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Research Article

Synthesis of ^{13}C- and ^{14}C-labelled catechol

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Summary
^{13}C- and ^{14}C-uniformly labelled catechol was synthesized from phenol in three steps. Phenol was derivatized with 2-chloro-5-nitrobenzophenone in THF containing NaH, followed by *ortho*-hydroxylation with 35% aqueous H_2O_2 in sulphuric acid/glacial acetic acid solution, and by cleavage with piperidine, giving an overall 80% yield with respect to phenol. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: preparation; U-^{13}C-catechol; U-^{14}C-catechol; isotope labelling

Introduction

Catechol and catechol derivatives are ubiquitous in the environment, as metabolic intermediates in the microbial degradation of many naturally occurring and anthropogenic aromatic substances.[1–3] Though being a constituent unit of many plant polyphenols, however, catechol is generally not found in free form in nature.[4] As one of the precursors of soil humic substances, catechol is preferentially used for the synthesis of model humic substances, especially when radioactive labelling is involved.[5] Experiments with ^{14}C-labelled catechol have shown that catechol is to a great extent immobilized in soil and incorporated into soil humic substances.[3,4] However, it is still not known how catechol is stabilized and in what chemical form. While ^{14}C-labelled substances

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serve well for studying the rate and extent of transformation and stabilization, stable isotope-labelled substances are a prerequisite to elucidate the stabilization mechanisms.[6] Commercial ^{13}C-labelled chemicals are highly expensive and the unavailability of ^{13}C-catechol is a big obstacle in elucidating the stabilization mechanisms. To facilitate such studies, a convenient and effective preparation of isotopically labelled catechol is very important.

Catechol is generally synthesized from phenol by hydroxylation with H_2O_2, mostly in the presence of a catalyst, e.g. titanosilicates,[7,8] by oxidation of phenol with O_2,[9] or by light irradiation.[10] These one step syntheses not only convert phenol incompletely but also produce a mixture of dihydroxy compounds with very low *ortho*-stereoselectivity. Chew and Heys[11] used a three-step pathway to prepare ^{14}C- and ^{13}C-labelled catechol from phenol, which gave ^{14}C- and ^{13}C-catechol in 3.1 and 7% overall yield, respectively. Haider[12] used a five-step preparation for ^{14}C-catechol, with a higher yield (44%). Loudon and Scott[13] described a pathway for *ortho*-hydroxylation of phenols with almost quantitative stereoselectivity and Kratzl and Vierhapper[14] modified the first derivatizing step for the preparation of ^{14}C-guaiacol in 70% yield.

In this study, we modified and improved the method of Loudon and Scott,[13] and prepared ^{14}C- and ^{13}C-labelled catechol from phenol in good yield.

Results and discussion

The preparation of catechol consisted of three steps (Figure 1): Phenol was firstly derivatized to 2-phenoxy-5-nitro-benzophenone (**2**) with 2-chloro-5-nitrobenzophenone in tetrahydrofuran (THF) under reflux, followed by quantitative hydroxylation in the *ortho*-position to 2-(2-hydroxyphenoxy)-5-nitrobenzophenone (**3**) with H_2O_2 after dissolution

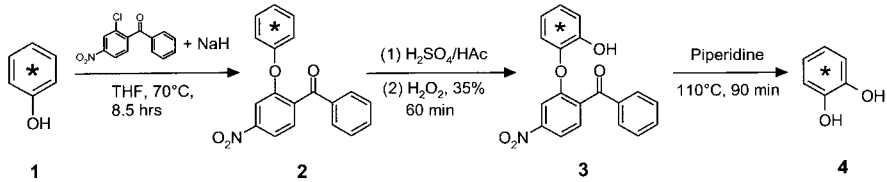

Figure 1. Scheme for the synthesis of catechol

in H_2SO_4. Catechol (**4**) was finally obtained by cleavage of the derivatizing group with piperidine under reflux in ca. 80% yield with respect to phenol (**1**). Table 1 summarizes the yields for the various synthetic steps, together with the yields and the recoveries of radioactivity in the case of U-^{14}C-catechol synthesis, showing that the reactions in steps 1 and 2 could be carried out almost quantitatively. Because the intermediates and the product have strong fluorescent properties, all synthetic reactions are easily monitored by thin layer chromatography (TLC).

The derivatization of phenol to **2** can be carried out in THF in 8.5 h or overnight, or instead in *N*,*N*-dimethylformamide (DMF) under reflux (110°C) for 30 min. Oily brown substances were formed in the reaction mixture in the case of the DMF-reflux method, probably reaction products of some mineral oil, which is commercially used for the dispersion of NaH. These contaminants had to be removed before sublimation of catechol (**4**) in step 3, e.g. by using column chromatography on silica gel at the expense of the yield (ca. 10%). When using THF as solvent for phenol derivatization, a purification procedure for the intermediate products (**2**) and (**3**) was not necessary.

It is important for the yield of step 2 that **2** is carefully dried and completely dissolved in H_2SO_4 before oxidation with H_2O_2. After oxidation, the product (**3**) becomes sticky, if it is not immediately separated from the reaction mixture by precipitation with H_2O.

In the case of step 3, the amount of piperidine should not be too high, usually about 0.8–1.5 ml for 100 mg of **3**, because at the high reaction temperature piperidine forms dark brown substances, which contaminated further the crude catechol (**4**). Pure catechol could be obtained either by column chromatography or by sublimation *in vacuo*. Using sublimation, we obtained white crystals of catechol in 99.7% purity as analysed by high-performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC-MS). Purification by TLC should be conducted under an O_2-free atmosphere.

Table 1. Recovery and yields for the synthetic steps

Synthetic step	^{14}C recovery (%)	Yield (%)	
		^{14}C	Mass
1–2	97.6	94.3	102
2–3	96.7	96.6	96.1
3–4	100	88.2	82.3
Total	94.4	80.3	80.7

Because there are no data of nuclear magnetic resonance (NMR) spectroscopy and MS for **2** and **3** found in literature, non-labelled **2** and **3** (purified by recrystallization in acetic acid and methanol, respectively) were analysed by NMR and GC-MS to facilitate the control of the syntheses (data see section Experiment). The results of MS analysis of ^{13}C-labelled catechol are similar to natural catechol in the mass distribution pattern (data not shown). ^{13}C-NMR of ^{13}C-labelled catechol showed that the signals for 2- and 4-C of the catechol ring are highly irregular multi-peaks in a broad range of 116.9–114.1 ppm, while the signals for the other C atoms are relatively orderly multi-peaks (data not shown). HPLC and TLC results showed that the synthetic ^{14}C-catechol was 99.7% radiochemically pure.

Experimental

TLC was performed on silica gel 60 with fluorescence indicator (Sil G-25 UV254, 0.25 mm; Macherey-Nagel, Düren, Germany) and viewed under UV light (254 nm). Preparative column chromatography was conducted with silica gel 60 (particle diameter 0.064–0.200 mm, Merck). For autoradiography of the TLC plate, a bioimaging analyzer (Fujifilm BAS-1000; Tokyo, Japan) was used. Quantitative determination of radioactivity was performed with a liquid scintillation counter LS-5000 TD (Beckman Instrument; CA, USA) using the cocktail Lumasafe Plus (Lumac LSC; Groningen, The Netherlands).

NMR was performed on an AC-300 (Bruker; Rheinstetten, Germany) equipped with a 10 mm type broad-band probe head or on a DPX-300 (Brucker) equipped with a 5 mm QNP probe head using $CDCl_3$ or DMSO-d_6 as solvents. For ^{13}C-NMR, proton broad band decoupling was used, and line broadening was 1.0 and 3.0 Hz for unlabelled substances and ^{13}C-catechol, respectively.

The GC-MS analysis was conducted on a Hewlett-Packard 5890 Series II gas chromatograph (Agilent Technologies; Waldbronn, Germany) equipped with an FS-SE-54-NB-0.5 column (25 m $\times$ 0.25 mm, 0.46 μm film thickness; CS Chromatographie Service; Langerwehe, Germany) and connected to a Hewlett-Packard 5971 A mass selective detector (MSD), which was operated in the scan mode (mass range m/z 50–600) with an electron energy of 70 eV. The temperature program of the GC was 50°C for 5 min, 10°C/min to 280°C, then 280°C for 5 min. The injection volume was 1 μl. Carrier gas

was helium (1 ml/min). For **3** and **4**, samples (5 μg) were derivatized with 100 μl *N*,*O*-bis-(trimethylsilyl)-trifluoracetamide (BSTFA, 98% purity, Merck, Darmstadt, Germany) at 60°C for 30 min in closed crimp cap vials.

HPLC was conducted on a RP-18 column (Nucleosil 100-5 C18; CS Chromatographie Service) using a Hewlett-Packard Series 1100 chromatograph system equipped with a diode array UV-detector and an on-line radioactivity detector (Ramona 2000; Raytest, Straubenhardt, Germany) with a 2420 quartz cell (0.37 ml; Raytest). For elution (1 ml/min), the following gradient was applied: solution A (1% acetic acid in water) and B (1% acetic acid in methanol): A:B (60:40/v:v) for 5 min, linear gradient to 100% B for 20 min, isocratic B (100%) for 5 min, return to initial conditions within 5 min.

The U-^{14}C-phenol (**1**), with a specific activity of 1.62×10^{11} Bq/mmol, was supplied in petrol ether by Hartmann Analytic (Braunschweig, Germany). The U-^{13}C-phenol with 99% atom ^{13}C was supplied as a solid by Euriso-Top (Saarbrücken, Germany). 2-Chloro-5-nitrobenzophenone (99% purity) was purchased from Acros (Geel, Belgium). The remaining chemicals were purchased from commercial sources.

*^{14}C-labelled 2-phenoxy-5-nitro-benzophenone (**2**)*

U-^{14}C-phenol (4.35×10^9 Bq, 2.53 mg) diluted with unlabelled phenol (91.5 mg) (**1**) in dry petrol ether (boiling point 60–95°C, 5 ml) was added at room temperature to a suspension of NaH (60%, 50 mg) in dry petrol ether (3 ml) in a 50-ml round bottom flask, which was connected to a reflux condenser. Then 2-chloro-5-nitrobenzophenone (277 mg, in 1 ml dry THF) was added dropwise to the flask with stirring. The mixture turned dark brown and H_2 was released. The reaction mixture was heated under reflux at 70°C and samples monitored by GC-MS and TLC on silica gel with pentane:ethyl acetate (5:1/v:v) as eluent (R_f of **1**, 2-chloro-5-nitrobenzophenone, and **2**: 0.39, 0.49, and 0.41, respectively). During the reaction, oily 'precipitates' with brown-red colour were formed in the bottom of the flask. After 8.5 h reaction, GC-MS and TLC showed that all the phenol and 2-chloro-5-nitrobenzophenone has reacted. Autoradiography indicated that the product (**2**) contained 94.5% of the total radioactivity. The reaction was terminated by adding 1 M NaOH (6 ml) to the flask, resulting in a yellow organic phase and a red-brown aqueous phase. The aqueous phase was extracted with CH_2Cl_2 four times (first 10 ml, then 6–7 ml). The extract and the organic

phase were combined and dried over Na_2SO_4 overnight. Yellow crude product (**2**) of 0.326 g was obtained after rotary evaporation of the organic solvents and drying over P_2O_5 in a desiccator overnight, containing 6.87×10^7 Bq of radioactivity. NMR and MS data of non-labelled **2**: **^{1}H-NMR** (δ, $CDCl_3$): 8.40, 8.39, 8.29, 8.26, 8.25, 7.89, 7.86, 7.62, 7.51, 7.49, 7.46, 7.39, 7.37, 7.34, 7.21, 6.98, 6.95, 6.94, 6.91; **^{13}C-NMR** (δ, $CDCl_3$): 193.23, 160.61, 154.24, 142.40, 136.53, 133.94, 130.37, 130.31, 129.76, 128.64, 127.21, 125.92, 125.70, 120.36, 116.45; **MS** (m/z, (%)): 319 (M^+, 17.1), 302 (15.5), 272 (11.5), 242 (8.0), 226 (5.9), 196 (18.0), 180 (2.5), 168 (10.2), 139 (16.8), 126 (1.5), 105 (100), 77 (93.8), 51 (28.2).

*^{14}C-labelled 2-(2-hydroxyphenoxy)-5-nitrobenzophenone (**3**)*

All of the ^{14}C-labelled **2**, without purification, was completely dissolved in 1.2 ml of concentrated H_2SO_4 in a 50-ml round bottom flask. Glacial acetic acid (4 ml) was then added to dilute the solution. H_2O_2 (1.0 ml, 35%) was added dropwise to the solution within 1 min with strong stirring. The reaction mixture was stirred for 60 min at room temperature. Then the mixture was poured onto ice (150 g), forming a fine precipitate, which was separated by filtration under vacuum and washed thoroughly with H_2O. The filter pellet was completely dissolved in ethyl acetate (EtOAc). Crude product (**3**) was obtained by evaporation of the EtOAc dried over Na_2SO_4 (0.329 g, containing 6.63×10^7 Bq of radioactivity). TLC (pentane:EtOAc = 3:1/v:v, R_f of **2** and **3**: 0.72 and 0.44, respectively) showed 97.5% of ^{14}C was located on the spot of **3**. NMR and MS data of non-labelled **3**: **^{1}H-NMR** (δ,$CDCl_3$): 8.32, 8.31, 8.29, 8.26, 8.25, 7.96, 7.94, 7.93, 7.70, 7.57, 7.55, 7.52, 7.16, 7.14, 7.13, 7.11, 7.06, 7.05, 7.03, 6.91; **^{13}C-NMR** (δ, $CDCl_3$): 194.39, 161.40, 148.83, 142.24, 141.61, 135.66, 134.75, 130.46, 129.00, 127.84, 127.53, 125.92, 122.27, 120.38, 118.24, 116.94; **MS** (m/z (%)), derivatized with BSTFA: 407 (M^+, 13.7), 392 (35.4), 346 (3.5), 318 (1.8), 300 (0.9), 253 (4.8), 223 (1.8), 207 (3.0), 166 (24.2), 151 (39.2), 136 (13.8), 105 (100), 77 (68.7), 73 (67.6), 51 (11.6).

*U-^{14}C-labelled catechol (**4**)*

^{14}C-labelled **3** (88.1 mg, 1.78×10^7 Bq), without purification, was dissolved in dry piperidine (1 ml) in a 50-ml pear-shaped flask connected to a water condenser. Under the protection of argon, the solution was

heated at 110°C in an oil bath with stirring for 90 min. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2 (3 ml), then cooled in an ice bath, and HCl (2 M, 12 ml) was added dropwise with stirring. The aqueous phase was extracted with a mixture of CH_2Cl_2 and EtOAc (1:1/v:v) five times each with 6 ml. The extract and the organic phase were combined and dried over Na_2SO_4. TLC ($CHCl_3$:EtOAC = 5:1/v:v) showed a yellow spot (R_f: 0.94) as a contaminant; 92.9% radioactivity was located in the catechol spot (R_f: 0.41). Solvents were removed by rotary evaporation. The remaining liquid was dissolved in CH_2Cl_2 (14 ml) and extracted with 1 mM HCl (6×8 ml). Catechol (**4**) was extracted from the HCl solution with a mixture of EtOAc:CH_2Cl_2 (1:1/v:v) (7×6 ml). The extract was dried over Na_2SO_4. TLC ($CHCl_3$:EtOAc = 5:1/v:v; R_f of **3** and **4**: 0.86 and 0.41, respectively) and autoradiography showed 98.6% radioactivity purity. After evaporation of the solvents, **4** (R_f: 0.94) was obtained. The crude product was sublimed under 0.05 mbar at 55°C for 3 h, cooled at 0°C, resulting in 23.8 mg of crystalline catechol (**4**) with a specific radioactivity of 7.28×10^7 Bq/mmol.

*Synthesis of U-^{13}C-catechol (**4**)*

The synthesis of U-^{13}C-catechol was conducted under the same reaction conditions as for U-^{14}C-catechol except that phenol was derivatized in dry DMF as solvent at 110°C for 30 min.. The product of the first synthetic step, ^{13}C-labelled 2-phenoxy-5-nitro-benzophenone (**2**), was separated from the reaction mixture by precipitation with H_2O. Before sublimation of crude ^{13}C-catechol (**4**), it was purified using column chromatography with silica gel 60 ($CHCl_3$:EtOAc = 5:1/v:v). For 500 mg U-^{13}C-phenol, 297 mg U-^{13}C-catechol was obtained (65% yield with respect to phenol).

Conclusion

U-^{13}C- and U-^{14}C-catechol were successfully synthesized from phenol in an overall 80% yield, considerably higher than previously reported in the literature. This method uses temperate reaction conditions and the product intermediates are sufficiently pure for the next synthetic steps.

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