昆虫におけるシアノヒドリン類の作用機構

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Original Article

Mode of Action of Cyanohydrins in Insects

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Fumigation toxicity of hydrogen cyanide (HCN) was compared to very potent natural and synthetic cyanohydrins against the house fly and the lesser grain borer. The concentration of cyanide ion was much higher in the house flies exposed to HCN fumigation than in the flies that were exposed to cyanohydrin fumigation or to topical application of cyanohydrins. The results indicate that the toxic action of cyanohydrins in insects is due to the combined effects of cyanide ion, the aldehyde or ketone produced by the decomposition of the cyanohydrin, and possibly the intact cyanohydrin molecule. © Presticide Science Society of Japan

Keywords: fumigant, insecticide, mode of action, insects.

INTRODUCTION

Cyanogenesis, liberation of hydrogen cyanide (HCN), is well known in a wide variety of species in the plant kingdom. For protection from herbivores, many types of plants can synthesize cyanogenic compounds. The distribution, characteristics, and role of these cyanogenic glycosides were reviewed by Park.[1] In this laboratory, we found that natural and synthetic cyanohydrins were potent fumigants to house flies and stored-product pests and are as effective as commercial fumigants.[1–3] Commercial fumigants such as methyl bromide, dichlorvos, and chloropicrin have been used for many years to control insects and pathogens. Those fumigants are being restricted or phased out due to their adverse effects to humans and the environment, so, alternative fumigants are needed for reasons of safety and biodegradability. The insect LC_{50} values of cyanohydrins were obtained with the assumption that the natural and synthetic cyanohydrins had been totally volatilized in the chamber and available to enter the insect body and to release cyanide ion in the insect body.[2]

The objective of this study is to determine the components which kill the insects; that objective addresses whether the cyanohydrins decompose to release sufficient cyanide ion to kill the insects or whether other mechanisms may be involved. Fumigation toxicity of hydrogen cyanide (HCN) against the house fly and the lesser grain borer was compared to natural and synthetic cyanohydrins. Concentrations of the cyanohydrins in the headspace of the chamber and in exposed house fly were investigated by gas chromatography. After topical or fumigation exposure of house fly and lesser grain borer to LC_{50} concentrations of cyanohydrins, the total amount of cyanide ion in the insect body was determined by a spectrophotometric method. The total amount of cyanide ion in the house fly was also measured after a fumigation exposure at the LC_{50} concentration of HCN. This total concentration of cyanide was then compared with the concentration of cyanide ion in the house fly body as a result of exposure to cyanohydrins to determine whether or not the cyanide ion alone in the insect was sufficient to cause death. If not, then other metabolites or the parent cyanohydrin molecule itself must contribute to their insect toxicity.

MATERIALS AND METHODS

1. *Chemicals*

The chemical structures of cyanohydrins tested in this study are shown in Fig. 1. 1-Cyano-1-hydroxy-propene (CHP) and dimethylketone cyanohydrin (DMK) were synthesized in this laboratory.[4] CHP-acetate (CHP-ace), the acetate ester of CHP and also called 2-acetoxy-3-butenenitrile, was purchased from Aldrich (Milwaukee, WI). DMK is a cyanohydrin that occurs naturally, e.g., in flax, as is methylethylketone cyanohydrin (MEK).

2. *Fumigation Toxicity of Hydrogen Cyanide to House Fly and Lesser Grain Borer*

2.1. *Fumigation of* Musca domestica *and* Rhyzopertha dominica

Fumigation toxicity of HCN against the house fly and lesser grain borer was tested by the method of previous research in this laboratory.[4] Ten to 15 adult house fly or lesser grain

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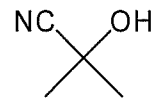
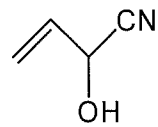
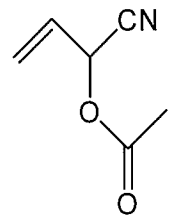


dimethylketone cyanohydrin (DMK)
[a naturally occurring cyanohydrin]



1-cyano-1-hydroxy-2-propene (CHP)
[a synthetic cyanohydrin]



CHP-acetate (CHP-ace)
[an acetate derivative]

Fig. 1. Structures of two cyanohydrins and one derivative which were tested.

borer were placed in a small glass jar (35 ml) with food (powered sugar and dried milk for the house fly or wheat for the lesser grain borer). One ml of 100,000 μg/ml of KCN solution (100,000 μg KCN) was put into a 35-ml glass french square bottle. Both the small jar and french square bottle were put into a 490-ml glass mason jar. The lid was capped immediately after 50 μl of concentrated sulfuric acid was put into the 35-ml french square jar to adjust the pH to 3. The concentration of KCN used was based on an approximate LC_{99} (24-hr). Mortalities were recorded after 24 hr. The fumigation LC_{50} values were calculated by using Proc Probit on SAS.[5] Three replications were conducted in this test.

3. *Determination of Cyanohydrins in the Headspace of the Chamber and in the CHP-Ace-exposed House Fly*

3.1. *Gas chromatography*

A Hewlett-Packard 5890 GC equipped with a flame ionization detector and a carbowax column (25 m×0.25 mm) was used for determining cyanohydrins. For CHP-ace, the oven temperature was programmed at 40°C for 4 min, then increased at 10°C/min to 120°C, and then held for 15 min. The injection and detector temperatures were 180°C and 280°C, respectively. For CHP and DMK, the oven temperature was programmed at 80°C for 4 min, then increased at 10°C/min to 220°C, and then held for 15 min. The injection and detector temperatures were 180°C and 280°C, respectively.

3.2. *Cyanohydrins in the headspace of the chamber*

The highest concentration of CHP-ace and pure CHP and DMK cyanohydrins was used for testing in the headspace of the chamber due to the detection limits. One milliliter of 100,000 μg/ml of CHP-ace solution (100,000 μg of CHP-acetate) in acetone was applied to filter paper in a glass french square bottle (260 ml). After 24 hr, 25 μl of air was taken by a gas-tight syringe through a septum in the lid and was injected onto the GC. For CHP and DMK, pure compound was used, with quantification by the same method as for CHP-ace. After analysis of CHP and DMK with the GC, the chromatogram was compared with a standard curve of CHP-ace to obtain an estimate of their concentrations.

3.3. *Cyanohydrin in CHP-ace-exposed adult house fly*

Fifty adult house flies were put into a small mason jar with food. Filter paper was treated with 100 μl of 10,000 μg/ml of CHP-ace (1000 μg CHP-ace). After 24 hr, adult house flies were ground and extracted with 100 ml of methanol and centrifuged. Two microliters of the supernant was injected onto the GC.

4. *Concentration of Cyanide Ion in* Musca domestica *and* Rhyzopertha dominica

The determination of cyanide ion concentrations in insects was conducted following the method of Epstein.[6] One milliliter of each supernant solution was taken in a tube with 0.2 ml of 1% aqueous chloramine-T solution. Then 6 ml of a pyridine-pyrazolone reagent (500 ml of saturated water of phenyl methyl pyrazolone with 100 ml of pyridine and 0.1 g of bis-pyrazolone) were added. After 20 min, absorbance was measured in a spectrophotometer at 630 nm. A Bausch & Lomb Spectronic®21 spectrophotometer was used for determining the cyanide ion concentrations in insects. Standard curves were prepared by using Microsoft Excel®.

4.1. *Topical*

One microliter (μl) of 10,000 μg/ml of CHP-ace (10 μg CHP-ace) and 100,000 μg/ml of CHP and DMK (100 μg) were dosed onto the dorsal thorax of the house fly. The amounts to be applied were based on approximate LD99 values. After 24 hr, 50 house flies were ground with 100 ml of distilled water and centrifuged. The concentration of cyanide ion in the extracts was measured by the spectrophotometric method.

4.2. *Fumigation*

The fumigation exposure test used the same method as for previous research in this laboratory.[4] Filter paper in a mason jar was treated with 100 μl of 10,000 μg/ml of CHP-ace (1000 μg CHP-ace) and 100,000 μg/ml of CHP and DMK (10,000 μg CHP or DMK). The concentrations were based on an approximate LC_{99}. After 24 hr, 50 house flies were ground and extracted with 100 ml of distilled water and centrifuged. The concentration of cyanide ion in the extracts was analyzed by using the spectrophotometric method. For the lesser grain borer, 1 g of adult lesser grain borer (approximately 800 of the beetles) was used in the same method as used for the house fly. After the fumigation test with 100 μl of 100,000 μg/ml of KCN (10,000 μg KCN) to the house fly,

Table 1. Fumigation 24-hr LC_{50} values ($\mu g \cdot ml^{-1}$) of natural and synthetic cyanohydrins, HCN and commercial fumigants

	M. domestica	95% F.L.[a)]	*R. dominica*	95% F.L.[a)]
Naturally occurring cyanohydrins				
DMK	0.07	0.06, 0.09	0.40	0.35, 0.46
MEK[b)]	0.09	0.07, 0.10	0.41	0.34, 0.51
Synthetic cyanohydrin				
CHP	0.056	0.049,0.063	0.37	0.14, 0.42
Cyanohydrin ester				
CHP-ace	0.26	0.23, 0.30	0.37	0.32, 0.45
Hydrogen cyanide (HCN)	0.02	0.015, 0.033	0.16	0.144, 0.172
Commercial fumigants				
Chloropicrin	0.08	0.076, 0.099	1.30	1.20, 1.42
Dichlorvos	0.011	0.009, 0.013	0.29	0.21, 0.41
Dichloropropene	0.90	0.82, 1.15	5.47	4.76, 6.17

[a)] 95% fiducial limits.

[b)] MEK=methylethylketone cyanohydrin.

which generated HCN gas at an approximate LC_{99} concentration, the concentration of cyanide ion was also measured in the flies.

RESULTS

1. *Fumigation Toxicity of Cyanohydrins and HCN against the House Fly and Lesser Grain Borer*

LC_{50} values for the fumigation toxicity of hydrogen cyanide and some natural and synthetic cyanohydrins are shown in Table 1. Two natural cyanohydrins (DMK and MEK) from flax (*Linum usitatissimum*) and the synthetic analog, CHP, were all very effective fumigants with LC_{50}'s similar to chloropicrin and nearly as potent as HCN.

2. *Cyanohydrins in the Headspace of the Chamber and in the CHP-Ace-exposed House Fly*

The percentage of each cyanohydrin in the headspace of the chamber was as follows: DMK 1%, CHP 1%, CHP-ace 7%. The mean percentage of cyanohydrin in the CHP-ace-exposed adult house fly was 53%.

3. *Concentration of Cyanide Ion in House Fly and Lesser Grain Borer*

Table 2 shows the amount of total cyanide ion in the insect body after the topical and fumigation tests with cyanohydrins and after a fumigation test with hydrogen cyanide.

DISCUSSION

Comparison of the fumigation toxicity (LC_{50}) of hydrogen cyanide to the cyanohydrins showed that hydrogen cyanide was somewhat more toxic than the cyanohydrins against the house fly and lesser grain borer. It is possible that cyanohydrins could have been transformed to degradation products other than HCN or could have remained on the filter paper or the glass jar wall of the exposure jar. It is well known that HCN is a highly toxic fumigant that has been used to control insects. Hansen reported, for example, that HCN fumigation was highly effective against pests of Hawaiian cut flowers and foliage.[7)]

As a result of determination of the cyanohydrins in the headspace of the chamber, less than 10% of the total cyanohydrin in the air indicates that the rest of the cyanohydrins could have stayed in the filter paper or on the jar wall or could have degraded. Filter papers treated with cyanohydrins were extracted with acetone, and the cyanohydrins were measured by GC. Residual cyanohydrins were not detected in the filter paper. This may be due to volatilization, transformation or reaction with the filter paper. 53% of CHP-ace was detected in the house fly body after the fumigation exposure. The 7% in the headspace and 53% in the house fly bodies together only provide a mass balance of 60%.

Table 2. Concentration of cyanide ion in insects, as μg CN^-/g insect body (ppm), following topical application at the LD_{99} or fumigation at the LC_{99}

Compound	House fly		Lesser grain borer	
	Topical	Fumigation	Topical	Fumigation
DMK	56	48	NT	43
CHP	52	38	NT	35
CHP-ace	54	39	NT	33
HCN	NT	680	NT	NT

NT: not tested.

The release of hydrogen cyanide is not confined to higher plants. Cyanogenic glycosides are released by millipedes[8] and larvae of *Zygaena* spp. (the Burnet moth). Detecting 53% of CHP-ace in the house fly body after the fumigation exposure means that some of cyanohydrin stayed on the filter paper or the jar wall, the same assumption as for the headspace experiment, or was transformed or excreted by the house flies.

After the house fly and the lesser grain borer were treated with cyanohydrins or hydrogen cyanide at the approximate LC_{99} concentrations, it was determined that the total amount of cyanide ion in the insect body exposed to hydrogen cyanide was higher than in those that were exposed to a cyanohydrin. This indicates that the cyanohydrins entered the insect body but generated less cyanide ion than hydrogen cyanide. The conversion of the cyanohydrins to CN^- in the body was obviously not 100%; they may bind to tissues or be detoxified and/or excreted.

At the approximate LC_{99} (for fumigation), or LD_{99} (for topical application) exposure levels, the three cyanohydrins only generated 33–56 ppm CN^- in the insect bodies, while the HCN exposure at the approximate LC_{99} resulted in 680 ppm CN^- in the insect bodies (Table 2). This result implies that other modes of toxic action must contribute to the efficacy of the cyanohydrins. Possible explanations are 1) the ketone or aldehyde that results from degradation of a cyanohydrin (this laboratory, unpublished results) is also toxic, either additive or synergistic, 2) cyanohydrins may be toxic themselves (before degradation), or 3) cyanohydrins may penetrate further into lipoid tissue of the insect than HCN (due to greater lipophilicity) before releasing cyanide ion, *i.e.*, delivering the toxic moiety more efficiently than HCN does.

Comparing fumigation LC_{50} values to total cyanide ion for the CHP- and DMK-exposed house fly and lesser grain borer at the same treatment concentration (LC_{99}), the total cyanide ion from the CHP-exposed should be higher than for the DMK-exposed because the CHP LC_{50} was lower than the DMK LC_{50}, because CHP should decompose to release cyanide in vivo more readily than DMK does. However, the data indicated that the quantity of cyanide ion in the DMK-exposed insects was higher than for the CHP-exposed insects (except for the topical application for the house fly). This might be due to different rates of detoxification of the molecule in the insect body, prior to cyanogenesis.

Cyanide ion concentrations generated in the house fly were higher than in the lesser grain borer. This result supports the fact that potency for the house fly was lower than for the lesser grain borer. Responsibility for toxicity of the cyanohydrins apparently depends partially on the quantity of cyanide ion released in the insect body, but rates of decomposition to CN^- and a small carbonyl compound can differ, and they may account for part of the difference in toxicities observed.

These low-molecular-weight natural and synthetic cyanohydrins are highly volatile. Although the measured concentrations in the headspace were low, significant quantities of the cyanohydrin reach the insect body through the vapor phase. The natural and synthetic cyanohydrins were almost as potent as hydrogen cyanide against house fly and lesser grain borer. Although the cyanohydrins were not totally broken down to cyanide ion in the insect body, the cyanohydrins generated some quantities of cyanide ion and apparently kill the house fly and lesser grain borer through a combination of two or more mechanisms of toxicity.

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REFERENCES

1) D.-S. Park and J. R. Coats: *The Korean J. Pestic. Sci.* **6**, 51–57 (2002).
2) C. J. Peterson, R. Tsao and J. R. Coats: *Pest Manag. Sci.* **56**, 615–617 (2000).
3) C. J. Peterson, R. Tsao, A. L. Eggler and J. R. Coats: *Molecules* **5**, 648–654 (2000).
4) D.-S. Park, C. J. Peterson, S. Zhao and J. R. Coats: *Pest Manag. Sci.* **60**, 833–838 (2004).
5) SAS Institute (1991).
6) J. Epstein: *Anal. Chem.* **19**, 272–274 (1947).
7) J. D. Hansen, A. H. Hara, H. T. Chan, Jr. and V. L. Tenbrink: *J. Econ. Entomol.* **84**, 532–536 (1991).
8) S. S. Duffey: "Cyanide and Arthropods," ed. by B. Vennesland, E. E. Conn, C. J. Knowles, J. Westly and F. Wissing, Academic Press, London, pp. 385–414, 1981.