A Practical Synthesis and Estrogenic Activity of 5-Hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan, a Contaminant in Industrial Grade Bisphenol A

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(Received November 8, 2004; CL-041333)

A practical synthesis of 5-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan was achieved from α-methylstyrene. The starting compound was dimerized in the presence of trifluoroacetic acid to produce 1-phenyl-1,3,3-trimethylindan. Aromatic nitration of the phenylindane was carried out using a mixture of nitric acid and acetic anhydride. The nitro group was then converted to hydroxyl via reduction and a diazonium salt sequence to form 5-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan. The estrogenicity of the product was 8.7 times that of BPA. Our finding has demonstrated the endocrine-disrupting properties of the product.

Bisphenol A (4,4′-isopropylidenediphenol, BPA; CAS No. 80-05-7) (Chart 1) is used as a monomer in the manufacture of polycarbonate and epoxy resins, as a stabilizer or antioxidant for many types of plastics such as polyvinyl chloride (PVC), and as an inhibitor of end oxidation in PVC.[1] Annual production capacity of this compound in Japan is about 490 000 tons.[2] Recently, considerable attention has been focused on BPA as well as other phenolic compounds as endocrine-disrupting chemicals. In particular, numerous in vivo and in vitro studies have demonstrated the estrogenic properties of this compound.[3] We have recently identified 5-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan, as one of impurities in samples of an industrial grade of bisphenol A.[4] This compound, like BPA itself, possesses a phenolic hydroxy group *para* to other substituents, and thus resembles 17β-estradiol (Chart 1), the main female hormone, and might also have estrogenic properties. Synthesis was necessary to provide sufficient material for estrogenic activity assays. However, no appropriate synthetic intermediate was commercially available, and no convenient synthetic method for the compound has hitherto been reported. In this study, we established a facile synthetic route to 5-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan from commercially available α-methylstyrene and assessed the estrogenic activity of this compound using yeast two-hybrid assays incorporating the human estrogen receptor or the medaka fish (*Oryzias latipes*) estrogen receptor.[5]

Synthesis of 5-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan: In the synthetic path illustrated in Scheme 1, we selected all reagents and solvents volatile enough to be easily evaporated, except for the filtration required for the separation of Pd/C in the third step, in order to attain a facile synthesis. α-Methylstyrene (**3**) (10 mL) was added dropwise to trifluoroacetic acid (TFA) (30 mL) and the mixture, which rapidly turned red, was stirred for 12 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. ^{1}H, ^{13}C NMR, and high resolution (HR) FAB MS examination of the residue showed 1-phenyl-1,3,3-trimethylindan (**4**), a dimer of α-methylstyrene.[6]

The key step was the substitution reaction of phenylindan (**4**), which introduced two nitro groups at the required positions on the benzene ring. Although formation of the desired dinitro compound, 5-nitro-1-(4′-nitrophenyl)-1,3,3-trimethylindan (**5**), is sterically disadvantageous, nitration gave an appreciable amount of **5**. Thus, to the reaction product containing **4** in chloroform (20 mL), a previously mixed solution of nitric acid (3.3 mL) and acetic anhydride (22.5 mL) was added dropwise at 0 °C. The reaction temperature was maintained for an additional 2 h. The product was revealed to contain dinitroindans **5** and **6** in the ratio of 4 to 6, as analyzed by ^{1}H NMR and GC–MS.[7] Nitric acid and acetic anhydride were removed under reduced pressure, and the dissolution and evaporation cycle was performed 5 times, at first using water (50 mL each) and then 5 times using benzene (30 mL each) to remove water as an azeotropic mixture, according to the method of Nomoto et al.[8]

The yellow residue containing dinitroindans **5** and **6** in 2-propanol (50 mL) was heated to reflux. Once it dissolved, Pd/

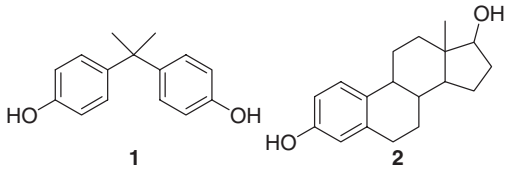
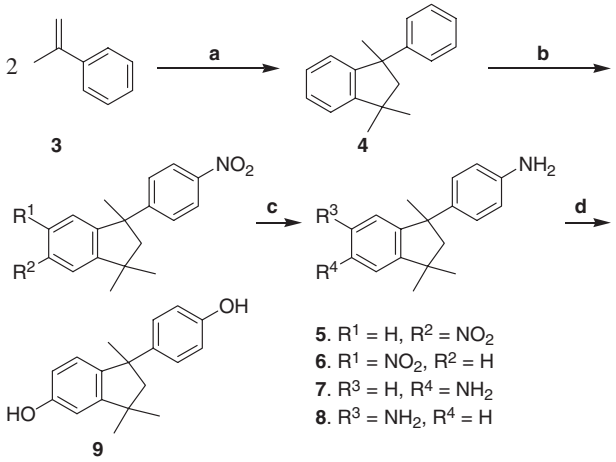


Chart 1. Chemical structures for bisphenol A (**1**) and 17β-estradiol (**2**).



Scheme 1. (a) TFA, rt 12 h; (b) $HNO_3/(Ac)_2O$, 0 °C, 4 h; (c) Pd/C, hydrazine, reflux, 4 h; (d) H_2SO_4, $NaNO_2$, H_2O, 0 °C/heat.

C (0.25 g) was added followed by 15-mL hydrazine monohydrate, which was slowly added dropwise. The mixture was refluxed for 4 h, and then the catalyst was removed by filtration under nitrogen. The reaction mixture was concentrated under reduced pressure and subjected to the dissolution and evaporation cycle 5 times using benzene (50 mL each). This residue was considered to contain diaminoindans **7** and **8** as analyzed by GC–MS.[9]

In the final step of synthesis, the above residue was dissolved in 10% sulfuric acid (20 mL) to give a dark red solution. To the mixture, cooled sodium nitrite (3 g) in H_2O (10 mL) was added dropwise at 0 °C, and then the mixture was heated under reflux for 20 min. The product from this reaction was concentrated under reduced pressure and the residue was applied to a silica-gel column (300 × 30 mm). Solvent was removed by rotary evaporation from each fraction separately and the residues were examined by ^{1}H NMR. The desired **9** was eluted by dichloromethane–acetone (9:1) together with a by-product. This by-product could not be separated from the main product on silica-gel TLC using dichloromethane–acetone (9:1) as a developing solvent. The mixture was finally separated by HPLC to afford pure **9**.[10] The total yield from α-methylstyrene was 376 mg (3.6%). The structure of this product was confirmed by NMR spectra, as shown in Figure 1.[11]

Estrogenic activity of 5-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan (9) by yeast two-hybrid assays: According to the synthetic scheme described here, **9** can be easily obtained in a practical yield without using any special reagents. This synthetic work facilitated definitive estrogenicity assays of **9**, which is found as one of impurities in industrial grade BPA. Estrogenicity of **9** and purified laboratory reagent BPA are presented in Table 1. The assay using the mERα showed that the estrogenicity of **9** was 8.7 times that of BPA. In the assay using hERα, **9** had 1.2 times the activity of BPA.

BPA is manufactured by the condensation of phenol with acetone in the presence of an acid catalyst. Different manufacturing processes are likely to yield different relative quantities of the product and of impurities. Trace impurities present in low concentrations, arising either during synthesis or storage, may lead to markedly different activities than might be expected from the activities of the individual components.[12]

The work reported in the current paper has demonstrated the endocrine-disrupting properties of **9**, a contaminant in industrial grade BPA. Confirmation of the in vivo activity of **9** would indicate that the potential estrogenicity of industrial grade BPA should be re-examined.

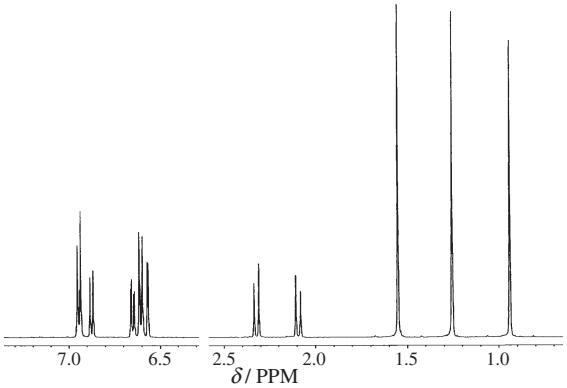


Figure 1. ^{1}H NMR spectra of 5-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan (**9**).

Table 1. Estrogenic activity of **9** and BPA in yeast two-hybrid assays for hERα and mERα

Compd.	hERα assay		mERα assay	
	ECx10; nM[a]	R. A.[b]	ECx10; nM[a]	R. A.[b]
9	2400	1.2	230	8.7
BPA	2800	1.0	2000	1.0

[a] Estrogenic activity was recorded as the ECx10 which was defined as the concentration of test chemical solution producing a chemiluminescent signal 10x that of the blank control. [b] Activity relative to BPA.

References and Notes

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5 a) F. Shiraishi, H. Shiraishi, J. Nishikawa, T. Nishihara, and M. Morita, *J. Environ. Chem.*, **10**, 57 (2000). b) T. Nishikawa *et al.*, unpublished data. The amplification of the gene for the medaka estrogen receptor and its introduction to a yeast recombinant plasmid for use in the yeast two-hybrid assay has shown that, in general, the relative estrogenic activities to xenoestrogens of the medaka estrogen receptor (ER) are higher than to the human ER.
6 ^{1}H NMR (500 MHz, methanol-d_4): δ 0.98, 1.32, 1.65 (s, 3H, Me), 2.18 (d, 1H, J = 13.2 Hz), 2.42 (d, 1H, J = 12.6 Hz), 7.06–7.26 (m, 9H, Ar–H); ^{13}C NMR (125 MHz, methanol-d_4): δ 151.89, 150.95, 148.47, 127.63, 126.99, 126.37, 126.31, 125.18, 124.62, 122.23, 59.12, 50.52, 42.43, 30.11, 29.72, 29.27; HRFAB MS positive mode (M + H): 237.1641 (calcd. for 237.1643; $C_{18}H_{22}$).
7 ^{1}H NMR (500 MHz, methanol-d_4): δ 1.08, 1.41, 1.78 (6H, Me), 2.38 (2H), 2.57 (2H), 7.38 (d, 1H, J = 8.0 Hz), 7.42 (d, 4H, J = 9.0 Hz), 7.50 (d, 1H, J = 8.5 Hz), 8.01 (d, 1H, J = 1.5 Hz), 8.12 (d, 1H, J = 1.5 Hz), 8.15 (d, 4H, J = 8.8 Hz), 8.17 (dd, 1H, J = 2.5 and 9.0 Hz), 8.23 (dd, 1H, J = 2.5 and 8.5 Hz); The mass chromatogram by GC–MS showed two peaks, which gave the same mass fragment at m/z 326 (M^+), 311 (M–CH_3), 265 (M–CH_3, –NO_2).
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9 MS (EI) data for both **7** and **8**: m/z 266 (M^+), 251, 236, 221, 208, 193, 180, 165, 158, 143, 130, 118, 106, 93, 77, 65.
10 a) Column: Inertsil ODS-3 (250 × 20 mm; 5 μm; GL Sciences, Tokyo, Japan); Elution: acetonitrile-H_2O (5:5); Detector: UV 270 nm. b) The by-product was identified as 6-hydroxy-1-(4′-hydroxyphenyl)-1,3,3-trimethylindan as judged from MS, NMR spectra and Nuclear Overhauser Effect (NOE) difference experiments (methanol-d_4). Selected physical data for this compound: mp 182–184 °C; ^{1}H NMR (500 MHz, methanol-d_4): δ 0.98, 1.27, 1.58 (s, 3H, Me), 2.11 (d, 1H, J = 13.8 Hz), 2.33 (d, 1H, J = 13.8 Hz), 6.47 (d, 1H, J = 2.3 Hz), 6.63 (d, 2H, J = 9.2 Hz), 6.68 (dd, 1H, J = 1.7 and 8.0 Hz), 6.97 (d, 2H, J = 9.2 Hz), 6.98 (d, 1H, J = 8.0 Hz). NOE experiment: enhancements at δ 6.47, 6.97, and 2.11 (irradiation at Me, δ 1.58), at 6.98, 2.11, and 0.98 (at Me, δ 1.27), at 6.98, 2.33 and 1.27 (at Me, δ 0.97); HR FAB MS positive mode $[M + H]^+$: 269.1539 (calcd for 269.1542; $C_{18}H_{21}O_2$).
11 Selected physical data for **9**: mp 181–183 °C; ^{1}H NMR (500 MHz, methanol-d_4): δ 0.96, 1.27, 1.57 (s, 3H, Me), 2.11 (d, 1H, J = 13.2 Hz), 2.33 (d, 1H, J = 12.6 Hz), 6.57 (d, 1H, J = 2.3 Hz), 6.62 (d, 2H, J = 9.2 Hz), 6.66 (dd, 1H, J = 2.3 and 8.6 Hz), 6.87 (d, 1H, J = 8.1 Hz), 6.95 (d, 2H, J = 9.2 Hz). NOE experiment: enhancements at δ 6.87, 6.95, and 2.11 (irradiation at Me, δ 1.57), at 6.57, 2.11, and 0.96 (at Me, δ 1.27), at 6.57, 2.33, and 1.27 (at Me, δ 0.96); HR FAB MS positive mode $[M + H]^+$: 269.1540 (calcd for 269.1542; $C_{18}H_{21}O_2$).
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Published on the web (Advance View) January 15, 2005; DOI 10.1246/cl.2005.188