Syntheses and Estrogenic Activity of 4-Nnylphenols

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Introduction

4-Nonylphenol (NP) is known as an environmental endocrine disruptor[1] and widely used as plastic additive and antioxidant.[2] Commercial NP prepared by the alkylation of phenol with nonene isomers ("propylene trimer") is a mixture of more than 31 components.[2] Wheeler *et al*. identified 22 NP isomers using a high-resolution mass spectrometry-gas chromatography with 100 m capillary column.[3] In our previous studies[4a-c], we described preparative fractionation of a commercial NP mixture using high performance liquid chromatography (HPLC) to afford fourteen NP isomers (structures were shown in Fig. 1): 4-(2,4-dimethyl-heptan-4-yl)phenol (**NP-A**), 4-(2,4-dimethylheptan-2-yl)phenol (**NP-B**), 4-(3,6-dimethylheptan-3-yl)phenol (**NP-C**), 4-(4-ethyl-2-methylhexan-2-yl)phenol (**NP-D**), 4-(3,5-dimethylheptan-3-yl)phenol (**NP-E**; diastereomer of **NP-G**),4-(2,5-dimethylheptan-2-yl)phenol (**NP-F**), 4-(3,5-dimethylheptan-3-yl)phenol (**NP-G**; diastereomer of **NP-E**), 4-(4-methyloctan-4-yl)phenol (**NP-H**), 4-(3-ethyl-2-methylhexan-2-yl)phenol (**NP-I**), 4-(3,4-dimethyl-heptan-4-yl)phenol (**NP-J**; diastereomer of **NP-L2**), 4-(3,4-dimethylheptan-3-yl)phenol (**NP-K**), 4-(3,4-dimethylheptan-4-yl)phenol (**NP-L2**; diastereomer of **NP-J**) 4-(2,3-dimethyl-heptan-2-yl)phenol (**NP-M**), 4-(3-methyloctan-3-yl)phenol (**NP-N**) and a decylphenol, 4-(3,6,6-trimethylheptan-3-yl)phenol (**DP-L**). In the present report, we describe the synthesis of seven NP isomers (**NP-C**, **NP-E+G**, **NP-F**, **NP-D**, **NP-I**, **NP-M**, **NP-N**) by two different synthetic methods and the estrogenic activities of synthetic NP isomers.

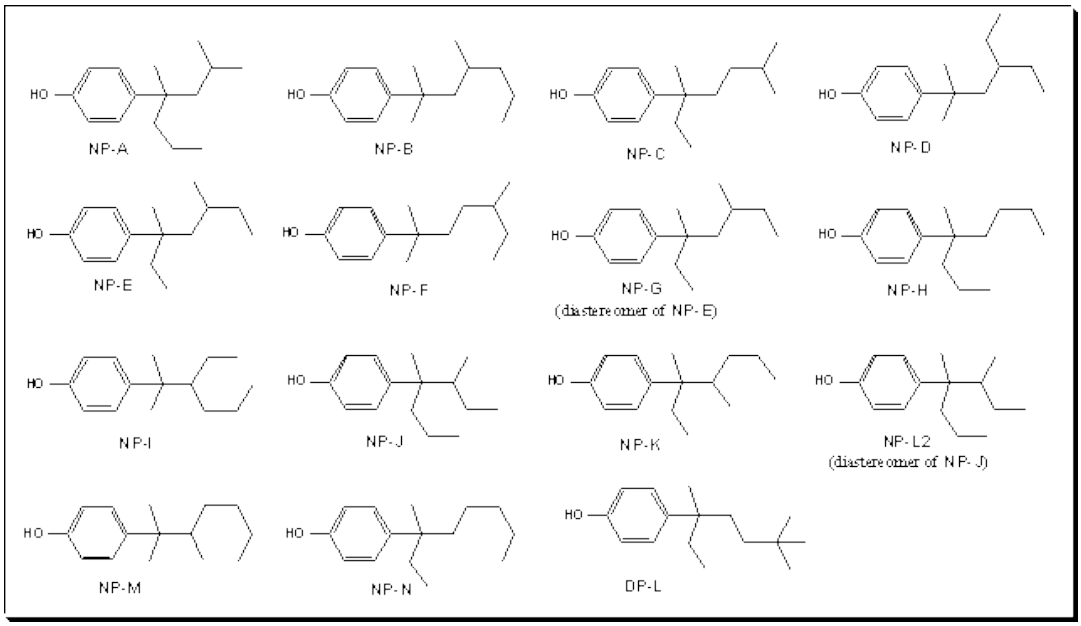

Fig. 1 Chemical structures of isolated nonylphenol isomers and decylphenol

Materials and Methods

Materials

The NP mixture (N0300, CAS No. 84852-15-3) was obtained from Tokyo Kasei Kogyo Co. For the synthetic method

A, *p*-hydroxyacetophenone, benzyl bromide, potassium carbonate, lithium and magnesium were obtained from Wako Pure Chemical Industries. 3-Bromohexane (90% purity by GC), 2-bromohexane (contain ca. 26% 3-bromohexane), 1-bromo-2-ethylbutane (95% purity by GC) and trimethylaluminum (15% in toluene) were obtained from Tokyo Kasei Kogyo Co. Titanium(IV) chloride (1.0 M solution in toluene) was obtained from Aldrich Chemical Co. 1-Bromo-3-methylpentane was prepared by the reaction of 3-methyl-1-pentanol (99% purity by GC, Tokyo Kasei Kogyo Co.) with triphenylphosphine (PPh_3)/*N*-bromosuccinimide (NBS). For the synthetic method B, phenol (99% purity by GC), boron trifluoride diethyl ether complex and petroleum ether were obtained from Wako Pure Chemical Industries. 3-Methyl-3-octanol (99% purity by GC) was obtained from Aldrich Chemical Co. 3,5-Dimethyl-3-heptanol (99% purity by GC) and 3,6-dimethyl-3-heptanol (99% purity by GC) were obtained from Avocado Research Chemicals. 3-Ethyl-2-methyl-2-hexanol was prepared by the reaction of 3-bromohexane with acetone in the presence of lithium under ultrasonication. For the biological assay, 17b-estradiol (E2) was purchased from Tokyo Kasei Kogyo Co.

Methods

1. Synthetic methods of NPs:

Method A (Fig. 2); *p*-Hydroxyacetophenone containing methyl group at a-position in the structure was chosen for reasonable starting material to synthesize the proposed NP isomers. Phenolic hydroxyl group of the starting material was protected with benzyl group using benzyl bromide and potassium carbonate in methanol. The protected compound was converted into corresponding tertiary alcohol with various C_7-alkyl bromide and lithium ("Barbier reaction") or magnesium under ultrasonication. Finally, treatment of tertiary alcohol with trimethylaluminum /titanium tetrachloride accompanied by concomitant deprotection of the benzyl group gave corresponding a,a-dimethyl NP isomers. Total yields were 1~30 % after purification with silica gel column chromatography.

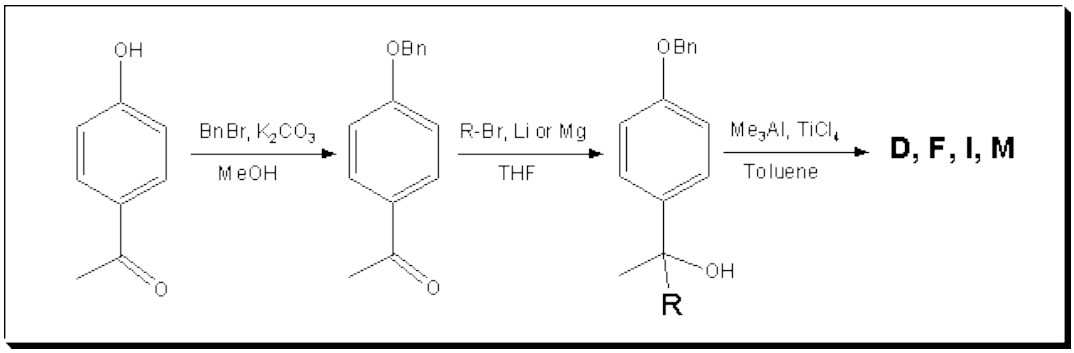

Fig. 2 Method A

Method B (Fig. 3); Vinken *et al*. reported that the reaction of phenol and tertiary nonylalcohol in the presence of BF_3-ether complex as a catalyst gave a corresponding NP isomer. [5] According to this manner (Friedel-Crafts alkylation), four NP isomers (**NP-C**, **NP-E**+**G**, **NP-I**, **NP-N**) were synthesized in 50~90 % yields after purification by silica gel column chromatography. All of the synthesized NPs gave satisfactory spectral data consistent with assigned structures.

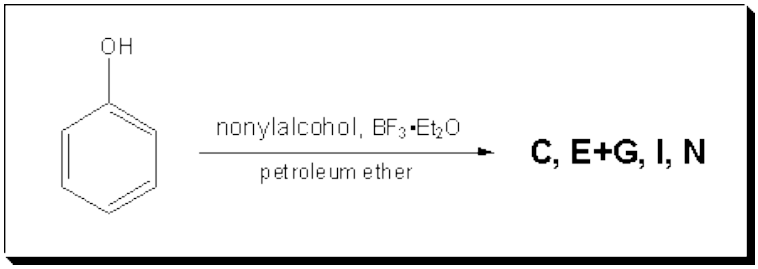

Fig. 3 Method B

2. Estrogenic activity:

Estrogenic activity of each synthetic NP isomer was tested by the recombinant yeast screen assay. The yeast was kindly supplied by Dr. Sumpter, Brunel University, UK. In this system, human estrogen receptor (hER) is expressed in a form capable of binding to estrogen-responsive sequence (ERE). The yeast cells also contain expression plasmids carrying the reporter gene, *lacZ*, which is regulated by the ERE. Activation of the receptor by binding of ligand causes expression of the reporter gene *lacZ*, which produces the enzyme b-galactosidase. The activity of the estrogen-inducible b-galactosidase was measured by the coloration of chlorophenyl-red-b-galactosylpyranoside (CPRG). Synthetic NP isomer was diluted with dimethylsulfoxide (DMSO) and added to the yeast culture media, which contained CPRG in wells of micro-titer plates. Plates were incubated for 4 days at 28˚C. Color development was measured at 540 and 620 nm and the difference in the measurements was taken to represent the activity of b-galactosidase, which correlated well with the estrogenicity of standard E2. The amounts of color development were plotted against the molar concentrations of sample to give a dose-response curve. From this curve, the minimal effective concentration was calculated from half of the maximum effect. Under conditions in the laboratory, the minimal effective concentrations varied by 20 to 50% depending on samples. Hence 4 to 6 independent experiments were carried out to calculate the mean value. The activities of the synthetic samples were determined by comparison of the minimal effective concentration of each sample relative to that of E2 (being included in all assay plates as the standard).

Results and Discussion

All of fourteen NP isomers and a decylphenol isolated from NP mixture possessed tertiary a-carbon having at least one methyl group in their chemical structures. The two sets of NPs (**NP-E** and **-G**, **NP-J** and **-L**) with the same plane structures were diastereomeric with each other. Furthermore, seven isomers (**NP-C**, **NP-E**+**G**, **NP-F**, **NP-G**, **NP-I**, **NP-M**, **NP-N**) of fourteen NPs were chemically synthesized by two synthetic methods. Routledge and Sumpter have shown that the structural difference of alkyl chain in NPs affected the estrogenic activity on the recombinant yeast screen assay. [6] Estrogenic activities of our synthesized NP isomers were shown in Fig. 4.

Two isomers (**NP-D** and **NP-I**) exhibited stronger activities (1/500~1/250 corresponding to that of E2) than the others and some isomers showed lower activities than the commercial NP mixture. These results indicated that the residual analysis of NPs in environment should be carried out in isomer specific manner. In spite of the low contents of **NP-D** and **NP-I**, the commercial NP mixture exhibited rather high estrogenicity. Therefore the mixture should contain some other highly estrogenic NP isomers. The isolation and structure elucidation of highly estrogenic NPs from the commercial NP mixture are now in progress.

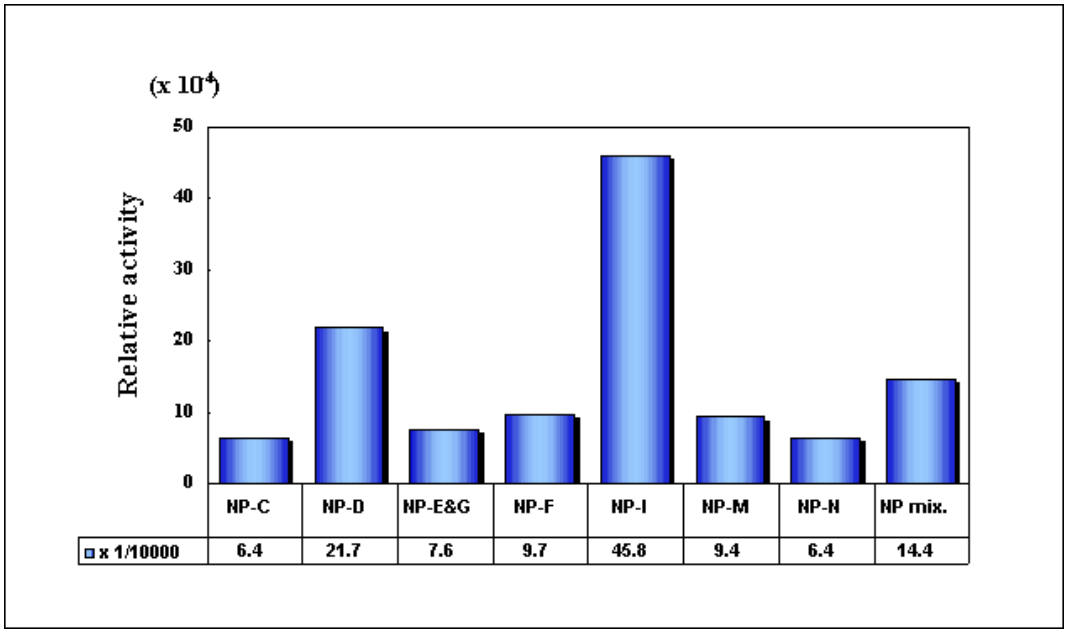

Fig. 4 Estrogenic activities of synthetic NPs relative to E2

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