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Synthesis of *trans*-dihydronaphthalene-diols and evaluation of their use as standards for PAH metabolite analysis in fish bile by GC-MS

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HIGHLIGHTS

- Evaluation of qualitative analysis for metabolites in bile.
- Limitation of GC-MS analysis for PAH metabolites.
- Synthesis of *trans*-1,2-dihydro-1,2-diols.

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ABSTRACT

Phenols and *trans*-1,2-dihydro-1,2-diols are metabolites commonly formed *in vivo* in fish upon exposure to polycyclic aromatic hydrocarbons (PAHs). These metabolites are excreted via the bile and gas chromatography-mass spectrometry (GC-MS) analysis of bile is becoming more frequently used for evaluating PAH exposure levels in fish. Current protocols focus on the detection and quantification of phenols formed during *in vivo* oxidation of PAHs, leaving out analyses and quantification of other oxidation products such as *trans*-1,2-dihydro-1,2-diols, potentially underestimating exposure levels. Herein, four *trans*-1,2-dihydro-1,2-diols, namely *trans*-1,2-dihydronaphthalene-1,2-diol, *trans*-6-methyl-1,2-dihydronaphthalene-1,2-diol, *trans*-5,7-dimethyl-1,2-dihydronaphthalene-1,2-diol, and *trans*-4,6,7-trimethyl-1,2-dihydronaphthalene-1,2-diol, were successfully prepared and used as standards in the GC-MS analysis, aiming to further develop this qualitative and quantitative analytical method for the determination of PAH exposures. This study shows that the currently used GC-MS analysis, including sample workup, is not suitable for determining the quantity of the corresponding diols derived from naphthalene and methylated naphthalenes. Alternative approaches are needed to provide a correct estimate of PAH exposure levels.

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1. Introduction

Petrogenic polycyclic aromatic hydrocarbons (PAHs), toxic components of crude oil, enter the marine environment through natural oil seeps, accidental oil spills, and produced water discharges (Pampanin and Sydnes, 2013; Sydnes, 2017). Post uptake in vertebrates, PAHs are metabolized by enzymatic oxidation processes, forming molecules that are often more toxic than the parent compounds, i.e. phenols, dihydrodiols, triols, tetraols, and various epoxides (Fig. 1) (Boyd et al., 1987; Jacob, 2008; Davies and Vethaak, 2012; Pampanin and Sydnes, 2013; Pampanin et al., 2016a, 2016b). Additional metabolites (e.g. glucuronides, sulfates) are formed in the phase II part of the xenobiotic metabolism, when PAHs are converted into more water soluble conjugates to facilitate their subsequent excretion from the organism (Beyer et al., 2010).

The analysis of PAH metabolites in fish bile is included in many international monitoring programs, using various species (HELCOM, 2015; Nyberg et al., 2013; Kammann et al., 2017), and is currently used as a biological marker of exposure to oil (Ariese et al., 2005; Beyer et al., 2010; Pampanin and Schlenk, 2020). Determination methods include fixed wavelength synchronous fluorescence scanning (SFS) (Ariese et al., 1993) or fluorescence (FF) (Lin

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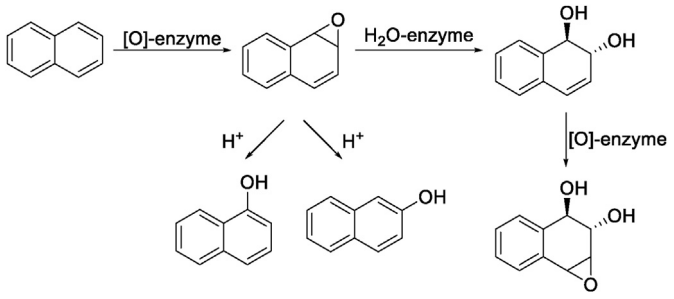


Fig. 1. General outline of the *in vivo* metabolic degradation of naphthalene.

et al., 1996), high performance liquid chromatography (HPLC) (Kammann et al., 2013), and gas chromatography-mass spectrometry (GC-MS) (Jonsson et al., 2004), where the GC-MS analysis is the most commonly used for quantitative analyses (Beyer et al., 2010; Davies and Vethaak, 2012; Pampanin, 2017). Although FF and SFS are easy to carry out and not very costly (Aas et al., 1998, 2000; Beyer et al., 1998; Dissanayake and Galloway, 2004; Pathiratne et al., 2010; Sundt et al., 2012; Elcoroaristizabal et al., 2014), our previous studies have shown that results from FF and SFS should be treated with great care prior to drawing conclusions concerning exposure levels (Pampanin et al., 2016a, 2016b). Therefore, GC-MS or various LC analyses are required in order to verify results from the former methods (Sundt et al., 2011; Pampanin et al., 2016a, 2016b). In addition, the GC-MS analysis is the most suitable method for applied research in biomonitoring programs, as outlined by Davies and Vethaak (2012) and Iversen et al. (2015). More advance approaches, including double mass spectrometry analyses are currently under evaluation in the research community, and could provide an alternative in the long run. However, it is not expected that most laboratories can use this approach for routine monitoring activities at present.

The GC-MS method has its limitations and is only suitable for analysing metabolites derived from lighter PAHs found in crude oil, namely naphthalene, methylated variations of naphthalene (one, two, and three methyl groups), phenanthrene, and chrysene and some of their methylated variations (Beyer et al., 2010). The analytical method development focus has only been on the evaluation of metabolites that are easy to obtain analytical standards of, e.g. PAHs and their corresponding phenols (Fig. 2). However, it is well known that *trans*-1,2-dihydro-1,2-diols are commonly formed metabolites *in vivo* in fish upon exposure to PAHs (Pangrekar et al., 2003; Jonsson et al., 2004; Jacob, 2008; Pampanin and Sydnes, 2013). In order to evaluate the presence of these environmentally relevant metabolites in fish bile, synthetic standards need to be prepared, since they are not commercially available.

In the work presented herein, we have synthetically prepared four *trans*-1,2-dihydronaphthalene-1,2-diols, namely *trans*-1,2-dihydronaphthalene-1,2-diol (**1(±)**), *trans*-6-methyl-1,2-dihydronaphthalene-1,2-diol (**2(±)**), *trans*-5,7-dimethyl-1,2-dihydronaphthalene-1,2-diol (**3(±)**), and *trans*-4,6,7-trimethyl-1,2-dihydronaphthalene-1,2-diol (**4(±)**) (Fig. 3) and evaluated them as standards for the GC-MS analysis. This work represents a natural follow up of our previous research activities on this topic (Lorentzen et al., 2014; Pampanin et al. 2014, 2016a, 2016b; Enerstvedt et al., 2017). Bile samples (31 individuals) from Atlantic cod exposed to different doses of dispersed crude oil (0.01, 0.05 and 0.1 ppm) were analysed in order to verify our analytical method (details regarding the exposure study have already been reported by Enerstvedt et al. (2018)).

2. Materials and methods

2.1. General experimental

Automated flash chromatography was performed on an Interchim PuriFlash® 215 chromatography system, detection at 254 nm. Infrared absorption spectroscopy was performed on a Cary 630 FTIR from Agilent Technologies. Proton (^{1}H) and carbon (^{13}C) NMR spectra were conducted on an AscendTM 400 NMR spectrometer from Bruker, which operated at 400 MHz and 100 MHz for proton and carbon, respectively. Chemical shifts (d) are reported relative to residual chloroform ($CHCl_3$) in deuterated chloroform ($CDCl_3$) (δ 7.26 ppm, ^{1}H; δ 77.16 ppm, ^{13}C) and residual methanol (CH_3OH) in deuterated methanol (CD_3OD) (δ 3.31 ppm, ^{1}H; δ 49.0 ppm, ^{13}C) as references. ^{1}H NMR data are reported by the following sequence: chemical shift (δ) [multiplicity, coupling constant(s) J (Hz), relative integral], in which the multiplicity is reported as: s = singlet; d = doublet; dd = doublet of doublett; t = triplet; m = multiplet; bs = broad singlet. For ^{13}C NMR spectra, data are reported as chemical shift (d). Melting points (mp) were determined on a Stuart SMP20 melting point apparatus and are uncorrected. The GC-MS analysis was run on an Agilent 6890 N gas chromatograph, Gerstel MPS autosampler and an Agilent 5975 mass spectrometer. Bile samples from exposed Atlantic cod were obtained from a previously reported study (Enerstvedt et al., 2018).

***trans*-1,2-Dihydronaphthalene-1,2-diol (1(±)).** Ethanol (EtOH) (12 mL) was added to a flask containing 1,2-naphthoquinone (**5**) (100 mg, 0.63 mmol) and sodium borohydride ($NaBH_4$) (255 mg, 6.74 mmol, 10.7 equiv.). The atmosphere was changed to oxygen (O_2), and the reaction mixture was left to stir overnight. The resulting reaction mixture was evaporated onto Celite® and transferred to a prepacked flash column for purification (silica, pet. ether → 75:25 v/v petroleum ether (pet. ether)/ethyl acetate (EtOAc)). Concentration of relevant fractions (R_f = 0.5 in 75:25 v/v pet. ether/EtOAc) resulted in compound **1(±)** (Kundu, 1979), (40 mg, 39%) as a white solid, mp 105.7–106.0 °C (lit.[19] 105–106 °C) IR ν_{max} 3271, 3034, 2924, 2852, 2320, 2105, 1919, 1475, 1376, 1246, 1215, 1188, 1158, 1039, 975 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.53–7.51 (m, 1H), 7.25–7.19 (m, 2H), 7.09–7.07 (m, 1H), 6.43 (dd, J = 9.9 and 2.1 Hz, 1H), 5.92 (dd, J = 9.8 and 2.6 Hz, 1H), 4.68 (d, J = 9.9 Hz, 1H), 4.37–4.33 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) 138.3, 133.9, 131.7, 128.7, 128.7, 128.5, 127.3, 126.6, 75.5, 73.9.

***trans*-6-Methyl-1,2-dihydronaphthalene-1,2-diol (2(±)).** 2-Iodoxybenzoic acid (IBX) (194 mg, 0.69 mmol, 4.4 equiv.) was added in portions to a solution of 6-methyl-1-tetralone (**6**) (25 mg, 0.16 mmol) in dry dimethyl sulfoxide (DMSO) (3 mL). After 21 h the reaction mixture was quenched with distilled water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with saturated sodium bicarbonate ($NaHCO_3$) solution (2 × 20 mL), dried over sodium sulphate (Na_2SO_4), filtered and concentrated under reduced pressure. The residue, which contained compound **7**, was placed on a vacuum line for 3 h prior to the next step. The residue was then dissolved in EtOH (15 mL) and $NaBH_4$ (60 mg, 1.6 mmol, 11 equiv.) was added to the solution in portions (3 × 20 mg). The atmosphere was changed to O_2, along with protection against light. After 17 h the reaction mixture was quenched with distilled water (H_2O) (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (15 mL). The solvent was evaporated onto Celite® followed by purification on a Puriflash Interchim 215 (silica, 97:3 v/v/v $CHCl_3$/EtOAc → 90:10 v/v $CHCl_3$/EtOAc). Concentration of relevant fractions (R_f = 0.2 in 85:15 v/v $CHCl_3$/EtOAc) resulted in compound **2(±)**, (16.1 mg, 59%) as a white solid, mp. 114–116 °C. IR ν_{max} 3295, 2923, 2852, 1717, 1600, 1574, 1498, 1462, 1443, 1375, 1310, 1252, 1168, 1093, 1045, 1029, 971 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42

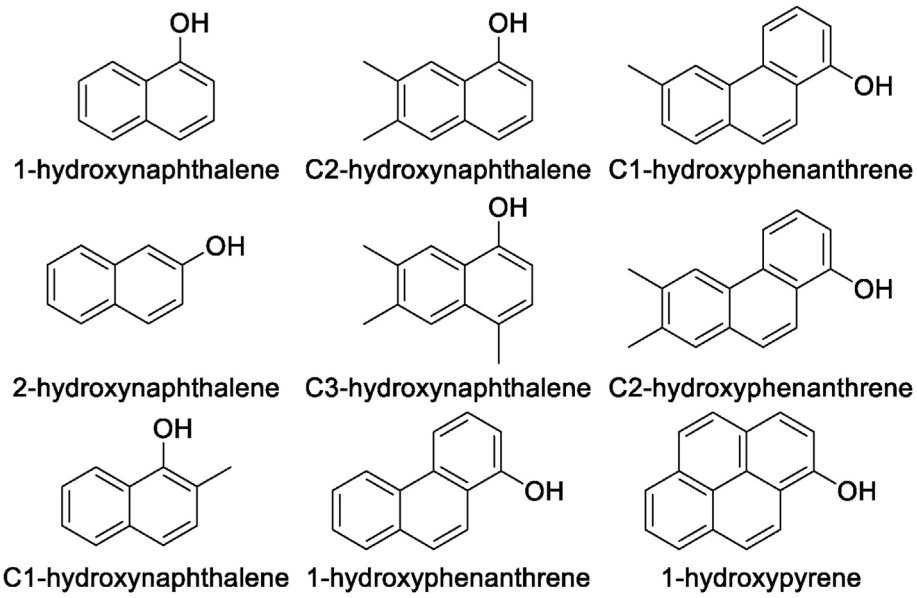


Fig. 2. Targeted hydroxyl-PAH metabolites in currently run standard gas chromatography-mass spectrometry analysis of fish bile.

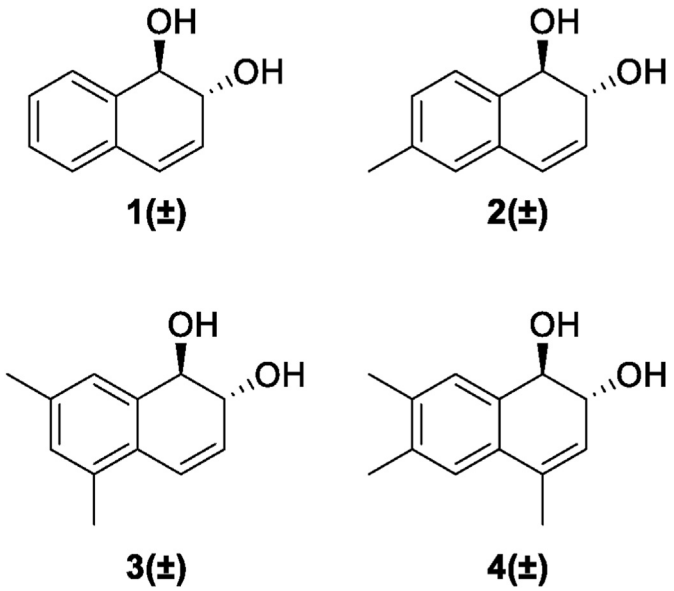


Fig. 3. Structure of *trans*-1,2-dihydronaphthalene-1,2-diol (**1**(±)), *trans*-6-methyl-1,2-dihydronaphthalene-1,2-diol (**2**(±)), *trans*-5,7-dimethyl-1,2-dihydronaphthalene-1,2-diol (**3**(±)), and *trans*-4,6,7-trimethyl-1,2-dihydronaphthalene-1,2-diol (**4**(±)).

(d, J = 8.3, 1H), 8.74 (dd, J = 8.4 and 2.4, 1H), 6.60 (d, J = 2.3, 1H), 6.33–6.31 (m, 1H), 5.93 (dd, J = 10.0 and 2.0, 1H), 4.69 (d, J = 10.4, 1H), 4.38 (d, J = 10.4, 1H), 2.83 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 133.7, 131.2, 128.7, 127.6, 126.2, 112.5, 112.3, 74.6, 73.7, 55.4.

5,7-Dimethylnaphthalene-1,2-dione (9). IBX (0.96 mg, 3.4 mmol, 4 equiv.) was added in portions to a solution of 5,7-dimethyl-1-tetralone (**8**) (150 mg, 0.86 mmol) in dry DMSO (10 mL). After 21 h the reaction mixture was quenched with distilled water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (2 × 20 mL), dried ($MgSO_4$), filtered and evaporated onto Celite®. Purification was performed on a Puriflash Interchim 215 (silica, 95:5 v/v pet. ether/EtOAc → 50:50 v/v pet. ether/EtOAc). Concentration of relevant fractions (R_f = 0.5 in 75:25 v/v pet. ether/EtOAc) resulted in compound **9**, (96.3 mg, 86%) as an orange solid, mp. 96–99 °C. IR ν_{max} 2921, 2853, 1659, 1611, 1380, 1298, 1260, 1211, 1163 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.70 (d, J = 10.4 Hz, 1H), 7.27–7.26 (m, 1H), 6.36 (d, J = 10.4 Hz, 1H), 2.44 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.2, 179.9, 142.0, 141.5, 138.6, 137.8, 132.2, 130.2, 129.5, 126.2, 21.3, 18.8.

***trans*-5,7-Dimethyl-1,2-dihydronaphthalene-1,2-diol (3(±))**. 5,7-Dimethylnaphthalene-1,2-dione (**9**) (85 mg, 0.46 mmol) was dissolved in dry EtOH (12 mL) and $NaBH_4$ (190 mg, 5.0 mmol, 11 equiv.) was added to the solution in portions (3 × 63.3 mg). The atmosphere was changed to O_2, along with protection against light. After 17 h the reaction mixture was quenched with distilled H_2O (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (1 × 15 mL), followed by evaporation onto Celite®, and purified on a Puriflash Interchim 215 (silica, 80:17:3 v/v/v pet. ether/EtOAc/$CHCl_3$ → 40:57:3 v/v/v pet. ether/EtOAc). Concentration of relevant fractions (R_f = 0.4 in 50:50 v/v pet. ether/EtOAc) resulted in compound **3(±)**, (28.3 mg, 0.15 mmol, 32%) as a white/light yellow solid, mp. 127–129 °C. IR ν_{max} 3347, 2922, 2854, 1609, 1454, 1376, 1302, 1253, 1166, 1064, 980 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1H), 6.90 (s, 1H), 6.60 (dd, J = 10.1 and 2.0 Hz, 1H), 5.94 (dd, J = 10.1 and 2.1 Hz, 1H), 4.74 (d, J = 10.6 Hz, 1H), 4.46–4.42 (m, 1H), 2.61 (bs, 2H), 2.30 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.4, 136.3, 133.9, 130.6, 128.9, 127.9, 124.6, 123.4, 75.6, 73.6, 21.5, 18.9; HRMS (ESI): calced. for $C_{12}H_{14}O_2$ [M + Na^+] 213.08915, found 213.08934.

4,6,7-Trimethylnaphthalene-1,2-dione (12). 4-Methyl-1,2-benzenediol (**11**) (0.1 g, 0.81 mmol) was dissolved in cold acetate buffer (20 mL, 0.1 M, pH 4.5), containing Laccase (120 mg, 113 U). The mixture was added dropwise to a solution containing 2,3-dimethyl-1,3-butadiene (**10**) (0.7 g, 8.1 mmol, 10 equiv.) in acetate buffer (20 mL) placed in ice bath over a stirring plate and exposed to air. The reaction mixture was protected from light, within the next 3 h of reaction; Laccase (110 mg, 103 U) was added each h and allowed to stir at room temperature after the last addition. After 10 h the mixture was extracted with EtOAc (3 × 15 mL). The organic layers were evaporated onto Celite® and transferred to a prepacked flash column for the purification (silica, pet. ether → 75:25 v/v pet. ether/EtOAc). Concentration of relevant fractions (R_f = 0.44 in 80:20 v/v pet. ether/EtOAc) resulted in compound **12** (Wozniak

et al., 1989), (61 mg, 38%) as an orange solid, mp 94–97 °C (decomposed) (lit (Wozniak et al., 1989). 112 C° (decomposed)) IR ν_{max} 2921, 2854, 1902, 1740, 1685, 1654, 1599, 1550, 144, 1401, 1377, 1310, 1278, 1262, 1238, 1195, 1128, 1024, 968, 928, 900 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.20 (s, 1H), 6.24 (d, $J = 0.9$ Hz, 1H), 2.34 (s, 3H), 2.32 (d, $J = 0.9$ Hz, 3H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 179.7, 154.1, 145.6, 140.0, 133.5, 131.3, 129.2, 128.0, 126.8, 20.6, 20.6, 19.6.

***trans*-4,6,7-Trimethyl-1,2-dihydronaphthalene-1,2-diol (4(±)).** 4,6,7-Trimethylnaphthalene-1,2-dione (**12**) (46 mg, 0.23 mmol) was dissolved in dry EtOH and $NaBH_4$ (96 mg, 2.53 mmol, 11 equiv.) was added to the solution in portions (3 × 32 mg). The atmosphere was changed to O_2, along with the protection against light, and after 17 h the reaction was complete. The solvent was removed under reduced pressure and the crude residue was evaporated onto Celite® and transferred to a pre-packed flash column for the purification (silica, pet. ether → 60:40 v/v pet. ether/EtOAc). Concentration of relevant fractions ($R_f = 0.5$ in 50:50 v/v pet. ether/EtOAc) resulted in compound **4(±)**, (3.3 mg, 10%) a white solid, mp. 129–131 °C. IR ν_{max} 3363, 2920, 2852, 1735, 1458, 1376, 1249, 1186, 1110, 1077, 1028, 969 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.26 (s, 1H), 7.03 (s, 1H), 5.67–5.66 (m, 1H), 4.54 (d, 1H), 4.24–4.20 (m, 1H), 2.27 (s, 3H), 2.25 (s, 3H), 2.04 (t, $J = 1.7$, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 136.8, 136.5, 135.7, 133.9, 132.9, 128.1, 127.3, 125.7, 75.4, 73.7, 19.7, 19.6, 19.1.

2.2. Preparation of the synthetic metabolites for GC-MS analysis

Triphenylamine (TPA) (98%) and 2,6-dibromophenol (99%) were purchased from Chiron AS. *N,O*-Bis(Trimethylsilyl)trifluoroacetamid (BSTFA), β-glucuonidase 5% sulphatase activity and anhydrous sodium acetate were purchased from Sigma-Aldrich. Glacial acetic acid and EtOAc for GC analysis was obtained from VWR.

The four synthetic metabolites, compounds **1(±)**-**4(±)**, were prepared as calibration standards for the GC-MS by adding EtOAc at seven concentration levels (10–1000 ppb). The surrogate internal standard, 2,6-dibromophenol (100 μL, 2.24 ppm), was added before the derivatization with BSTFA (200 μL) for 2 h at 60 °C, and TPA (40 μL, 4.23 ppm) was added as a GC internal standard prior to the analysis.

2.3. Preparation of bile for GC-MS analysis

The preparation of hydrolyzed bile samples was performed as described in the standard operating procedure developed at NORCE and is based on previous work by Krahn et al. (1987), Jonsson et al. (2003), and Aas et al. (1998, 2000). Bile samples were obtained from freezer (−80 °C) and thawed on ice for about 30 min before the hydrolysis.

In brief, bile samples (30 μL) and surrogate standard 2,6-dibromophenol (2.24 ppm, 100 μL) were treated with 300 μL of β-glucuronidase (100 000 units/mL) with sulphathase activity (7500 units/mL) diluted by in 1:10 sodium acetate buffer (0.4 M, pH 5) for 2 h at 40 °C. Hydrolyzed metabolites were extracted with EtOAc (0.5 mL x 4) and extracts were dried with sodium sulphate. Extracts were then transferred to clean scintillation vials and evaporated until approximately 500 μL EtOAc was remaining, the BSTFA (200 μL, 0.19 g, 0.75 mmol) was added. The mixture was incubated at 60 °C for 2 h the TPA as a GC internal standard (4.23 ppm, 20 μL) was added prior to the GC-MS analysis.

2.4. GC-MS conditions

TMS derivatives of naphthalene *trans*-1,2-dihydro-1,2-diols were analysed by the GC-MS system. Helium was used as carrier gas and the applied column was HP 5MS (30 m × 0.25 mm and 0.25 μm from Agilent Technologies). Samples and calibration standards (1 μL) were injected on a split/splitless injector with splitless mode for 1 min. Temperatures for the injector, transferline and ion source were held at 280, 300 and 250 °C, respectively, and the GC oven temperature program was: 85 °C for 1 min, 85–120 °C at 20 °C/min, 120–300 °C at 8 °C/min, and held at 300 °C for 7 min. The quantitative determination was done in selected ion mode (SIM). Targeted mass to charge (*m/z*) ratios were selected on the basis of the preliminary analysis in full scan mode (SCAN) at 70 eV, in order to identify the most abundant ions.

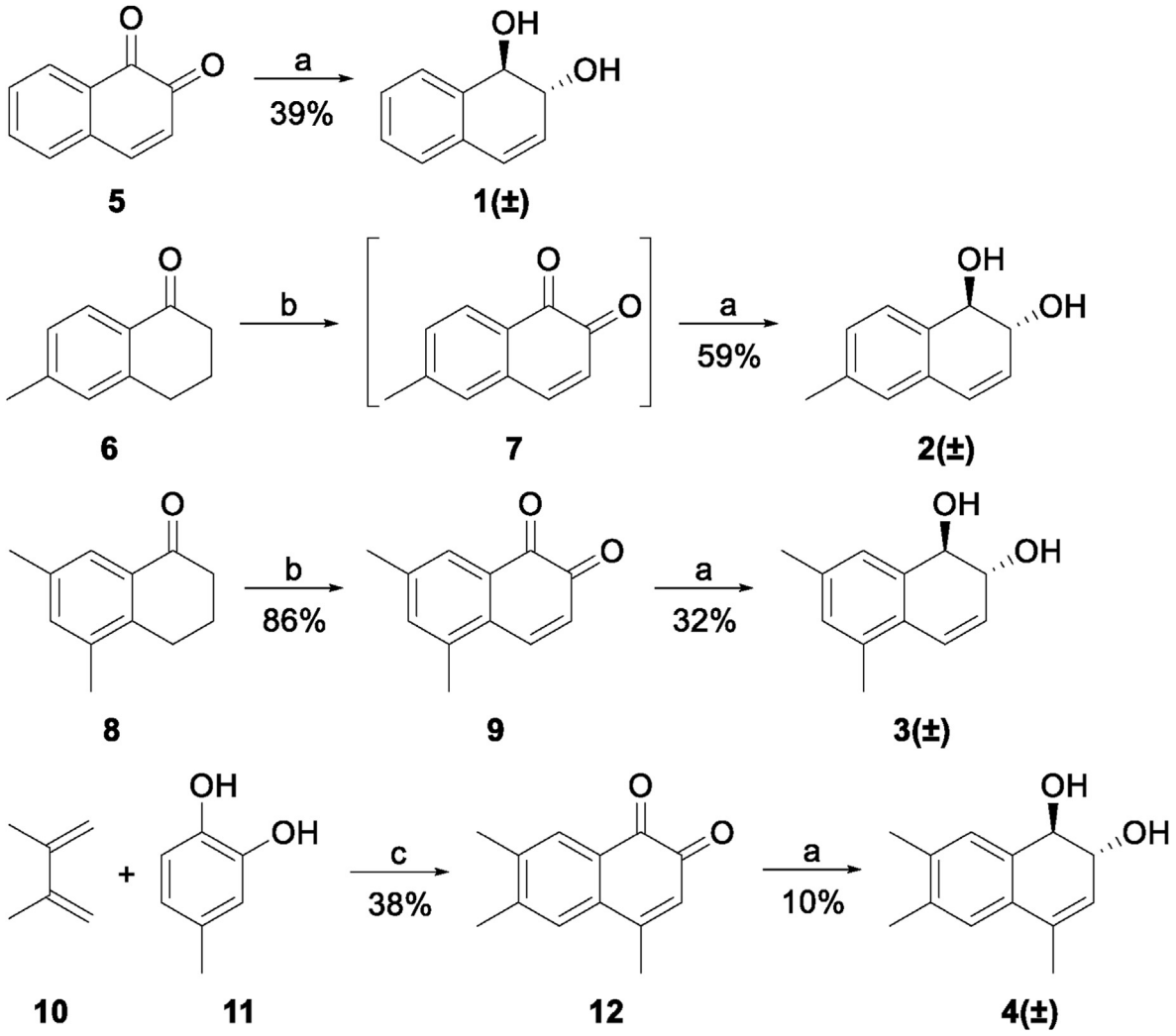
3. Results and discussion

3.1. Synthesis of PAH trans-diols

Our synthetic strategy for the formation of the four *trans*-1,2-dihydro-1,2-diols (**1(±)**-**4(±)**) was based on the reduction of the relevant 1,2-naphthoquinones, which were either commercially available or readily prepared from suitable starting materials, with $NaBH_4$, under an oxygen atmosphere, using the method reported by Platt and Oesch (1983). Treating commercially available 1,2-naphthoquinone (**5**) with $NaBH_4$, under an oxygen atmosphere in ethanol, gave the desired product **1(±)** (Scheme 1). Compound **2(±)** was formed by first converting 6-methyl-1-tetralone (**6**) to 6-methylnaphthalene-1,2-dione (**7**), by the IBX promoted oxidation, using a method reported by Ren et al. (2015), followed by a reduction according to the method just described for the formation of compound **1(±)**. *trans*-5,7-Dimethyl-1,2-dihydronaphthalene-1,2-diol (**3(±)**) was prepared by the same method described for diol **2(±)**, take for the fact that 1,2-naphthoquinone **9** was isolated and purified by flash chromatography prior to the reduction by $NaBH_4$. The final required 1,2-naphthoquinone **12** was formed by treating diene **10** and catechol **11** with Laccase (113 U) in acetate buffer, following a procedure by Witayakran et al. (2007). Under these conditions, the catechol **11** was converted to the corresponding benzoquinone *in situ* (Cannatelli and Ragauskas, 2017), which then engaged in the Diels-Alder reaction resulting in the formation of 4,6,7-trimethylnaphthalene-1,2-dione (**12**) in 38% yield after purification. Compound **12** was then finally converted to the corresponding *trans*-1,2-dihydro-1,2-diol **4(±)**, upon reduction with $NaBH_4$, under an oxygen atmosphere. Although yields for the reduction of the 1,2-naphthoquinones in general were relatively poor for the formation of *trans*-1,2-dihydro-1,2-diols **1(±)**, **3(±)**, and **4(±)**, it did provide the desired *trans*-1,2-dihydro-1,2-diols **1(±)**-**4(±)** in sufficient quantity for the analytical study; and in good purity as can be seen from the ^1H NMR spectra (see supporting information for NMR spectra). The synthetic methods developed herein can also be scaled up in order to provide larger quantities of reference materials.

3.2. Preparation of trans-dihydronaphthalene-diols *1(±)-4(±)* for the GC-MS analysis

With the four dihydro-diols (**1(±)**-**4(±)**) in hand, the work shifted towards the evaluation of their ability to function as standards for the GC-MS analysis. In order to improve the currently used GC-MS method for PAH metabolites in bile, the diols also needed to be readily converted to the corresponding trimethylsilyl (TMS) ethers in the same efficient way as the phenols depicted in Fig. 2 are converted to the corresponding TMS-ethers in the work by Krahn et al. (1987) and Jonsson et al. (2003). By treating the *trans*-dihydronaphthalene-diols with BSTFA in EtOAc at 60 °C for 2 h, the standard method used for conversion of phenols to the

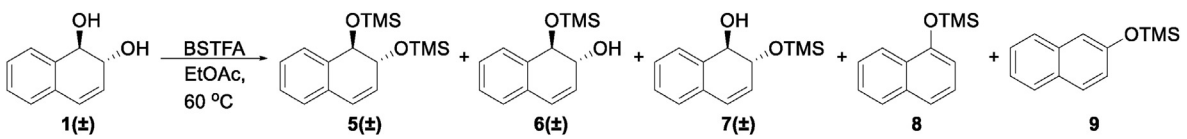


Scheme 1. Synthesis of *trans*-dihydronaphthalene-diols **1(±)**-**4(±)**. Reaction conditions: (a) $NaBH_4$, O_2, EtOH, rt; (b) IBX, dry DMSO, rt; (c) Laccase (113 U), acetate buffer (0.1 M, pH 4.5), 0 °C → rt (% = chemical yield for the reaction).

corresponding TMS-ethers, a range of products were formed from compounds **1(±)**, **3(±)**, and **4(±)**, as highlighted by the products detected by the GC-MS analysis (Scheme 2) and as exemplified by the chromatogram of compound **1(±)** in Fig. 4b. One of the products formed was the desired di-TMS-ether **5(±)**, in addition to both the possible mono TMS-ethers **6(±)** and **7(±)**. Moreover, small amounts of compounds **8** and **9**, derived from the loss of water followed by the conversion of the resulting phenols to the corresponding TMS-ethers, were also detected. A similar product distribution was also detected when compounds **3(±)** and **4(±)** were subjected to the same reaction conditions. Products derived from *trans*-6-methyl-1,2-dihydronaphthalene-1,2-diol **2(±)** were not detected in the GC-MS analysis, most likely due to the decomposition of the derived compound on the GC column.

Attempts to improve the results from the derivatization, by enhancing the reaction time, increasing the temperature, or increasing the amount of BSTFA, did not increase the outcome. In order to verify our reaction conditions, we utilized the same derivatization conditions on 1-naphthol, which resulted in, as expected, a clean conversion to the corresponding TMS-ether **8**, as shown in the GC chromatogram (mass confirmed by the MS analysis) (Fig. 4a). This confirmed that our standard reaction conditions were providing the desired result for the phenols.

The lack of conversion to single products, when compounds **1(±)**, **3(±)**, and **4(±)** were treated under standard derivatization conditions (i.e. conditions used on bile samples), highlighted the fact that sample workup conditions used for the GC-MS analysis of fish bile has limitations when it comes to the quantification of lighter PAH metabolites. Small quantities of diol are converted to the two naphthol derivatives, *viz* compounds **8** and **9**, which are



Scheme 2. Products formed upon reaction with *N,O*-bis(trimethylsilyl-fluoro)acetamide (BSTFA) exemplified with *trans*-1,2-dihydronaphthalene-1,2-diol (**1(±)**).

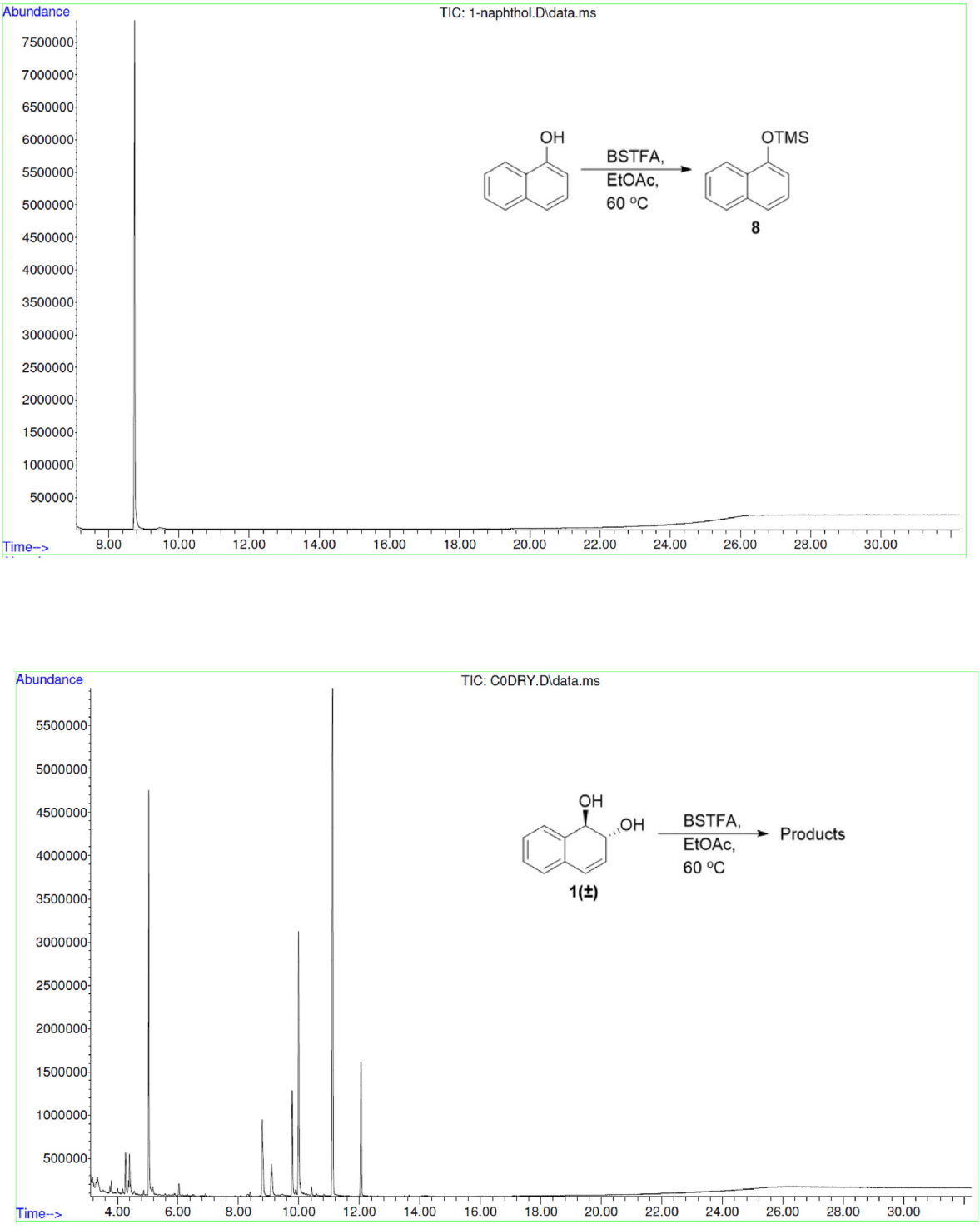


Fig. 4. a) Chromatogram of derivatization product of 1-naphthol **8**; b) chromatogram after derivatization of *trans*-1,2-dihydronaphthalene-1,2-diol **1(±)**.

quantified in the method used today. However, the full overview is lost since the majority of the product mixture derived from the diols are other derivatives that are not included in the current analytical scheme.

3.3. The GC-MS analysis of fish bile samples

Although the derivatization and analysis of the three standard compounds indicated that it would not be possible to utilize the method for quantitative analysis of compounds **1(±)**, **3(±)**, and **4(±)** in bile, we did investigate their use as standards for the qualitative analysis of fish bile. Therefore, 31 bile samples from Atlantic cod exposed to dispersed crude oil were analysed (Enerstvedt et al., 2018). The dispersed crude oil contained 6.7 μg/L naphthalene, 23 μg/L C1-naphthalene, 29 μg/L C2-naphthalene (containing two methyl groups), and 44 μg/L C3-naphthalene (containing three

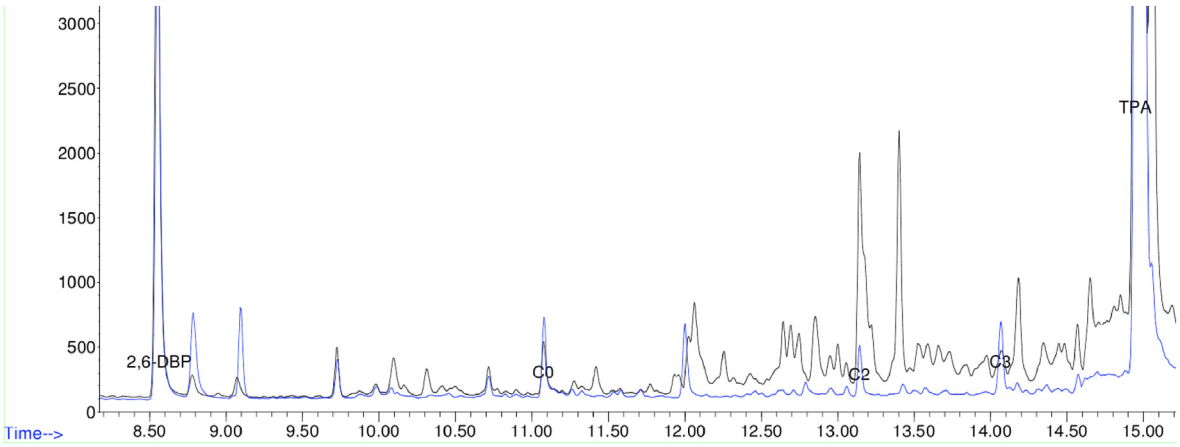


Fig. 5. The overlay total ion chromatograms (TICs) of two samples analysed by gas chromatography-mass spectrometry (GC-MS). The blue line represents a control bile sample spiked with the standards (1(±)-4(±)), and the black line shows a bile sample from fish exposed to medium concentration level of crude oil. C0, C2 and C3 represents the standards 1(±), 3(±) and 4(±), respectively (TPA = triphenylamine; 2,6-DBP = 2,6-dibromophenol). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

methyl groups) (Enerstvedt et al., 2018). The GC-MS analysis confirmed the presence of derivatives derived from *trans*-dihydronaphthalene-diols **1(±)**, **3(±)**, and **4(±)** in samples from Atlantic cod exposed to medium and high concentrations of crude oil (see Fig. 5 for an example and supporting information for the full data set). The presence of TMS-ether derivatives from the different naphthalenes could be distinguished from the GC chromatogram and confirmed by their mass. However, a proper quantification of the parent compounds, *viz.* the *trans*-dihydronaphthalene-diols, could not be obtained due to the challenges outlined above.

Naturally the diols described in this work could have been detected by LC-MS techniques with plenty of examples of that being reported (Beyer et al., 2010). However, the aim of this study was to investigate the possibility to broaden the scope of the commonly used GC-MS method for analysis of fish bile for petrogenic PAH metabolites.

4. Conclusion

Four *trans*-1,2-dihydro-1,2-diols (**1(±)-4(±)**) were successfully prepared by synthesis, providing new standard compounds for PAH metabolite analyses. Unfortunately, the conversion of these standards to the corresponding single TMS-ethers failed, resulting in a range of products. These results show that the currently used sample workup conditions for the GC-MS analysis are not suitable for determining the quantity of the corresponding naphthalene *trans*-1,2-dihydro-1,2-diols metabolites in fish bile. In order to successfully be able to conduct quantification of *trans*-1,2-dihydro-1,2-diols (**1(±)-4(±)**), other analytical methods are therefore required. LC-MS techniques (e.g. atmospheric pressure chemical ionization in positive ionization mode ($APCI^+$) LC/MS/MS) are recommended as preferable tools for studying PAH metabolites in fish bile, considering the limitation of the GC-MS method and the preliminary positive results obtained in recent studies (Sette et al., 2013). Our results also open up the possibility for further studies where other sample preparation methods for converting PAH metabolites in bile to suitable derivatives for the GC-MS analysis could be considered to facilitate the analysis of both phenols and diols.

Credit author statement

Conceptualization: DMP and MOS. Methodology: ICV, DMP, and MOS. Validation: ICV, DMP, and MOS. Investigation: ICV. Writing original draft: ICV, DMP, and MOS. Writing, review & editing: DMP and MOS. Supervision: MOS. Project administration: MOS. Funding acquisition: MOS

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126928.

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