0.01 ppm was obtained for all three flow rates tested (Table I). High concentrations of 3000 ppm could also be analyzed by using a 10-μL sample size at 100× attenuation.

When ethylene oxide was analyzed, a 1-mL sample of 0.04 ppm gave a full-scale deflection on the recorder. Higher concentrations could be determined with smaller samples and by attenuation of up to 100 times. The retention time for ethylene oxide was 2.2 min at 15 cm^3/min carrier gas.

Analysis of the higher boiling fumigants ethylene dibromide, carbon tetrachloride, and 1,1,1-trichloroethane proved to be more difficult under the conditions tested here. The retention time of these materials on the Carbopak BHT column was more than 1 h, indicating the need for selection of more suitable column packings for workable retention times. Further investigation was done with the fumigant ethylene dibromide; however, as sorption factors and the establishment of precise standards were found to be somewhat complex, these results are being published separately.

The instrument performed satisfactorily for all three compounds (phosphine, methyl bromide, and ethylene oxide), even at very low concentrations. Fluctuations of the surrounding air temperature had no observable adverse effects on the performance of the instrument. The only problem that arose concerned interfering substances in the atmosphere. These contaminants had no effect on accuracy or reliability, but they did influence the number of samples that could be taken in any given period of time. According to the manufacturer a simple modification will be made in the instrument to overcome this problem. With such modification we expect that this instrument would be quite satisfactory for the detection and analysis of both high and low levels of phosphine and several other fumigants in commercial situations.

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Received for review December 28, 1982. Revised manuscript received May 18, 1982. Accepted June 2, 1982.

Helenin Inhibition of Liver Microsomal Enzymes

When helenin in the presence of NADPH was incubated with male rate liver microsomes prepared from the phenobarbital-pretreated animals, there occurred a significant loss of the microsomal cytochrome P-450 as well as the activity of benzphetamine *N*-demethylase. However, no inhibition of the hemoprotein was noted in the microsomal incubations with helenin minus NADPH, suggesting that the metabolic product(s) of the sesquiterpene lactone may have been responsible for the inhibition of the microsomal enzyme activities.

Several species of the *Helenium* (sneezeweed) and *Hymenoxys* (bitterweed, rubberweed) are well-known poisonous plants that are sometimes responsible for heavy losses of livestock (U.S. Department of Agriculture, 1968). A number of structurally related sesquiterpene lactones such as helenalin and hymenoxon have been isolated from these plants, and their toxicity in test animals has been confirmed (Kim, 1980). Although some of these lactones have been found to be useful in medicine as anthelmintic, bactericidal, fungicidal, and antitumor agents (Dalvi et al., 1971; Kim, 1980; Kupchan et al., 1971; Lee et al., 1971, 1977), they are highly toxic compounds, and the plants containing them may cause severe losses of food-producing animals, especially sheep and cattle (Kingsbury, 1964). Furthermore, a species of helenium has been reported to have caused poisoning of human beings who consumed bread made with flour that was contaminated with large quantities of seeds of the plant (Kingsbury, 1964). It is also interesting to note that consumption of even a small quantity of bitter sneezeweed by lactating cows makes the milk very bitter in taste and virtually unpalatable (Radeleff, 1970). Thus, in view of the economic importance of these plants in food and agriculture and since these lactones are toxic to liver and information with regard to their effect on hepatic microsomal enzymes is lacking, the following report on the toxicity of helenin as a representative of sesquiterpene lactones is presented.

EXPERIMENTAL SECTION

Chemicals. Helenin was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were analytical or reagent grade.

Isolation and Incubation of Liver Microsomes. Male Sprague-Dawley rats weighing 150–200 g were given a daily ip dose of 50 mg/kg phenobarbital for 4 successive days to induce microsomal enzymes. Twenty-four hours after the last injection, the animals were sacrificed and liver microsomes isolated following the established procedure (Dalvi and Howell, 1977). These microsomes containing cytochrome P-450 as the terminal oxidase were resuspended in 0.05 M Hepes buffer, pH 7.8, and the suspensions were incubated with helenin dissolved in methanol (1 mM final concentration) in the presence or absence of the NADPH-generating system (Dalvi and Howell, 1977). At the end of the 15-min incubation period, reactions were stopped by placing the incubation flasks in ice-cold water. The contents of the flasks were transferred to corresponding centrifuge tubes and centrifuged, and the microsomal pellets after washing twice with the buffer were resuspended in a known amount of 0.1 M phosphate buffer, pH 7.4. An aliquot from each of the resuspended microsomal suspensions was removed to determine the

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Table I. In Vitro Effect of Helenin in the Presence and Absence of NADPH on the Activity of Rat Liver Microsomal Enzymes

incubation conditions		benzphetamine N-demethylase act.,[a] nmol min^{-1} (mg of protein)$^{-1}$	cytochrome P-450,[a] nmol (mg of protein)$^{-1}$
helenin	NADPH		
–	–	15.9 ± 0.16	2.4 ± 0.01
–	+	12.7 ± 0.85	1.7 ± 0.07
+	–	4.6 ± 0.02[b]	2.1 ± 0.09
+	+	4.1 ± 0.07[b]	0.9 ± 0.05[b]

[a] Values represent means ± SD of three replicates.
[b] Significantly different ($P < 0.05$) from the corresponding control.

Table II. Effect of Higher Concentrations of Benzphetamine on the Reversal of Helenin-Induced Inhibition of Microsomal Benzphetamine N-Demethylase Activity

incubation conditions		benzphetamine N-demethylase act., % of control
helenin[a]	benzphetamine, mM	
–	5	100
+	5	22
+	10	24
+	20	8

[a] The concentration of helenin in the appropriate incubations was 1 mM.

cytochrome P-450 level and the remainder used to determine benzphetamine *N*-demethylase activity as described previously (Dalvi and Howell, 1977). Microsomal protein was determined by the biuret method modified to include deoxycholate in the samples (Dalvi et al., 1975).

Statistical Analysis. The data were analyzed by using the standard *t* test. Significance of treatment mean differences was based on a *P* value of 0.05.

RESULTS AND DISCUSSION

For determination of the nature of its effect on the hepatic mixed-function oxidase enzyme system, the sesquiterpene lactone was preincubated with rat liver microsomes with the incubation conditions shown in Table I. After the preincubation, the microsomes were sedimented and resuspended in phosphate buffer, and their ability to catalyze the demethylation of benzphetamine (5 mM) was examined. In addition, the cytochrome P-450 level in the resuspended microsomes was also determined. An examination of these data (Table I) indicates that there was some loss of cytochrome P-450 and the activity of benzphetamine *N*-demethylase in the microsomes preincubated with NADPH alone as compared to those preincubated in the absence of both NADPH and helenin. Such decrease in the microsomal incubations containing NADPH is not unusual and is attributed to NADPH (Dalvi and Peeples, 1978). However, there was a profound loss of the amount of cytochrome P-450 and the activity of benzphetamine *N*-demethylase in the microsomal incubations containing NADPH, a cofactor required for the activity of microsomal enzymes, and helenin, suggesting that the observed inhibition of the microsomal enzymes might have been caused by the helenin metabolite(s). The results showing that helenin in the absence of NADPH did not affect cytochrome P-450 support this contention.

The apparent decreased rate of benzphetamine metabolism in the microsomes preincubated with helenin minus NADPH may be attributed to the incomplete removal of helenin from the preincubated microsomes that were later centrifuged, resuspended in buffer, and incubated with benzphetamine and the NADPH-generating system. This inhibition of benzphetamine metabolism could be attributed either to residual helenin acting as a competitive inhibitor of benzphetamine (Norman et al., 1974) or to the metabolite(s) of helenin being strongly bound to the active site of the microsomal enzymes, thus effecting their inactivation. The former possibility was studied by incubating excess amounts of benzphetamine in the microsomal incubations containing helenin (1 mM), and it was found that the higher concentrations of benzphetamine failed to overcome the helenin-induced enzyme inhibition (Table II). Therefore, it appears that helenin per se may not be the inhibitor of microsomal benzphetamine *N*-demethylase activity. On the other hand, the results suggest that the inactivation of the drug-metabolizing enzyme system could have occurred through the helenin metabolism by hepatic microsomal enzymes.

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Received for review February 26, 1982. Revised manuscript received May 26, 1982. Accepted June 2, 1982.