# Nitrosation of Piperine Using Different Nitrosating Agents: Characterization and Mutagenicity of the Products

Narmada R. Shenoy,*,†,‡ Ahmed S. U. Choughuley,† Tulsi K. Shetty,§ and Rathin K. Bhattacharya§

Bio-organic Division and Radiation Biology and Bio-Chemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India

Piperine, an N,N-disubstituted amide possessing a methylenedioxy moiety, is the main pungent principle of pepper, a spice consumed by people throughout the world. On nitrosation the reaction mixture exhibits mutagenic activity toward *Salmonella typhimurium* strains. In the present work we have nitrosated piperine using nitrous acid ($HNO_2$), nitrosyl chloride (NOCl), and dinitrogen tetraoxide ($N_2O_4$). The compounds identified from the nitrosation of piperine are piperonal (PA), 6-nitropiperonal (NPA), 3,4-(methylenedioxy)cinnamaldehyde (MDCA), 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2(*E*),4(*E*)-pentadienyl]piperidine (MNAP), 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]-piperidine (MNOP), 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine (DNP), and *N*-nitrosopiperidine (NPIP). The mutagenicity of the unfractionated nitrosated reaction mixture (UPNM) and compounds NPA, MNAP, MNOP and DNP toward *S. typhimurium* TA 98 and TA 100 strains was studied. Compounds NPA and DNP and the unfractionated reaction mixture were found to be mutagenic toward both the strains.

Epidemiological studies have indicated the importance of environmental factors, especially dietary causes, of cancer and dietary protective factors (Doll and Peto, 1981). A number of human cancers, especially those of the digestive system and liver, may be caused by *N*-nitroso compounds formed during endogenous nitrosation (Magee, 1982; Hotchkiss and Cassens, 1987). It is of importance, therefore, to identify the substances that can undergo facile nitrosation to form mutagens and carcinogens.

Piperine is the major pungent principle of pepper, a spice consumed by people all over the world. It is the trans-trans isomer of 1-piperoylpiperidine and contains the methylenedioxy moiety. Nitrosation of piperine is interesting because unidentified mutagenic products have been obtained from this reaction (Nakamura et al., 1981; Osawa et al., 1982). This is of concern as human beings are exposed to substantial amounts of nitrites either directly or indirectly. Nitrate as it is present in vegetables, water, and other food supplies is essentially nontoxic. However, it can be reduced to nitrite by microbial conversion in saliva, under adverse storage conditions of food, in an achlorhydric stomach, and in an infected urinary bladder (Archer and Hathcock, 1982; Lijinsky, 1977; Lijinsky and Conrad, 1972; Tannenbaum, 1979). As the physiological conditions prevalent in the human stomach are ideal for nitrosation reactions to occur from innocuous precursors, viz., amines present in foods, we thought it important to investigate the nitrosation of piperine. In our present study we have nitrosated piperine using nitrous acid ($HNO_2$) in aqueous solutions, nitrosyl chloride (NOCl), and dinitrogen tetraoxide ($N_2O_4$) and have isolated and characterized several *C*-nitro compounds that could be the hitherto unknown mutagens. Both NOCl and $N_2O_4$ were found to be very efficient nitrosating agents and gave increased yields of the products. Consequently, we have been able to isolate enough quantities to elucidate their structure and study the mutagenic activity of the *C*-nitro derivatives of piperine to *Salmonella typhimurium* TA 98 and TA 100.

* Author to whom correspondence should be addressed.
† Bio-organic Division.
‡ Present address: Division of Immunology, Beckman Research Institute of the City of Hope, 1450 E. Duarte Rd., Duarte, CA 91010-0269.
§ Radiation Biology and Bio-Chemistry Division.

## MATERIALS AND METHODS

**Chemicals.** Piperine and sodium nitrite were obtained from Sigma Chemical Co., St. Louis, MO, and British Drug House, Bombay, India, respectively. Dimethyl sulfoxide (DMSO), aflatoxin $B_1$, nicotinamide adenine dinucleotide phosphate (NADP), and glucose 6-phosphate were purchased from Sigma. Nutrient broth and agar were purchased from Difco Labs, Detroit, MI. All other chemicals were of analytical grade, and solvents were distilled before use.

**Apparatus.** The melting points were recorded on a Fischer John apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer Model 783 IR spectrophotometer, UV spectra on a Shimadzu UV240 UV-visible spectrophotometer, PMR spectra on a Varian XL 300-MHz spectrometer, and mass spectra on a Shimadzu GCMS-QP 1000.

Chromatographic analyses were carried out on a Waters Associates HPLC using a μBondapak $C_{18}$ column equipped with a 254-nm UV detector. The mobile phase used for the separation was water/methanol (80:20) at a flow rate of 0.5 mL/min. Gas chromatography was carried out on a Shimadzu 7C GC using a 3% OV 17 on gas chrom Q (80/100) glass column equipped with a FID detector. The column temperature was programmed between 120 and 250 °C, the injection port temperature was 250 °C, and the nitrogen gas flow rate was 40 mL/min.

**Caution.** Utmost caution was used during the workup of the experiments described as these involve mutagenic/carcinogenic reaction products. Contact with skin and inhalation of vapors were avoided.

**Methods.** *Nitrosation of Piperine Using $HNO_2$.* Piperine (2.85 g, 10 mmol) was reacted with an excess of sodium nitrite (13.8 g, 200 mmol) in acetic acid solution (pH 3.5, 500 mL, 4 h). The reaction was arrested by adding 200 mmol of ammonium sulfamate and then extracted with ethyl acetate (100 × 5 mL). The organic extract was washed with 15% sodium bicarbonate solution (25 × 5 mL) and brine solution (25 × 2 mL), dried over anhydrous sodium sulfate, and concentrated on a rotary evaporator.

*Nitrosation of Piperine Using NOCl and $N_2O_4$.* Nitrosyl chloride was generated by reacting sodium nitrite with concentrated HCl (Morton and Wilcox, 1954) and $N_2O_4$ by the thermal decomposition of lead nitrate (Digenis and Issidorides, 1979).

0021-8561/92/1440-2211$03.00/0 

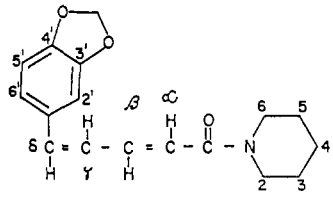

Figure 1. Structure of piperine.

Nitrosyl chloride was passed through a cold solution (0 °C) of piperine (1 g, 3.5 mmol) in $CCl_4/CHCl_3$ (1:1, 500 mL) for 10 min. The reaction mixture was neutralized with bicarbonate solution (15%), treated with ammonium sulfamate solution 25% (25 × 3) followed by brine (25 × 2 mL), dried over anhydrous sodium sulfate, and concentrated on a rotary evaporator.

Nitrosation of piperine using dinitrogen tetraoxide was carried out in exactly the same way as described above.

Separation of the reaction products obtained from the different reaction mixtures was carried out as follows: The reaction mixture was fractionated on a silica gel column (4 × 30 cm) using stepwise gradient from *n*-hexane to ethyl acetate (0:100, 10:90, ..., 100:0). Fractions were monitored by micro-TLC and visualized at 245 nm. Like fractions were pooled and concentrated on a rotary evaporator. Solid compounds obtained were crystallized in *n*-hexane/ethyl acetate. Only those compounds that were present in all three nitrosation mixtures were isolated, identified, and quantified by HPLC and GLC.

*Assay Procedure for Mutagenic Activity. S. typhimurium* strains TA 98 and TA 100 of histidine-dependent type were kindly supplied by Prof. B. N. Ames, University of California, Berkeley, CA. The assay to determine the mutagenic activity of the *C*-nitro derivatives NPA, MNOP, MNAP, and DNP and the nitrosated reaction mixture (UNOM) (obtained using $HNO_2$ as the nitrosating agent) was carried out using the plate incorporation test according to the revised procedure described by Maron and Ames (1983) and as adopted previously (Bhattacharya et al., 1987; Francis et al., 1989). Metabolic activation was provided by liver postmitochondrial supernatent (S-9) obtained from Arochlor 1254 induced male Wistar rats. To 2 mL of molten top agar (45 °C) was added 0.1 mL of the bacterial culture grown overnight in nutrient broth, 0.5 mL of S-9 mix, and 0.1 mL of the test chemical in DMSO (various concentrations). The mixture was poured onto a minimal-glucose agar plate and incubated at 37 °C for 48 h, after which time the number of histidine-independent ($His^+$) revertant colonies of *S. typhimurium* (strains TA 98 and TA 100) was scored. The compounds were tested with and without S-9 mix. Triplicate plates were run for each dose of the test compound. Spontaneous reversion of the tester strains to histidine independence was measured and expressed as the number of spontaneous revertants per plate. The average number of spontaneous revertants was subtracted with each mean value. Positive control plates containing aflatoxin were included in each assay.

## RESULTS AND DISCUSSION

**Characterization of the *C*-Nitro Derivatives of Piperine.** The compounds identified from the nitrosation of piperine are piperonal (PA), 6-nitropiperonal (NPA), 3,4-(methylenedioxy)cinnamaldehyde (MDCA), 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2(*E*),4(*E*)-pentadienyl]piperidine (MNAP), 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine (MNOP), 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine (DNP), and *N*-nitrosopiperidine (NPIP). The structure of piperine is illustrated in Figure 1 and that of the other derivatives in Figure 2. The physical and spectral characteristics of compounds PA, NPA, MDCA, and NPIP matched with those obtained from their respective authentic samples and literature values. The structures of the three *C*-nitro compounds MNAP, MNOP, and DNP are discussed below and, to the best of our knowledge, have not been reported previously in the literature.

Compound MNAP showed a molecular ion peak at $m/z$ 330 and fragment peaks at $m/z$ 300 $(M - NO)^+$ and 285 $(M - NO_2)^+$. The PMR spectrum in DMSO-$d_6$ showed a two-proton singlet at 6.1 ppm (methylenedioxy), a six-proton multiplet at 1.6 ppm (piperidine 3,4,5 protons), a four-proton multiplet at 3.54 ppm (piperidine 2,6 protons), two singlets of the aromatic protons at 7.51 and 7.12 ppm, a one-proton doublet at 6.5 ppm ($\alpha$-olefinic proton), a one-proton double doublet at 7.21 ppm ($\beta$-olefinic proton), a one-proton double doublet at 6.92 ppm ($\gamma$-olefinic proton), and a one-proton doublet at 7.3 ppm ($\delta$-olefinic proton). When the lanthanide chemical shift reagent $Eu(fod)_3$ was added, the olefinic protons shifted as follows: 8.8 (dd, $\beta$-proton), 8.53 (d, $\delta$-proton), 7.8 (d, $\alpha$-proton), and 8.2 ppm (dd, $\gamma$-proton). The compound has an absorption at 1320 and 1530 (nitro), 1640 (carbonyl), 3010, and 980 $cm^{-1}$ (trans double bond) in the IR spectra. From these spectroscopic data it was deduced that the compound was a nitro derivative of piperine. The position of the nitro group was established to be in the 6′ position of the aromatic ring as the other two aromatic protons gave singlets in the PMR spectrum. The compound (MNAP) was assigned the structure 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2(*E*),4(*E*)-pentadienyl]piperidine.

Compound MNOP showed a molecular ion peak at $m/z$ 330 and fragment peaks at $m/z$ 300 $(M - NO)^+$ and 285 $(M - NO_2)^+$. The PMR spectrum in $CDCl_3$ showed a two-proton singlet at 6.1 ppm (methylenedioxy), two six-proton multiplets at 1.7 ppm (piperidine 3,4,5 protons split magnetically due to addition of nitro group), two four-proton multiplets at 3.29–3.63 ppm (piperidine 2,6 protons split magnetically due to nitro addition), a one-proton doublet of the aromatic proton at 6.83 ppm (C-5′ aromatic proton), a one-proton double doublet of the aromatic proton at 7.02 ppm (C-6′ aromatic proton), a one-proton doublet of the aromatic proton at 6.69 ppm (C-2′ aromatic proton), a one-proton doublet at 7.79 ppm ($\beta$-olefinic proton), a one-proton double doublet at 6.62 ppm ($\gamma$-olefinic proton), and a one-proton doublet at 7.11 ppm ($\delta$-olefinic proton). When the chemical shift reagent $Eu(fod)_3$ was added, the olefinic protons shifted as follows: 8.22 (d, $\beta$-proton), 7.26 (d, $\delta$-proton), and 7.65 ppm (dd, $\gamma$-proton). The compound has an absorption at 1320 and 1530 (nitro), 1630 (carbonyl), 3010, and 980 $cm^{-1}$ (trans double bond) in the IR spectra. These data indicated the compound to be a mononitro derivative of piperine with the nitro substitution at the olefinic $\alpha$ position. The compound (MNOP) was assigned the structure 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine.

Compound DNP showed a molecular ion peak at $m/z$ 375 and fragment peaks at $m/z$ 330 $(M - NO_2)^+$ and 285 $(M - NO_2$ and $NO)^+$. The PMR spectrum in $CDCl_3$ showed a two-proton singlet at 6.18 ppm (methylenedioxy), two six-proton multiplets at 1.64 ppm (piperidine 3,4,5 protons split magnetically due to addition of nitro group), two four-proton multiplets at 3.3–3.52 ppm (piperidine 2,6 protons split magnetically due to nitro addition), two singlets of the aromatic protons 7.55 (C-5′ proton) and 7.03 ppm (C-2′ proton), a one-proton doublet at 7.78 ppm ($\beta$-olefinic proton), a one-proton double doublet at 6.64 ppm ($\gamma$-olefinic proton), and a one-proton doublet at 7.55 ppm ($\delta$-olefinic proton). When the chemical shift reagent $Eu(fod)_3$ was added, the olefinic protons shifted as follows: 8.66 (d, $\beta$-proton), 8.0 (d, $\delta$-proton), and 8.45 ppm (dd, $\gamma$-proton). The compound has an absorption at 1320 and 1530 (nitro), 1640 (carbonyl), 3010 and 980 $cm^{-1}$ (trans

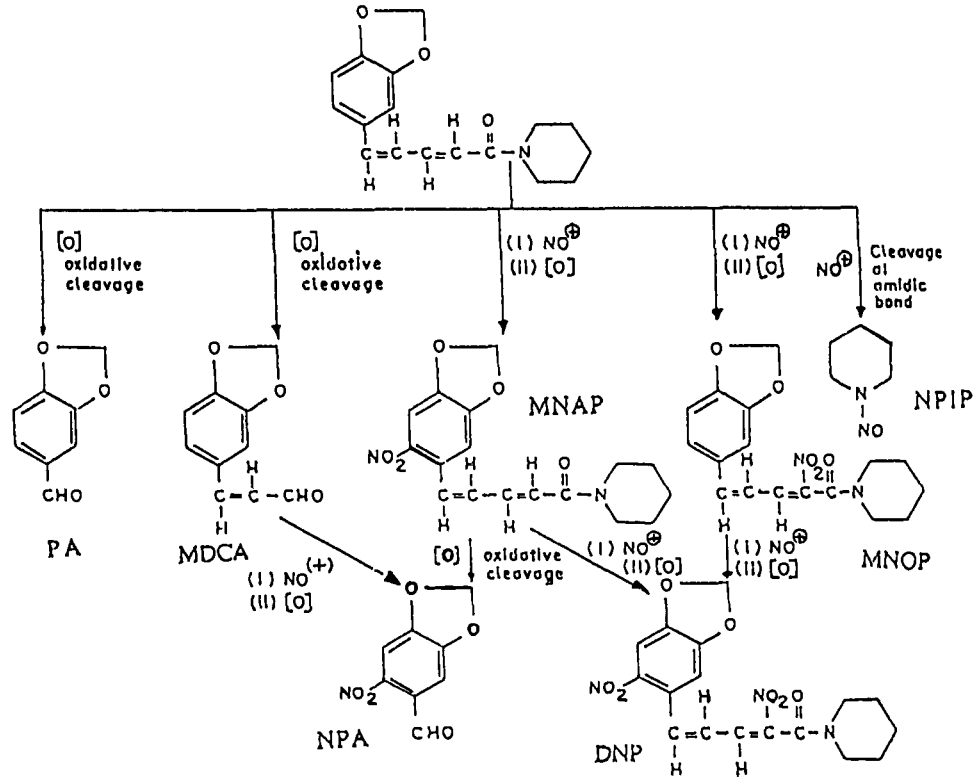


**Figure 2.** Mechanism of nitrosation of piperine. The compounds identified from the nitrosation of piperine are piperonal (PA), 6-nitropiperonal (NPA), 3,4-(methylenedioxy)cinnamaldehyde (MDCA), 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2(*E*),4(*E*)-pentadienyl]piperidine (MNAP), 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine (MNOP), 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine (DNP), *N*-nitrosopiperidine (NPIP).

**Table I. Yield of the Nitrosation Products of Piperine**

| | % yield on nitrosation using | | |
|---|---|---|---|
| compd formed | $HNO_2$ | NOCl | $N_2O_4$ |
| PA | 0.93 | 3.23 | 2.67 |
| NPA | 1.48 | 3.66 | 2.93 |
| MDCA | 0.63 | 1.95 | 1.14 |
| MNAP | 0.55 | 2.25 | 1.65 |
| MNOP | 1.54 | 6.75 | 5.54 |
| DNP | 0.64 | 1.81 | 1.60 |
| NPIP | 2.71 | 5.26 | 4.76 |

double bond) in the IR spectra. From these spectroscopic data it was deduced that the compound was a dinitro derivative of piperine with one of the nitro groups substituted at the $\alpha$-olefinic position and the other at the 6′ position of the aromatic ring. The compound (DNP) was assigned the structure 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine.

The elemental analysis of compounds MNAP, MNOP, and DNP matched within 0.3% of the required value. The yields of the compounds identified from the nitrosation of piperine using the different nitrosating agents are listed in Table I. As can be seen from this table, both NOCl and $N_2O_4$ are very efficient nitrosating agents and give higher yields of the nitro/nitroso compounds than nitrous acid.

The mechanism of nitrosation of piperine is outlined in Figure 2. Nitrous acid, NOCl, and $N_2O_4$ behave as both nitrosating and oxidizing agents in this reaction. They give $NO^+$ as follows (which reacts with piperine):

$$2HNO_2 \rightleftharpoons NO^+\text{-}NO_2^- + H_2O$$

$$NOCl \rightleftharpoons NO^+ + Cl^-$$

$$N_2O_4 \rightleftharpoons NO^+ + NO_2^-$$

Piperine undergoes oxidative cleavage at the amide bond and at the olefinic double bonds to form piperonal and 3,4-(methylenedioxy)cinnamaldehyde. Both compounds probably undergo nitrosation followed by oxidation to form 6-nitropiperonal. Although the nitro derivative of MDCA has not been isolated, it might very well be formed and may be present in small amounts. Cleavage of piperine at the amide bond and subsequent N-nitrosation of the cleaved piperidine molecule result in the formation of the carcinogenic *N*-nitrosopiperidine. The three nitro derivatives formed from uncleaved piperine are MNAP, MNOP, and DNP. Two of these three, MNAP and MNOP, are formed by C-nitrosation of the aromatic ring at the 6′ position and across the olefinic bond at the $\alpha$ position, respectively, followed by oxidation forming mononitro derivatives of piperine. The dinitro derivative of piperine, DNP, is formed by C-nitrosation followed by oxidation at two positions, the aromatic 6′ position and the olefinic $\alpha$ position. The dinitro derivative could also result from nitrosation followed by oxidation of the mononitro derivatives, MNOP and MNAP, at the aromatic 6′ position and the olefinic $\alpha$ position, respectively.

**Mutagenicity Studies of the *C*-Nitro Derivatives of Piperine.** Earlier researchers have detected *N*-nitrosopyrrolidine and NPIP, two carcinogenic nitrosamine in cured meats (Sen et al., 1973, 1974). The study linked the presence of NPIP to the pepper present in the curing mixture and urged a full investigation of the reaction between the ingredients used in the curing mixture, viz., between nitrite, nitrate, and the amine components present in the spices, viz., pepper and paprika. Since then, others have observed mutagenic activity in the piperine nitrite reaction mixture and have attributed it to NPIP, NPA and other unidentified nitro derivatives. *N*-Nitrosopiperidine is a known carcinogen, and NPA has been proven to be a mutagen to *S. typhimurium* (Osawa and Namiki,

Table II. Mutagenic Activity of UPNM, NPA, MNOP, MNAP, and DNP to *S. typhimurium* TA 98 and TA 100

| concn of mutagen, μg/plate | His+ revertants per plate | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UPNM | | | | NPA | | | | MNOP | | | | MNAP | | | | DNP | | | |
| | TA 98 | | TA 100 | | TA 98 | | TA 100 | | TA 98 | | TA 100 | | TA 98 | | TA 100 | | TA 98 | | TA 100 | |
| | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 | +S-9 | -S-9 |
| 40 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 070 | 380 | 045 | 105 |
| 60 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 075 | 525 | 060 | 120 |
| 80 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 085 | 690 | 115 | 175 |
| 100 | 085 | 110 | 051 | 261 | 055 | 065 | 010 | 720 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 090 | 710 | 120 | 205 |
| 200 | 165 | 210 | 060 | 440 | 105 | 125 | 035 | 2159 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | | | | |
| 300 | 250 | 385 | 070 | 725 | 120 | 218 | 065 | 2990 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | 000 | | | | |

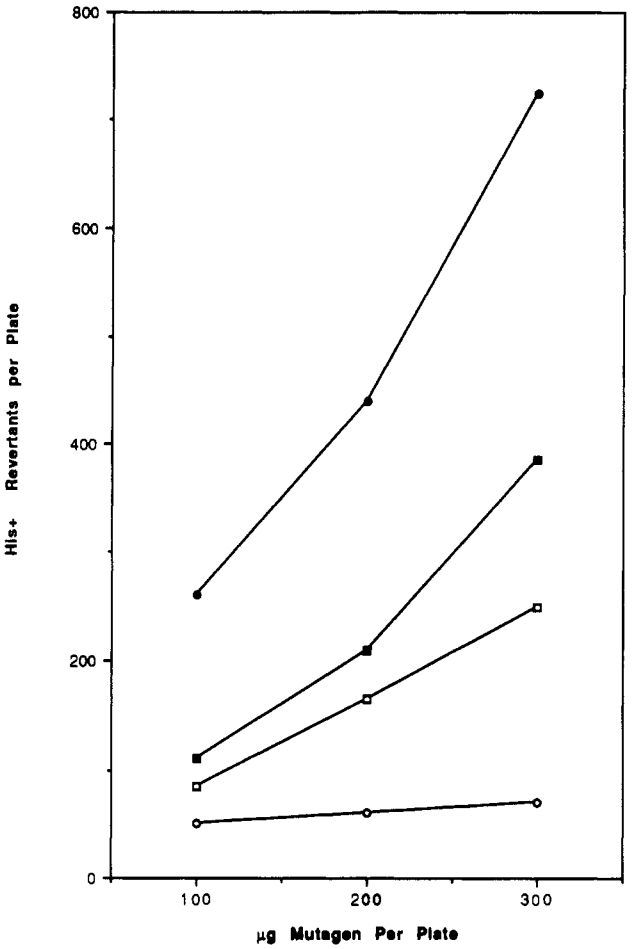


**Figure 3.** Mutagenic activity of the piperine nitrosation reaction mixture toward *S. typhimurium* strains TA 98 with S-9 (□) and without S-9 (■) and TA 100 with S-9 (○) and without S-9 (●).

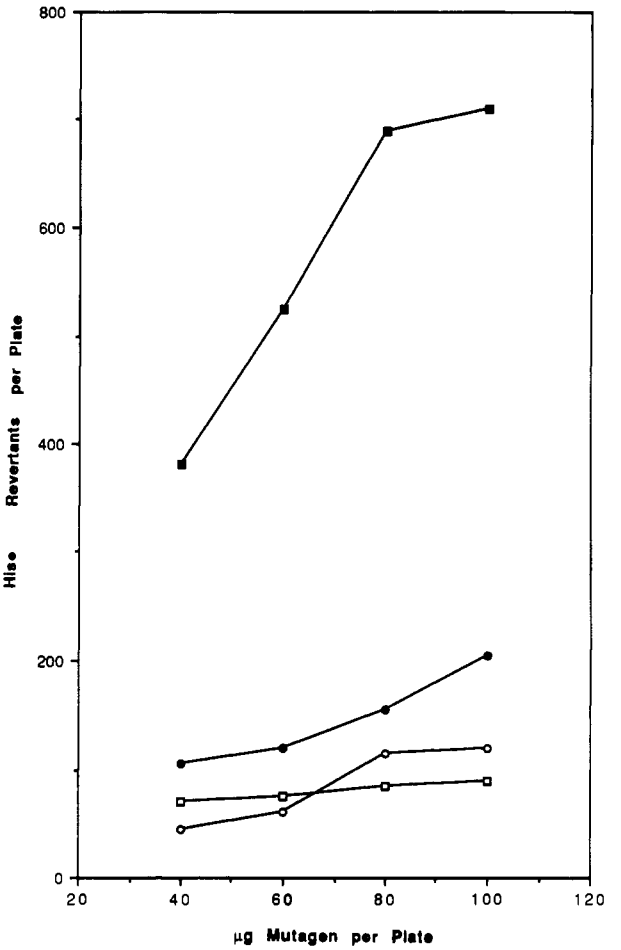


**Figure 4.** Mutagenic activity of DNP toward *S. typhimurium* strains TA 98 with S-9 (□) and without S-9 (■) and TA 100 with S-9 (○) and without S-9 (●).

1982; Osawa et al., 1982). There are no studies indicating the mutagenicity of PA and MDCA. We studied the mutagenicity of the unfractionated reaction mixture and the four nitro derivatives of piperine, NPA, MNAP, MNOP, and DNP. Their mutagenic activities toward *S. typhimurium* strains TA 98 and TA 100 with and without S-9 mix are given in Table II.

Figure 3 shows the mutagenic activity of the unfractionated ethyl acetate extract of the nitrosation reaction mixture of piperine (with nitrous acid). The results indicate that the total reaction mixture is mutagenic to both *S. typhimurium* strains TA 98 and TA 100 without S-9 mix, exhibiting a dose-dependent activity. The mutagenicity of the reaction mixture decreased considerably on addition of the S-9 mix.

Figure 4 indicates the mutagenic activity of DNP to *S. typhimurium* strains TA 98 and TA 100 with and without S-9 mix. As can be seen, a dose–response curve was obtained between 40 and 100 μg/plate. DNP exhibits mutagenic activity toward both *S. typhimurium* strains without S-9 mix. On addition of S-9 mix, the activity was reduced.

The two mononitro derivatives of piperine, MNOP and MNAP, did not exhibit any mutagenic activity toward the strains used by us. Only the dinitro derivative indicated mutagenic activity in our assay system. Researchers have suggested that the *C*-nitro group at the conjugated double bond or aromatic *C*-nitro groups are essential structural features for mutagenicity of dienoic carbonyl compounds (Nakamura et al., 1981; Osawa and Namiki, 1982; Osawa et al., 1982). All three nitro derivatives MNOP, MNAP, and DNP exhibit the structural features considered necessary for mutagenicity. However, our data show both the mononitro aromatic MNAP and mononitroolefinic MNOP to be nonmutagenic. Studies using other types of assays will assist in proving their nonmutagenicity. The inactivation of the mutagenic activity of DNP as well as that of the reaction mixture by

S-9 mix is of interest from the viewpoint of the detoxification systems in the liver. A more detailed study is required to justify this supposition.

A number of other food constituents, viz., ascorbic acid, α-tocopherols, vegetable juices, plant phenolics, flavanoids, etc., are known to modify the mutagenic potential of preformed or endogenously formed mutagens (Archer, 1984; Bhattacharya et al., 1987; Shetty et al., 1988; Francis et al., 1989; Shenoy and Choughuley, 1989). There are several naturally occurring compounds that render protective action against mutagens. Furthermore, the amount of nitrite and piperine used in our experiments exceeds the amount consumed in normal diets. Along with the protective factors present in the diet, formation of the nitro derivatives of piperine will be a multifactorial and competitive event.

Future goals for this research project are detailed in-vivo studies as well as mutagenicity studies of the piperine nitrosation products using different assay systems, characterization and then synthesis of the many unidentified minor products detected in the piperine and aqueous nitrite reaction mixture, simulation of the piperine–nitrite reaction under physiological conditions, and development of high-sensitivity analytical techniques to detect the minor reaction products.

## ABBREVIATIONS USED

Piperonal (PA); 6-nitropiperonal (NPA); 3,4-(methylenedioxy)cinnamaldehyde (MDCA); 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2(*E*),4(*E*)-pentadienyl]piperidine (MNAP); 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)pentadienyl]piperidine (MNOP); 1-[5-(1,3-benzodioxol-6-nitro-5-yl)-1-oxo-2-nitro-2(*E*),4(*E*)-pentadienyl]piperidine (DNP); *N*-nitrosopiperidine (NPIP); unfractionated piperine nitrosation reaction mixture (UPNM); dimethyl sulfoxide (DMSO); infrared (IR); ultraviolet (UV); proton magnetic resonance (PMR); high-pressure liquid chromatography (HPLC); gas chromatography (GC); flame ionization detector (FID).

## ACKNOWLEDGMENT

We are thankful to Dr. Padmanabh Pradhan, Bioorganic Div., Bhabha Atomic Research Centre, Bombay, for helpful discussions on the structural elucidation of some compounds reported in the study.

## LITERATURE CITED

Archer, M. C. Catalysis and inhibition of N-nitrosation reactions. In *N-nitroso compounds: Occurrence, biological effects and relevance to human cancer*; O'Neill, I. K., VonBorstel, R. C., Bartsch, H., Eds.; IARC Scientific Publication 57; IARC: Lyon, France, 1984; pp 263–270.

Archer, M. C.; Hathcock, J. N. Hazards of nitrate, nitrite and N-nitroso compounds. *Nutr. Toxicol.* **1982**, *1*, 327–380.

Bhattacharya, R. K.; Francis, A. R.; Shetty, T. K. Modifying role of dietary factors on the mutagenicity of aflatoxin $B_1$: In vitro effect of vitamins. *Mutat. Res.* **1987**, *188*, 121–128.

Digenis, G. A.; Issidorides, C. H. Some Biochemical aspects of N-nitroso Compounds. *Bio-Org. Chem.* **1979**, *8*, 97–137.

Doll, R.; Peto, R. The causes of Cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66*, 1191–1308.

Francis, A. R.; Shetty, T. K.; Bhattacharya, R. K. Modifying role of dietary factors on the mutagenicity of aflatoxin $B_1$: In vitro effect of plant flavanoids. *Mutat. Res.* **1989**, *222*, 393–401.

Hotchkiss, J. H.; Cassens, R. G. *Nitrate, Nitrite and Nitroso Compounds in Foods, A Scientific Status Summary*; Institute of Food Technologists, Expert Panel on Food Safety and Nutrition: Chicago, IL, 1987; pp 1–9.

Lijinsky, W. Standard setting for nitrites and nitrosamines. In *Origins of Human Cancer*; Magee, P. N., Ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1977; pp 1807–1811.

Lijinsky, W.; Conrad, E.; Van-de-Bogart, R. Nitrosamines formed by drug/nitrite interaction. *Nature* **1972**, *239*, 165–167.

Magee, P. N. *Nitrosamines and Human Cancer*; Banbury Report 12; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1982.

Maron, D.; Ames, B. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* **1983**, *113*, 173–215.

Morton, M. S.; Wilcox, K. Synthesis of nitrosyl chloride. *Inorg. Synth.* **1954**, *4*, 48–50.

Nakamura, M.; Katoh, K.; Kawabata, T. Precursors to nitrosopyrrolidine in black pepper treated with nitrite. *Agric. Biol. Chem.* **1981**, *45*, 1257–1259.

Osawa, T.; Namiki, M. Mutagen formation in the reaction of nitrite with the food components analogous to sorbic acid. *Agric. Biol. Chem.* **1982**, *46*, 2299–2304.

Osawa, T.; Namiki, M.; Namiki, K. Mutagen formation in the nitrite piperic acid reaction. *Agric. Biol. Chem.* **1982**, *46*, 3105–3108.

Sen, N. P.; Miles, W. F.; Donaldson, B.; Panalaks, T.; Iyengar, R. J. Formation of nitrosamines in a meat curing mixture. *Nature* **1973**, *245*, 104–105.

Sen, N. P.; Miles, W. F.; Donaldson, B.; Panalaks, T.; Iyengar, R. J. Effect of sodium nitrite concentration on the formation of NPY and NDMA on fried bacon. *J. Agric. Food Chem.* **1974**, *22*, 540–541.

Shenoy, N. R.; Choughuley, A. S. U. Effect of certain plant phenolics on N-nitrosamine formation. *J. Agric. Food Chem.* **1989**, *37*, 721–725.

Shetty, T. K.; Francis, A. R.; Bhattacharya, R. K. Modifying role of vitamins on the mutagenicity of N-methyl-N'-nitro-N-nitrosoguanidine. *Carcinogenesis* **1988**, 1513–1515.

Tannenbaum, S. R. Endogenous formation of nitrite and N-nitroso compounds. In *Naturally occurring carcinogens, mutagens and modulators of carcinogenesis*; Miller, J. S., et al., Eds.; University Park Press: Baltimore, MD, 1979; pp 211–220.

Received for review April 27, 1992. Accepted July 27, 1992.

**Registry No.** MNAP, 143706-35-8; MNOP, 143706-36-9; DNP, 143706-37-0; PA, 120-57-0; NPA, 712-97-0; MDCA, 14756-00-4; NPIP, 100-75-4; $HNO_2$, 7782-77-6; NOCl, 2696-92-6; $N_2O_4$, 10544-72-6; piperine, 94-62-2.