Table III. Recoveries of Furazolidone and Furaltadone from a Premix Fortified with Graded Amounts of Nitrofurans

concn, mg/g	recovery, %	
	furazolidone	furaltadone
1	100	111
2	96	101
3	99	102
4	96	96

furazolidone and furaltadone at 1, 2, 3, and 4 mg/g, gave recovery percentages compiled in Table III. It appears that nitrofuran extraction from premixes was complete in such experimental conditions.

Extraction tests of furazolidone and furaltadone, from silica gel of the chromatoplates, were realized with stirring times from 5 to 30 min in the DMF. The results showed the extraction of these nitrofurans was achieved within 10 min and the recovery was entire.

Because of the well-established sensibility of nitrofurans to light, a study of the decomposition of these products in DMF was carried out. Degradation rates of furazolidone and furaltadone after sunlight exposures of 5, 15, and 30 min, respectively, reached the values of 18, 36, and 42%.

The extraction of nitrofurans from feed premixes was complete in the precited conditions. An evaluation of the losses which may happen after this extraction was attempted; four samples containing a mixture of 2 mg of each nitrofuran in 10 mL of DMF were treated according to the above mentioned conditions. These assays allowed recoveries of 98.3% for furaltadone and 95% for furazolidone. The so determined small losses were probably due to light decomposition which cannot be completely avoided.

This assay method of furaltadone and furazolidone was applied to the analysis of commercial feed premixes. The reproducibility of the results from such products was satisfactory as well as that obtained with premixes in which nitro drugs were added by ourselves. Thus, standard deviations of the results were the equivalent of about 5% of the amount of furaltadone or furazolidone when present at 800 ppm and reduced to about 1.5% when present at 2000 ppm, the mean quantity of nitrofurans in commercial premixes.

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Effect of Vegetable Juices and Milk on Alkylating Activity of *N*-Methyl-*N*-nitrosourea

Effect of vegetable juices and milk, which are regarded to be the low-risk foods for gastric cancer, on the alkylating activity of *N*-methyl-*N*-nitrosourea (MNU) toward 4-(*p*-nitrobenzyl)pyridine was investigated to gain some information about dietary factors for stomach cancer. The juices and milk effectively decomposed MNU and consequently decreased its alkylating activity. The results suggest that they may play an important role to prevent human stomach cancer caused by alkylating agents.

Carcinogenic activity of *N*-nitroso compounds is believed to be due to their alkylating properties toward nucleophilic groups in proteins and nucleic acids (Magee and Barnes, 1967; McCalla, 1968; Swann and Magee, 1968). Among these compounds, alkylnitrosamides, which have powerful carcinogenic activity without metabolic activation, are the most possible carcinogens to cause human stomach cancer (Marquardt et al., 1977). In our previous work we studied the alkylating activity of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) to 4-(*p*-nitrobenzyl)pyridine (NBP) in some vegetable juices and found that they decomposed MNNG to noncarcinogenic *N*-methyl-*N'*-nitroguanidine, suggesting the presence of an anticarcinogenic effect in some fresh vegetables (Yano and Morita, 1977). This result was also in good agreement with earlier findings of the antimutagenic effect of vegetables in other systems (Kada, 1977).

Since a negative correlation between the consumptions of some fresh vegetables (Haenszel et al., 1972) or milk (Hirayama, 1963) and gastric cancer has been reported, it seems to be of interest to study the effect of these foods on the alkylating activity of alkylnitrosamides. Thus I investigated the alkylating activity of *N*-methyl-*N*-nitrosourea (MNU) on NBP in milk and the following vegetable juices: garden pea (*Pisum sativum* L.), tomato (*Lycopersicum esculentum* var. *commune*), celery (*Apium graveolens* var. *dulce*), radish (*Raphanus sativus* var. *hortensis*), lettuce (*Lactuca scariola* var. *sativa*), cucumber (*Cucumis sativus* L.), and cabbage (*Brassica oleracea* var. *capitata* L.). In this communication, the results of the above alkylations as well as some factors which influence the alkylating activity are reported.

METHODS AND MATERIALS

MNU was prepared by the method developed by Arndt (1943) and recrystallized from ether. NBP (Tokyo Kasei Co.) and 3-amino-1-propanol (Tokyo Kasei Co.) were used without purification. Vegetables and milk were purchased

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Table I. Alkylating Activity of MNU in Vegetable Juices and Milk

sample	pH		absorbance			alkylating activity[f]	freeze-drying[g]
	initial[a]	final[b]	initial[c]	final[d]	blank[e]		
garden pea	6.31	5.99	1.102 ± 0.038[h]	0.223 ± 0.018[h]	0.088	22.2	7.27
tomato	4.15	4.19	0.850 ± 0.003	0.385 ± 0.056	0.045	55.9	6.34
celery	5.96	6.49	1.170 ± 0.042	0.445 ± 0.035	0.020	69.9	4.05
radish	5.92	5.85	1.065 ± 0.035	0.383 ± 0.011	0.020	59.7	1.98
lettuce	5.96	5.74	1.102 ± 0.019	0.533 ± 0.032	0.030	82.7	2.08
cucumber	6.15	6.21	1.182 ± 0.033	0.480 ± 0.014	0.030	74.0	3.30
cabbage	5.96	5.85	1.087 ± 0.031	0.394 ± 0.023	0.058	55.3	3.97
milk	6.49	6.15	1.177 ± 0.089	0.159 ± 0.014	0.040	19.6	11.24
water	4.46	4.85	1.164 ± 0.019	0.623 ± 0.053	0.015	100	0

[a] pH value of a mixture of MNU (17.7 μmol) in 1 mL of sample before incubation. [b] pH value of its corresponding mixture after incubation (37 °C, 200 min). [c] Absorbance of a mixture of MNU (7.2 μmol) in 400 μL of sample before incubation (zero-time value). [d] Absorbance of its corresponding mixture after incubation (37 °C, 200 min). [e] Absorbance of sample without MNU after incubation (37 °C, 200 min). [f] Net absorbance (final-blank) of sample was divided by that of water (%). [g] Weight percentage. [h] Average ± SD.

from a local market. The alkylating activity of MNU toward NBP (Friedman and Boger, 1961) was measured by essentially the same method as modified by Colvin et al. (1976) using a Shimadzu UV-200 or SP-20 spectrophotometer. The pH values were measured on a Toadenpa HM-5BS.

Measurements of Alkylating Activity of MNU (or MNNG) in Vegetable Juices and Milk. Fresh vegetable juice was prepared by a juicer, followed by filtration through three layers of gauze. A mixture of MNU (0.742 mg, 7.2 μmol) in 400 μL of the juice, milk, or water was incubated in a silicone-stoppered tube at 37 °C for 200 min, which corresponds to half of the half-life of decomposition of MNU in water ($t_{1/4}$). The zero-time run was carried out directly to the next step without this incubation. To the tube were added 0.15 mL of 0.10 M acetate buffer (pH 4.76) and 0.50 mL of 5% NBP in acetone. The mixture was then incubated again at 37 °C for 2 h. After the mixture was cooled with ice, 1.0 mL of 25% 3-amino-1-propanol in *tert*-butyl alcohol was added. The resulting mixture was shaken vigorously for 1 min, followed by addition of 3 mL of acetone. The absorbance of the solution which was obtained by filtration of the mixture through No. 2 Toyo filter paper was measured at 540 nm in a dark room. Its blank was a run without MNU. The absorbance of MNNG (7.2 μmol) in milk was measured by the same method as described above except that it was incubated for 12 h ($t_{1/4}$).

Determination of Dried Materials in Vegetable Juices and Milk. The weight of 2.0 mL of juice or milk was measured, and it was subjected to freeze-drying in the usual fashion. Its weight percentage was determined.

pH Measurements. The pH of a mixture of MNU (1.825 mg) in 1 mL of juice, milk, or water was determined as an initial pH value and that of its corresponding incubated mixture (37 °C, 200 min) as a final pH value (Table I).

WARNING: MNU and MNNG, both of which are powerful carcinogens, should be handled with great caution.

RESULTS AND DISCUSSION

The effect of the vegetable juices and milk on MNU was examined by a previous procedure (Yano and Morita, 1977), and the results are summarized in Table I. The initial absorbances (zero-time values) are essentially identical in all the samples studied here, except tomato juice in which the low pH (4.15) might influence the alkylation at the initial stage. But the final absorbances (incubated run values) are quite variable in the range from 20% of milk to 83% of lettuce juice when compared to water as a reference (100%). To explain this observation,

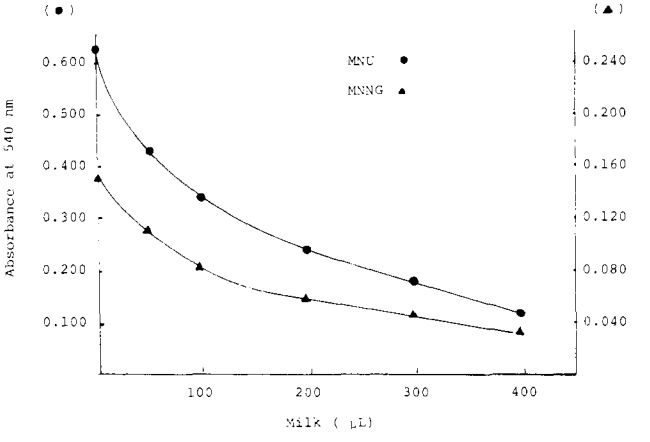

Figure 1. Effect of milk on alkylating activities of MNU and MNNG. Alkylating activities of MNU (7.2 μmol) and MNNG (7.2 μmol) as a function of added milk (0, 50, 100, 200, 300, and 400 μL) (total volume was adjusted to 400 μL with water) were determined as described in the Methods section.

at least two factors must be considered; the pH of the reaction mixture and the content of the freeze-dried materials in the sample. The former, however, does not seem to be the predominant factor because there is no correlation between the absorbances and pH values in Table I. On the other hand, when one compares the decreased alkylating activity, which can be obtained by subtracting each percent of the final absorbance from 100%, with the content of the dried material, an ordering of their effectivenesses (per dried materials) is estimated to be radish (20.4) > cabbage (11.3) > garden pea (10.7) > lettuce (8.3) > cucumber (7.9) > celery (7.4) > milk (7.2) > tomato (7.0). As seen from the above data, their variabilities are much less than those of the final absorbances, indicating that the latter factor must be significant in terms of the alkylation.

To further generalize the above results, the alkylating activities of MNU as well as MNNG were examined as a function of the added milk (Figure 1). Although the activity of MNU is much greater than that of MNNG, their curves are very similar to each other, suggesting that the mechanism of decomposition of MNU may also be similar to that of MNNG, which was discussed in a previous paper (Yano and Morita, 1977).

In summary, the results of this work suggest that the low-risk foods for gastric cancer, especially some fresh vegetables and dairy products, may play an important role to prevent stomach cancer by reducing the alkylating properties of carcinogens to cellular nucleophiles. Furthermore, these findings provide another interesting ob-

servation possibly related to the fact that the high incidence of stomach cancer in Japan may be related to dietary factors (Marquardt et al., 1977).

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Diterpenoid Acids, (−)-*cis*- and (−)-*trans*-Ozic Acid, in Wild Sunflower, *Helianthus occidentalis*

Two diterpenoid acids, (−)-*cis*- and (−)-*trans*-ozic acid, were isolated from the wild sunflower species, *Helianthus occidentalis*. This is the first report of the levorotatory optical enantiomorph of *trans*-ozic acid and the first isolation of both acids from *Helianthus*. Because *H. occidentalis* is resistant to sunflower insect pests and various diterpenoid acids have shown antibiotic activity to several insect species, the presence of *cis*- and *trans*-ozic acid may contribute to host plant resistance.

The wild sunflower *Helianthus occidentalis* subsp. *plantagineus* (T. & G.) Heiser is resistant to several insect pests (Thompson and Rogers, 1977; Rogers and Thompson, 1978). No feeding and complete larval mortality of the sunflower beetle [*Zygogramma exclamationis* CF.] were noted on *H. occidentalis* compared to only 22% mortality on susceptible *H. annuus* L., and total mortality of an aphid [*Masonaphis masoni* (Knowlton)] occurred on this wild species of sunflower. Early investigators further noted that individual plants of *H. occidentalis* exhibited autotoxic action (allelopathy) against other plants of the same species (Curtis and Cottam, 1950).

In an effort to identify a chemical basis for resistance to insects, we compared the composition of extracts of florets of *H. occidentalis* with those from a susceptible check, Hybrid 896 (*H. annuus*). Two diterpenoid acids were isolated from the extracts of *H. occidentalis* but not from the extracts of Hybrid 896. These diterpenoid acids were also present in extracts of *H. occidentalis* leaves and roots.

Two diterpenoid acids, 16-kauren-19-oic acid and trachyloban-19-oic acid, were isolated from florets of *H. annuus* (Pyrek, 1970) and inhibited the growth of the sunflower moth [*Homeosoma electellum* (Hulst)] (Waiss et al., 1977). These and several other diterpenoid acids also inhibited the growth of *Heliothis virescens* (Fab.), *Heliothus zea* (Boddie), and *Pectinophora gossypiella* (Saynders) (Elliger et al., 1976).

In view of the general toxicity of diterpenoid acids to insects, we investigated two diterpenoids from *Helianthus occidentalis* that were absent in Hybrid 896. These are identified as (−)-*cis*- and (−)-*trans*-ozic acids.

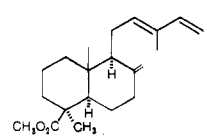
EXPERIMENTAL SECTION

Materials and Isolation. *H. occidentalis* was grown at the USDA, Southwestern Great Plains Research Center, Bushland, TX. Leaves and florets from mature plants were frozen, lyophilized, and ground to a powder with a mortar and pestle. Leaves (20 g) were stirred with a mixture of ethyl acetate/petroleum ether (bp 35–60 °C) (1:3, 120 mL) and water (2 mL) for 30 min. The slurry was poured into a glass column (2.5 cm diameter) and extracted successively with hexane (400 mL), ethyl acetate/petroleum ether (1:3, 200 mL), and ether (110 mL). Because all extracts contained the desired acids, they were combined and evaporated to dryness to give 1.0 g of crude material. The leaf residue was further extracted in a Soxhlet with refluxing ether and gave an additional 0.3 g of crude material. The combined crude material was chromatographed on thin layers of silica gel with chloroform/acetone/formic acid (94:5:1). The UV (254 nm) absorbing band (R_f ~0.7) was collected. This band, which gave an acid reaction with bromocresol green, was chromatographed as above with ether/hexane (1:1; solvent 1) and gave 230 mg of material consisting primarily of *cis*- and *trans*-ozic acids.

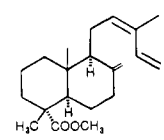
The mixed ozic acids (75 mg) were esterified with diazomethane (see safety precautions: Fales and Jaouni, 1973) and chromatographed on thin layers of silica gel containing 3% silver nitrate (solvent 1). The top UV absorbing band was *cis*-methyl ozate (25 mg) and the lower band was *trans*-methyl ozate (23 mg).

RESULTS AND DISCUSSION

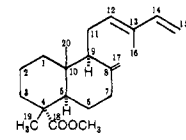
The methyl esters of the diterpenoid acids from *H. occidentalis* gave parent ions at *m/e* 316. High-resolution



trans-Methyl Communate



cis-Methyl Ozate



trans-Methyl Ozate

measurement of these ions indicated that the molecular formula was $C_{21}H_{32}O_2$ for each ester. Thus six degrees of unsaturation are indicated. The ^1H and ^{13}C NMR spectra