produce edible cheese due to bitterness. Our finding of a renin-like activity in safflower seed is important because it makes sweet cheese and is present in the defatted meal of the seeds which are available in high quantities as a byproduct of oil production. Since the defatted meal is already in use for food fortification, this enzyme can be used in cheesemaking without being considered a potential threat to human health.

LITERATURE CITED

Green, M. L., *J. Dairy Res.* **39**, 261 (1972).
Iwasaki, J. Yu., Tamur, G., Arima, K., *Annu. Meet. Agric. Chem. Soc. Jpn.* **203** (1968).
Kosikowski, F., "Cheese and Fermented Milk Food", 2nd ed, Edward Brothers, Ann Arbor, Mich., 1977, p 419.
Thomasow, J., *Milchwissenschaft* **26**, 276 (1971).
Webb, B. H., Johnson, A. H., Alford, J. A., "Fundamentals of Dairy Chemistry", Avi Publishing Co., Westport, Conn., (1974.

Badri Tavasolian*
Forogh Shabbak

Department of Nutrition
School of Pharmacy
University of Tehran
Tehran, Iran

Received for review February 8, 1978. Accepted August 22, 1978. This work was supported through the University of Tehran research funds.

Determination of the Herbicide Dinoseb in Fababeans

A analytical method for determining residues of the herbicide dinoseb (2-*sec*-butyl-4,6-dinitrophenol) in fababean (*Vicia faba* L.) green tissue and mature beans has been developed using electron-capture gas chromatographic detection. The fababeans were treated with preemergence applications of dinoseb amine at 4, 6, and 8 kg/ha. The maximum dinoseb residue found in the mature beans was 8 ppb, the limit of detection being 2 ppb based on a 1-g fresh-weight equivalent. Recoveries were in the order of 60% at the 50 ppb fortification level.

Fababeans (*Vicia faba* L.) are grown on a limited hectarage in Canada (less than 10 000 ha in 1977; Longmuir, 1978). Because of its high protein content, much of the fababean crop is harvested as silage (Winia, 1977). Dinoseb (2-*sec*-butyl-4,6-dinitrophenol)amine is presently registered in Canada for selective weed control in fababeans as a postemergence treatment. It has also shown potential for weed control in fababeans when used as a preemergence treatment, both in Prince Edward Island (Ivany and Sanderson, 1974; Ivany, 1975, 1976) and in Ontario (Anderson, 1975). However, residue data for this treatment are not available for registration purposes.

The present paper describes a highly sensitive method of analysis for the determination of dinoseb residues in fababeans. The method, based on that of McKellar (1970) for the determination of dinoseb residues in alfalfa, utilizes electron-capture gas chromatographic detection of dinoseb either as the free phenol or as the methyl ether. The method was used to determine dinoseb residues in both green tissue and mature beans after preemergence applications of dinoseb amine.

MATERIALS AND METHODS

Herbicide Treatments. Fababeans, variety Diana, were seeded on May 19, 1976, in 1.25 m × 6.0 m plots at the Agriculture Canada Research Station, Charlottetown, Prince Edward Island. Preemergence treatments of 4, 6, and 8 kg of dinoseb amine/ha were applied to the soil surface on May 26 using a hand-held small plot sprayer. The same treatments were similarly applied on May 27 to 12.6 m^2 plots located at the Nova Scotia Agricultural College, Truro, Nova Scotia, which had been seeded the previous day to fababeans (variety Acupearl). Each treatment at both locations was replicated four times and the herbicide was not incorporated into the soil.

Sampling. Green foliage samples were harvested at both locations on August 20 with additional sampling at Charlottetown on September 8. Ten plants, removed at random from each replicate, were chopped and thoroughly mixed, and the sample for residue analysis (approximately 0.25 kg) was taken. Mature beans were harvested on October 25 at Charlottetown. Composite samples (approximately 0.75 kg) for each treatment, made up of subsamples taken from the whole plot yield of each replicate, were taken for residue analysis. At Truro the mature beans, harvested on October 26, were picked at random from each replicate until the sample size (approximately 0.5 kg) was obtained.

Green foliage and mature bean samples from both locations were frozen in sealed polyethylene bags immediately after harvest, shipped to Regina in dry ice, and upon arrival, stored in a freezer at −10 °C until extraction.

Chemicals. All solvents were pesticide grade (Caledon Laboratories Ltd., Georgetown, Ontario, Canada). All steps involving benzene, which is very toxic to bone marrow, were carried out in or in front of a well-ventilated fume hood. The analytical grade dinoseb and dinoseb methyl ether were supplied by Dow Chemical of Canada, Ltd., Sarnia, Ontario, Canada.

The NaOH solution (pH 11) was prepared using a pH meter.

Sample Extraction. (a) *Beans.* Ten grams of mature fababeans (milled through a 1-mm screen), 50 mL of methanol, and 10 mL of 1.2 N H_2SO_4 were mechanically stirred in a 125-mL Erlenmeyer flask for 30 min at 70 °C (wax bath). The sample was cooled with stirring in an ice bath, filtered through a fritted glass Büchner funnel (porosity number 3), and washed twice with 20 mL of methanol, and the combined filtrates were taken to volume (100 mL) with methanol. Ten milliliters of the combined filtrates (equivalent to 1 g of plant tissue) and 10 mL of 2.5% NaCl solution were combined, extracted with 10 mL of benzene in a 60-mL separatory funnel, and centrifuged at 3000 rpm for 2 min, and the benzene layer was recovered

0021-8561/79/1427-0191$01.00/0

with a disposable pipet. The extraction was repeated as above with another 10 mL of benzene, and the benzene extracts were combined.

(b) *Green Samples.* Twenty-five grams of green plant tissue was cut into 1.3 cm lengths and blended in a 1-L stainless steel blender jar with 150 mL of methanol and 5 mL of 6 N H_2SO_4 at high speed for 2 min. The plant material was transferred to a 250-mL Erlenmeyer flask, including a 5-mL methanol rinse of the blender jar, and heated with stirring, cooled and filtered as described above. The plant material was washed three times with 20 mL of methanol, and the combined filtrates were taken to volume (200 mL) with methanol. Eight milliliters of the combined filtrates (equivalent to 1 g of plant tissue) and 8 mL of 2.5% NaCl solution were combined and extracted twice with benzene as described above.

Basic Alumina Cleanup. Basic alumina (1 mL; heated at 600 °C for 24 h and stored at 130 °C, Fisher Scientific Co., Ltd.) was added to 5 mL of benzene in a 7 mm i.d. × 200 mm pressure column (Kontes Glass Co., Vineland, N.J.; chromaflex pressure column, K 420520, size 22). The benzene was drained from the column, and the combined benzene extracts were passed through the column followed by a 15-mL benzene wash which was discarded. Fifteen milliliters of NaOH solution (pH 11) were then run through the column under approximately 21 kPa nitrogen pressure into a 60-mL separatory funnel. The eluate was acidified with 2 drops of 6 N H_2SO_4 and extracted twice with 10 mL of benzene. The benzene extracts were combined, concentrated with a rotary evaporator to approximately 1 mL, and either taken to volume (1 mL) with benzene prior to quantitation of dinoseb by GLC (mature beans only) or methylated with diazomethane (green tissue and mature beans) followed by acidic alumina cleanup.

Diazomethane Derivatization. Diazomethane is very toxic and was prepared and used in a well ventilated fume hood. Contact of ground glass apparatus with diazomethane, which is also explosive, was avoided.

Diazomethane was prepared by the addition of 100 mg of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG; Aldrich Chemical Co., Inc., Milwaukee, WI) to 10 mL of 5 N NaOH solution and 25 mL of benzene in a 50-mL graduated cylinder. The concentrated benzene extract was transferred to an 18 mm × 150 mm test tube and approximately 3 mL of diazomethane solution was added (using a flame polished disposable pipet). The mixture was allowed to react for 20 min at room temperature and then was immersed in a water bath (35 °C), and the mixture was concentrated to approximately 1 mL with a stream of nitrogen.

Acidic Alumina Cleanup. Acidic alumina (1 mL; heated at 600 °C for 24 h and stored at 130 °C, Fisher Scientific Co., Ltd.) was added to 5 mL of benzene in a 17 mm i.d. × 200 mm column. The benzene was drained from the column and a 25-mL RB flask placed under the column. The methylated plant extract was transferred to the column (plus three 1-mL benzene rinses of the test tube) and the column was eluted with 15 mL of benzene. The eluate was concentrated with a rotary evaporator and taken to volume (1 mL) with benzene prior to quantitation of the dinoseb methyl ether by GLC.

Fortification. Recoveries of dinoseb were determined by extraction of green fababean foliage fortified at 0.1 ppm and mature fababeans fortified at both 0.1 and 0.05 ppm. The green fababean foliage was fortified as follows: 25 g of frozen untreated foliage was cut into 1.3 cm lengths and allowed to thaw in a 250-mL Erlenmeyer flask. Dinoseb (5 mL, 0.5 ppm in benzene) was added to the green tissue and the flask was sealed with parafilm to prevent drying of the plant tissue. Mature fababeans were fortified as follows: 2 mL or 1 mL of 0.5 ppm dinoseb in benzene was added to 10 g of milled (1 mm screen) untreated fababeans in a 125-mL Erlenmeyer flask. The fortified samples were left in darkness at room temperature for 48 h before extraction and analysis.

Gas Chromatography. A Hewlett-Packard Model 5733A gas chromatograph, equipped with a ^{63}Ni detector, was used with a Honeywell Electronic 194 1 m-V recorder. The 1.2 m × 4 mm i.d. coiled glass column was packed with 2% LAC-2R-446 (diethylene glycol adipate; DEGA)/0.4% H_3PO_4 on Chromosorb W, HP. Retention times were 3.8 and 4.0 min for dinoseb and dinoseb methyl ether, respectively, with the following operating conditions: 95% argon–methane (carrier gas), 35 mL/min; injector and column, 190 °C; detector, 300 °C. Under these conditions, 0.82 ng of dinoseb (attenuator set at ×16) and 0.55 ng of dinoseb methyl ether (attenuator set at ×32) gave full-scale recorder deflections. A linear response was observed over the range 0.04 to 4.0 ng for both dinoseb and its methyl ether. To prevent reduced sensitivity due to broadening of the dinoseb peak, 4 μL of 0.3% H_3PO_4 in acetone was injected initially each day [in contrast to the 0.2% H_3PO_4 in ether chaser used with each injection by Getzendaner (1969) for the gas chromatography of dalapon (2,2-dichloropropionic acid)].

RESULTS AND DISCUSSION

The LAC-2R-446/H_3PO_4 column, which has been used by several workers (Smith et al. 1964; Hirvnak and Stota, 1968; Clifford and Watkins, 1968, 1970) for the gas chromatography of free phenols, is also suitable for dinoseb as well as its methyl ether (Clifford and Watkins, 1970). Thus, methylation followed by acidic alumina column cleanup is unnecessary for dinoseb determinations where coextracted plant substituents do not interfere with the detection of dinoseb (after basic alumina column cleanup), or when such interference is effectively removed by dilution because of large dinoseb concentrations.

It was possible to determine dinoseb residues in the mature beans of both varieties by either procedure. GLC analysis of the plant extract, when subjected to basic alumina column cleanup only, produced a small peak on a sloping baseline with the same retention time as dinoseb (see Figure 1, chromatogram b). Based on a 1-g fresh-weight equivalent, the limit of detection for dinoseb was 5 ppb. However, methylation followed by acidic alumina column cleanup removed any plant extract interferences (see Figure 2, chromatogram b). This, coupled with the fact that dinoseb (due to a broader peak shape) was three times less sensitive than its methyl ether (as noted in the description of the gas chromatographic conditions), resulted in a limit of detection of 2 ppb. The same limit of detection, also based on a 1-g fresh-weight equivalent, was observed for the green tissue samples for which dinoseb had to be determined as the methyl ether.

Recoveries of dinoseb as the methyl ether from both green foilage and mature beans were determined from a standard calibration curve constructed by plotting nanograms of dinoseb methyl ether against peak height. Six replicates were analyzed at each fortification level, the recovery from the green foliage being 68.2 ± 6.6% at the 100 ppb level and 65.7 ± 6.3% and 57.1 ± 5.9% from the mature beans at the 100 and 50 ppb levels, respectively.

Dinoseb residues were found in the green tissue and mature beans from both locations (Table I). A single analysis was done for each replicate, except that two replicates (mature beans) harvested at Truro were analyzed

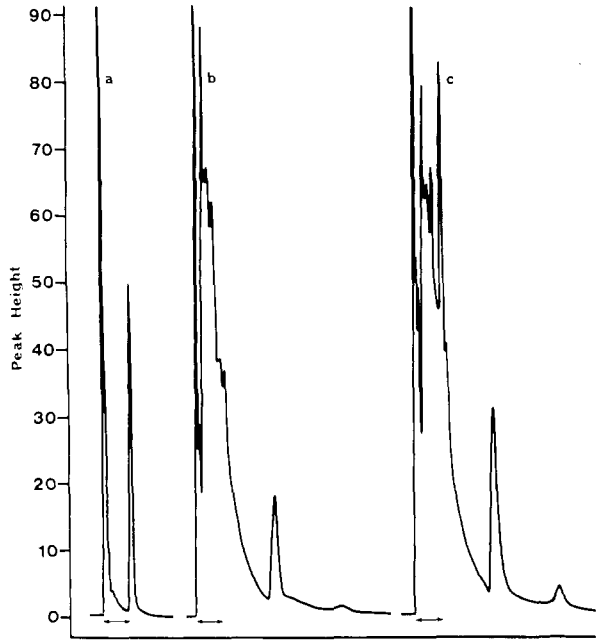

Figure 1. Chromatogram a, 0.4 ng of dinoseb in benzene (attenuator: ×16); chromatogram b, unmethylated extract from mature beans; chromatogram c, recovery of dinoseb as the free phenol from a fortified mature bean sample.

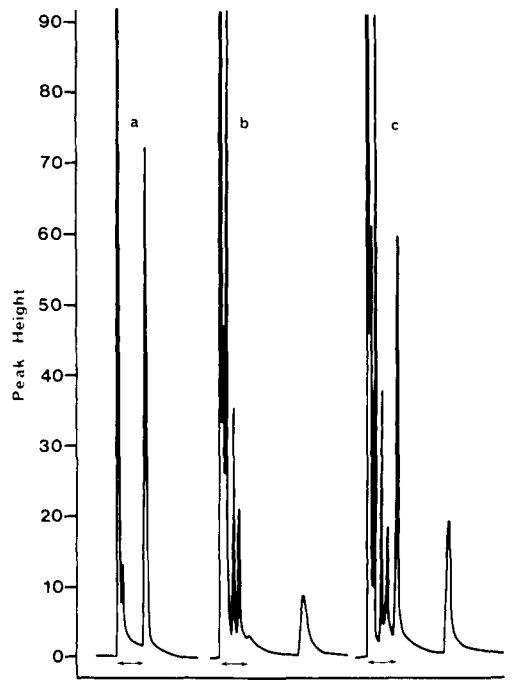

Figure 2. Chromatogram a, 0.4 ng of dinoseb methyl ether in hexane (attenuator: ×32); chromatogram b, methylated extract from mature beans; chromatogram c, recovery of dinoseb as the methyl ether from a fortified mature bean sample.

for dinoseb as both the free phenol and methyl ether. Of the six subsamples (mature beams) harvested at Charlottetown, three were analyzed for dinoseb as the free phenol and three as the methyl ether.

The registration of the postemergence treatment of dinoseb amine for selective weed control in fababeans in Canada was based on dinoseb residues in the mature grain being less than 100 ppb (Huston, 1977). The dinoseb residues observed from the preemergence applications of dinoseb amine used in the present study are well below this level both in the mature grain and in the green tissue which, collected 93 and 112 days after seeding, was representative of that used for silage production.

Table I. Dinoseb Residues Found in Fababean Samples after Preemergence Application of Dinoseb Amine at 4, 6, and 8 kg/ha

application rate, kg/ha	dinoseb residues, ppb[a]			
	Charlottetown[b]		Truro[c]	
	av[d]	max	av	max
	August 20			
4	<2	<2	8.25 ± 8.5	17
6	<2	5	11.25 ± 15.1	33
8	<2	<2	15.75 ± 20.8	46
	September 8			
4	<2	<2		
6	<2	<2		
8	<2	<2		
	Mature Beans			
4	5.4 ± 1.6	8	<2	<2
6	4.0 ± 1.1	6	<2	5
8	<2	<2	<2	<2

[a] Dinoseb residues are uncorrected for recoveries. [b] Moisture content of fababean samples (Charlottetown): 79.8 ± 1.0% (August 20); 71.6 ± 1.9% (September 8); 11.3 ± 0.2% (October 25). [c] Moisture content of fababean samples (Truro): 69.0 ± 3.6% (August 20); 7.1 ± 0.5% (October 26). [d] Average of four replicates, except for mature beans (Charlottetown) where the average is of six subsamples.

ACKNOWLEDGMENT

The author wishes to thank M. Aldred and M. Bishop for their technical assistance and D. G. Palfrey, Nova Scotia Agricultural College, Truro, Nova Scotia, and J. A. Ivany, Agriculture Canada Research Station, Charlottetown, Prince Edward Island, for conducting the field treatments and collecting and forwarding the fababean samples to Regina.

LITERATURE CITED

Anderson, G. W., "Research Report: Canada Weed Committee (Eastern Section)", 1975, p 130 (abstract only).
Clifford, D. R., Watkins, D. A. M., *J. Gas Chromatogr.* **6**, 191 (1968).
Clifford, D. R., Watkins, D. A. M., *J. Gas Chromatogr.* **48**, 523 (1970).
Getzendaner, M. E., *J. Assoc. Off. Anal. Chem.* **52**, 824 (1969).
Hirvnak, J., Stota, Z., *J. Gas Chromatogr.* **6**, 9 (1968).
Huston, B., Health and Welfare Canada, Health Protection Branch, Additives and Pesticides Division, Ottawa, Ontario, Canada, private communication, Dec 1977.
Ivany, J. A., "Research Report: Canada Weed Committee (Eastern Section)", 1975, p 132 (abstract only).
Ivany, J. A., "Research Report: Canada Weed Committee (Eastern Section)", 1976, p 156 (abstract only).
Ivany, J. A., Sanderson, J. B., "Research Report: Canada Weed Committee (Eastern Section)", 1974, p 161 (abstract only).
Longmuir, N., Agriculture Canada, Economics Branch, Special Crops Economist, Ottawa, Ontario, Canada, private communication, March 1978.
McKellar, R. L., The Dow Chemical Co., Midland, Mich., Feb 1970.
Smith, J. R. L., Norman, R. O. C., Radda, G. K., *J. Gas Chromatogr.* **2**, 146 (1964)
Winia, G., "Proceedings of the Saskatchewan Pulse Crop Grower's Association Pulse Crop Production Conference", Saskatoon, Saskatchewan, Canada, Feb 1977, p 43.

Allan J. Cessna

Agriculture Canada
Research Station
Regina, Saskatchewan, Canada S4P 3A2

Received for review April 4, 1978. Accepted July 24, 1978.