LIQUID CHROMATOGRAPHY/MASS SPECTROSCOPY AND QUANTUM CHEMICAL MODELING ANALYSIS OF AQUEOUS CHLORINATED BISPHENOL A. AN EVALUATION ON ESTROGEN RECEPTOR BINDING AFFINITY OF BYPRODUCTS

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Introduction

Phenolic compounds such as bisphenol A (BPA), alkylphenolic compounds, hydroxybiphenyl etc. have been demonstrated to have high estrogenicity or ER binding affinity[1)], and were detected in raw water in Japan[2]. To evaluate the endocrine disruption potential for residual phenolic compounds in raw water, it is necessary to identify thechlorination byproducts due to their higher reactivity. GC/MS and LC/MS methods are widely used to identify unknown compounds, however, it is difficult for the reaction of which mechanism is not clarified. LC/NMR method is expected to be of a valuable tool for this case; however, there are difficulties for the trace compound in a multi-component environmental sample. In this study, the APCI-LC/MS method and quantum chemical modeling analysis were used to investigate the reaction mechanism between bisphenol A and HOCl. Finally, the estrogen receptor binding affinities of aqueous chlorinated bisphenol A were detected.

Method and Material

Computational Chemistry. Ver.6 of MOPAC was used as adapted by CAChe Scientific Inc. (Oxford). The AM1 parameter was served to optimize stable and transition states (TS) structures. The program was used to obtain highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) energy and their density, optimum and transition stategeometries, atom partial charges, vibrational spectra and the intrinsic reaction coordinates (IRCs).

Chlorination procedures. The experiments were carried out in glass reactor which was placed in a water bath to keep the reaction temperature at 25°C. Synthetic raw water was prepared by dissolving 3.5 mg of a standard bisphenol A into 7 L Milli-Q pure water with pH of 7.5 adjusted by phosphoric acid. After taking out one liter, which was used to detect the estrogen receptor binding affinity before chlorination, HOCl (1.46 mg/l) was added to the above solution. The sample (1L) was taken out at 10, 30, 60min, respectively. After decomposing the residualHOCl by adding $Na_2S_2O_3$, the samples were concentrated by solid phase extraction (SPE-GLF, HP), and the final volume was made to 0.1ml(DMSO). The 2*f*Êl was used to detect the ER binding affinity using the assay as previously described[3)], and 2*f*Êl to analyze the byproduct using APCI-LC/MS (Model M-1200H, Hitachi, Japan). Bisphenol A was purchased from Kanto Chemical Co. (Tokyo, Japan).

Results and Discussion

Analysis of products by APCI-LC/MS. Table 1 shows the spectra of each peak. From the Table 1, it can be found that the A, D, F, and H peaks are corresponding the monochloro-, dichloro-, trichloro-, and tetrachloro-BPA, and peak C are corresponding the trichlorophenol. No way, however, can characterize their structures just based on the information from LC/MS analysis. According to the literature (4), the other peaks were postulated to be of dimmers of phenolic compounds, however, their structures could not also be located due to lack of mechanistic study.

Table 1 APCI-LC/MS Spectra of chlorination products of BPA

	RT	m/z (%, RI)		
		10min	30min	60min
A	**21.31**	**261(100%)**	-	-
B	22.71	311(100), 295(70%)	311(100%), 293(65%)	313(100), 295(65%)
C	23.30	-	197(100%)	197(100%)
D	**24.59**	**295(100%)**	**295(100%)**	**295(100%)**
E	26.0	278(100%), 347(15%)	278(100%), 347(15%)	278(100%), 347(15%)
F	**28.5**	**331(100%)**	**331(100%)**	**331(100%)**
G	29.49	381(100%), 363(60%)	381(100%), 363(60%)	381(100%), 363(60%)
H	**34.72**	**365(100)**	**365(100%)**	**365(100)**
I	51.2	421(100), 457(100)	491(100%), 455(20%), 331(100%)	491(100%), 455(20%), 331(20%)
J	60.38	457(100)	457(100%)	457(100%)
K	63.5		357(100%)	357(100%)
L	65.1		525(100%)	525(100%)

Quantum Chemical Analysis for Chlorination Pathways. To characterize the structures of byproducts and propose a feasible reaction pathway, the analysis based on the molecular orbital theory was carried out. According to the frontier orbital theory, the reaction is dominated by the interaction of HOMO and LUMO with the smallest difference in orbital energy. The C6 or C16 and C2 or C21 atoms with higher HOMO density as shown in Figure 1(a) will be attacked by Cl atom of HOCl with higher LUMO density due to the smallest HOMO-LUMO gap between the LUMO on HOCl and the HOMO on the bisphenol A. This figure simultaneously shows the atom partial charges of C atoms on the benzene ring of bisphenol A. The C4 or C19 will be a region susceptible to electrophilic attack due to its comparatively large and negative atom partial charge. To locate energetically feasible pathways, the activation energies when HOCl attack the C2 with the large negative atom

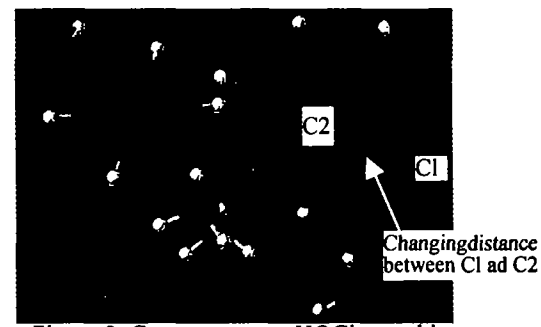

Figure 2 Geometry upon HOCl attacking on the C2 of bisphenol A

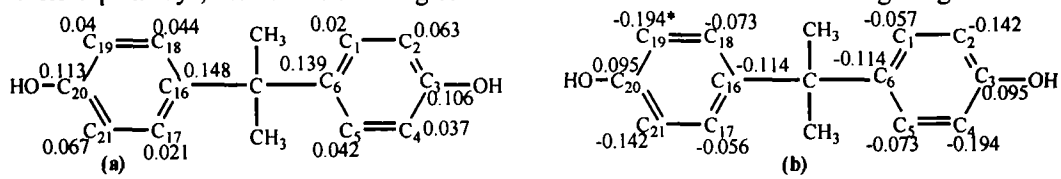

Figure 1 The HOMO density and atom partial charge of bisphenol A

partial charge and C6 with higher HOMO density were calculated.

The reaction coordinate of HOCl/BPA interaction involves the initial attack and subsequent dehydration. For this study the only the attack step is considered, and the geometry upon HOCl

attacking on the C2 atom was postulated as shown in Figure 2. The reaction coordinate was located using the *Optimized Search* method in MOPAC by changing the distance between C2 and Cl as shown in Figure 2. The structure at 3.6 Å distance with maximum energy at *minimum energy path* was used as possible TS, and was optimized by using *Minimize Gradient*. The vibration frequency calculation using the *Vibrational Spectrum (FORCE)* method showed this TS structure to have only one imaginary frequency which identify these TS is appropriate. Finally, the intrinsic reaction coordinate (IRC) calculations were performed with a positive, then negative, direction along the both negative energy and positive energy vibration mode which led to the expected reactants and products. The activated energy is 0.08kcal/mol and the product will be monochloro-BPA.

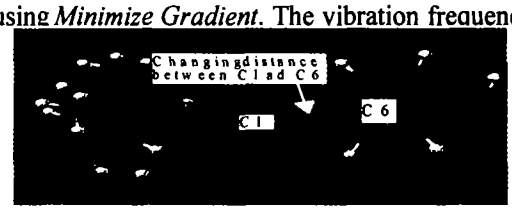

Figure 3 Geometry upon HOCl attacking on the C6 of bisphenol A

On the other hand, the postulated geometry where HOCl attacks on the C6 was shown in Figure 3. The transition state was also located using the similar to the above calculation. As a result, 4-(1-hydroxy-isopropyl) phenol (No.2 in Figure 5-1) and 4-chloro-phenol (No.3 in Figure 5-1) will be formed, and the activated energy was 0.04 kcal/mol, which is lower than that of the above reaction. The reaction where HOCl attacks on C6 is energetically more favorable than that where HOCl attacks on C2, which suggest the HOCl/BPA reaction is comparably dependant on the orbital overlap (chemical bond).

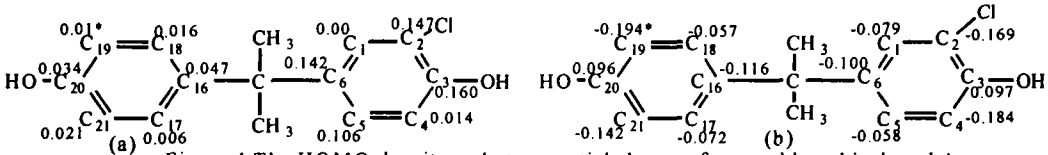

Figure 4 The HOMO density and atom partial charge of monochloro-bisphenol A

The three products formed in HOCl/BPA interaction will subsequently react with HOCl. To clarify the feasible reaction pathways, the similar computational chemistry analysis for the HOCl/monochloro-BPA interaction was also carried out. Figure 4 shows the HOMO density and atom partial charges of monochloro-BPA. The C6 or C5 is with higher HOMO density, and the C19, C4, and C21 with larger negative atom partial charges. To obtain energetically feasible pathways, the activation energies when HOCl attacks the C19 and C5 were calculated and the activated energy was 0.07 and 0.14 kcal/mol, respectively. It is clear that the Cl atom of HOCl attacking the C19 is energetically more favorable, and the atom partial charge may be of a main factor, which dominates the reaction.

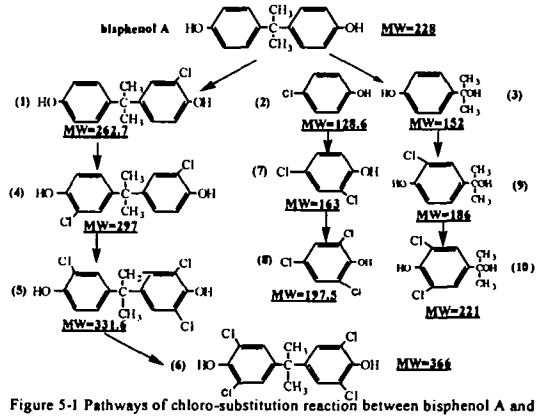

Figure 5-1 Pathways of chloro-substitution reaction between bisphenol A and HOCl

Based on a series of mechanistic calculation, the energetically feasible pathway was proposed as Figure 5-1. The products in the proposed pathway were located by the LC/MS analytical result as

shown in Table 1.

Formation of Polychlorinated phenoxyphenols. It was reported that polychlorinated phenoxyphenols (PCPP) would be formed in the reaction of phenol with hypochlorite in water. In this study, the mechanistic calculation could not be successes, but 6 kinds of polychlorinated phenoxyphenols in Figure 5-2 were proposed based on the pathways in Figure 5-1 and the LC/MS spectra in Table 1.

Effects of Chlorination on the Estrogen Receptor Binding Activity of BPA. It was found that the many kinds of products were formed in the chlorination of BPA. To evaluate the estrogen disruptor activity of BPA occurred in raw water for water supply, it is necessary to investigate the estrogen disruptor activity of their products. Figure 6 shows the variation of estrogen receptor binding activity with chlorination time. It was found that the binding activity was become to higher with increased reaction time. The EC50 was 11610(0 min.), 2992(10 min.), 1094(30 min.) and 473.9 (60min.) concentration times. The activity at 60 min. is as higher as 24 times than that before chlorination. In order to obtain further information on the estrogen disruptor compounds, it is necessary to analyze quantitatively estrogenicity of each byproduct.

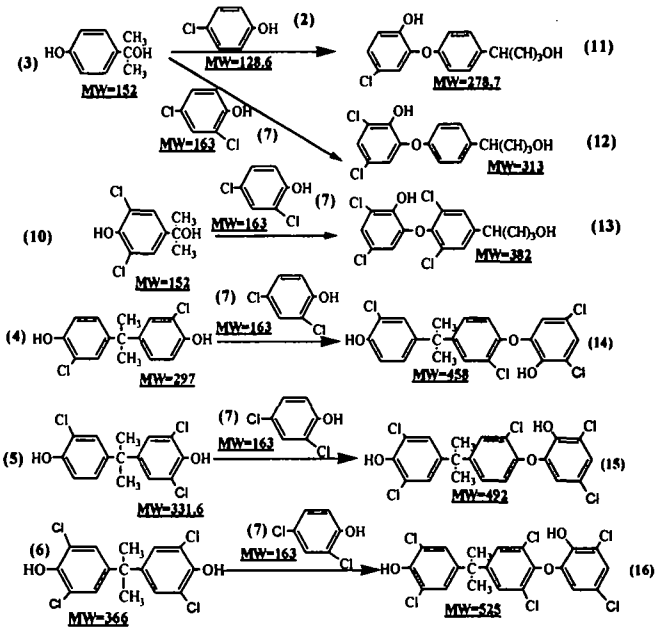

Figure 5-2 Formation of polychlorinated phenoxyphenols

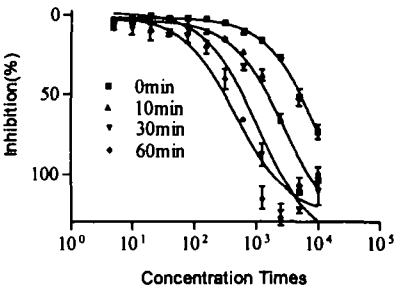

Figure 6 Effects of chlorinization time on the estrogen receptor binding affinity of bishphenol A

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