Table I. Aflatoxin Content of Some Peanuts that Are Currently Cultivated in Iran

Varieties	Millicolumn, ppb	Spectrophotometric, ppb
Local Gilan Iran	0	0
Flori Spanish 334 A	10	12.50 ± 0.18
Georgia 119-20	50	60.50 ± 0.91

(because of its higher concentration of aflatoxin) of peanut III prepared by the method described by Holaday and Barnes (1973) were applied to the plates, respectively. The chromatograms were developed and then observed by a mercury lamp. The blue fluorescent spots were scraped off, and the aflatoxin was extracted by ethanol, filtered, and dried. To the dried sample 5 mL of ethanol was added, and aflatoxin concentration was measured by an ultraviolet spectrophotometer (Beckman DB-GT) in 233 nm. To make a standard solution, a 0.1-μL aliquot of 5% aflatoxin solution (Makors Chemical LTD) was applied to the plates, chromatographed, extracted, and measured. The data are averages of three replicate analyses unless otherwise indicated.

RESULTS AND DISCUSSION

The blue fluorescent bands in the millicolumns prepared from peanut II and III extracts and the TLC prepared from the same extracts indicated the presence of the aflatoxin in these samples. Such spots, in comparison to the standard, were not observed on TLC plates prepared from extract of Local Gilan Iran variety (Figure 1). Besides the spectrophotometric data, some degrees of quantitation was possible with the method described by Holaday and Barnes. Table I shows the absence of aflatoxin in Local Gilan Iran and the concentration of the toxin in the other two samples. It should be mentioned that not only the kernel but also the shell of the samples from either 1974 or 1975 were not contaminated with aflatoxin.

Significance. Research on prevention (Beuchat and Smith, 1974) or removing (Goldblatt, 1969; Kensler and Natoli, 1969) of aflatoxin from peanuts and peanut products has not been successful. Further increases in commercially available peanut and peanut products (peanut butter, oil, and defatted meal) calls for detoxification of this nutrient. Thus, our finding, peanut resistance to seed infection, is important in viewpoint of yielding detoxified rich protein and sulfur amino acid peanut that could be used as such in nutrition without being considered a potential threat to food safety and human health, and problem for producers, handlers, and users of peanut and peanut products.

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Received for review July 23, 1976. Accepted May 13, 1977. Financial support was received from The University of Tehran Research Funds.

Analysis of *N*-Nitrosoproline in Raw Bacon. Further Evidence that Nitrosoproline Is Not a Major Precursor of Nitrosopyrrolidine

Nitrosoproline (NPro) has been suggested as a precursor of nitrosopyrrolidine (NPyr) in fried bacon. We have developed a procedure for isolation of NPro from raw bacon for subsequent chromatographic analysis. Analysis has been done by both gas and high-performance liquid chromatography. The two methods have included different specific detectors. Results indicate that preformed NPro is not a major source of NPyr.

Nitrosopyrrolidine (NPyr) is a carcinogen which has been consistently found in fried, but not raw, bacon (Crosby et al., 1972; Fazio et al., 1973). The mechanism causing the formation of NPyr has been the subject of several investigations (Pensabene et al., 1974; Fiddler et al., 1974), but it is still unclear. Lijinsky and Epstein (1970) suggested that NPyr is formed by nitrosation and decarboxylation of proline, though not necessarily in that order. The presence of nitrosoproline (NPro) in raw bacon would suggest nitrosation of proline during bacon curing and storage, followed by decarboxylation to NPyr on frying. The substantial decrease of NPyr found when ascorbyl palmitate is added prior to frying, however, implies that nitrosation during cooking is responsible for much of the NPyr formed (Sen et al., 1976b).

A few studies have focused on either the presence of NPro in raw bacon (Kushnir et al., 1975; Nakamura et al., 1976) or the yield of NPyr from NPro in bacon or in model

systems (Bills et al., 1973; Sen et al., 1976a; Eisenbrand et al., 1976). No definite conclusions have been drawn by these investigators. The lack of a rapid, simple analytical technique for NPro has made extensive studies difficult.

The development of the Thermal Energy Analyzer (TEA, Thermo-Electron Corp., Waltham, Mass.), which is very sensitive and selective for *N*-nitroso compounds (Fine et al., 1975a), has facilitated routine analysis of such compounds in food samples. We have been using a TEA as a gas chromatography (GC) detector for analysis of volatile *N*-nitroso compounds, including NPyr in bacon.

In order to analyze NPro in raw bacon, we have developed a clean-up procedure that is simple and very efficient. Samples put through this procedure have been analyzed by two different chromatographic methods. One method uses GC–TEA after conversion of NPro to its trimethylsilyl ester; the other uses high-performance liquid chromatography (HPLC), with a specific photohydrolysis (PH) system that was developed in this laboratory (Iwaoka and Tannenbaum, 1976). This PH system causes photolytic cleavage of the NO group from *N*-nitroso compounds and the subsequent formation of nitrite. The nitrite is then coupled with a Griess reagent, and the resultant dye is measured by absorbance at 550 nm.

This paper presents the results obtained from a bacon sample containing both large amounts of NPro before frying and NPyr after frying. The results indicate that preformed NPro is unlikely to be a major precursor of NPyr.

EXPERIMENTAL SECTION

Preparation of Bacon Samples. A commercial 1-lb package of sliced bacon was used. The slices were separated and mixed, and two 100-g samples were removed. One sample was fried 3 min on each side at 170 °C and then drained for NPyr analysis. The other sample was extracted and further treated for NPro analysis, as described in Table I.

NPyr Analysis. The drained bacon strips were weighed and ground with an equal amount of mineral oil to a homogeneous paste in a stainless steel blender. The sample was distilled by a procedure similar to that of Fine et al. (1975b). The slurry was heated to 120 °C under vacuum, and volatile components were collected in a liquid nitrogen trap. Contents of the trap were then rinsed with water. The water was extracted with dichloromethane (DCM), which was then dried and reduced to a volume of 1 mL for GC–TEA analysis. By spiking a similar sample with a known amount of NPyr, we found the recovery of NPyr by this method to be greater than 95%. Analysis was carried out on a 10 ft × 0.125 in. column packed with 10% FFAP on Chromosorb G 80/100 with helium carrier at 20 mL/min. Oven temperature was 190 °C and injection port temperature was 200 °C.

NPro Analysis. The final 2-mL extract was divided into two portions, one of which was analyzed by GC, the other by HPLC. Recovery of NPro was found to be 85–90%, by recovery of activity in a similar sample spiked with ^{14}C-labeled NPro.

The portion for GC analysis was evaporated to dryness and derivatized with 1 mL of isopropenyloxytrimethylsilane (Applied Science, State College, Pa.). Nitrosoproline trimethylsilyl ester (NPro–Me_3Si) was analyzed on a 6 ft × 0.125 in. column packed with 3% OV-17 on Chromosorb G 80/100 with helium carrier at 30 mL/min. Oven temperature was 120 °C and injection port temperature was 175 °C.

The portion for HPLC analysis was also evaporated to dryness and then taken up in 0.5 mL of water. Analysis of this sample was done on a prepacked 1 ft × 0.25 in. column of μ Bondapak C_{18} (Waters Associates, Milford, Mass.), with a mobile phase of 1% Na_2HPO_4 in distilled water at 1.5 mL/min. Two different detectors were used for this sample. One was a Model 440 ultraviolet absorption (UV) detector (Waters) at 254 nm; the other was our PH detector described earlier.

Table I. Procedure for Extraction of Nitrosoamino Acids from Bacon

1. Homogenize 100 g of raw bacon in 1.5 volume of distilled water. Make sure a representative sample of the bacon is obtained. Use 0.5 volume of water to rinse out homogenizer.
2. Centrifuge homogenate at 5000 rpm and 0 °C for 5–10 min or until the fat solidifies in the centrifuge bottle. Carefully pour off supernatant and refrigerate.
3. Repeat homogenization and centrifugation steps twice more with bacon solids and combine three supernatant fractions. Discard solids.
4. Filter chilled supernatant fraction with Buchner funnel to remove remaining solidified fat and meat particles.
5. Add filtered supernatant to ion-exchange column at a flow rate of 3–5 mL/min. (Column is a 20–30 in. × 1 in. i.d. glass tube filled with 100 mL of Dowex 2X8-100 strongly basic anion-exchange resin in the chloride form.) Discard column eluate.
6. Wash column with 300 mL of distilled water or until eluate is clear at a flow rate of 5–7 mL/min. Discard eluate.
7. Elute nitrosoamino acids from ion-exchange column with 100 mL of 1.0 M NaCl adjusted to pH 1.0 with HCl and at a flow rate of about 5–7 mL/min.
8. Remove water from column eluate on rotary evaporator connected to an aspirator vacuum. Keep water bath temperature at 50 °C. Be sure residual salts are absolutely dry. If salts are not completely dried, the nitrosoamino acids are difficult to extract.
9. Extract nitrosoamino acids from salts with ten 50-mL portions of dichloromethane (DCM).
10. Combine all DCM extractions and concentrate sample in a 500-mL Kuderna-Danish evaporator with a graduated test tube bottom. Use a straight glass chimney instead of a Snyder column on top of the evaporator. Keep water bath temperature at 60 °C.
11. Concentrate sample in evaporator until about 5 mL of DCM remains. Remove apparatus from water bath and cool the external surface of the evaporator with cold water to condense volatile DCM. Remove graduate test tube and evaporate DCM to 2 mL.

RESULTS AND DISCUSSION

Analysis of the fried bacon by GC–TEA indicated the presence of 44 μg of NPyr/kg of bacon. Figure 1 shows the GC–TEA chromatogram of the raw bacon extract. The peak occurring at about 7 min corresponds to NPro-Me_3Si. The concentration of NPro-Me_3Si was determined by comparing peak area with that of a known amount of dibutylnitrosamine, assuming equal molar response. This method gave an original concentration of 70 μg of NPro/kg of raw bacon.

Figure 2a shows the same raw bacon extract analyzed by HPLC–PH. The first two peaks correspond to NPro. The double peak also appears in an NPro standard (Figure 2b) and is due to the separation of the syn and anti conformers of NPro (Iwaoka et al., 1975). Comparison of the absorbance values of the bacon extract with an NPro standard indicate an original concentration of 80 μg of NPro/kg of raw bacon.

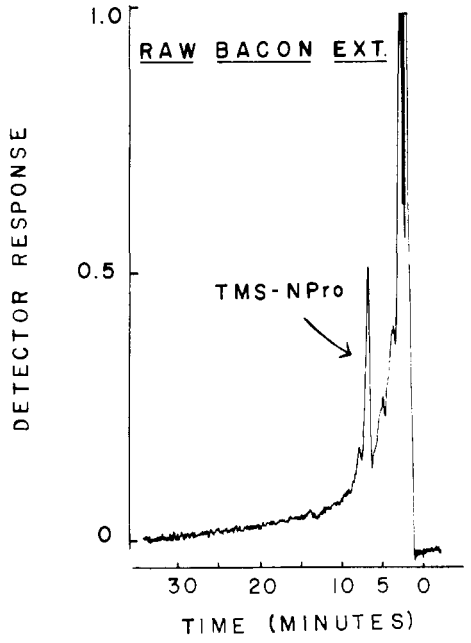

Figure 1. A 1.7-μL sample of DCM extract from raw bacon after workup for NPro analysis and trimethylsilylation; it was separated by GC on OV-17, with TEA detector.

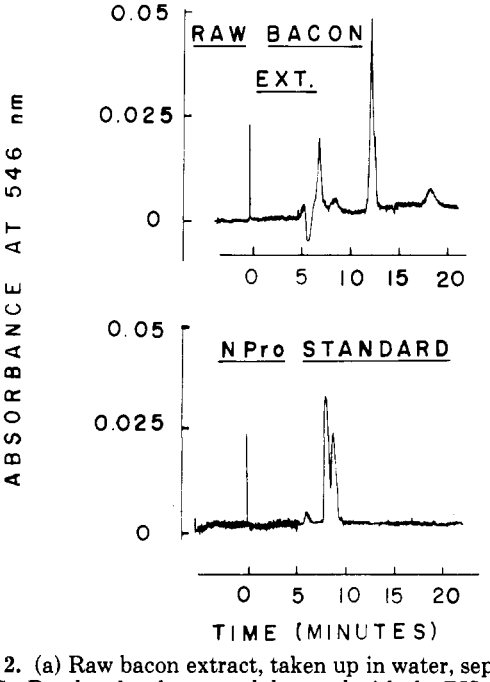

Figure 2. (a) Raw bacon extract, taken up in water, separated on the C_{18} Bondapak column, and detected with the PH system. The mobile phase was 1% Na_2HPO_4 in distilled water, and the flow rate was 1.5 mL/min. The injection size was 30 μL. (b) NPro standard in water, separated on the C_{18} Bondapak column, and detected with the PH system. The mobile phase was 1% Na_2HPO_4 in distilled water, and the flow rate was 1.5 mL/min.

Two other peaks, which possibly correspond to *N*-nitroso compounds, are also apparent in Figure 2a. They did not appear when GC–TEA analysis was used and have not been identified.

Figure 3 is the same raw bacon extract analyzed by the same HPLC conditions, but with the UV detector. A comparison of Figures 3 and 2a shows that several peaks interfering with NPro analysis by HPLC–UV are eliminated by the use of the PH detector. This selectivity makes further cleanup unnecessary. Moreover, the PH detector is sensitive enough to detect less than 50 ng of NPro/injection.

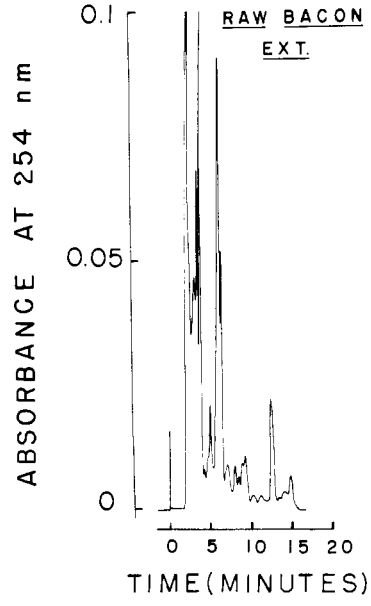

Figure 3. Raw bacon extract, taken up in water, separated on the μ C_{18} Bondapak column, and detected at 254 nm. The mobile phase was 1% Na_2HPO_4 in distilled water, and the flow rate was 1.5 mL/min. The injection size was 30 μL.

The agreement between GC–TEA and HPLC–PH procedures on the concentration of NPro in the raw bacon sample was very good. Either procedure could be used to investigate systematically the role of NPro in the production of NPyr in fried bacon.

Several other bacon samples we have studied had no detectable NPro, but conclusions were difficult to draw since they also contained 10 μg of NPyr/kg or less upon frying. Although no general conclusions can be made from the small number of samples studied here, our results cast further doubt on the significance of preformed NPro as a precursor of NPyr, since they show a requirement for at least 80% of the NPro to be converted to NPyr during frying. Consideration of the sizable amount of NPyr present in both the cooking fumes and cooked-out fat (Warthesen et al., 1976; Sen et al., 1976b), in addition to the lean portion we have analyzed, reveals that even decarboxylation of all of the preformed NPro is insufficient to account for the quantity of NPyr formed. Furthermore, results from model systems indicate a conversion of less than 3% (Bills et al., 1973). In agreement with the findings of Hwang and Rosen (1976) in which ^{14}C-labeled precursors were employed, our results lend increasing credence to the role played by free proline in determining NPyr formation at the time of frying. The expected difference in concentration between NPro and proline would be much greater than the approximate sixfold difference in yield of NPyr, and a concentration of 20 ppm of proline would suffice to produce the 50 ppb NPyr found in our bacon (Hwang and Rosen, 1976).

Much further work is needed before the mechanism of NPyr formation is understood, and such an understanding is required before occurrence of NPyr in food can be eliminated.

ACKNOWLEDGMENT

This research was supported by PHS contract NO1-CP-33315 from the National Cancer Institute and by a grant-in-aid from the American Meat Institute.

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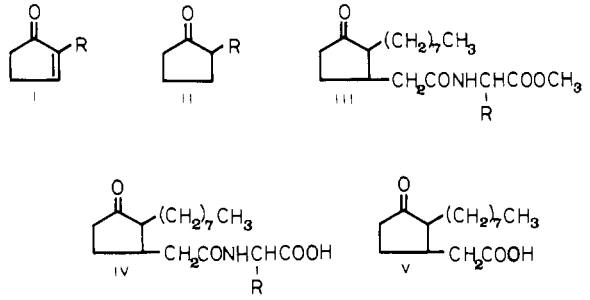
Received for review March 28, 1977. Accepted July 1, 1977.

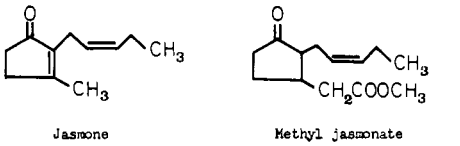
Effect of Substituted Cyclopentenones and Cyclopentanones on Lettuce Seed Germination and Radicle Growth

A series of 2-alkylcyclopent-2-ene-1-ones (I), 2-alkylcyclopentan-1-ones (II), and amides of 2-octyl-3-oxocyclopentyl-1-acetic acid (IV) were synthesized. The data suggest that 2-propylcyclopentan-1-one and *n*-[2′-octyl-3′-oxocyclopentyl-1′-acetyl)isoleucine are potent inhibitors of lettuce seedling growth as compared to other compounds described in Table I.

In a recent study (Ravid et al., 1975) we reported the effect of jasmonoids on the lettuce seedling growth.

In the following communication we wish to report the effect of alkylcyclopentenones (I), alkylcyclopentanones (II), as well as amides of methyl 2-octyl-3-oxocyclopentylacetate (III), and 2-acetyl-3-oxocyclopentyl-1-acetic acid (IV), which are structurally related to naturally occurring jasmonoids such as jasmone and methyl jasmonate, on lettuce seedling growth.





Jasmone

Methyl jasmonate

EXPERIMENTAL SECTION

Synthesis. The structures of compounds studied in this work (Table I) are shown under the general formulae I to V. The general synthetic route for synthesizing these compounds was outlined by Katsin and Ikan (1977).

2-Propylcyclopent-2-ene-1-one (I, R = C_3H_7). IR (liquid) 1700, 1630, 1442, 1403, 1378, 1295, 1198, 1100, 1046, 1000, 785 cm^{-1}; NMR (CCl_4) 0.85 (3 H, t), 1.45 (2 H, m), 1.90–2.70 (6 H, m), 7.3 (1 H, m). Anal. Calcd for $C_8H_{12}O$: C, 77.42; H, 9.68. Found: C, 77.39; H, 9.73.

2-Octylcyclopent-2-ene-1-one (I, R = C_8H_{17}). IR (liquid) 1705, 1630, 1465, 1343, 1205, 998, 785 cm^{-1}; NMR ($CDCl_3$) 0.86 (3 H, t), 1.00–1.60 (12 H, m), 1.8–2.65 (6 H, m), 7.1 (1 H, m). Anal. Calcd for $C_{13}H_{22}O$: C, 80.41; H, 11.34. Found: C, 80.63; H, 11.45.

1-Propylcyclopentan-1-one (II, R = C_3H_7). IR (liquid) 1735, 1465, 1453, 1155 cm^{-1}. Anal. Calcd for $C_8H_{14}O$: C, 76.19; H, 11.21. Found: C, 76.92; H, 11.73.

1-Octylcyclopentan-1-one (II, R = C_8H_{17}). IR (liquid) 1735, 1465, 1452, 1150 cm^{-1}; NMR ($CDCl_3$) 0.85 (3 H, t), 1.19 (12 H, m), 1.65–2.50 (7 H, m).

***N,N′*-(2′-Octyl-3′-oxocyclopentyl-1′-acetyl)glycine Methyl Ester (III, R = H).** A solution of glycine methyl ester hydrochloride (0.51 g, 2.04 mmol) in triethylamine (0.28 mL, 4.08 mmol) and *N,N*-dicyclohexylcarbodiimide (0.56 g, 2.72 mmol) in acetonitrile (4 mL) were added to a cooled (0 °C) solution of 2-octyl-3-oxocyclopentyl-1-acetic acid (0.69 g, 2.72 mmol) in acetonitrile (4 mL). The mixture was allowed to stay for 1 h at 0 °C and then stirred for 2 days at room temperature. A few drops of acetic acid are then added to the reaction mixture and the solvents removed under reduced pressure. Ethyl acetate was then added and the solid filtered off. The residue was washed with dilute hydrochloric acid, sodium bicarbonate solution, and water. Removal of ethyl acetate left an oily product: yield, 0.5 g (56.3%); IR (liquid) 3310, 1735, 1655, 1535, 1438, 1408, 1369, 1205 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (3 H, t),