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Natural Antioxidants. III.[1] Antioxidative Components Isolated from Rhizome of *Curcuma longa* L.

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Antioxidative components in the methanol extract of the rhizome of *Curcuma longa* L. were investigated by using our evaluation method based on the air oxidation of linoleic acid. Curcuminoids such as curcumin, 4-hydroxycinnamoyl(feruloyl)methane and bis(4-hydroxycinnamoyl)methane were found to be active components. Curcumin was the most active component and its 50% inhibitory concentrations for the air oxidation of linoleic acid were 1.83×10^{-2}% (thiobarbituric acid value) and 1.15×10^{-2}% (peroxide value). These values of curcumin are superior to those of *dl*-α-tocopherol.

Keywords——*Curcuma longa*; antioxidant; air oxidation; linoleic acid; curcumin; 4-hydroxycinnamoyl(feruloyl)methane; bis(4-hydroxycinnamoyl)methane; *dl*-α-tocopherol

We have investigated the natural antioxidants in methanol extracts of crude drugs by using our evaluation method[2] based on the air oxidation of linoleic acid. We have already reported[3] that methanol extracts of 20 kinds crude drugs (Zingiberis rhizoma, Plantaginis semen, Schizandrae fructus, Crucumae rhizoma, *etc.*) showed antioxidative activities which were stronger than that of *dl*-α-tocopherol at the same concentration. Thus, we have been attempting to identify the antioxidative components in such methanol extracts of crude drugs. We have identified several antioxidative components such as caffeine, *d*-catechin and *l*-epicatechin in the leaves of *Thea sinensis* L.,[4] and geniposidic acid in the seeds of *Plantago asiatica* L.[1] The activities of these components were found to be comparable to those of butyl hydroxyanisole (BHA) and butyl hydroxytoluene (BHT).

In this paper, we describe the isolation and identification of active components in the antioxidative methanol extract or rhizome of *Curcuma longa* L. (=*C. domestica* VALETON; Curcumae rhizoma (turmeric), Japanese name: Ukon) by using our evaluation method.[2] We also invetigated the correlation between chemical structures and antioxidative effects of antioxidative components of the rhizome of *Curcuma longa* L. by testing various related compounds.

Experimental

All melting points were determined on a Yanaco MP-500 micromelting point apparatus and are uncorrected. The infrared (IR) spectra were recorded with a JASCO IRA-202 spectrophotometer, mass spectra (MS) with a JEOL JMS-D/100 spectrometer and proton nuclear magnetic resonance (^{1}H-NMR) spectra with a JEOL LNM-FX 900 FT spectrometer (internal standard, tetramethylsilane). Thin layer chromatography (TLC) was conducted on Kieselgel $60F_{254}$ (Merck). The developing solvent for TLC was chloroform–methanol (19 : 1, v/v) mixture. The spots on TLC plates were detected by spraying 50% H_2SO_4 solution followed by heating.

Reagents——BHA, BHT, cinnamic acid and vanillic acid were purchased from Wako Pure Chemical Industries Co. *dl*-α-Tocopherol, *p*-coumaric acid, caffeic acid, ferulic acid and protocatechuic acid were obtained from Tokyo Kasei Co., Ltd. Other chemicals used were of special grade of equivalent quality.

Antioxidative Test——Antioxidative test were carried out under the same conditions as described in the previous paper.[2] Inhibitory ratios of samples tested were similarly calculated from the peroxide value (POV) and thiobarbituric acid value (TBAV) of linoleic acid after air oxidation with or without the addition of samples.

Extraction and Separation——The dried rhizomes of *Curcuma longa* L. (500 g, Japanese commercial product) were extracted three times with 1.5 l of *n*-hexane under reflux for 6 h, then with methanol in the same way. Both extracts were concentrated to dryness under reduced pressure. The methanol extract, which was more active than the *n*-hexane extract, was fractionated repeatedly by silica gel (Malinckrodt) column chromatography with chloroform–methanol (19 : 1, v/v) mixture as an eluent. Three active components were obtained, and were identified as curcumin (15 g, yield: 3%) by mixed mp determination and comparison of the IR and ^{1}H-NMR spectra with those of an authentic sample, and 4-hydroxycinnamoyl(feruloyl)methane (0.09 g, yield: 0.018%) and bis(4-hydroxycinnamoyl)methane (0.68 g, yield: 0.136%) whose data were in accord with published values (mp, elemental analysis, IR, MS, ^{1}H-NMR).[5-7]

Results and Discussion

The rhizomes of *Curcuma longa* L. were extracted first with *n*-hexane and then with methanol as described in the experimental section. As shown in Table I, the inhibitory ratio of the *n*-hexane extract added to linoleic acid at 0.1% concentration was low, whereas that of the methanol extract was high. Thus, the methanol extract was fractionated repeatedly by silica gel column chromatography with chloroform–methanol (19 : 1, v/v) mixture as the eluent. The fractions were monitored by TLC and measurements of inhibitory ratios on the air oxidation of linoleic acid when they were added at 0.1% concentration. The active fractions were purified by recrystallization. The active components were identified as curcumin, 4-hydroxycinnamoyl(feruloyl)methane and bis(4-hydroxycinnamoyl)methane on the basis of mp, elemental analysis and spectral comparisons. The antioxidative activities of these components at 0.1% concentration are shown in Table I. Curcumin was the most active of these three components. Even the least active component, bis(4-hydroxycinnamoyl)methane, was more potent than *dl*-α-tocopherol at the same concentration.

Next, the relationships between inhibitory ratios and the added concentrations of test samples were examined. All the active samples showed concentration–dependent inhibitory

TABLE I. Effects of Fractions and Components of the Rhizome of *Curcuma longa* L. and Related Compounds on Air Oxidation of Linoleic Acid

Sample (0.1% added)	Inhibitory ratio	
	TBAV (%)	POV (%)
n-Hexane ex.	42	42
Methanol ex.	100	100
Curcumin	100	100
4-Hydroxycinnamoyl(feruloyl)methane	94	86
Bis(4-hydroxycinnamoyl)methane	80	77
Cinnamic acid	58	37
p-Coumaric acid	65	42
Caffeic acid	100	100
Ferulic acid	100	100
Protocatechuic acid	88	83
Vanillic acid	80	79
BHA	100	100
BHT	100	100
dl-α-Tocopherol	17	20

TABLE II. The 50% Inhibitory Concentration (IC_{50}) Values of Antioxidative Components of the Rhizome of *Curcuma longa* L. and Related Compounds on Air Oxidation of Linoleic Acid

Sample	50% inhibitory concentration (IC_{50})	
	TBAV (%)	POV (%)
Methanol ex.	1.22×10^{-2}	1.21×10^{-2}
Curcumin	1.83×10^{-2}	1.15×10^{-2}
4-Hydroxycinnamoyl(feruloyl)methane	1.88×10^{-2}	2.79×10^{-2}
Bis(4-hydroxycinnamoyl)methane	2.80×10^{-2}	3.17×10^{-2}
Caffeic acid	5.63×10^{-3}	5.30×10^{-3}
Ferulic acid	8.95×10^{-3}	5.41×10^{-3}
Protocatechuic acid	1.85×10^{-2}	1.54×10^{-2}
Vanillic acid	2.01×10^{-2}	1.83×10^{-2}
BHA	3.37×10^{-3}	3.75×10^{-3}
BHT	1.92×10^{-3}	2.24×10^{-3}
dl-α-Tocopherol	1.95×10^{-1}	2.48×10^{-1}

effects on the air oxidation of linoleic acid. Thus, the 50% inhibitory concentration (IC_{50}) of each samples was calculated from the results. The values obtained are listed in Table II. The IC_{50} values of curcumin, 4-hydroxycinnamoyl(feruloyl)methane, and bis(4-hydroxycinnamoyl)methane were higher than that of BHA or BHT, but lower than that of *dl*-α-tocopherol. Therefore, the main antioxidative components in the rhizome of *Curcuma longa* L. were proved to be curcuminoids, of which curcumin is a typical example.

We next investigated the correlation between the chemical structures and antioxidative activities of curcuminoids by using the same evaluation method.[2] The antioxidative activities of the test compounds added to linoleic acid at 0.1% concentration are also shown in Table I. The IC_{50} values (except for those of cinnamic acid and *p*-coumaric acid) are listed in Table II. Since cinnamic acid showed weaker antioxidative activity than *p*-coumaric acid, as shown in Table I, a phenolic hydroxy group is important for the antioxidative activity. From a comparison of IC_{50} values of three curcuminoids in Table II, it is evident that the presence of a methoxy group next to the phenolic hydroxy group also contributes to the antioxidative activities of curcuminoids. However, from the IC_{50} values of caffeic acid and ferulic acid, it seems that a phenolic hydroxy group is preferable as the group adjacent to the phenolic hydroxy group. Further, it appears that double bonds in curcuminoids also contribute to the antioxidative activities on the basis of the relative activities of vanilic acid and ferulic acid, or of protocatechuic acid and caffeic acid. Therefore, it is understandable on the basis of these results that eugenol and zingerone, which possess the partial active chemical structure of curcumin, are very strong antioxidative agents.[8,9]

Although Hirahara *et al.*[10] reported the antioxidative activity of alcohol extract of turmeric (spice; rhizome of *Curcuma longa* L.), they did not identify the active components. On the other hand, Kiso *et al.*[11] recently reported antihepatoxic principles of the rhizome of *Curcuma longa* L. against carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes. In that paper, they reported that curcuminoids possess significant antihepatoxic action. However, our paper is the first report on the antioxidative components of the rhizome of *Curcuma longa* L.

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References

1) Part II: S. Toda, T. Miyase, H. Arichi, H. Tanizawa, and Y. Takino, *Chem. Pharm. Bull.*, **33**, 1270 (1985).
2) H. Tanizawa, Y. Sazuka, A. Komatsu, S. Toda, and Y. Takino, *Chem. Pharm. Bull.*, **31**, 4139 (1983).
3) S. Toda, H. Tanizawa, S. Arichi, and Y. Takino, *Yakugaku Zasshi*, **104**, 394 (1984).
4) H. Tanizawa, S. Toda, Y. Sazuka, T. Taniyama, T. Hayashi, and Y. Takino, *Chem. Pharm. Bull.*, **32**, 2011 (1984).
5) K. R. Schrinavasan, *J. Pharm. Pharmacol.*, **5**, 488 (1953).
6) M. Kuroyanagi and S. Natori, *Yakugaku Zasshi*, **90**, 1467 (1970).
7) P. J. Roughley and D. A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, **1973**, 2379.
8) T. Hasegawa, *Nippon Eiyo Shokuryo Gakkaishi*, **32**, 267 (1979).
9) H. Fujio, A. Hiyoshi, T. Asari, and K. Sumie, *Nippon Shokuhin-Kogyo Gakkaishi*, **16**, 241 (1964).
10) F. Hirahara, Y. Takagi, and H. Iwao, *Eiyogaku Zasshi*, **32**, 1 (1974).
11) Y. Kiso, Y. Suzuki, N. Watanabe, Y. Oshima, and H. Hikino, *Planta Med.*, **49**, 185 (1983).