METABOLISM OF 2,7-DICHLORODIBENZO-P-DIOXINS IN RATS : IDENTIFICATION AND EXCRETION OF HYDROXYLATED METABOLITES IN RAT FECES

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ABSTRACT

Hydroxylated and sulfur-containing metabolites in the feces of rats fed 2,7-dichlorodibenzo-p-dioxin (DCDD) were investigated by gas chromatography (GC) and GC/mass spectrometry. A total of seven phenolic metabolites, that is, hydroxylated and dihydroxylated monochlorodibenzo-p-dioxins/DCDDs, were determined, while no sulfur-containing metabolites could be detected. Two further metabolites were detected from the hydroxylated metabolites fraction and determined to be a dihydoxy-dichlorobiphenyl(DCB) and a trihydroxy-DCB by comparison with mass spectra of synthetic standards.

INTRODUCTION

Several studies have shown that polychlorinated dibenzo-p-dioxins(PCDDs) are mainly metabolized to give monohydroxy- and dihydroxy-PCDDs in animals (1-3). In addition to these hydroxylated metabolites, sulfur-containing metabolites such as methylthio(MeS)-PCDDs have also been found in lower chlorinated PCDD metabolism (1). Our previous study has demonstrated that 2,8-dichlorodibenzofuran(DCDF) is metabolized to a series of sulfur-containing metabolites, MeS-, methylsulfoxide(MeSO)- and methylsulfone($MeSO_2$)-DCDF as major metabolites, along with phenolic metabolites (4-5). Therefore, in the metabolism of PCDD, MeSO- and $MeSO_2$-PCDD could be other potential metabolites. In the present study, we examined for phenolic and sulfur-containing metabolites in the feces of rats given 2,7-DCDD in order to clarify the metabolic pathways of PCDD.

MATERIALS AND METHODS

Chemicals: 2,7-DCDD was purchased from the Analabs (North Haven,USA). 3,3'-dihydroxy-4,4'-dichlorobiphenyl(DCB) was obtained from the Ultra Scientific (Hope, USA). Methoxy-, dimethoxy-, MeS- and $MeSO_2$-PCDDs were synthesized from catechols and chloronitrobenzenes according to the method of Gray et al. (6). Dimethoxy-DCBs were synthesized from 4-chloro-2-aminoanisole and 4-chloroanisole by the method of Cadogan (7). The hydroxy-, dihydroxy-PCDDs and dihydroxy-DCBs were prepared from the corresponding methoxy-, dimethoxy-PCDDs and dimethoxy-DCBs by demethylation with BBr_3 (8).

Animals: 2,7-DCDD dissolved in corn oil was given orally to male Wistar rats(body wt. about 210 g) at a single dose of 10 mg/kg. Feces were collected daily for 5 days after dosing.

Analytical Procedures: Extraction and clean-up procedures for metabolites were performed by the same procedures described previously (5).

Instruments: GC and GC/MS were carried out as previously described (11).

RESULTS AND DISCUSSION

Mass spectral properties of hydroxylated PCDD

In the dibenzo-p-dioxin molecule, there are two different substituted positions: the 1-(identical to the 4-, 6- and 9-) and the 2-(identical to the 3-, 7- and 8-) positions. The mass spectra of trimethylsilylated(TMS) derivatives of 1-hydroxy- and 2-hydroxy-dibenzo-p-dioxin are shown in Fig. 1. The major fragment ion of both isomers was $[M-CH_3]^+$. However, different relative intensity of this fragment peak was observed. As seen in Fig. 1, if the hydroxy group is in the 1-position, fragment $[M-CH_3]^+$(at m/z 257) was observed as base peak, while the 2-hydroxy-dibenzo-p-dioxin did not give this fragment as base peak, molecular ion M^+(at m/z 272) being the base peak. This diagnostic difference in mass spectra of the two different hydroxy substituted dibenzo-p-dioxin was also recognized in the case of monochlorodibenzo-p-dioxins(MCDDs) and DCDDs. As an example, Fig. 2 demonstrates the mass spectra of hydroxy-MCDDs synthesized. Again, the relative intensity of $[M-CH_3]^+$ fragment differed significantly, depending on the positions of hydroxy group. MCDDs with a hydroxy group in the 1-position showed fragment $[M-CH_3]^+$ as base peak. On the other hand, the isomers having hydroxy group at the 2-position gave M^+(at m/z 306) as base peak. It appears that the position of a hydroxy group in the dibenzo-p-dioxin molecule can be determined by the mass spectra of TMS derivative of hydroxy-PCDD.

It is noteworthy that the mass spectrum of TMS derivative of hydroxy-PCDD is useful not only for the position of a hydroxy group, but also for the position of substituted chlorine atom. As can be seen in Fig. 2, when the chlorine atom being adjacent to a hydroxy group, fragment $[M-51]^+$(at m/z 255) was characteristic for this case, while the chlorine atom being not adjacent, $[M-50]^+$(at m/z 256) was observed as major fragment ion. In addition, fragments of m/z 93 and 95 due to migration of chlorine atom to silicone indicated the presence of chlorine atom and a hydroxy group in the same benzene ring.

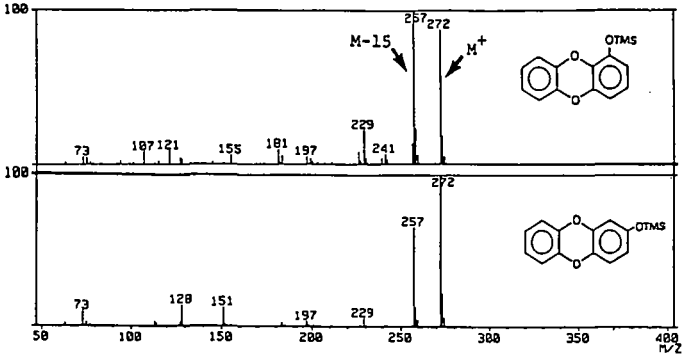

Fig. 1 Mass spectra of TMS derivatives of hydroxy-dibenzo-p-dioxins

Hydroxylated metabolites

Fig.3 shows the mass spectra of a main metabolite (I), an accompanying isomer (II) and an authentic reference compound. The both metabolites (I and II) exhibited diagnostic fragment ions such as weak $[M-CH_3]^+$ and $[M-51]^+$, which are characteristic for 2-hydroxy-PCDD having chlorine atom adjacent to the hydroxy group as described above. Comparison of the retention times on GC and GC/MS revealed that main metabolite (I) was identical with the reference compound (the exact structure is not determined so far) in Fig. 3. Thus, I and II were assumed to be 3-OH-2,7-DCDD and 3-OH-2,8-DCDD, respectively, indicating the NIH shift following an 2,3-aren oxide formation.

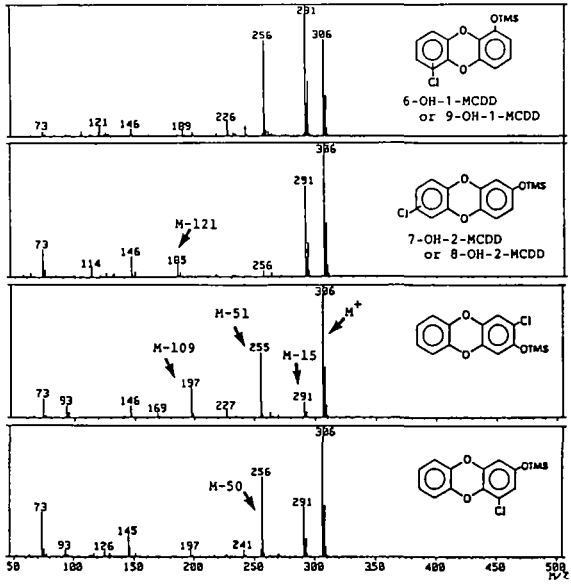

Fig. 2 Mass spectra of TMS derivatives of hydroxy-MCDD standards

A dechlorinated phenolic metabolite (III) was determined. In comparison with mass spectra and GC retention times of synthesized hydroxy-MCDDs, III was identified as 7-hydroxy-2-MCDD or 8-hydroxy-2-MCDD (See Fig. 2). The fecal excretion of this metabolite (III) amounted to 0.01 % of dose during 5 days after administration. Then, it appears that this hydrolytic dechlorination is not a major metabolic pathway in the metabolism of 2,7-DCDD. The additional metabolites were one dihydroxy-MCDD (IV) and three isomeric dihydroxy-DCDDs (V, VI and VII), but we were not able to elucidate the structures of these dihydroxylated metabolites at present. Furthermore, two minor metabolites (VIII and IX) were determined from the hydroxylated metabolites fraction at the first day. The mass spectra of TMS derivatives of VIII and IX showed intense M^+ at m/z 398 and 486, respectively, with diagnostic isotope peaks due to 2 chlorine substituents. These molecular weights (MW) do not correspond to those of hydroxy-DCDD(MW: 340 as TMS derivative) or dihydroxy-DCDD(MW:428 as TMS derivative). Metabolites VIII and IX were presumed to be dihydroxy-DCB and trihydroxy-DCB, respectively, from their molecular ions. To determine the postulated these metabolites and the sites of the hydroxy groups substituted to the biphenyl rings, some dihydroxy-DCBs were synthesized and their mass spectra were investigated. The mass fragmentation patterns of TMS derivatives of dihydroxy-DCBs were very specific, depending on the positions of the two hydroxy groups. 3,3'-$(OH)_2$-4,4'-DCB gave M^+ at m/z 398 as base peak, major fragment ion $[M-15]^+$(at m/z 383), minor fragment ions $[M-50]^+$(at m/z 348), $[M-123]^+$(at m/z 247) and fragment ions of m/z 93 and 95. 5,6'-$(OH)_2$-2,3'-DCB also showed intense M^+, major fragment $[M-123]^+$, minor fragment $[M-15]^+$ and fragment ions of m/z 93 and 95. On the other hand, 6,6'-$(OH)_2$-3,3'-DCB which has the two hydroxy groups at the 2(2')- and 6(6')-positions, exhibited intense M^+ and a few weak fragment ions such as $[M-15]^+$. The mass fragmentation pattern and the retention time of metabolite VIII were quite similar to those of 6,6'-$(OH)_2$-3,3'-DCB. Thus, VIII was determined to be a dihydroxy-DCB having the two hydroxy groups at 2(2')- and 6(6')-positions of

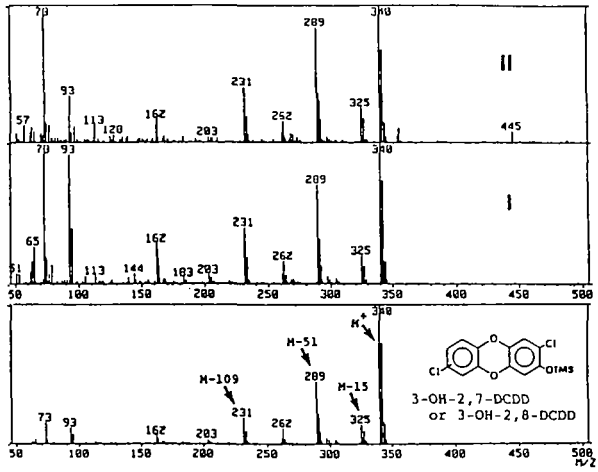

Fig. 3 Mass spectra of metabolites (I and II) and an authentic hydroxy-DCDD as TMS derivatives

biphenyl ring. This metabolite was tentatively considered to be formed by cleavage of the C-O bond of the dibenzo-p-dioxin ring, because the two hydroxy groups attached at the ortho positions of the biphenyl ring. Formation of such a compound resulting from cleavage of C-O bond has also been found in the metabolism of polychlorinated dibenzofurans (9-11), but not in PCDD metabolism. The fecal excretion of metabolite VIII was estimated to be 0.01 % of dose. This metabolic pathway from PCDD still remains to be elucidated.

Sulfur-containing metabolites

No sulfur-containing metabolites could be detected in the feces, even though the chemical structure of 2,7-DCDD is very similar to that of 2,8-DCDF, which has been found to give rise to sulfur-containing metabolites (4-5).

Excretion of 2,7-DCDD, hydroxy- and dihydroxy-MCDDs/DCDDs

Fecal excretion of 2,7-DCDD amounted to 23.9 % of the dose during the first three days. However, the unchanged 2,7-DCDD was not detected on four and five days. Hydroxylated metabolites were detected at the first two days and the fecal excretion of hydroxy- and dihydroxy-MCDDs/DCDDs were 1.4 % and 0.3 % of dose, respectively.

REFERENCES

1. M.Th.M.Tulp and O.Hutzinger, Chemosphere, 7, 761-768 (1978)
2. H.Poiger, H.R.Buser, H.Weber, U.Zweifel and Ch.Schlatter, Experientia, 38, 484-486 (1982)
3. T.Sawahata, J.R.Olson and R.A.Neal, Biochem. Biophys. Res. Commun., 105, 341-346 (1982)
4. H.Kuroki, R.Hattori, K.Haraguchi and Y.Masuda, Chemosphere, 16,1641-1647 (1987)
5. H.Kuroki, R.Hattori, K.Haraguchi and Y.Masuda, Chemosphere, 19, 803-808 (1989)
6. A.P.Gray, S.P.Cepa, I.J.Solomon and O.Aniline, J.Org. Chem., 41, 2435-2437 (1976)
7. J.I.G.Cadogan, J. Chem. Soc., 4257-4258 (1962)
8. S.H.Safe and L.M.Safe, J. Agric. Food Chem., 32, 68-71 (1984)
9. H.Poiger, H.R.Buser and Ch.Schlatter, Chemosphere, 13, 351-357 (1984)
10. N.Pluess, H.Poiger, Ch.Schlatter and H.R.Buser, Chemosphere, 17, 209-216 (1987)
11. H.Kuroki, K.Haraguchi and Y.Masuda, Chemosphere, in press