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Cyanogenic glycosides : Alternative insecticides?

Dong-Sik Park and Joel R. Coats

Department of Entomology, Iowa State University, Ames, IA 50011, USA

Abstract : Cyanogenic glycosides are secondary plant metabolites that are known as plant defense chemicals. They are found in cassava, bamboo, flax, and other plants. In this paper, the role of cyanogenic glycosides, their characteristics, and their interactions with insects are discussed. Previous and current research in our laboratory found that several natural and synthetic cyanohydrins were effective against stored-product insects as fumigants. Due to their insecticidal activity to insects, cyanohydrins can be used as an alternative fumigant and also as soil fumigants. Risk assessment, however, should be done to account for possible environmental problems, non-target wildlife effects, and human health effects.(Received June 4, 2002; accepted June 26, 2002)

Key words : Cyanogenic glycosides, stored-product insects, fumigant, insecticidal activity.

Introduction

Agrochemicals (insecticides, herbicides, and fungicides, etc.) are used for safeguarding the food supply and decreasing pest populations. It is well known that the large-scale use and repeated application of synthetic insecticides, chlorinated hydrocarbon, organophosphorus esters, carbamates, and synthetic pyrethroids, have caused widespread concern regarding environmental problems (air, water and soil), increased resistance, as well as serious acute and chronic toxicity to non-target organisms, sometimes including humans. In addition, some of their metabolites can also be of concern as contaminants in groundwater or as carcinogens or mutagens. One of the types of insecticides used to eliminate pests is the fumigant, which is categorized by its delivery mechanism, i.e., route of exposure. For their fumigation activity, fumigants should be volatile, so they mainly have low molecular weight and are nonpolar, frequently containing chlorine, bromine, or phosphorus. However, several current leading commercial fumigants, methyl bromide, chloropicrin, and dichlorvos, are considered to be contaminants in the environment. For that reason, those commercial fumigants are becoming severely restricted or will be phased out. Those risks and the need for new types of insecticides led us to consider possible reduced-risk insecticides.

Rapid biodegradability in the environment, safety to non-target organisms, and selectivity are considered as good properties of new alternative pesticides. One possible alternative is to use naturally-based pesticides and their close derivatives or analogs. Since the time of the ancient Romans, extracts of plants have been used as insecticides. Phytochemicals are defined as materials that plants make for growth, reproduction and defense from plant feeding animals. Numerous plants produce metabolites for physiological development, namely, growth and reproduction, called primary metabolites such as carbohydrates, proteins, fats, hormones, *etc.* They also make

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*Corresponding author (Fax : +1-515-294-4757,
E-mail : dpark@iastate.edu, jcoats@iastate.edu)

secondary metabolites which are less abundant or derived from primary metabolites in the plant and have a role in defense against herbivores, pests and pathogens. The secondary plant metabolites can function as feeding attractants, repellents, feeding stimulants, feeding deterrents, oviposition stimulants and deterrents, and toxicants (Hedin, 1991). Due to their insecticidal characteristics, we have been using plant secondary metabolites as insecticides for many years, for example, pyrethrins extracted from pyrethrum (*Chrysanthemum cinerariaefolium*) flower heads, nicotinoids (*Nicotiana*), rotenonoids isolated from Leguminosae (*Derris*) and terpenoids (mints, pine, and cedar) (Adityachaudhury, 1985; Klocke, 1987; Benner, 1993). Still, many higher plant species have not been surveyed and exploited for insecticidal activity of their secondary metabolites. Discovering and developing plant-based insecticides is a major concern for finding new insecticides in the future.

This overview article will focus on the characteristics and role of cyanogenic glycosides in plants, and their interaction with insects and will cover some aspects of cyanogenic glycosides as alternative insecticides.

Distribution and characteristics of cyanogenic glycosides

Because the cyanogenic glycosides are not ubiquitous in nature, they must be classified as secondary plant products, providing defense mechanisms (allelopathy). Cyanogenic glycosides are polar and water soluble compounds, and are one group of the potentially toxic constituents which is found in some root and seed crops. Those plant species include bitter almonds (*Prunus amygdalus*), apricot (*Prunus armeniaca*), and cherry (*Prunus avium*), all of which contain amygdalin (a source of HCN); sorghum (*Sorghum bicolor*), which contains dhurrin; cassava (*Manihot esculenta*), lima beans (*Phaseolus lunatus*), and flax (*Linum usitatissimum*), which contain linamarin; and many other vascular plant groups. These plants are grown and used for starch, protein, oil or fiber sources, and as spices or crude drugs. Fig. 1 shows the three major cyanogens in plants.

The cyanogenic glycosides are biosynthesized by the plants from aromatic or branched-chain amino acids, namely, valine, isoleucine, leucine, phenylalanine and tyrosine. Cyanogenic glycosides can be defined as glycosides of α-hydroxynitriles (cyanohydrins); the cyanohydrins are called aglycones, meaning that the sugar(s) have been hydrolyzed off the glycoside leaving only the cyanohydrin. The glucose with a β-configuration of glycosidic linkage is the sugar which is directly attached to the α-hydroxynitriles (cyanohydrins) of most cyanogenic glycosides (Seigler, 1992; Nahrstedt, 1993). These substances are not themselves toxic; however, the formation of free HCN, a process called cyanogenesis, is associated with a cyanogenic glycoside that is hydrolysed by a β-glycosidase to give a hydroxynitrile, which then decomposes to a carbonyl compound and HCN when tissues of the plants are crushed or destroyed by animals feeding on them. Their general structure and metabolism is shown in Fig. 2.

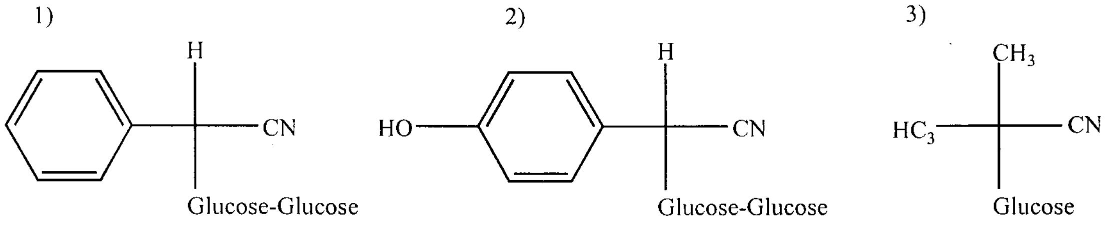


Fig. 1. Three major cyanogens in plants (1: amygdalin, 2: dhurrin, 3: linamarin).

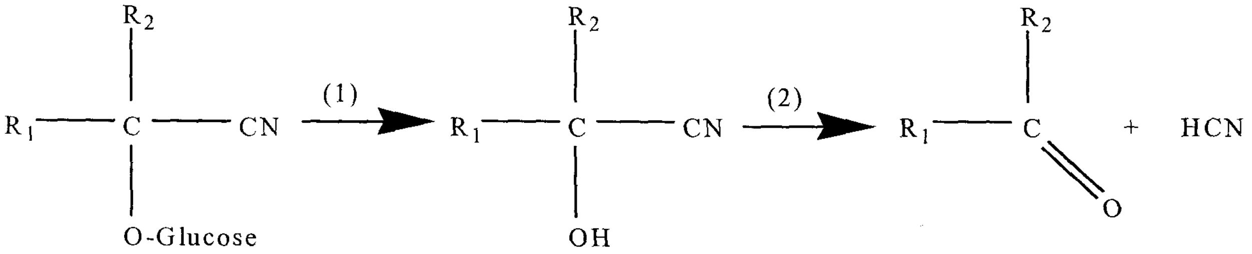


Fig. 2. The structure of cyanogenic glycosides, and their metabolism to release hydrogen cyanide (HCN); (1) *β*-glycosidase, (2) hydroxynitrile lyase.

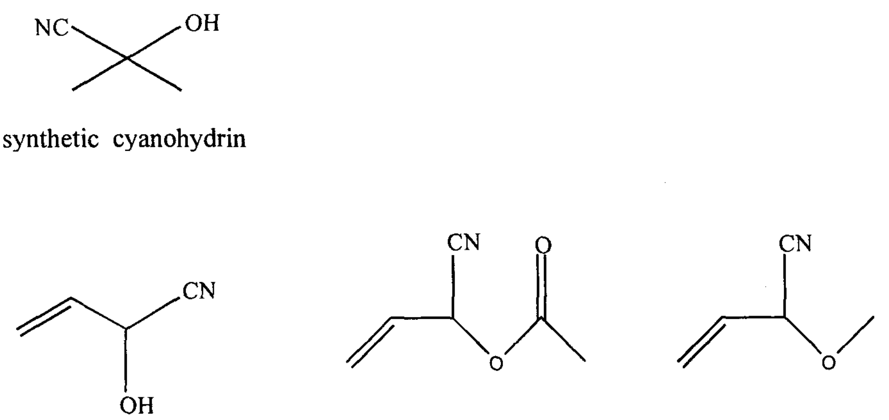
The pH and temperature influence the reactions that yield carbonyl components and hydrogen cyanide (Eyjolfsson, 1970; Conn, 1981; Nahrstedt, 1993). At an alkaline pH and temperatures in excess of 60 ºC, cyanogenesis is faster. The chiral (asymmetric) cyanohydrin carbon in cyanogenic glycosides can be epimerized by dilute base; there are two types of epimers, (R)- and (S)-, in many series of compounds (Eyjolfsson, 1970; Ettlinger et al., 1977; Conn, 1979; Seigler, 1992).

Role of cyanogenic glycosides

Many scientists have already mentioned the role of cyanogenic glycosides in plants (Conn, 1981; Poulton, 1990; Vetter, 2000). In their physiological role, it is clear that cyanogenic glycosides in a plant are related to the production of HCN which is toxic to herbivores; therefore, the plant can be protected from the attack. Several studies have shown that cyanogenic glycosides can act as either feeding deterrents or phagostimulants (Brattsen, et al. 1983; Ellsbury, et al. 1992; Alborn, et al. 1992; Belloti, et al. 1993). In fact, cyanogenesis is not confined to plants. For example, millipedes produce cyanoglycosides (Duffey, 1981), and a cyanogenic glycoside is released by larvae of *Zygaena* spp. (Burnet moth) when they are attacked. This secretion by arthropods is evidence in support of the function of cyanogenic glycosides in plants. Robinson (1930) reviewed the early ideas, which included the cyanogens being nitrogen reserves and precursors for protein synthesis, excretory waste products or protective substances. Consumption of foods containing cyanogenic glycosides by humans has very rarely resulted in cyanide poisoning. However, major livestock losses have occurred due to their consuming cyanogenic glycosides. Cattle and other ruminants are more sensitive to these cyanogenic glycosides because the near-neutral pH in their stomachs favors the release of HCN, compared to the acidic stomachs in other mammals. Several research papers and reviews already provide a look at the interaction between cyanogenic glycosides and insects (Jones, 1962, 1966, 1971, 1988; Crawford-Sidebotham, 1972; Ennos, 1981; Compton, 1985; Nahrstedt, 1985; Hruska, 1988). Their research has helped explain the biochemical role of cyanogenic glycosides against herbivores.

Mode of action

Hydrogen cyanide (HCN) is a toxic substance that generates CN^-, and this causes the inhibition of cytochrome oxidase and other respiratory enzymes. The primary mode of action of cyanide is based on its affinity for the ferric heme form of cytochrome a_3 (cytochrome oxidase) in the electron transport system (Buck, et al., 1976; Klaassen, 1996). This reaction forms a relatively stable cytochrome oxidase-CN complex in the mitochondria. When iron is maintained in the trivalent state, electron transport along the cytochrome chain is stopped, and the chain of cellular respiration is brought to a halt. As a result, hemoglobin cannot release its



Naturally occurring cyanohydrin

synthetic cyanohydrin

1-cyano-1-hydroxy-2-propene (CHP)

CHP-acetate (CHP-ace)

CHP-methyl ether (CHP-me)

Fig. 3. Volatile natural and synthetic cyanohydrins and derivatives.

oxygen to the electron transport system.

Alternative insecticides?

As we mentioned above, cyanogenic glycosides can be considered as possible insect control materials by means of their distribution in many plants, their role, and interaction with insects. Previous research (Peterson, 2000a) in our laboratory found that several natural and synthetic cyanohydrins were effective against insects as fumigants. Also, they (Peterson, 2000b) found that topical application of several synthetic cyanohydrin compounds were effective against house flies and mosquitoes. For that reason, we synthesized natural cyanohydrins and some derivatives in the laboratory to test the insecticidal activity against the house fly (*Musca domestica*), and several stored-grain pests (lesser grain borer, *Rhyzopertha dominica*; maize weevil, *Sitophilus zeamais*; sawtoothed grain beetle, *Oryzaephilus surinamensis*; red flour beetle, *Tribolium castaneum*) and to determine which chemical structure is the best one to explain the toxicity, using quantitative structure-activity relationships (QSAR). The structures of 4 representative natural and synthetic cyanohydrins tested in our study are shown in Fig. 3. Other compounds and data are available in Park 2002a and Park 2002b. In addition, various concentrations of 1-cyano-1-hydroxy-2-propene acetate (acetoxybu-

Table 1. Fumigation LC_{50} values (g ml^{-1}) of volatile natural and synthetic cyanohydrins

	M. domestica	95% F.L.[a]	*R. dominica*	95% F.L.[a]
Naturally occurring cyanohydrin				
DMK	0.07	0.06, 0.09	0.40	0.35, 0.46
Synthetic cyanohydrin				
CHP	0.056	0.049,0.063	0.37	0.14, 0.42
Cyanohydrin ether				
CHP-me	0.41	0.34, 0.49	0.88	0.75, 1.04
Cyanohydrin ester				
CHP-ace	0.26	0.23, 0.30	0.37	0.32, 0.45

[a] 95% fiducial limits

tenenitrile) were tested as soil fumigants. Specifically, anti-bacterial and anti-fungal activity, as well as inhibition of weed-seed germination, were measured in treated soils.

As a result, we found that natural and synthetic cyanohydrins are quite effective against stored-product pests (Park, 2002a). Fumigation LC_{50} values of 4 volatile natural and synthetic cyanohydrins against stored-product pest and house fly are shown in Table 1. Three parameters (log P, polarizability, and molar refractivity) are related to fumigation toxicity to the house fly (Park, 2002b). These parameters explain the chemical properties such as lipid solubility, London dispersive forces, and molar volume, respectively. The R^2 values of three parameters and molecular descriptors for cyanohydrins are shown in Table 2 and 3.

Table 2. The R2 of three parameters to house fly and lesser grain borer

Parameters	R^2	
	House fly	Lesser grain borer
Log P	0.86	0.62
Polarizability	0.79	0.53
Molar refractivity	0.80	0.40

Table 3. Molecular descriptors for cyanohydrins and derivatives

Compounds	Log P	Polarizability	Molar refractivity
DMK	0.354	7.035	22.5
CHP	0.550	7.796	22.2
CHP-ace	0.679	8.356	31.4
CHP-me	0.828	9.330	27.0

This result means that the degree of adverse effects on the insects depend on the chemical properties. 1-Cyano-1-hydroxy-2-propene acetate reduced the total soil bacterial and fungal counts significantly, and was effective in inhibiting the germination of weed seeds in soil. Twelve different cyanohydrins or their derivatives were also shown to be nematicidial in laboratory trials and in soil. The possibility of a cyanohydrin (or ester of one) serving as a soil fumigants to replace methyl bromide is intriguing, since some can effectively kill insects, nematodes, weeds, fungi, and bacteria. These results tell us that cyanohydrins might be a useful alternative insecticide.

With laboratory tests and soil tests, we have only limited data for fumigation toxicity and biological activity as a soil fumigant for natural and synthetic cyanohydrins. Mammalian toxicity testing has not been conducted on any of the cyanohydrins discussed here. For a potential commercial fumigant aimed at controlling pests in storage bin, buildings, ships, stored products, soil, on food, or in any closed areas, risk assessments should be done for environmental problems, wildlife effects, and human health. Those include acute and chronic toxicity to mammals, birds, or movement to ground water, and persistence in soil. Also, testing should be done for carcinogenicity, mutagenicity or teratogenecity in mammals.

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