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A Research Note

Squash Containing Toxic Cucurbitacin Compounds Occurring in California and Alabama

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ABSTRACT

A highly toxic, extremely bitter compound was found in canned zucchini squash from a large California cannery. The same toxin occurred in yellow straightneck squash grown in two different home gardens in Alabama. The compound was determined as cucurbitacin E and the quantities found in both squash types were potentially hazardous to humans.

The cucurbitacins are a group of tetracyclic triterpenes which occur naturally in the plant family *Cucurbitaceae*. Over twenty different compounds have been isolated from the resin, cork, or cutin of various species (*9,15*). Cattle and sheep have been poisoned from consuming fruit of wild species (*17*). While the existence of these toxic compounds in wild species has long been known, their presence in fruit of cultivated species is extremely rare, and probably represents a mutation to an ancestral condition or a chance outcross with a wild species.

Historical records as early as 1552 BC documented the use of colocynth (presumably an extract of *Citrullus colocynthis* - a wild species of watermelon) as a medicinal preparation by an Egyptian physician (*2*). Pharmacologically these compounds have been used as purgatives (*1,11*), anti-tumor agents (*14*), and growth inhibitors for solid tumors in vitro and in vivo (*7*). The extremely high toxicity of these compounds has resulted in the abandonment of pharmaceutical uses in recent years (*3*).

Cucurbitacin E, which is the compound found in squash (*16*), has an LD_{50} i.p. in mice of 2.0 mg/kg (*12*), and other cucurbitacins have similar toxicities: cucurbitacin A, 1.2 mg/kg i.p. in mice, 2.0 mg/kg i.p. in rats; cucurbitacin B, 1.1 mg/kg i.p. in mice; cucurbitacin C, 6.8 mg/kg i.p. in mice (*4*).

The cucurbitacins as a group are thought to be the most bitter substances known to man (*13*). Cucurbitacin B has been detected by taste panel in dilutions as low as 1 ppb and the glycoside of cucurbitacin E at 10 ppb. Brucine alkaloid was previously thought to be the bitterest substance when it was detected at 1 ppm (*10*).

These bitter toxins may be concentrated in fruits and roots of mature plants and the leaves and stems are then only slightly bitter. The roots are the only bitter parts in a number of species. With few exceptions, bitter roots appear to be a prerequisite for bitterness in other parts of the plant. However, there may be any combination of cucurbitacin concentration between vegetative parts and fruit. Watermelon and squash are among those species which are characterized by the presence of mainly one cucurbitacin, cucurbitacin E (*16*).

The objectives of this study were to identify and quantitatively determine the bitter substances occurring in squash fruit occurring in California and Alabama, to determine if other plant parts contained the substances, and to obtain preliminary information on the genetic aspects of the substances in the plants.

MATERIALS AND METHODS

Materials

Samples of bitter canned zucchini pieces in tomato sauce were obtained directly from the canner's warehouse in California during the summer of 1981. The samples were part of a large pack, which was not released for sale since the presence of the bitter compounds had been detected during quality control testing. Frozen raw zucchini fruit pieces of the Castleverde cultivar (Castle Seed Co.) were also obtained from California as well as mature seed from the bitter fruit.

The yellow straightneck squash fruits were obtained from home gardens in two widely separated counties in Alabama (Fayette and Lee) during the same growing season, through county extension agents. The garden in Lee county was visited and the plant photographed. Seed were purchased of the same cultivar (Early Prolific Improved, Hollar Seed Co.). The seeds for the yellow straightneck squash with bitter fruit planted in Fayette county were obtained from the Guerney Seed Co. (Guerney's Early Improved).

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Methods

The following analysis is a modification of the method of Enslin (*5*). Fresh weights of squash samples were taken and analyzed as follows: (*a*) blend fruit with 70% ethanol, equal w/v; (*b*) centrifuge 15 min at 5000 rpm; (*c*) decant and extract solids again with 70% ethanol; (*d*) partition 3 times with equal volumes chloroform; (*e*) evaporate at 50°C and wash residue 3 times with petroleum ether; (*f*) read absorption spectrum in chloroform from 180-300 nm and calculate cucurbitacin content at 242 nm from standard curve prepared with cucurbitacin E.

Seeds from the same source (Hollar Seed Co.) which produced the bitter yellow squash were planted in a growth chamber and cotyledons from over 300 seedlings were tasted for bitterness. A sample of 20 seeds from a bitter zucchini squash fruit self-pollinated in the greenhouse in California was planted in a greenhouse and seedlings were tasted for bitterness. The seedlings were grown to maturity and the fruits were also tasted for bitterness.

RESULTS

No bitterness was detected in any of the cotyledons from over 300 of the yellow straightneck squash seedlings tasted. Germination was 90% on the 20 seeds planted from one of the self-pollinated bitter zucchini fruit from California. Thirteen of the 18 seedlings had bitter cotyledons and five were non-bitter. Self-pollinated fruit were obtained from 15 of the 18 seedlings of which 12 were bitter and three non-bitter. Two of the non-bitter fruits came from plants having bitter seedlings and the other non-bitter fruit came from a plant having a non-bitter seedling. Thus one of the bitter fruit came from a seedling having non-bitter cotyledons. The 15 fruits from the bitter zucchini showed the variation in size, shape and coloring expected of the segregation pattern for a hybrid. The seeds that produced the bitter plants probably resulted from a mutation (atavism) or from a chance pollination (outcross) from a plant with bitter fruit. In both the home gardens in Alabama, only one plant in the garden had bitter fruit and in California it was estimated that only one plant in 3,000 was bitter. (However, in regard to the commercial canned zucchini, the entire pack had to be destroyed because one small section of bitter fruit made an entire can extremely bitter). There was no difference in appearance between the bitter or non-bitter plants or fruit of the yellow straightneck squash in the gardens. A complete genetic study is in progress to determine the genetic basis for the occurrence of these bitter squashes.

The quantity of cucurbitacin E found in the bitter yellow straightneck squash fruit averaged 3.10 mg/g of fresh fruit, the canned zucchini sample contained 0.93 mg/g of can contents, and the frozen fresh zucchini sample contained 7.20 mg/g of flesh at the stem end with 2.7 mg/g in the central portion of the fruit. One would assume that since the cucurbitacins are among the most bitter compounds known to man it would not be likely that anyone would consume bitter fruit unless that person were insensitive to the bitter taste. However during the same period (November 1981 to December 1982) that the bitter zucchini and yellow straightneck squash occurred in California and Alabama, 22 cases of severe food poisoning associated with commercially produced zucchini were reported in Queensland and Australia (*6,8*). Tracebacks were to the Blackjack cultivar (Petoseed Co.) in 21 cases and Castleverde in the other. In case studies symptoms of severe cramps, persistent diarrhea and collapse occurred within 1 to 2 h after ingestion of about 3 g of zucchini.

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