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Synthesis of Octadecachloroquaterphenyls and the Ratio of Six Types of Polychlorinated Quaterphenyl Isomers in the Blood of "Yusho" Patients

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Six types of octadecachloroquaterphenyls (ODCQPs) were prepared by chlorination of the corresponding quaterphenyls (QPs), and the QP skeletons of polychlorinated quaterphenyls (PCQs) in the blood of "Yusho" patients were investigated. The results are as follows: (1) The six types of ODCQPs showed characteristic mass spectra. (2) PCQs in the blood of Yusho patients were found to be a mixture of six types of PCQ isomers by gas chromatography and mass spectrometry. (3) The ratios of six types of PCQ isomers in the Kanechlor 400 used as the thermotransfer medium and in the blood of Yusho patients were different.

Keywords——octadecachloroquaterphenyl; polychlorinated quaterphenyl; quaterphenyl; Yusho patient; Kanechlor 400

Recently, Miyata *et al.*[1,2] reported the presence of polychlorinated quaterphenyls (PCQs) in an amount equal to that of PCBs in the rice oil implicated as the causative agent of the disease "Yusho." PCQs are still detectable in the blood of patients who had ingested the causal rice oil 14 years ago, but little is detectable in the blood of ordinary persons.[3] Thus, PCQs are of great interest in relation to the etiology of Yusho, together with polychlorinated dibenzofurans (PCDF), *etc.*

PCQs are dimers of PCBs, and have six skeletal isomers and more than 100000 chlorine-bonding isomers theoretically. PCQs in the blood of patients are estimated quantitatively by ECD-GC after perchlorination, by using PCQ obtained from used Kanechlor 400 as the standard.[2,4] However, the gas chromatograms of the standard PCQs after perchlorination were different from those of patients' blood. Thus, in order to determine PCQs more accurately and to evaluate their toxicity, it is necessary to elucidate the structures of PCQs in the blood of patients and standard PCQs. In this paper, we report the synthesis of six types of octadecachloroquaterphenyls (ODCQPs), and the investigation of the skeletons of standard PCQs and PCQs in the blood of Yusho patients.

Results and Discussion

1. Synthesis of ODCQPs

Quaterphenyls (QPs) have six types of isomers involving the bonding positions of benzene. *o*-Quaterphenyl (**3**), *o,p*-quaterphenyl (**4**), *o,m*-quaterphenyl (**5**), *m,p*-quaterphenyl (**7**) and *m*-quaterphenyl (**8**) were synthesized according to the method reported by Kern *et al.*[5,6] *p*-Quaterphenyl (**6**) was synthesized by Ullmann's method.[7] (Chart 1)

Chlorination of polyphenylenes is usually achieved by using $SbCl_5$, Cl_2–$AlCl_3$, SO_2Cl_2–$AlCl_3$, *etc.*,[8,9] but these conditions did not give ODCQPs in high yield. However, with Cl_2–$AlCl_3$/$SnCl_4$, ODCQPs **9**, **10**, **11**, **12**, **13** and **14** could be readily synthesized from the corresponding QPs **3**, **4**, **5**, **6**, **7** and **8**, respectively.

2. Skeletons of PCQs in the Blood of Yusho Patients and Standard PCQs

Figure 1 shows gas chromatograms of a mixture of six synthetic ODCQPs, and also of ODCQPs derived from PCQs in the blood of Yusho patients and standard PCQs by the method of Kashimoto *et al.*[4] For usual quantitative analysis of PCQs in the blood, gas chromatography using SE-52 or OV-210 column was applied after perchlorination, and three peakes, A′, B′ and C′, were observed. The skeletons which gave these peaks have not been elucidated.[4] By comparison with retention times of synthetic ODCQPs, we assigned the *o*-isomer (**9**) to A′, *o,p*-isomer (**10**) and *o,m*-isomer (**11**) to B′, and *p*-isomer (**12**), *m,p*-isomer (**13**)

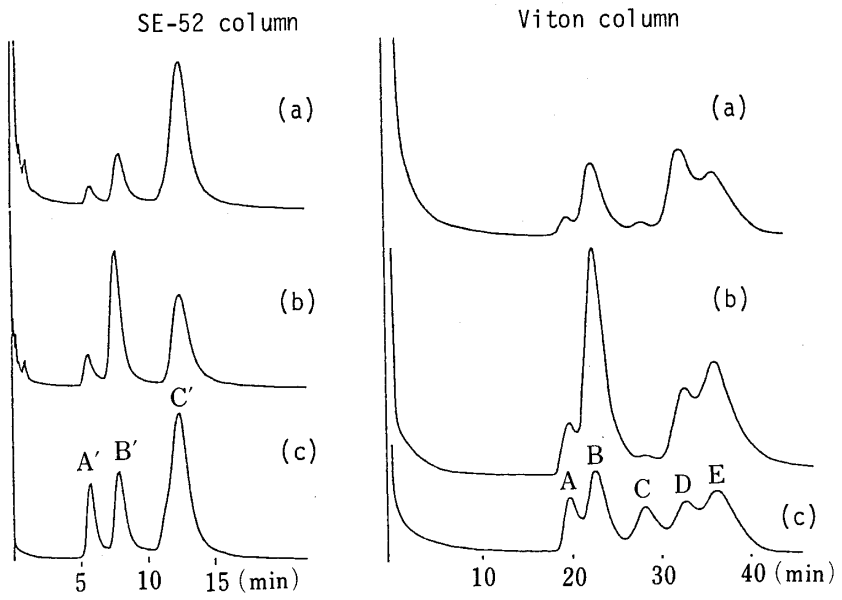

Fig. 1. Gas Chromatograms of Octadecachloroquaterphenyls (ODCQPs)

(a) blood from a typical Yusho patient, (b) standard PCQs, (c) synthetic ODCQP isomers. Peak A (*o*-ODCQP), peak B (*o,p*-+*o,m*-ODCQP), peak C (*p*-ODCQP), peak D (*m,p*-ODCQP), peak E (*m*-ODCQP).

TABLE I. Gas Chromatographic Data for ODCQP Isomers

ODCQP	Retention time		
	SE-52	Viton	Dexsil 300GC
o- ODCQP (**9**)	6.38	19.78	59.3
o,p- ODCQP (**10**)	8.59	22.81	75.8
o,m- ODCQP (**11**)	8.83	22.09	73.8
(**10**)+(**11**) [1 : 1]	8.63	22.61	74.8
p- ODCQP (**12**)	12.62	27.62	103.0
m,p-ODCQP (**13**)	13.72	32.16	122.8
m- ODCQP (**14**)	13.71	36.10	131.2
Standard	6.38	19.73	59.2
	8.66	22.50	74.8
	13.71	—	—
	—	32.63	123.4
	—	36.13	131.0
Blood	6.30	19.74	59.6
	8.61	22.49	74.8
	13.70	22.78	103.4
	—	32.41	123.0
	—	35.95	131.1

and *m*-isomer (**14**) to C′. On the other hand, when a Viton or Dexsil 300 GC column was used, five peaks, A, B, C, D and E, were observed. The retention times of peaks A, C, D and E were the same as those of the *o*-isomer (**9**), *p*-isomer (**12**), *m,p*-isomer (**13**) and *m*-isomer (**14**), respectively. The retention time of peak B was slightly different from those of the *o,p*-isomer (**10**) and *o,m*-isomer (**11**), and the same as that of a mixture of **10** and **11**. These results suggested

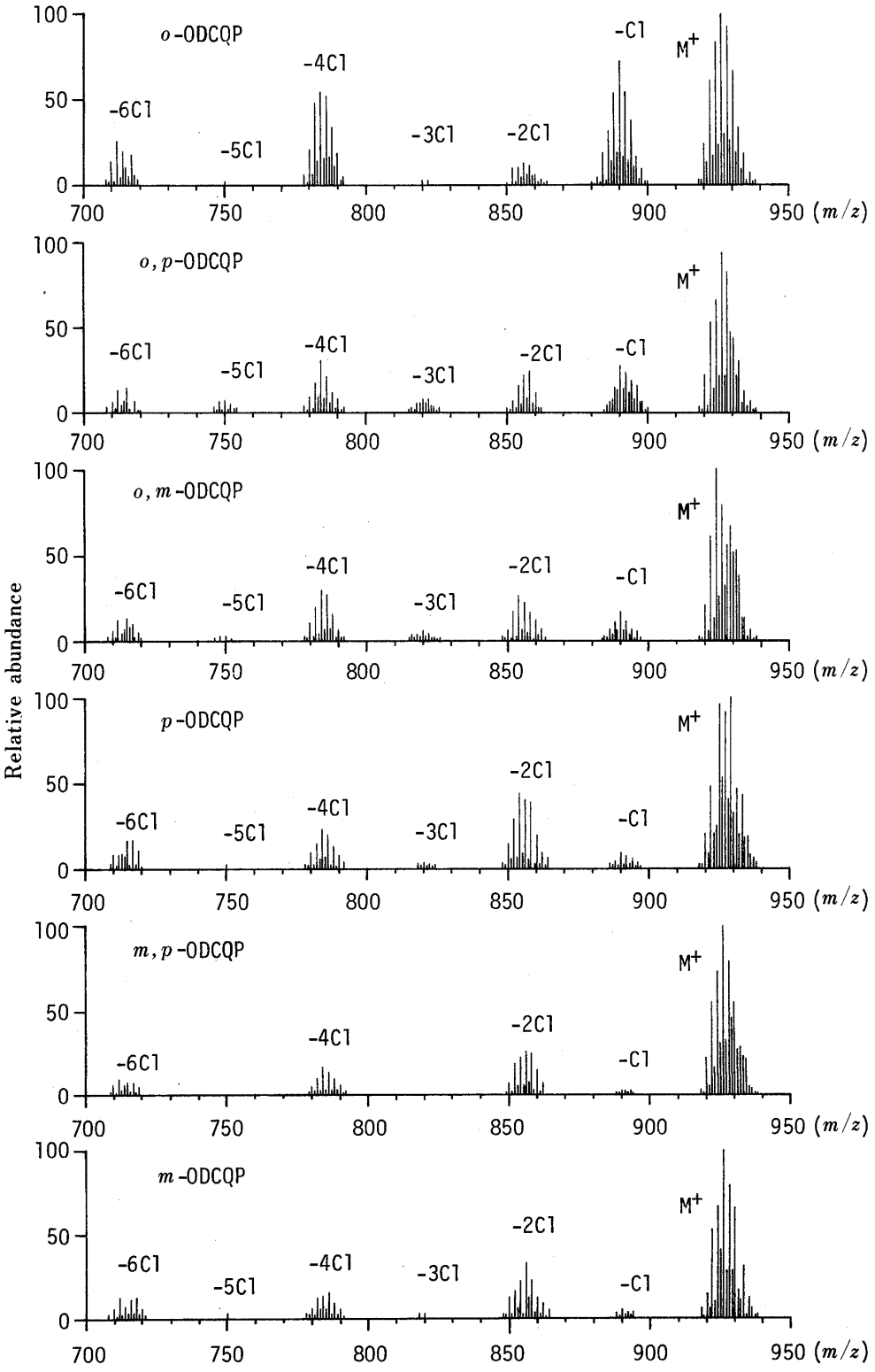

Fig. 2. Direct Inlet Mass Spectra of Synthetic ODCQP Isomers

that standard PCQs (obtained from Kanechlor 400 used as a thermotransfer medium[2,4]) and PCQs in the blood of Yusho patients were mixtures of six types of PCQ isomers.

As illustrated in Fig. 2, the mass spectra of ODCQPs show fragment ions such as $[M-Cl]^+$ and $[M-2Cl]^+$, which are characteristic of PCBs.[10] The intensity of $[M-Cl]^+$ of the *p*-isomer (**12**), *m,p*-isomer (**13**) and *m*-isomer (**14**), having only *meta* and/or *para* chains, was smaller than that of *o*-isomer (**9**), *o,p*-isomer (**10**) or *o,m*-isomer (**11**), which contain *ortho* chains. In particular, the intensities of $[M-Cl]^+$ and $[M-4Cl]^+$ of the *o*-isomer (**9**) were larger than those of the other isomers. As shown in Fig. 3, the mass spectrum of peak A′ was very similar to that of the *o*-isomer (**9**), while peak B′ corresponded to the *o,p*-isomer (**10**) or

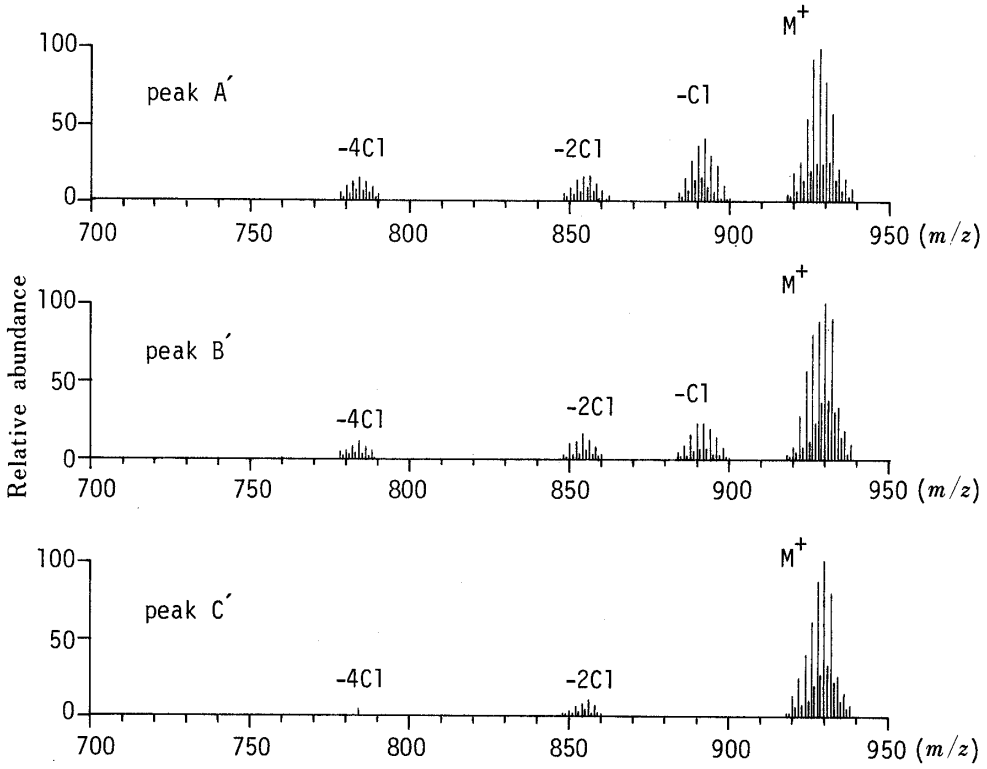

Fig. 3. GC-MS Spectra of Peaks A′, B′ and C′

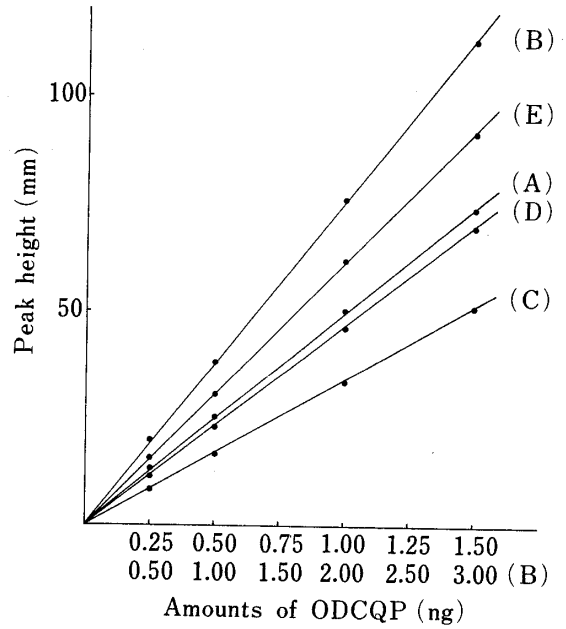

Fig. 4. Calibration Curves of ODCQP

(A) peak A (*o*-ODCQP), (B) peak B (*o,p*-+*o,m*-ODCQP), (C) peak C (*p*-ODCQP), (D) peak D (*m,p*-ODCQP), (E) peak E (*m*-ODCQP).

o,m-isomer (**11**), and peak C′ to the *m,p*-isomer (**13**) or *m*-isomer (**14**). These mass spectra supported the above-mentioned results of ECD-GC.

3. The Ratios of the Six Types of PCQ Isomers in Patients' Blood

Six types of PCQ isomers in patients' blood and standard PCQs were analyzed (after perchlorination) by ECD-GC with a Viton column, using synthetic ODCQPs as the standards. The ODCQPs corresponding to peak B were determined by use of an equivalent mixture of the *o,p*-isomer (**10**) and *o,m*-isomer (**11**) as the standard. As illustrated in Fig. 4, calibration curves showed good linearity in the range of 0.25—3.0 ng, and could be extrapolated through zero. The ratios of the six perchlorinated PCQ isomers in patients' blood and in standard PCQs were as follows. The values represent the mean ± S.D. of 5 samples. *o*-Isomer (**9**): *o,p*-isomer (**10**) + *o,m*-isomer (**11**) : *p*-isomer (**12**) : *m,p*-isomer (**13**) : *m*-isomer (**14**) = 5.4 ± 1.2 : 39.2 ± 3.5 : 1.8 ± 0.9 : 34.9 ± 3.2 : 18.7 ± 0.8 (%), and 9.2 : 57.8 : 0.6 : 16.2 : 16.2 (%), respectively. The ratio of the mixture of the *o,p*-isomer and *o,m*-isomer in the patients' blood was lower than in standard PCQs and that of the *m,p*-isomer was conversely higher. Consequently, the ratios of PCQ isomers were found to be different in patients' blood and in standard PCQs. These differences suggest that skeletal isomers of PCQs may be differentially metabolized in humans. Further experimental work will be necessary to confirm this and to elucidate its significance.

Experimental

1. Materials——The standard PCQ sample was given by Dr. T. Kashimoto of Osaka Prefectural Institute of Public Health. The blood samples were collected in October, 1981 from 5 Yusho patients in Hiroshima Prefecture. The other chemicals were of the grade suitable for polychlorinated biphenyl (PCB) analysis and residual pesticide analysis (Katayama Chemical Industries Co., Ltd.).

2. Preparation of Sample——Samples were prepared according to the method of Kashimoto *et al.*[4]

3. Apparatus and Conditions——(1) Gas Chromatography: A Yanaco G-2800 gas chromatograph equipped with a ^{63}Ni-ECD detector was used. The conditions were as follows:

i) Column: 3.4 mm × 1.0 m glass column packed with 3% Viton on Uniport HP. Temperature (°C): column 270, injection port 300, detector 310. Carrier gas: N_2, 90 ml/min.

ii) Column: 3.4 mm × 0.75 m glass column packed with 2% Dexsil 300GC on Uniport HP. Temperature (°C): column 310, injection port 330, detector 340. Carrier gas: N_2, 80 ml/min.

iii) Column: 3.4 mm × 0.75 m glass column packed with 2% SE-52 on Gaschrom Q. Temperature (°C): column 295, injection port 310, detector 320. Carrier gas: N_2, 80 ml/min.

(2) Gas Chromatography/Mass Spectrometry: A JEOL JMS D-300 machine was used, and the conditions were as follows:

Column: 3.4 mm × 0.75 m glass column packed with 2% OV-210 on Gaschrom Q. Temperature (°C): column 220, injection port 250, separator 280, ion source 290. Ionization voltage: 20 eV. Carrier gas: He, 30 ml/min.

4. Synthesis of Octadecachloroquaterphenyls——(1) Preparation of *o*-Quaterphenyl (**3**): 2-Phenylcyclohexanone[5] (**1**) (8.7 g, 0.05 mol) in dry ether (100 ml) was added to 250 ml of ether solution containing *o*-biphenyllithium [prepared from 1.26 g of lithium and 21 g of *o*-bromobiphenyl]. The reaction mixture was stirred for 48 h at room temperature and then refluxed for 2 h with continued stirring. Ethyl alcohol was added to decompose unreacted lithium, and the mixture was washed with water, and dried over magnesium sulfate. The solvent was evaporated off. A solution of oxalic acid (20 g) in dioxane (100 ml) was added to the residue and the whole was refluxed for 6 h. This mixture was subjected to steam distillation until all the biphenyl had been removed, and the residue was extracted with benzene. The extract was washed with dilute sodium bicarbonate and water, and dried over magnesium sulfate. The solvent was evaporated off. A solution of chloranil (17.4 g) in xylene (250 ml) was added to the residue and the mixture was refluxed for 24 h. The solvent was evaporated off, then 100 ml of a solution of sodium hydroxide (6 g) and sodium hydrosulfite (15 g) was added to the residue, and the mixture was refluxed for 30 min. The mixture was extracted with benzene, and the benzene layer was washed with water, then dried over magnesium sulfate. The solvent was evaporated off and the residue was chromatographed on silica gel with cyclohexane as the eluent. Recrystallization of the product from petroleum ether–ether gave 6.3 g (41%) of **3**, mp 118—119 °C (reported[11] 118—119 °C).

(2) Preparation of *o,p*-Quaterphenyl (**4**): A solution of *p*-iodobiphenyl[12] (28 g, 0.1 mol) in dry ether (100 ml) was added dropwise with stirring at 0 °C into 100 ml of an ether solution of butyllithium [prepared from lithium

TABLE II

Compounds	Yield (%)	mp (°C)	Recrystallization solvent	Crystal form	Analysis (%) Calcd for $C_{24}Cl_{18}$: C, 31.10 Found C,
o- ODCQP (**9**)	72	311—312	Benzene–EtOH	Colorless prisms	31.36
o,p- ODCQP (**10**)	77	331—332	Benzene	Colorless needles	30.98
o,m- ODCQP (**11**)	75	263—264	Benzene–EtOH	Colorless prisms	31.55
p- ODCQP (**12**)	98	258—259	Benzene–EtOH	Colorless needles	30.85
m,p-ODCQP (**13**)	71	359—360	Benzene–EtOH	Yellow prisms	31.52
m- ODCQP (**14**)	95	309—310	Benzene	Colorless needles	30.87

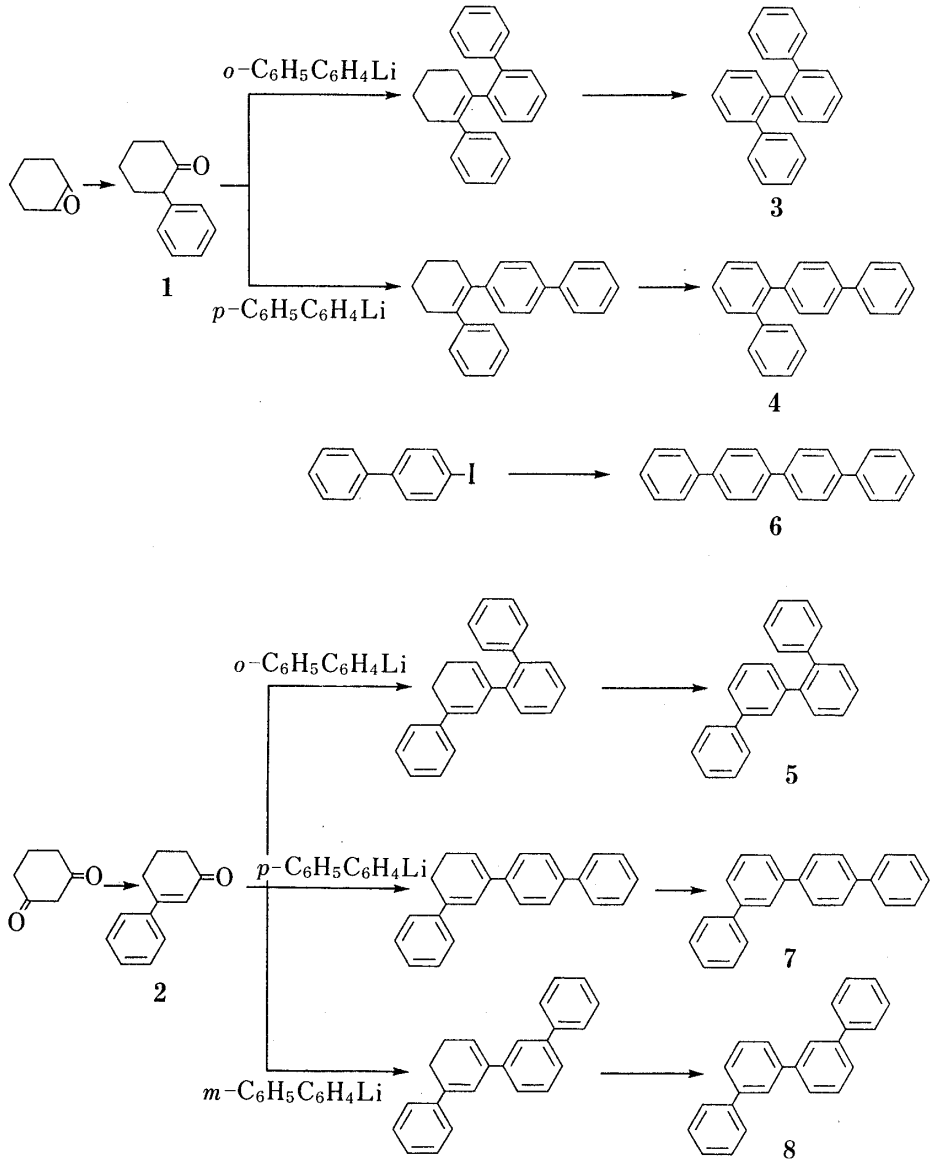

Chart 1

(2.1 g) and butyl bromide (16.5 g)], and the mixture was stirred for 3 h at room temperature. A solution of 2-phenylcyclohexanone (8.7 g, 0.05 mol) in dry ether (100 ml) was added to the solution of *p*-biphenyllithium. The reaction mixture was stirred for 48 h at room temperature, refluxed for 2 h, and treated by the same procedure as described in (1). Recrystallization of the product from petroleum ether–ether gave 7.5 g (49%) of **4**, mp 119—120 °C (reported[5] 118—119 °C).

(3) Preparation of *o,m*-Quaterphenyl (**5**): A solution of 3-phenyl-Δ^2-cyclohexenone[13] (**2**) (8.3 g, 0.05 mol) in dry ether (100 ml) was added dropwise with stirring into an ethereal solution of *o*-biphenyllithium (0.09 mol). The mixture was stirred for 24 h at room temperature and then was refluxed with continuous stirring for 2 h. A small volume of ethanol was added to decompose unreacted lithium and the lithium complex was decomposed with cold 5% sulfuric acid. This solution was subjected to steam distillation until all the biphenyl had been removed, and then extracted with benzene. The benzene layer was washed with dilute sodium bicarbonate and water, and dried over magnesium sulfate. The solvent was evaporated off, and the residue was treated by the same procedure as described in (1). Recrystallization of the product from ethanol gave 8.2 g (54%) of **5**, mp 89—90 °C (reported[14] mp 88—89 °C).

(4) Preparation of *p*-Quaterphenyl (**6**): The procedure followed that of Ullmann.[7] mp 319—320 °C.

(5) Preparation of *m,p*-Quaterphenyl (**7**): An ethereal solution of *p*-biphenyllithium (0.05 mol) and **2** (6.0 g, 0.035 mol) was treated by the same procedure as described in (3). Recrystallization from benzene–ethanol gave 3.7 g (34%) of **7**, mp 169—170 °C (reported[6] mp 165—166 °C).

(6) Preparation of *m*-Quaterphenyl (**8**): *m*-Bromobiphenyl[15] (21 g, 0.09 mol) and **2** (8.6 g, 0.05 mol) were treated by the same procedure as described in (3). Recrystallization of the product from benzene–ethanol gave 8.0 g (52%) of **8**, mp 87—88 °C (reported[11] mp 85—86 °C).

(7) General Procedure for the Chlorination of Quaterphenyl: Chlorine gas was passed for 2.5 h at 50—70 °C into a mixture of quaterphenyl (0.5 g), anhydrous aluminium chloride (1.34 g) and stannic chloride (10 ml) with stirring. The mixture was poured into ice water, and the precipitate was collected by filtration, then washed with conc. HCl and water. The product was purified by recrystallization. The results are listed in Table II.

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