[Chem. Pharm. Bull. 27(7)1675—1678(1979)]

UDC 547.833.8.04.09 : 547.554.04

Antioxidant Properties of N-Methyl-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid

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(Received September 28, 1978)

N-Methyl-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (MDTI), a new chemical compound, was prepared by cyclization of α-methyl-α-(3,4-methylenedioxybenzyl)-α-aminoacetonitrile with formaldehyde and formic acid, followed by hydrolysis with hydrochloric acid. The compound (MDTI) showed extremely high electron-donating activity and was found to have excellent antioxidant properties.

Keywords——N-methyl-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; antioxidant; electron donating power; 1,1-diphenyl-2-picrylhydrazyl; active oxygen method; corn oil; linoleic acid

Various derivatives of partially hydrogenated quinoline or isoquinoline are known to have antioxidant activity. Of these, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin, EQ) is widely used to prevent the oxidative deterioration of feeds. Organic acid salts of ethoxyquin have been patented as antioxidants.[2] Lin and Olcott[3] found that ethoxyquin nitroxide was slightly superior to ethoxyquin as regards antioxidant activity. Sheinkman *et al.*[4] described the antioxidant effects of compounds having a dihydroquinoline or tetrahydroquinoline skeleton. Derivatives of partially hydrogenated isoquinoline having two hydroxyl groups in an unsaturated ring were found to be effective in inhibiting the oxidation of soybean oil.[5]

In view of these reports, we decided to prepare a new compound, N-methyl-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (MDTI) and study its effectiveness as an antioxidant. MDTI (**2**) was prepared by cyclization of α-methyl-α-(3,4-methylenedioxybenzyl)-α-aminoacetonitrile (**1**), as shown in Chart 1. The present report describes the synthesis of MDTI, and its antioxidant properties together with its electron-donating activity.

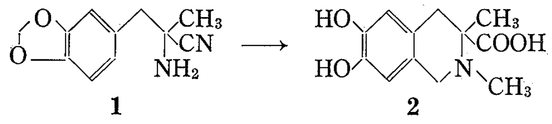

Chart 1

Experimental

Materials——Butylated hydroxyanisol (BHA, a mixture of the 2- and 3-isomers of tert-butyl-4-methoxyphenol), butylated hydroxytoluene (BHT), propylgallate (PG), linoleic acid (peroxide value: 0.3 meq/kg), and corn oil (peroxide value: 0.9 meq/kg) were obtained from Katayama Chemicals; 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Tokyo Kasei. *tert*-Butylhydroquinone (TBHQ) was prepared from BHA by the method described in the previous report.[6]

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Synthesis of N-Methyl-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (MDTI)——A mixture of α-methyl-α-(3,4-methylenedioxybenzyl)-α-aminoacetonitrile[7] (6.12 g), 80% formic acid (10 g) and 37% formaldehyde (6 g) was heated at 85—90° for 3 hr with stirring. After formic acid and formaldehyde had been removed *in vacuo*, the residue was dissolved in ethyl acetate. The solution was washed with water and then evaporated down to remove the solvent. The resulting product was dissolved without purification in a mixture of 20% hydrochloric acid (70 ml) and phenol (2 g). The mixture was heated under reflux for 13 hr with stirring. When the reaction was complete, the reaction mixture was extracted with ethyl acetate, and the aqueous layer was concentrated to dryness *in vacuo*. The residue was recrystallized from water-acetone to yield MDTI hydrochloride (2.4 g, 29%), mp 139—142° (dec.). MDTI hydrochloride (2.0 g) was dissolved in water (5 ml), the solution was adjusted to pH 6.0 with 1 N sodium hydroxide, and the mixture was allowed to stand overnight in an ice box. The precipitate was collected by filtration, washed with methanol, and dried; 1.6 g of MDTI was obtained as the monohydrate, mp 268—270° (dec.). *Anal.* Calcd. for $C_{12}H_{15}NO_4 \cdot H_2O$: C, 56.46; H, 6.71; N, 5.49. Found: C, 56.42; H, 6.56; N, 5.34. IR ν_{max}^{Nujol} cm^{-1}: 3500, 3150, 2600, 1620. NMR (in CF_3COOD) δ: 6.85 (2H, d, arom-H), 4.96—3.3 (4H, m, C_1–H_2 and C_4–H_2), 3.19 (3H, s, NCH_3), 1.88 (3H, s, C_3–CH_3).

MDTI was also synthesized starting from α-methyl-(3,4-methylenedioxy)-phenylalanine[8] as follows. A mixture of α-methyl-(3,4-methylenedioxy)-phenylalanine (9.6 g), 85% formic acid (12 ml) and 37% formaldehyde (9 ml) was heated at 80—85° for 3 hr with stirring. When the reaction was over, the reaction mixture was evaporated to dryness. A mixture of 20% hydrochloric acid (300 ml) and phenol (10 g) was added to the residue. Hydrolysis and work-up were carried out as described above to afford the desired product (7.6 g, 69%). The physicochemical properties of this compound were identical with those of the compound obtained above.

Determination of Electron-donating Power——The electron-donating power of antioxidative compounds was determined using a stable free radical, DPPH, according to the method reported by Mitsuda *et al.*[9] To 0.5 ml of 50 μM or 100 μM sample solution, 1.5 ml of 99% ethanol, 2 ml of 0.1 M phosphate buffer (pH 6.5) and 1 ml of 5×10^{-4} M DPPH ethanolic solution were added with thorough mixing. The final concentration of sample was 5 μM or 10 μM. After storage at room temperature for 1 hr, the absorbance at 525 nm was read with a Varian Techtron 635D spectrophotometer.

Evaluation of Antioxidant Effectiveness in an Oil System——Antioxidant activity in an oil system was measured by the active oxygen method.[10] Corn oil (20 ml) was placed in each tube (24 mm × 200 mm) of an active oxygen apparatus (Kuramochi Kagaku Co.). The tubes were maintained at 97.8±0.1°. Air was bubbled into the oil at a constant rate of 2.33 ml/sec for 8 hr, then the peroxide value of the oil was determined by the conventional KI-$Na_2S_2O_3$ titration procedure.

Evaluation of Antioxidant Effectiveness in an Aqueous System——Antioxidant activity in an aqueous system was measured by the method of Mitsuda *et al.*[9] with slight modifications. Using a test tube (15 mm × 130 mm), 2 ml of 2.5% linoleic acid in ethanol, 4 ml of 0.1 M phosphate buffer (pH 7.0), 2 ml of water and 2 ml of ethanol were mixed. An antioxidant sample was dissolved in either water or ethanol, then added. The resulting clear solution was kept at 37°, and the extent of oxidation was measured by the ferric thiocyanate method: 4.7 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid were added to 0.1 ml of the oxidized solution. Precisely 3 min after the Fe^{2+} addition, the absorbance of the red color was measured at 500 nm. The induction period was expressed in terms of the time required to reach an absorbance of 0.3.

Results

Electron-donating Ability of MDTI

Electron donating abilities of antioxidant compounds was evaluated by the DPPH method. Table I shows the degree of decolorization of DPPH by phenolic compounds and quinoline or isoquinoline derivatives, including common antioxidants.

As can be seen from the table, decolorization of DPPH was greatest with MDTI. Among the conventional antioxidants, PG was most effective, followed by BHA, BHT, EQ, and TBHQ (decreasing order). Tyrosine ethylester had little effect.

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Table I. Electron-Donating Ability of Antioxidants

Antioxidants	Absorbance 525 nm		Decolorization (%)*	
	5 μM	10 μM	5 μM	10 μM
None	0.911	0.966	0	0
BHT	0.787	0.716	13.6	21.8
BHA	0.765	0.649	16.0	28.9
PG	0.652	0.465	28.4	49.2
TBHQ	0.805	0.743	11.6	18.9
EQ	0.803	0.730	11.9	20.3
MDTI	0.638	0.398	30.3	57.0
p-Hydroxyanisole	0.796	0.726	12.6	20.4
Tyr·OEt·HCl	0.895	0.898	1.8	2.0

1.5 ml of 99% ethanol, 2 ml of 0.1 M phosphate buffer (pH 6.5) and 1 ml of 5×10^{-4} M DPPH ethanolic solution were added to 0.5 ml of 50 μM or 100 μM antioxidant solution. After 1 hr, absorbance at 525 nm was read.

* Decolorization (%) was calculated as follows: $\frac{\text{absorbance of control}-\text{absorbance of sample}}{\text{absorbance of control}}\times100$

Antioxidant Activity of MDTI

The antioxidant activity of MDTI was examined in both oil and water systems. BHA and EQ (commonly used as phenolic and quinoline antioxidants, respectively) were employed as the active controls.

The results with the active oxygen method are shown in Table II. Antioxidant activity of MDTI in the oil system was demonstrated at levels from 0.002 to 0.05%. At a level of 0.02%, MDTI was more effective than BHA or EQ. The results of antioxidant tests in a water-containing system are shown in Table III. MDTI was quite effective in protecting linoleic acid from oxidation, like BHA and EQ. At a level of 0.04%, the activity was almost equal to that of BHA. At a level of 0.02%, MDTI appeared to be more effective than BHA.

Table II. Effects of MDTI, BHA and EQ on the Oxidation of Corn Oil as Determined by the Active Oxygen Method*

Additive (%)	Peroxide value (meg/kg)	Additive (%)	Peroxide value (meg/kg)
None	52.3	MDTI (0.002)	34.9
BHA (0.01)	34.2	MDTI (0.005)	26.2
BHA (0.02)	31.6	MDTI (0.01)	18.2
EQ (0.01)	29.7	MDTI (0.02)	11.3
EQ (0.02)	25.4	MDTI (0.05)	8.5

* Heated at 97.8° for 8 hr with aeration (2.33 ml /sec).

Table III. Antioxidant Activities of MDTI, BHA and EQ in a Water System

Additive (%)	Induction period*	Additive (%)	Induction period*
None	12.0	MDTI (0.01)	18.9
BHA (0.02)	17.5	MDTI (0.02)	20.0
BHA (0.04)	23.0	MDTI (0.04)	22.2
EQ (0.02)	16.8	MDTI (0.1)	28.7
EQ (0.04)	19.4	MDTI (0.2)	38.6

* Days required to reach an absorbance of 0.3 at 500 nm as determined by the ferric thiocyanate method.

Discussion

Recently, antioxidant properties of partially hydrogenated isoquinoline derivatives have been reported by several workers.[2–5] We therefore synthesized a new isoquinoline derivative, N-methyl-3-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (MDTI), and studied its electron-donating power and effectiveness as an antioxidant.

It is considered that the electron-donating power of a compound having a phenolic moiety depends on many factors, *e.g.*, location, number and steric hindrance of hydroxyl groups, and the quantum chemical properties of the whole molecule. In the present experiments, MDTI at 5 μM was found to be more effective as an electron donor than BHA at 10 μM, TBHQ at 10 μM or PG at 5 μM. As the numbers of hydroxyl groups in MDTI, BHA, TBHQ and PG are two, one, two and three, respectively, the electron-donating activity per hydroxyl group was greatest with MDTI. BHA, BHT or TBHQ may be less effective than MDTI partly because hydroxyl groups in the former three compounds are hindered by the adjacent *tert*-butyl group, which is not the case in the latter.

As described above, it is clear that MDTI has strong electron-donating power, and this suggested that the compound would probably be an effective antioxidant. The antioxidant activity of MDTI was confirmed in both oil and water systems. It seems noteworthy that MDTI at 0.01% was more active than BHA or EQ at 0.02% in the AOM test. The order of the antioxidant activity was different from that of the electron-donating activity. One possible explanation is that BHA is less stable to heat treatment than MDTI and EQ. It should be noted that antioxidant effectiveness evaluated in the AOM test corresponds to the combined effect with tocopherols, which occur naturally in corn oil. MDTI was also more effective than BHA in the water system, although the difference was less marked than in the oil system.

In general, conventional phenolic antioxidants such as BHA and BHT have practically no water solubility. They are employed together with an emulsifier when used in water-fat systems. One disadvantage of phenolic antioxidant is that they are quite volatile and easily decomposed at high temperature. Fortunately, MDTI is slightly soluble and its hydrochloride is readily soluble in water. MDTI is also expected to be relatively stable at high temperature. Thus, in view of its excellent antioxidant activity, MDTI seems promising as a potential antioxidant for practical use. However, further studies, especially toxicological studies will be necessary.

Acknowledgement The authors thank Miss Toshiko Osaki for technical assistance.