Table 1. Results of tests for carbohydrase activity in the digestive tract of *B. craniifer*.

Substrate	Salivary glands	Foregut	Midgut	Malpighian tubules	Hindgut	Type of enzyme
Glycogen	+	+	+		−	Amylase
Starch	+	+	+		−	Amylase
Cellulose	−	−	−		−	
Melezitose	−	−	+	−	−	α—Glucosidase
Raffinose		+	+	+	+	β—Fructofuranosidase
Sucrose		+	+	+	+	α—Glucosidase or β—Fructofuranosidase
Methyl glucoside		−	+	+	+	α—Glucosidase
Cellobiose	−	+	+	−	+	β—Glucosidase
Lactose	+	+	+	−	+	β—Galactosidase
Maltose	+	+	+	+	+	α—Glucosidase
Melibiose		+	+	−	+	α—Galactosidase
Control No. 1	+	+	+	−	+	β—Fructofuranosidase
Control No. 2	−	−	−	−	−	

moved and cleared of surrounding tissues. Like tissues from five cockroaches were pooled, and ground with clean white sand in a small amount of saline (0.9 percent NaCl, 0.02 percent KCl, 0.02 percent $CaCl_2$, 0.4 percent glucose). The resulting brei was filtered into test tubes, each suspension then being made up to 6.0 ml by the addition of saline.

To test for the presence of carbohydrases, experiments were conducted in vitro with substrates consisting of a di-, tri-, or polysaccharide, or a glycoside. Reaction mixtures were put in incubation tubes, each of which contained 0.5 ml of suspension, 1.0 ml of 2-percent substrate, 1.0 ml phosphate buffer (0.05*M*) adjusted to *p*H 6.5, and 5 drops of toluene, a bacterial inhibitor. To serve as controls, one tube contained the complete reaction mixture, sucrose substrate, but no buffer: another contained the complete reaction mixture and sucrose substrate, but was inactivated by boiling for 3 minutes (Table 1, Nos. 1 and 2, respectively). All tubes were incubated 24 hours at 36°C. To test for cellulase activity, finely divided filter paper was used as the substrate and the incubation time extended to 72 hours. Benedict's reagent was used to test for hydrolysis of non-reducing carbohydrates, while the hydrolytic products of reducing disaccharides were detected by the osazone method.

The fates of carbohydrates in the alimentary tract of *B. craniifer* were followed by means of paper chromatography. Pairs of cockroaches were provided with cotton swabs soaked with a 20-percent solution of the desired substrate. One pair (controls) were provided with swabs dampened with tap water. The substrates were left with the cockroaches for 3 hours, after which the animals were anesthetized and opened. Ligatures were applied at the juncture of the fore- and midgut, and between the mid- and hindgut. Each section was then aspirated, after puncture with a fine glass capillary, and the intestinal juice thus collected was applied in drops, 1 inch apart, to a sheet of 9- by 11-inch Whatman No. 1 filter paper. Chromatograms were run by the ascending method. The solvent system was *n*-butanol, pyridine, and water (3:1:3 vol/vol). Benzidine trichloroacetic acid (*5*) was the developing reagent. Presence of the original substrate or of its hydrolytic products was determined by comparing their positions with those of reference sugars.

Results are summarized in Table 1. Results obtained with Benedict's solution indicated the presence of amylase which hydrolyzes starch and glycogen to maltose and glucose. An α-glucosidase, which cleaves methyl glucoside and melezitose, as well as β-fructofuranosidase, which splits sucrose and raffinose, were present. There was no evidence of a cellulase.

Positive osazone tests indicated the presence of α- and β-galactosidases and α- and β-glucosidases, which catalyze the hydrolysis of melibiose, lactose, maltose, and cellobiose, respectively. Results obtained with the buffer-free control suggest that the influence of the buffer was negligible. The negative test results in the control that was heated (No. 2, Table 1) show that any enzymes that may have been present were inactivated.

Chromatograms of aspirated intestinal juices yielded spots which support the above findings. Indeed, definite evidence of melibiase activity in the hindgut, of lactase in the fore and hindgut, and of cellobiase in all regions, was obtained only by this procedure. By contrast, results of osazone tests, in the instances cited, were often inconclusive.

The distribution of carbohydrases in *B. craniifer* resembles that reported for *B. discoidalis* and *Leucophaea maderea* (*3*). It differs from the latter two species but resembles most other insects in its apparent lack of cellulase (*2, 6*). The midgut is the site of the most vigorous enzymatic activity, there being a gradual decrease in activity in other parts of the tract. This is consistent with histological findings of a preponderance of secretory epithelium in the midgut, slight extension of secretory cells into the caeca, and none in the other areas. It seems reasonable, then, to ascribe the carbohydrase activity of the fore- and hindgut regions partly to overflow from the midgut, and partly to enzymes exuded from macerates of the two sections (*7*).

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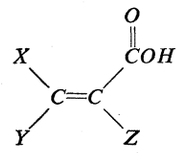
cis-3-Chloroacrylic Acid: A New Cotton Defoliant and Crop Desiccant

Abstract. *cis-3-Chloroacrylic acid is a potent cotton defoliant and a crop desiccant. Relationships between structure and activity indicate a relatively high degree of specificity, since minor modifications in structure result in loss of activity.*

Halogenated, short-chain aliphatic acids are known to have effective herbicidal properties. Examples include sodium trichloroacetate and dalapon (sodium 2,2-dichloropropionate) (*1*).

Unusual plant-growth regulating properties of *cis*-3-chloroacrylic acid and its salts have been discovered. Sodium *cis*-3-chloroacrylate (*2*) is an efficient cotton defoliant (*3*) and crop

Table 1. Cotton defoliating properties of various derivatives and analogs of *cis*-3-chloroacrylic acid



applied at the rate of 2 kg per hectare (2 lb/acre).

X	*Y*	*Z*	Defoliation 10 days after application
Cl	H	H	Complete
H	Cl	H	None
Cl	Cl	H	None
Br	H	H	Complete
Cl	H	CH_3	Complete
Cl	H	C_2H_5	None
Cl	H	C_6H_5	None
Cl	H	Cl	None
Cl	CH_3	H	None
Cl	COOH	H	None
COOH	Cl	H	None
CH_3	H	H	None
CH_3	Cl	H	None
H	CH_3	H	None
H	Cl	CH_3	None

desiccant (*4*). It also has a relatively low mammalian toxicity (LD_{50} rat: oral, 320 mg/kg; LD_{50} rat: skin penetration, 400 mg/kg) (*5*).

cis-3-Chloroacrylic acid can be synthesized by reacting sodium acetylide with CO_2 with the formation of sodium propiolate, which, when treated with aqueous HCl in the presence of Cu_2Cl_2, forms *cis*-3-chloroacrylic acid almost exclusively. Recrystallization from hexane gives a good yield of product, melting point 60° to 62°C, which may be compared to 63° to 64°C (*6*). Calculated for $C_3H_3ClO_2$ are C, 33.83 percent; H, 2.84 percent; Cl, 33.29 percent; found: C, 33.68 percent; H, 2.95 percent; Cl, 33.23 percent.

The defoliating activity of *cis*-3-chloroacrylic acid and several closely related compounds was determined by applying approximately 2.0 kg per hectare (2.0 lb/acre) to the foliage of greenhouse-grown cotton (*Gossypium hirsutum*, L. var. Coker 100A) at the stage when four to eight leaves had appeared. The comparative defoliating properties of these compounds are presented in Table 1.

Activity is associated with the *cis*-isomer and its salts. The corresponding *trans*isomer and its sodium salt are virtually without activity. Substitution of bromine for chlorine does not alter activity. Replacement of the α-hydrogen with an α-methyl does not reduce activity. However, when the α-substituent is ethyl, phenyl, or chloro, activity is eliminated. Replacement of the β-hydrogen with a methyl, chloro, or carboxyl group also results in inactivation. Chloromaleic acid, *cis*- and *trans*-crotonic acid, and halogenated saturated aliphatic acids, such as 3-chloropropionic acid, 2,2-dichloropropionic acid, and 3,3-dichloropropionic acid are all without activity.

Amides, substituted amides, and esters of *cis*-3-chloroacrylic acid, were ineffective. Subsequent studies indicated that hydrolysis by the plant to the acid was negligible; however, soil organisms were capable of hydrolyzing both the ester and the amide to the active acid.

From these data, it is possible to construct structural criteria for defoliation activity in this series of related compounds. (i) The beta carbon of the acrylic acid derivative must be substituted with a halogen oriented *cis* to the carboxyl group and a hydrogen oriented *trans* to the carboxyl group. (ii) Unsaturation must be present for activity. (iii) The carboxyl group must be readily available. Esters and amides will not be effectively hydrolyzed by the plant but may be by soil organisms. (iv) Substitution in the α-position is limited to size since substitution of groups larger than methyl results in inactive molecules.

The coincidence of defoliation activity with a high degree of structural and stereospecificity leads us to propose that *cis*-3-chloroacrylic acid and the few other active analogs act as inhibitors of a specific biochemical mechanism in the regulation of leaf abscission.

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Thymectomy: Prolongation of Immunological Tolerance in the Adult Mouse

Abstract. *The loss of acquired immunological tolerance of mice to bovine gamma globulin depended on the presence of the thymus. Mice were repeatedly injected with bovine γ-globulin from birth until the age of 5 to 10 weeks and then thymectomized or sham operated. After 130 to 160 days without antigen, an accelerated (immune) disappearance of I^{125} bovine γ-globulin could be uniformly induced in controls while thymectomized mice remained tolerant. Adult thymectomized mice made tolerant by a single injection of bovine γ-globulin lost tolerance more slowly than sham-operated controls.*

The thymus is thought to be essential for the development of immunologically competent cells capable of producing specific antibody. Mammals thymectomized at birth have a reduced capacity to produce antibodies when later stimulated with antigen. They fail to reject skin homografts and have fewer small lymphocytes in their blood and tissues (*1*). The thymus is also active in adult life. Adult animals treated with whole-body radiation will not recover immunological reactivity if they are thymectomized before x-ray is given (*2*). It is not known whether immunologically competent cells are produced in the thymus and transported to the lymphoid tissues or whether the development of these lymphoid cells is under the indirect, perhaps humoral, influence of the thymus.

A state of immunological unresponsiveness to soluble complex antigens may be induced in normal animals by injecting these antigens at birth. Such unresponsiveness may also be produced in adult animals if the antigen is injected in large enough doses, or if the animal is treated with x-ray or radiomimetic drugs (*3*). Although the mechanism is unknown, the duration of this form of immunological tolerance is proportional to the dose and persistence of antigen. When tolerance disappears, the capacity to respond to antigen reappears and in some instances antibody may be formed without further antigenic stimulation (*4*). The waning of tolerance may be explained on a cellular basis either by the loss of unresponsiveness by cells capable of producing specific antibody or by the development of new uninhibited cells.