Bitter Compounds in Tubers of *Dioscorea bulbifera* L.

Bitter and related nonbitter compounds were extracted from tubers of *Dioscorea bulbifera*, purified by column chromatography, and separated on thin layers of silica gel. Color reactions to spray reagents, solubilities, and uv absorption peaks show the compounds to be terpenoid, and they appear to be related to previously described furanoid norditerpenes. Varieties of *D. bulbifera* vary in bitterness, either cooked or uncooked, but all contain some of the bitter and nonbitter compounds.

Naturally occurring bitter substances are widely distributed among plants. In foods they lend characteristic flavors that are rarely appreciated and more often avoided. The bitter substances of carrots have been described as isocoumarin (Sondheimer, 1957) and as a sesquiterpene (Shallenberger *et al.*, 1960). Bitterness increases during storage of the roots. Ethylene increases bitterness by inducing synthesis of isocoumarin (Carlton *et al.*, 1960). The bitter principles of cucurbits are diterpenoids called cucurbitacins (Enslin, 1954). The occurrence and structure of terpenoid bitter principles have been reviewed (Courtney, 1961).

Bitterness in yams (*Dioscorea* spp.) has been observed by persons familiar with these widespread edible tropical tubers. The bitter yam (*D. dumetorum* (Kunth) Pax) contains toxic alkaloids, removed by soaking and boiling (Corkill, 1948). It is used as an occasional food in many parts of Africa. The bitter substance in *D. tokoro* Makino was found by Tsukamoto and Ueno (1936) to be a saponin, dioscin. This substance is now known to occur in many yams and is the chief source of diosgenin for the steroid industry. Tannins in quantities large enough for commercial purposes (Endres *et al.*, 1962) occur in several species of yams. These taste bitter also.

The bitter substances of the common edible yams (*D. alata* L., and *D. rotundata* Poir.) have not been investigated. Casual observations show that bitterness is common, that it is stronger in upper or older parts of the tuber, and that it increases during storage. Cooked as well as raw preparations are sometimes bitter, and bitterness may be strong enough to render the dish unpalatable. Because bitterness occurs in the older parts that are often exposed to light and in stored yams, it is commonly believed that bitterness is induced by light.

In a search for steroidal sapogenins in the bitter and sometimes poisonous yam (*D. bulbifera* L.) Kawasaki *et al.* (1968) found at least three new compounds, identified as furanoid norditerpenes. These unusual compounds proved to be bitter and thus constitute a new class of bitter compounds. Because varieties of *D. bulbifera* vary in their bitter flavor, we are not sure how widely these compounds are distributed through the species or whether these are the only or the principal bitter compounds of *D. bulbifera*. The structure of these compounds has been carefully elucidated (Komori *et al.*, 1968), and more recently fine structural details were determined by Kamiya and Wada (1972).

In the present studies we developed simple chemical methods for revealing the bitter components of several varieties of *D. bulbifera* in preparation for studies of bitterness in other edible species.

MATERIALS AND METHODS

Varieties of *D. bulbifera*, native to Africa and Asia and introduced to Puerto Rico, were used for these studies. Their origins and some of their characteristics are given in Table I. This species produces both aerial and underground tubers. The aerial tubers were used for most of the observations reported here because they are more readily available throughout the growing season of the plants. The underground tubers of some varieties were compared with the aerial tubers. The newly formed stems of some germinating tubers were also checked for bitterness. Bitterness was observed by tasting before and after cooking.

Bitter compounds were extracted as follows. One-hundred grams of fresh, peeled, and sliced aerial or underground tubers or sprouts of *D. bulbifera* varieties was extracted in a blender with 125 ml of methanol for 10 min. The slurry was centrifuged. The clear supernatant extract was collected in a 500-ml flask. The sediments were washed back to the blender with 100 ml of methanol and reextracted. After the third extraction, the pooled extracts were evaporated to about 100 ml. The cooled solution was passed through a polyamide column of ½-in. diameter and 6-in. length (Polyclar AT, GAF Corp., New York, N. Y., larger than 80 mesh). The column impeded the passage of phenolic components. It was washed with methanol until the dark eluate reached the lower tip of the column. Thus, a slightly yellow solution was obtained. The solution was dried with 40 g of sodium sulfate, filtered, and concentrated in a rotary evaporator to about 0.5 ml. The concentrated sample was removed with a small quantity of hot methanol and stored in a stoppered vial until used.

After extraction, residues of the tuber were dried and retasted for bitterness.

The methanol extract was spotted on a 20 × 20 cm glass plate coated with silica gel G. The plates were activated by heating at 110° for 30 min. The compounds were separated by an ascending method with a solvent mixture of ethyl acetate–hexane (90:10). Several other solvent mixtures were also useful (Table II). The plates were air dried. Common spray reagents were used in an effort to detect specific classes of chemical substances. As a standard treatment, plates were sprayed with 1% anisaldehyde in glacial acetic acid containing 2% concentrated sulfuric acid. The plates were then heated to 105° for 10 min and examined for spots in visible and ultraviolet light (uv). The spots were first purple, then turned to dark blue, and finally faded. The color of spots could be preserved by covering the chromatogram with a glass plate and sealing the edges of the plates with paraffin.

We covered a large plate with a small glass plate and then sprayed only the edges. The spots at the sides were used as guides to remove the silica gel containing the separated substances. The silica gel was eluted with boiling methanol in small flasks. The methanol was then reduced to 5-ml volumes by heating in a water bath. Strips of filter paper were immersed in this solution and then dried. These strips were tasted for bitterness.

Bitter compounds were partly characterized by their solubilities, their chromatographic mobilities, and their ultraviolet (uv) absorption spectra. The findings were compared with reports available in the literature (Kawasaki *et al.*, 1968).

RESULTS

Aerial tubers of different varieties of *D. bulbifera* varied in intensity of bitterness and therefore in palatability (Table I). When underground tubers were compared with aerial tubers, they were similar in color, texture, flavor,

Table I. *Dioscorea bulbifera* **Varieties Tested for Bitterness, Their Origins, and Some of Their Characteristics**

Identification number	Geographical source	Size of aerial tuber	Form of aerial tuber	Flesh color	Taste	
					Uncooked	Cooked
14861	Puerto Rico	Large	Sharp angled	Yellow	Slightly bitter	Slightly bitter
15216	Puerto Rico	Medium	Spherical	Light green	Not bitter	Not bitter
15330	India	Medium	Spherical	Dull pink	Bitter	Bitter
15335	Puerto Rico	Large	Angled	Yellow	Good	Slightly bitter
15338	Puerto Rico	Large	Smooth angled	Yellow	Not bitter	Slightly bitter
15472	Hawaii	Small	Spherical	Dull pink	Bitter	Bitter
15492	New Caledonia	Medium	Smooth, oblate	Yellow	Not bitter	Not bitter
15500	Ivory Coast	Medium	Sharp angled	Pink grey	Bitter	Bitter
15501	Ivory Coast	Medium	Smooth angled	Light yellow	Not bitter	Not bitter
15772	Ivory Coast	Small	Flattened	Yellow	Bitter	Inedible
15773	Nigeria	Medium	Smooth angled	Yellow	Slightly bitter	Slightly bitter
15774	Nigeria	Small	Flattened	Yellow	Bitter	Inedible
15776	Nigeria	Medium	Sharp angled	Yellow	Bitter	Bitter
15777	Nigeria	Medium	Spherical, rough	Light yellow	Not bitter	Slightly bitter
15778	Sierra Leone	Small	Spherical	Light yellow	Bitter	Bitter
15779	Sierra Leone	Large	Smooth angled	Light yellow	Not bitter	Slightly bitter

Table II. Compounds Extracted from the Bitter Yam 15330, Their Tastes, R_f Values, and Ultraviolet Absorption Peaks

Compound number	Taste	R_f values in different solvent systems			Uv absorption peaks	
		Ethyl acetate–hexane, 90:10	Ethyl acetate–hexane–methanol, 90:5:15	Ethyl acetate–acetone, 90:10	Principal	Minor
1	Not bitter	0.31	0.13	0.13	228	280
2	Not bitter	0.34	0.20	0.20	228	280
3	Bitter	0.36	0.26	0.36	226	276
4	Bitter	0.47	0.45	0.51	226	276
5	Bitter	0.53	0.53	0.54	226	276
6	Not bitter	0.60	0.61	0.57	226	276
7	Not bitter	0.71	0.68	0.60		
8	Bitter	0.76	0.76	0.78		

Table III. Bitter Compounds and Related Nonbitter Components in Ten Varieties of *Dioscorea bulbifera*

Variety	Presence of bitter compounds							
	1, not bitter	2, not bitter	3, bitter	4, bitter	5, bitter	6, not bitter	7, not bitter	8, bitter
15216	−	+	−	+	+	+	+	+
15330	+	+	+	+	+	+	+	+
15335	−	−	−	+	+	+	+	+
15338	−	−	−	−	+	−	+	+
15472	+	+	+	+	+	+	+	+
15773	+	−	+	−	−	−	+	+
15774	+	−	−	−	−	+	+	+
15775	−	−	−	+	+	+	+	+
15776	−	+	−	+	+	+	+	+
15778	−	−	−	−	+	+	+	+

and degree of bitterness. Newly sprouted stems from bitter aerial and underground tubers were also bitter.

The first extraction of fresh tissue with methanol removed only a part of the bitterness. The bulk was removed in the second and third extractions. After the fourth extraction the residue was essentially free of bitterness and was palatable.

Taste tests of media taken from chromatograms reliably located the bitter compounds. Once the compounds were located, the spots could be revealed with I_2 vapor absorption on some but not all chromatographic media, by uv absorption on plates with background fluorescence and by the anisaldehyde reagent. The latter is specific for sugars, steroids, and terpenes.

The following specific reagent sprays did not reveal the bitter substances: ferric chloride test for phenolics; aluminum chloride test for flavonoids; ammonium hydroxide vapor test; potassium permanganate test for reducing compounds; vanillin–phosphoric acid test for steroids.

The anisaldehyde spray revealed eight principal uv absorbing purple spots in the most bitter variety. The characteristic R_f values of these spots are given in Table II for three solvent systems. The compounds associated with four spots were bitter in taste. Those with the four other spots were not bitter. No relationship was found between the intensity of the spots and the intensity of the bitterness. The most bitterness occurred at the site of component 4.

The bitter and nonbitter compounds found in ten varieties of *D. bulbifera* are listed in Table III. All the varieties, including those not particularly bitter to the taste, contained at least one of the bitter compounds. The most bitter tubers contained all. None of the bitter spots appeared to be a predominant or principal determinant of bitterness. The subjective taste is probably always associated with several different substances.

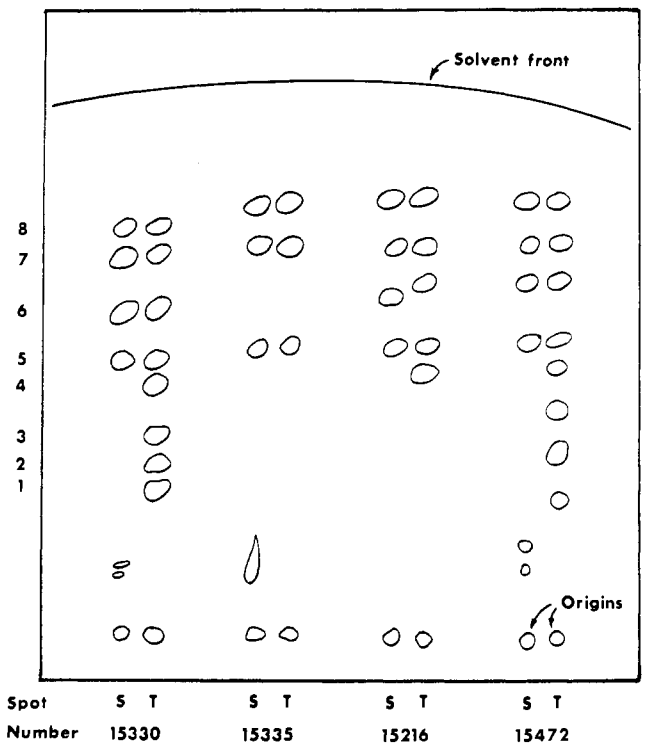

Figure 1. Chromatogram showing bitter and nonbitter components extracted from four varieties of *D. bulbifera* L. stems (S) and aerial tubers (T). Numbers correspond to variety identification numbers in Table III.

Young stems from the aerial tubers of four bitter varieties were tested for bitterness (Figure 1). The two bitter components 5 and 8, when present in the tubers, were also invariably present in the new stem. The less mobile components 3 and 4, when present in the tubers, were not present at detectable levels in the stem. Several of the nonbitter compounds, 6 and 7 when present in the tubers, were usually present in the stem. Bitterness in young stems of growing plants can be detected easily and permits a rapid evaluation of the potential taste of the tuber itself.

All components eluted from purple spots and dissolved in acetonitrile showed identical absorption spectra. The chief absorption occurred at about 226–228 nm. In addition, strong absorption peaks occurred at 276 or 280 nm. The ratio of the first to the second peak was about 12:1. Slight modifications of the peaks were seen in a few cases (Table III). Confirming the observations of Kawasaki *et al.* (1968), the chief absorption peaks in methanol are about 210 nm, an absorption associated with the furanoid ring of the molecule.

DISCUSSION

Bitterness in *D. bulbifera* has thus been found to be associated with several substances easily extracted from fresh or dried tuber by methanol. These bitter compounds are found in all varieties of *D. bulbifera* tested, even when the tubers are quite palatable. In addition to the bitter compounds themselves, nonbitter substances with similar solubility, chromatographic, and uv absorption properties are found. Thus, nonbitter and bitter compounds apparently constitute a family of related compounds, probably differing only in structural details.

Bitter compounds had previously been isolated from *D. bulbifera* (Kawasaki *et al.*, 1968). These were characterized as furanoid norditerpenes and constitute a new class of bitter substances. Although about 20 such compounds have been described in other plants, their bitter characteristics have not been previously observed. Because nonbitter compounds with similar properties to the bitter compounds are found in yams, very probably bitterness or the lack of it is associated with simple changes in one principal type of molecule. Because no other bitter compounds were found in *D. bulbifera*, we believe that the bitter compounds we isolated are some of the same compounds isolated from the same species by Kawasaki *et al.* (1968) and thus are terpenoid. Further characterization is necessary.

The problem of interest that remains with *D. bulbifera* is the nature of the bitter *vs.* nonbitter compounds. We expect from further analysis to be able to recognize the molecular configurations that cause bitterness and from there to deduce the chain of events that makes some yams bitter whereas others are not. The chromatographic and uv absorption methods developed should be useful in detecting and measuring bitter compounds and their nonbitter analogs in the nonbitter or only slightly bitter yams.

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