Antioxidant Effect and Antimicrobial Activity of Phenolic Sulfides

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Some phenolic compounds containing both phenolic hydroxyl groups and sulfide groups in the molecules were prepared by Michael addition and radical addition reactions of ethanethiol and ethanedithiol with *o*-, *m*- and *p*-vinylphenol. The antioxidant effect of these products on the autoxidation of lard was examined by an oven test at 60°C. The antimicrobial activities of these products were evaluated by an agar dilution method. Three kinds of gram-positive and three kinds of gram-negative bacterial strains, as well as six kinds of fungal strains were used for the test. It was found that the Michael addition products showed considerably better antioxidant effects than BHT, whereas the radical addition products did not show any antioxidant effect. The antioxidant effects of these compounds were influenced by the positions of the sulfide groups in their molecules. The phenolic sulfides tested in this report showed good antimicrobial activities against bacteria and fungi.

Organic substrates such as oils, plastics and rubbers are deteriorated by the action of atmospheric oxygen and microbes in the environment. It is well known that phenolic antioxidants act as inhibitors for radical chain reactions on autoxidation of such organic substrates, and the sulfuric antioxidants act as decomposers for hydroperoxides (1,2). Moreover, some phenolic compounds are known to show antimicrobial activities in addition to their antioxidant effects (3). Previously, we characterized some methylene-bridged phenolic oligomers and polyvinylphenols as good antioxidants (4-6).

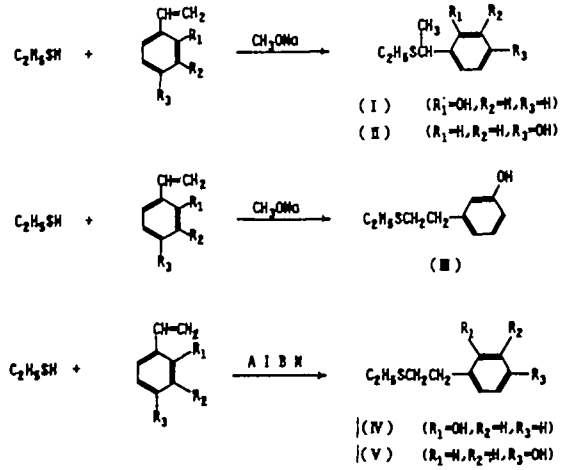
In this report, some phenolic compounds containing both phenolic hydroxyl groups and sulfide groups in the molecules were prepared by Michael addition and radical addition reactions of thiols with *o*-, *m*- and *p*-vinylphenol. The antioxidant effects on the autoxidation of lard and antimicrobial activities were evaluated.

EXPERIMENTAL

Materials and measurements. *o*-, *m*- and *p*-Vinylphenols were prepared by the method described in our previous report (6), and characterized by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. ^{1}H-NMR spectra were determined with a JEOL model JNM-FX90A (90 MHz) spectrometer using TMS as an interanl standard. ^{13}C-NMR spectra were recorded on a JNM-FX90A Fourier Transform Spectrometer operating at 22.5 MHz with complete proton decoupling. Chemical shifts were referred to internal TMS as the standard. IR spectra were measured using a BIO-RAD model DIGILAB FTS-60 spectrometer.

Refined lard was obtained from a commercial refinery with POV:0.20; AV:0.70; SV:196.5; and IV:60.9.

Preparation of ethanethiol derivatives. Ethanethiol derivatives were prepared as follows:



Michael addition products (I) and (II) were prepared by the reaction of ethanethiol with *o*- or *p*-vinylphenol in the presence of sodium methoxide as a catalyst. A mixture of ethanethiol (0.93 g, 15 mmol), *o*- or *p*-vinylphenol (0.60 g, 5.0 mmol) and sodium methoxide (60 mg) was stirred at 40°C for 12 hr. After the reaction was over, the mixture was neutralized with hydrochloric acid, and then extracted with ether. Ether was evaporated *in vacuo*, and the residue was passed through a chromatographic column on silica gel to obtain (I) in 79.4% yield (0.66 g) and (II) in 53.7% yield (0.49 g). In the case of *m*-vinylphenol, no Michael addition product was obtained, but a small amount of radical addition product (III) was obtained in 1.2% yield (11 mg). Radical addition products (IV) and (V) were prepared by reaction of ethanethiol with *o*- or *p*-vinylphenol using 2,2'-azobis(isobutyronitrile) (AIBN) as a catalyst. A mixture of ethanethiol (1.20 g, 20 mmol), *o*- or *p*-vinylphenol (0.24 g, 2.0 mmol) and AIBN (48 mg) was stirred at 40°C for 12 hr. After the reaction, the reaction mixture was passed through a chromatographic column on silica gel to obtain (IV) in 40.1% yield (0.15 g) and (V) in 68.5% yield (0.25 g).

The molecular structure of these products was determined by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The results are as follows:

(I):IR (Capi., cm $^{-1}$):3600-3200, 1230 (Ar-OH), 2970, 1373 (CH_3-), 2927 (-CH_2-), 1600, 1581, 1485, 752 (arom.); ^{1}H-NMR ($CDCl_3$, δ):1.2 (3H, t, $\underline{CH_3}$-CH_2-), 1.6 (3H, d, $\underline{CH_3}$-CH<), 2.4 (2H, q, -CH_2-), 4.2 (1H, q, -CH<), 6.6-7.5 (4H, arom.); ^{13}C-NMR ($CDCl_3$, δ):14.4 ($\underline{CH_3}$-CH_2-), 20.5 ($\underline{CH_3}$-CH<), 25.2 (-CH_2-), 42.0 (-CH<), 117.9, 120.5, 127.1, 128.9, 155.4 (arom.)

(II):^{1}H-NMR ($CDCl_3$, δ):1.2 (3H, t, $\underline{CH_3}$, CH_2-), 1.5 (3H, d, $\underline{CH_3}$-CH<), 2.3 (2H, q, -CH_2-), 3.9 (1H, q, -CH<), 6.7-7.3 (4H, arom.); ^{13}C-NMR ($CDCl_3$, δ):14.4 ($\underline{CH_3}$-CH_2-), 22.5 ($\underline{CH_3}$-CH<), 25.1 (-CH_2-), 43.1 (-CH<), 115.3, 128.3, 135.8, 154.6 (arom.)

(III):^{1}H-NMR ($CDCl_3$, δ):1.2 (3H, t, CH_3-), 2.0 (2H, q, CH_3-$\underline{CH_2}$-S-), 2.8 (4H, -S-$\underline{CH_2}$-$\underline{CH_2}$-), 5.8 (1H, s, OH-), 6.5-7.4 (4H, arom.); ^{13}C-NMR ($CDCl_3$, δ):14.8 (CH_3-), 26.2, 33.1,

36.3 (-CH_2-), 113.4, 115.6, 121.0, 129.7, 142.7, 155.8 (arom.)

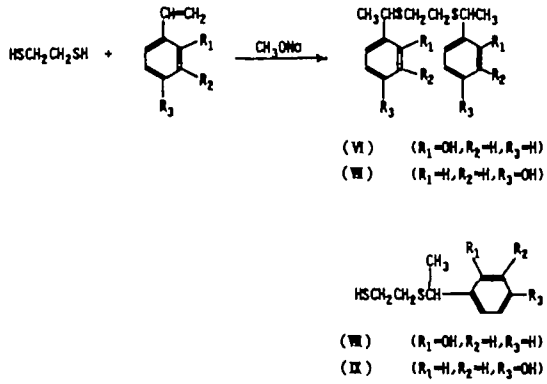
(IV):^{1}H-NMR ($CDCl_3$, δ):1.2 (3H, t, CH_3-), 2.6 (2H, q, CH_3-$C\underline{H}_2$-S-), 2.9 (4H, -S-$C\underline{H}_2$-$C\underline{H}_2$-), 6.1 (1H, s, OH-), 6.7-7.3 (4H, arom.); ^{13}C-NMR ($CDCl_3$, δ):14.7 (CH_3-), 26.3, 31.5, 32.2 (-CH_2-), 116.2, 121.0, 127.4, 127.9, 130.7, 153.9 (arom.)

(V):IR (Capi., cm^{-1}):3600-3100, 1219 (Ar-OH), 2967, 1373 (CH_3-), 2924 (-CH_2-), 1613, 1512, 1447 (arom.); ^{1}H-NMR ($CDCl_3$, δ):1.2 (3H, t, CH_3-), 2.0 (2H, q, CH_3-$C\underline{H}_2$-S-), 2.8 (4H, -S-$C\underline{H}_2$-$C\underline{H}_2$-), 5.2 (1H, s, OH-), 6.6-7.2 (4H, arom.); ^{13}C-NMR ($CDCl_3$, δ):14.8 (CH_3-), 26.1, 33.5, 35.5 (-CH_2-), 115.4, 129.6, 132.9, 154.1 (arom.)

Preparation of ethanedithiol derivatives. 1,2-Ethanedithiol derivatives were prepared as follows:

Using sodium methoxide (60 mg) as a catalyst, Michael addition of *o*- or *p*-vinylphenol (0.60 g, 5.0 mmol) to 1,2-ethanedithiol (0.27 g, 3.0 mmol) resulted in the disubstituted products of (VI) in 13.1% yield (0.11 g) and (VII) in 23.7% yield (0.20 g), and monosubstituted products (VIII) in 11.2% yield (0.12 g) and (IX) in 22.4% yield (0.24 g), respectively. The reaction procedure was the same as those of (I).

(VI):IR ($CDCl_3$, cm^{-1}):3500-3100, 1225 (Ar-OH), 2980 (CH_3-), 2920 (-CH_2-), 1605, 1580, 1480 (arom.) ^{1}H-NMR ($CDCl_3$, δ):1.5 (6H, d, CH_3-) 2.5 (4H, -CH_2-), 4.1 (2H, q, -CH<), 6.6-7.3 (8H, arom.); ^{13}C-NMR ($CDCl_3$, δ):20.5 (CH_3-), 30.6, 30.7 (-CH_2-), 40.4, 40.6 (-CH<), 117.2, 120.8. 127.5, 128.5, 128.7, 154.4 (arom.)



(VII):^{1}H-NMR ($CDCl_3$, δ):1.5 (6H, d, CH_3-), 2.4 (4H, s, -CH_2-), 3.8 (2H, q, -CH<), 6.3 (1H, s, OH-), 6.6-7.3 (8H, arom.); ^{13}C-NMR ($CDCl_3$, δ):22.8 (CH_3-), 31.3 (-CH_2-), 43.8 (-CH<), 115.5, 128.5, 135.9, 154.5 (arom.)

(VIII):IR ($CDCl_3$, cm^{-1}):3600-3200, 1230 (Ar-OH), 2990, 1460 (CH_3-), 2920, 1460, (-CH_2-), 2580 (-SH), 1615, 1590, 1495, 750 (arom.); ^{1}H-NMR ($CDCl_3$, δ):1.6 (3H, d, CH_3-), 2.6, 2.7 (4H, -CH_2-), 4.3 (1H, q, -CH<), 6.7-7.3 (4H, arom.); ^{13}C-NMR ($CDCl_3$, δ):20.6 (CH_3-), 24.3, 34,9 (-CH_2-), 40.3 (-CH<), 117.0, 120.7, 127.7, 128.4, 128.6, 154.2 (arom.)

(IX):^{1}H-NMR ($CDCl_3$, δ):1.5 (3H, d, CH_3-), 2.5, 2.6 (4H, -CH_2-), 3.9 (1H, q, -CH<), 6.6-7.3 (4H, arom.); ^{13}C-NMR ($CDCl_3$, δ):22.7 (CH_3-), 24.6, 35.3 (-CH_2-), 43.7 (-CH<), 115.5, 128.5, 135.8, 154.6 (arom.)

Antioxidant effect. The antioxidant effects of these compounds were evaluated by an oven test at 60°C (4-7). 2.0 mg of phenolic sulfide and 4.0 mg of citric acid were dissolved in 26 mg of glycerol monooleate and 6.0 mg of propylene glycol, respectively. Then 20 g of refined lard was added and completely mixed with stirring (8,9). These samples were placed in the oven at 60°C in the dark, and peroxide values (POV) were determined periodically in accordance with the standard method of the Japan Oil Chemists' Society (10). Results were expressed as the number of hours required to reach POV of 30 and 100 meq of peroxide per kg of lard.

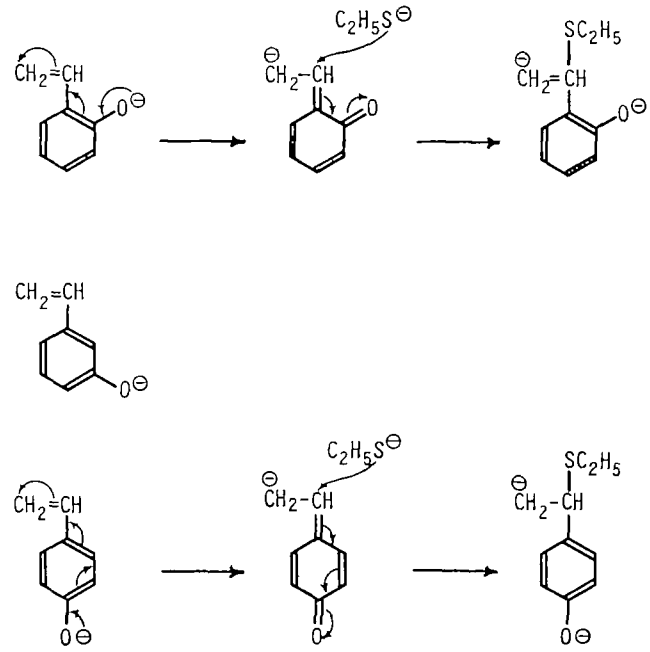


FIG 1. Reaction mechanism of phenolic sulfide.

Antimicrobial activity. The antimicrobial activities of these compounds were evaluated by an agar dilution method (11). Three kinds of gram-positive bacterial strains, *Staphylococcus aureus* FDA-209P, *Bacillus subtilis* PCI-219 and *Micrococcus lutea* ATCC-1001, and three kinds of gram-negative bacterial strains, *Escherichia coli* 0-80, *Salmonella typhi* H-901W and *Pseudomonas aeruginosa* IFO-3080, and six kinds of fungal strains, *Candida albicans* ATCC-7491, *Saccharomyces cerevisiae* KF-25, *Trichophyton interdigitale* KF-62, *Microsporium gypseum* KF-64, *Penicillium chrysogenum* KF-97 and *Aspergillus niger* ATCC-6275 were used for the tests. Nutrient agar and Sabouraud dextrose agar were used for bacteria and fungi, respectively. These bacteria were cultured at 37°C for 48 hr, and fungal strains were incubated at 25°C for five days. Antimicrobial activities were represented in terms of minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Preparation of phenolic sulfide. It was found that thiols reacted with *o*- and *p*-vinylphenols to form Michael addition products, but not with *m*-vinylphenol. This shows that the thiols add to carbon-carbon double bonds having an electron withdrawing group. Figure 1 shows the proposed reaction mechanism of phenolic sulfide. It seems that *o*- and *p*-vinylphenols contribute such resonance structures, and each carbonyl group activates the compounds towards conjugate addition. On the other hand,

TABLE 1

Anioxidant Effect of Phenolic Sulfide

Compounds	Days to reach POV	
	30 (meq/kg)[a]	100 (meq/kg)[a]
(I)	32.8	41.8
(II)	33.8	43.0
(III)	15.5	22.8
(IV)	14.7	22.2
(V)	14.8	22.2
(VI)	37.6	46.4
(VII)	37.5	46.8
(VIII)	37.3	45.9
(IX)	31.6	41.4
Control	14.7	22.1
BHT	20.2	28.0

[a]Meq peroxide per kg lard.

m-vinylphenol does not contribute such resonance structure. This is the reason why thiols form Michael addition products only with *o*- and *p*-vinylphenols.

Antioxidant effect of phenolic sulfide. Table 1 shows the antioxidant effects of phenolic sulfides on the autoxidation of lard. The antioxidant effects of these products were exhibited in terms of days to reach POV of 30 and 100, respectively.

From Table 1, it was found that the Michael addition products (I), (II), (VI), (VII), (VIII) and (IX) showed excellent antioxidant effects which were superior to BHT, whereas radical addition products (III), (IV) and (V) did not exhibit any antioxidant effect.

It seems that the difference in the antioxidant effects of these compounds is ascribed to the positions of the sulfide groups in the molecules. In the antioxidant process, Michael addition products are stabilized by their sulfide groups, retaining their radical trapping abilities. On the other hand, radical addition products cannot be stabilized by their sulfide groups, because the sulfide groups are too far separated from the aromatic rings.

Among the Michael addition products, disubstituted phenolic products showed slightly better antioxidant effect than monosubstituted ones. However, the influence of the position of the hydroxyl group on the aromatic ring on their antioxidant effect was uncertain.

Antimicrobial activity of phenolic sulfide. Table 2 shows the antimicrobial activities of phenolic sulfides.

All these compounds exhibited higher antimicrobial activities than that of phenol itself, and 1,2-ethanedithiol derivatives were the best against gram-positive bacterial strains. However, 1,2-ethanedithiol derivatives did not have good activity against gram-negative bacterial strains. All these compounds showed better antimicrobial activities against fungal strains than against bacterial strains. Their antimicrobial activities were affected by the positions of hydroxyl groups on the aromatic rings. *o*-Vinylphenol derivatives showed slightly stronger antimicrobial activities than *p*-vinylphenol derivatives.

TABLE 2

Antimicrobial Activity of Phenolic Sulfide[a]

Compounds	MIC (μg/mL)[b]								
Organism	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	(IX)
S. aureus	200	100	100	200	400	10	50	10	25
B. subtilis	200	100	100	200	400	10	50	5	25
M. lutea	200	100	100	200	400	10	50	5	25
E. coli	200	200	100	200	200	400	>400	>400	>400
S. typi	200	400	100	200	400	>400	>400	>400	>400
P. aeruginosa	>400	>400	>400	>400	>400	>400	>400	>400	>400
C. albicans	400	400	100	200	400	100	400	400	400
S. cerevisiae	200	200	100	200	400	10	50	10	25
T. interdigitale	100	100	25	50	100	10	50	25	25
M. gypseum	100	100	25	25	50	10	100	25	25
P. chrysogenum	400	200	50	200	200	200	>400	400	400
A. niger	400	400	50	400	200	200	>400	400	>400

[a]Control always produced growth of the microorganism.
[b]Minimum inhibitory concentration.

ACKNOWLEDGMENTS

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