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Research Article

Synthesis of tertiary ^{14}C-labelled nonylphenol isomers

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Summary

The ring-^{14}C-labelled *p*-nonylphenol (NP) isomers 4(3′,5′-dimethyl-3′-heptyl)-phenol (*p*353NP), 4(3′,6′-dimethyl-3′-heptyl)-phenol (*p*363NP) and 4(2′,6′-dimethyl-2′-heptyl)-phenol (*p*262NP) were synthesized for application in metabolism and sorption studies. Friedel–Crafts alkylation of ^{14}C-labelled phenol and the corresponding tertiary nonylalcohol with BF_3 as catalyst was used. After clean-up of *p*262NP and *p*363NP by preparative thin-layer chromatography radiochemical yields amounted to 62.8 and 64.6%, specific radioactivities were 332 and 88.2 MBq/mmol, and radiochemical purities 97.6 and 99.0%. For both isomers, a large-scale synthesis with non-labelled phenol was additionally developed, which led to pure products (96 and 99%, respectively) without further purification steps. In the case of *p*353NP, which was formed as a diastereomeric mixture, the crude synthetic product had a radiochemical purity of 96.9% (radiochemical yield: 76.0%; specific activity: 298 MBq/mmol); thus, purification was not necessary. All products were characterized by means of gas chromatography-mass spectroscopy, ^{1}H- and ^{13}C-NMR, as well as IR. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: ^{14}C-labelling; synthesis; Friedel–Crafts alkylation; branched nonylphenol isomers

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Introduction

The technical nonylphenol (NP) consists of a mixture of more than 20 branched *p*-alkylated isomers in which the tertiary branching in the alpha-position of the nonyl chain dominates (85%).[1,2] It is a well known and important intermediate in the production of many commercial and industrial materials, e.g. detergents, polymers, pesticide formulations, water-based paints, cosmetics and antioxidants.[2,3] Above all, the majority of NP is used for the production of NP polyethoxylates. These nonionic surfactants are widely utilized for industrial applications, such as the pulp, paper, and textile industries, as well as for the production of household and industrial detergents.[3,4] The global annual consumption amounts to more than 600 000 t.[5,6]

After its use, the NP polyethoxylates can arrive at sewage treatment plants via wastewater. In both wastewater and sewage treatment plants, NP polyethoxylates are microbially degraded to form NP and its derivatives.[6,7] The persistence of branched NP isomers is dependent on their degree of branching. In general, highly branched isomers are rather stable with only low biodegradation rates.[8,9] Concentrations of 2–4000μg/l of NP have been determined in treated wastewaters.[5,10] As a consequence, NP is known as an ubiquitous pollutant in urban aquatic environments, where concentrations are in the μg/l range.[11,12]

Acute adverse toxic effects of NP have been reported concerning invertebrates, fish, mammals, and algae.[6,13] Recent studies demonstrated that NP can accumulate in aquatic organisms due to its lipophilic properties.[14,15] Additionally, there is growing evidence resulting from both field and laboratory experiments that NP exerts estrogenic activity.[12,16] Furthermore, reports on the increasing incidence of breast, prostate and testicular cancer, besides decreasing sperm counts and semen volume as affected by NP, calls for an increasing appreciation of endocrine disruption by environmental chemicals in general.[17,18]

However, information on the microbial degradation and on the environmental fate of NP is incomplete. Many questions remain to be answered regarding the effective treatment of NP-contaminated wastewater in municipal and industrial wastewater treatment plants. Therefore, during the last few years, the endocrine disrupting chemical NP has been the subject of increasing environmental fate and toxicology investigations. For further detailed studies on the binding, metabolism

and sorption of NP, well-defined branched NP isomers are a necessary prerequisite. By synthesizing various isomers of NP, we aim at a thorough evaluation of its ecochemical and ecotoxicological behaviour in the environment as affected by the degree of side-chain branching.

To accelerate these processes, we have developed an easy and effective micro-scale synthesis of defined tertiary ^{14}C-labelled *p*-NP isomers.

Results and discussion

Three different ^{14}C-*p*-NP isomers were synthesized from ^{14}C(U)-labelled phenol and the corresponding nonylalcohols. Before the ^{14}C-labelling experiments, the influence of different synthesis parameters on product yield was investigated using non-labelled phenol. These parameters included molar ratios of the reaction substrates, the reaction time, and the temperature. For the individual nonylalcohols, optimization led to different reaction conditions shown in Table 1. To obtain the respective products in highest yields and purities, it was necessary to carry out all micro-scale reactions at a constant temperature of 50°C. This deviation from published procedures performed under cooling[19,20] was probably due to the low concentrations of reactants used.

Table 1 also shows the resulting yields and purities of the crude synthetic products obtained from the ^{14}C-labelling experiments. For each *p*-NP, the corresponding dialkylated phenol was the main side product, followed by *o*-NP. In the case of *p*262NP, the gas chromatography-mass spectroscopy (GC–MS) analysis indicated the presence of some aliphatic components as further impurities. Isomer *p*353NP, which was formed as a diastereomeric mixture (1:1), exhibited a purity of about 97%, and thus, no further purification step was necessary. The radiochemical purities and further data obtained from GC–MS after preparative thin-layer chromatography (TLC) are displayed in Table 1. Total yields of the *p*-NPs were between 60 and 70%. The specific radioactivities of the products resulted from the amounts of ^{14}C-labelled phenol introduced into the reactions (Table 1), and ranged from 90 to 330 MBq/mmol. For identification and characterization of the reaction products, GC–MS was used for both the ^{14}C-labelled and non-labelled *p*-NPs. In addition, IR, ^{1}H-NMR and ^{13}C-NMR spectra were recorded to identify the non-labelled products.

Table 1. Syntheses of three ring-^{14}C-labelled *p*-nonylphenol isomers. *M* (*X* : *Y*) = molar ratio of *X* : *Y*; radiochem. = radio-TLC analysis (*p*-NP: R_f = 0.49); chem. = GC–MS analysis; *xyz*OH = *x*,*y*-dimethyl-*z*-heptanol; Di-NP = *o*,*p*-dinonylphenol

Isomer (alcohol/ phenol)	Total activity of phenol (MBq)	*M* (alcohol: phenol)	*M* (catalyst: phenol)	Yield of *p*-NP isomer in product; radiochem./ chem. (%)	Content of *p*-NP isomer in product; radiochem./ chem. (%)	Side products; chem. (%)	Specific activity (MBq/mmol)
Crude products							
353OH *p*353NP	25.74	1.8 : 1	11 : 1	76.0/60.8	96.9/96.9	Di-NP (2.5) *o*-NP (0.5)	298
363OH *p*363NP	9.316	1 : 1	4.4 : 1	73.6/70.7	93.8/92.0	Di-NP (6.1) *o*-NP (1.9)	—
262OH *p*262NP	34.34	1 : 1	2.2 : 1	71.1/66.6	79.4/77.7	Di-NP (16.3) *o*-NP (4.7) aliphatics (1.4)	—
Purified							
*P*363NP	—	—	—	64.6/62.5	99.0/100.0	—	88.2
*P*262NP	—	—	—	62.8/56.2	97.6/98.8	*o*-NP (1.2)	332

The mass spectra of the *p*-NP isomers, *m/z* (relative intensity) details are as follows:

p353NP; 1st *diastereomer*: 220 (6, $M^{+\bullet}$), 191 (13), 163 (1), 150 (11), 149 (1 0 0), 135 (11), 122 (7), 121 (81), 107 (59), 105 (3), 93 (2), 91 (8), 77 (9), 55 (13).

p353NP; 2nd *diastereomer*: 220 (6, $M^{+\bullet}$), 191 (14), 163 (1), 150 (11), 149 (1 0 0), 135 (10), 122 (7), 121 (84), 107 (58), 105 (3), 93 (2), 91 (8), 77 (9), 55 (13).

p363NP: 220 (6, $M^{+\bullet}$), 205 (1), 191 (24), 150 (11), 149 (1 0 0), 135 (10), 121 (60), 107 (88), 97 (4), 91 (7), 77 (9), 55 (22).

p262NP: 220 (3, $M^{+\bullet}$), 205 (1), 191 (1), 149 (1), 136 (10), 135 (1 0 0), 121 (5), 107 (15), 95 (4), 91 (3), 77 (4).

The main MS-signals of the individual *p*-NP isomers agree with those reported in the literature.[1]

The infrared spectrum of all the synthesized *p*-NP isomers showed strong and very broad peaks between 3100 and 3600 cm^{-1} (O–H), and very strong absorption bands for the C–H valence vibrations of the aliphatic CH_3-, CH_2-, and CH-groups between 2850 and 3000 cm^{-1}. The aromatic rings were unequivocally identified by their typical three C=C-valence vibration signals between 1513 and 1612 cm^{-1}. In addition, the following signals in the fingerprint region were detected (cm^{-1}; s = strong (< 30% Transmittance), m = medium (30–70% T), w = weak (> 70% T)):

p353NP: 1460s, 1379s, 1292w, 1240s, 1182s, 1115w, 1049w, 1013 m, 965w, 937w, 884w, 829s, 783w, 751w, 729w, 663w, 591 m, 546w, 523w.

p363NP: 1465 m, 1381 m, 1335 m, 1295w, 1242 m, 1181 m, 1115w, 1013w, 929w, 828s, 782w, 756w, 727w, 662w, 586w, 562w, 522w, 501w.

p262NP: 1466 m, 1442 m, 1384 m, 1366 m, 1295w, 1242s, 1180 m, 1116 m, 1015w, 919w, 831s, 738w, 667w, 568 m, 509w.

The ^{1}H-NMR chemical shifts recorded for the *p*-NP isomers are in ppm relative to the TMS at 400 MHz (for positions of atoms see Figure 1):

p353NP (diastereomers): 0.49, 0.76 (each *d*, $J = 6.6$, 3 H-(CH_3 at C5′)); 0.62, 0.78 (each *t*, $J = 7.4$, 3 H–C7′); 0.66 (*t*, $J = 7.4$, 3 H–C1′); 0.87–0.95, 1.00–1.12, 1.15–1.35, 1.43–1.54, 1.64–1.76 (each br. m, 10 H–C(2′, 4′–6′, CH_3 at C3′)); 5.61 (s, OH); 6.76 (*d*, $J = 8.8$, 2 H–C(2, 6)); 7.13 (*d*, $J = 8.8$, 2 H–C(3, 5)).

p363NP: 0.65 (*t*, $J = 7.4$, 3 H–C1′); 0.80 (*t*, $J = 6.5$, 6 H–C(7′, 7″)); 0.78–0.88, 0.95–1.05 (each br. m, 2 H–C5′); 1.20 (m, 3 H–(CH_3 at C3′));

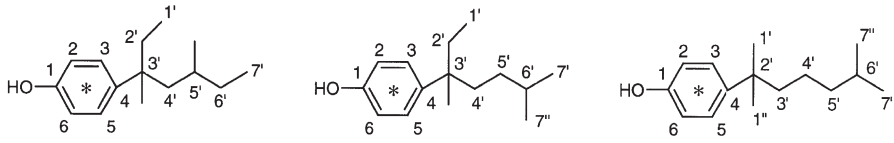


Figure 1. Chemical structures of the three synthesized ^{14}C(U)-ring-labelled *p*-NP isomers. From left to right: 4(3′,5′-dimethyl-3′-heptyl)-phenol (*p*353NP), 4(3′,6′-dimethyl-3′-heptyl)-phenol (*p*363NP) and 4(2′,6′-dimethyl-2′-heptyl)-phenol (*p*262NP). * = position of ^{14}C-label

1.25–1.71 (br. m, 5 H–C(2′, 4′, 6′)); 4.81 (s, OH); 6.76 (*d, J* = 8.8, 2 H–C(2, 6)); 7.13 (*d, J* = 8.8, 2 H–C(3, 5)).

*p*262*NP*: 0.80 (*d, J* = 6.6, 6 H–C(7′, 7″)); 1.00–1.11 (br. m, 4 H–C(4′, 5′)); 1.25 (s, 6 H–C(1′, 1″)); 1.37–1.54 (br. m, 3 H–C(3′, 6′)); 5.13 (s, OH); 6.76 (*d, J* = 9.0, 2 H–C(2, 6)); 7.18 (*d, J* = 8.8, 2 H–C(3, 5)).

The ^{13}C-NMR chemical shifts of the *p*-NP isomers are in ppm relative to the TMS at 100 MHz (for positions of atoms see Figure 1):

*p*353*NP* (*diastereomer*): 8.542, 8.603 (C1′); 11.212, 11.402 (C7′); 21.234, 21.689 (CH_3 at C5′); 22.918, 23.548 (CH_3 at C3′); 30.527, 30.747 (C5′); 31.293, 31.801 (C6′); 36.019, 36.467 (C2′); 40.624, 40.814 (C3′); 50.661, 50.714 (C4′); 114.498 (C2, C6); 127.653, 127.683 (C3, C5); 140.018, 140.041 (C4); 152.604 (C1).

*p*363*NP*: 9.021 (C1′); 23.010, 23.025 (C7′, C7″, diastereotopes); 23.943 (CH_3 at C3′); 29.026 (C6′); 33.562 (C5′); 36.066 (C2′); 40.610 (C3′); 40.966 (C4′); 114.909 (C2, C6); 127.866 (C3, C5); 140.528 (C4); 152.984 (C1).

*p*262*NP*: 22.813 (C4′); 23.025 (C7′, C7″); 28.130 (C6′); 29.519 (C1′, C1″); 37.461 (C2′); 40.018 (C5′); 45.230 (C3′); 115.023 (C2, C6); 127.214 (C3, C5); 142.454 (C4); 153.037 (C1).

On the whole, the chemical structures of the three *p*-NP isomers synthesized could be confirmed by their MS, IR, ^{1}H- and ^{13}C-NMR spectra.

By comparison with the other published methods,[15,21,22] the advantages of the presented synthesis are the one-step preparation of the *p*-NP isomers, the micro-scale applicability (reactants about 10 mg), and the high yields and purities of the products. Ekelund *et al.*[15] synthesized a ^{14}C-labelled mixture of NPs corresponding to technical NP, from phenol and the commercially available nonene mixture utilized for the industrial production of NP. They introduced about 300 mg of phenol into the reaction and obtained a yield of 46%.

Lalah *et al.*[22] synthesized *p*363NP in a two-step reaction with 3-bromo-3,6-dimethyl heptane and 90 mg of ^{14}C(U)-ringlabelled anisole as starting material and $AlCl_3$ as catalyst. After Friedel–Crafts coupling of the reactants, a 98% yield of the intermediate para product was obtained. Deprotection of the methoxy function, however, also led to splitting of the phenol-alkyl bond. Correspondingly, the final yield after preparative TLC purification was only 24% (purity >95%). Meldahl *et al.*[21] using ^{14}C(U)-labelled phenol and 1-nonene obtained a mixture of 4(2′-nonyl)-phenol, 4(3′-nonyl)-phenol and 4(4′-nonyl)-phenol as main products. The difficulty of synthesizing a single 4-*n*-NP isomer was traced back to the relative instability of the primary nonyl carbocation.

Wheeler *et al.*[1] examined in detail the technical mixture of NP by GC-MS. Besides five secondary, 17 tertiary *p*-NP isomers were identified, amounting to 84.5% of the technical product. These 17 isomers were organized into three isomer-type groups related to their alpha- and beta-branching. The three isomers synthesized in our laboratory belong to two of these groups of tertiary *p*-NP isomers. Thus, we will examine the behaviour of NP in degradation, ecotoxicological, and sorption studies using three different single, defined and representative constituents of the technical mixture.

Experimental

Materials

The ^{14}C(U)-labelled phenol (73 mCi/mmol, radiochemical purity 99.7% by HPLC) in petroleum ether was supplied by Hartmann Analytic (Braunschweig, Germany). Unlabelled phenol with a purity of 99.5% was supplied by Fluka (Buchs, Switzerland). 3,6-Dimethyl-3-heptanol and 3,5-dimethyl-3-heptanol were obtained from Avocado (Heysham, UK), and 2,6-dimethyl-2-heptanol from Acros (New Jersey, USA), all with 99% purity. Boron trifluoride was used as the ether complex (for synthesis; Merck, Hohenbrunn, Germany). Anhydrous sodium sulphate with a purity of 99% was supplied by Acros and potassium hydroxide in technical grade by Merck. Petroleum ether, boiling range from 60 to 95°C, was received from Acros. For the micro-scale syntheses, stock solutions of unlabelled phenol and the respective alcohol were prepared in petroleum ether. The petroleum ether and all stock solutions were dried over molecular sieve 4 A (Merck) prior to use.

Analytical methods

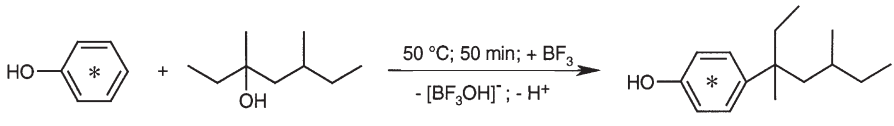
The preparative TLC was performed on silica gel plates (SIL G-200; Macherey-Nagel, Düren, Germany) and developed using toluene-methanol, 9:1 (v/v). For analytical TLC, silica gel 60 plates (Sil G-25 UV254, 0.25 mm; Macherey–Nagel) were used and developed in the same solvent system. Radioactive TLC spots were detected by means of a Fujifilm Bio-imaging Analyzer BAS-1000 (Fuji Photo Film, Tokyo, Japan), while the non-labelled compounds were visualized under UV light (254 nm wavelength). ^{13}C-and ^{1}H-solution state NMR results were recorded in $CDCl_3$ on a Varian Inova-400 spectrometer (Varian Inc., Palo Alto, USA) at 100 and 400 MHz, respectively; chemical shifts are given in ppm relative to the internal standard tetramethylsilane (TMS), coupling constants J in Hz. The IR spectra in cm^{-1} were recorded on a Perkin–Elmer PE-1760 FT (Perkin–Elmer Life Sciences, Boston, USA). The GC–MS studies were carried out on an HP 5890 Series II gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a FS-SE-54-NB-0.5 column (25 m × 0.25 mm, 0.46 μm film thickness; CS Chromatographie Service, Langerwehe, Germany), coupled to an HP 5971A mass selective detector (Agilent Technologies). The mass selective detector (EI) was operated in the scan mode (mass range m/z 50–600) with an electron energy of 70 eV. The temperature program was 50°C for 5 min, 10°C per minute to 280°C, then 280°C for 5 min. The injector temperature was 250°C and the interface temperature 280°C. The injection volume was 1 μl (splitless injection). The carrier gas was helium (1 ml/min). The IR, NMR and MS measurements were performed with non-labelled reference compounds. Liquid scintillation counting (LSC) was performed by means of a Beckman LS-5000 TD (Beckman) using the cocktail Lumasafe (Lumac LSC, Groningen, The Netherlands).

Large-scale synthesis

For spectroscopic and chromatographic characterization of the reaction products, a large-scale synthesis with non-labelled reactants was carried out. The aim was to produce a pure product with no need for further purification. For *p*353NP, the conditions for highest yield, as optimized for the radioactive synthesis, also led to a product with high purity (about 97%). In contrast, optimum conditions for highest purity had to be determined individually for the two other isomers.

According to Friedel–Crafts alkylations in general,[19,20] the reaction for *p*363NP and *p*262NP was carried out under a four-fold molar excess of phenol by comparison with the corresponding nonylalcohol. In contrast to literature data, the reaction temperature was set to 50°C. The most important condition for obtaining pure products were low concentrations of the reactants.

Experimentally 0.55 g of non-labelled phenol, 0.25 ml (*p*363NP and *p*262NP), respective 1.0 ml (*p*353NP) of the corresponding nonylalcohol, 0.25 ml (*p*363NP), 1.0 ml (*p*262NP), or 4 ml (*p*353NP) of BF_3-ether complex and 100 ml of petroleum ether were placed in a 250 ml two-necked flask equipped with a reflux condenser and a drying tube (potassium hydroxide). The reaction (Scheme 1) was allowed to run for 15 min with stirring. After stopping the reaction, the mixture was worked up as described below. The resulting residue contained the desired NP isomers; *p*363NP: 73 mg (99% purity), *p*262NP: 283 mg (96% purity), and *p*353NP: 385 mg (97% purity). The products were characterized by means of TLC, GC-MS, IR, and NMR.



Scheme 1. Reaction of ^{14}C(U)-labelled phenol with the tertiary nonylalcohol 3, 5-dimethyl-3-heptanol. * = position of ^{14}C-label

Micro-scale synthesis

All micro-scale reactions were carried out using flame-dried glassware under a positive pressure of dry N_2. A mixture of labelled and non-labelled phenol (10 mg; specific activities see Table 1), the respective nonylalcohol, BF_3-ether complex (for details see Table 1), and petroleum ether (total reaction volumes 1–1.5 ml) were placed in a 25 ml two-necked reaction flask connected to a reflux condenser and a drying tube (potassium hydroxide). The reaction (Scheme 1) was allowed to run for 50 min at 50°C, and then stopped by adding 3 ml of water. After intensive stirring for 15 min, 3 ml of petroleum ether was added, and the petroleum ether phase was removed with a Pasteur pipette. The organic phase was washed seven times with 3 ml of distilled

water to remove the non-reacted phenol, and dried over sodium sulphate. Subsequently, the petroleum ether was removed under vacuum and the mass of the remaining product was determined. The crude product was redissolved in a small volume of methanol (concentration of about 5 μg/μl). The product was analysed by TLC and GC–MS (Table 1).

The crude products of the reactions to *p*363NP and *p*262NP were purified by preparative TLC. Aliquots of the crude product solutions in methanol were transferred manually to preparative silica gel plates. After development, ^{14}C zones were located using the Bio-Imager. The respective zones were scrapped from the plate and extracted extensively with chloroform. Then the solvent was removed under vacuum and the mass of the resulting product was determined. Radiochemical and chemical purities of the product were determined by TLC and GC–MS, respectively (Table 1).

For complete balance of radioactivity (Table 1), the amounts of ^{14}C recovered in potassium hydroxide (contained in the drying tube), water phases resulting from wash steps, solvents removed under vacuum and in the crude product were determined.

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