samples were all quite toxic. The clam sample from Tanakee Beach, Alaska, which was the most toxic sample encountered, was taken 2 months after a number of people became seriously ill by consuming clams collected at that location.

The fluorescence of the unoxidized blank generally corresponds to less than 0.02 μg of saxitoxin per g of shellfish for samples of *S. giganteus*. A somewhat higher blank is found of *M. californianus* and for clam samples subjected to prolonged cold storage. Reproducibility better than ±5% is easily obtained for the fluorescence of the oxidized portion and the unoxidized blank. The presence of compounds in the shellfish extracts capable of quenching the fluorescence of 1 was ruled out by adding a known amount of 1 to the fluorescent solution. No quenching was ever observed.

The bioassay compares reasonably well with the chemical assay, considering the modest number of test animals used and the detrimental effect of NaCl on the bioassay. The chemical assay is at least as expeditious as the bioassay, it eliminates the problems inherent in the bioassay, it is 100 times more sensitive, and it can detect any concentration of saxitoxin likely to be encountered without dilution.

The chemical method described here should allow for the specific rapid, routine, and reliable assay of shellfish for toxicity as a desirable substitute for quarantine by calendar.

ACKNOWLEDGMENT

We are indebted to R. E. Reynolds (Department of Economic Development, Juneau, Alaska) for the samples of *Saxidomus giganteus*.

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Received for review July 25, 1974. Accepted November 6, 1974. This work is a result of research sponsored in part by NOAA Office of Sea Grant, Department of Commerce, under Grant No. USDC 04-3-58-122. The U.S. Government is authorized to produce and distribute reprints for governmental purposes.

The Detection and Quantitative Determination of Ipomeamarone in Damaged Sweet Potatoes (*Ipomoea batatas*)

Garnett Wood* and Anita Huang

A relatively simple procedure is described for the quantitative determination of ipomeamarone in damaged sweet potatoes. This compound is produced along with other toxic furanoterpenoid compounds as a general response of the sweet potato to certain exogenous stimuli. The toxic compounds were extracted from the tissue with ether and separated by column chromatography, using increasing percentages of ethyl acetate in hexane as the eluting solvent mixture. Thin-layer chromatography was used for qualitatively analyzing the separation fractions. Those fractions containing ipomeamarone were analyzed quantitatively by gas–liquid chromatography, using hexadecane as the internal standard. The lower limit of detection of ipomeamarone by this procedure was 2 ng.

Ipomeamarone is one of several toxic furanoterpenoid compounds produced in sweet potatoes in response to various exogenous stimuli such as fungal infection (Kubota, 1958), insect invasion (Akazawa *et al.*, 1960), and the presence of certain chemicals (Uritani *et al.*, 1960). Ipomeamarone is a hepatotoxic agent (Watanabe and Iwata, 1952) and is usually produced in larger amounts than the other furanoterpenes which are produced simultaneously. These compounds are not found in sound, healthy sweet potatoes; however, their presence in some sweet potatoes that contained minor blemishes and the observation that they are not destroyed by normal cooking procedures (Wilson *et al.*, 1970) suggested that they might pose a health hazard for humans. It was therefore of interest to develop a relatively simple, yet quantitative procedure for screening sweet potatoes for the presence of ipomeamarone and other furanoterpenes. Although procedures have been reported for several of these (Boyd and Wilson, 1972), our procedure involves several modifications of the one reported by Boyd and Wilson (1971) so that it could be used for our specific needs.

Thin-layer chromatography (tlc) was used to detect the presence of the toxin in crude extracts; column chromatography and gas–liquid chromatography (glc) employing an internal standard were used for isolating the ipomeamarone and determining its concentration.

EXPERIMENTAL SECTION

The sweet potatoes used in this study were carefully selected and purchased from local grocery stores. Sound, healthy sweet potatoes without any apparent blemishes or bruises were used for control purposes, while those that contained blemishes and showed evidence of bruises or other damages were used as possible sources of ipomeamarone. In some instances, peelings of healthy sweet potatoes (skin plus 2–3 mm of adjoining tissue) were sliced into small pieces, placed in a beaker, and covered loosely with aluminum foil overnight. The beaker plus contents were stored 5–7 days at room temperature in a sealed

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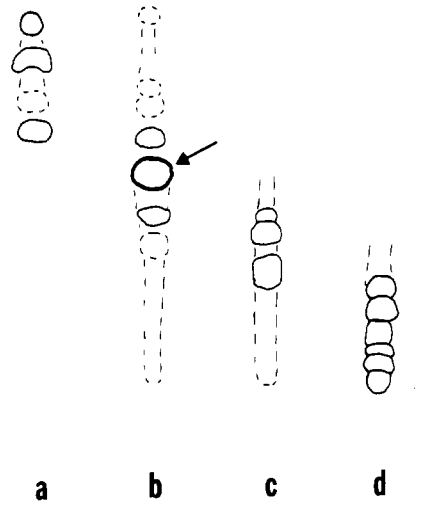

Figure 1. Thin-layer chromatogram of fractionated extract from a damaged sweet potato. Fractions a–d eluted from silica gel column with the following mixtures of ethyl acetate in hexane: 10, 15, 20, and 100% ethyl acetate. Arrow indicates ipomeamarone.

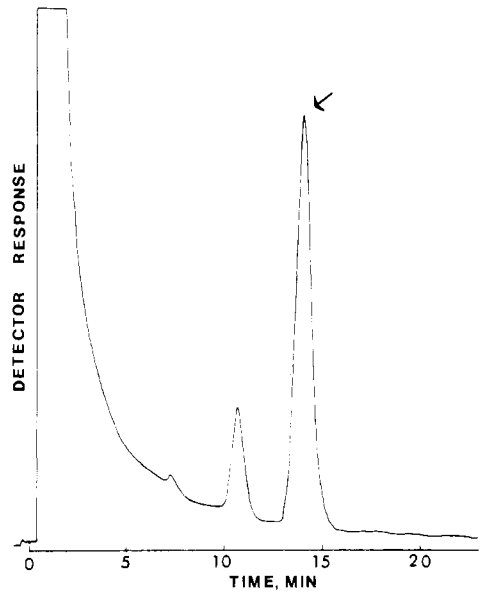

Figure 2. Gas chromatogram at 160° of a 15% ethyl acetate in hexane fraction obtained from an extract of damaged sweet potatoes. Arrow indicates ipomeamarone. Operating conditions are given in text.

plastic bag. This tissue was then used as a possible source of ipomeamarone.

Extraction of Tissue. Whole sweet potatoes or stored slices were cut into small pieces and minced by blending with crushed Dry Ice in a food blender. The resultant material was thawed, homogenized with diethyl ether (300 ml/100 g of material), and extracted by the procedure described by Boyd and Wilson (1971).

Thin-Layer Chromatography. Thin-layer chromatography was used as a screening technique to determine the presence of ipomeamarone and other furanoterpenes in crude extracts and fractionated extracts eluted from silica gel columns. Baker-Flex silica gel IB-F thin-layer sheets (J. T. Baker Chemical Co.) were used. Alternatively, glass plates were prepared in the laboratory, coated with 250-μ silica gel G (Brinkmann), air dried, and stored in a desiccator until needed. A Gelman chromatography chamber (Model 51325) was used for the development of the thin-layer sheets; the laboratory-prepared plates were developed in a lined chromatography tank containing a stainless steel trough. In each case, the tanks were allowed to equilibrate 20 min with the developing solvent, 20% ethyl acetate in *n*-hexane (Oguni *et al.*, 1969), prior to placing the plates in the chamber. The extracts to be examined were dissolved in chloroform, applied to the plates with a micropipet, and allowed to develop to 10 cm. The developed plates were air dried and sprayed with Ehrlich's reagent.

In initial studies, ipomeamarone isolated in this laboratory was identified by comparing its R_f value on thin-layer plates with that obtained with pure ipomeamarone chromatographed at the same time. The identity of the isolated ipomeamarone was also confirmed by glc. The retention time of the isolated compound was identical with that of the pure ipomeamarone. The identity of the isolated ipomeamarone was further confirmed by infrared spectroscopy. The spectrum of the isolated compound exhibited the characteristic bands of pure ipomeamarone as reported by Akazawa (1960) and Boyd and Wilson (1971).

Column Chromatography. In view of the many constituents in damaged sweet potatoes in addition to ipomeamarone, and the time required to pack and condition new glc columns, it was considered necessary to clean up the crude extract by at least one passage through a silica gel column before analyzing by glc. A 3.4 × 19 cm column of 0.05–0.2 mm silica gel was prepared. The gel was slurried in hexane, carefully added to the column, and allowed to settle under gravity. One centimeter of 0.2–0.5 mm silica gel and 1 cm of anhydrous sodium sulfate, respectively, were added to the top of the packed column. The column described above and the elution scheme indicated below represent modifications of techniques reported by Boyd and Wilson (1971) and Akazawa (1960). Up to 3 g of crude extract was dissolved in a minimum amount of ethyl acetate and transferred to the top of the column. The column was eluted at the rate of 120–130 ml/hr with the following solvents: 200 ml of hexane, 200 ml of 5% ethyl acetate in hexane, 230 ml of 10% ethyl acetate in hexane, 300 ml of 15% ethyl acetate in hexane, 200 ml of 20% ethyl acetate in hexane, and finally 200 ml of ethyl acetate. The eluate was collected in 30-ml amounts beginning after the appearance of a yellow tint in the eluate (in the 10% fraction). Each fraction was evaporated to dryness on a rotary evaporator, weighed, and analyzed by tlc. Those fractions containing mainly ipomeamarone were combined and analyzed quantitatively by glc.

Gas–Liquid Chromatography. A Nuclear-Chicago Selecta-System 5000 chromatograph equipped with a flame ionization detector was used. A U-shaped glass column (8 ft × 4 mm i.d.) was packed with 10% UC-W98 (Boyd and Wilson, 1971) on 100–120 mesh Gas-Chrom Q and conditioned for 21 hr at 250°. Operating temperatures were: injector 300°, column 160°, and detector 290°. At the analytical level of interest in this study, an electrometer setting of 1×10^{-10} A full-scale deflection on a 5-mV recorder was used. Nitrogen was used as the carrier gas at a flow rate of 60 ml/min.

For the preparation of a standard curve, four solutions containing different levels of ipomeamarone were prepared from a stock solution that contained 14 mg of ipomeamarone in 10 ml of carbon disulfide. The concentration of ipomeamarone in the diluted solutions ranged from 0.07 to 0.42 μg/μl. Hexadecane was used as the internal standard and its concentration in each solution was 0.021 μg/μl. Three analyses of each diluted standard were made and the average value was used in plotting the standard curve. The peak height ratio of ipomeamarone to hexadecane was linear at ipomeamarone concentrations ranging from 0.1 to 0.5 μg/μl. Under the conditions used, ipomeamarone had a retention time of 1.3 relative to hexadecane, or an absolute retention time of 13.7 min.

Usually, the sample to be analyzed was concentrated to dryness on a rotary evaporator, dissolved in a minimal

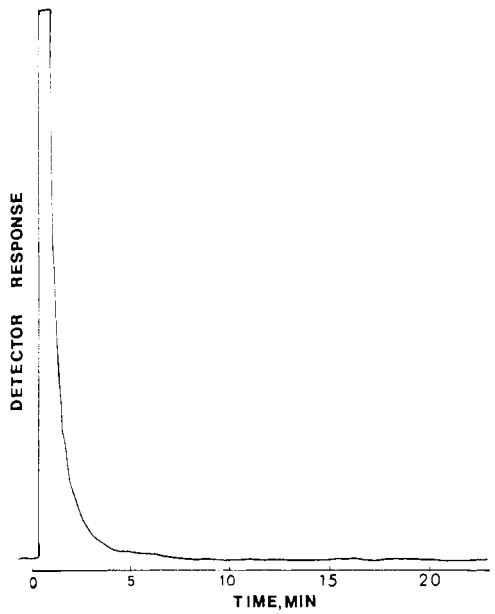

Figure 3. Gas chromatogram at 160° of a 15% ethyl acetate in hexane fraction obtained from an extract of healthy sweet potatoes. Operating conditions are given in text.

amount of carbon disulfide, and transferred to a 10-ml volumetric flask. An amount of hexadecane equal to that used in the preparation of the standard curve was added to the flask, and the contents were diluted to volume with carbon disulfide. When further dilution of the sample was necessary for the ipomeamarone peak to fall on-scale, a diluting solution containing 21 μg of hexadecane/ml of carbon disulfide was used. Initially 1 ml of the original solution is mixed with 1 ml of the diluting solution, thus giving a dilution factor of 2. Additional measured amounts of diluting solution can be added when necessary to get the peak on-scale. The amount of ipomeamarone present in the sample was determined by multiplying the results obtained from the standard curve by the appropriate dilution factor. The use of the diluting solution assures that the linearity of the ipomeamarone/hexadecane peak height ratio will not be changed by dilution. Ethyl acetate or carbon disulfide may be used as solvents for the glc analysis; carbon disulfide was preferred because it gave a smaller solvent front on the chromatogram.

RESULTS AND DISCUSSION

Ipomeamarone appeared as a pinkish-orange spot which soon turned dark bluish-gray when thin-layer plates were sprayed with the Ehrlich reagent. The other furanoterpenes appeared as red-purple spots with R_f values lower than ipomeamarone.

Ipomeamarone had an R_f of 0.4 on laboratory-prepared plates; it was slightly higher on Baker-Flex plates. To determine the lowest level of the compound detectable in extracts from damaged sweet potatoes by tlc, varying amounts of the relatively pure toxin were added to a crude extract from healthy sweet potatoes and each was analyzed. The lower limit of detection of ipomeamarone in 1-2 μl of extract applied to the thin-layer plate was 200 ng. Hence, it is feasible to use tlc as a rapid qualitative test for the presence of ipomeamarone in slightly damaged sweet potatoes that may have a high level of the toxin.

Figure 1 shows the distribution of various components of the crude extract in the fractions eluted from the column by increasing the polarity of the solvent mixture. All of the ipomeamarone was eluted with the 15% ethyl acetate in hexane mixture. No colored material was eluted with pure hexane or with 5% ethyl acetate in hexane. Thus, for routine quantitative analysis, it is possible to collect the 15% fraction in bulk, evaporate to dryness, dissolve in carbon disulfide along with the internal standard, and inject into the gas chromatograph. A typical glc pattern is shown in Figure 2. Ipomeamarone had a retention time of 1.3 relative to hexadecane. A glc pattern of the 15% fraction obtained from healthy sweet potato tissue is shown in Figure 3. The column used for the separation was stable for several months. The lower limit of detection of ipomeamarone was determined to be 2 ng at attenuation 1, range 10^{-10}. This setting gave a 0.3% full-scale pen deflection. To determine the efficiency of the extraction and chromatographic procedures, known weights of relatively pure ipomeamarone were added to two weighed portions of healthy sweet potato tissue that had been blended with crushed Dry Ice. These samples, along with control (unspiked) tissue, were carried through the entire extraction and analytical procedures. The recoveries of ipomeamarone, based on the weight of the 15% fraction eluted from the silica gel column, were 89.3 and 100.9% (95.1% average); recovery based on glc analysis was 105% for each sample.

Table I. Ipomeamarone Content of Selected Damaged Sweet Potatoes

Sample	Wt of whole sweet potatoes, g	Ipomeamarone, ppm, by glc
1	176	2.6
2	194	4.3
3	290	7.6
4	372	12.9
5	200	46.3

Using the procedure described in this paper, selected damaged sweet potatoes for sale in local stores were examined for the presence of ipomeamarone. The data shown in Table I are representative of the results obtained. In view of the limited toxicological data on ipomeamarone, it is not possible to predict the significance of the levels of the toxin shown in the table. These results do suggest that current procedures used for the handling and storage of sweet potatoes should be given serious consideration.

The literature seems to reflect an increasing awareness of the presence of small amounts of toxic or deleterious materials in many food products resulting from processing and/or storage conditions. Unfortunately, the development of relatively simple procedures for the quantitative determination of some of these materials in foods for human consumption has been neglected. Hopefully, the revelation of toxic materials in common food items, such as sweet potatoes that have been damaged, will stimulate rapid progress in methodological and toxicological studies designed for isolating, identifying, and characterizing previously unknown components of foods.

ACKNOWLEDGMENT

We thank B. J. Wilson, Vanderbilt University, Nashville, Tenn., for the pure ipomeamarone.

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Received for review July 24, 1974. Accepted December 2, 1974.