Environmental Health Perspectives
Vol. 67, pp. 135-142, 1986

Chemical Constituents of Peppers (*Piper* spp.) and Application to Food Preservation: Naturally Occurring Antioxidative Compounds

by Nobuji Nakatani,* Reiko Inatani,* Haruko Ohta,* and Atsuko Nishioka*

In a structure analysis of the compounds of the genus *Piper* (Family Piperaceae), we identified five phenolic amides from *Piper nigrum*, seven compounds from *P. retrofractum*, and two compounds from *P. baccatum*. All the phenolic amides possess significant anitoxidant activities that are more effective than the naturally occurring antioxidant, α-tocopherol. One amide, feruperine, has antioxidant activity as high as the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Naturally occurring antioxidants, therefore, may surpass BHA and BHT in their ability to inactivate mutagens in food.

Introduction

Much research has involved finding ways to prevent or to delay deterioration in foods. Recently, our research (*1,2*) has focused on developing safe and effective compounds from natural sources (especially from edible plants) that will prolong the storage life of food.

We studied the genus *Piper* of the Family Piperaceae, to identify the structure of the compounds in its species that give organoleptic (*3–5*), medicinal (*6–9*), and insecticidal (*10–13*) properties. Many species, among the 700 in this genus, are used not only as spices but also as folk medicines (*14*). This paper reveals the structure of the compounds and their resulting properties for *Piper nigrum* L., *P. retrofractum* Vahl., and *P. baccatum* Blume.

Structural Determination of Constituents

Powdered dry fruits of white pepper (*P. nigrum*) were extracted with methylene chloride and fractionated as shown in Figure 1. The fractions determined were neutral (83.80%), weakly acidic (1.72%), strongly acidic (0.55%), and basic (0.22%). We found piperine [1] (a pungent principle) and more than 40 constituents isolated from *P. nigrum* to be neutral compounds. The weakly acidic fraction showed significant anitoxidative activity, and we subjected it to column chromatography on silica gel, using a solution of CH_2Cl_2 and MeOH as an eluant. The major compound [2a], purified upon recrystallization from hot chloroform (mp 144.0–144.5°C), produced colorless needles.

Mass spectral and elemental analyses of [2a] indicated the molecular formula $C_{18}H_{19}NO_4$.

Three absorption bands at 3500, 3360, and 3240 cm^{-1} in the infrared (IR) spectrum showed the presence of hydroxy or amino groups. The absorptions at 1645, 1615, and 980 cm^{-1} represented a *trans*-conjugated amide carbonyl group.

The λ_{max} at 295 and 321 nm in the ultraviolet (UV) spectrum indicated the presence of feruloyl moiety in the molecule, as shown in the spectrum of ferulic acid (λ_{max} 294 and 320 nm). This was also confirmed by the base peak at *m/z* 177 in the mass spectral (MS) data.

Further evidence for the structural characteristics was obtained from nuclear magnetic resonance (NMR) spectroscopy. One methoxy group was observed at δ 3.88, and a typical set of two doublets at δ 5.47 and δ 7.47 (both J = 16 Hz) was assigned to *trans*-α and *trans*-β-protons conjugated to the carbonyl group, respectively. A triplet (2H) centered at δ 2.75, (J = 7 Hz) and

*Department of Food and Nutrition, Faculty of Science of Living, Osaka City University, 3-3-138, Sugimoto, Sumiyoshi-ku, Osaka 558, Japan.

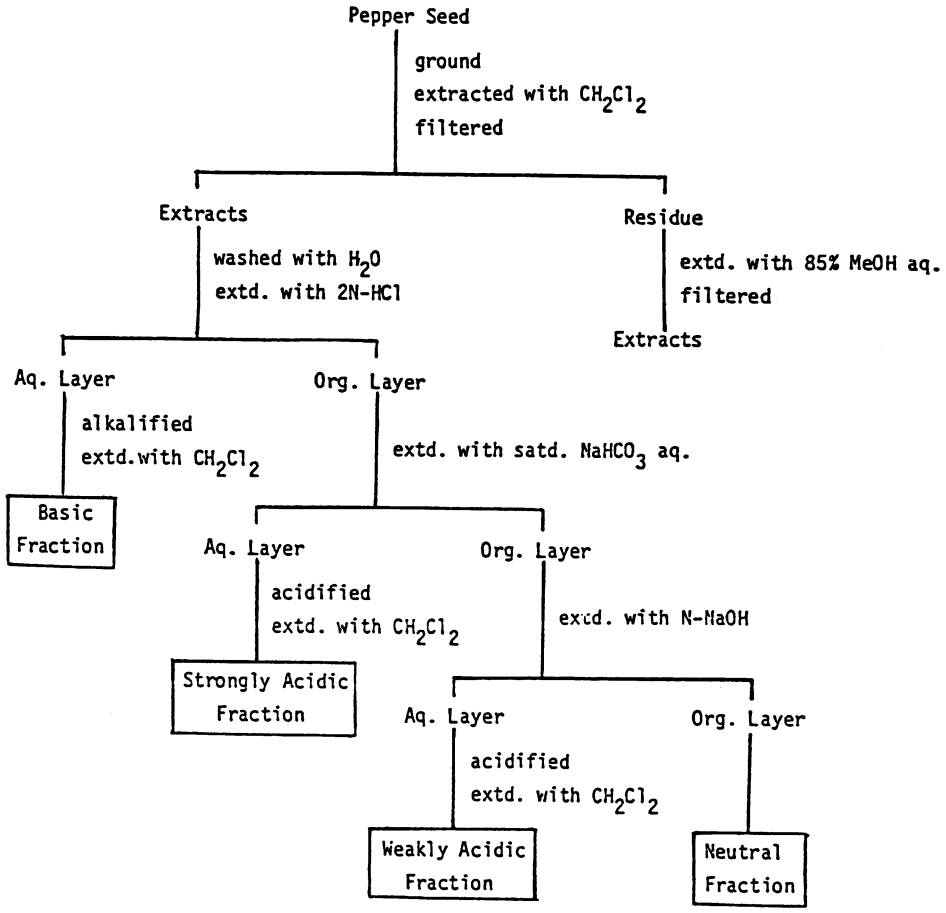

FIGURE 1. Extraction and fractionation of pepper seed.

a multiplet (2H) at δ 3.2 to 3.7 were attributed to the partial structure of Ar-C*H*$_2$-CH$_2$-R and RCH$_2$-C*H*$_2$-NH-R, respectively. Seven aromatic protons and an N*H* were observed in the range δ 6.65 to 7.2.

To confirm the structure, we prepared two derivatives. The acetylated compound [2b] showed the acetate absorption at 1760 cm^{-1} and the disappearance of two hydroxyl groups. The methyl protons of acetyl groups at δ 2.30 (6H) in the NMR spectrum and the molecular ion at *m/z* 397 in the MS were good evidence for the existence of two hydroxyl groups in the structure of [2a]. Using the spin decoupling technique in the NMR analysis, irradiation of a triplet of [2b] at δ 2.84 caused an apparent quartet at 3.58 to collapse to a doublet, with a coupling constant of J = 7 Hz; whereas irradiation on the signal at δ 3.58 caused a triplet at δ 2.84 to collapse to a singlet and a triplet at δ 6.00 (NH, J = 7 Hz) to collapse to a singlet. These data supported the partial structure of this amide, -CONH-CH$_2$-CH$_2$Ar.

Methylation of [2a] with methyl iodide and sodium methoxide resulted in the addition of two methyl groups and a new molecular ion [2c] at *m/z* 341, a base peak at *m/z* 191, and a major fragment at *m/z* 134 (175%), indicating the feruloyl moiety and the tyramine moiety, respectively (Fig. 2). All of these data indicate the structure of the main amide [2a] to be *N*-feruloyl tyramine.

The second major component, coumaperine, yielded a molecular formula of $C_{16}H_{19}NO_2$, a base peak at *m/z* 173, and a strong fragment at *m/z* 84, suggesting acyl moiety and piperidine moiety, respectively. The IR spectrum revealed a hydroxyl group at 3200 cm^{-1}, an amide carbonyl at 1630 cm^{-1}, and conjugated olefinic groups at 1605 cm^{-1}. The conjugated olefinic system was also revealed in the NMR and by λ_{max} at 241, 315, and 338 nm in the UV spectrum, close to that of piperine [1].

Acetylation of [3a] produced a monoacetyl derivative [3b], which showed an acetate absorption at 1755 cm^{-1} in the IR spectrum and acetyl methyl protons at λ 2.25 as a 3H singlet in the NMR spectrum.

We isolated three minor phenolic amides from the fractions that were less polar than [2a] and [3a]. Each was isolated by repeated chromatography on silica gel and Sephadex LH-20. All were piperidine amides and identified by spectroscopic analyses to be *N-trans*-feruloyl piperidine [4], *N*-5-(4-hydroxy-3-methoxyphenyl)-2E,4E-pentadienoyl piperidine [5], and *N*-5-(4-hydroxy-3-methoxyphenyl)-2E-pentenoyl piperidine [6].

The structures of all five compounds obtained from the weakly acidic fraction of *P. nigrum* were confirmed by synthesis (*15,16*) (see Fig. 3).

We examined another *Piper* species, the Javanese

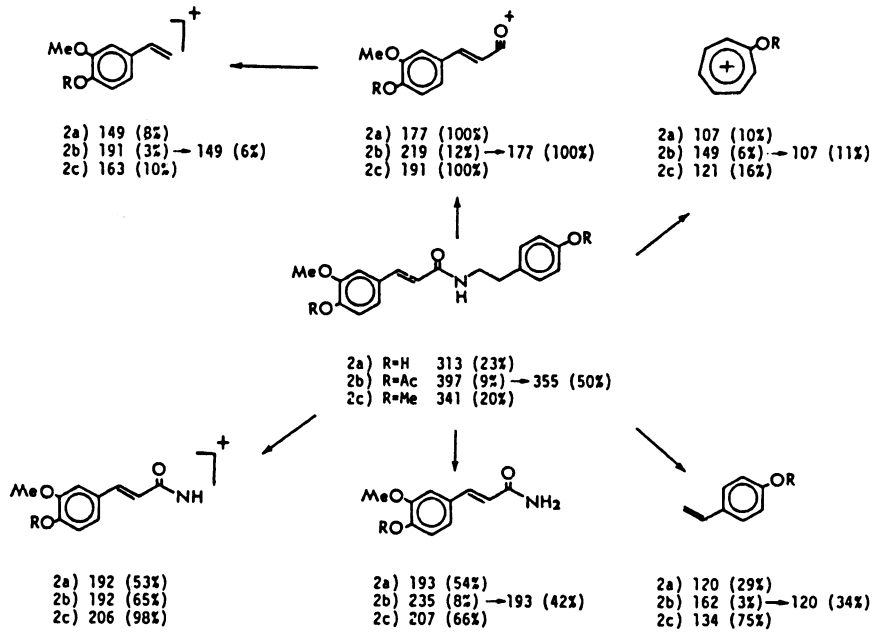

FIGURE 2. Mass fragmentation pattern of *N*-feryloyl tyramine [2a] and its acetyl [2b] and methyl [2c] derivatives. Numbers given are *m/z* and proportions relative to most abundant ion (in parentheses).

long pepper, *P. retrofractum* Vahl. (*17*). The fruits of this species are used as spice for pickling and in medicine for digestive and intestinal disorders. Extractions and purification were performed as previously described (see Fig. 1). Six compounds from the neutral fraction and piperic acid [7a] from a strongly acidic fraction were

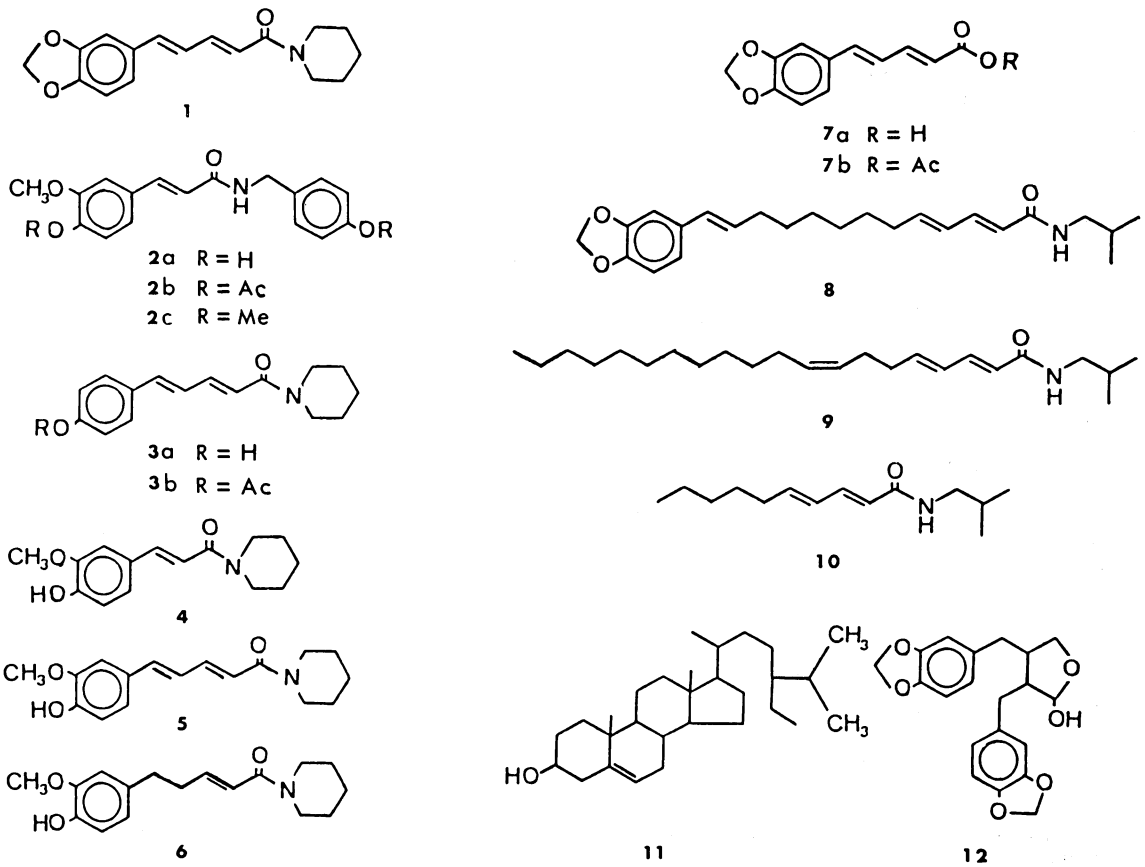

FIGURE 3. Compounds isolated from peppers.

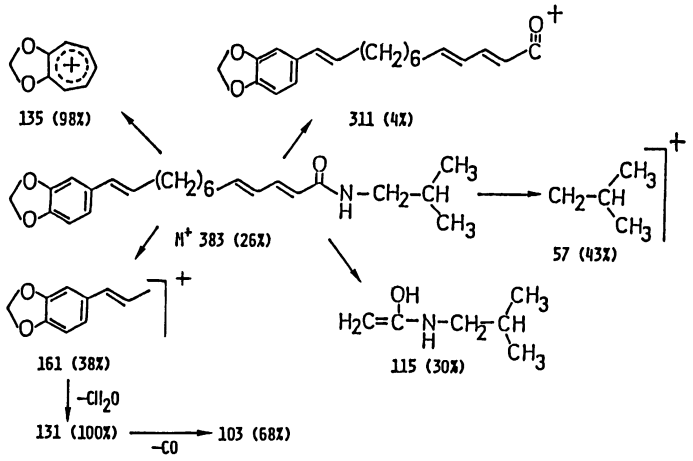

FIGURE 4. Mass fragmentation pattern of *N*-isobutyl-13-(3,4-methylenedioxyphenyl)-2E,4E,12E-tridecatrienamide. Notations are as in Figure 2.

isolated. One of the neutral compounds recrystallized from hexane and produced needles [8] (mp 116°C). We determined the molecular formula to be $C_{24}H_{33}NO_3$.

The IR absorption bands indicated a conjugated amide carbonyl at 1655, piperonyl moiety at 1255, 1040, and 970 cm^{-1}, which was also supported by a two-proton singlet at δ 5.92 in the NMR spectrum. A doublet (6H) at δ 0.91 and a triplet (2H) at δ 3.16 suggested the presence of isobutylamino moiety in the structure of [8].

Mass spectral analysis of this amide showed a molecular ion peak at *m/z* 383 and a prominant ion at *m/z* 135, indicating a stable methylenedioxy tropyrium ion. We observed a typical fragmentation pattern, a series of fragments of *m/z* 161, 131 (base peak), and 103. All of these data suggested the structure of [8] to be *N*-isobutyl-13-(3,4-methylenedioxyphenyl)-2E,4E,12E-tridecatrienamide (guineensine) (*18*) (Fig. 4).

Repeated column chromatography produced yet another compound (mp 84°C); we determined the molecular formula to be $C_{14}H_{25}NO$. A λ_{max} of 258 nm suggested the presence of *trans*-α,β,γ,δ-double bond conjugated to a carbonyl group; it was confirmed by NMR analysis. The data of this compound were identical with those of authentic pellitorine, *N*-isobutyl-2E,4E-decadienamide [9] (*19*). We identified other components from the neutral fraction to be piperine [1], methyl piperate [7b], *N*-isobutyl-2E,4E,8Z-eicosatrienamide [10], and β-sitosterol [11].

Another species, *P. baccatum* Blume (known as "rinu" in Indonesia), contained abundant glyceride. Fatty acid composition was determined by gas chromatography following hydrolysis and methylation with BF_3-MeOH. Palmitic acid (26.9%), linoleic acid (26.1%), oleic acid (9.2%), stearic acid (5.6%), and other acids were identified. The ratio of constituents of *P. baccatum* differed from those of the two preceding species: the volatile component was as high as 40%, and the piperine component was only a trace. We isolated two compounds from the neutral fraction of the nonvolatile component; one was a lignan recrystallized from methanol (mp 128°C). Mass spectral analysis showed a molecular ion peak at *m/z* 356, a dehydrated ion at *m/z* 338, a base peak at *m/z* 203 ($M^+ - 18 - 135$), and a methylenedioxytropyrium ion at − 135. The NMR and IR spectra also supported the structure of hydroxy furan substi-

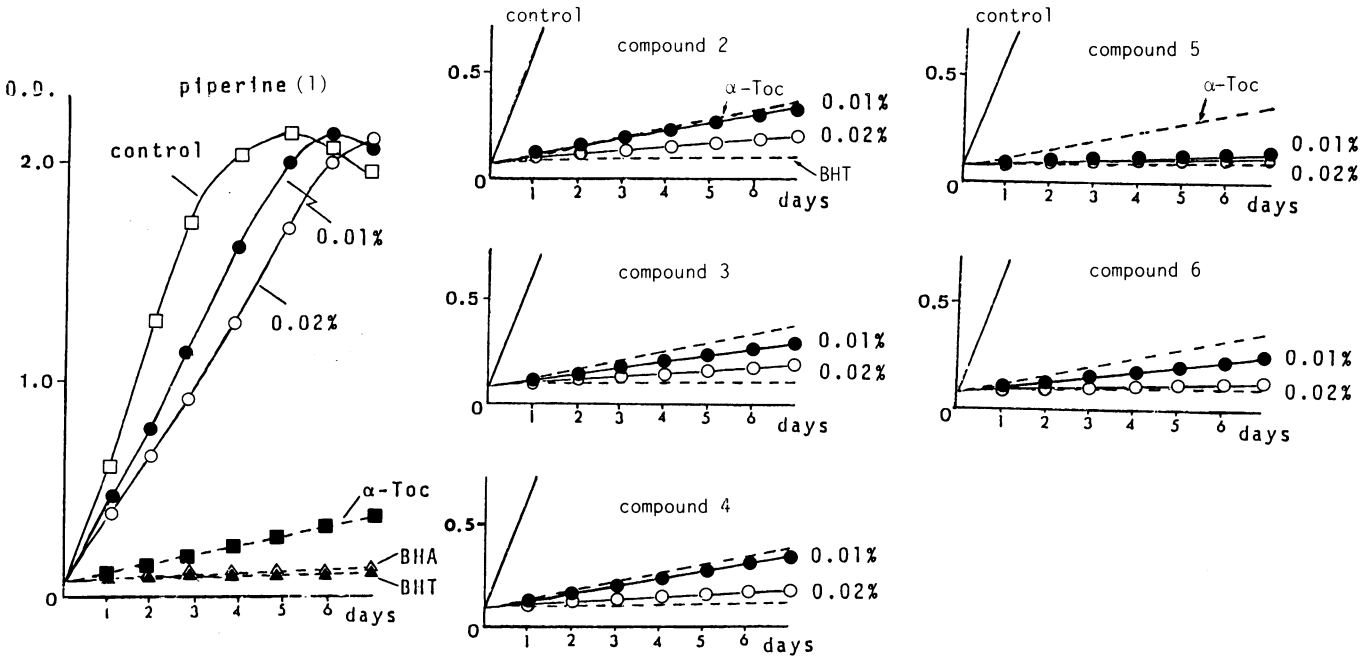

FIGURE 5. Antioxidative activity of compounds isolated from *P. nigrum* (measured by ferric thiocyanate method). Abscissa, reaction time; ordinate, optical density.

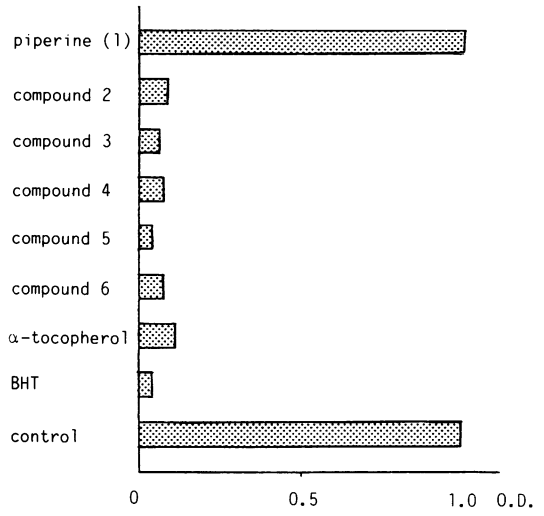

FIGURE 6. Antioxidative activity of compounds isolated from *P. nigrum* (measured by thiobarbituric acid method). Abscissa, optical density.

tuted with two methylenedioxy benzyl groups. We identified this compound as (-)-cubebin [12] (*20,21*). On the basis of its spectral data we determined the other compound (mp 85.0–85.5°C) to be a 2,3-*seco*-7,11-dehydrogermacranolide sesquiterpene lactone.

Antioxidative Activity

Today, to depress and delay the formation of peroxide and rancidity of oils and fats, antioxidants are widely used in processed foods. The most commonly used antioxidants are synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), and a natural antioxidant, α-tocopherol. However, the use of BHA and BHT as food additives is restricted in several countries because of undesirable effects these additives produce on the enzyme systems of liver and lung (*22,23*). The antioxidative ability of α-tocopherol is less active. Therefore, finding and preparing antioxidants from natural foodstuffs is a logical alternative.

We measured antioxidative activity on the compounds isolated from pepper. Figures 5 and 6 show the antioxidative activity of piperine and five amides from *P. nigrum*, measured by the ferric thiocyanate method and thiobarbituric acid method, respectively. In these experiments, linoleic acid was used for the oxidation substrate. Piperine [1] showed no activity. All phenolic amides [2–6] showed significant activity, more effective than α-tocopherol at the same concentration (0.01%). The activity of compound [5] was as high as that of synthetic antioxidants, BHA and BHT, at the same level. Piperic acid [7a] and some of the neutral components isolated from *P. retrofractum* and *P. baccatum* showed slight antioxidative activity, but they were less active than α-tocopherol (Fig. 7).

Antimicrobial Activity

It is known that certain pepper spices possess antifungal and antibacterial activities. Antibacterial activities of compounds [2–6] from *P. nigrum* and [7a], [7b], and [8] from *P. retrofractrum* were measured against eight microorganisms, including *Staphylococcus aureus* and *Streptococcus faecalis*, to determine minimum inhibition concentrations. The results of these studies revealed that the antibacterial activity of these compounds was low.

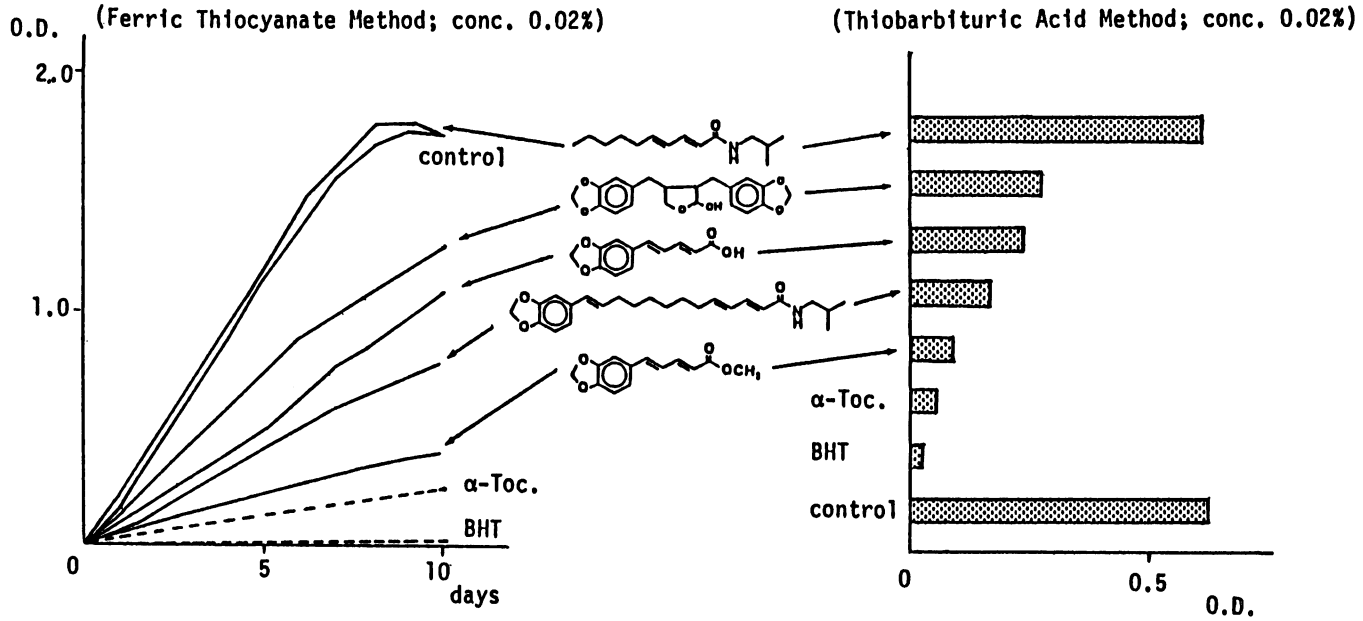

FIGURE 7. Antioxidative activity of compounds isolated from *P. retrofractum* and *P. baccatum*. α-Toc. is α-tocopherol.

Antioxidants and Mutagens

In recent years, chemicals with mutagenic and carcinogenic activities have been isolated in foods (*24,25*). These chemicals are either naturally occurring constituents, substances formed during processing or storage, or contaminants (*26*). Lipid peroxidation, a radical chain oxidation of unsaturated fatty acid, causes oxidation damage in living organisms. Mutagenesis and carcinogenesis is induced by lipid peroxide and by malondialdehyde produced by oxidative cleavage of fatty acid (*27*). Antioxidants such as BHA, BHT, ascorbic acid, and selenium are known to reduce mutagenicity (*28-33*). Plant phenol ellagic acid, which possesses high antioxidative property, reacted with an ultimate carcinogen, benzo[a]pyrene-7,8-diol 9,10-epoxide, to form inactive *cis* and *trans* adducts (*34*) (Fig. 8).

Mutagenesis in food is an obvious hazard. Continued study of the relationship between mutagens and naturally occurring antioxidants isolated from peppers and other spices is important if methods are to be developed to prevent the formation of mutagens or to inactivate them.

Experimental

Melting points, measured with a Yanagimoto micro melting-point apparatus, were left uncorrected. The UV absorption spectra were determined on Hitachi 323 and 220 spectrophotometers, and IR spectra were recorded by a Jasco IR-S. The ^{1}H-NMR spectra were determined on a Hitachi R-600S instrument tetramethylsilane (TMS) being used as an internal standard. The MS data were obtained on a Shimadzu GCMS-7000. Column chromatography was performed using Merck silica gel-60 (70-230 mesh) and Pharmacia Sephadex LH-20; thin layer chromatography (TLC) was completed using silica gel GF-254.

Extraction and Isolation

Dried and powdered (20–60 mesh) fruits of Sumatran white pepper (*P. nigrum*, 1 kg) were extracted three times with CH_2Cl_2 (1000 mL) at room temperature. After the solvent evaporated from the combined extracts, the residue (82 g) was dissolved again in CH_2Cl_2 (300 mL). This solution was washed with H_2O and successively extracted with 2 N HCl, saturated $NaHCO_3$ aqueous, and 1 N NaOH solution to separate the basic, strongly acidic, weakly acidic, and neutral fractions. The crude syrup (1.25 g) of the weakly acidic fraction was subjected to column chromatography on silica gel, eluted with CH_2Cl_2–MeOH (99:1). Samples (20 mL each) were collected and monitored by TLC. Samples 15 and 16 (325 mg) were combined and concentrated to form the main compound [2a]. Samples 11–13 (133 mg) yielded the second major compound [3a]. Less polar fractions, samples 5–10 (140 mg) were combined and rechromatographed on a silica gel (12 g) column using benzene-methanol (95:5, v/v) as the eluant. Samples 6–12 (78 mg) were subsequently purified with isopropyl alcohol on a Sephadex LH-20 column to produce three pure compounds: [4], [5], and [6]. Extraction and purification procedures for *P. retrofractum* and *P. baccatum* were performed in the same manner.

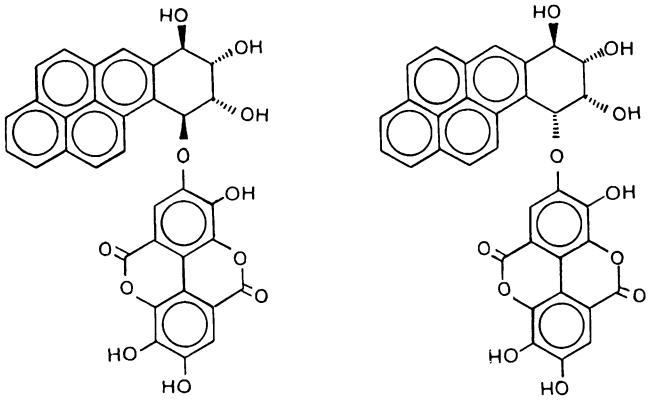

FIGURE 8. Adducts (*trans* and *cis*) of benzo[a]pyrene-7,8-diol 9,10-epoxide with ellagic acid.

Antioxidative Assay

Antioxidative activity of each sample to protect linoleic acid was measured by the ferric thiocyanate method and thiobarbituric acid method (TBA) (*2*). The sample solution, prepared according to our method at a concentration of 0.01% or 0.02%, was kept in a dark oven at 40°C.

Properties of Isolated Compounds

Piperine [1]. The neutral fraction of the CH_2Cl_2 extract of *P. retrofractum* was triturated with EtOH to produce crystals of piperine, mp 129.0°C (EtOH), M^+ *m/z*285. After separation of compound [1], the neutral fraction was subjected to column chromatography on silica gel, using a mixture of benzene-acetone (98:2) as eluant. Methyl piperate [7b] was obtained from samples 14–18, compounds [10] and [11] were obtained from samples 21–26, and compounds [8] and [9] were obtained from samples 32–40.

N-Trans-feruloyl tyramine [2a]. The major compound (325 mg), purified by rechromatography on a silica gel column and recrystallized from $CHCl_3$, formed colorless needles, mp 144.0–144.5°C. ANAL. Found: C, 68.87%; H, 6.00%; N, 4.52%. Calcd. for $C_{18}H_{19}NO_4$: C, 68.99%; H, 6.11%; N, 4.47%. UV λ_{max} (EtOH) nm (log ϵ); 219 (4.29), 228 (4.21), 295 (4.13), 321 (4.22). IR ν_{max} (Nujol) cm^{-1}: 3500, 3360, 3240, 1645, 1615, 1240, 1220, 1025, 980. NMR (d_6-acetone) δ: 2.75 (2H, t, J = 7 Hz), 3.2–3.7 (2H, m), 3.88 (3H, s), 6.47 (1H, d, J = 16 Hz), 6.65–7.2 (8H), 7.47 (1H, d, J = 16 Hz), 8.00 (1H, s, OH), 8.19 (1H, s, OH).

Diacetyl N-trans-feruloyl tyramine [2b]. Compound [2a] was acetylated with acetic anhydride in pyridine, mp 159.5°C (from benzene). UV λ_{max}x (EtOH)

nm (log ε): 278 (4.37), 305 sh (4.11). IR λ_{max} (Nujol) cm^{-1}: 3250, 1760, 1650, 1610, 1260, 1210, 1030, 995. NMR ($CDCl_3$) δ: 2.30 (6H, s), 2.84 (2H, t, J = 7 Hz), 3.58 (2H, q, J = 7 Hz), 3.80 (3H, s), 6.00 (1H, t, J = 7 Hz), 6.28 (1H, d, J = 16 Hz), 6.9–7.3 (7H), 7.54 (1H, d, J = 16 Hz).

Trimethyl N-trans-cafferoyl tyramine [2c]. This compound yielded an mp of 135.0°C (benzene). UV λ_{max} (EtOH) nm (log ε): 279 sh (4.17), 286 (4.23), 292 (4.24), 318 (4.28). IR ν_{max} (Nujol) cm^{-1}: 3280, 1646, 1616, 1257, 1240, 1025, 970. NMR ($CDCl_3$) δ: 282 (2H, t, J = 7 Hz), 3.64 (2H, m), 3.79 (3H, s), 3.88 (6H, s) 5.75 (1H, t, J = 7 Hz), 6.21 (1H, d, J = 16 Hz), 6.7–7.3 (7H), 7.56 (1H, d, J = 16 Hz).

Coumaperine [N-5-(4-hydroxyphenyl)-2E,4E-pentadienoyl piperidine] [3a]. The second compound was rechromatographed on a silica gel column and produced pure crystals, mp 199.5–200.5°C (acetone). UV λ_{max} (EtOH) nm (log ε): 241 (3.97) 315 sh (4.38), 338 (4.58). IR ν_{max} (Nujol) cm^{-1}: 3200, 1630, 1605, 1275, 1250, 1010, 845. NMR (d_6-acetone) δ: 1.58 (6H, br s), 3.57 (4H, br s), 6.60 (1H, d, J = 16 Hz), 6.7–7.7 (7H), 8.64 (1H, s, OH). MS, $C_{16}H_{19}NO_2$, *m/z* (%); 257 (M^+, 54), 173 (100), 145 (17), 127 (29), 115 (36), 107 (6), 91 (15), 84 (81).

Acetyl coumaperine [3b]. This compound yielded an mp of 127.0–128.0°C (ether). M^+ 299.

N-Trans-feruloyl piperidine [4]. This compound yielded an mp of 135°C (benzene–isopropyl ether). IR ν_{max} (Nujol) cm^{-1}: 3560, 1640, 1260, 1140, 1125, 1030, 1020, 980. NMR ($CDCl_3$) δ: 1.64 (6H, br s), 3.60 (4H, br s), 3.93 (3H, s), 5.94 (1H, s), 6.70 (1H, d, J = 15 Hz), 6.9–7.35 (3H), 7.60 (1H, d, J = 15 Hz). MS, $C_{15}H_{19}NO_3$, *m/z* (%); 261 (M^+, 96), 177 (100), 145 (50), 117 (20), 89 (18), 84 (95).

Feruperine [N-5-(4-hydroxy-3-methoxyphenyl)-2E,4E-pentadienoyl piperidine] [5]. This compound yielded an mp of 159.0°C (benzene). IR ν_{max} (Nujol) cm^{-1}: 3300, 1628, 1611, 1280, 1255, 998. NMR ($CDCl_3$) δ: 1.54 (6H, br s), 3.50 (4H, br s), 3.84 (3H, s), 6.35 (1H, d, J = 14.4 Hz), 6.6–7.0 (6H), 7.25–7.70 (1H, m). MS, $C_{17}H_{21}NO_3$, *m/z* (%); 287 (M^+, 91), 203 (100), 175 (40), 171 (12), 143 (16), 137 (15), 115 (30), 84 (68).

N-5-(4-hydroxy-3-methoxyphenyl)-2E-pentenoyl piperidine [6]. This compound yielded an mp of 78.0°C (ether). IR ν_{max} (Nujol) cm^{-1}: 3570, 1655, 1605, 1260, 1035, 970. NMR ($CDCl_3$) δ: 1.60 (6H, br s), 2.3–2.8 (4H, m), 3.48 (4H, br, s), 3.88 (3H, s), 6.08 (1H, br s), 6.20 (1H, d, J = 15 Hz), 6.6–7.1 (4H). MS, $C_{17}H_{23}NO_3$, *m/z* (%); 289 (M^+, 30), 153 (60), 138 (70), 137 (100), 84 (40).

Piperic acid [7a]. This compound yielded an mp of 208.0°C (EtOH), M^+ 218.

Methyl piperate [7b]. This compound yielded an mp of 142.5°C (EtOH), M^+ 232.

N-Isobutyl-13-(3,4-methylenedioxyphenyl)-2E,4E,-12E-tridecatrienamide [8] (guineensine). This compound yielded an mp os 116.0°C (*n*-hexane/acetone). UV λ_{max} (MeOH) nm (log ε): 262 (4.08), 305 (3.21), IRν_{max} (Nujol) cm^{-1}: 3300, 1655, 1615, 1550, 1255, 1040, 970.NMR ($CDCl_3$) δ: 0.91 (6H, d, J = 6 Hz), 1.2–1.7 (9H, m), 1.9–2.4 (4H, m), 3.16 (2H, t, J = 6.5 Hz), 5.3–5.8 (1H, m), 5.73 (1H, d, J = 15 Hz), 5.93 (2H, s), 5.8–6.3 (4H, m), 6.7–6.9 (3H), 7.0–7.4 (1H, m). MS,$C_{24}H_{33}NO_3$, M^+ 383.

N-Isobutyl-2E,4E-decadienamide [9] (pellitorine). This compound yielded an mp of 84.0°C (*n*-hexane). UV λ_{max} (EtOH) nm (log ε): 258 (4.57), IR ν_{max} Nujol) cm^{-1}: 3300, 1655, 1615. NMR ($CDCl_3$) δ: 0.90 (6H, d, J = 6 Hz), 0.8–1.1 (3H), 1.1–1.6 (7H, m), 1.7–2.4 (2H, m), 3.15 (2H, t, J = 6 Hz), 5.75 (1H, d, J = 15.6 Hz), 6.0–6.2 (2H, m), 6.9–7.3 (2H, m). MS, $C_{14}H_{25}NO$, *m/z* (%): 223 (M^+, 30), 208 (7), 180 (7), 152 (36), 151 (100), 96 (49), 81 (60).

N-Isobutyl-2E,4E,8Z-eicosatrienamide [10]. This compound yielded an mp of 64.0°C (*n*-hexane). UV λ_{max} (MeOH) nm (log ε): 259 (4.49), IR ν_{max} (Nujol) cm^{-1}: 3300, 1655, 1625, 1258, 997. NMR ($CDCl_3$) δ: 0.91 (9H, d, J = 6 Hz), 1.29 (19H), 1.7–2.3 (6H, m), 3.15 (2H, t, J = 6 Hz), 5.34 (2H, t, J = 5 Hz), 5.77 (1H, d, J = 15.6 Hz), 5.6–6.2 (3H, m), 7.2 (1H, m). MS, $C_{24}H_{43}NO$, *m/z* (%): 361 (M^+, 33), 289 (11), 180 (19), 152 (44), 115 (32), 95 (33), 81 (78).

β-Sitosterol [11]. This compound yielded an mp of 138.0°C (acetone). MS, $C_{29}H_{50}O$, M^+ 414.

(-)-Cubebin [12]. Repeated chromatography on the neutral fraction of the CH_2Cl_2 extract of *P. baccatum* produced needles, mp 128.0°C, recrystallized from MeOH. UV λ_{max} (EtOH) nm: 235.0, 287.5. IR ν_{max} (Nujol) cm^{-1}: 3350, 1609, 1242, 1040, 928. NMR ($CDCl_3$) δ: 1.9–2.8 (6H, m), 3.7–4.0 (2H, m), 5.24 (1H, s), 5.64 (1H, s), 5.91 (4H, s), 6.5–6.9 (6H, m). MS, $C_{20}H_{20}O_6$, *m/z* (%): 356 (M^+, 36), 338 (32), 203 (100), 202 (59), 161 (30), 135 (55).

Conclusions

Our investigation of the constituents of peppers and their properties is summarized as follows.

The chemical structures of five phenolic amides from *Piper nigrum*, seven compounds from *P. retrofractum*, and two compounds from *P. baccatum* were determined by chemical and spectroscopic methods.

All phenolic amides possessed significant antioxidative activities that were more effective than α-tocopherol at the same concentration (0.01%). At this concentration, one of the amides had activity as high as the synthetic antioxidants, BHA and BHT.

Naturally occurring antioxidants do inactivate the mutagens in food, and continued study is warranted.

REFERENCES

1. Nakatani, N., and Inatani, R. Two antioxidative diterpenes from rosemary (*Rosmarinus officinalis* L.) and a revised structure for rosmanol. Agr. Biol. Chem. 48: 2081–2085 (1984).
2. Inatani, R., Nakatani, N., and Fuwa, H. Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis* L.) and their derivatives. Agr. Biol. Chem. 47: 521–528 (1983).

3. Muller, C. J., and Jennings, C. G. Constituents of black pepper. Some sesquiterpene hydrocarbons. J. Agr. Food Chem. 15: 762–766 (1967).
4. Muller, C. J., Creveling, R. K., and Jennings, W. G. Some minor sesquiterpene hydrocarbons of black pepper. J. Agr. Food Chem. 16: 113–117 (1968).
5. Traxler, J. T. Piperanine, a pungent component of black pepper. J. Agr. Food Chem. 19: 1135–1138 (1971).
6. Chatterjee, A., and Dutta, C. P. Alkaloids of *Piper longum* Linn.—Structure and synthesis of piperlongumine and piperlonguminine. Tetrahedron 23: 1769–1781 (1967).
7. Hänsel, R., Weiss, D., and Schmidt, B. Fungistatische Wirkung der Kavadroge und ihrer Inhaltsstoffe. Planta Med. 14: 1–9 (1966).
8. Loder, J. W., Moorhouse, A., and Russell, G. B. Tumour inhibitory plants. Amides of *Piper novae-hollandiae* (Piperaceae). Austral. J. Chem. 22: 1531–1538 (1969).
9. Shin, K. H., Yun, H. S., Woo, W. S., and Lee, C. K. Pharmacologically active principle of *Piper retrofractum*. Korean J. Pharmacol. 10: 69–71 (1979).
10. Matsui, K., Wada, K., and Munakata, K. Insect antifeeding substances in *Parabenzoin praecox* and *Piper futokadzura*. Agr. Biol. Chem. 40: 1045–1046 (1976).
11. Su, H. C. F. Insecticidal properties of black pepper to rice weevils and cowpea weevils. J. Econ. Entomol. 70: 18–21 (1977).
12. Miyakado, M., Nakayama I., Yoshioka, H., and Nakatani, N. The Piperaceae amides I: structure of pipercide, a new insecticidal amide from *Piper nigrum* L. Agr. Biol. Chem. 43: 1609–1611 (1979).
13. Su, H. C. F., and Horvat, R. Isolation, identification, and insecticidal properties of *Piper nigrum* amides. J. Agr. Food Chem, 29: 115–118 (1981).
14. Atal, C. K., Dhar, K. L., and Singh, J. The chemistry of Indian *Piper* species. Lloydia 38: 256–264 (1974).
15. Nakatani, N., Inatani, R., and Fuwa, H. Structures and syntheses of two phenolic amides from *Piper nigrum* L. Agr. Biol. Chem. 44: 2831–2836 (1980).
16. Inatani, R., Nakatani, N., and Fuwa, H. Structure and synthesis of new phenolic amides from *Piper nigrum* L. Agr. Biol. Chem. 45: 667–673 (1981).
17. Burkill, I. H. A dictionary of the economic products of the Malay peninsula. Governments of Malaysia and Singapore, Kuala Lumpur, Malaysia, 1966, pp. 1781–1782.
18. Okogun, J. I., and Ekong, D. E. U. Extracts from the fruits of *Piper guineense* Schum. and Thonn. J. Chem. Soc. Perkin Trans. 1: 2195–2198 (1974).
19. Nakatani, N., and Inatani, R. Isobutyl amides from pepper (*Piper nigrum* L.). Agr. Biol. Chem. 45: 1473–1476 (1981).
20. Chatterjee, A., Basa, S. C., and Ray, A. B. Spectral properties of the lignan cubebin. J. Indian Chem. Soc. 45: 723 (1968).
21. Batterbee, J. E., Burden, R. S., Crombie, L., and Whiting, D. A. Chemistry and synthesis of the lignan (-)-cubebin. J. Chem. Soc. (C): 2470–2477 (1969).
22. Branen, C. L. Toxicology and biochemistry of BHA and BHT. J. Am. Oil Chemists Soc. 52: 59 (1975).
23. Anon. Food Chemical News 17: 00 (March 1, 1976).
24. Sugimura, T., Nagao, M., Kawachi, T., Honda, M., Yahagi, T., Seino, Y., Sato, S., Matsukura, N., Matsushima, T., Shirai, A., Sawamura, M., and Matsumoto, H. Mutagen-carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. In: Origins of Human Cancer (H. H. Hiatt, J. D. Watson, and J. A. Winsten, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, N Y, 1977, p. 1561.
25. Taylor, S. L. Mutagenesis vs. carcinogenesis. Food Technol. 36 (3): 65–127 (1982).
26. Pariza, M. W. Mutagens in heated foods. Food Technol. 36 (3): 53–56 (1982).
27. Yonei, S., and Furui, H. Lethal and mutagenic effects of malondialdehyde, a decomposition product of peroxidized lipids, on *Escherichia coli* with different DNA-repair capacities. Mutat. Res. 88: 23–32 (1981).
28. Shamberger, R. J., Corlett, C. L., Beaman, K. D., and Kasten, B. L. Antioxidants reduce the mutagenic effect of malonaldehyde and β-propiolactone. Part IX, Antioxidants and cancer. Mutat. Res. 66: 349–355 (1979).
29. Wattenberg, L. W. Inhibition of chemical carcinogen-induced pulmonary neoplasia by butylated hydroxyanisole. J. Natl. Cancer Inst. 50: 1541–1544 (1973).
30. McKee, R. H., and Tometsko, A. M. Inhibition of promutagen activation by the antioxidants butylated hydroxyanisole and butylated hydroxytoluene. J. Natl. Cancer Inst. 63: 473–477 (1979).
31. Clapp, N. K., Bowles, N. D., Satterfield, L. C., and Klima, W. C. Selective protective effect of butylated hydroxytoluene against 1,2-dimethylhydrazine carcinogenesis in BALB/c mice. J. Natl. Cancer Inst. 63: 1081–1085 (1979).
32. Guttenplan, J. B. Inhibition by L-ascorbate of bacterial mutagenesis induced by two *N*-nitroso compounds. Nature 268: 368–370 (1977).
33. Shamberger, R. J. Relationship of selenium to cancer. I. Inhibitory effect of selenium on carcinogenesis. J. Natl. Cancer Inst. 44: 931–936 (1970).
34. Sayer, J. M., Yagi, H., Wood, A. W., Conney, A. H., and Jerina, D. M. Extremely facile reaction between the ultimate carcinogen benzo[a]pyrene-7,8-diol 9,10-epoxide and ellagic acid. J. Am. Chem. Soc. 104: 5562–5564 (1982).