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Metabolism of 2,4',5-Trichlorobiphenyl by the Mercapturic Acid Pathway

Abstract. *Carbon-14–labeled 2,4',5-trichlorobiphenyl was found to be metabolized by the mercapturic acid pathway to metabolites that are excreted in bile. About 57 percent of the carbon-14 was excreted in the bile; 30 to 35 percent was present as mercapturic acid pathway metabolites. Mercapturic acid was also isolated from the urine (0.3 percent of the dose).*

Although polychlorinated biphenyls (PCB's) have not been shown to be metabolized by the mercapturic acid pathway (MAP), there is evidence that the MAP may be involved. Biphenyl and 2,2',5,5'-tetrachlorobiphenyl are metabolized to dihydrodiols (*1*, *2*), and the NIH shift occurs in the metabolism of 4-chloro- and 4,4'-dichlorobiphenyl (*3*, *4*). Both of these metabolic routes usually indicate that an arene oxide precursor was formed, and compounds that form arene oxides are often metabolized in part by conjugation with glutathione, that is, by the MAP. Also, biphenyl is known to be metabolized by the MAP (*5*).

The most common indication that a xenobiotic was metabolized by the MAP is the isolation of the appropriate mercapturic acid from the excreta; however, this may also be indicated by formation of metabolites that contain metabolically introduced thiol, *S*-glucuronyl, methylthio, methylsulfinyl, or methylsulfonyl groups (*6*–*8*). Several chlorinated biphenyls were found to be excreted by mice as metabolites containing methylthio and methylsulfonyl groups (*9*), and chlorinated biphenyl methyl sulfones were also isolated from various tissues (*10*–*12*) and from milk from a lactating female (*13*). The radioactivity from intraperitoneally administered [^{35}S]cysteine was incorporated into 2,4',5-trichlorobiphenyl (triCB) methyl sulfones that accumulated in the lungs of mice given oral doses of triCB (*14*).

The evidence cited above indicated that some chlorinated biphenyls are metabolized by the MAP and that the common products of this pathway (the corresponding mercapturic acid and its precursors) are metabolized further before excretion. The mechanism was thought to be similar to that described for pentachlorothioanisole, where the biliary MAP metabolites were excreted mainly in the feces as bis-(methylthio)tetrachlorobenzene and nonextractable residues (*15*) and about 1 percent of the dose was present in the urine as *N*-acetyl-*S*-(methylthiotetrachlorophenyl)cysteine. The excretion of triCB methyl sulfide and methyl sulfone in feces from mice given triCB (*9*) prompted a search for MAP metabolites in bile from rats given ^{14}C-labeled triCB. In addition, triCB is a significant component of technical PCB containing 42 to 48 percent chlorine.

Bile collected from four bile duct–cannulated rats given single oral doses of ^{14}C-labeled triCB (*16*) (4 mg, 2.94 μCi per rat) contained 52.7 ± 19.2 percent of the dose after 48 hours, and 84 to 90 percent of the radioactivity was extracted from the bile (*17*). The radioactivity in the extract was separated into six fractions by reversed-phase high-performance liquid chromatography (HPLC) (*18*). The fractions were examined for possible MAP metabolites by converting the xenobiotic moieties to the corresponding triCB-*S*-acetates (*19*). Fractions 4 and 5, which contained 4.5 and 33.5 percent of the biliary ^{14}C, respectively, yielded significant quantities of triCB-*S*-acetates. Small quantities were obtained from fractions 1, 2, and 3. Two isomeric triCB-*S*-acetates were separated by gas chromatography (*20*) and found to have retention times and mass spectra identical with those of authentic triCB-3-*S*-acetate and triCB-4-*S*-acetate (*21*). After derivatization (*22*) of fraction 4, the derivatized triCB-*S*-cysteinylglycine and -cysteine conjugates were isolated by HPLC. After derivatization of fraction 5, the methyl ester of triCB-*S*-(*N*-acetyl)cysteine was isolated by HPLC. From the mass spectral data (*23*), structures were assigned to these derivatives and to the underivatized mercapturic acid as outlined in Fig. 1 (*21*).

About 30 to 35 percent of the radioactivity in the bile was present as MAP metabolites, showing that the MAP is a major metabolic pathway for this chlorinated biphenyl and that significant quantities of the metabolites are available for further metabolism by intestinal enzyme systems.

The fate of biliary triCB MAP metabolites in the intestine could not be deduced from the identities of the metabolites reported previously (*24*); therefore, the metabolic fate of ^{14}C-labeled triCB in

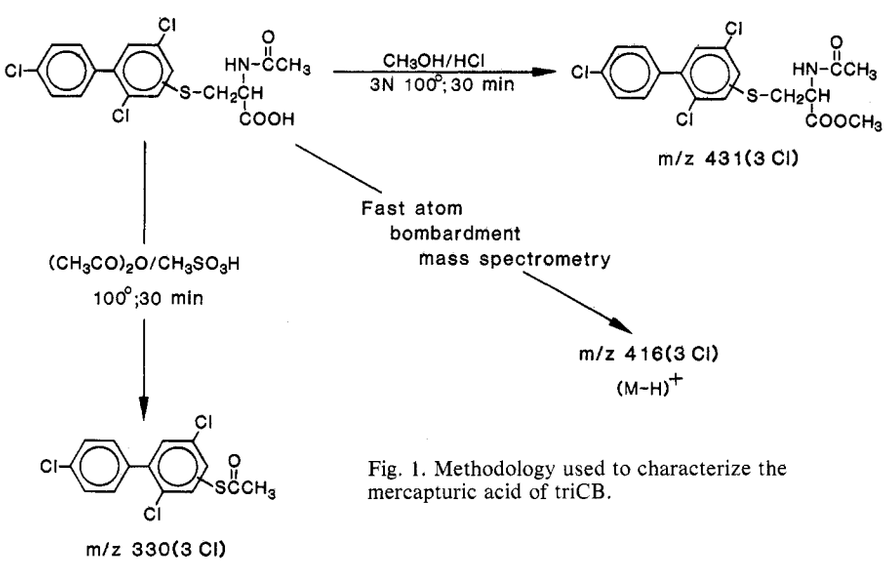

Fig. 1. Methodology used to characterize the mercapturic acid of triCB.

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rats was reexamined (*21*). Recoveries of ^{14}C from eight rats given single oral doses of ^{14}C-labeled triCB (4 mg, 2.94 μCi per rat) are given in Table 1. The feces were the major route of excretion and about 23 percent of the fecal ^{14}C was not extractable with methanol.

TriCB mercapturic acid was the only sulfur-containing metabolite found in the urine and it accounted for about 0.3 percent of the dose. A glucuronide of monohydroxy-triCB was also isolated as the trimethylsilyl derivative (2.4 percent of the dose). Three other minor metabolites (0.5 percent of the dose) were separated from the urine but were not characterized.

Three types of sulfur-containing ^{14}C-labeled metabolites—methylthio-, methylsulfinyl-, and methylsulfonyl-triCB (Fig. 2)—were isolated from methanol extracts of the feces by HPLC and gas chromatography. They accounted for about 22 percent of the dose. Because the molecular ions of the methylthio and methylsulfinyl metabolites are isobaric with those of the monomethoxymonohydroxy- and monomethoxydihydroxy-triCB metabolites, respectively, reported by Lay *et al.* (*24*), the elemental compositions of the metabolites were confirmed by high-resolution mass spectrometry and the metabolites were synthesized (*21*). The total radioactivity in sulfur-containing triCB metabolites isolated from the feces plus the nonextractable radioactivity in the feces (36 percent of the dose) accounted for most of the radioactivity recovered from the bile as MAP metabolites (35 percent).

The amounts of methylsulfonyl-triCB in the lungs of the bile duct–cannulated rats and control rats were also determined 48 hours after dosing (*11*). The lungs from control rats had much higher concentrations of residues than the lungs from cannulated rats (160 to 436 ppm and < 1 to 17 ppm, respectively). This indicates that the xenobiotic moieties of the biliary triCB conjugates undergo enterohepatic circulation to supply most of the methylsulfonyl-triCB residues that appear in the lung tissue. The remaining methylsulfonyl-triCB residues are probably produced by a tissue enzyme system similar to that described by Tateishi *et al.* (*7*). These proposed metabolic pathways for catabolism of the MAP metabolites of triCB in rats are outlined in Fig. 2.

Table 1. Recoveries of radioactivity from single oral doses of ^{14}C-labeled triCB given to rats (N = 8; rats were killed 8 hours after dosing).

Source	Percent of dose
Feces (total radioactivity)	58.9 ± 3.4
Feces (nonextractable radioactivity)	13.8 ± 2.3
Urine	4.3 ± 1.6
Lungs	< 0.3
Gastrointestinal tract with contents	5.3 ± 1.1
Body without gastrointestinal tract	22.0 ± 2.8
Total recovery	90.5 ± 3.5

We propose that the metabolic production of methylsulfonyl-triCB residues takes place through two chemically identical but physiologically different pathways. In one pathway, triCB MAP metabolites are cleaved by a tissue C-S lyase (*6*, *7*) and the resulting triCB-thiol is methylated (*7*) and oxidized to triCB methyl sulfones. In the other, triCB MAP metabolites are excreted from the liver with the bile, where they are cleaved by an intestinal C-S lyase (*8*); the thiol is then methylated and oxidized to the sulfone, which appears in the feces and as lung residues. We do not know whether the methylation occurs before or after reabsorption. Intestinal absorption of the biliary MAP metabolites and subsequent tissue metabolism as described above is not indicated (disregarding possible species differences) because involvement of the intestinal microflora in the production of methylsulfonyl-triCB residues in lung tissue of mice has been reported; control mice had lung residues that were 16 times greater than those of germfree mice given the same doses of triCB (*25*).

The toxicological significance of the existence of these pathways for catabolism of triCB MAP metabolites is not known. However, victims of a PCB intoxication in Japan exhibited respiratory distress that persisted, in most cases, for more than 10 years (*26*), and the pulmonary vital capacity of workers exposed daily to PCB's (in capacitor manufacturing) was reported to be 14 percent less than that of controls (*27*).

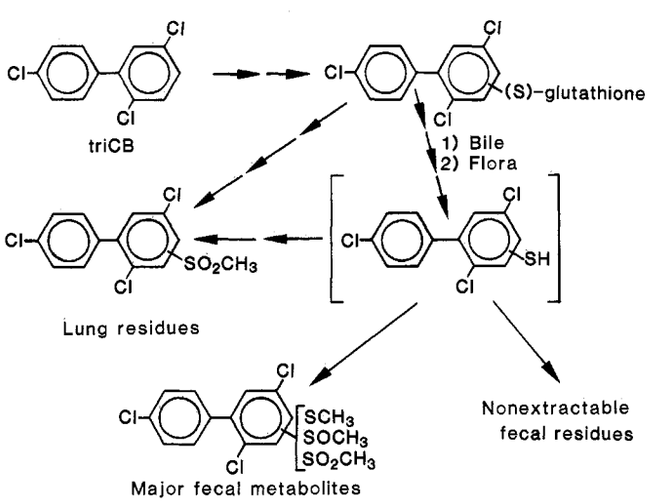

Fig. 2. Proposed metabolic pathways for catabolism of MAP metabolites of triCB in rats.

J. E. BAKKE
Metabolism and Radiation Research Laboratory, State University Station, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota 58105
Å. L. BERGMAN
Section of Organic Chemistry, Wallenberg Laboratory, University of Stockholm, Stockholm, Sweden
G. L. LARSEN
Metabolism and Radiation Research Laboratory, State University Station, Agricultural Research Service, U.S. Department of Agriculture, Fargo

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22. Heating in 3*M* HCl in methanol for 30 minutes at 100°C followed by heating in acetic anhydride at 100°C for 30 minutes.
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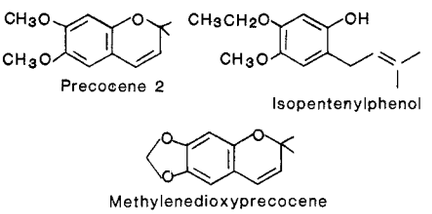
Natural and Synthetic Allatotoxins: Suicide Substrates for Juvenile Hormone Biosynthesis

Abstract. *Cytotoxic agents with antijuvenile hormone activity in insects have been discovered. Their mechanism of action may involve an oxidative bioactivation into a reactive quinone methide.*

The precocenes are simple chromenes derived from plants in the genus *Ageratum* (*1, 2*). Many insect species on contact, feeding, or fumigation with the precocenes undergo physiological responses suggestive of the induced absence of juvenile hormone. These responses include precocious metamorphosis, sterilization, inhibition of sex attractant production, embryogenetic damage, interrupted circadian feeding rhythms, diapause induction, and disruptive actions on caste and morph determination (*3*).

In micromorphological studies, we found that precocene treatment inhibited the normal postimaginal development of the corpus allatum. Similarly, the treatment of mature reproducing females induced permanent sterility and caused a diminution in the size of the corpus allatum (*4*). Although the brain regulates allatal function in many insects, surgical denervation techniques (*5*) and culture in vitro (*6, 7*) have shown that precocenes act directly on the corpus allatum. Histological examination of glands from insects treated with precocenes gave evidence of direct cytotoxic destruction of the parenchymal cells of the corpus allatum (*8–11*). Studies of the relation of their chemical structure to their biological activity and studies of the metabolism of the precocenes (*12–18*) have indicated that these compounds undergo oxidative activation within the corpus allatum and form highly reactive epoxides that alkylate nucleophilic substrates (Fig. 1). It seemed to us that the precocenes were serving as "suicide" substrates (that is, substrates capable of enzymatic destabilization, leading to destructive alkylation of cellular constituents) for oxidative enzymes in the corpus allatum that participate in juvenile hormone biosynthesis. This oxidative bioactivation seemed to parallel the course of activation of certain other plant-derived compounds, such as obtusaquinone (*19, 20*) in which oxidative activation results in the formation of a quinone methide that subsequently reacts with nucleophiles. Since, reactive epoxides and quinone methides result from the action of monooxygenase enzymes on related aromatic substrates (*3*), we synthesized some simple analogous isopentenylphenols (*21*), which might more readily be converted into tautomeric quinone methide intermediates (Fig. 1), and discovered that they displayed physiological activity indistinguishable from that of the precocenes (Table 1). The methylenedioxy analog of precocene inhibits the biological activity of precocene (*15*) through competitive inhibition of the allatal oxidases necessary for its activation (for example, through epoxidation). When we combined methylenedioxyprecocene and 3-ethoxy-4-methoxy-6-*iso*-pentenylphenol, we observed complete inhibition of the antihormonal activity (*22*).



Although the isopentenylphenols could be acting by conversion through ring closure to the related precocene and formation of a reactive epoxide, this seems unlikely because of the complexity of the transformations required. The inhibition of the biological activity of precocene and the isopentenylphenols by methylenedioxyprecocene indicates a similar oxidative activation into allatotoxic agents. The corpora allata of newly emerged milkweed bug females treated with the isopentenylphenols, as when they were treated with precocenes, failed to undergo postimaginal development. Oxidative bioactivation of the isopentenylphenols into quinone methides appears to be the most reasonable explanation for their biological activity.

Although the full spectrum of biologi-

Table 1. Induction of precocious metamorphosis and sterilization in the milkweed bug with 3-ethoxy-4-methoxy-6-*iso*-pentenylphenol and precocene 2.

Allatotoxin	Precocious metamorphosis*		Sterilization†	
	Concentration ($\mu g/cm^2$)	Precocious adults (%)	Concentration ($\mu g/cm^2$)	Sterile females (%)
Precocene 2	1.0	100	8.0	100
Isopentenylphenol	1.0	100	80.0	100

*Twenty second-stage nymphs were confined to a 9-cm petri dish coated with the test compound residue. †Ten newly emerged females were confined to a treated 9-cm petri dish for 48 hours. Ovaries were examined for development after 6 days.

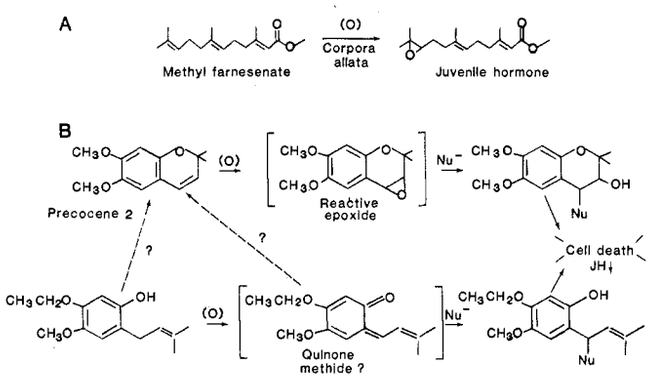


Fig. 1. Oxidative interactions occurring within the insect corpus allatum resulting (A) in juvenile hormone biosynthesis or (B) in the generation of cytotoxic agents from proallatotoxins through the formation of reactive epoxides or quinone methides capable of alkylating nucleophilic (*Nu*) substrates. *JH*, juvenile hormone.

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