Bull. Environ. Contam. Toxicol. (1986) 37:866–873

Bulletin of Environmental Contamination and Toxicology

Chlorination of 4-Hydroxycinnamic Acid and Its Toxic Risk as a Natural Occurring Water Contaminant

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During the decay process of plant material a number of phenolic compounds are released into the environment. Previous studies on water courses have shown that five phenolic acids derived from plants predominate with 4-hydroxycinnamic acid as the major compound (Kickuth 1982). Some phenolics are potentially hazardous and these compounds are important water contaminants (Korte 1980; Le Blanc 1980).

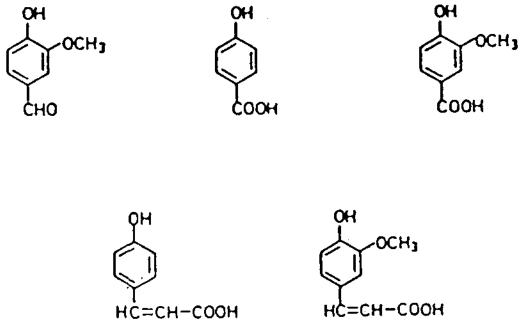

Figure 1: Phenolic acids derived from plants identified in a stream in Sudniedersachsen (FRG) (after Kickuth 1982).

As can be seen from Figure 1 the substitutions pattern and C-3-methylation possible confirms their origin as lignin fragmentation products of litter decay. This is important, since the edaphone and other microorganisms are capable of biosynthesis of aromatics, and therefore potential contributors to the environmental contamination (e.g. soil, water) with phenolic compounds (Haider and Martin 1967). Many of these compounds can travel through the soil matrix and enter a water stream. However, water supplies are routinely chlorinated which leads to the chemical chlorination of phenolic water contaminants. These naturally occurring phenolics may therefore change their chemical and biological behaviour and probably become, after chlorination, increasingly toxic. Although the occurrence of water compounds in tap water have been increasingly

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investigated with focus on industrial water pollutants, it is not fully understood to what extent the decay of plant material contributes to phenolic water contamination. Great concern about routine water chlorination has been expressed, because of the chlorination of aliphatic and aromatic water contaminants possibly accompanied by a change in their chemical and biological behaviour and the synthesis of organochlorine derivatives. The present situation does not allow any prediction of the chemical and biological behaviour of the parent compound nor is it possible to anticipate the amount and potential risk of its organochlorine derivatives. The objective of the present work was to study the chemical behaviour of plant-derived phenolic acids under conditions similar to the routine water chlorination. The toxicity of the chlorophenolic mixtures was assessed on a Escherichia coli strain similar to that found in the human intestine.

MATERIALS AND METHODS

Chemicals and chlorophenol synthesis. The 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, 3,4 dihydroxybenzoic acid, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzoic acid and the 4-hydroxy-3-methoxycinnamic acid were obtained from Fluka, Swiss with a purity > 99%. The 4-hydroxycinnamic acid was obtained from Merck-Schuchardt, West Germany with a purity of > 99%. The synthesis of the chlorophenols was carried out by weighing 1/100 molar chemical into a Erlenmeyer flask and the addition of 10 ml glacial acetic acid. After briskly shaking, the chemicals dissolved well and were ready for a five minute excessive chlorine incubation. After chlorine treatment the mixtures were incubated for 30 minutes at room temperature in the dark. The solvent is removed with a rotary evaporator in a water bath at 60°C. For 48 h the chlorophenol mixtures were dried with phosphorus pentoxide at room temperature, vacuum and protection against illumination.

Analysis of single chlorophenol derivatives. One and two dimensional thin-layer separations of the chlorophenol mixtures was carried out by using for the first dimension a mixture of 4 ml paraffin, 11 ml benzene, 33 ml glacial acetic acid, 77 ml cyclohexane and 10 ml chloroform and for the second dimension a mixture of 174 ml benzene, 20 ml dichloromethane and 6 ml glacial acetic acid. For the present work thin layer plates (Merck, FRG) with the size 20 x 20 cm and a film thickness between 250 µm to 1000 µm appeared to be advantageous. Single chlorophenol derivatives were recognized by either using thin layer plates containing the fluorescence indicator 60F for 254 nm or the following coupling agents as described by Hinney (1972): diazotization of sulphanilic acid (1), silver nitrate reagent (2), 4-4'-Bi(2-methoxybenzene diazoniumchloride) (3).

Single spots of the thin-layer plates were gently isolated with a scalpel and several times washed with glacial acetic acid. After removal of solvent, the chlorophenol derivatives were dried and further spectroscopic analysis was carried out employing UV, IR and ^{1}HNMR-spectropscopy.

Measurement of chlorophenol toxicity: The toxicity of the chlorophenol mixture was measured on E. coli DSM 613. The bacterial toxicity was correlated to the inhibition of growth at various concentrations.

RESULTS AND DISCUSSION

The thin-layer separation of the chlorophenol mixtures appeared to be a good and relatively simple method. As it can be seen from Table 1 there are remarkable differences in the synthesis of chlorophenol derivatives. In the case of the 4-hydroxybenzoic acid only two chloroderivatives were apparent, whereas the 4-hydroxy-3-methoxybenzoic acid produced more than 15 derivatives. The electrochemical stability of the parent compound (non-chlorinated compound) probably counts as an important factor in the capability of producing a large number of chloroderivatives. Indeed it appears that the vicinal substitution in the C-3 and C-4 position are likely to influence the chemical reactivity. In each case these chemicals demonstrated comparable high reactivity under the chlorination condition and produced a large number of chlorophenol derivatives. Best detection of single chloroderivatives were obtained by using thin-layer plates containing a fluorescence indicator. In some cases two-dimensional thin-layer separation achieved further isolation of chloroderivatives as in the case of the 4-hydroxycinnamic acid. Five additional derivatives were separated in the second dimension. These additional derivatives exert a changed absorption behaviour for 254 nm and for some spots had a distinct absorption at 366 nm (results not shown). The use of all 3 coupling agents were proved to be less sensitive in the detection of single chlorophenol derivatives compared to the U.V. detection at 254 nm. The present results of the chlorination of 7 plant-derived phenolics provide indication of the synthesis of > 60 chloroderivatives (see Table 1). Many of these compounds individually are likely to be acceptable in low concentration, however, with the formation of such a large number of derivatives the accumulative concentration is relatively high. Moreover it has yet to be proved that individual derivatives are important toxicants or whether there are synergistic effects when they are applied in combination with other derivatives and water contaminants. This could lead to an unacceptable level of toxicity.

Table 2 lists the average concentration of some plant-derived phenolic water contaminants. Because of its function as a major contaminant, it appeared reasonable to choose the 4-hydroxycinnamic acid for model reactions. After chlorination of the 4-hydroxycinnamic acid, the chlorophenol mixture was separated on a thin-layer system and the major chlorophenol derivative was isolated.

Infrared spectroscopical analysis (Fig. 2) revealed the following structure:

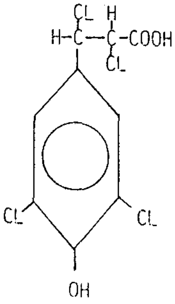

Figure 2. 3,5 Dichloro-4-hydroxyphenyl-$\alpha\beta$-dichloropropionic acid

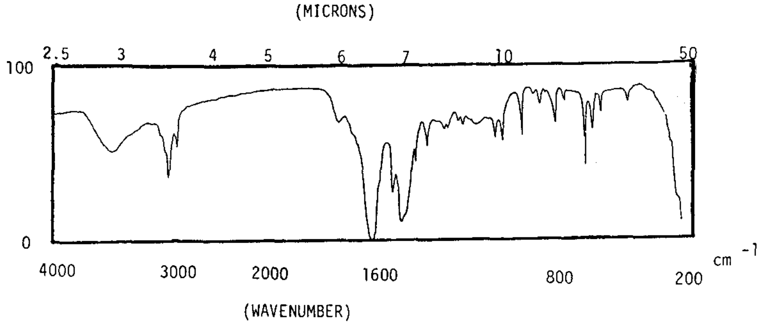

Figure 3. Infrared spectroscopic analysis of the main chlorophenol derivative of 4-hydroxycinnamic acid.

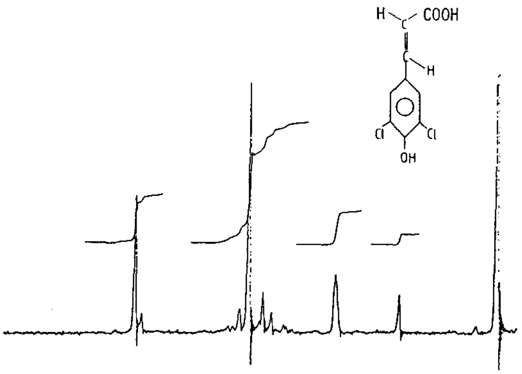

Figure 4. ^1HNMR analysis of the main chlorophenol derivative of 4-hydroxycinnamic acid.

Table 1. Number of separated chlorophenol mixtures on a one-dimensional thin-layer system with comparison of different detection methods

Chlorophenol mixture	UV 254 nm	Coupling Agents (as described by Hinney 1972) (1)	(2)	(3)
4-hydroxybenzaldehyde	6	2	2	2
4-hydroxybenzoic acid	2	2	2	2
3,4-dihydroxybenzoic acid	10	8	8	10
4-hydroxy-3-methoxy benzaldehyde	13	-	-	-
4-hydroxy-3-methoxy benzoic acid	15	8	9	9
4-hydroxycinnamic acid	8	7	7	7
4-hydroxy-3-methoxy cinnamic acid	11	9	-	10

Table 2. Average extraction of plant derived phenolics from the 'Auschwippe', a water stream in Sudniedersachsen (FRG) (after Kickuth 1982).

Chemical	Quantity
4-hydroxycinnamic acid	380 µg/l
4-hydroxybenzoic acid	210 µg/l
3-methoxy-4-hydroxybenzoic acid	165 µg/l
3-methoxy-4-hydroxybenzaldehyde	40 µg/l

The infrared spectroscopic analysis (Fig. 3) was not entirely confirmed, since the proton nuclear magnetic resonance of the same compound lacked the halogen addition reaction of the double bond of the side chain (Fig. 4). According to the ^1HNMR-analysis of this compound only the 3,5 dichloro substitution of the ring can be confirmed. However, the UV absorption shows a hypsochromic effect (blue shift), which is typical for losses of double bonds (Fig. 5). Moreover, elementary analysis confirmed the tetrachlorine substitution leading to the formula of $C_9H_6CL_4O_3$ (data not shown). From the results obtained the authors believe that the tetrachlorine

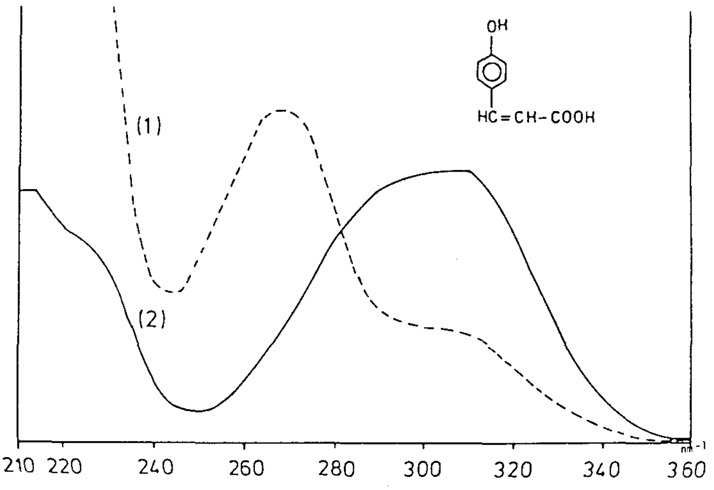

Figure 5. Ultraviolet absorption of the main chlorophenol derivative of 4-hydroxycinnamic acid. (1) chlorinated; (2) non-chlorinated.

substitution is the most probable reaction product. Difficulties in the preparation of this compound, its isolation and its photochemical instability may account for the discrepancy of the NMR-spectrum compared with other results.
Many investigators have studied the occurrence of organic contaminants in the tap water. Two hundred and eighty nine organic compounds have been extracted and isolated from the tap water of the United States (Tardiff et al. 1978). Approximately 40% of these compounds were halogenated. In a study by the U.S. Environmental Protection Agency in 1975 five representative cities were chosen for analysis of the tap water. Up to 50% of the extracted organic contaminants were halogenated, including the compounds chloroform, bromoform, chloroethane, vinyl chloride and vinylidene chloride. Twenty six organic chemicals in the drinking water have been identified as either carcinogens (twenty one) or potential carcinogens and promoters (five). Additionally, twelve carcinogens have been analysed in raw water supplies (Kraybill 1978). Other workers studied the genetical risk of some chlorinated water contaminants including the 5-chloruracil which is known to interact with DNA (Witkin and Parisi 1974). Because of its potential risk as an organic halogenated water contaminant the toxicity of the chlorophenol mixture of the 4-hydroxycinnamic acid was studied. The toxicity of this compound was assessed on an E. coli strain DSM 613 which resembles the E. coli flora of the human intestine.

As can be seen from Figure 6 there is a good linear relationship between the concentration of the chlorophenol mixture and the inhibition of growth. At a concentration of 1250 ppm the growth of the bacterial colonies were inhibited. It was necessary to assess the toxicity of the chlorinated 4-hydroxycinnamic acids as a mixture to mimic their occurrence in water supplies.

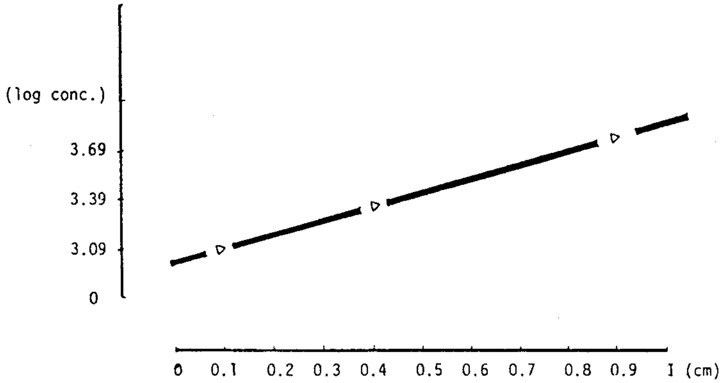

Figure 6. Growth inhibition of Escherichia coli DSM 613 with the chlorophenol mixture of 4-hydroxycinnamic acid at various concentrations.

The present work does not not imply an immediate risk for the E. coli flora of the human intestine. Assuming an average concentration of about 380 µg extractable 4-hydroxycinnamic acid per liter tap water (see Table 2), the concentration of its chlorophenol mixture must rise x3,000 to show acute oral toxicity. However, the effects of a long term exposure to low concentrations have not been studied, nor the dose response relationship with respect to other water contaminants.

Phenolic compounds have a high partition coefficient for lipids and hydrophobic compounds and are known to be membrane poisoning (Riley and Seal 1974). Although bacterial systems are useful in toxicological screenings they lack sensitivity. Certainly, the bacterial cell wall differs from the more sensitive and vulnerable mammalian cell membrane. However, future studies must focus on the limitation of a simple toxicological screening method and incorporate studies on the mammalian intestine. This would provide important information of the toxicological potential of the chlorinated 4-hydroxycinnamic acid and would help to evaluate the risk of plant-derived phenolic water contaminants in drinking water.

Acknowledgements. This research work was supported by the DFG Deutsche Forschungsgemeinschaft. The technical assistance of Irene Hutter is gratefully acknowledged.
Dedication: This paper is dedicated to Professor H. Kilian's retirement.

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Received February 10, 1986; accepted June 18, 1986.