OPTICAL RESOLUTION OF SYNTHETIC BRANCHED 4-NONYLPHENOL ISOMER AND ABSOLUTE STRUCTURE

Uchiyama T[1], Saito H[1], Makino M[2], Fujimoto Y[2], Katase T[3], Hashizume D[4]

[1]College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba, 274-8555, Japan; [2]College of Humanities and Sciences, Nihon University, 3-25-40 Sakurajosui, Setagaya, Tokyo, 156-8550, Japan; [3]College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa, 252-8510, Japan; [4]The Institute of Physical and Chemical Research (RIKEN) Hirosawa 2-1, Wako-shi, Saitama, 351-0198 Japan

Introduction

4-Nonylphenol (NP) is known as an environmental endocrine disruptor[1] and widely used in many industrial applications, as plastic additive, antioxidant and raw material of nonylphenol polyethoxylate (NPEO), which is a nonionic surface-active agent.[2,3,4] Commercial NP prepared by the alkylation of phenol with nonene isomers ("propylene trimer") is a mixture of more than 31 components.[5] Several articles have been published with descriptions of the mass spectral fragmentation of NP isomers to identify the isomeric structure.[5,6,7,8,9] For example, Wheeler *et al*. identified 22 NP isomers using a high-resolution mass spectrometry-gas chromatography with 100 m capillary column.[6] Ieda *et al*. recently reported analysis of nonylphenol isomers by comprehensive two-dimensional GC-MS.[8]

The estrogenic activity, *in vitro*, of commercial NP mixture was reported to be 10^{-6} times less than 17β-estradiol (E2) at the minimum to 2 x 10^{-3} times less at the maximum.[10,11] Routledge and Sumpter examined structure-activity relationship of alkylphenols and reported that the estrogenic activity of alkylphenols was dependent on the numbers of carbon atoms in the alkyl chain.[12] Although a few groups had reported the synthesis of NP isomers[13,14,15], the exact structure of estrogenic active NP isomer(s) in the commercial NP mixture were not identified yet. In our previous studies, we described preparative fractionation of a commercial NP mixture using high performance liquid chromatography (HPLC) to afford fourteen NP isomers[16] (structures were shown in Fig. 1) : 4-(2,4-dimethylheptan-4-yl)phenol (**NP-A**), 4-(2,4-dimethylheptan-2-yl)phenol (**NP-B**),

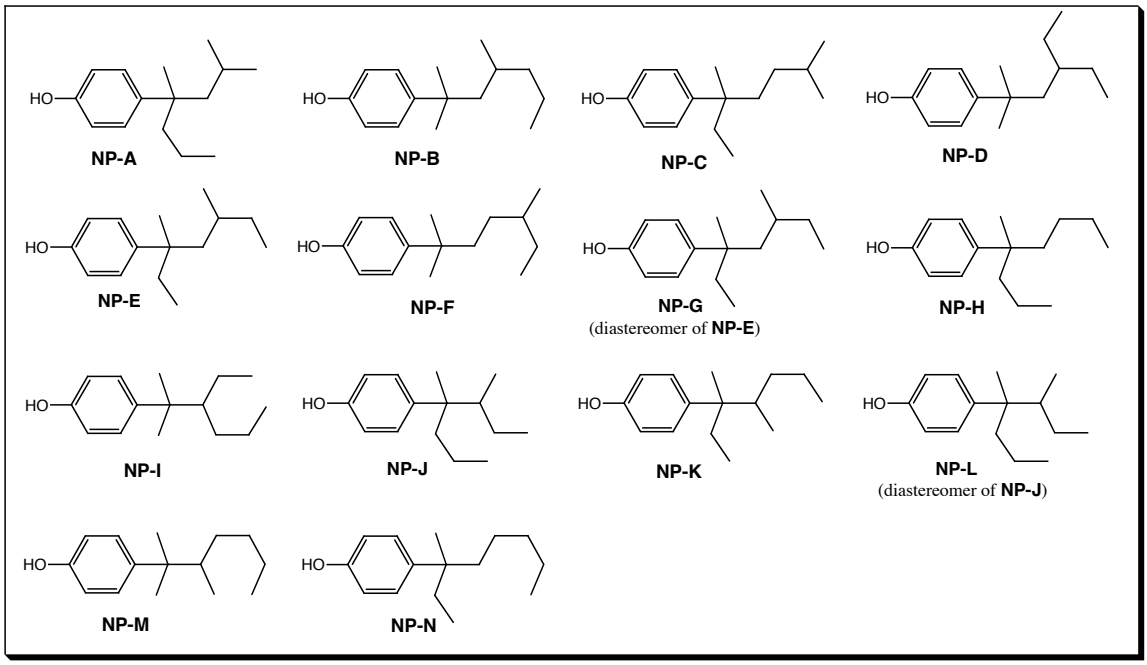
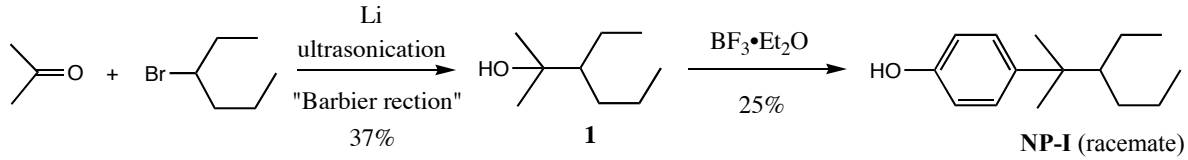


Fig. 1. Chemical structures of branched 4-nonylphenol isomers identified from a commercial NP mixture in our previous work.[16]

4-(3,6-dimethylheptan-3-yl)phenol (**NP-C**), 4-(4-ethyl-2-methylhexan-2-yl)phenol (**NP-D**), 4-(3,5-dimethyl-heptan-3-yl)phenol (**NP-E**; diastereomer of **NP-G**), 4-(2,5-dimethylheptan-2-yl)phenol (**NP-F**), 4-(3,5-dimethyl-heptan-3-yl)phenol (**NP-G**; diastereomer of **NP-E**), 4-(4-methyloctan-4-yl)phenol (**NP-H**), 4-(3-ethyl-2-methyl-hexan-2-yl)phenol (**NP-I**), 4-(3,4-dimethylheptan-4-yl)phenol (**NP-J**; diastereomer of **NP-L**), 4-(3,4-dimethyl-heptan-3-yl)phenol (**NP-K**), 4-(3,4-dimethylheptan-4-yl)phenol (**NP-L**; diastereomer of **NP-J**) 4-(2,3-dimethyl-heptan-2-yl)phenol (**NP-M**), 4-(3-methyloctan-3-yl)phenol (**NP-N**). All of fourteen NP isomers isolated from NP mixture possessed tertiary α-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 compounds with each other. In DIOXIN 2005 (Toronto), we presented the syntheses and estrogenic activities of seven branched NP isomers (**NP-C**, **NP-D**, **NP-E(G)**, **NP-F**, **NP-I**, **NP-M**, **NP-N**) existed in the commercial NP mixture.[17] **NP-I** (racenate), one of synthetic NP isomer, showed strong activity than the others. We report herein optical resolution of racemic **NP-I** using HPLC and elucidation of its absolute structure to clear the optical isomeric effect for estrogenic activity.

Materials and Methods

For the synthesis of **NP-I** (Scheme 1), phenol (99% purity by GC), boron trifluoride (BF_3) diethyl ether complex and petroleum ether were obtained from Wako Pure Chemical Industries. Commercially non-available nonylalcohols, 3-ethyl-2-methyl-2-hexanol (**1**), was prepared by the reaction of anhydrous acetone (Wako Pure Chemical Industries) with 3-bromohexane (Tokyo Kasei Kogyo Co.) and Li in anhydrous tetrahydrofuran (THF, Wako Pure Chemical Industries) under ultrasonication (Barbier reaction). Friedel-Crafts alkylation of phenol with tertiary nonylalcohol (**1**) in the presence of BF_3-ether complex as a catalyst gave **NP-I**.



Scheme 1. Synthesis of racemic **NP-I**

*Preparation of 3-ethyl-2-methyl-2-hexanol (**1**)*

To a suspension of Li (134 mg, 19.1 mmol) in THF (10 ml), anhydrous acetone (404 μl, 5.50 mmol) and 3-bromohexane (1.152 ml, 8.16 mmol) in THF (10 ml) were added under argon atmosphere. The reaction mixture was ultrasonicated for 30 min. at 0~5˚C. Saturated NH_4Cl solution (10 ml) was added to the reaction mixture, and extracted with ethyl acetate (40 ml). The organic layer was washed successively with water (20 ml x 3) and brine (20 ml), and dried with Na_2SO_4. After filtration, the filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 9 : 1) to afford tertiary alcohol (**1**) (290 mg, 37%).

*Synthesis of **NP-I** (racemate)*

To a solution of **1** (203 mg, 1.41 mmol) and phenol (550 mg, 5.80 mol) in petroleum ether (100 ml), BF_3 diethyl ether complex (250 μl, 1.45 mmol) was added at room temperature under argon atmosphere. The reaction mixture was stirred at room temperature over night, and then crushed ice and water (100 ml) were added. The organic layer was washed several times with water (100 ml) to remove excess phenol, and dried over Na_2SO_4. After filtration, the filtrate was concentrated *in vacuo* and residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 15 : 1) to give **NP-I** (78 mg, 25%).

*Optical resolution of **NP-I***

We ran the HPLC (pump; SHIMADZU LC-10AD, UV detector; SHIMADZU SPD-10A, column; DAICEL CHEMICAL INDUSTRIES, LTD. CHIRALCEL OJ-H. length 250 mm x i.d. 10 mm, flow rate; 3 ml/min.) eluted with *n*-hexane-*iso*-propyl alcohol (9:1) to give chiral **NP-I-1** (retention time: 7.9 min.) and **NP-I-2** (retention time: 10.1 min.) for preparative scale.

Results and Discussion

Optical resolution of racemic **NP-I** (4-(3*SR*-ethyl-2-methylhexan-2-yl)phenol) was achieved by HPLC equipped with a chiral column (CHIRACEL OJ-H) to give **NP-I-1** and **NP-I-2**. (Fig. 2) CHIRALPAK AS-H, AD-H, CHIRALCEL OD-H were ineffective in optical resolution of **NP-I** (data were not shown). After the optical resolution of racemic **NP-I**, faster eluted **NP-I-1** was 2-bromobenzoylated and recrystallized from isopropyl alcohol - H_2O. The absolute configuration of **NP-I-1** was proved to be 2*R* (Fig. 2, Fig. 3) by X-ray crystallo- graphic analysis of the benzoate, threfore **NP-I-2** was 2*S*. The evaluation of estrogenic activity for both the chiral **NP-I**s and synthesis of the other NP isomers are now in progress.

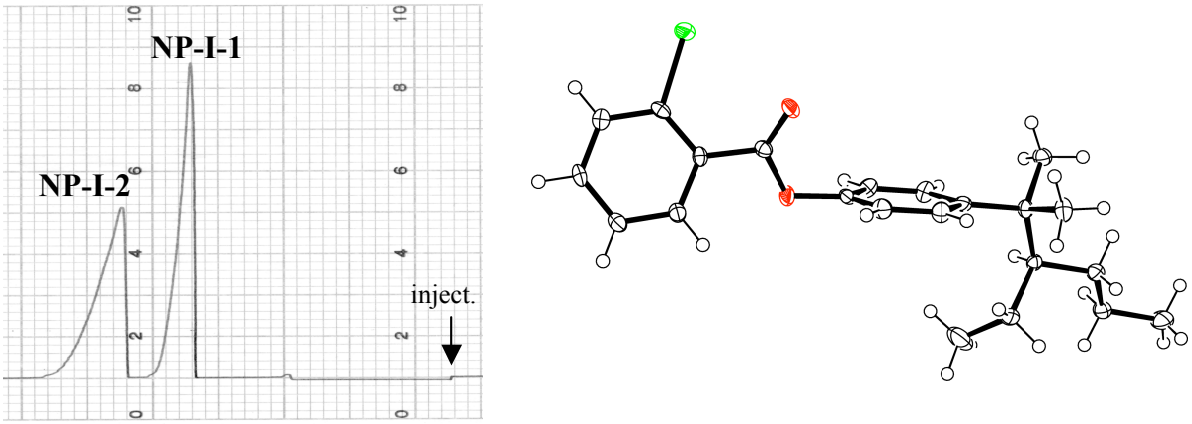


Fig. 2. HPLC analysis of synthesized racemic **NP-I** with CHIRALCEL OJ-H and X-ray crystal structure of 2-bromobenzoylated **NP-I-1**.

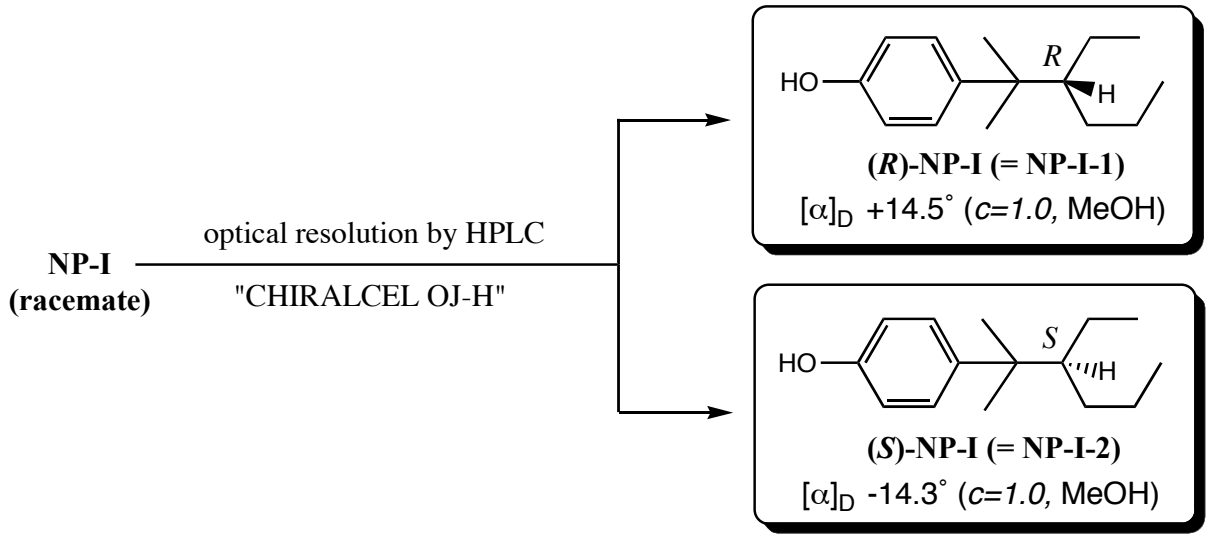


Fig. 3. Chemical structures of **(*R*)-NP-I** and **(*S*)-NP-I**

Acknowledgements

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