Treatment of 1,2,3,4-Tetrahydroquinoline with Sodium Borohydride in Acetic Acid——Tetrahydroquinoline (5 mmol) was treated as described for **4b** to give a mixture of **3a** [40%] and **4a** [60%] on GLC. This mixture was separated by acid extraction into neutral (0.4 g) and basic (0.3 g) fractions. The neutral product was **4a**, IR ν_{max} cm^{-1}: 1655. NMR δ: 1.95 (2H, quintet, $J=6.5$ Hz, C-3-H), 2.23 (3H, s, NCOMe), 2.72 (2H, t, $J=6$ Hz, C-4-H), 3.78 (2H, t, $J=6$ Hz, C-2-H), 7.0—7.3 (4H, m, Ar–H). The basic fraction was purified on an alumina column with dichloromethane to give 0.2 g of **3a**.[3b] Compounds **3a** and **4a** were identical with appropriate authentic specimens prepared from 1,2,3,4-tetrahydroquinoline by alkylation and acylation.

Reduction of Isoquinoline 6——Isoquinoline **6** was reduced as described for quinoline in the presence of acetic anhydride. The product was chromatographed on an alumina column with dichloromethane to give N-acetyl-1,2-dihydroisoquinoline **7**[4a] [80% yield], IR ν_{max} cm^{-1}: 1663, 1627. NMR δ: a mixture of two rotational isomers of the amide bond in a ratio of 88: 12. 2.22 (3H, s, NCOMe), 4.95 and 4.83 (2H, each s, C-1-H), 5.85 and 5.86 (1H, each d, $J=8$ Hz, C-3-H), 6.66 (1H, d, $J=8$ Hz, C-4-H), 6.9—7.4 (4H, m, Ar–H), N-acetyl-1,2,3,4-tetrahydroisoquinoline **9** [9% yield], IR ν_{max} cm^{-1}: 1638. NMR δ: a mixture of two rotational isomers of the amide bond in a ratio of 64: 36. 2.17 (3H, s, N–COMe), 2.90 (2H, m, C-4-H), 3.67 and 3.82 (2H, each t, $J=5$ Hz, C-3-H), 4.73 and 4.62 (2H, each s, C-1-H), 7.17 (4H, s, Ar–H), and N-ethyl-1,2,3,4-tetrahydroisoquinoline **8**[3b] [6% yield], IR ν_{max} cm^{-1}: 1601, 1572. NMR δ: 1.16 (3H, t, $J=7$ Hz, N–CH_2–Me), 2.55 (2H, q, $J=7$ Hz, N–$C\underline{H}_2$–Me), 2.88 (4H, m, C-3-H+C-4-H), 3.60 (2H, s, C-1-H), 7.05 (4H, s, Ar–H). Compounds **8** and **9** were identical with appropriate authentic specimens prepared from 1,2,3,4-tetrahydroisoquinoline by alkylation and acylation.

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Studies on the Antioxidants. XII.[1] Combination Effects of Antioxidants on the Reduction of Ferric Ions

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The reduction of ferric ions by phenolic antioxidants was investigated by the Emmerie–Engel method. Diphenolic and triphenolic compounds reduced 2—2.5 and 5 molar equivalents of ferric ions, respectively. Monophenolic compounds reduced different amounts of ferric ions: BHT, 0.6; tocopherol, 2; BHA, 3 and sesamol, 4 molar equivalents. When two compounds were used in combination, the reducing potency was enhanced or decreased depending on the antioxidants used. The combination of BHA and BHT produced 1.5 times more ferrous ions than the calculated amount, and the combination of BHA and PG produced only 0.75 times the calculated amount of ferrous ions. In the combined use of BHA and BHT, BHT was more rapidly lost than it was in the reaction of BHT alone, probably due to some interaction of the two compounds.

Keywords——Emmerie–Engel reagent; phenols; antioxidants; butylated hydroxyanisole (BHA); butylated hydroxytoluene (BHT)

It has been demonstrated by Bolland[3] that the peroxidation of lipids involves the free radical-forming propagation reactions, in which various peroxy free radicals are formed. Phenolic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tocopherol and nordihydroguaiaretic acid (NDGA), are used as inhibitors of lipid peroxidation, and several theories have been proposed regarding the mech-

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anism of their antioxidative effects.[4] BHA, BHT and PG are frequently used in combination[4] since their excellent synergistic antioxidative effects have been demonstrated with lard.[5] It seems important to investigate the hydrogen- or electron- donating ability of antioxidants, alone and in combination, with stalbe hydrogen or electron acceptors. Production of the ferrous ion from the ferric ion can be colorimetrically estimated in the presence of α,α′-dipyridyl by the Emmerie–Engel method, which is practically used to estimate tocopherol[6] and several other antioxidants.[7,8] This paper deals with the combined effects of common antioxidants on the reduction of ferric ions.

Experimental

Materials——α,α′-Dipyridyl and PG were commercial products obtained from Tokyo Kasei Kogyo Company, Ltd. Ferric chloride ($FeCl_3 \cdot 6H_2O$) and ferrous chloride ($FeCl_2 \cdot 4H_2O$) were products of Wako Pure Chemical Industries, Ltd. NDGA and sesamol were obtained from K and K Laboratories, Inc. and Aldrich Chemical Company, Inc., respectively. *dl*-α-Tocopherol (Toc; 100% purity) was kindly supplied by Eisai Company, Ltd. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), both supplied by Nikki-Universal Company, Ltd., were recrystallized from petroleum ether to remove the 3-isomer[9] and from ethanol, respectively. Ethyl protocatechuate (EP) was synthesized from protocatechuic acid according to the method of Hesse.[10]

Estimation of the Color Intensity of Emmerie-Engel Reagent——A 10.0 ml aliquot of freshly prepared 6.4 mM (0.10%) α,α′-dipyridyl ethanol solution and 10.0 ml of 1.5 mM ferric chloride (0.04% $FeCl_3$–$6H_2O$) ethanol solution were added successively to 10.0 ml of ethanolic solution containing 20—100 μM of each test compound in a test tube. The mixtures were kept at 20° in the dark. The absorbances of the mixture at 520 nm were measured at selected times with a Hitachi 101 spectrophotometer. A control experiment without test compound was simultaneously performed, and the constant absorbance (0.06 at 520 nm) of this was substracted.

Loss of BHA and BHT by Emmerie-Engel Reagent——A mixture of equal volumes (5.0 ml) of ethanolic solutions of 3 or 6 mM BHA and/or 3 or 6 mM BHT, 64 mM α,α′-dipyridyl and 15 mM ferric chloride was treated at 20° for 60 min. Aliquots (5.0 ml) of the reaction mixtures were extracted with 5.0 ml of benzene in the presence of 30 ml of water. The organic phase (5 μl) was subjected to high performance liquid chromatography (HPLC) using a Shimadzu LC-2 liquid chromatograph with a stainless steel column of Zorbax ODS (4.6 mm i.d. × 0.25 m) and a Shimadzu SPD-1 spectrophotometric detector. HPLC was carried out by elution with ethanol–methanol–water (5 : 3 : 2) at 0.5 ml/min. The amounts of BHA and BHT were estimated from their peak heights. The retention times of BHA and BHT were 7.6 and 29.5 min, respectively. They were detected at 290 nm (absorption maximum of BHA) and at 280 nm (absorption maximum of BHT).

Results and Discussion

Phenolic antioxidants such as BHA, BHT, sesamol, Toc, EP, PG and NDGA were treated with a large excess of ferric ions in the presence of α,α′-dipyridyl. Color developments by Toc, EP and NDGA rapidly reached the maxima within 5 min. BHA, sesamol and PG reacted with the reagent rather slowly and the color developments reached the maxima after about 10 min. BHT gradually reacted with the reagent, but the color development did not reach the maximum even after 60 min. The color intensities with the antioxidants were proportional to their concentrations when measured after 60 min. Representative profiles of concentration dependence are shown in Fig. 1 together with that of the standard ferrous chloride. EP,

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having a diphenolic function, and NDGA, having two diphenolic functions, yielded 2 and 4 molar equivalents of ferrous ions, respectively. The triphenolic antioxidant, PG, produced 5 molar equivalents of ferrous ions. The results were consistent with those obtained with water-soluble phenols in terms of the numbers of phenolic hydroxyl functions. Thus, 1 mol of pyrocatechol, protocatechuic acid, pyrogallol and gallic acid produced 2.5, 2.0, 5.0 and 5.1 mol of ferrous ions after treatment for 60 min, respectively. Quite different molar equivalents of ferrous ions were, however, produced by the monophenolic antioxidants: BHT, 0.6 mol; Toc, 2 mol; BHA, 3 mol; and sesamol, 4 mol. The amounts of ferrous ions produced by Toc and sesamol were identical with those reported earlier.[6,11,12]

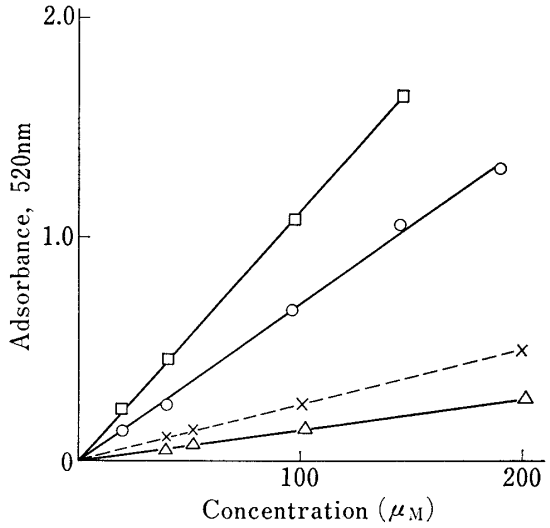

Fig. 1. Calibration Plots for the Ferrous–Dipyridyl Complex

Mixtures of equal volumes of ethanolic solutions of the antioxidant, 6.4 mM α,α'-dipyridyl and 1.5 mM ferric chloride were treated for 60 min. ○, BHA; △, BHT; and □, PG. × indicates the absorbances of a mixture of equal volumes of ethanol, 6.4 mM α,α'-dipyridyl and 0—200 μM ferrous chloride.

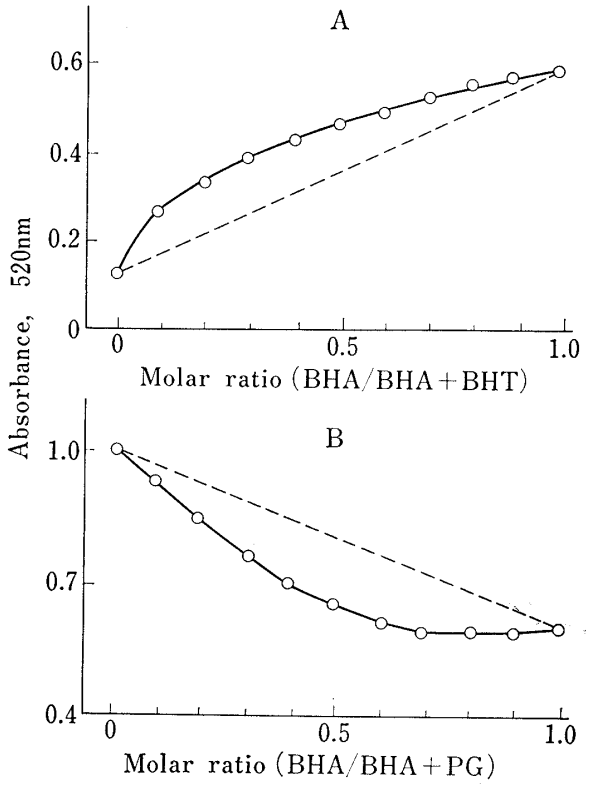

Fig. 2. Color Intensity of Mixtures of the Combined Antioxidants, 6.4 mM α,α'-Dipyridyl and 1.5 mM Ferric Chloride Treated for 60 min

A: BHA+BHT (total concentration, 80 μM). B: BHA+PG (total concentration, 80 μM). Calculated values are indicated by dashed lines.

Table I. Color Intensity Ratios of Emmerie–Engel Reagent with Combined Antioxidants Relative to the Calculated Mean Values

	BHT	Sesamol	Toc	EP	PG	NDGA
BHA	1.15	1.03	0.93	0.97	0.77	0.91
BHT		0.98	0.89	0.96	0.89	0.94
Sesamol			1.00	0.99	0.91	0.95
Toc				1.00	1.00	1.00
EP					1.20	1.02
PG						1.00

Two antioxidants were mixed in a molar equivalent ratio (total concentration: 40 μM) and treated with 6.4 mM α,α'-dipyridyl and 1.5 mM $FeCl_3$ for 60 min. The ratios of the observed color intensity to that calculated (see Fig. 2) are listed in the table.

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Various pairs from seven antioxidants were combined in a molar equivalent ratio, and the ratios of the color intensities of the 21 combinations at 60 min to the calculated mean values are listed in Table I. If the ratio is larger or smaller than 1.0, some combination effects of the two antioxidants on the color intensities are present. Such effects were observed with several combinations. Marked deviations of the ratio from 1.0 were observed with three combination systems: BHA+BHT (1.15), PG+EP (1.20) and BHA+PG (0.77). When BHA was combined with BHT in various ratios (Fig. 2A), the enhancement of the color intensity of the Emmerie–Engel reagent was greatest at a molar ratio of BHA/BHT=3/7, exhibiting 1.52-fold increase of the color intensity. When BHA was combined with PG in several ratios (Fig. 2B), inhibition was greatest at a molar ratio of BHA/PG=6/4, and the combination decreased the color intensity to 75% of the calculated value. There were no apparent regularities with regard to the structures of the antioxidants to explain the combination effects.

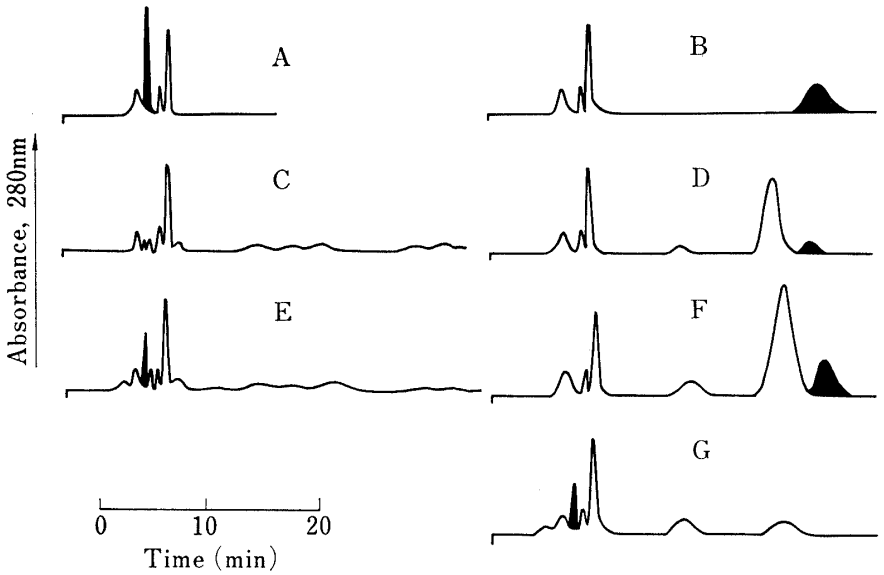

Fig. 3. High Performance Liquid Chromatograms of BHA and BHT Treated with Ferric Ions (15 mM)

A: BHA (3 mM) without $FeCl_3$. B: BHT (3 mM) without $FeCl_3$. C: BHA (3 mM). D: BHT (3 mM). E: BHA (6 mM). F: BHT (6 mM). G: BHA (3 mM)+BHT (3 mM). The peaks corresponding to BHA and BHT are filled (black).

The losses of BHA and BHT with ferric ions were studied by HPLC (Fig. 3). Complete loss of 3 mM (Fig. 3C) and the loss of 5 mM from 6 mM BHA (Fig. 3E) were observed when BHA was treated with an excess of ferric ions (15 mM). When BHT was treated similary, 1.8 mM and 3.5 mM BHT were lost from the initial concentrations of 3 mM (Fig. 3D) and 6 mM (Fig. 3F), respectively. When a mixture of BHA (3 mM) and BHT (3 mM) was treated with 15 mM ferric ion, the loss of BHA was 2 mM and that of BHT was complete, as shown in Fig. 3G. It is evident that the loss of BHT was significantly enhanced by BHA, when compared with that of BHT alone (Fig. 3D and F). Distributions of oxidation products of BHT in the reaction of the combined antioxidants were different from those in the reaction of BHT alone. The effects of enhacement of the color development with a combination of BHA and BHT may be explained by the increase of the rate of loss of BHT through the interaction with BHA. There might be similar interactions between pairs of antioxidants in the combimnations of BHA and PG, and PG and EP.

The effectiveness of an antioxidant depends on the substrate oil used. With lard, the antioxidative effect increases in the order NDGA, PG, BHA, BHT and EP on a weight basis.[13]

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Judging from the reducting power of ferric ions described here, the antioxidative potency increased in the order PG, NDGA, BHA, EP and BHT. It has been reported that the combinations of BHA with BHT or of BHA with PG showed synergism in the protection of lard from peroxidation.[5] In this study, the combination of BHA and BHT showed synergism, while that of BHA and PG showed inhibitory effects. Thus, the potencies of these antioxidants towards the reduction of ferric ions were not in accord with their antioxidative effects on lard.

As early as 1938, Emmerie and Engel[6] proposed an elegant colorimetric determination of α-tocopherol on the basis of its reducing power against ferric chloride, and this method has been applied to the analysis of several antioxidants with slight modifications.[7,8,14,15] It was noted that application of the Emmerie–Engel method to the estimation of antioxidants was not suitable when more than two antioxidants are present, since several combinations greatly modified the reducing potency of the antioxidants against ferric ions.

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Inhibition of S-Adenosylmethionine Decarboxylase from Rat Liver by Synthetic Decarboxylated S-Adenosylmethionine and Its Analogs

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Synthetic decarboxylated S-adenosylmethionine and six analogs were tested in a decarboxylating system based on rat liver S-adenosylmethionine decarboxylase. All the compounds inhibited the putrescine-activated decarboxylase activity and were competitive with respect to S-adenosylmethionine. Among the compounds tested, S-5′-deoxyadenosyl-(5′)-2-methylthioethylamine was the most potent inhibitor. The K_i value of the inhibitor was seven times lower than that of decarboxylated S-adenosylmethionine.

Keywords——S-adenosylmethionine decarboxylase; polyamines; new inhibitor; product inhibition; decarboxylated S-adenosylmethionine analogs

S-Adenosylmethionine decarboxylase (EC 4.1.1.50) is one of the two key decarboxylases in polyamine biosynthesis. The enzyme from rat liver has been purified and characterized as having a pyruvoyl prosthetic group.[2,3] The product, decarboxylated S-adenosylmethionine, is known to inhibit the enzyme reaction activated by putrescine *in vitro*,[4] since it binds to the enzyme more tightly than the substrate. Recently, we succeeded in synthesizing various analogs of decarboxylated S-adenosylmethionine,[5] and we have studied the effects of these analogs in a spermidine synthesizing system from rat prostate.[6] The present paper deals with the effects of these analogs in the decarboxylating system.

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