Estrogenic Activities of UV Stabilizers Used in Food Contact Plastics and Benzophenone Derivatives Tested by the Yeast Two-Hybrid Assay

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UV stabilizers used in food contact plastics were tested for their estrogenic activity by the yeast two-hybrid assay. Among 11 kinds of UV stabilizers, 2-hydroxy- 4-methoxybenzophenone and 2,2′-dihydroxy-4-methoxybenzophenone displayed estrogenic activity, while salicylate, benzoate and benzotriazole derivatives and a benzophenone derivative had no activity. Therefore, benzophenone and 19 kinds of hydroxylated derivatives were further studied. Of these, 15 chemicals showed estrogenic activity. The strongest activity was by 2,4-dihydroxybenzophenone, 4-hydroxy-4′-chlorobenzophenone, 4-hydroxybenzophenone and 2,3,4-trihydroxybenzophenone. Their activities were stronger than that of bisphenol A which was recognized as a potential endocrine disruptor. The following structure-activity relationships of benzophenones were obtained. The activity of the benzophenones with a hydroxyl group at the 3 or 4-position was positive and rather strong, though that of other benzophenones without a hydroxyl group at the 3 or 4-position was negative or weakly positive. The effect of the hydroxyl group in the phenol moiety were in order of 4- > 3- >> 2-position. A hydroxyl group added at the 2-position of the 4-hydroxylated benzene ring enhanced the activity. On the other hand, a hydroxyl group added to the benzene ring of the hydrophobic moiety reduced the binding, while the chloro group enhanced it. Some of these relationships might possibly hold for other estrogenic chemicals that possess two benzene rings.

Key words —— estrogenic activity, UV stabilizer, benzophenone derivatives, yeast two-hybrid assay

INTRODUCTION

UV stabilizers are used in food packages as plastic additives, their function being mainly to prevent polymer degradation and/or a change in the quality of the packed food due to UV rays. Some UV stabilizers in sunscreen products including 2-hydroxy-4-methoxybenzophenone were reported to have estrogenicity in an MCF-7 breast cancer cell assay and in an immature rat uterotrophic assay,[1] and another UV stabilizer, 2,2′-dihydroxy-4-methoxybenzophenone, was also reported to show estrogenicity in an MCF-7 assay.[2]

In the present study, 11 kinds of UV stabilizers used in food contact plastics were tested for estrogenic activity by a yeast two-hybrid assay.

The assay was performed according to Nishikawa,[3,4] based on the ligand-dependent interaction of estrogen receptor (ER) α and the coactivator transcriptional intermediary factor 2 (TIF2), and the estrogenic activity was detected as β-galactosidase activity. Two expression plasmids, pGBT9-estrogen receptor ligand binding domain (pGBT9-ERLBD) and pGAD424-TIF2, were introduced into yeast cells (*Saccharomyces cervisiae* Y190), which carry a β-galactosidase reporter gene and require tryptophan and leucine for growth. By this method, more than 500 chemicals have already been tested for estrogenic activity, and structure-activity relationships have been proposed.[5]

Furthermore, twenty kinds of benzophenones were tested using the same assay to demonstrate their estrogenic activities. Their structure-activity relationships are also discussed.

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Table 1. Estrogenic Activities of UV Stabilizers Used in Food Contact Plastics Tested by Yeast Two-Hybrid Assay

Class	Chemical name	CAS No.	Trade name	REC_{10} (M)
Benzophenone	2-Hydroxy-4-methoxybenzophenone	131-57-7	Cyasorb UV-9, Seesorb 101	6.6×10^{-4}
Benzophenone	2,2′-Dihydroxy-4-methoxybenzophenone	131-53-3	Cyasorb UV-24	1.0×10^{-3}
Benzophenone	2-Hydroxy-4-*n*-octyloxybenzophenone	1843-05-6	Cyasorb UV-531, Seesorb 102	$> 1.0 \times 10^{-3}$
Salicylate	4-*tert*-Butylphenylsalicylate	87-18-3	Sumisorb 90, Seesorb 202	$> 1.0 \times 10^{-3}$
Benzoate	2,4-Di-*tert*-butylphenyl-3,5-di-*tert*-butyl-4-hydroxybenzoate	4221-80-1	Tinuvin 120	$> 1.0 \times 10^{-3}$
Benzotriazole	2-(2′-Hydroxy-5′-methylphenyl) benzotriazole	2440-22-4	Tinuvin P, Seesorb 701, JF-77	$> 1.0 \times 10^{-3}$
Benzotriazole	2-(2′-Hydroxy-3′,5′-di-*tert*-amylphenyl) benzotriazole	25973-55-1	Tinuvin 328, Sumisorb 350	$> 1.0 \times 10^{-4}$
Benzotriazole	2-(2′-Hydroxy-3′-*tert*-butyl-5′-methylphenyl)-5-chlorobenzotriazole	3896-11-5	Tinuvin 326, Seesorb 703	$> 1.0 \times 10^{-4}$
Benzotriazole	2,5-Bis(5′-*tert*-butyl-2′-benzoxazolyl)thiophene	7128-64-5	Uvitex OB	$> 1.0 \times 10^{-4}$
Benzotriazole	2-(2′-Hydroxy-3′,5′-di-*tert*-butylphenyl)-5-chlorobenzotriazole	3864-99-1	Tinuvin 327, Seesorb 702	$> 1.0 \times 10^{-4}$
Benzotriazole	2-[2′-Hydroxy-3′,5′-bis(α, α-dimethylbenzyl) phenyl]-2H-benzotriazole	70321-86-7	Tinuvin 234	$> 1.0 \times 10^{-4}$

MATERIALS AND METHODS

Reagents —— 17β-Estradiol (E_2), and benzophenone were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), 3-hydroxybenzophenone was purchased from Sigma-Aldrich Japan Co. (Tokyo, Japan), 3 kinds of UV stabilizers (CAS No. 4221-80-1, 25973-55-1, 70321-86-7) were obtained from the manufacturers, and the other UV stabilizers, benzophenone derivatives, and 4-nonylphenol were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). The UV stabilizers and benzophenones used in this study are shown in Tables 1 and 2, and the structures of the benzophenones are shown in Fig. 1. Zymolyase 20T was purchased from Seikagaku Co. (Tokyo, Japan), and used as the Z buffer [0.1 M sodium phosphate (pH 7.0), 10 mM KCl and 1 mM $MgSO_4$] solution. *o*-Nitrophenyl-β-D-galactoside (ONPG) was purchased from Sigma-Aldrich Japan Co. and dissolved in 0.1 M phosphate buffer pH 7.0.

Preparation of Test Chemicals —— Test chemicals were dissolved in dimethylsulfoxide (DMSO) at 10^{-1} to 10^{-5} M (final concentrations: 10^{-3} to 10^{-7} M). When the chemical could not be dissolved at 10^{-1} M, the concentration was changed to 10^{-2} M (final concentration: 10^{-4} M). The concentration of DMSO was 1% in the assay, which did not inhibit the yeast growth. Each experiment was accompanied by E_2 as a positive control and DMSO as a negative control.

Measurement of Estrogenic Activity by Yeast Two-Hybrid Assay —— The cells were preincubated overnight at 30°C with vigorous shaking in a synthetic dropout (SD) medium free from tryptophan and leucine. The culture was diluted with 4 volumes of fresh SD medium (finally 250 μl) in a small test tube, a test chemical solution (2.5 μl) was added and the mixture was incubated for 4 hr at 30°C.

After incubation, 150 μl of the culture solution was placed into each of the 96 wells of a microplate and the absorbancy measured at 595 nm. The rest of the culture was centrifuged at 10000 rpm for 7 min, and the supernatant was removed. The cells were digested enzymatically by incubation with 1 mg/ml of Zymolyase 20T (200 μl) at 30°C for 15 min. The cell lysate was mixed with 4 mg/ml of ONPG (40 μl) and incubated at 30°C for 30 min exactly. The reaction was stopped by the addition of 1 M Na_2CO_3 (100 μl). After centrifugation at 10000 rpm for 5 min, the supernatant (150 μl) was placed into each well of a microplate. The absorbances at 420 and 570 nm were read on a microplate reader. β-Galactosidase activity was calculated with the following equation: $U = 1000 \times ([OD_{420}] - [1.75 \times OD_{570}])/([t] \times [v] \times [OD_{595}])$ where t = time of reaction (min), v = volume of culture used in the assay (ml), OD_{595} = cell density at the start of the assay, OD_{420} = absorbance by *o*-nitrophenol at the end of the reaction, and OD_{570} = light scattering at the end of the reaction. β-Galactosidase activity was expressed as the means and standard deviations of the results from three independent test tubes.

Table 2. Estrogenic Activities of Benzophenones and Other Chemicals Tested by Yeast Two-Hybrid Assay

No.	Chemical name	CAS No.	REC_{10} (M)
E_2	17β-Estradiol	87-18-3	3.4×10^{-10}
BPA	Bisphenol A	50-28-2	1.1×10^{-5}
NP	4-Nonylphenol	80-05-7	4.6×10^{-7}
1	Benzophenone	119-61-9	$> 1.0 \times 10^{-3}$
2	2-Hydroxybenzophenone	117-99-7	6.2×10^{-4}
3	3-Hydroxybenzophenone	13020-57-0	1.0×10^{-5}
4	4-Hydroxybenzophenone	1137-42-4	4.5×10^{-6}
5	2,2′-Dihydroxybenzophenone	835-11-0	$> 1.0 \times 10^{-3}$
6	2,4-Dihydroxybenzophenone	131-56-6	1.8×10^{-6}
7	4,4′-Dihydroxybenzophenone	611-99-4	3.8×10^{-5}
8	2,3,4-Trihydroxybenzophenone	1143-72-2	9.0×10^{-6}
9	2,4,4′-Trihydroxybenzophenone	1470-79-7	1.8×10^{-5}
10	2,2′,4,4′-Tetrahydroxybenzophenone	131-55-5	1.4×10^{-5}
11	2,3,4,4′-Tetrahydroxybenzophenone	31127-54-5	3.6×10^{-5}
12	2,3′,4,4′-Tetrahydroxybenzophenone	61445-50-9	2.4×10^{-4}
13	2-Hydroxy-4-methoxybenzophenone	131-57-7	6.6×10^{-4}
14	2,2′-Dihydroxy-4-methoxybenzophenone	131-53-3	1.0×10^{-3}
15	2,2′-Dihydroxy-4,4′-dimethoxybenzophenone	131-54-4	$> 1.0 \times 10^{-3}$
16	4-Hydroxy-2′,4′-dimethoxybenzophenone	41351-30-8	4.0×10^{-5}
17	2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid	4065-45-6	$> 1.0 \times 10^{-3}$
18	2-Hydroxy-4-*n*-octyloxybenzophenone	1843-05-6	$> 1.0 \times 10^{-3}$
19	2-Hydroxy-5-methylbenzophenone	1470-57-1	1.3×10^{-4}
20	4-Hydroxy-4′-chlorobenzophenone	42019-78-3	2.2×10^{-6}

The results were evaluated based on relative activity, expressed as 10% relative effective concentration (REC_{10}), which is the concentration of the test chemical showing 10% of the agonist activity of 10^{-6} M E_2, the highest activity level of E_2. When the activity of the test chemical was higher than the REC_{10} within the concentration tested, the chemical was judged to be positive. When it was judged to be negative, more than the highest dose tested was indicated.

RESULTS

Estrogenic Activity of UV Stabilizers

Eleven kinds of UV stabilizers used in food contact plastics in Japan were tested for estrogenic activity in the yeast two-hybrid assay (Table 1). Two chemicals, 2-hydroxy-4-methoxybenzophenone and 2,2′-dihydroxy-4-methoxybenzophenone showed such activity, and their REC_{10} values were 6.6×10^{-4} and 1.0×10^{-3} M, respectively. The estrogenicity of 2-hydroxy-4-methoxybenzophenone agreed with that of the MCF-7 cell assay and the uterotrophic assay by Schlumpf,[1] and that of 2,2′-dihydroxy-4-methoxybenzophenone agreed with that of the MCF-7 cell assay by Nakagawa.[2] These two benzophenones, however, did not show estrogenicity in an ER competitive binding assay.[2,6]

The other UV stabilizers, namely one salicylate, one benzoate and one benzophenone derivative, and six benzotriazole derivatives, had no estrogenic activity.

Estrogenic Activity of Benzophenone Derivatives

Many hydroxylated benzophenones are popular as UV stabilizers not only for food contact plastics but also general purpose plastics, sunscreens and cosmetics. Moreover, some of them, *e.g.* 4-hydroxybenzophenone and 2,4-dihydroxy benzophenone, are also produced inside the body as metabolic products of benzophenone and its derivatives.[2,7–10] However, their estrogenic activities and structure-activity relationships were not clear.

Therefore, benzophenone and 16 of its hydroxylated derivatives were further tested for estrogenic activity using the yeast two-hybrid assay. The results are shown in Table 2 and dose–response curves are shown in Figs. 2–7, including those for the three kinds of benzophenones described above as UV sta-

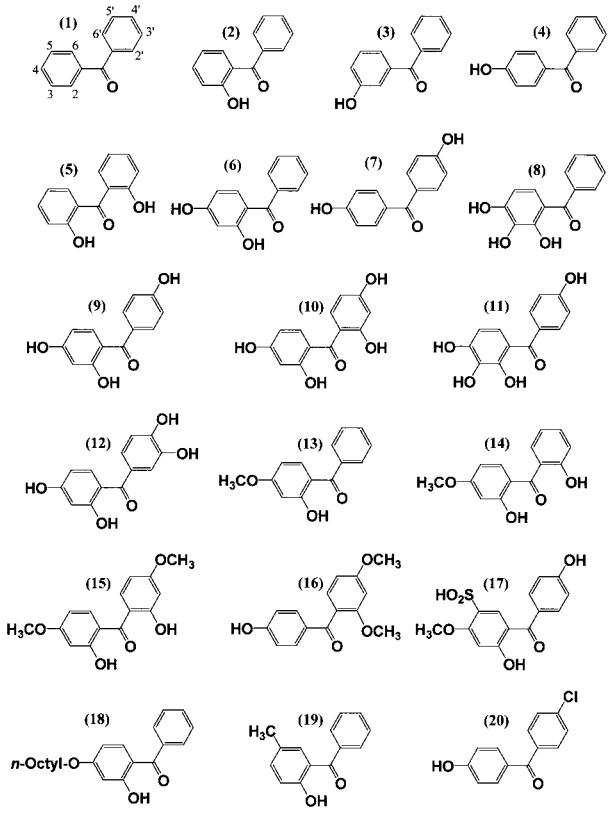

Fig. 1. Structures of Benzophenone and Hydroxylated Derivatives

Each number in parentheses shows the same compound as in Table 2 and 3.

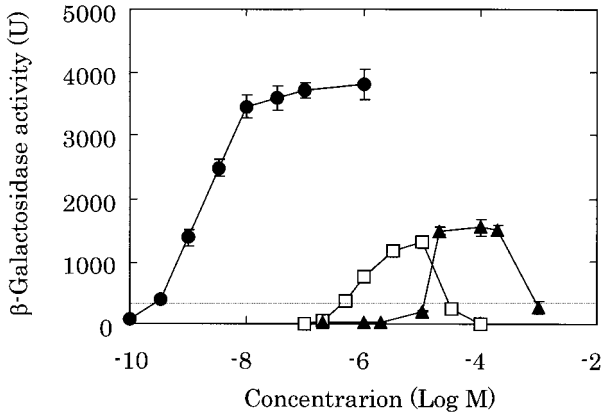

Fig. 2. Dose–Response Curves of Estrogenic Activity of 17β-Estradiol (●), 4-Nonylphenol (□) and Bisphenol A (▲)

Each point is the mean ± S.D. (n = 3). The dotted line shows 10% activity of 10^{-6} M 17β-estradiol.

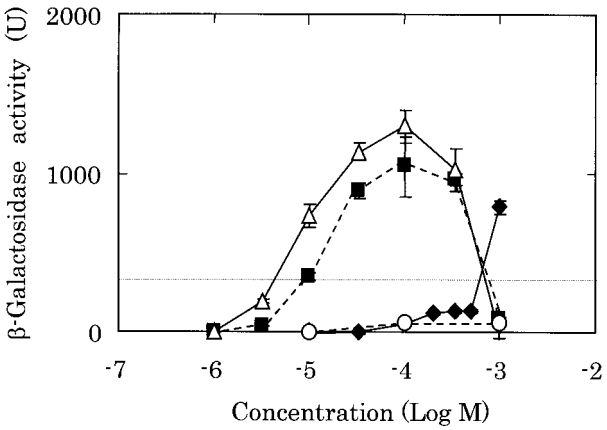

Fig. 3. Dose–Response Curves of Estrogenic Activity of Benzopheone and Mono-Hydroxylated Benzophenones

Each point is the mean ± S.D. (n = 3). The dotted line shows 10% activity of 10^{-6} M 17β-Estradiol. benzophenone (○) , 2-hydroxybenzophenone (◆), 3-hydroxybenzophenone (■), 4-hydroxybenzophenone (△).

bilizers.

The REC_{10} values of 4-nonylphenol and bisphenol A obtained in this study were consistent with those in previous reports using the same assay method.[3–5)]

Benzophenone (No. 1) was found to have low estrogenicity below the REC_{10}, it was then judged to be negative. However, all the mono-hydroxylated benzophenones were shown to have estrogenic activity (Fig. 3). Based on their REC_{10} values, they ranked in the order 4-hydroxybenzophenone (No. 4, 4.5×10^{-6} M) > 3-hydroxybenzophenone (No. 3, 1.0×10^{-5} M) >> 2-hydroxybenzophenone (No. 2, 6.2×10^{-4} M), and this tendency agreed with the results of the recombinant yeast assay by Schultz.[11)]

Regarding the di-hydroxylated benzophenones, the activity of 2,4-dihydroxy- benzophenone (No. 6) was the strongest among the benzophenone derivatives we tested, although 2,2′-dihydroxybenzophenone (No. 5) exhibited no activity (Fig. 4). The REC_{10} values were in the order of 2,4-dihydroxybenzophenone (1.8×10^{-6} M) > 4,4′-dihydroxybenzophenone (No. 7, 3.8×10^{-5} M) >> 2,2′-dihydroxybenzophenone (> 1.0×10^{-3} M).

The activities of 2,3,4- and 2,4,4′-trihydroxybenzophenone (No. 8, 9) were rather strong (Fig. 4), and their REC_{10} values (9.0×10^{-6} and 1.8×10^{-5} M) were between that of 2,4-dihydroxybenzophenone and 4,4′-dihydroxybenzophenone.

The activities of 2,2′,4,4′- and 2,3,4,4′-tetrahydroxybenzophenone (No. 10, 11) were rather strong, though that of 2,3′,4,4′-tetrahydroxybenzophenone (No. 12) was weak (Fig. 5). Their REC_{10} values were 1.4×10^{-5}, 3.6×10^{-5} and 2.4×10^{-4} M, respectively.

Regarding both hydroxylated and methoxylated benzophenones, the activity of 4-hydroxy-2′,4′-

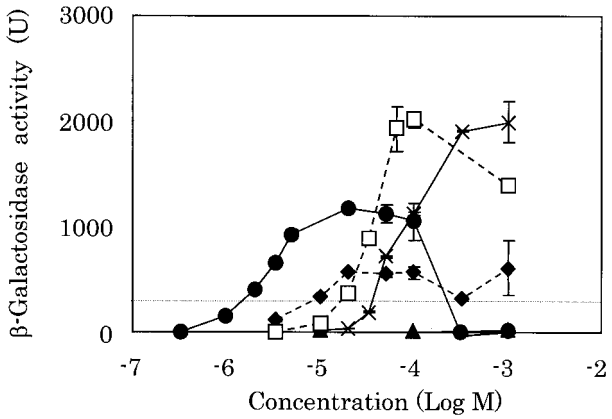

Fig. 4. Dose–Response Curves of Estrogenic Activity of Di- and Tri-Hydroxylated Benzophenones

Each point is the mean ± S.D. (n = 3). The dotted line shows 10% activity of 10^{-6} M 17β-estradiol. 2,2′-dihydroxybenzophenone (▲), 2,4-dihydroxybenzophenone (●), 4,4′-dihydroxybenzopenone (□), 2,3,4-trihydroxybenzophenone (◆), 2,4,4-trihydroxybenzophenone (×).

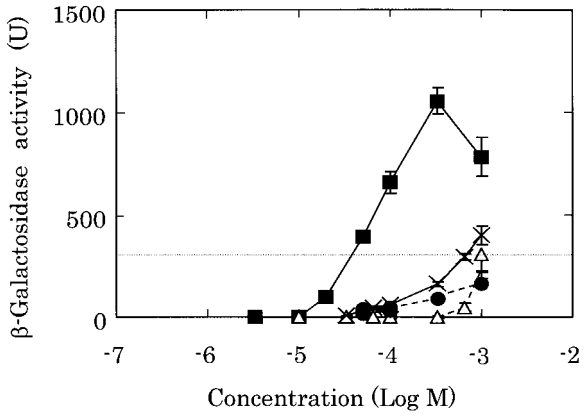

Fig. 6. Dose–Response Curves of Estrogenic Activity of Hydroxylated and Methoxylated Benzophenones

Each point is the mean ± S.D. (n = 3). The dotted line shows 10% activity of 10^{-6} M 17β-estradiol. 2-hydroxy-4-methoxybenzophenone (×), 2,2′-dihydroxy-4-methoxybenzophenone (△), 2,2′-dihydroxy-4,4′-dimethoxybenzophenone (●), 4-hydroxy-2′,4′-dimethoxybenzophenone (■).

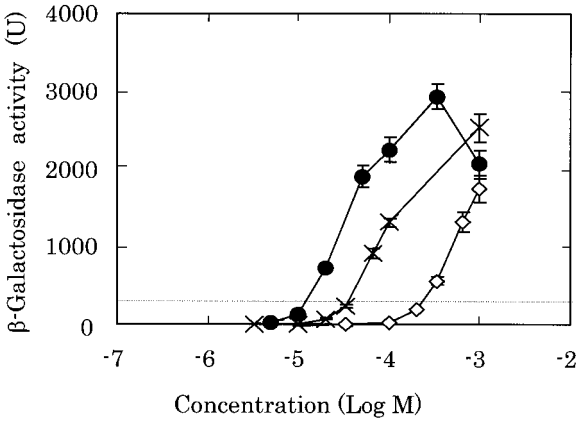

Fig. 5. Dose–Response Curves of Estrogenic Activity of Tetra-Hydroxylated Benzophenones

Each point is the mean ± S.D. (n = 3). The doted line shows 10% activity of 10^{-6} M 17β-estradiol. 2,2′,4,4′-tetrahydroxybenzophenone (●), 2,3,4,4′-tetrahydroxybenzophenone (×), 2,3′,4,4′-tetrahydroxybenzophenone (◇).

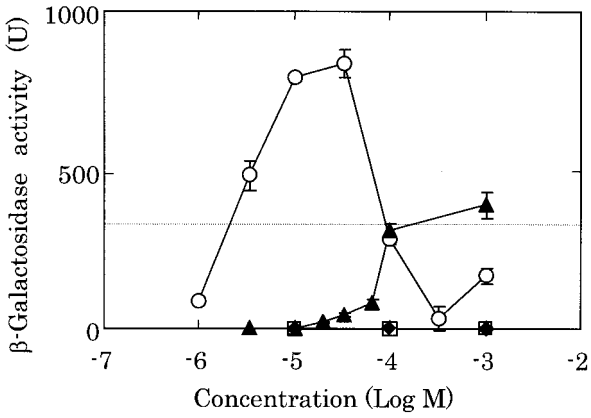

Fig. 7. Dose–Response Curves of Estrogenic Activity of the Other Hydroxylated Benzophenones

Each point is the mean ± S.D. (n = 3). The dotted line shows 10% activity of 10^{-6} M 17β-estradiol. 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (□), 2-hydroxy-4-n-octyloxybenzophenone (◆), 2-hydroxy-5-methylbenzophenone (▲), 4-hydroxy-4′-chlorobenzophenone (○).

dimethoxybenzophenone (No. 16) was rather strong (4.0×10^{-5} M), but the others which did not possess a hydroxyl group at the 4-position had weak or negative activity (Fig. 6).

The activities of 2-hydroxy-4-methoxybeozophenone-5-sulfonic acid and 2-hydroxy-4-n-octyloxybenzophenone (No. 17, 18) were not determined, and 2-hydroxy-5-methylbenzophenone (No. 19) had weak activity. However, the activity of 4-hydroxy-4′-chlorobenzophenone (No. 20, 2.2×10^{-6} M) was as strong as that of 2,4-dihydroxybeozophenone (Fig. 7).

DISCUSSION

As shown in Table 3, 13 benzophenones were already reported to be estrogenic using other assay methods.[1,2,6,9–11] Comparing the present results to those, findings 8 relatively strong compounds for which REC_{10} values were under 10^{-4} M (No. 3, 4, 6–10, 20), and two negative compounds (No. 1, 5) were the same as in all the previous papers regarding positive or negative activity. Only three weakly positive compounds whose REC_{10} values were over

Table 3. Comparison of Estrogenic Activities of Benzophenones with Present and Previous Investigations

No.	Compound	Present investigation	Previous investigation						
		Yeast two-hybrid assay	MCF-7 cell assay		Competitive binding assay		Recombinant yeast assay[11]	Uterotropic assay	
		REC_{10} (M)	EC_{50}[1] (M)	positive[2,9] (M)	IC_{50}[2,10] (M)	IC_{50}[6] (M)	EC_{50} (M)	ED_{50}[1] (mg/kg/day)	positive[10] (mg/kg/day)
1	Benzophenone	$> 1.0 \times 10^{-3}$		$> 10^{-4}$	$> 5 \times 10^{-4}$		Nonactive		> 400
2	2-Hydroxy	6.2×10^{-4}					Nonactive		
3	3-Hydroxy	1.0×10^{-5}					2.57×10^{-6}		
4	4-Hydroxy	4.5×10^{-6}		10^{-5}	5×10^{-5}		1.12×10^{-6}		100
5	2,2′-Dihydroxy	$> 1.0 \times 10^{-3}$				$> 1.00 \times 10^{-4}$	Nonactive		
6	2,4-Dihydroxy	1.8×10^{-6}		10^{-8}	5×10^{-5}	3.65×10^{-5}			
7	4,4′-Dihydroxy	3.8×10^{-5}				2.60×10^{-5}	2.53×10^{-6}		
8	2,3,4-Trihydroxy	9.0×10^{-6}		10^{-7}	5×10^{-4}		5.08×10^{-6}		
9	2,4,4′-Trihydroxy	1.8×10^{-5}					5.64×10^{-7}		
10	2,2′,4,4′-Tetrahydroxy	1.4×10^{-5}					7.92×10^{-7}		
11	2,3,4,4′-Tetrahydroxy	3.6×10^{-5}							
12	2,3′,4,4′-Tetrahydroxy	2.4×10^{-4}							
13	2-Hydroxy-4-methoxy	6.6×10^{-4}	3.73×10^{-6}	$> 10^{-5}$	$> 1 \times 10^{-4}$	$> 1.00 \times 10^{-4}$		1000–1500	
14	2,2′-Hydroxy-4-methoxy	1.0×10^{-3}		10^{-7}	$> 1 \times 10^{-4}$	$> 1.00 \times 10^{-4}$			
15	2,2′-Hydroxy-4,4′-methoxy	$> 1.0 \times 10^{-3}$							
16	4-Hydroxy- 2′,4′-methoxy	4.0×10^{-5}							
17	2-Hydroxy-4-methoxy-5-sulfonic acid	$> 1.0 \times 10^{-3}$							
18	2-Hydroxy-4-*n*-octyloxy	$> 1.0 \times 10^{-3}$							
19	2-Hydroxy-5-methyl	1.3×10^{-4}							
20	4-Hydroxy-4′-chloro	2.2×10^{-6}					2.88×10^{-7}		

1, 2, 6, 9–11) : Refernce No.

10^{-4} M (No. 2, 13, 14), were found to be negative in some reports mainly by the competitive binding assay. These discrepancies seemed to be due to differences in sensitivity of methods. Thus, the present results were very consistent with previous results, though the strength of activity showed some variation.

The present study is the first report on the estrogenicities of 9 kinds of UV stabilizers and 7 kinds of hydroxylated benzophenones (No. 11, 12, 15–19). Among them, 4 kinds of benzophenones (No. 11, 12, 16, 19) were positive for estrogenic activity.

To predict estrogenic potency based on molecular structure, rules have been proposed and one was applied to benzophenone derivatives.[11] The predictions, however, did not fit our results well, especially regarding compounds hydroxylated only at the 2-position and symmetrical compounds.

A common structure for estrogenic compounds tested by the yeast two-hybrid assay was proposed by Nishihara[5] that most positive compounds have a phenol ring with a moiety of appropriate hydrophobicity at the *p*-position. The present results were a very good match with this rule. All of the 4-hydroxylated benzophenones (No. 4, 6–12, 16, 20), which had a phenol ring, and also had a benzoyl group as a hydrophobic moiety at the *p*-position, had rather strong activity. However, benzophenones without a hydroxyl group at the 4-position were negative (No. 1, 5, 15, 17, 18) or weakly positive (No. 2, 13, 14, 19). One exception was 3-hydroxybenzophenone which had no hydroxyl group at the 4-position, though its activity was rather strong. It was presumed that the two benzene rings of benzophenone were not fixed; therefore, the 3-hydroxyl group could bind the receptor to nearly the same degree as the 4-hydroxyl group. The hydroxyl group of the phenol moiety affects estrogenic activity in the order of the 4-position > 3-position >> 2-position of benzophenone.

Furthermore, when one more hydroxyl group at the 2-position was added to 4-hydroxylated benzophenones, the activity was enhanced such that No. 4 (REC_{10}: 4.5×10^{-6} M) < No. 6 (1.8×10^{-6} M), and No. 7 (3.8×10^{-5} M) < No. 9 (1.8×10^{-5} M). It was presumed that the 2-hydroxyl group interacted with the carbonyl group; the benzophenone structure then became fixed and the length of the hydrophobic moiety fitted the receptor. A hydroxyl group at the 3-position added to 4-hydroxylated benzophenones, however, might reduce the activity slightly such that No. 6 (1.8×10^{-6} M) > No. 8 (9.0×10^{-6} M) and No. 9 (1.8×10^{-5} M) > No. 11 (3.6×10^{-5} M).

On the other hand, when a hydroxyl group was added to the benzoyl moiety, the estrogenic activity was reduced drastically, with No. 2 (6.2×10^{-4} M) > No. 5 (nonactive), No. 4 (4.5×10^{-6} M) > No. 7 (3.8×10^{-5} M), No. 6 (1.8×10^{-6} M) > No. 9 (1.8×10^{-5} M) > No. 12 (2.4×10^{-4} M), No. 8 (9.0×10^{-6} M) > No. 11 (3.6×10^{-5} M), and No. 13 (6.6×10^{-4} M) > No. 14 (1.1×10^{-3} M). Commonly, estrogenic compounds possess two hydroxyl groups on opposite sides, *e.g.* estradiol, distylbesterol, bisphenol A, and both hydroxyl groups were presumed to contribute to the estrogenicity. However, our results indicated that the second hydroxyl group at the opposite ring would not only contribute to the estrogenicity but also reduce activity in the case of benzophenone derivatives.

One exception was No. 9 (1.8×10^{-5} M) < No. 10 (1.4×10^{-5} M), and it was presumed that the 2′-hydroxyl group interacted with the carbonyl group the same as the 2-hydroxyl group described previously, and the benzophenone structure might be fixed. While a chloro group added to the benzoyl moiety enhanced the activity, such that No. 4 (4.5×10^{-6} M) < No. 20 (2.2×10^{-6} M).

Regarding the methoxy group, the estrogenic activity was unchanged when it was added to phenol ring. While the activity might be reduced when the methoxy group was added to the benzoyl moiety, though examples were a few.

With these structure-activity relationships, the estrogenic activity of most of the benzophenones tested in the present study could be explained clearly. Moreover, it is presumed that some of these relationships have potential applications in other estrogenic chemicals which possess two benzene rings.

Fifteen hydroxylated benzophenones showed estrogenic activity, and four of them were more active than bisphenol A. The majority of these chemicals are used as UV stabilizers in plastics, sunscreens, cosmetics and others. They are also produced in the body as the metabolic products of benzophenone and its derivatives. Therefore, more investigations of hydroxylated benzophenones and their endocrine disrupting effects are needed.

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