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C-Nitrosation of Sesamol and Its Effects on N-Nitrosamine Formation *in Vitro*

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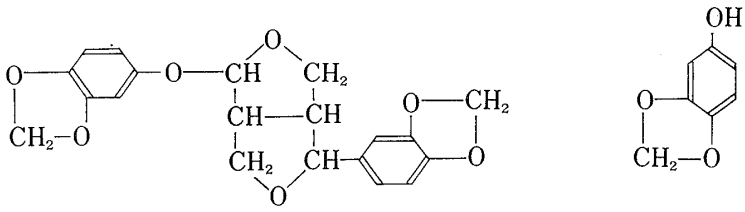
Sesamol (II), a monophenolic antioxidant present in sesame oil, was found to interact readily with nitrite. In dilute aqueous solutions with II in excess, it rapidly and completely removed nitrite to produce an equivalent amount of 3,4-methylenedioxy-6-nitrosophenol (III); the rate was fastest at pH 2.0, followed by pH 3.0 and 4.0 in that order. In dilute solutions with nitrite in excess, the amount of III formed, which corresponded well to that of nitrite lost, was less than that of II initially present, and some side reactions may have occurred. Compound II either prevented the N-nitrosation of dimethylamine, as did pyrocatechol and L-ascorbic acid, or accelerated the reaction, depending upon the conditions. Compound III showed a catalytic effect on the N-nitrosation.

Keywords——sesamol; 3,4-methylenedioxy-6-nitrosophenol; nitrite; N-nitrosodimethylamine; C-nitrosation; N-nitrosation; pyrocatechol; L-ascorbic acid

Nitrite is used in many countries as a food additive, but it readily produces nitrosamines by reaction with secondary amines at gastric pH,[2] and these compounds are potential carcinogens.[3] Several compounds that are endogeneous to foodstuffs or may be added to food for preservative purposes are known to influence nitrosamine formation. Ascorbic acid,[4] sorbic acid[5] and unsaturated fatty acids[6] are known to inhibit the formation of nitrosamines. With phenolic compounds, including polyphenolic constituents of plants and antioxidants of oils, two opposing effects have been reported on the rate of nitrosamine formation, depending upon the conditions,[7–11] but the details are still obscure.



I
sesamolin

II
sesamol

Chart 1

1) Location: *1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.*
2) J. Sander and F. Seif, *Arzneim.-Forsh.*, **19**, 1091 (1969).
3) H. Druckrey, R. Preussman, S. Ivankovic, and D. Schmaehl, *Z. Krebsforsh.*, **69**, 103 (1967).
4) S.S. Mirvish, L. Wallcave, M. Eagen, and P. Shubik, *Science*, **177**, 65 (1972).
5) K. Tanaka, K.C. Chung, H. Hayatsu, and T. Kada, *Fd. Cosmet. Toxicol.*, **16**, 209 (1978).
6) T. Kurechi and K. Kikugawa, *J. Food Sci.*, **44**, 1263 (1979).
7) J.I. Gray and L.R. Dugan, *J. Food Sci.*, **40**, 981 (1975).
8) B.C. Challis and C.D. Bartlett, *Nature (London)*, **254**, 532 (1975).
9) E.A. Walker, B. Pignatelli, and M. Castegnaro, *Nature* (London), **258**, 176 (1975).
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Sesame oil, which is widely used as an edible oil and as a solvent for injections, contains three constituents: sesamin, sesamolin (I) and sesamol (II).[12,13] Sesamol (3,4-methylenedioxyphenol, II), a monophenolic antioxidant, is formed from I during the processing of sesame oil. During our investigations on this antioxidant, it was found that II readily reacted with nitrite to undergo C-nitrosation, and affected the N-nitrosation of secondary amines. This paper describes the loss of nitrite by reaction with II and its effects on *in vitro* N-nitrosodimethylamine formation.

Experimental

Sesamol (II) was purchased from Aldrich Chemical Company and recrystallized from $CHCl_3$–petroleum ether.[14] Pyrocatechol (Wako Pure Chemical Industries, Ltd.), L-ascorbic acid (Daiichi Pure Chemicals Company, Ltd.), and dimethylamine hydrochloride (Kanto Chemical Company, Inc.) were also commercial products. Phenol (Wako Pure Chemical Industries, Ltd.) was used after distillation *in vacuo*. N-Nitrosodimethylamine (standard for gas chromatography) showing $\lambda_{max}^{CHCl_3}$ 351 nm (ε: 110) was a product of Wako Pure Chemical Industries, Ltd. Griess reagent was prepared as follows. A solution of 1% sulfanylic acid in 30% glacial acetic acid and a solution of 1% α-naphthylamine in 30% glacial acetic acid were separately prepared and combined in equal volumes before use.

Absorption spectra were taken with a Shimadzu UV-200S double-beam spectrophotometer. Nuclear magnetic resonance spectra were taken with a JEOL SP-100 machine. Thin-layer chromatography was performed with Wakogel B-5F. A Yanaco G80 gas chromatograph equipped with a hydrogen flame ionization detector and a glass column (3 mm I.D. × 3 m) of polyethylene glycol 6000 on 80—100 mesh Chromosorb W AW was used to determine N-nitrosodimethylamine. The chromatograph was operated isothermally at 130° (column temperature) and at 150° (injection temperature) with a carrier nitrogen gas flow of 20 ml/min. The amount of nitrosamine was determined by comparing the peak areas of samples with that of an authentic standard solution (5 μl, 0.40 mg/ml).

Nitrososesamol (3,4-Methylenedioxy-6-nitrosophenol, III)——Method A: A solution of 100 mg (0.725 mmol) of sesamol (II) in a mixture of 2.0 ml of ethanol and 0.2 ml of glacial acetic acid was treated with 1.2 ml (1.2 mmol) of 1 M $NaNO_2$. The solution, which became amber-colored spontaneously, was allowed to stand at room temperature. After 5 min a green precipitate began to separate, and thin-layer chromatography (solvent: AcOEt) of the supernatant revealed one major yellow spot (*Rf* 0.5) in addition to a 2,4-dichloroquinone chloroimide-positive spot (*Rf* 0.8) corresponding to II. After one hour the precipitate was collected by filtration and dried over P_2O_5 *in vacuo*. Ninety-two milligrams (yield, 76.0%) of dark green needles of III was obtained. Recrystallization from ethanol gave pure dark green needles, which decomposed at 216—217° (softening around 210°). Nuclear magnetic resonance spectrum (d_6-dimethylsulfoxide; internal standard, tetramethylsilane) δ, ppm: 5.90 (1H, s, Ph), 6.08 (2H, s, $-CH_2-$), 6.48 (1H, s, Ph) and 12.97 (1H, bs, OH or NOH). Absorption spectra: $\lambda_{max}^{0.7N\ HCl}$ nm ($\varepsilon \times 10^{-3}$), 226 (9.6), 315 (10.5), 370 (3.1), 395 (3.0); $\lambda_{max}^{0.01N\ NaOH}$ 240 (9.2), 345 (11.9), 423 (6.6); $\lambda_{max}^{H_2O}$ 225 (8.5), 315 (10.3), 370 (3.1), 395 (3.0). The pK_a value of III determined spectrophotometrically was 7.9, while that of II was 10.3. *Anal.* Calcd. for $C_7H_5NO_4$: C, 50.30; H, 3.02; N, 8.38%. Found: C, 50.26; H, 3.00; N, 8.09%.

Method B: A solution of 56 mg (0.40 mmol) of II in 19 ml of 0.15 M citrate buffer (pH 3.0) was treated with 1.0 ml of 1.0 M $NaNO_2$ (20 mM II + 50 mM $NaNO_2$). The mixture was incubated at 37° for 5 min. The crystalline precipitate was collected by filtration and redissolved in 2.0 liters of 0.01 N NaOH. The absorption spectrum of the solution was recorded as soon as possible, and showed the characteristic profile of III. The absorbancy at 423 nm was 0.861, which corresponded to 0.26 mmol of III. Thus, the yield of III precipitated was estimated to be 65%.

Measurement of the Loss of Nitrite——A solution of $NaNO_2$ in citrate buffer (10 ml) was treated with phenol, sesamol (II), pyrocatechol or L-ascorbic acid for a selected period in a thermostatically controlled water-bath. The buffers were 0.05 M or 0.15 M sodium citrate adjusted to the required pH by addition of concentrated hydrochloric acid. Portions of 1.0 ml of the reaction mixture or the 1:250-diluted mixture were mixed with 5.0 ml of water containing 0.4 ml of Griess reagent. The absorbancy at 520 nm was recorded as a measure of nitrite content of the solution. The control of 0.1 mM $NaNO_2$ showed an absorbancy of 0.380. Measurement of the loss of nitrite above pH 4 was not reproducible, probably because the compounds reacted with nitrite during the azo-dye producing process with acidic Griess reagent.

Measurement of the Formation of Nitrososesamol (III)——A solution of $NaNO_2$ and sesamol (II) in 0.05 M citrate buffer (30 ml) at pH 2.0, 3.0 or 4.0 was allowed to stand at 20° for a selected period. Portions of 2.0

12) P. Budowski and K.S. Markley, *Chem. Rev.*, **48**, 125 (1951).
13) P. Budowski, *J. Am. Oil Chemists' Soc.*, **41**, 280 (1964).
14) E. Haslam and R.D. Haworth, *J. Chem. Soc.*, **1955**, 827.

ml were removed from the reaction mixture and mixed in 1.0 ml of 0.5 N NaOH. The absorbancies of the alkaline solution, which were stable for 10—15 min, were recorded as soon as possible; the ratio of the absorbancies at 420 nm to those at 350 nm was in the range of 0.4—0.55 (III showed a ratio of 0.55; Fig. 4). The observed absorbancies at 420 nm were multiplied by 1.5, which reflected the amount of III formed. The absorption spectra of $NaNO_2$ showed $\lambda_{max}^{H_2O,\ 0.1N\ NaOH}$ 356 nm (ε: 23) and $\lambda_{max}^{1N\ HCl}$ 356 (27), 361 (44), 390 (27). The absorbancies of 0.1 mM $NaNO_2$ and 1 mM $NaNO_2$ in alkaline medium were less than 0.002 and 0.023, respectively, and could thus be neglected.

Determination of N-Nitrosodimethylamine——Each of the test compounds, 1 M dimethylamine and 1 M $NaNO_2$ were added to 15.0 ml of citrate buffer. The mixtures (total volume, 20 ml) were incubated at 37° for 5.0 hr in stoppered flasks. The buffers used were 0.2 M sodium citrate buffers adjusted to the required pH with concentrated hydrochloric acid. Portions of 10 ml were removed from the reaction mixture and poured into 5 ml of 5 N NaOH containing 2.0 g of NaCl. The mixtures were extracted with 40 ml of $CHCl_3$. The extracts were directly subjected to ultraviolet and gas chromatographic analysis for N-nitrosodimethylamine (5 μl).

Results

Decrease of Nitrite due to Reaction between Sesamol (II) and Nitrite

Decreases in nitrite concentration on mixing with sesamol (II) in aqueous solutions were measured in terms of the production of azo-dye with Griess reagent. The rate of loss was measured and compared with those of known nitrite-consuming conpounds under three sets of conditions (A, B and C). The compounds used for comparison were phenol, which is of the parent structure of II and is known to undergo nitrosation,[15–17] diphenolic pyrocatechol, which might from a corresponding quinone by reaction with nitrite,[8] and L-ascorbic acid which is transformed into dehydroascorbic acid.[18]

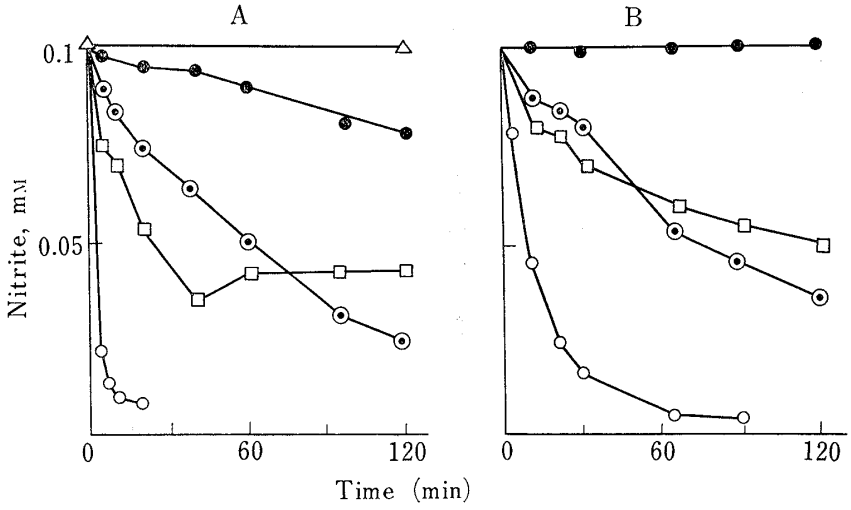


Fig. 1. Time Course of Loss of Nitrite in Mixtures of 0.1 mM $NaNO_2$ and 1.0 mM Test Compound in 0.05 M Citrate Buffer at 20°

Sesamol (II) (○), phenol (●), pyrocatechol (⊙), L-ascorbic acid (□) and control (△). A, pH 2.0; B, pH 3.0.

Figure 1 shows the time course of the loss of nitrite in mixtures of 0.1 mM $NaNO_2$ and 1.0 mM of each compound (Condition A: low concentrations with the compound in excess) at 20°. The rate of loss with every compound at pH 2.0 was faster than that at pH 3.0. The rate of loss with II was the fastest, and II had consumed most of the nitrite after 20 min

15) S. Veibel, *Chem. Ber.*, **63**, 1577 (1930).
16) B.C. Challis and A.J. Lawson, *J. Chem. Soc. B*, **1970**, 770.
17) B.C. Challis, *Nature* (London), **244**, 466 (1973).
18) H. Dahn, L. Loewe, and C.A. Bunton, *Helv. Chim. Acta*, **43**, 320 (1960).

at pH 2.0 or 60 min at pH 3.0. The rates with pyrocatechol and L-ascorbic acid were slower than that with II at both pH values. The rate with phenol was much slower than that with II, indicating that the 3,4-methylenedioxy function in II contributed to the rapid interaction of II with nitrite. Nitrite concentration with L-ascorbic acid increased after the concentration had fallen to a relatively low value (Fig. 1A). This effect was reproducible but the reasons for it are not clear.

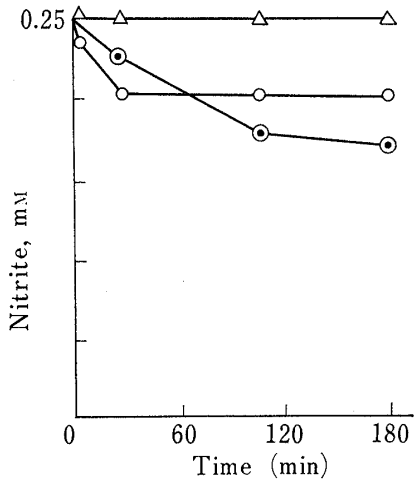


Fig. 2. Time Course of Loss of Nitrite in Mixtures of 0.25 mM $NaNO_2$ and 0.10 mM Test Compound in 0.05 M Citrate Buffer (pH 3.0) at 20°

Sesamol (II) (○), pyrocatechol (⊙) and control (△).

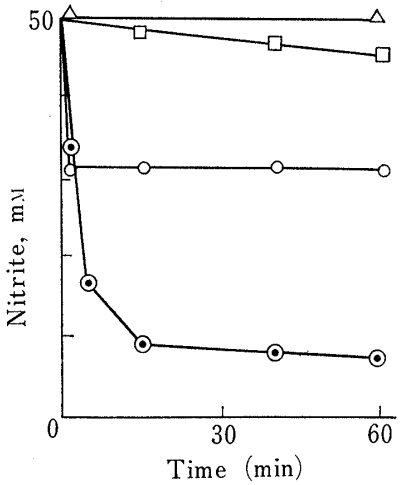


Fig. 3. Time Course of Loss of Nitrite in Mixtures of 50 mM $NaNO_2$ and 20 mM Test Compound in 0.15 M Citrate Buffer (pH 3.0) at 37°

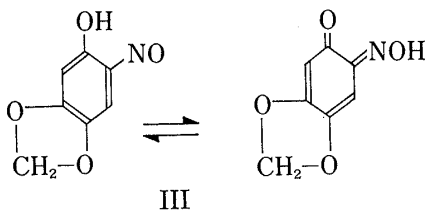
Sesamol (II) (○), pyrocatechol (⊙), L-ascorbic acid (□) and control (△).

Figure 2 shows the loss in a mixture of 0.25 mM $NaNO_2$ and 0.10 mM II or pyrocatechol (Condition B: low concentrations with nitrite in excess) at pH 3.0 and 20°. The loss with II was very rapid, but the concentration of nitrite did not decrease below 0.20 mM. Pyrocatechol, on the other hand, removed nitrite slowly.

Figure 3 shows the losses in mixture of 50 mM $NaNO_2$ and 20 mM of each compound (Condition C: high concentrations with nitrite in excess) at pH 3.0 and 37°. Sesamol (II) reacted with an equivalent of nitrite within 1 min, producing a dark green precipitate. Pyrocatechol reacted with more than 2 equivalents of nitrite during 15 min, forming a brown precipitate. L-Ascorbic acid gradually reacted with nitrite, evolving a brown gas. Under these conditions, the amount of nitrite lost with pyrocatechol was larger than that with II.

Identification and Properties of Nitrososesamol (III)

The nitrosated product (III) of II was readily obtained by the reaction of II and nitrite in a mixture of ethanol and glacial acetic acid. Compound (III) was isolated as dark green needles in a yield of 76%. Nitrosation of phenols occurs at the *para* or *ortho* position,[15,19] and the structure of III was proved to be 3,4-methylenedioxy-6-nitrosophenol by elemental analysis and from the nuclear magnetic resonance spectrum. Nitrososesamol (III) might exist in solution as a tautomeric mixture of nitrosophenol and oximequinone.[19] The com-



III

Chart 2

19) Rodd's Chemistry of Carbon Compounds, III, 2nd edition by S. Coffey, 346 (Elsevier, Amsterdam, 1971).

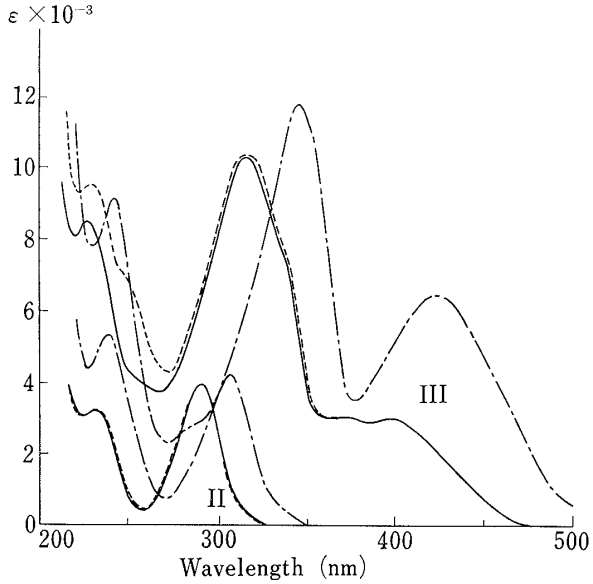


Fig. 4. Full Spectra of Sesamol (II) and Nitrososesamol (III)

Spectra were taken in water (——), 0.7 N HCl (– – –) and 0.01 N NaOH (—·—).

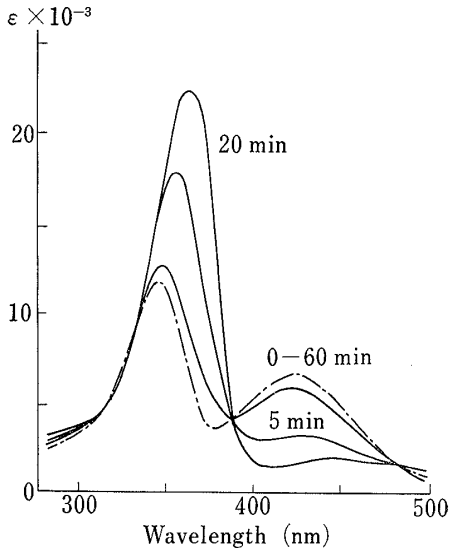


Fig. 5. Changes of the Spectra of 0.05 mM Nitrososesamol (III) in 0.01 N NaOH (—·—) and 1 N NaOH (——) at Room Temperature

ε values calculated on the molar basis of III.

pound showed a characteristic absorption spectrum having an absorption maximum (0.01 N NaOH) at 423 nm (ε: 6600) (Fig. 4). It was stable in a neutral or acidic medium but unstable in an alkaline medium, depending on the hydroxide ion concentration. In 0.01 N NaOH the spectrum of III did not change during 60 min, but in 1 N NaOH III changed within 20 min into an unidentified compound having absorption maxima at 367 (1 N NaOH) and 320 nm (0.1 N HCl). The spectral change showed isosbestic points at 310, 338 and 389 nm (Fig. 5).

When III was treated with nitrite in an acidic medium, the spectrum changed slightly. The absorption maximum of the solution at alkaline pH shifted from 423 to 435 nm, with a slight decrease in the absorbancy, when a mixture of 1.0 mM $NaNO_2$ and 0.1 mM III in citrate buffer (pH 3.0) was treated at 37° for 5 hours, while that of a control without $NaNO_2$ did not. This suggests that III interacts with nitrite in acidic media.

Formation of III by Reaction between II and Nitrite

The rate of formation of III in the reaction of II with nitrite was mesure in terms of the absorbancy at 420 nm under the three sets of conditions described above.

Figure 6A shows the time course of the formation of III in mixtures of 0.1 mM $NaNO_2$ and 1.0 mM II (Condition A) at 20°. The rate of increase in the absorbancy at 420 nm was faster with a lower pH. The concentration of III formed was 0.10 mM at pH 2.0, 0.086 mM at pH 3.0 and 0.006 mM at pH 4.0 after 20 min. At pH 2.0 the yield of III formed was quantitative and equivalent to the amount of nitrite lost (Fig. 1). The kinetics of formation of III were investigated at pH 4.0 at 20° in mixtures of 0.1 mM $NaNO_2$ and 5—20 mM II. Plots of $\log a_\infty/a_\infty - a_t$ against time (Fig. 7), indicated pseudo-first order kinetics with a rate coefficient, k, of 1—3 $M^{-1} \cdot min^{-1}$ (Table I).

Under condition B (0.25 mM $NaNO_2$ and 0.10 mM II), the concentration of III formed was 0.04 mM after 10 min at pH 3.0 and 20°, which was close to the loss of nitrite (Fig. 2). Figure 6B shows the time course under similar conditions (1.0 mM $NaNO_2$ and 0.10 mM II). The formation of III reached a maximum (0.04 mM) after 5 min at pH 2.0 and 3.0, and the absorbancy decreased thereafter. With nitrite in excess, the formation of III was not quantitative and the amount of the nitrite lost was less than that of II initially present. It is

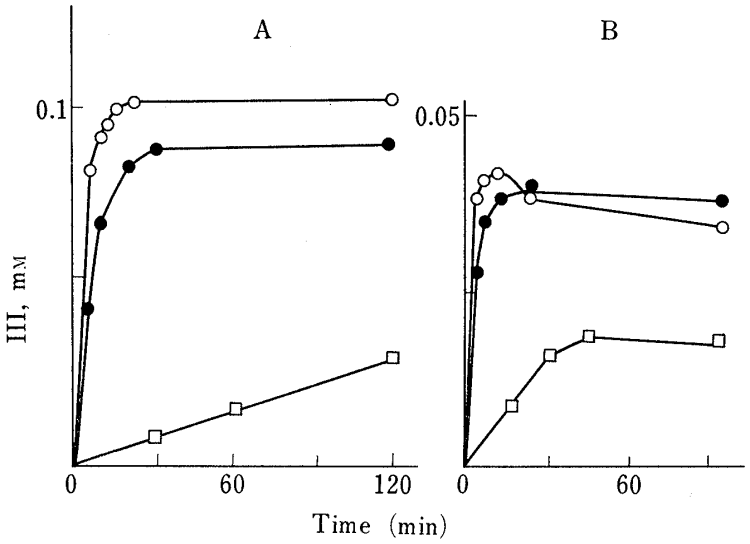


Fig. 6. Time Course of the Formation of Nitrososesamol (III) in Mixtures of $NaNO_2$ and II in 0.05 M Citrate at 20°, and pH 2.0 (○), 3.0 (●) and 4.0 (□)

An absorbancy at 420 nm in the alkaline medium of 0.660 corresponds to 0.1 mM III. Control experiments (0.1 mM $NaNO_2$ or 1.0 mM II in citrate, pH 2.0) did not show any increase in absorbancy during 120 min.
A: 0.1mM $NaNO_2$ and 1.0mM II, and B: 1.0mM $NaNO_2$ and 0.1mM II.

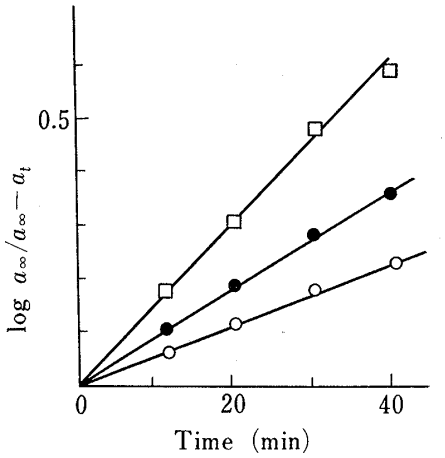


Fig. 7. Reaction Kinetics for Sesamol (II) and Nitrite (0.1 mM) in 0.05 M Citrate Buffer (pH 4.0) at 20°

II: 5 mM (○), 10 mM (●) and 20 mM (□). The absorbancy at 420 nm in the alkaline medium was measured. Plots of log $a_\infty/a_\infty - a_t$ against time were made; a_∞ is the experimentally determined infinite time absorbancy at 420 nm (0.660) and a_t is the absorbancy at "t" min.

Table I. Rate Coefficient of Formation of III at pH 4.0 and 20°[a)]

II Concentration (M)	k'	k ($M^{-1} \cdot min^{-1}$)
0.005	0.0127	2.54
0.010	0.0207	2.07
0.020	0.0345	1.73

a) Calculations were based on the results in Fig. 7.
$[NO_2^-] = 10^{-4}$ M.
$\log a_\infty/a_\infty - a_t = k't/2.303 = k[II]t/2.303$.

possible that III might be transformed into active nitrites by interaction with an excess nitrite, or that II might be converted into other active derivatives. Decreases in absorbancy on prolonged treatment (Fig. 6B) suggest the former possibility.

Under condition C, III readilly precipitated owing to its low solubility. The yield of precipitated III was estimated to be 65% based on II. Removal of III by precipitation resulted in increased formation of III, even though excess nitrite was present.

Effects of Sesamol (II) on Nitrosamine Formation

The effects of II on the N-nitrosation of dimethylamine were investigated. Dimethylamine undergoes N-nitrosation slowly in the presence of nitrite under acidic conditions.[20)] Dimethylamine (50 or 200 mM) and nitrite (50 or 200 mM) were incubated at pH 3.0 and 5.0, using various reactant concentrations, for 5.0 hours at 37°. For comparison, pyrocatechol and L-ascorbic acid were also tested under the same conditions. The results are listed in Table II. At pH 3.0, the presence of more than 100 mM II largely prevented the formation of N-nitrosodimethylamines; 20 mM II inhibited the formation by 40—50%, but the rate

20) S.S. Mirvish, *J. Natn. Cancer Inst.*, 44, 633 (1970).

was lower than expected. Thus, the formation of the nitrosamine with 50 mM $NaNO_2$ and 20 mM II was greater than that of the control with 30 mM $NaNO_2$, even though 50 mM nitrite would have been rapidly decreased to about 30 mM by II (20 mM). It was found that 1 mM II clearly accelerated nitrosamine formation at pH 5.0, although the same concentration of II had no effect at pH 3.0.

Table II. Effects of Sesamol (II), Nitrososesamol (III), Pyrocatechol and L-Ascorbic Acid on N-Nitrosodimethylamine Formation

Test compound	Reaction conditions[a]				N-Nitrosodimethylamine[b] mM		Ratio A/B
	pH	mM			A	B	
		$NaNO_2$	$(CH_3)_2NH$	Test compd.	Formed	Control	
II	3.0	50	200	100	0.3	16	0
	3.0	50	200	20	8 —11	16[c]	0.5—0.6[c]
	3.0	50	200	1	16 —18	16—18	1.0
	5.0	200	50	50	3.0	4.2	0.7
	5.0	50	200	1	2.5— 3	1— 1.5	2 —2.5
	5.0	200	50	1	6.0	4.9	1.2
III	3.0	50	200	1[d]	16 —18	16—18	1.0
	5.0	50	200	1[d]	1.5— 2	1— 1.5	1 —1.5
	5.0	200	50	1[d]	5.9	4.9	1.2
Pyrocatechol	3.0	50	200	20	0.3	16	0
	3.0	50	200	5	9.5—10	16	0.6—0.7
	5.0	200	50	50	0.2	2.4	0.1
	5.0	50	200	0.5	1 — 1.5	1— 1.5	1.0
	5.0	200	50	1	4.8	4.9	1.0
L-Ascorbic acid	3.0	50	200	100	0.3	16	0
	3.0	50	200	20	12 —13	16	0.7—0.8

a) Reaction conditions were as described in "Experimental."
b) Analysis of the N-nitrosodimethylamine was done by ultraviolet absorption measurement and/or gas chromatography. The results of several experiments are shown as ranges.
c) Control values with 30 mM $NaNO_2$ were 7.5—8.0 mM and the ratio was 1.1—1.4.
d) III was not completely dissolved because of its low solubility (<0.5 mM).

Nitrososesamol (III) at 1 mM also acted as an accelerator of nitrosamine formation at pH 5.0, although no effect was observed at pH 3.0. This acceleration might be explained by a catalytic action of III through interaction with nitrite under acidic conditions. III was not active for N-nitrosation, since it did not nitrosate the amine in the absence of $NaNO_2$. As the extraction of the nitrosamine was performed in a strongly basic medium, it is possible that alkali-labile III promoted N-nitrosation during the extraction process. However, this possibility can be eliminated, because nitrosamine could not be detected in the extract from a strongly basic mixture of 200 mM $NaNO_2$, 50 mM dimethylamine and 1 mM III.

Pyrocatechol and L-ascorbic acid inhibited nitrosamine formation; there was no acceleration even at low concentrations. At high concentrations, the inhibitory effect of pyrocatechol was greatest.

Discussion

Sesame oil is an edible oil, and is used as a solvent for injections (The Pharmacopoeia of Japan IX, The United States Pharmacopeia XIX). Sesamol (II) is a monophenolic antioxidant derived from sesamolin (I) and present in sesame oil, protecting the oil from autoxidation.[12,13]

Sesamol (II) was found to reduce nitrite rapidly in mildly acidic systems. The reaction was, however, a very complex one. In dilute mixtures with excess II, nitrite was removed

rapidly, forming an equivalent amount of nitrososesamol (III). The rate of removal of nitrite by II was fastest at pH 2.0, followed by pH 3.0 and 4.0 in that order. Kinetic studies on the reaction showed pseudo-first order kinetics with a rate coefficient of $k=1—3\,\text{M}^{-1}\cdot\text{min}^{-1}$ ($[NO_2^-]=10^{-4}\,\text{M}$, pH 4.0, 20°). The rate of reaction was much faster than that of the parent compound, phenol, which undergoes nitrosation in aqueous perchloric acid[15–17] with a rate coefficient of $k=0.65\,\text{M}^{-1}\cdot\text{min}^{-1}$ ($[NO_2^-]=3\times10^{-3}\,\text{M}$, pH 1.5, 25°).[17] The 3,4-methylenedioxy function in II may thus permit a more effective interaction with nitrite. The rate was also faster than those with pyrocatechol and L-ascorbic acid, which yield a corresponding quinone[8] and dehydroascorbic acid,[18] respectively.

In dilute mixtures with nitrite in excess, loss of nitrite was rapid but the amount was relatively small, corresponding to a half of II initially present. The amount of nitrite lost was close to that of III formed, indicating the side reactions did not involve removal of nitrite activity. In concentrated mixtures, the amount of nitrite lost was equivalent to that of II initially present, even when nitrite was present in excess.

The effects of II on the N-nitrosation of dimethylamine were also complex. A large amount of II suppressed the N-nitrosation almost completely through the complete removal of nitrite. A small amount of II, which removed only a little nitrite, accelerated the reaction. Nitrososesamol (III) was shown to accelerate the reaction catalytically at pH 5.0 at low concentrations.

Opposing effects of phenolic compounds on the rate of formation of nitrosamines have already been reported.[7–11] In the case of monophenols, Gray and Dugan[7] reported that tocopherol, vanillin and thymol inhibited the rates of N-nitrosations. Davies and McWeeny[10] have shown that *p*-cresol, a smoke phenol, accelerated the rate of N-nitrosation, and they assumed that the effect might be due to the nitrosated cresol produced, since the closely related *p*-nitroso-*o*-cresol catalytically accelerated the reaction. The present results obtained with II support their results and assumption.[10]

It should be noted that naturally occurring monophenols such as II readily interact with nitrite to remove available nitrite for N-nitrosation, but catalyze the N-nitrosation by producing the C-nitrosated phenol. Although the interactions of II with nitrite and its effects on N-nitrosation were largely dependent on the conditions, it should be emphasized that II could promote the formation of potentially carcinogenic nitrosamines even at a low concentration and relatively high pH.

With regard to polyphenols, inhibitory effects by propyl gallate, hydroquinone, mono-*tert*-butylhydroquinone,[7] pyrocatechol, pyrogallol, 4-methylcatechol and gallic acid[11] have been reported. However, gallic acid,[9] chlorogenic acid and 4-methylcatechol[8] showed accelerating effects, depending upon the conditions. In the present experiments, pyrocatechol did not show any accelerating effect on the formation of N-nitrosodimethylamine.