INCREASING 5-LIPOXYGENASE INHIBITORY ACTIVITIES BY OXIDATIVE CONVERSION OF *o*-METHOXYPHENOLS TO CATECHOLS USING A Cu^{2+} – ASCORBIC ACID – O_2 SYSTEM

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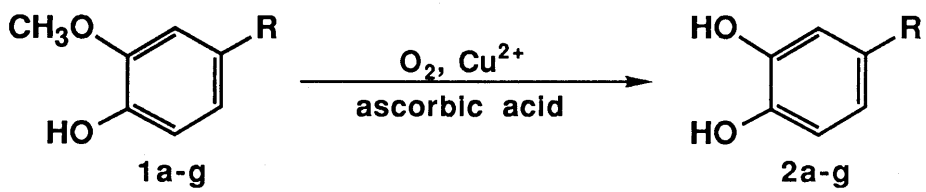
Several complicated *o*-methoxyphenols were oxidized with high selectivity to catechols by a Cu^{2+} – ascorbic acid – O_2 system. In this way, the RBL-1 5-lipoxygenase inhibitory activities of *o*-methoxyphenols were greatly increased. [6]-Norgingerol (**4**), a novel compound derived from [6]-gingerol (**3**), shows promise as a lead compound for new drugs because of its high inhibitory potency ($IC_{50} = 5.0 \times 10^{-8}$ M).

KEYWORDS 5-lipoxygenase inhibitor; [6]-norgingerol; esculetin; copper ion; ascorbic acid; oxygen; biomimetic oxidation; cytochrome P-450; *o*-methoxyphenol; catechol

o-Methoxyphenol compounds occur abundantly in nature as metabolites of bioactive catechols deactivated by catechol O-methyl transferase (COMT).[1] Hence, O-demethylation of the natural *o*-methoxyphenols regenerates bioactive catechols. O-Demethylation is conducted oxidatively by cytochrome P-450 *in vivo*,[1,2] hitherto considered difficult to do in purely chemical systems.[3] However, we recently reported that the oxidation system of Cu^{2+} – ascorbic acid – O_2 in aqueous solution can effectively convert *o*-methoxyphenols to the corresponding catechols, which are quite stable and suffer no further oxidation under this condition.[4] Though this oxidation system is known to degrade biomolecules[5] such as amino acid, protein, and DNA *in vitro* by generating active oxygen species, this is the first example of it being used as a synthesis tool. The high mildness of this oxidation couple facilitates the chemoselective conversion of natural *o*-methoxyphenols to reproduce their potent bioactivities or produce new activities.

Here we report that selective conversion of some complicated *o*-methoxyphenols such as gingerol and coumarins successfully proceeded using a Cu^{2+} – ascorbic acid – O_2 system to afford the corresponding catechols; as expected, the inhibitory activities of RBL-1 5-lipoxygenase[6] were greatly increased compared with their precursors.

Oxidative Conversion of *o*-Methoxyphenols to Catechols Typical examples of the reaction are shown in Chart 1. The most representative reaction was as follows; after ascorbic acid (30 mmol) was added to an aqueous solution of guaiacol (**1a**, 1 mmol) and $Cu(ClO_4)_2$ (3 mmol) in water (20 ml), the reaction mixture was stirred under pure oxygen atmosphere for 24 hours to afford catechol (**2a**) in moderate yield (33%[7]) and with high selectivity (99%[8]).[4] This converted ferulic acid (**1b**), with an α,β–unsaturated carboxylic acid moiety, to caffeic acid (**2b**, 24% isolated yield), with high selectivity (96%[8]) without further oxidation, hydroxylation or epoxidation. Another *o*-methoxyphenol (**1c**) having a β-nitroolefin moiety was also converted to the corresponding catechol (**2c**). Several other *o*-methoxyphenols, which have other functional groups such as carboxylic ester, carbamates (**1d** and **1e**) and imide (**1f**), likewise underwent selective catechol formation.[4] Dopamine (**2g**), a neurotransmitter, was effectively derived from 3-O-methyldopamine (**1g**) with 19% yield[7] and high selectivity (up to 100%[8]). Acetone, acetic acid or N,N-dimethylformamide was useful as a cosolvent when the substrate solubility in water was low.



1a-g 2a-g

a. R: H; b. R: CH=CHCOOH(trans); c. R: CH=CHNO$_2$(trans); d. R: CH$_2$CH$_2$NHCbz; e. R: CH$_2$CH$_2$NHBoc; f. R: CH$_2$CH$_2$NPht; g. R: CH$_2$CH$_2$NH$_2$

Chart 1

As Chart 2 shows, chemoselective conversion of several natural *o*-methoxyphenols proceeded *via* the Cu^{2+} – ascorbic acid – O_2 system to give the corresponding catechols. [6]-Gingerol (**3**) was converted selectively to a novel catechol compound[9] which we named [6]-norgingerol (**4**, 11% isolated yield); this conversion is not possible with a conventional demethylating reagent such as a strong acid or a Lewis acid, because **3** and **4** have an acid-labile β-hydroxyketone moiety which results from aldol condensation of arylethyl methyl ketone with *n*-hexanal. An *o*-methoxy group of an alkaloid (**5**) was also removed to obtain the catechol compound (**6**, 11% isolated yield); however in this case oxidative N-demethylation did not occur. Another alkaloid (**7**) having a methylenedioxy group underwent chemoselective catechol formation by removal of only a methoxy group, whereas the methylenedioxy group is susceptible to a Lewis acid.[3a] From both scopoletin (**9**) and isoscopoletin (**11**), this oxidation couple derived the same product, esculetin (**10**, 10% yield[7] from **9** and 9% isolated yield from **11**).[10]

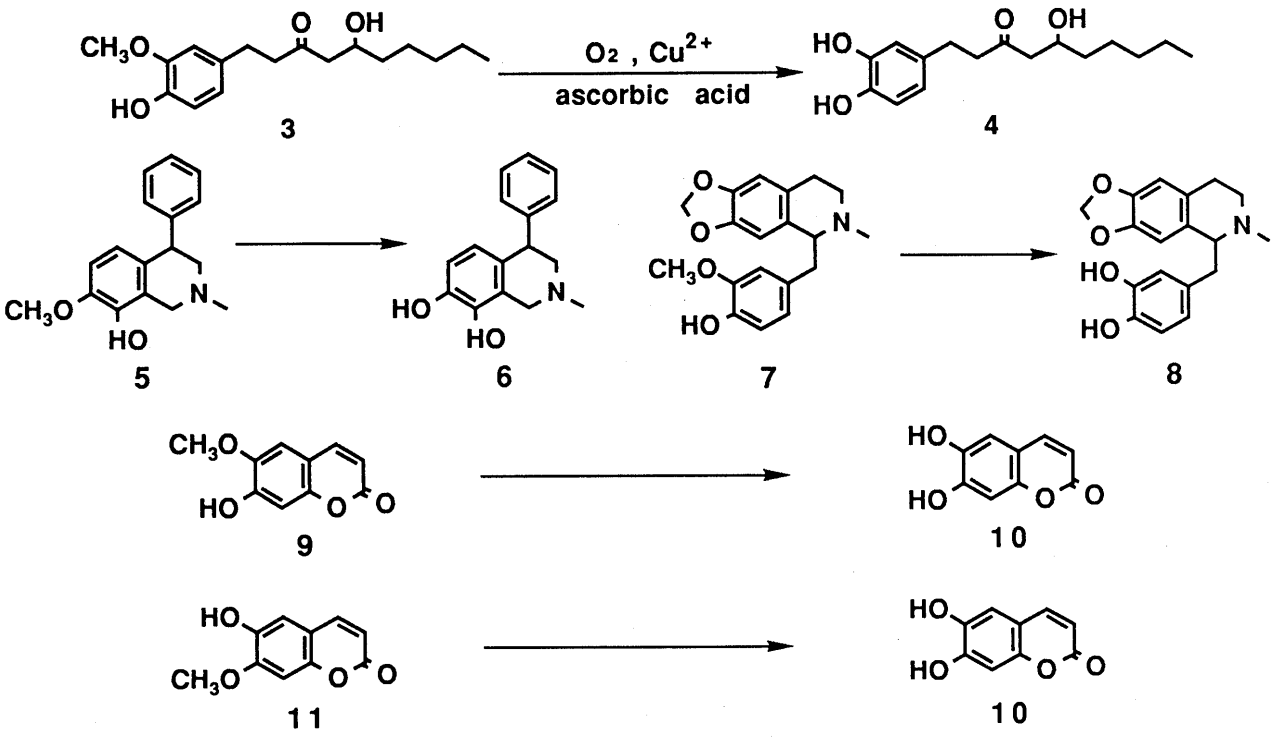


Chart 2

Table I. Increase of the RBL-1 5-Lipoxygenase[6] Inhibitory Activities due to Conversion of *o*-Methoxyphenols **1c-f**, **3**, **9**, and **11** to Catechols **2c-f**, **4**, **10** by a Cu^{2+} – Ascorbic Acid – O_2 System

o-Methoxyphenols	IC_{50}[a], μM	Catechols	IC_{50}[a], μM
1c	39[b]	**2c**	2.9[b]
1d	120[b],[c]	**2d**	3.7[b]
1e	1%[b],[d]	**2e**	47%[b],[d]
1f	90[b],[e]	**2f**	5.1[b]
3	10[b](3)[f]	**4**	0.8[b](0.050)[f]
9	6%[b],[g]	**10**	1.9[b](0.15)[f]
11	0%[b],[g]	**10**	1.9[b](0.15)[f]

a) 50% inhibitory concentration. b) The enzyme is the 10,000 × g supernatant from the centrifuged mixture of the homogenized RBL-1 cells. c) IC_{59}. d) Inhibition at 27 μg/ml. e) IC_{42}. f) The enzyme is the 105,000 × g supernatant. g) Inhibition at 25 μg/ml.

5-Lipoxygenase Inhibition We assayed the RBL-1 5-lipoxygenase[6] inhibition of some *o*-methoxyphenols **1c-f**, **3**, **9**, and **11** and their catechol derivatives **2c-f**, **4**, and **10** (Table I).[11] As expected, the activities of the catechols prepared by the Cu^{2+} – ascorbic acid – O_2 system were greatly enhanced in comparison with their precursors. **4** should serve as a lead compound for new drugs because of its high inhibitory potency ($IC_{50} = 5.0 \times 10^{-8}$ M) compared with that of known active substances.[12]

In general, catechols chelate to iron ions more strongly than *o*-alkoxyphenols, thereby affecting the redox reaction. Consequently, enhancement of the 5-lipoxygenase inhibitory activity of the catechols **2c-f**, **4**, and **10** is understandable as these catechols can attain higher chelation to the non-heme iron at the active center of the enzyme and, therefore, more effectively inhibit its enzyme redox reaction.[12]

A number of drugs and xenobiotics are known to be converted to more bioactive substances *in vivo* by metabolic reaction which proceeds chiefly in the presence of cytochrome P-450's.[1,2] The Cu^{2+} – ascorbic acid – O_2 system successfully produced several bioactive catechols from the corresponding *o*-methoxyphenols with high selectivity. Thus, we could reproduce "metabolic activation" of *o*-methoxyphenols by the biomimetic oxidation system containing molecular oxygen activation, as the enzyme also does. We are now interested in the organic chemistry of this reaction and are studying the mechanistic details.

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6) The enzyme is the 10,000 × g or 105,000 × g supernatant from the centrifuged mixture after homogenization and sonication of rat basophilic leukemia cells.
7) Determined by HPLC.
8) Based on the substrate consumed.
9) 1-[3',4'-Dihydroxyphenyl]-5-hydroxydecan-3-one (**4**) exists as colorless needles with mp 89-90°C; ^{1}H-NMR (400 MHz; $CDCl_3$/TMS) δ 6.76 (d 1H, J = 8.1 Hz; 5'-Ar-H), 6.67 (s 1H; 2'-Ar-H), 6.59 (d 1H, J = 8.1 Hz; 6'-Ar-H), 5.73 and 5.43 (br s × 2, 1H × 2; 3'- and 4'-Ar-OH), 4.04 (m 1H; -C<u>H</u>(OH)-), 3.16 (br s 1H; -CH(O<u>H</u>)-), 2.81-2.77 and 2.73-2.69 (m × 2, 2H × 2; Ar<u>CH$_2$CH$_2$</u>C(O)-), 2.57 (dd 1H, J_{gem} = 17.6 Hz, J_{vic} = 2.9 Hz; C(O)C<u>H$_2$</u>CH(OH)-), 2.48 (dd 1H, J_{gem} = 17.6 Hz, J_{vic} = 9.5 Hz; C(O)C<u>H$_2$</u>CH(OH)-), 1.50-1.27 (m 8H; CH_2 × 4), 0.88 (t 3H; CH_3); IR (KBr) cm^{-1} 3400 and 3220 (OH), 2920 and 2850 (alkyl C-H), 2320, 1703 and 1693$_{sh}$, (C=O), 1605, 1516; Mass spectra were analyzed for **4** and its per-O-trimethylsilyl (per-O-TMS) derivative: MS (**4**) m/z = 280 (M^+), 262 ($M^+ - H_2O$), 191, 180, 165, 164, 137, 136, 123, 122; MS (per-O-TMS derivative of **4**) m/z = 496 (M^+; this derivative contains three O-TMS moieties); Anal. calcd for $C_{16}H_{24}O_4$: C 68.54, H 8.63. Found: C 68.34, H 8.57.
10) Simultaneously, **9** and **11** underwent hydroxylation at the *3-position* to yield 3-hydroxyscopoletin and 3-hydroxyisoscopoletin respectively, which are novel compounds. The report on the hydroxylation and their bioactivities is submitted.
11) Assays to determine 5-lipoxygenase activity were performed at 37°C for 5 min in 200-μl incubation mixtures composed of phosphate buffer (100 mM, pH 7.4), reduced glutathione (1.0 mM), ATP (2.0 mM), $CaCl_2$ (2.0 mM), the enzyme,[6] and various concentrations of test compounds. Reactions were initiated by adding 1-^{14}C-arachidonic acid (30 μM, 35,000 cpm), and terminated by acidification with an aqueous solution of citric acid (0.2 M, 20 μl) and extraction with ethyl acetate (300 μl). After evaporation of the extracts (200 μl), the reaction products of each sample were separated by TLC[13),14)] and measured by liquid scintillation spectroscopy. Inhibition of 5-lipoxygenase activity was calculated as the ratio of the product formed in the presence and absence of inhibitor.
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13) TLC was purchased from Whatman (60A LK6D).
14) The mobile phase was the mixed solvent composed of ether : petroleum ether : acetic acid = 50 : 50 :1 (v/v).

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