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Antioxidant Activity of Tetrahydrocurcumins†

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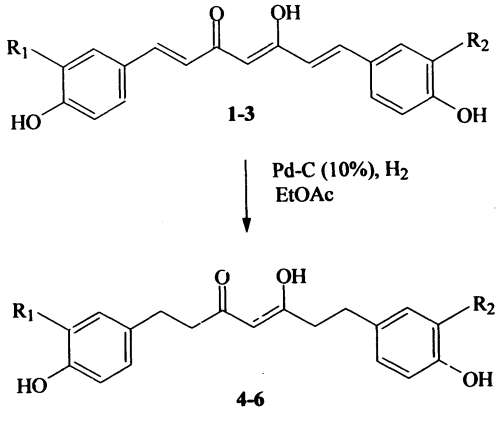
Tetrahydrocurcumins (**4–6**), obtained through the hydrogenation of naturally occurring curcumins (**1–3**), have shown weak antioxidant activity by superoxide scavenging ability and inhibition of lipid peroxidation.

Key words: Curcumins; tetrahydrocurcumins; antioxidant activity.

INTRODUCTION

Natural antioxidants have been receiving more attention, in recent years, due to their ability to inhibit free radical mediated biological processes[1–3]. Curcuminoids (**1–3**), constituents of turmeric (the powder from the roots/rhizomes of *Curcuma longa* L.) have exhibited strong antioxidant[4] and anti-inflammatory[5] activities. As the naturally occurring curcumins possess intense yellow colour, their use as general food additives and in cosmetics is restricted. We have recently synthesised tetrahydrocurcumins (**4–6**), which are not only colourless, but have also shown enhanced antibacterial activity.[6] In continuation of this study, we have examined the antioxidant activity of **4–6** by superoxide scavenging ability (nitroblue tetrazolium reduction method) and inhibition of lipid peroxidation (thiobarbituric acid method) and the results are presented in this paper.



1-3

4-6

1, 4: $R_1 = R_2 = OCH_3$
2, 5: $R_1 = H$; $R_2 = OCH_3$
3, 6: $R_1 = R_2 = H$

†Laila Impex communication # 8.

EXPERIMENTAL

Synthesis of tetrahydrocurcumins: Compounds (**4–6**) were prepared by the procedure reported earlier.[6]

1,7-Bis(4-hydroxy-3-methoxyphenyl)-3-hydroxy-3-hepten-5-one (**4**): Colourless amorphous powder, 69% yield, m.p. 94–96°C (Lit. 95–96°C); IR (Neat) ν_{max}: 3440, 2930, 1722, 1699, 1611, 1515, 1271, 1033, 816 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.6–3.0 (8H, m), 3.83 (6H, s), 5.4 (1H, s), 6.64 (2H. d, J = 8 Hz), 6.66 (2H, s), 6.81 (2H, d, J = 8 Hz).

1-(4-hydroxyphenyl)- 7-(4-hydroxy-3-methoxyphenyl) - 3-hydroxy-3-hepten-5-one (**5**): Colourless oil, 70% yield; IR (Neat) ν_{max}: 3398, 2936, 1721, 1697, 1613, 1515, 1269, 1033, 823 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.5–2.9 (8H, m), 3.81 (3H, s), 5.4 (1H, s), 6.6–7.0 (7H, m).

1,7-Bis-(4-hydroxyphenyl)-3-hydroxy-3-heptene-5-one (**6**): Colourless powder, 70% yield, m.p. 104–105°C; IR (Neat) ν_{max}: 3153, 2936, 1720, 1602, 1517, 1447, 1248, 1099, 826 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.52 (4H, t, J = 7.5 Hz), 2.84 (4H, t, J = 7.5 Hz), 5.36 (1H, s), 6.73 (4H, d, J = 8.3 Hz), 7.01 (4H, d, J = 8.3 Hz).

Antioxidant activity

Superoxide scavenging ability: Inhibition of superoxide scavenging ability was determined by the nitroblue tetrazolium (NBT) reduction method[8, 9]. The reaction mixture comprises of EDTA (6 μM) containing 3 μg NaCN, riboflavin (2 μM), NBT (50 μM), various concentrations of the sample (**4** or **5** or **6**), in ethanol and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical density was measured at 560 nm before and after the illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and compound treated tubes.

Lipid peroxidation inhibiting activity: The inhibition of lipid peroxidation was determined by the thiobarbituric acid method[10]. Different concentrations of the compound were incubated at 37°C with a 20% rat liver homogenate (0.1 mL) containing 30 mM KCl, tris-HCl buffer (0.04 M, pH 7.0), ascorbic acid (0.06 mM) and ferrous iron (0.16 mM) (total volume 0.5 mL) for 1 h. At the end of incubation period, 0.4 mL of the reaction mixture was treated with 0.2 mL sodium dodecyl sulphate (8.1%), 1.5 mL thiobarbituric acid (0.8%) and 1.5 mL acetic acid (20%, pH 3.5). The total volume was made up to 4 mL by adding distilled water and kept in a water bath at 95°C for 1 h. After cooling, 1 mL distilled water and 5 mL butanol-pyridine mixture (15 : 1 v/v) were added. After vigorous shaking, the tubes were centrifuged and the upper layer containing the chromophore was read at 532 nm. The percentage inhibition of lipid peroxidation by the compounds was determined by comparing the absorbance values of the control and compound treated tubes.

RESULTS AND DISCUSSION

Tetrahydrocurcumins (**4–6**) were prepared by the hydrogenation of naturally occurring curcumins (**1–3**) in ethyl acetate over palladium-charcoal (10%) in

good yield, as described earlier[6]. **4–6** showed decreased antioxidant activity by superoxide scavenging ability (NBT method) and inhibition of lipid peroxidation (thiobarbituric acid method using liver homogenate) compared to their natural analogues **(1–3)** (Table-1). Our results corroborate with the literature report[11] in which tetrahydrocurcumin **4** was reported to be a weaker antioxidant compared to **1** by 12-O-tetradecanoylphorbol-13-acetate (TPA) induced O_2^- generation in differentiated HL-60 cells. However, it may be noted that tetrahydrocurcumin **(4)** was claimed to be a stronger antioxidant than **1** in the inhibition of lipid peroxidation (thiobarbituric acid method using linoleic acid)[12, 13]. It is known that lack of extended conjugation reduces antioxidant activity. For example, the flavanones in which the extended conjugation is absent, are weaker antioxidants compared to the corresponding flavones[14].

TABLE 1
ANTIOXIDANT ACTIVITY OF **1–6**

Compound	IC_{50} (μg/mL)	
	Superoxide (NBT) scavenging method	Lipid peroxidation (Thiobarbituric acid) method
1	15	140
2	17	190
3	15	185
4	> 200[a]	> 200[b]
5	> 200[a]	> 200[b]
6	> 200[a]	> 200[b]

[a]The percentage inhibition of 4, 5 and 6 at 200 μg/mL were 37, 28 and 27 respectively.
[b]The percentage inhibition of 4, 5 and 6 at 200 μg/mL were 24, 12 and 7 respectively.

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