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Antioxidant Activity of Diarylbutanes

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Abstract—Antioxidant activity of diarylbutane type lignans was evaluated in TBA-reactant assay to elucidate the structure-activity relationship. The antioxidant potency of lignans increased with increasing the number of hydroxyl groups, with the exception of macelignan(**I**), which showed a more potent activity than demethyl *meso*-dihydroguaiaretic acid(**III**).

Keywords—Lignans • antioxidant activity

Although DuBois reported that mace exhibited an antioxidant activity in 1943[1], it was not until 1986[2] that one of the responsible substances was found to be 9-(2, 5-dihydroxyphenyl) nonanoyl-2, 6-dihydroxybenzene. Recently, macelignan[3], a 1, 4-diarylbutane derivative, isolated from mace was demonstrated to elicit an antioxidant activity[4]. Moreover, *nor*-dihydroguaiaretic acid (NDGA), the mother substance of macelignan has been used as an antioxidant in fats and oils.[5]†

This paper deals with the investigation to help us elucidate the structure-activity relationship on the antioxidant activity of diarylbutane type lignans.

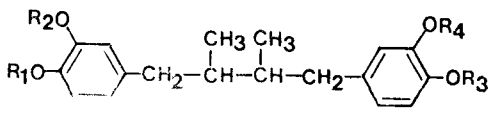
Results and Discussion

Macelignan, also called anwulignan[7] (**I**) and *meso*-dihydroguaiaretic acid(**II**) were isolated and purified from the arils of *Myristica fragrans* according to the procedure described previously[3]. Compound **III** was prepared from macelignan(**I**) by selective cleavage of a methylenedioxy group with borontrichloride by the method of Teitel, *et al.*[8] Compound **IV** and **VI** were synthesized by methylation of macelignan (**I**) and NDGA(**V**) with dimethyl sulfate in dry acetone, respectively.

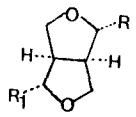
Machilin-A[9] (**VII**) was synthesized by cupric oxide plus dibromomethane catalyzed reaction from NDGA(**V**) according to the procedure described by Brossi, *et al.*[10] The identity and purity of the isolated lignans and of the synthesized lignan congeners were established by EI-MS, ^{1}H-NMR and other spectral comparisons.

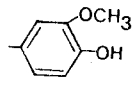
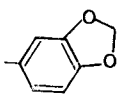
Compond **I**~**VII** were evaluated for their capacity to inhibit lipid peroxidation in mouse liver homogenate *in vitro*. The results were summarized in Table I. NDGA(**V**) was shown to be the most potent among the lignans tested with an IC_{50} of 1.5 μM in TBA-reactant assay. The inhibitory potency of lignans increased with increasing the number of hydroxyl groups in the benzene ring, i.e., in the order of com-

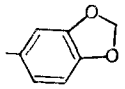
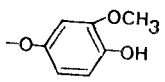
†Recently NDGA is banned for this purpose in the U.S.A. because it is suspected that it induces kidney cysts.[6]

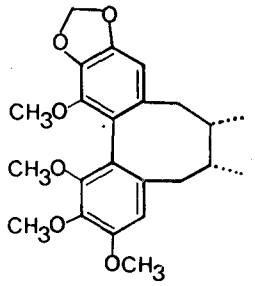


Compounds	R_1	R_2	R_3	R_4
I	-CH_2-		H	CH_3
II	H	CH_3	H	CH_3
III	H	H	H	CH_3
IV	-CH_2-		CH_3	CH_3
V	H	H	H	H
VI	CH_3	CH_3	CH_3	CH_3
VII	-CH_2-		-CH_2-	



VIII, R =  ; R_1 = 

IX, R =  ; R_1 

X, 

pound **V**>**III**>**II**>**IV**, indicating that the number of hydroxyl groups essentially determine the potency of lipid peroxidation. Thus it is clear from the result of this investigation that the ability of lignans to elicit an antioxidant activity depends to a large extent on their hydrophyllic character.

Surprisingly, macelignan(**I**) carrying a methylenedioxyphenyl moiety and a hydroxyl and a methoxyl group in a benzene ring, however, was exceptionally potent, its IC_{50} being 2.8 μM which was more potent than compound **III** having three hydroxyls and a methoxyl group. This phenomenon was also observed in (−)-piperitol(**VIII**) and sesamolinol(**IX**)[11] which are furofuran type lignans carrying similar aryl moieties as macelignan (**I**). They have been demonstrated to be more efficient than vitamin E in tests with microsomes *in vitro*.[12]

As expected, compound **VI** and **VII** which are devoid of a hydroxyl group were found to elicit a complete loss of the activity. Though there is an exception, that gomicin N(**X**), a dibenzocyclo-octadiene type lignan devoid of a hydroxy group was reported to be a more active antioxidant.[13]

Phenolic compounds are generally considered to be the primary antioxidants present in all natural fats, and in laboratory test systems NDGA has been shown to be one of the most effective antioxidants known. However, chief disadvantage of NDGA have been stated to be its low solubility in fats.[14] These methylated lignans would make up this deficiency in solubility.

Experimental

Melting points were determined on a Mitamura-Riken melting point determining apparatus and are uncorrected. IR spectra were taken on a Perkin Elmer 281B spectrometer. ^{1}H-NMR spectra were determined on a Varian FT-80A instrument. Mass spectra were obtained on a Hewlett Packard 5985B GC/MS spectrometer. Silica gel 60F-254 and silica gel(Art 7734) were used for TLC and column chromatography, respectively.

Isolation of macelignan(I) and *meso*-dihydroguaiaretic acid(II)-Dried mace(500 g) was coarsely powdered and extracted with ether four times. The ether extract on removal of solvent gave reddish brown viscous solids(260 g). The non-volatile residue (50 g) after removal of volatile essential oils by steam distillation was chromatographed on a silica gel column by

gradient elution with mixtures of benzene/ether (1 : 0→2 : 1) to give 7 fractions.

From fraction 1, macelignan(**I**) was obtained as pale yellow oil which recrystallization from n-hexane/ether(1 : 1) gave colorless prisms (3 g). mp 70∼72°; $[\alpha]_D^{20}$=+5.28(C=1.8, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1} : 3480(OH), 1610, 1514, 1500, 1486(aromatic C=C), 926(O−CH_2−O); ^{1}H-NMR ($CDCl_3$) δ : 0.83(6H, d, J=6.4 Hz, H-9/H-9′), 1.61∼1.88(2H, m, H-8/H-8′), 2.27∼2.72(4H, m, H-7/H-7′), 3.86(3H, s, OCH_3), 5.42(1H, s, OH), 5.91(2H, s, O-CH_2-O), 6.59∼6.81(6H, m, aromatic H); MS, m/z(rel. int. %): 328(M^+, 11), 137($C_8H_9O_2^+$, 100), 135($C_8H_7O_2^+$, 68).

Fraction 3(6.8 g) on crystallization from methanol gave *meso*-dihydroguaiaretic acid(**II**) as colorless prisms (500 mg).

Mp. 84∼86°; $[\alpha]_D^{20}$=0°(C=1.0, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1} : 3480(OH), 1610, 1514, 1486(aromatic C=C); ^{1}H-NMR($CDCl_3$) δ : 0.84(6H, d, J=6.4 Hz, H-9/H-9′), 1.57∼1.84(2H, m, H-8/H-8′), 2.13∼2.85(4H, m, H-7/H-7′), 3.85(6H, s, OCH_3×2), 5.42(2H, s, OH×2), 6.60∼6.88(6H, m, aromatic H); MS, m/z(rel. int. %) : 330(M^+, 2.6), 165($C_{10}H_{13}O_2^+$, 2.0), 137($C_8H_9O_2^+$, 100), 122($C_7H_6O_2^+$, 9.8).

Preparation of lignan derivatives

3-demethyl *meso*-dihydroguaiaretic acid (III)-To 600 mg of macelignan(**I**) dissolved in 40 ml of methylene chloride was added at room temperature 25 ml of methylene chloride solution containing 1.5 g of borontrichloride. The solution was stirred at ambient temperature for 1 hr: 6 ml of methanol was added slowly and then evaporated to dryness. The residue was suspended in water and extracted with ether. The ether extract on removal of solvent gave amorphous solids which was chromatographed on a silica gel column by elution with benzene/ether (5 : 1) to obtain white amorphous solids(200 mg).

Mp. 88∼89°; $[\alpha]_D^{20}$=0°(C=0.1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1} : 3480(OH), 1610, 1514, 1486(aromatic C=C); ^{1}H-NMR($CDCl_3$) δ : 0.84(6H, d, J=6.4 Hz, H-9/H-9′), 1.66∼1.83(2H, m, H-8/H-8′), 2.09∼2.83(4H, m, H-7/H-7′), 3.85 (3H, s, OCH_3), 5.20∼5.47(3H, m, OH×3), 6.60∼6.88(6H, m, aromatic H); MS m/z(rel. int. %) : 316(M^+, 1.6), 179($C_{11}H_{15}O_2^+$, 0.9), 165($C_{10}H_{13}O_2^+$, 1.4), 151($C_9H_{11}O_2^+$, 2.3), 137 ($C_8H_9O_2^+$, 100), 123($C_7H_7O_2^+$, 39.6).

1-(3, 4-methylenedioxyphenyl)-2, 3-dimethyl-4-(3′, 4′-dimethoxyphenyl) butane(IV) —To a solution of 200 mg of macelignan(I) in acetone(10 ml) was refluxed with Me_2SO_4(1.6 ml) in the presence of dry K_2CO_3(4.8 g) at 50° for 1 hr. The reaction mixture was diluted with distilled water, extracted with ether, washed, dried and evaporated. The crude solid was chromatographed on a silica gel column by elution with benzene to give compound **IV** as pale yellow oil (150 mg).

$[\alpha]_D^{20}$=−4.2°(C=0.1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 1608, 1514, 1500, 1486(aromatic C=C); 926 (O-CH_2O); ^{1}H-NMR($CDCl_3$) δ : 0.83(6H, d, J=6.3 Hz, H-9/H-9′), 1.67∼1.77(2H, m, H-8/H-8′), 2.11∼2.85(4H, m, H-7/H-7′), 3.85 (6H, s, OCH_3×2), 5.89(2H, s, O-CH_2-O), 6.64∼6.74(6H, m, aromatic H); MS m/z(rel. int. %) : 342(M^+, 15.3), 179($C_{11}H_{15}O_2^+$, 2.1), 151($C_9H_{11}O_2^+$, 100), 135($C_8H_7O_2^+$, 40.4).

***Meso*-dihydroguaiaretic acid dimethyl ether(VI)**—To a solution of 500 mg of NDGA in dry acetone(10 ml) was refluxed with Me_2SO_4 (2 ml) in the presence of K_2CO_3(6 g) at 50° for 1 hr. The product was worked up in the usual manner; chromatographed and recrystallized from methanol to give 300 mg of compound **VI** as colorless needle crystals. mp. 101∼102°; $[\alpha]_D^{20}$=0°(C=0.8, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1};1608, 1514, 1470(aromatic C=C); ^{1}H-NMR($CDCl_3$) δ : 0.85(6H, d, J=6.4 Hz, H-9/H-9′), 1.64∼

1.88(2H, m, H-8/H-8′), 2.15～2.93(4H, m, H-7/H-7′), 3.84(12H, s, $OCH_3\times4$), 6.64～6.85(6H, m, aromatic H); MS *m/z*(rel. int. %) : 358(M^+, 9.6), 179($C_{11}H_{15}O_2^+$, 3.0), 151 ($C_9H_{11}O_2^+$, 100).

Machilin-A (VII)—To a mixture of NDGA (500 mg), anhydrous potassium carbonate(2 g) and dibromomethane(0.6 ml) was added cupric oxide(15 mg) dissolved in dimethylformamide (8 ml). The mixture was refluxed at 170～180° in oil bath for 7 hr, cooled, diluted with H_2O and extracted with ether. The ether extract was washed with H_2O, dried and concentrated *in vacuo*. Elution with benzene from a silica gel column and recrystallization from methanol gave compound **VII** as colorless needle crystals(300 mg).

Mp. 60～61°; $[\alpha]_D^{20}=+1°$(C=0.1, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 1608, 1500, 1486(aromatic C=C), 926(O-CH_2-O); ^{1}H-NMR($CDCl_3$) δ : 0.82 (6H, d, J=6.4 Hz, H-9/H-9′), 1.65～1.78 (2H, m, H-8/H-8′), 2.11～2.84(4H, m, H-7/H-7′), 5.91(4H, s, O-CH_2-O×2), 6.53～6.68(6H, m, aromatic H); MS *m/z*(rel. int. %) : 326(M^+, 7.6), 163($C_{10}H_{11}O_2^+$, 3.0), 148 ($C_9H_8O_2^+$, 1.0), 135($C_8H_7O_2^+$, 100).

Assay of antioxidant activity *in vitro*— The antioxidant activity was estimated by measuring lipid peroxidation with liver homogenate of male mice(18～25 g) *in vitro*.

0.3 ml of pooled liver homogenate(1 g of liver was homogenized in 10 ml of iced 0.9% saline) was incubated at 37° for 5 hr in the presence or absence of 0.5 ml of lignans dissolved 2% DMSO.

Peroxidation was assayed by the formation of thiobarbituric acid reactants as previously described. Graded concentrations of the lignan samples were added in the incubation mixture dissolving in 2% DMSO. IC_{50} values, the concentration of compounds that cause 50% inhibition of lipid peroxidation were calculated from regression equations.

Table I. Antioxidant potency of lignans

Compounds	IC_{50}*(μM)
I	2.8
II	68
III	7.3
IV	100
V	1.5
VI	—
VII	—

*Concentration of compounds that cause 50% inhibition of lipid peroxidation.

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