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Douglas E. Stack & Bejan Mahmud

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Efficient access to bisphenol A metabolites: Synthesis of the monocatechol, mono-*o*-quinone, dicatechol, and di-*o*-quinone of bisphenol A

Douglas E. Stack

Department of Chemistry, University of Nebraska, Omaha

Bejan Mahmud

Department of Chemistry, University of Nebraska, Omaha

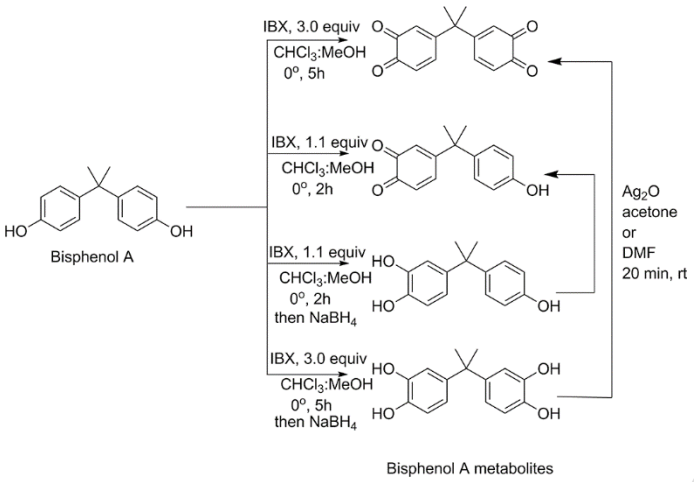
Address correspondence to Douglas E. Stack, Department of Chemistry, University of Nebraska, Omaha 68182. E-mail: dstack@unomaha.edu

ABSTRACT

2-Iodoxybenzoic acid (IBX) oxidation of bisphenol A (BPA) is described. The selective production of either the mono-*o*-quinone or the di-*o*-quinone can be controlled by IBX stoichiometry. Isolated yields of either quinone was greater than 80%. Previous synthesis of the BPA-di-*o*-quinone using a large excess of Fremy's salt produced only trace amounts of product. In addition to *o*-quinone products, both mono- and dicatechols of BPA can synthesize in high yield and isolated without chromatography. The more stable catechols can be quantitatively

converted back to *o*-quinones using silver oxide oxidation in either acetone or DMF. These one-pot reactions provide access to four different BPA metabolites in high yield and significant scale.

Graphical Abstract



KEYWORDS: bisphenol A, bisphenol A metabolites, catechols, genotoxic, o-quinones

Introduction

Bisphenol A (BPA) is a widely used industry chemical prominent in the manufacture of plastics and epoxy resins.[1] BPA toxicity is controversial and is the subject of many publications over the past 20 years. BPA and its metabolites can act as estrogen mimics and are classified as endocrine disrupting chemicals (EDC).[2] In addition, evidence for genotoxic effects *in vitro* have been revealed in studies involving mouse lymphoma,[3] MCF-7[4] and CHO-K1[5] cells. *In vivo* studies showed structural changes in bone marrow cells of mice.[6]

The major metabolites of BPA include glucuronic acid conjugates and catechols.[7] Catechols can undergo further oxidation to *o*-quinones (Scheme 1). *o*-Quinones are genotoxic and can react with DNA nucleophiles.[8] BPA metabolism *in vivo* produced hydroxylated BPA

similar to the metabolism of other phenols including β-estradiol. Atkins and Roy found that incubating BPA in the presence of rat hepatic microsomal cytochrome P450 activation system produced DNA adducts as assayed by ^{32}P-postlabeling.[9] A similar ^{32}P-postabling chromatographic profile was obtain when they exposed DNA to the *o*-quinone of BPA, BPAQ (**2**), made synthetically.[10]

Atkins and Roy synthesized BPAQ from BPA using potassium nitrosodisulfonate (Fermy's salt) oxidation in an acetone:acetic acid solvent system.[10] The synthesis is inefficient with isolated yields around 25–50%. Although using a 10–15 equiv excess of Fermy's salt, little to no formation of the di-*o*-quinone of BPA, BPADQ (**3**), is observed. Edmonds's NMR analysis of the crude, chloroform extract showed Femy's salt oxidation produce 70% BPAQ, 3% BPADQ, 3% unreacted BPA, 15% 1,4-benzoquinone and 10% unidentified by-products.[11] BPADQ has also been synthesized using 70% *tert*-butyl hydroperoxide in water (T-HYDRO) in the presence of a dirhodium catalyst but isolated yields were only 44%. Although 8 equiv of T-HYDRO was employed, formation of BPADQ was not observed.[12]

Using ethereal solution of BPA, mixed with an aqueous solution of Fermy's salt containing NaH_2PO_4, Yoshida was able to isolate BPADQ in 4% yield allowing NMR structural analysis.[13] Yoshida observed small amounts of BPADQ form from BPA oxidation by polyphenol oxidase (potato extract) in the presence of O_2 establishing BPADQ as metabolite of BPA.[14] Imanaka showed that fruit homogenates produce 3-hydroxyBPA which is further oxidized to 3,3'-dihydroxyBPA a possible precursor to BPADQ.[15] Other studies directed at identifying BPAQ-DNA adducts and BPAQ-sulfur adducted adducts have employed Femy's salt oxidation to obtain the BPAQ electrophile.[16,17]

2-Iodoxybenzoic acid (IBX) oxidation of phenols has proven an effective method that can produce *o*-quinones directly from phenols.[18–20] *In situ* reductions of the *o*-quinones by sodium borohydride produces catechols from phenols in a one-pot reaction. We have used this method to produced catechols of estrone and β-estradiol whose *o*-quinones are less stable than BPAQ.[21] The catechols are easily converted back to *o*-quinones via oxidation typically with activated MnO_2 or Ag_2O. We sought to explore IBX oxidation of BPA as a way to control production of BPAQ and BPADQ by regulation of IBX stoichiometry. This work presents the results of IBX oxidation of BPA to form compounds **1** through **4**, isolated conveniently in a one-pot, high yield processes (Scheme 2).

Results and Discussion

Initial oxidation of BPA with IBX was done in a 3:2 solvent mixture of $CHCl_3$:CH_3OH at –20ºC using 1.1 equiv of IBX over a period of 12 h. Low temperatures were initially attempted to prevent over oxidation of **2** to **3**. Using 1.1 equiv of IBX produced a deep red solution after 1 h at –20ºC. HPLC analysis showed complete consumption of BPA and formation of **2** after 12h with only trace amounts of **3**. After aqueous extraction of the *o*-iodobenozic acid byproduct from chloroform using a 0.1 M phosphate buffer at pH 6.0, **2** was purified by flash chromatography using hexanes and ethyl acetate solvents as in prior synthesis.[11] Isolated yields of pure compound were typically 80–85%. The reaction produces similar results when conducted at 0ºC for 2 h.

We observed that pre-absorption of the crude mixture on silica gel for even short periods of time generated a unknown green compound with diminished isolated yields. Best results were obtain by directly applying the crude mixture on the flash column using dichloromethane

immediately before separation. We hypothesize the green compound may involve some type of hydrolyzed byproduct perhaps catalyzed by silica gel. NMR analysis of purified **2**in acetone-*d6* show the compound stable at room temperature in solution for over a one month period. A more efficient purification method can be had by converting **2** to catechol **1** which can be conveniently oxidized back to **2** quantitatively using Ag_2O (*vide infra*).

The oxidation of BPA to monoquinone **2** must occur faster than further oxidation of **2** to **3** at -20°C. The lack of any diquinone product using 1.1 equiv of IBX suggest that a difference in the rate of oxidation must be in effect. Magdziak et al.[19] showed that phenols with electron-withdrawing groups undergo IBX oxidation slowly or not at all. While the two arene π-systems in BPA are separated by a saturated carbon, the formation of the first *o*-quinone can impart an inductive withdraw to the saturated carbon connecting the two arene rings thus slowing the second oxidation.

To examine the rate difference in these two oxidation, the reaction was followed by HPLC using 2.05 equiv of IBX at rt, 0°C, and –20°C. **Figure 1** shows the oxidation of BPA at -20°C using 2.05 equiv of IBX at various times. Monitoring at 280 nm (**Figure 1A**) shows the formation of an intermediate with slightly longer retention time than BPA after 15 min. IBX oxidation of phenols is postulated to first involve ligand exchange onto the hypervalent iodane.[18] IBX is notoriously insoluble and is not observed directly in the HPLC chromatographs. IBX forms a suspension in the $CHCl_3$:CH_3OH solvent system used in the reaction; however, the reaction mixture does become homogenous after the addition of BPA to the IBX suspension. When a suspension of IBX in methanol is mixed, filter and analyzed by HPLC, the two peaks flanking compound **3** (220min chromatograph, **Figure 1A**) are observed in the same ratio. These observations indicate that several IBX-alkoxy species may be present

during the course of the reaction. The change in chromatographs a t = 220min and t = 18.5h (**Figure 1A**) shows the consumption of **2** with simultaneous increase in **3** and the *o*-iodobenzoic acid byproduct without any other significant peaks. The same analysis was observed at shorter wavelengths (250 and 230 nm). Thus the oxidizing species is not observed in the HPLC chromatographs. Monitoring the same chromatographs a 400 nm clearly shows the formation of **2** followed by **3** (**Figure 1B**).

Table 1 shows the percentage of **3** as a total of quinone products when 2.05 equiv of IBX is used at various temperatures. When using 1.1 equiv of IBX, selective formation of **2**in lieu of **3** can be done conveniently at 0^{o}C. Complete conversion of **2** to **3** is observed if 3.0 equiv of IBX is used and the reaction conducted at 0^{o}C for 5h (see Supplementary material for HPLC analysis at 0^{o}C and rt).

The diquinone **3** is difficult to purify via flash chromatography due to formation of the aforementioned, green byproduct and broad elution from a hexane-ethyl acetate solvents system. The product was easily isolated by recrystallization from chloroform:hexane and isolated yields above 80% were routinely obtained. The work-up involves the same extraction of the *o*-iodobenzoic acid byproduct, drying the chloroform layer with magnesium sulfate, reducing the chloroform volume (50 mL/g of **3**) and adding hexane to the hot chloroform extract. This represents a convenient and efficient synthesis of **3** allowing further exploration of this novel electrophilic BPA metabolite.

A more convenient access to **2** that avoids flash chromatography and associated byproducts (*vide supra*) is shown in Scheme 3. After IBX oxidation of BPA, the monoquinone **2** is reduced to monocatechol **1**with sodium borohydride causing the red-colored quinone solution to become colorless in less than two minutes. After extraction of the *o*-iodobenzoic byproduct, **1**

is easily isolated by recrystallization in hot dichloromethane. The oxidation of **1** back to **2** can be conducted with silver oxide in acetone to furnish pure solutions of **2**. HPLC analysis of Ag_2O oxidation shows complete conversion of **1** to **2**in 20minutes at rt in either acetone or DMF solvent. A small-scale oxidation in acetone-*d6*, followed by ^1H NMR analysis, showed complete formation of **2**in high purity. The dicatechol **4**, recrystallized from chloroform:hexane, can be made in a similar manner as that of **1** using 3.0 equiv of IBX and 4.0 equiv of $NaBH_4$. Dicatechol **4** is also quantitatively oxidized to diquinone **3** using Ag_2O in either acetone or DMF.

Conclusion

A convenient, high yield synthesis of mono- or di-*o*-quinones of BPA using IBX has been developed. In addition, monocatechol **1** and dicatechol **2** can be made in high yield and conveniently isolated. Conversion of these catechols to their corresponding *o*-quinones occurs quantitatively using Ag_2O oxidation. This flexible synthetic approach to BPA metabolites should be of use to researchers investigating the toxicological properties of BPA.

Experimental

All reagents were commercial grade and used without further purification IBX was synthesized by the method of Frigerio,[22] CAUTION! IBX is explosive under impact if heated to more than 200^{o}C. NMR analysis was obtained on a Bruker 400MHz Avance III spectrometer. HPLC was conducted on a Waters 2690 Separations Module equipped with a Waters 2487 dual absorbance detector. Flash column purification of compounds was conducted using a Biotage Isoler One automated system.

Preparation of 4, 4,4'-(propane-2,2-diyl)bis(benzene-1,2-diol)

BPA (0.500 g, 2.190 mmol) was dissolved in 100mL of a solvent mixture consisting of 3:2 $CHCl_3$:CH_3OH in a 250 mL round bottom, equipped with a magnetic stir bar and brought to 0°C with an ice bath. To this solution was added IBX 1.840g (6.57 mmol) causing the solution to turn deep red after approximately 15min . The mixture was stirred at 0°C for 5h . While still at 0°C, $NaBH_4$ 0.322g (8.76 mmol) was added and after two minutes the solution turned colorless and 0.5mL of acetic acid was added to consume excess $NaBH_4$. All solvents were remove by rotovap evaporation and the mixture re-dissolved in 100mL of ethyl acetate. The solution was then transferred to 250mL separatory funnel and washed six times with 100mL of a buffer consisting of 400mL 0.1 M phosphate buffer, pH 6.0, 200mL of brine, and 50g of sodium dithionite. The organic layer was died over $MgSO_4$ and solvents removed. The crude was dissolved in 30mL of hot chloroform, made turbid with 5mL of hexane and kept overnight at -20°C, filtered through a sintered glass frit resulting in 0.477g (86%) of **4.** Powdery white solid; mp 146–148°C; R_f = 0.20 (EtOAc/hexane 1:1); ^{1}H NMR (acetone-*d6*, 400MHz): δ 1.53 (s, 6H), 6.61 (dd, J = 8.2 and 2.3Hz, 1H), 6.64 (d, J = 2.3Hz, 1H), 6.72 (d, J = 8.2Hz, 1H), 7.64 (bs, 3H); ^{13}C NMR (acetone-*d6*, 100MHz): δ 32.1, 42.8, 115.9, 116.0, 119.2, 144.1, 144.6, 145.7; HRMA (EI): calcd for $C_5H_{16}O_4$: 260.1049; found 260.1046.

Acknowledgment

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Supporting Information

Experimental details, ^{1}H and ^{13}C NMR spectra, HPLC traces and flash column chromatographs. This material can be found via the "Supplementary Content" section of this article's webpage.

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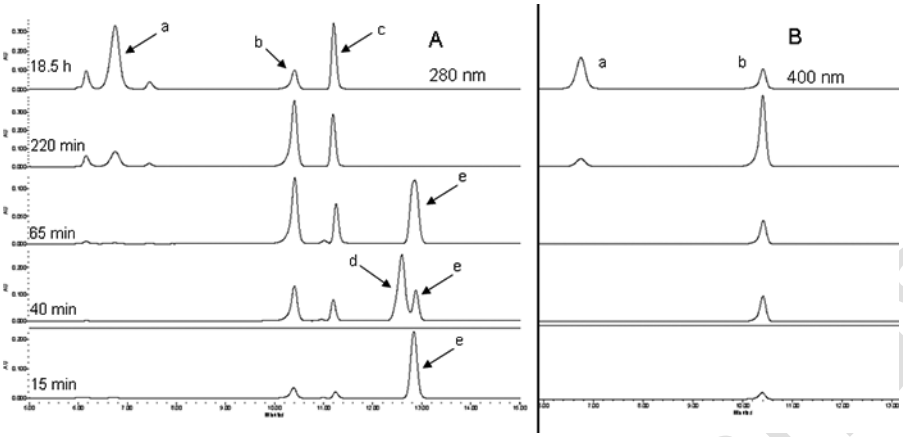
Table 1. Selective conversion of BPA to **2** at various temperatures using 2.05 equiv of IBX.

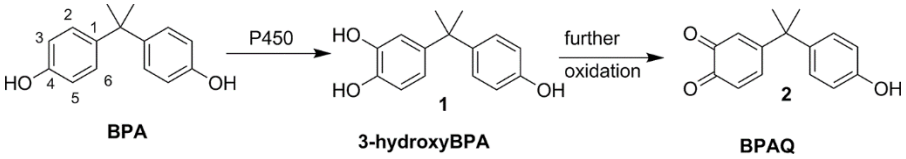
Temperature	Percent **3** of total quinone products **2** and **3**[a]			
	15 min	40 min	65 min	220 min
–20°C	0	0	0	13
0°C	0	1	25	67
rt	49	66	68	68

[a]Monitored at 400 nm

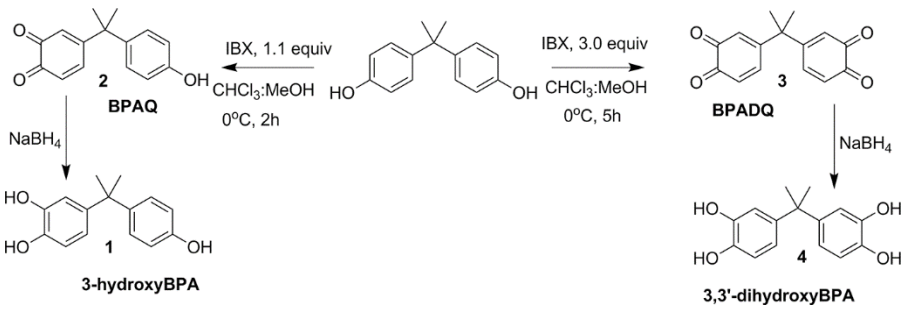
Figure 1. A. HPLC analysis of the reaction of BPA (100 mg) with 2.05 eq. of IBX (250 mg) in a 100mL solvent mixture of 3:2 CHC13:CH3OH at 0°C. Chromatographs monitored at 280 nm. a: BPADQ **(3), b:** BPAQ(2), c: o-iodobenzoic acid, **d:** BPA, e: unknown reaction intermediate. Instrument, Waters Alliance 2690: column, YMC-C18 ODS-AQ (5 µm, 10 × 250 mm); mobile phase, initial, 60% CH_3OH and 40% 0.5% acetic acid then linear gradient to 100% CH_3OH at 15 min, 3 ml/min. **B.** Same chromatographs monitored at 400 nm showing only quinone products.



Scheme 1. Metabolism of BPA to BPAQ.



Scheme 2. IBX controlled conversion of BPA to BPAQ, BPADQ, 3-hydroxyBPA and 3,3'-dihydroxyBPA.



Scheme 3. Conversion of BPA to 3-hydroxyBPA followed by silver oxide oxidation to monoquinone 2.