a lactating cow resulted in bitter milk, and chromatographic and mass spectral studies indicated that unmetabolized tenulin was secreted into the milk. However, of the total tenulin given the cow, not more than 0.1% appeared in the milk. These studies suggest that human consumption of bitterweed milk resulting from dairy animals grazing on *H. amarum* will not constitute a significant health hazard.

Helenium amarum (Rafin.) H. Rock. (Figure 1), also known as *H. tenuifolium* Nutt. and commonly called bitter sneezeweed, bitterweed, sneezeweed, or yellow dog fennel, is widely distributed in the eastern and southern United States. The plant is of economic importance to the dairy industry because when eaten by cattle, it imparts a bitter taste to the milk and renders it unpalatable. *H. amarum* is also a livestock poison (Kingsbury, 1964) but seems to affect horses and mules to a greater extent than other species (Dollahite et al., 1972; West and Emmel, 1952). Laboratory studies with sheep have shown that fresh *H. amarum* causes death when force fed at 2% of body weight per day for 2 days (Dollahite et al., 1972).

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The major bitter principle of *H. amarum* was apparently first isolated by MacDonald and Glaser (1929) who obtained a crystalline, very bitter, unidentified substance from both fresh and dried plant samples. This bitter compound, when present in milk at levels as low as 1 ppm, caused sufficient bitterness to make the milk unsalable (Herzer, 1942). The chemistry of the bitter principle of *H. amarum* was studied by Buehler et al. (1937) and in greater detail by Clark (1939), who named the compound tenulin. Tenulin has subsequently been shown to be an epimeric mixture of the sesquiterpene lactone derivative I (Herz, 1975; Herz et al., 1962, 1963; Rogers and Haque, 1963). When treated with weak base, tenulin is isomerized to isotenulin (II), which itself may be a minor constituent of *H. amarum* (Ungnade and Hendley, 1948).

The possibility that tenulin may contribute to the toxicity of *H. amarum* was suggested by recent studies in which the major toxic principle of *Hymenoxys odorata* DC.