Polyhalodibenzo-*p*-dioxins as Anti-tumor-promoting Agents

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1. Introduction

In 1959, Ueda and coworkers established the chemical structures of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) (1) through synthetic and spectral evidence[1]. Halogenated dibenzo-p-dioxins including 2,3,7,8-tetrabromo- (2), octachloro- (3), and octabromodibenzo-p-dioxin (4) were also synthesized and their structures were unequivocally corroborated. The dibenzo-p-dioxin skeleton was first recognized in nature in the structure of bisbenzylisoquinoline alkaloids trilobine and isotrilobine[2] isolated from Cocculus trilobus. The roots of this plant have been used as analgesics. Later, eckol[3] and related phlorotannins with a dibenzo-p-dioxin skeleton were isolated from a brown alga Ecklonia kurome as plasma α_2-macroglobulin inhibitors. Compounds bearing a dibenzo-p-dioxin skeleton show a characteristic blue color in sulphuric acid with an oxidizing agent such as sodium nitrate. Dibenzo-p-dioxin and its octamethyl derivative produce a blue color in concentrated sulphuric acid solution without adding any other oxidizing agent. An ESR study revealed that this coloration is due to the formation of cation radicals.[4] Octachloro- and octabromodibenzo-p-dioxin in sulphuric acid, however, did not show the coloration reaction and ESR signals. In the course of the total synthesis of N-methyl-dihydromenisarine,[5] an alkaloid having a dibenzo-p-dioxin skeleton, significant information on dibenzo-p-dioxin derivatives was obtained. Based upon these findings, we attempted to examine the biological activity of dibenzo-p-dioxin derivatives.

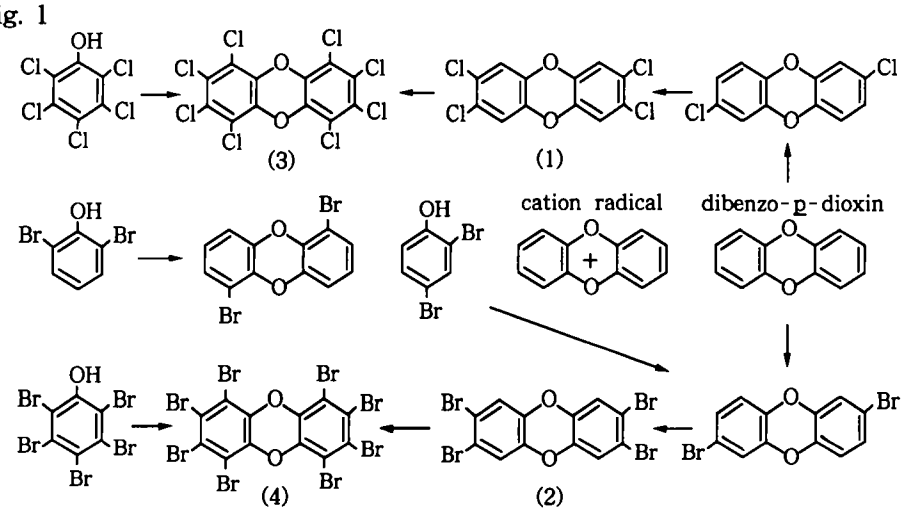
2. Objectives of the Study

Halogenated derivatives of dibenzo-p-dioxin formed at municipal incinerators [6] are of actual interest because of the risk of the contamination by these highly toxic substances. Among them, 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin (1)) is extremely toxic to animals. However, the lethal dose varies greatly from one species to another.[7] The objective of this study is the correct evaluation of the biological activity of dibenzo-p-dioxin derivatives and the detoxification of dioxin and related toxic compounds.

3. Approach and Method

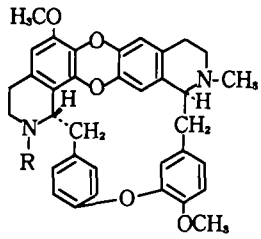
Dibenzo-p-dioxin congeners including dioxin and 2,3,7,8-tetrabromodibenzo-p-dioxin (2) were synthesized according to Fig. 1. The alkaloids trilobine and

Fig. 1



isotrilobine were extracted from plants and purified in the usual manner[8]. The anti-tumor-promoting and tumor-promoting activity were measured by a short-term in vitro assay utilizing the activation of Epstein-Barr virus (EBV) expression in EBV genome-carrying human lymphoblastoid cells (Raji cells). This assay system is composed of EBV-non-producer cells (Raji cells) as the indicator, n-butyrate as the trigger, 12-O-tetradecanoylphorbol-13-acetate (TPA)

as the EBV-activator and the test substance. Viability of Raji cells was measured through Trypan Blue staining, followed by counting of the surviving cells 48 hours after the concomittant treatment of the cells with TPA, n-butyrate and the test substances in a 0.25 % phosphate buffer solution (pH 7.2).



R = H : trilobine
R = CH_3 : isotrilobine

Table 1. Inhibitory effects of dibenzo-p-dioxin derivatives against TPA-induced Epstein-Barr virus early antigen (EBV-EA) activation

Compound	Concentration : molar ratio (test compound/TPA) 1000	500	100	10
dibenzo-p-dioxin (D)	88.8 (70)	100	100	100
2,7-dichloro-D	0 (70)	15.7	68.0	100
2,7-dibromo-D	24.6 (70)	59.1	77.2	100
1,6-dibromo-D	29.7 (70)	55.8	80.4	100
2,3,7,8-tetrachloro-D	21.0 (70)	42.1	88.2	100
2,3,7,8-tetrabromo-D	20.6 (70)	65.3	82.7	100
octachloro-D	10.5 (70)	38.3	79.1	100
octabromo-D	22.6 (70)	60.5	79.1	100
trilobine	0 (0)	0 (10)	39.6 (40)	81.8 (60)
isotrilobine	0 (0)	0 (20)	47.3 (60)	92.9

Values are EBV-EA activation (%) ± s.d. σ (± 5.0 %) in the presence of test compound (100). The activation was caused by TPA (32 pmol). Values in parentheses represent the viability % of Raji cells.

4. Results

Halogenated dibenzo-p-dioxins elicit a significant inhibitory effect against TPA-induced Epstein-Barr virus early antigen activation when the molar ratio relative to TPA is more than 100 to 1. At high concentrations the activities of tetrahalogenated derivatives suggest tumor-promoting activity, whereas octahalogenated compounds did not show any toxicity to Raji cells and are almost insoluble in ordinary solvents. Although the dehalogenation or cleavage of the dibenzo-p-dioxin skeleton has been attempted in order to eliminate dioxin congeners,[9] the results suggest that exhaustive halogenation could be more effective to detoxify the hazardous halogenated dibenzo-p-dioxins. based on the assay used in this stady : The halogenation can be achieved in chloroform in the presence of catalyst such as ferric chloride. Trilobine and isotrilobine, bisbenzylisoquinoline alkaloids of a menispermaceous plant Cocculus trilobus have stronger anti-tumor-promoting activity than that observed for the halogenated dibenzo-p-dioxins.

5. References

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