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CHLORINATION OF IRGASAN DP300 AND FORMATION OF DIOXINS FROM ITS CHLORINATED DERIVATIVES

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SUMMARY

Irgasan DP300 (2,4,4′-trichloro-2′-hydroxydiphenyl ether) (I), an antimicrobial agent for use with fabrics, was easily chlorinated with sodium hypochlorite to give 2′,3,4,4′-tetrachloro-2-hydroxydiphenyl ether (II), 2′,4,4′,5-tetrachloro-2-hydroxydiphenyl ether (III) and 2′,3,4,4′,5-pentachloro-2-hydroxydiphenyl ether (IV). Irgasan DP300 and its chlorinated derivatives were readily converted into polychlorinated dibenzo-*p*-dioxins (PCDDs) by heating: (1) Irgasan DP300 was converted into dichlorodibenzo-*p*-dioxin(s) (di-CDD, 42%); (2) II into two trichlorodibenzo-*p*-dioxins (tri-CDDs, 22%) and three tetrachlorodibenzo-*p*-dioxins (tetra-CDDs, 46%); (3) III into two tri-CDDs (44%), more than two tetra-CDDs (25%) and pentachlorodibenzo-*p*-dioxin(s) (penta-CDD, 1%); and (4) IV into two tetra-CDDs (16%), trace amounts of penta-CDD(s) and four hexachlorodibenzo-*p*-dioxins (hexa-CDDs, 40%).

Although UV irradiation of Irgasan DP300, II and III gave PCDDs, the amounts of PCDDs formed were much smaller than those obtained by heating. Moreover, PCDD was not detected in the UV irradiation of IV. The identified products suggested that disproportionation of chlorine atom(s) occurred in the photolysis.

INTRODUCTION

Irgasan DP300 (2,4,4′-trichloro-2′-hydroxydiphenyl ether) (I) is used as an antimicrobial agent for fabrics and as a bacteriostat for shampoo and toilet soap[1,2]. We have reported previously that Irgasan DP300 was detected in commercial textile products with a higher frequency than other agents[3].

It has been reported that the antibacterial activity of Irgasan DP300 in fabrics was reduced by bleaching with sodium hypochlorite[4] and the elimination and degradation of Irgasan DP300 by bleaching was postulated. This paper shows that Irgasan DP300 was easily chlorinated by bleaching with sodium hypochlorite to afford three chlorinated derivatives. Irgasan DP300 is regarded as a compound of low toxicity from studies on acute and subacute toxicity[5,6]. Although its percutaneous absorption[7] and metabolism[2] have also been studied, there are few reports on the toxicity of the three chlorinated derivatives.

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Nilsson *et al.*[8] reported on the formation of polychlorinated dibenzo-*p*-dioxins (PCDDs) by heating or UV irradiation of Irgasan DP300 and 2′,3,4,4′,5-pentachloro-2-hydroxydiphenyl ether (IV). In order to study the safety of Irgasan DP300 in detail, we carried out the identification and quantitation of the three chlorinated derivatives formed from Irgasan DP300 by bleaching with sodium hypochlorite. The conversion of the derivatives to PCDDs by heating and UV irradiation was also investigated.

EXPERIMENTAL

Fabrics

Of the commercial textile products treated with antimicrobial agents, fabrics in which Irgasan DP300 was detected[3] were used as samples.

Reagents

Irgasan DP300 was purchased from Ciba-Geigy. Sodium hypochlorite solution (10%) (Wako) was used after standardization of the available chlorine concentration by the method of the *Pharmacopoeia Japonica* XI[9]. Other reagents were of analytical-reagent grade.

Synthesis of chlorinated derivatives of Irgasan DP300

A 50-g amount of Irgasan DP300 was placed into a 500-ml flask and dissolved in 150 ml of chloroform, then chlorine was bubbled into the solution at the rate of 0.2 l/min. The reaction mixture was analysed every 20 min by high-performance liquid chromatography (HPLC). When the peak due to Irgasan DP300 disappeared from the chromatogram, the bubbling of chlorine was stopped and the solvent was evaporated. The resulting oily residue was dissolved in diethyl ether and the solvent was evaporated to remove residual chlorine. The mixture of chlorinated derivatives was subjected to preparative HPLC with methanol–water (7:3) as the eluent. The eluates were fractionated in 50-ml portions. Each fraction was analysed by thin-layer chromatography (TLC) on Kieselgel $60F_{254}$ aluminium sheets with light petroleum–diethyl ether (9:1) as the developing solvent and fractions exhibiting a single spot on TLC were collected and concentrated. Chlorinated derivatives were crystallized and recrystallized from light petroleum or *n*-hexane. The purity of each derivative was shown to be more than 99% by HPLC.

2′,3,4,4′-Tetrachloro-2-hydroxydiphenyl ether (II): yield, 8.3 g (14.8%); m.p., 91°C. NMR (C^2HCl_3): δ 6.65 (1H, d, J = 8.8 Hz, 6-H), 6.91 (1H, d, J = 8.8 Hz, 5-H), 6.98 (1H, d, J = 8.8 Hz, 6′-H), 7.23 (1H, d–d, J = 2.0, 8.8 Hz, 5′-H), 7.49 (1H, d, J = 2.0 Hz, 3′-H). Mass spectrum (MS): m/z 322 (M^+).

2′,4,4′,5-Tetrachloro-2-hydroxydiphenyl ether (III): yield, 10.0 g (17.9%); m.p., 76°C. NMR (C^2HCl_3): δ 6.75 (1H, s, 6-H), 7.01 (1H, d, J = 8.8 Hz, 6′-H), 7.16 (1H, s, 3-H), 7.28 (1H, d–d, J = 2.4, 8.8 Hz, 5′-H), 7.51 (1H, d, J = 2.4 Hz, 3′-H). MS: m/z 322 (M^+).

2′,3,4,4′,5-Pentachloro-2-hydroxydiphenyl ether (IV): yield, 20.8 g (33.6%); m.p., 104°C. NMR (C^2HCl_3): δ 6.67 (1H, s, 6-H), 6.98 (1H, d, J = 8.8 Hz, 6′-H), 7.28 (1H, d–d, J = 2.4, 8.8 Hz, 5′-H), 7.51 (1H, d, J = 2.4 Hz, 3′-H). MS: m/z 356 (M^+).

PCDD standards for quantitation

Trichlorodibenzo-*p*-dioxin (1,2,4-tri-CDD) and tetrachlorodibenzo-*p*-dioxin (2,3,7,8-tetra-CDD) were purchased from Gasukuro Kogyo.

Dichlorodibenzo-p-dioxin (di-CDD). A mixture of 20 g of Irgasan DP300 and 3 g of sodium hydroxide was heated in a 200-ml flask equipped with an air cooler. When the mixture became slightly colored, heating was stopped. After cooling, the residue was extracted with *n*-hexane and the solvent was evaporated to yield a colourless solid. Crystallization of the solid from *n*-hexane yielded a colourless product (amorphous); yield, 2.9 g (18.8%); purity, 99.5% (HPLC). MS: *m/z* 252 (M^+). Although capillary gas chromatography–mass spectrometry (capillary GC–MS) of the product showed a single peak at a retention time t_R of 11.6 min, its NMR data indicated a 3:1 mixture of two isomers. The mixture is assumed to consist of 2,8- and 2,7-di-CDD, which were formed by the Smiles rearrangement[10,11].

Hexachlorodibenzo-p-dioxin (hexa-CDD). Hexa-CDD, which had t_R = 27.8 min on capillary GC–MS, was separated by HPLC from the pyrolysate of IV. Crystallization and recrystallization of separated hexa-CDD from benzene yielded white needles; yield, 4 mg; purity, 98% (HPLC); m.p., 291°C (decomposition) (sealed capillary). NMR (C^2HCl_3): δ 7.11 (s). MS: *m/z* 388 (M^+). These physical and spectral data agreed well with those of 1,2,3,6,7,8-hexa-CDD reported previously[12,13].

HPLC conditions

Analysis of Irgasan DP300 and its chlorinated derivatives. A Shimadzu 3A HPLC system equipped with a Shimadzu R-3A Chromatopac was used with a TSK gel ODS-80TM stainless-steel column (250 mm × 4 mm I.D.). The mobile phase was acetonitrile–water–acetic acid (60:40:0.1) at a flow-rate of 1 ml/min. The temperature was 37°C and the wavelength was 240 nm.

Analysis and separation of dioxins. Stainless-steel columns of Nucleosil $5C_{18}$ (250 mm × 4 mm I.D. for analysis, 250 mm × 12 mm I.D. for separation) were used. The mobile phase was acetonitrile–water (4:1) at a flow-rate of 1.5 ml/min (analysis) or 7 ml/min (separation). The temperature and wavelength were as above.

Conditions for preparative HPLC

A Waters PrepLC/System 500 was used with a PrepPAK-500/C_{18} column (340 mm × 60 mm I.D.) and methanol–water (7:3) as the mobile phase at a flow-rate of 100 ml/min.

Conditions for capillary GC–MS

A Shimadzu DF2000 GC–MS system was used with an OV-1 FS-WCOT capillary column (25 m × 0.35 mm I.D.) and temperature programming from 120 to 240°C at 4°C/min. The injection temperature was 260°C and the separator temperature 280°C. The carrier gas was helium (1 kg/cm^2).

Quantitation of PCDDs

Amounts of di-, tri-, tetra- and hexa-CDDs were determined by gas chromatography with flame ionization detection (GC–FID). Pentachlorodibenzo-*p*-dioxin (penta-CDD) was quantified as tetra-CDD.

A Hitachi 073 GC–FID system equipped with a Shimadzu C-R1B Chroma-

topac was used with a 2% OV-1 on Chromosorb W AW DMCS glass column (2 m × 3 mm I.D.). The column temperature was programmed from 220 to 250°C at 3°C/min. The injection temperature was 260°C. The carrier gas was nitrogen at 40 ml/min.

NMR

A JEOL FX-100 NMR spectrometer was used with C^2HCl_3 or benzene-d_6 as solvent.

Chlorination of Irgasan DP300 in fabrics

A 1.0-g amount of fabric cut into pieces smaller than 1 cm^2 was placed in a 50-ml test-tube equipped with a cap, then 20 ml of sodium hypochlorite solution containing 0.02 or 0.2% of available chlorine were added and the tube was heated at 45°C for 30 min. After filtration through a glass filter (3G2), the fabric was washed with 50 ml of distilled water. The fabric and the filtrate were analysed as follows.

(a) The fabric was refluxed with 20 ml of methanol–acetic acid (9:1) for 30 min and filtered through a glass filter (3G2). The equipment and fabric were washed with 50 ml of methanol, then the filtrate and washings were combined and concentrated to 5 ml with a rotary evaporator. The concentrate was transferred into a 50-ml test-tube equipped with a cap and shaken with three 10-ml portions of diethyl ether after the addition of 10 ml of 0.1 *M* hydrochloric acid. The ether layer was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The residue was dissolved in 2 ml of methanol and analysed by HPLC.

(b) The filtrate and washings were transferred into a 100-ml separating funnel, then 1 ml of 20% hydroxylammine hydrochloride and 2 ml of 1 *M* hydrochloric acid were added and shaken with three 20-ml portions of diethyl ether. The ether layer was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The residue was dissolved in 2 ml of methanol and analysed by HPLC.

Pyrolysis

A 100-mg amount of Irgasan DP300 or its chlorinated derivative was placed in a glass bulb (25 mm diameter) fitted with an air cooler (40 cm × 6 mm I.D.) and heated at 400°C for 10 min on a heating mantle filled with glass-wool. After cooling, the pyrolysate was dissolved in benzene or diethyl ether. The aliquot was mixed with ethereal diazomethane and allowed to stand overnight. The mixture was evaporated to dryness. The residue was dissolved in a suitable volume of benzene and was analysed by capillary GC–MS.

UV irradiation

A 1-mg amount of Irgasan DP300 or its chlorinated derivative was dissolved in diethyl ether and placed on a glass plate (9 cm diameter). The solvent was evaporated at room temperature and the resulting thin layer was irradiated with a UV lamp (3.2 $J/m^2 \cdot s$) for 20 h. The photolysate was dissolved in ethereal diazomethane and treated as described above.

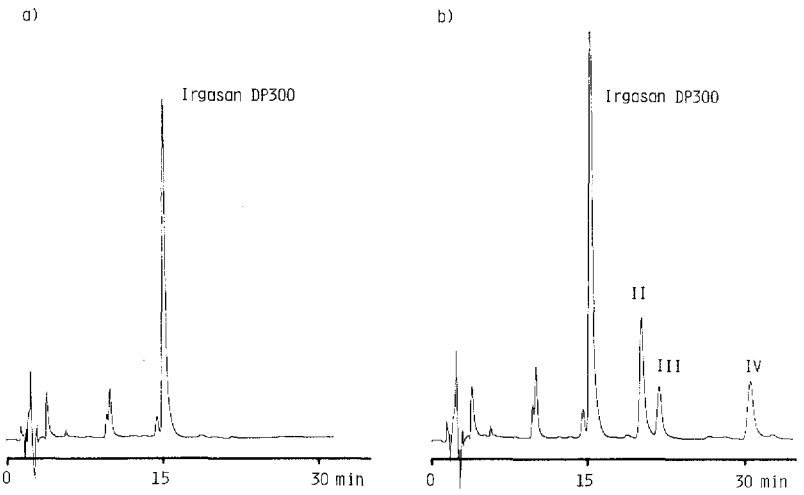

Fig. 1. HPLC traces for Irgasan DP300 and its derivatives. (a) Irgasan DP300 extracted from a commercial textile product; (b) three derivatives formed by bleaching with sodium hypochlorite solution containing 0.2% of available chlorine at 45°C for 30 min.

RESULTS

Identification of three chlorinated derivatives of Irgasan DP300

Fig. 1a shows a high-performance liquid chromatogram of Irgasan DP300 extracted from a commercial textile product (socks). When the textile was treated with sodium hypochlorite solution containing 0.2% of available chlorine at 45°C for 30 min, three peaks (II, III and IV) appeared on the chromatogram (Fig. 1b).

These three peaks were fractionated by HPLC and analysed by capillary GC–MS after methylation with diazomethane. Their mass spectra are shown in Fig. 2. The O-methyl derivative of Irgasan DP300 showed the molecular ion (M^+) peak at m/z 302. The M^+ peaks of methylated II and III were observed at m/z 336. Therefore, II and III were assumed to be formed by the substitution of a chlorine atom for a hydrogen atom in Irgasan DP300. On the other hand, the M^+ peak of methylated IV was found at m/z 370, indicating that IV is formed by the substitution of two chlorine atoms for two hydrogen atoms in Irgasan DP300. In order to elucidate the structures of II, III and IV, these three chlorinated derivatives were synthesized on a large scale by chlorination of Irgasan DP300 with chlorine. II was identified as 2′,3,4,4′-tetrachloro-2-hydroxydiphenyl ether by the absence of the 3′-proton of Irgasan DP300 in its NMR spectrum. Similarly, III and IV were identified as 2′,4,4′,5-tetrachloro-2-hydroxydiphenyl ether and 2′,3,4,4′,5-pentachloro-2-hydroxydiphenyl ether, respectively (Fig. 3). Further, the mass spectrum and t_R in the HPLC and capillary GC of II, III and IV obtained on bleaching agreed completely with those of the chlorinated derivatives synthesized.

Quantitation in fabrics of chlorinated derivatives formed from Irgasan DP300

Table I shows the amounts of the three chlorinated derivatives that were formed from Irgasan DP300 on bleaching with sodium hypochlorite solution con-

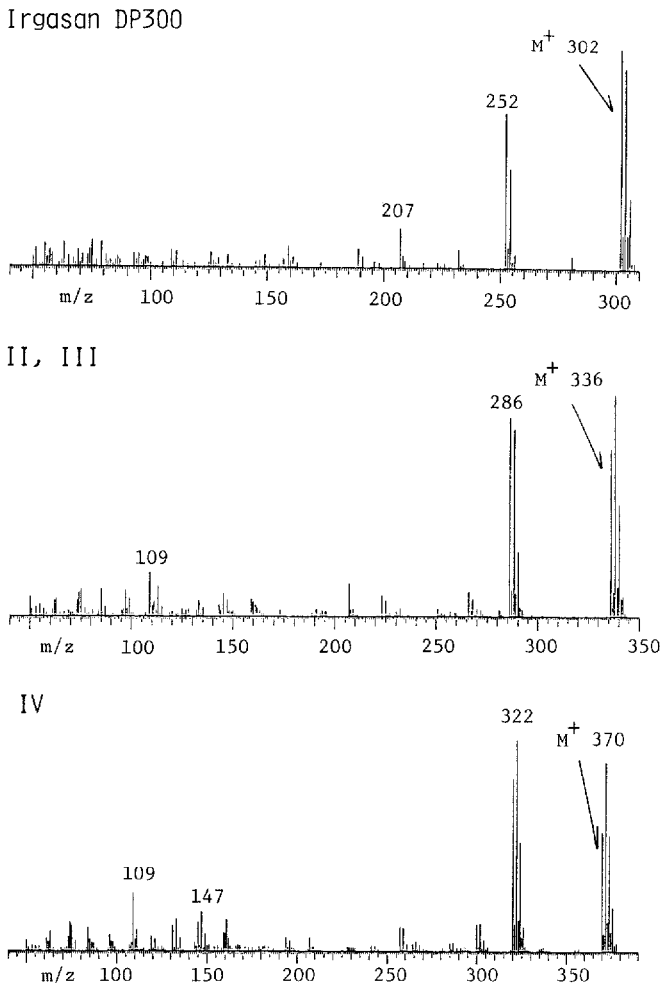

Fig. 2. Mass spectra of Irgasan DP300 and its derivatives after methylation.

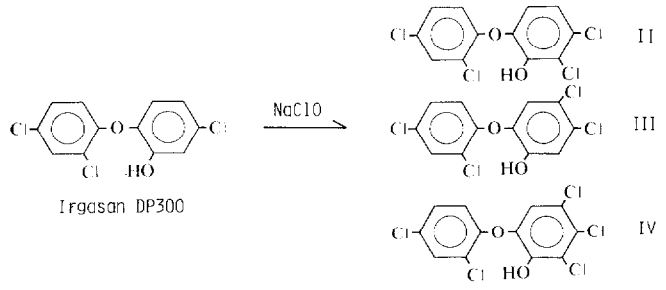

Fig. 3. Structures of Irgasan DP300 (I) and its chlorinated derivatives. II = 2′,3,4,4′-tetrachloro-2-hydroxydiphenyl ether; III = 2′,4,4′,5-tetrachloro-2-hydroxydiphenyl ether; IV = 2′,3,4,4′,5-pentachloro-2-hydroxydiphenyl ether.

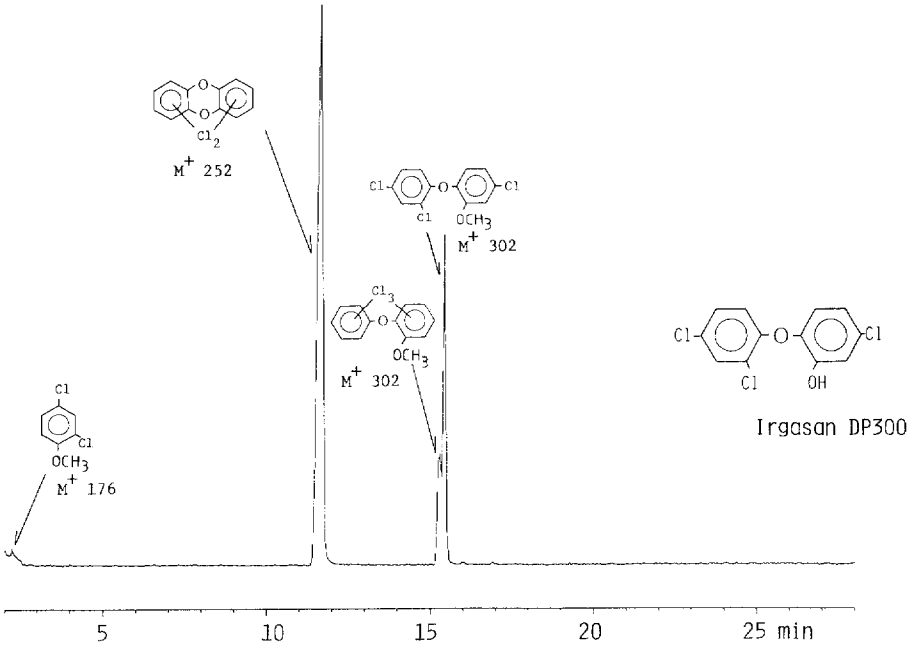

Fig. 4. TIM chromatogram of pyrolysate of Irgasan DP300 obtained by capillary GC–MS.

taining 0.02 and 0.2% of available chlorine at 45°C for 30 min. The available chlorine concentration in domestic sodium hypochlorite bleaching agent is 0.02% when it is ordinarily used for removal of a stain. Three chlorinated derivatives were detected in all samples tested after bleaching and the amounts were not proportional to the available chlorine concentration. As II and III were detected in sample No. 5 without bleaching, they may be formed during the treatment of fabrics with Irgasan DP300.

Formation of PCDDs by heating

Fig. 4 shows the total ion monitoring (TIM) chromatogram of pyrolysed Irgasan DP300 measured by capillary GC–MS. The major product was di-CDD(s), with t_R = 11.6 min and an M^+ peak at *m/z* 252. Under the conditions employed in this study, it was not able to separate 2,7- and 2,8-di-CDD. Irgasan DP300 was detected at 15.5 min (M^+ peak *m/z* 302) and its isomer, showing a similar fragmentation and M^+ peak to Irgasan DP300, was observed at 15.3 min. A trace amount of 2,4-dichlorophenol was also detected.

Fig. 5 shows the TIM chromatogram of the pyrolysate of II. Two peaks of tri-CDDs showing an M^+ peak at *m/z* 286 were observed at 15.3 and 16.2 min. The major peak at 16.2 min may be due to 1,2,8-tri-CDD derived from II. Further, at least three isomers of tetra-CDDs, which give an M^+ peak at *m/z* 320, were detected. A trace amount of residual II (methyl ether) was observed (18.5 min).

The TIM chromatogram of pyrolysed III is shown in Fig. 6. The major product may be 2,3,7-tri-CDD having an M^+ peak at *m/z* 286. Moreover, a small amount of tri-CDD was detected at 16.3 min. The peak of tetra-CDD at 20.3 min was broad, suggesting that more than two isomers contribute to the peak. 2,3,7,8-Tetra-CDD,

TABLE I

FORMATION OF II, III AND IV FROM IRGASAN DP300 BY BLEACHING WITH SODIUM HYPOCHLORITE

Bleaching was carried out at 45°C for 30 min. The results were obtained by HPLC.

Sample No.	*Amount (μg/g)*												
	Untreated					*Treated with 0.02% available chlorine*				*Treated with 0.2% available chlorine*			
	Irgasan	*II*	*III*	*IV*		*Irgasan*	*II*	*III*	*IV*	*Irgasan*	*II*	*III*	*IV*
1	1320	N.D.*	N.D.	N.D.	Fabric	495	18	14	67	474	20	10	62
					Aqueous	102	35	8	55	203	18	7	26
2	365	N.D.	N.D.	N.D.	Fabric	152	4	3	48	234	14	10	55
					Aqueous	2	6	1	22	48	7	2	13
3	367	N.D.	N.D.	N.D.	Fabric	5	N.D.	N.D.	5	5	N.D.	N.D.	3
					Aqueous	N.D.	6	N.D.	2	2	N.D.	N.D.	1
4	493	N.D.	N.D.	N.D.	Fabric	530	3	2	8	441	25	36	18
					Aqueous	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1
5	515	2	3	N.D.	Fabric	197	9	6	34	130	12	11	38
					Aqueous	N.D.	N.D.	N.D.	4	N.D.	N.D.	N.D.	1

* N.D. = not detected (< 1 μg/g).

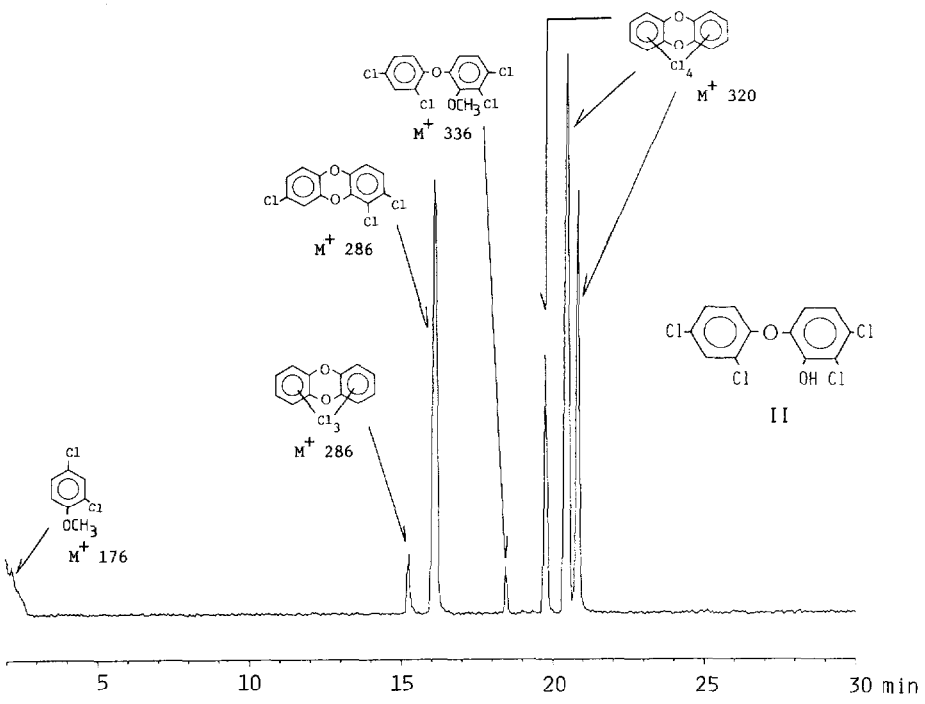

Fig. 5. TIM chromatogram of pyrolysate of II obtained by capillary GC–MS.

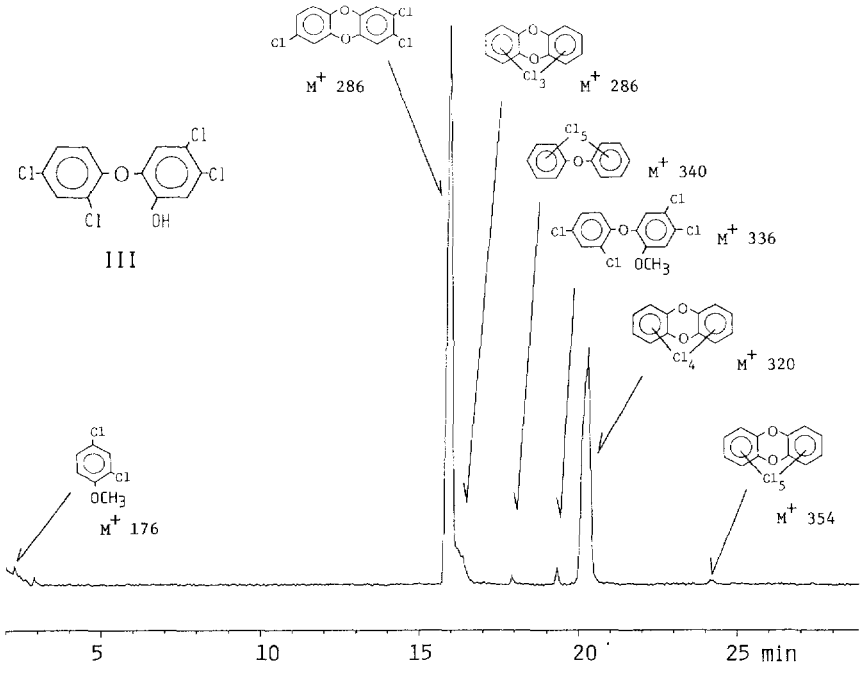

Fig. 6. TIM chromatogram of pyrolysate of III obtained by capillary GC–MS.

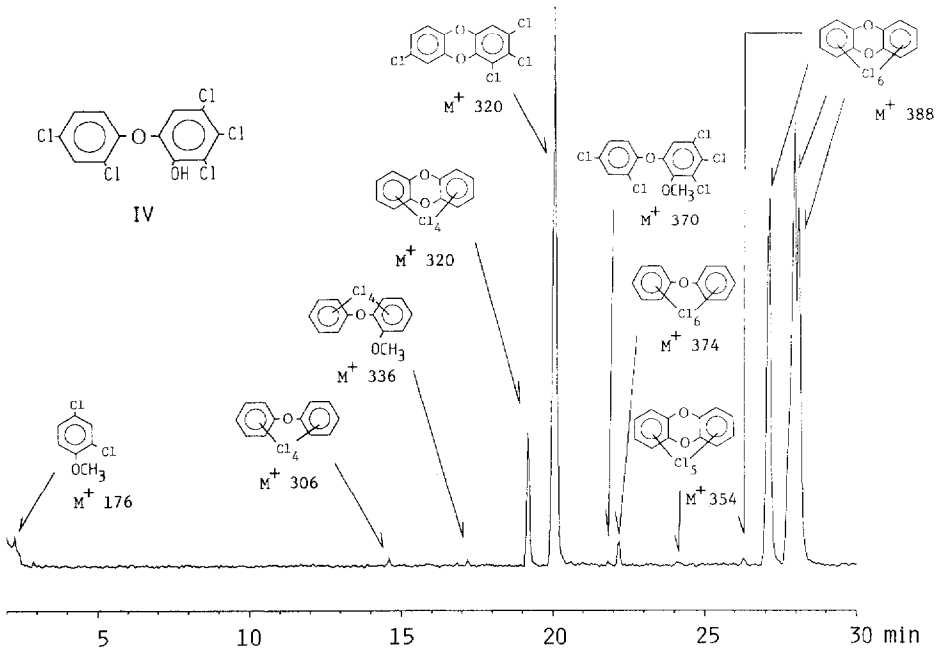

Fig. 7. TIM chromatogram of pyrolysate of IV obtained by capillary GC–MS.

the most toxic PCDD[14], had a retention time of 20.2 min under the same conditions. Small amounts of penta-CDD(s) (M^+ peak *m/z* 354, 24.2 min), residue of III (19.3 min) and pentachlorodiphenyl ether (M^+ peak *m/z* 340, 17.9 min) were also detected.

The TIM chromatogram of pyrolysed IV is shown in Fig. 7. Two peaks of tetra-CDDs were observed at 19.2 and 20.1 min. A major peak at 20.1 min may be

TABLE II

PYROLYSIS OF IRGASAN DP300 AND ITS CHLORINATED DERIVATIVES

The results were obtained by GC–FID. Penta-CDD was quantified as tetra-CDD. Values are averages of two experiments, with the range of the data in parentheses.

Compound	*Dioxin formed (%)**					
	Di-CDD	*Tri-CDD*	*Tetra-CDD*	*Penta-CDD*	*Hexa-CDD*	*Total*
Irgasan DP300	42 (39–44)					42
II		22 [2]** (17–26)	46 [3] (44–47)			68
III		44 [2] (31–56)	25 [2] (23–27)	1 (trace–1)		70
IV			16 [2] (11–20)	Trace	40 [4] (35–45)	56

* Calculated on the initial amounts of Irgasan DP300 and its derivatives.

** Values in brackets are the numbers of isomers detected.

assignable to 1,2,3,8-tetra-CDD. The amount of penta-CDD(s) was a trace, whereas four peaks of hexa-CDDs were observed at 26.2, 27.1, 27.8 and 28.1 min. Hexa-CDD of t_R = 27.8 min was purified by HPLC and crystallized from benzene; the melting point and the mass and NMR spectra agreed well with those of 1,2,3,6,7,8-hexa-CDD reported[12,13].

The quantitative analysis of PCDDs formed by heating was carried out by GC–FID and the results obtained are summarized in Table II; 42% of Irgasan DP300 was converted into di-CDD(s). II was converted into tri- and tetra-CDDs into extents of 22 and 46%, respectively. III was converted into tri-, tetra- and penta-CDD(s) to extents of 44, 25 and 1%, respectively. Further, IV was converted into tetra- and hexa-CDDs to extents of 16 and 40%, respectively. These results indicated that the three chlorinated derivatives of Irgasan DP300 are readily converted into various PCDDs by heating.

Formation of PCDDs by UV irradiation

Nilsson *et al.*[8] reported that PCDD(s) was formed from Irgasan DP300 only by heating, although it was formed from IV by both heating and UV irradiation in methanol. We therefore carried out UV irradiation (3.2 J/m² · s) of thin layers of Irgasan DP300 and chlorinated derivatives for 20 h. The photolysates were analysed by capillary GC–MS after methylation with diazomethane. Fig. 8 shows the TIM chromatogram of the photolysate of Irgasan DP300. The residue of Irgasan DP300 (19%, the average of two experiments) was observed as a major peak (15.5 min). Only 1% of di-CDD(s) was detected at 11.6 min. Further, a dechlorinated product

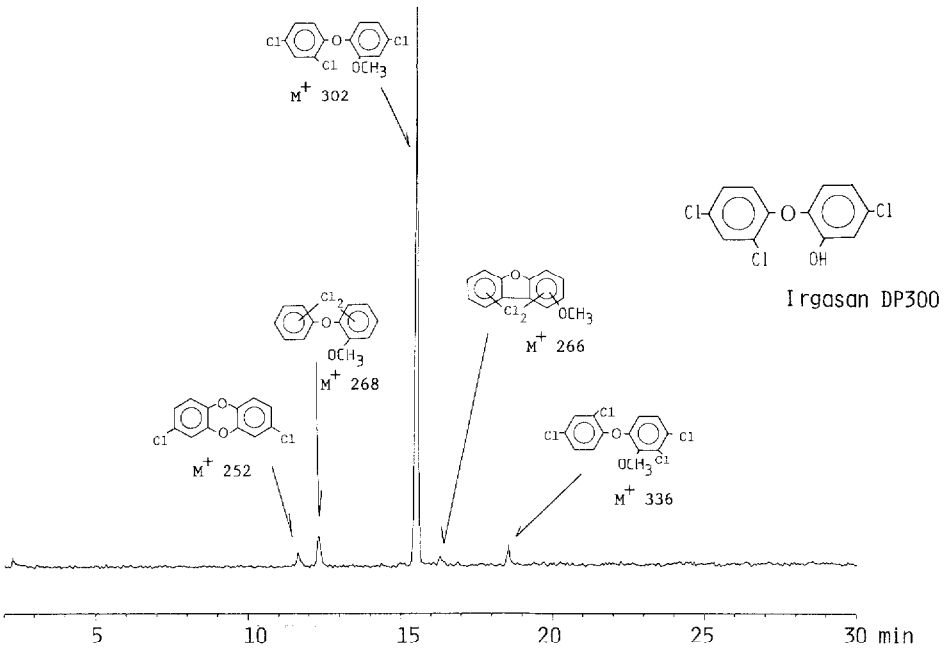

Fig. 8. TIM chromatogram of photolysate of Irgasan DP300 obtained by capillary GC–MS.

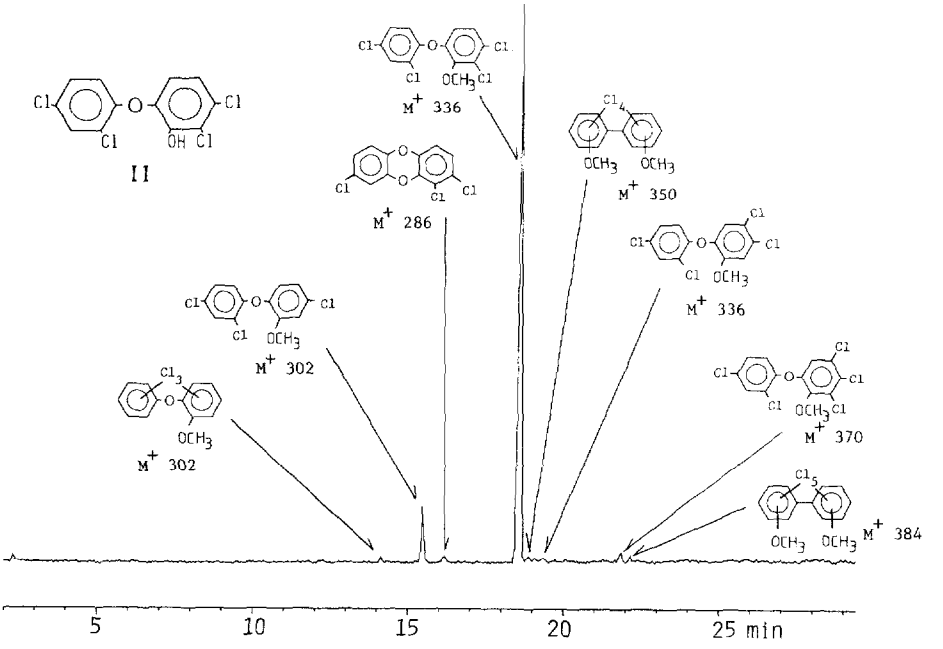

Fig. 9. TIM chromatogram of photolysate of II obtained by capillary GC–MS.

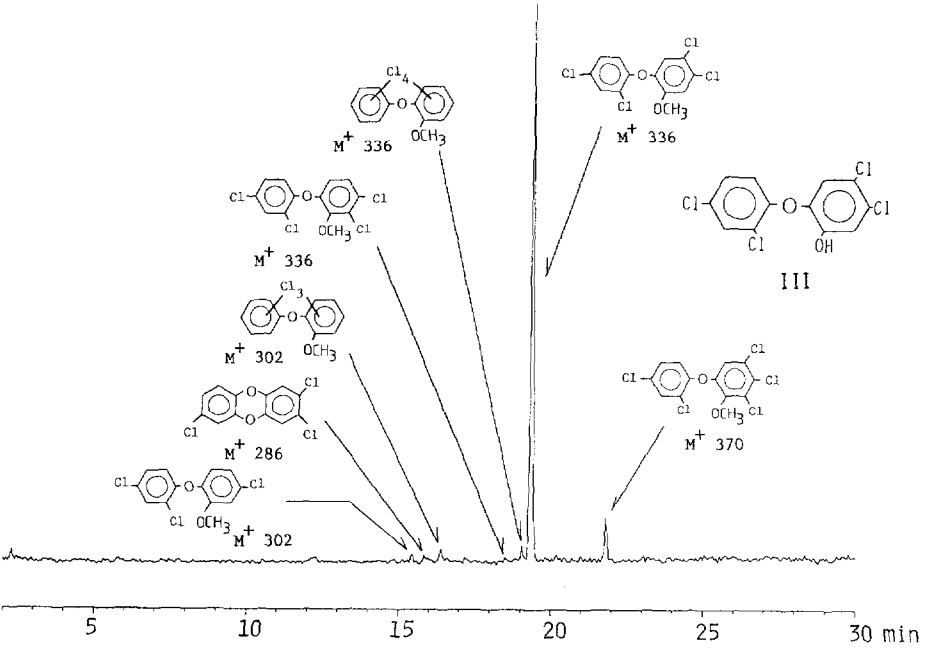

Fig. 10. TIM chromatogram of photolysate of III obtained by capillary GC–MS.

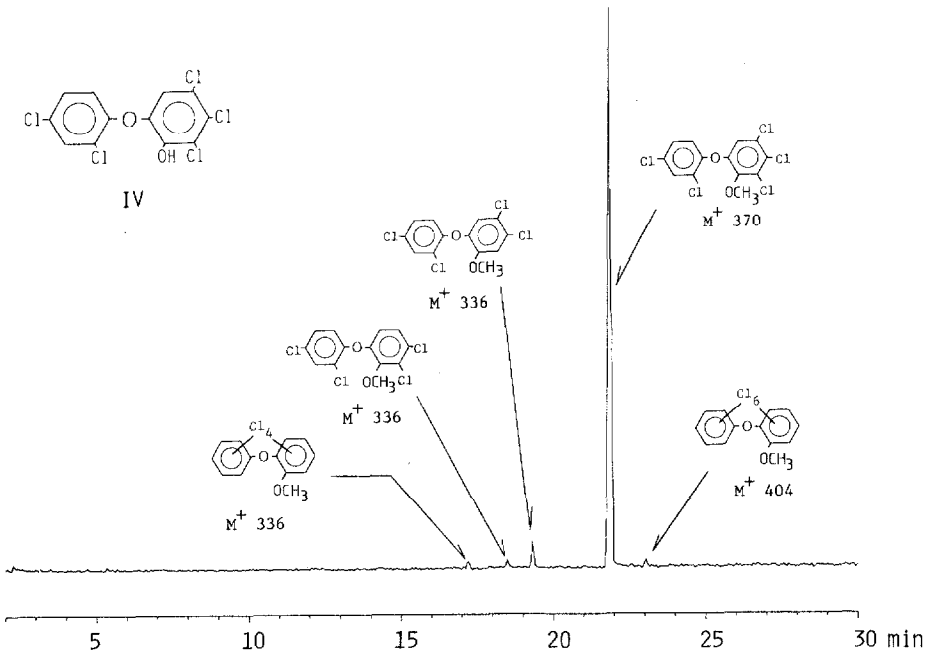

Fig. 11. TIM chromatogram of photolysate of IV obtained by capillary GC–MS.

of Irgasan DP300 (12.3 min, M^+ peak *m/z* 268), dichlorohydroxydibenzofuran (16.3 min, M^+ peak *m/z* 266) and II (18.5 min) were also detected.

The TIM chromatogram of the photolysate of II is shown in Fig. 9. The major peak (18.5 min) was due to the residue of II (16% of starting amount). Irgasan DP300 (15.5 min) was formed in about a 2% yield by dechlorination of II. Tri-CDD(s) was detected at 16.1 min (M^+ peak *m/z* 286) but only in a trace amount. Other detectable products were III, IV, tetrachlorodihydroxybiphenyl (18.9 min, M^+ peak *m/z* 350) and pentachlorodihydroxybiphenyl (22.1 min, M^+ peak *m/z* 384).

Fig. 10 shows the TIM chromatogram of the photolysate of III. The residue of III (19.3 min) was 12% of the starting amount and trace amounts of tri-CDD(s) were detected at 15.8 min. Other detectable products were Irgasan DP300, an isomer of Irgasan DP300 (16.4 min), II, an isomer of II or III (19.0 min) and IV.

The TIM chromatogram of the photolysate of IV is shown in Fig. 11. The major peak (21.9 min) was due to the residue of IV (22% of the starting amount). Detectable products were II, III, an isomer of II or III (17.2 min) and hexachlorohydroxydiphenyl ether (23.1 min, M^+ peak *m/z* 404). However, the formation of PCDD was not confirmed.

DISCUSSION

This work has shown that Irgasan DP300 in fabrics is easily chlorinated by sodium hypochlorite to give 2′,3,4,4′-tetrachloro-2-hydroxydiphenyl ether (II), 2′,4,4′,5-tetrachloro-2-hydroxydiphenyl ether (III) and 2′,3,4,4′,5-pentachloro-2-hy-

droxydiphenyl ether (IV). The formation of IV was especially notable. Of the commercial textile products treated with Irgasan DP300[3], only the half were labelled as "No bleaching with sodium hypochlorite". If the textile products had been used, it was difficult to distinguish between normal textile products and these treated with Irgasan DP300. Because there is every probability that the textile products treated with Irgasan DP300 are bleached with sodium hypochlorite, it is important to study the toxicities and biological effects of the three chlorinated derivatives.

Chlorinated phenols are known to be readily converted into PCDDs by heating[14–16]. Although 2,8-di-CDD derived from Irgasan DP300 was reported to be a low-toxicity dioxin[17], it is important to establish whether more toxic PCDDs (further chlorinated at the 3- and 7-positions) are formed or not under various conditions for the assessment of the safety of Irgasan DP300. Nilsson *et al.*[8] reported that Irgasan DP300 and IV (which was synthesized by Friedel–Crafts reaction from Irgasan DP300) were converted into 2,8-di-CDD and 1,2,3,8-tetra-CDD, respectively, by heating, and that the amounts of PCDDs formed by heating at 360–980°C for 15 s were 0–6.2%. When Irgasan DP300 and the three chlorinated derivatives were heated at 400°C for 10 min, Irgasan DP300 itself was converted only into di-CDD(s). However, the three chlorinated derivatives were converted into various PCDDs, *i.e.*, II was converted into two tri-CDDs and three tetra-CDDs, III produced two tri-CDDs, more than two tetra-CDDs and penta-CDD(s), and IV gave two tetra-CDDs, penta-CCD(s) and four hexa-CDDs. Under these conditions, Irgasan DP300, II, III and IV were converted into PCDDs to extents of 42, 68, 69 and 65%, respectively.

Further chlorinated PCDDs were also formed from the three chlorinated derivatives by pyrolysis. Lamparski and Nestrick[18] reported that 2,3,7,8-tetra-CDD was easily converted into hexa-, hepta- and octa-CDDs by surface chlorination with chlorine at 100°C for 30 min. Similarly, the further chlorination observed in this study may occur after conversion to PCCDs. If II or III was further chlorinated, primarily IV would be formed by *ortho-* and *para*-directing effects of the hydroxyl group and finally 1,2,3,8-tetra-CDD would be detected. However, 1,2,3,8-tetra-CDD was not detected in the pyrolysates of II and III.

It has been reported that the substitution of chlorine atoms at the 2-, 3-, 7- and 8-positions of the dibenzo-*p*-dioxin molecule is closely associated with the appearance of toxicity of PCDDs[19,20]. The pyrolysis studies suggested the possibility of the formation of highly toxic dioxins chlorinated at all the 2-, 3-, 7- and 8-positions. In fact, one of the hexa-CDDs formed from IV by pyrolysis was considered to be as 1,2,3,6,7,8-hexa-CDD. Therefore, the isomer-specific determination of the further chlorinated PCDDs is required for studies on the toxicity of the three chlorinated derivatives.

Although UV irradiation of Irgasan DP300, II and III gave PCDDs, the amounts of PCDDs formed were small or trace. In addition, the formation of tetra-CDD was not observed in the photolysis of IV (even if photolytic conversion occurred, it was less than 0.1% of the starting amount). These results on the formation of PCDDs by UV irradiation are different from those obtained by Nilsson *et al.*[8] in methanol solution (they detected PCDD in the photolysate of IV but not in that of Irgasan DP300). Most products identified in the photolysates suggested the disproportionation of chlorine atom(s).

Irgasan DP300 has been used in domestic products without restriction and

some used textile products treated with Irgasan DP300 have ultimately been incinerated. This appears to constitute a serious problem because the three chlorinated derivatives, which were readily converted into PCDDs by heating, are formed from Irgasan DP300 by bleaching with sodium hypochlorite.

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