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Reaction of *p*-Hydroxycinnamic Acid Derivatives with Nitrite and Its Relevance to Nitrosamine Formation

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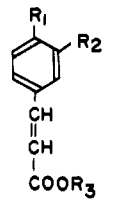
We studied loss of nitrite and inhibition of nitrosamine formation caused by naturally occurring *p*-hydroxycinnamic acid derivatives such as *p*-coumaric acid (I), ferulic acid (II), caffeic acid (III), and chlorogenic acid (IV). Compounds I, II, and III markedly reduced nitrite levels and inhibited nitrosamine formation. The potency of inhibition of nitrosamine formation was III > II > I > ascorbic acid ≫ cinnamic acid (V), and the order was almost the same as that of nitrite loss; III > II > I > IV > ascorbic acid > V. These effects are attributed to the chemical reactions of the phenolics with nitrite. From the reaction of monophenolics I and II with nitrite, many complicated products were isolated: product I(A), $C_8H_6N_2O_3$; I(B), $C_7H_6O_2$ (*p*-hydroxybenzaldehyde); I(D), $C_8H_5NO_3$; I(E), $C_8H_7NO_3$; II(A), $C_9H_8N_2O_4$; II(F), $C_9H_7NO_4$. Most of the reaction of nitrite with these monophenolics may be the addition reaction of nitrite to the olefinic group. Products I(A) and II(A) may be furoxan derivatives derived from dehydration of the nitroso–nitro or oxime–nitro compounds.

It has been well documented that nitrite reacts readily with secondary amines to produce carcinogenic nitrosamines (Sander and Seif, 1969; Druckrey et al., 1967). Nitrite may be present in some foodstuffs as an additive. It may be also yielded by salivary reduction from nitrate (Spiegelhalder et al., 1976). Certain compounds that are endogenous to foodstuffs or may be added as food additives exert effects on the nitrosamine formation from reaction of nitrite and secondary amines. Ascorbate (Mirvish et al., 1972), L-ascorbyl palmitate (Sen et al., 1976), acetals of ascorbate (Bharucha et al., 1980), unsaturated fatty acid (Kurechi and Kikugawa, 1979), soya products (Kurechi et al., 1981), and Japanese radish (Kurechi et al., 1980a) are known as inhibitors of the nitrosamine formation. Malondialdehyde (Kikugawa et al., 1980; Kurechi et al., 1980d) and alcohols (Kurechi et al., 1980c) are reported to be stimulatory on the nitrosamine formation. There are complicated effects, inhibition or stimulation, on the nitrosamine formation with the natural and synthetic phenolics. Polyphenols such as gallic acid and chlorogenic acid (Challis and Bartlett, 1975; Gray and Dugan, 1975; Nakamura and Kawabata, 1981; Sen et al., 1976; Walder et al., 1975; Yamada et al., 1978) and monophenolics such as α-tocopherol, sesamol, butylated hydroxyanisole, and cresol (Davies and McWeeny, 1977; Fiddler et al., 1978; Kurechi et al., 1979, 1980b; Pensabene et al., 1978; Walker et al., 1979) are inhibitory or stimulatory depending upon the conditions used.

In our previous paper (Kurechi et al., 1980a), it has been described that the inhibitory effect of Japanese radish on nitrosamine formation may be ascribed to unidentified unstable phenolics. This time, *p*-hydroxycinnamic acid derivatives which are widely distributed in plants and vegetables (Sosulski, 1979) were studied for nitrite loss, effects on nitrosamine formation, and reaction products between nitrite.

Chart I



I: *p*-coumaric acid; R_1=OH, R_2=R_3=H
II: ferulic acid; R_1=OH, R_2=OCH_3, R_3=H
III: caffeic acid; R_1=R_2=OH, R_3=H
IV: chlorogenic acid; R_1=R_2=OH, R_3=quinic acid
V: cinnamic aicd; R_1=R_2=R_3=H

MATERIALS AND METHODS

Materials. L-Ascrobic acid was the produce of Kanto Chemical Co., Inc. *p*-Coumaric acid (I), ferulic acid (II), caffeic acid (III), chlorogenic acid (IV), and *trans*-cinnamic acid (V) were the products of Tokyo Kasei Kogyo Co., Ltd. (see Chart I). Vanillin was purchased from Coso Chemical Co., Ltd. I, II, and V were dissolved at the indicated concentration by addition of 0.1 N or 1 N sodium hydroxide solution. Griess reagent was prepared by mixing an equal volume of 1.0% w/v sulfanilic acid in 30% glacial acetic acid and 1.0% w/v 1-naphtylamine in 30% glacial acetic acid just before use.

Analysis. Absorbances were measured with a Hitachi 101 spectrophotometer or a Shimadzu UV-200S double-

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beam spectrophotometer. Nitrosamines were determined by a Yanaco gas chromatograph and a glass column (3 mm i.d. × 2 m) of poly(ethylene glycol) 6000 (25%) on 80–100-mesh Chromosorb W AW. The chromatograph was operated isothermally at 120 °C (column temperature) and 140 °C (injection temperature) with a carrier nitrogen gas flow of 25 mL/min. The peaks were detected by a flame ionization detector. The amount of the nitrosamine was determined by comparing the peak area of the samples with that of each authentic standard (*N*-nitrosodimethylamine and *N*-nitrosodiethylamine; Wako Pure Chemical Industries, Ltd.) solution in chloroform (5 μL of solution of 5.0 mg/mL concentration).

Melting points were uncorrected. Mass spectra were obtained with a Hitachi RMU-7L double-focusing mass spectrometer. Nuclear magnetic resonance spectra (NMR) were taken in dimethyl-d_6 sulfoxide with a JEOL PS-100 machine with tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was performed on silica gel (Wako Pure Chemical Industries, Ltd.) by use of a solvent, benzene–ethanol (19:1). Spots were detected by irradiation with an ultravoilet (UV) light at 254 or 365 nm and by spraying 1% 2,6-dichloroquinone monochlorimide in ethanol (BQC reagent), specific to phenolic compounds (Gibbs, 1927). Silicic acid column chromatography was performed on silicic acid (100 mesh, analytical reagent) from Mallinckrodt, Inc.

Nitrite Disappearance Caused by the *p*-Hydroxycinnamic Acid Derivatives. The determination of nitrite concentration was performed as previously reported (Kurechi et al., 1980b). To 8.0 mL of 0.1 M citrate buffer (pH 2.0, 3.0, and 4.0) were added 1.0 mL of 1.0 mM sodium nitrite solution and 1.0 mL of a solution of each compound of the appropriate concentration. The mixture was incubated at 37 °C during the period of 90 min or 2 h in 50-mL content stoppered tubes. A 1.0-mL portion of each reaction mixture was poured into 5.0 mL of water containing 0.4 mL of Griess reagent. The absorbance at 520 nm of the solution was recorded after the solution was kept at room temperature for 15 min.

Effect of the *p*-Hydroxycinnamic Acid Derivatives on Nitrosamine Formation. The experiments were carried out as has been previously reported (Kurechi et al., 1980a). To 25 mL of 0.2 M citrate buffer (pH 3.0, 4.0, and 5.0) were added 1.25 mL of 2 M sodium nitrite, 1.25 or 2.50 mL of 0.2 M sample solution, and 5.0 mL of 1 M dimethyl- (or diethyl-) amine hydrochloride in that order. The pH values of the mixture were quickly readjusted by addition of concentrated hydrochloric acid, and the mixtures were made up to 50 mL with water. The mixtures were then incubated at 37 °C for 5 h in stoppered 100-mL flasks. The pH values of the reaction mixtures were constant throughout the reaction.

Portions of 10 mL were extracted with 40 mL of chloroform in the presence of 2.0 g of sodium chloride and 5.0 mL of 5 N sodium hydroxide (extraction 1) or 2.0 g of sodium chloride alone (extraction 2). Since Yamamoto et al. (1979) described that the amount of the nitrosamine in certain reaction mixtures was altered by alkalization before extraction, the extraction of the nitrosamine in the present study was performed in two ways. The amount of the nitrosamines in the extract (5 μL) was determined by gas chromatography as soon as possible. Recoveries of the nitrosamines in the extracts were quantitative.

Reaction of *p*-Coumaric Acid (I) with Nitrite. To a solution of 20.97 g (0.30 mol) of sodium nitrite in 125 mL of water was added a solution of 4.5 g (27 mmol) of *p*-coumaric acid (I) in 125 mL of ethanol. The mixture was adjusted at pH 3 with concentrated hydrochloric acid and kept at room temperature for 2 days. The reaction mixture was then extracted with 150 mL of chloroform twice and the extract was evaporated to dryness to obtain 5.8 g of gum. The gum was applied to a column (2 cm i.d. × 40 cm) of 55 g of silicic acid and eluted with chloroform. Fractions a, b, and c were obtained. Fraction a was rechromatographed on a column of 20 g of silicic acid and by stepwise elution with benzene and benzene–ethanol (49:1) to afford three products in crystalline forms: product I(A), needles from benzene, 137 mg, mp 194 °C dec; product I(B), yellow powderic crystals from chloroform, 435 mg; and product I(D), columns from benzene–ethanol, 40 mg, mp 183 °C dec. Fraction b gave product I(C), needles from benzene, 206 mg, mp 65–111 °C. Fraction c was rechromatographed on a column of 15 g of silicic acid, eluted with benzene–ethanol (19:1), and gave I(E), yellow leaflets from chloroform, 72 mg, mp 153–163 °C. Products I(A), I(B), I(C), I(D), and I(E) were recrystallized, and their physicochemical properties are listed in Table I.

Reaction of Ferulic Acid (II) with Nitrite. To a solution of 20.97 g (0.30 mol) of sodium nitrite in 125 mL of water was added a solution of 4.5 g (23 mmol) of ferulic acid (II) in 125 mL of ethanol. The mixture was adjusted at pH 3 with concentrated hydrochloric acid and kept at room temperature for 2 days. The reaction mixture was then extracted with 150 mL of chloroform twice and the extract was evaporated to obtain gum. The gum was applied to a column (4 cm i.d. × 33 cm) of 175 g of silicic acid, which was eluted with chloroform. Fractions a and b were obtained. Fraction a gave product II(A), 118 mg, mp 142–148 °C, after crystallization from chloroform–*n*-hexane. Fraction b was rechromatographed on a column of 30 g of silicic acid, eluted with chloroform, and gave product II(F), 177 mg, after crystallization from chloroform–*n*-hexane. Products II(A) and II(F) were recrystallized, and their physicochemical properties are listed in Table I.

Warning: *N*-Nitrosodimethylamine, *N*-nitrosodiethylamine, and 1-naphtylamine, all of which are carcinogens, should be handled with safety precautions.

RESULTS

Decrease in Nitrite Levels Caused by *p*-Hydroxycinnamic Acid Derivatives. The disappearance of nitrite caused by naturally occurring *p*-hydroxycinnamic acid derivatives such as *p*-coumaric acid (I), ferulic acid (II), caffeic acid (III), chlorogenic acid (IV), cinnamic acid (V), and vanillin was investigated. Decreases in nitrite concentration on mixing the compounds in aqueous solutions in the mild acidic ranges were measured in terms of the production of azo dye with Griess reagent. The rates of decrease in nitrite level by each of these compounds were measured and compared with those of the known nitrite-consuming substance, ascorbic acid (Mirvish et al., 1972). When a solution containing a concentration of 0.10 mM nitrite was incubated at 37 °C and at pH 3 with each of the compounds during the period of 90 min, the concentrations which lost nitrite completely were 1 mM (I), 0.2 mM (II), 0.1 mM (III), more than 2 mM (IV), and more than 2 mM (ascorbic acid), respectively (Figure 1). The compound (V) and vanillin did not decrease the nitrite level even at 2 mM concentration.

Time courses of nitrite decrease in treatment of 0.1 mM nitrite with I, II, III, and IV at three different pH values (pH 2, 3, and 4) during the period of 2 h exhibited the loss by every compound that was greater as the pH value decreased (Figure 2). These results indicated that the po-

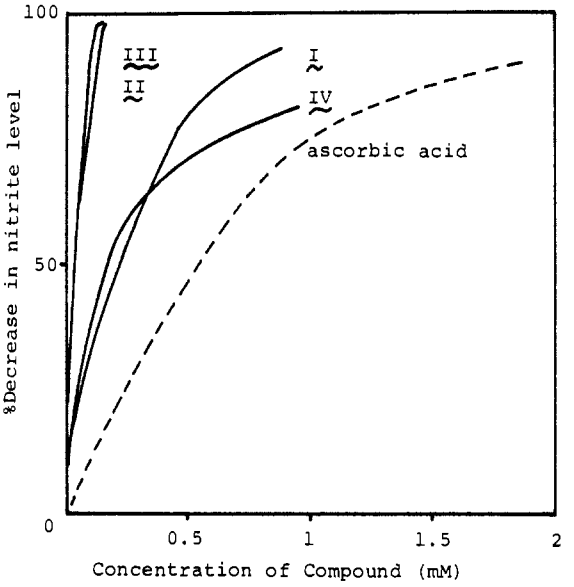


Figure 1. Decreases in nitrite concentration by I, II, III, IV, and ascorbic acid. A mixture of 0.10 mM sodium nitrite and I, II, III, IV, or ascorbic acid was treated at pH 3.0 and 37 °C for 90 min.

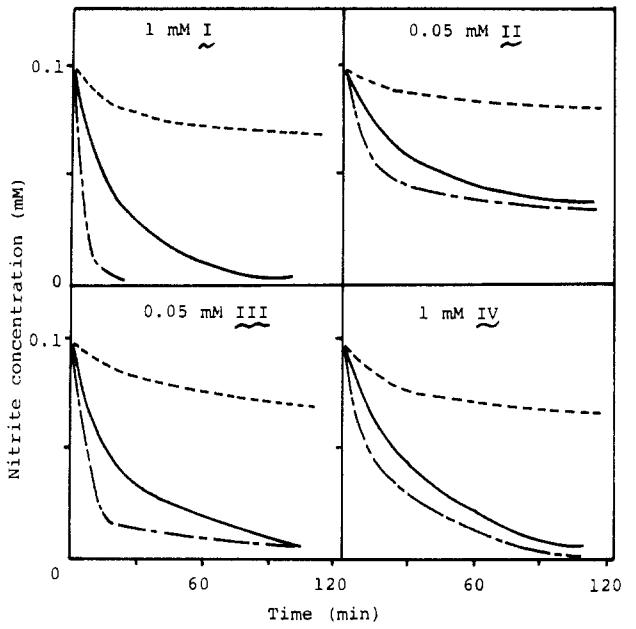


Figure 2. Time course of decrease in nitrite concentration by treatment with I, II, III, and IV. A mixture of 0.10 mM sodium nitrite and 1 mM I, 0.05 mM II, 0.05 mM III, or 1 mM IV was treated at pH 2.0 (---), pH 3.0 (—), or pH 4.0 (---) and at 37 °C.

tencies of nitrite reduction of I, II, and III were more powerful than that of ascorbic acid at every pH value tested, and the order of the potency was III > II > I > IV, ascorbic acid ≫ V, vanillin.

Effect of *p*-Hydroxycinnamic Acid Derivatives on Nitrosamine Formation. The effect of *p*-coumaric acid (I), ferulic acid (II), caffeic acid (III), and cinnamic acid (V) on the N-nitrosation of dimethylamine and diethylamine was investigated. The mixture containing concentrations of 100 mM dimethylamine and 50 mM nitrite was incubated in the presence of each of the phenolics at pH 3, 4, or 5 and at 37 °C for 5 h. *N*-Nitrosodimethylamine produced in the reaction mixture was extracted with chloroform in the presence (extraction 1) or absence (extraction 2) of caustic alkali. The yields of the nitrosamine were not significantly different by these two methods of

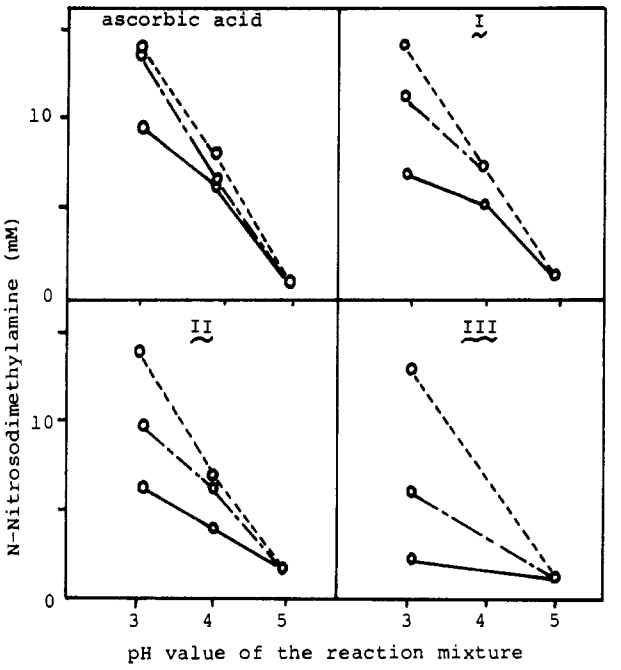


Figure 3. Effect of ascorbic acid, I, II, and III on *N*-nitrosodimethylamine formation at three different pH values. A mixture of 50 mM sodium nitrite and 100 mM dimethylamine was treated at 37 °C for 5 h in the absence (---) and presence of ascorbic acid, I, II, or III: 5 mM (-·-) and 10 mM (—). The nitrosamine was extracted by extraction method 1.

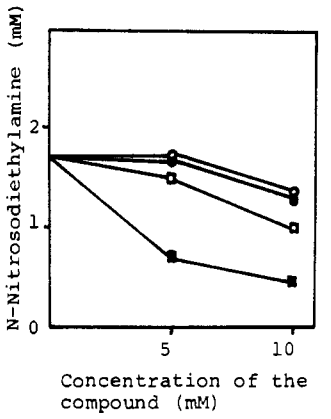


Figure 4. Effect of ascorbic acid, I, II, and III on *N*-nitrosodiethylamine formation. A mixture of 50 mM sodium nitrite and 100 mM diethylamine was treated at pH 3 and 37 °C for 5 h in the absence and presence of ascorbic acid (○), I (●), II (□), or III (■) of the indicated concentration. The nitrosamine was extracted by extraction method 1.

extraction. The effect of the phenolics on the formation of *N*-nitrosodimethylamine is shown in Figure 3, which indicated that I, II, and III had the inhibitory effect as well as the standard ascorbic acid, and the effects were dramatic at pH 3. The percent inhibitions of the nitrosamine formation at pH 3 by 10 mM test sample were 30% (ascorbic acid), 51% (I), 54% (II), and 81% (III). Under these conditions, V was quite inert against the nitrosamine formation. Thus, the inhibitory potency of the phenolics was in the order III > II > I > ascorbic acid ≫ V.

The inhibitory effect of these phenolics against the formation of *N*-nitrosodiethylamine was similar to that against the formation of *N*-nitrosodimethylamine (Figure 4).

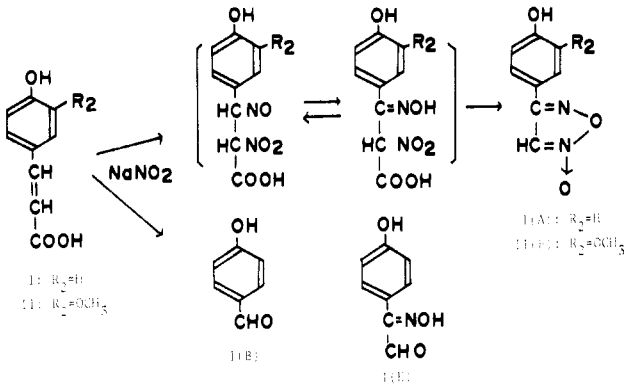
Reaction Products of I and II with Nitrite. The disappearance of nitrite and the inhibitory effect of nitrosamine formation by these phenolics may be due to

Table I. Physicochemical Properties of the Products

product	recrystallization solvent	appearance (mp)	TLC R_f	UV, nm: λ_{max} (H_2O) (ϵ)	UV, nm: λ_{max} (0.1 N HCl) (ϵ)	UV, nm: λ_{max} (0.1 N NaOH) (ϵ)	mass spectrum, m/e (M^+)	formula	NMR, ppm (Me_2SO-d_6)	analysis
I(A)	benzene	white needles (200 °C dec)	0.39[a]	238 (15 600) 286 (9400) 295 sh	239 (15 800) 286 (9500) 295 sh	248 (13 200) 352 (15 300)	178	$C_8H_6N_2O_3$[b]	10.17 (1 H, s, OH) 9.40 (1 H, s) 7.85 (2 H, d, J = 8 Hz) 6.94 (2 H, d, J = 8 Hz)	C, H, N
I(B)	chloroform–*n*-hexane	yellow crystalline powder (118 °C[c])	0.31[d]	217 (10 900) 279 (14 800)	217 (10 900) 279 (14 800)	233 (7500) 324 (26 400)	122	$C_7H_6O_2$	10.55 (1 H, s, OH) 9.77 (1 H, s, CHO) 7.74 (2 H, d, J = 8 Hz) 6.91 (2 H, d, J = 8 Hz)	C, H, N
I(C)	benzene–ethanol	white needles (87–91 °C)	0.18[d]	218 280	218 280	230 320				C, 60.16; H, 5.88; N, 0.00 (found)
I(D)	ethanol–benzene	amber crystals (186 °C dec)	0.13[d]	229 (13 000) 261 (3300) 318 (11 500)	229 (13 500) 317 (12 200)	268 (14 600) 291 (12 900)	163	$C_8H_5NO_3$[b]		C, H, N
I(E)	ethanol–chloroform	yellow leaflets (163–165 °C)	0.09[a]	228 (11 000) 304 (12 300)	225 (10 000) 299 (11 400)	256 (8000) 284 (9000) 360 (20 600)	165	$C_8H_7NO_3$[b]	12.32 (1 H, br s, NOH) 10.40 (1 H, br s, OH) 7.98 (1 H, s) 7.90 (2 H, d, J = 8 Hz) 6.83 (2 H, d, J = 8 Hz)	C, H, N
II(A)	chloroform	pale yellow leaflets (150–152 °C)	0.36[a]	241 (13 300) 309 (8400)	240 (13 400) 307 (8500)	254 (13 500) 365 (14 400)	208	$C_9H_8N_2O_4$[b]	9.83 (1 H, s, OH) 9.50 (1 H, s) 7.57 (1 H, s) 7.55 (1 H, d, J = 9 Hz) 6.95 (1 H, d, J = 9 Hz) 3.85 (3 H, s)	C, H, N
II(F)	chloroform–*n*-hexane	yellow needles (179 °C dec)	0.13[d]	351 (9900)	346 (10 200)	283 (5500) 376 (14 000)	193	$C_9H_7NO_4$	8.35 (1 H, s) 7.23 (1 H, s) 6.90 (1 H, s) 3.87 (3 H, s)	C, H, N

[a] Positively colored by BQC reagent. [b] Determined by high-resolution mass spectroscopy. [c] Authentic *p*-hydroxybenzaldehyde, mp 116 °C. [d] Negative to BQC reagent.

Scheme I



their chemical reactions with nitrite. The monophenolics, I and II, were reacted with more than 10 molar excess of sodium nitrite at pH 3 in 50% ethanol, and the mixtures were extracted with chloroform. Thin-layer chromatography of the extracts revealed several reaction products; *p*-coumaric acid (I) afforded five major products, I(A), I(B), I(C), I(D), and I(E), and ferulic acid (II) gave two major products, II(A) and II(F). The products were separated on silicic acid columns, and most of these products were isolated in crystalline form.

Product I(A) had an empirical formula of $C_8H_6N_2O_3$ with molecular weight of 178. Two nitrogen atoms were introduced and one carbon atom was eliminated in this compound. The nuclear magnetic resonance spectrum of the compound showed that four phenyl protons which appeared as doublets at 7.85 and 6.94 ppm with a coupling constant of 8 Hz were found intact when compared to those of the starting compound (I) which appeared as doublets at 7.46 and 6.78 ppm with a coupling constant of 8 Hz. A characteristic singlet with one proton appeared at 9.40 ppm and the olefinic protons of I, which appeared at 7.43 and 6.25 ppm with a coupling constant of 16 Hz, disappeared. The structure of product I(A) may be assigned to be furoxan derivative derived from the primary product with nitroso–nitro addition of nitrite to the olefinic group of I (Scheme I). It has been demonstrated that nitrite reacts with olefins to produce nitroso–nitro compounds, which derivatize into the dehydrated closed ring structure (Osawa et al., 1979).

Product I(B) was found to be *p*-hydroxybenzaldehyde bearing no nitrogen atoms and may be derived from the reaction products of nitrite to the olefinic group of I (Scheme I). Product I(C) had no nitrogen atoms, although its empirical formula was not determined by usual mass spectrometry owing to the rapid degradation. Ultraviolet absorption spectrum of I(C) was very close to that of product I(B), and it may be an analogue of I(B). The fluorescent product I(D), obtained in a relatively low yield, showed a molecular formula of $C_8H_5NO_3$ with molecular weight of 163. In this compound, one nitrogen atom was introduced and one carbon atom was eliminated. Product I(E) showed an empirical formula of $C_8H_7NO_3$ with molecular weight of 165. One nitrogen atom was introduced and one carbon atom was eliminated in this compound. Nuclear magnetic resonance spectrum revealed four protons due to the phenyl ring and one proton appeared as a characteristic singlet at 7.98 ppm. The structure of compound I(E) may be assigned to be the oxime–aldehyde as illustrated in Scheme I.

Product II(A) had an empirical formula of $C_9H_8N_2O_4$ with molecular weight of 208. Two nitrogen atoms were introduced and one carbon atom was eliminated. The nuclear magnetic resonance spectrum of compound II(A) showed that three phenyl protons appeared as doublets at 7.55 and 6.95 ppm with a coupling constant of 9 Hz and as a singlet at 7.57 ppm and three methoxy protons appeared as a singlet at 3.85 ppm. The signals of these protons were not markedly altered from those of the starting compound II, whose spectrum revealed three phenyl protons at 7.26, 7.10, and 6.77 ppm with ortho coupling by 8 Hz and meta coupling by 2 Hz and three methoxy protons at 3.82 ppm. The nuclear magnetic resonance spectrum of II(A) exhibited no olefinic protons and instead a sharp singlet at 9.50 ppm. The physical and spectroscopic characters of II(A) were very close to those of product I(A). The structure of II(A) may be assigned as a decarboxylated and dehydrated compound derived from the primary product with nitroso–nitro addition of nitrite (Scheme I). The fluorescent compound II(F) having molecular formula of $C_9H_7NO_4$ with molecular weight of 193 showed the loss of one carbon atom and the capture of one nitrogen atom.

Although the structure of all the reaction products of I and II with nitrite was not established, it is likely that most of these reaction products were derived from the reaction at the olefinic group of these phenolics with nitrite, which were subsequently converted into many products with complex structures. One of the reactions may be the addition reaction of nitrite to produce nitroso–nitro compound, which might be converted into its tautomer, oxime–nitro compound, and subsequently decarboxylated and dehydrated into a furoxan derivative (Scheme I).

o-Diphenolics such as caffeic acid (III) and chlorogenic acid (IV) may be readily converted into the corresponding *o*-quinones, but their olefinic group may also participate in both nitrite disappearance and inhibition of nitrosamine formation.

DISCUSSION

p-Hydroxycinnamic acid derivatives such as *p*-coumaric acid (I), ferulic acid (II), and caffeic acid (III) decreased nitrite levels and inhibited nitrosamine formation more effectively than the well-known inhibitor, ascorbic acid (Mirvish et al., 1972). The inhibitory potency of these phenolics against the nitrosamine formation was higher in the following order: III > II > I ascorbic acid ≫ V, and the order was almost the same as that of nitrite disappearance. These compounds are distributed in a wide variety of plants and vegetables (Sosulski, 1979), and they may participate in the nitrosamine formation of foodstuffs.

Naturally occurring phenolics such as gallic acid (Walker et al., 1975), chlorogenic acid (Challis and Bartlett, 1975), cresol (Davies and McWeeny, 1977), and sesamol (Kurechi et al., 1979) have been shown to enhance nitrosamine formation at rather higher pH values around 5. The reasons for the stimulation by monophenolics such as cresol and sesamol have been explained by the formation of *o*-nitroso derivatives which catalytically promoted the formation of nitrosamines. But the effect of polyphenolics, gallic acid, and chlorogenic acid is conflicting (Challis and Bartlett, 1975; Gray and Dugan, 1975; Nakamura and Kawabata, 1981; Walker et al., 1975; Yamada et al., 1978). The present studies demonstrated that *p*-hydroxycinnamic acid derivatives I, II, and III were inhibitory against the nitrosamine formation under the conditions tested.

Oxidation of diphenolics by nitrite to the corresponding quinones is well documented (Bruce, 1974; Fieser and Fieser, 1967). *o*-Diphenolics such as III and IV may be readily converted into the corresponding quinones, decreasing nitrite levels available for nitrosamine formation. Ortho nitrosation and ortho nitration of monophenolics

by nitrite is also demonstrated (Knowles et al., 1974; Kurechi et al., 1979, 1980b). Although I and II can undergo reaction with nitrite to suffer ortho nitrosation or ortho nitration, no such compounds were detected in the reaction mixtures of the present investigation.

The structural analysis of the reaction products of I and II with nitrite indicated that most of the reactions may be the addition of nitrite to the olefinic group of these compounds, giving the complicated reaction products. One of these complex reactions may be the addition reaction of nitrite to produce nitroso–nitro compounds, which might be readily transformed into its tautomer, oxime–nitro compounds, and subsequently decarboxylated and dehydrated into furoxan derivatives, I(A) and II(A). Nitrite has been shown to react with olefins such as sorbic acid to produce nitroso–nitro or its tautomeric form, the oxime–nitro compound, which might be dehydrated into the furoxan derivative as the closed ring structure (Osawa et al., 1979).

Formation of these complex products by reaction of I and II with nitrite must consume nitrite which would otherwise be available for the nitrosamine formation. Caffeic acid (III) and chlorogenic acid (IV) may suffer such kinds of reactions in their olefinic groups besides conversion into the corresponding quinones. The decrease in nitrite level and inhibition of nitrosamine formation by these diphenols may be attributed to the complex reaction of nitrite to the phenolics.

Although the formation of these complicated products by reaction of naturally occurring *p*-hydroxycinnamic acid derivatives with nitrite might retard formation of carcinogenic nitrosamines, carcinogenicity of the reaction products must be carefully investigated.

Registry No. I, 7400-08-0; I(B), 123-08-0; II, 1135-24-6; III, 331-39-5; IV, 327-97-9; nitrite, 14797-65-0.

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Quadrupole Mass Spectrometry/Mass Spectrometry of Ergot Cyclol Alkaloids

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The quadrupole MS/MS spectra of the ergot cyclol alkaloids are reported. The alkaloids were ionized by chemical ionization (CI) with isobutane as a reagent gas. Both parent and daughter experiments on major fragment ions in the isobutane CI spectra were used to differentiate all 12 ergot peptide alkaloids studied. Samples can be analyzed for these alkaloids with much less cleanup than required by other methods.

Considerable interest has been generated recently in using the rather new technique of tandem mass spectrometry or mass spectrometry/mass spectrometry (MS/MS) to analyze crude mixtures of organic compounds. This is particularly true when the compounds of interest are not amenable to separation by gas chromatography/mass spectrometry (GC/MS), because any mass spectrometric study of these compounds requires rigorous purification prior to mass spectrometric analysis. The approach of MS/MS is to utilize one stage of mass separation to isolate the compound of interest from the matrix and a second stage of mass separation for analysis. Usually

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