Preparative and Analytical Gas Chromatography of Ipomeamarone, A Toxic Metabolite of Sweet Potatoes (*Ipomoea batatas*)

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An improved procedure utilizing gas chromatography has been developed for the preparative scale separation and purification of ipomeamarone, a hepatotoxin produced by damaged sweet potatoes. Various liquid phases were tested and a methylvinyl silicone, UC-W98, was generally most useful for gas chromatography of this substance. Gc also served as an analytical tool for the quantitative estimation of the compound in sweet potato extracts. The method was applied to the analysis for ipomeamarone in sweet potato samples from local food stores.

Sweet potatoes damaged by various species of fungi (Kubota, 1958), invaded by insects (Akazawa *et al.*, 1960), or irritated by certain chemicals (Uritani *et al.*, 1960), turn black-brown and produce a considerable amount of furanoterpenoids, some of which are toxic to animals (Wilson *et al.*, 1970). In most cases, the major one produced is a hepatotoxin (Watanabe and Iwata, 1952) called ipomeamarone (Figure 1; Kubota and Matsuura, 1958). The recent clarification of the status of ipomeamarone as a naturally occurring toxicant (Wilson *et al.*, 1970) has demanded the development of more precise methods for both the isolation and quantitation of the compound.

The procedure for separation and purification of ipomeamarone used by early investigators (Akazawa *et al.*, 1960) consisted essentially of a distillation of the ethyl ether extract of the moldy sweet potatoes. Subsequently, Akazawa (1960) claimed that all previous investigators had obtained and reported only impure samples of ipomeamarone. Maintaining that distillation procedures alone were inadequate, he provided a new scheme for separation and purification which additionally included repeated silica gel column chromatography.

We have developed a method, using gas–liquid chromatography (glc), by which pure samples of ipomeamarone can be obtained on a preparative scale for detailed toxicological and chemical studies. An adaptation of Akazawa's method (1960) (omitting the initial distillation) was used for the initial crude separation of an ipomeamarone-rich oily fraction from infected sweet potatoes. Glc served to separate ipomeamarone from impurities in this fraction. In addition, it was shown that gas chromatography provides an easy and rapid method for quantitative analysis of this compound in sweet potatoes.

A previously reported method for the quantitative analysis of ipomeamarone by Akazawa and Wada (1961) involved a combination of thin-layer chromatography and colorimetric determination. Although these investigators did not provide data on the precision or accuracy of their method, the gas chromatographic procedure is probably superior in these respects. Additionally, much greater sensitivity was possible, as demonstrated by the fact that glc calibration curves for ipomeamarone were prepared in concentration ranges which would not give detectable thin-layer chromatograms.

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METHODS AND RESULTS

Preliminary Treatment of Crude Extract. Details of bioproduction of the furanoterpenoids used as source material will be published elsewhere.

Thirty kilograms of mold-damaged tubers was extracted with either chloroform or ether. The extract solution was dried over anhydrous sodium sulfate, filtered, and the solvent removed with a rotary evaporator. The sample (22 g) was a viscous, dark brown, spicy-smelling oil containing some small, dark-colored crystals.

Thin-Layer Chromatography. Crude extract solutions and column chromatography eluates were monitored for ipomeamarone using thin-layer technique. Baker-Flex (J. T. Baker Chemical Co.) Silica Gel, IB-F thin-layer sheets were used for all thin-layer chromatograms. The sheets were developed ascendingly in 10% methanol in benzene solution. The furanoterpenoids were detected on the developed plates by spraying with Ehrlich's reagent (prepared by dissolving 5.0 g of *p*-dimethylaminobenzaldehyde in 20 ml of concentrated HCl and 80 ml of 95% ethanol), followed by gentle warming to develop spot colors. Chromatograms (Figure 2) run on the crude residue were similar to those described by Akazawa *et al.* (1960). Ipomeamarone appeared immediately after spraying as a light pink spot which soon changed to a dark bluish-gray.

Partial Purification by Column Chromatography. Since crude extracts contained many nonvolatile impurities, column chromatography was required prior to the preparative glc step.

A 12 × 5 cm column was prepared using 11 cm of 0.05–0.20 mm Silica Gel (Brinkmann) topped by 1 cm of 0.2–0.5 mm of adsorbent suspended in *n*-hexane. Six grams of crude sweet potato extract was dissolved in 10 ml of ethyl acetate and applied to the column. The column was eluted successively, at a rate of 40 ml/hr, with 200 ml each of *n*-hexane, 5% ethyl acetate-hexane, 10% ethyl acetate-hexane, 15% ethyl acetate-hexane, 20% ethyl acetate-hexane, and finally 400 ml of ethyl acetate. Eluate was discarded until a yellow pigment began to appear, at which time collection of 40 ml fractions was begun. Thin-layer chromatograms showed that fractions 6–8 contained ipomeamarone as indicated by its characteristic R_f and color with Ehrlich's reagent (Figure 2). These fractions were combined, and the solvent was evaporated. To remove acidic impurities, the residue was dissolved in 30 ml of ether and washed three times with 15-ml portions of 0.05 *N* sodium hydroxide followed by three washings with 15-ml portions of water. The solution was then dried over

Table I. Gc Data and Results for Trial Columns

Stationary Phases	Dimensions	Injector/ Detector Temperature (°C)	Oven Temperature (°C)	Carrier Flow Rate (ml/min)	Ipomeamarone Retention Time (min)	No. of Observable Peaks with Smaller Retention Time Than Ipomeamarone[a]	No. of Observable Peaks with Greater Retention Time Than Ipomeamarone[a]	Resolution of Ipomeamarone (Completeness of Separation From Other Components)
SE-30, 18% (N)[b]	8 ft × 1/4 in.	310/320	235	80	10.0	10(1)	5(2)	Fair
UC-W98, 18% (N)	10 ft × 1/4 in.	300/310	250	85	4.5	8(2)	6(3)	Fair-Good
	8 ft × 3/8 in.	300/310	180	100	31.0	12(3)	8(3)	Good
Apiezon L, 18% (N)	10 ft × 1/4 in.	300/310	250	85	54.0	11(2)	2(0)	Poor
SF-96, 15% (N)	8 ft × 1/4 in.	300/310	220	80	13.5	11(1)	5(2)	Fair
Reoplex 400, 15% (I)	8 ft × 1/4 in.	300/300	200	80	15.0	14(8)	3(3)	Excellent
	4 ft × 1/4 in.	300/300	200	70	8.0	8(4)	4(3)	Fair-Good
DC-550, 15% (I)	8 ft × 1/4 in.	310/320	250	80	4.5	13(1)	5(2)	Fair
Carbowax 20M, 18% (P)	10 ft × 1/4 in.	300/310	250	85	30.0	14(3)	5(3)	Fair

[a] Number of resolved single peaks in parentheses. [b] P = polar; I = intermediate; N = nonpolar. Instrument, Varian Model 1520-B; Detector, Thermal Conductivity; Filament Current, 150 mV; Carrier Gas, Helium; Sample sizes, Analytical: 0.01–0.05 μl; Preparative: 70.0–350.0 μl; Columns, 1/4 in. and 3/8 in. o.d. Aluminum; Support, Chrom W 60/80 (NAW).

anhydrous sodium sulfate, filtered, and the ether removed with a rotary evaporator. The residue (0.4 g) was a light yellow viscous oil. The remaining fractions were saved for future studies.

GAS CHROMATOGRAPHY

Preparation of the Gc Columns. Several liquid phases were selected for trial from those used successfully by other investigators in the separation of structurally similar compounds.

Columns were prepared using the usual methods (Littlewood, 1970). Specifications for the test columns are given in Table I. Each column was preconditioned at a temperature of 250° C with a flow rate of 20 ml/min for 12 hr.

Evaluation of the Columns. Descriptive data and results are also given in Table I. In each case, the glc peak corresponding to ipomeamarone was tentatively identified by comparison of its retention time with that of authentic samples of the compound provided by Takashi Kubota and Ikuzo Uritani of Japan. Further verifications of the peak assignments were obtained in several cases (Reoplex 400, D.C. 550, UC-W98, and SE-30 columns) by collection of sufficient material for comparison of the infrared spectra with that of authentic compound. In all chromatograms, ipomeamarone comprised about 35–45 peak-area percent of the partially purified mixture.

Most columns tested gave fairly good separations of ipomeamarone from the other components. Results obtained with UC-W98, SE-30, and Reoplex 400 (Applied Science Laboratories, Inc.) columns were most acceptable. UC-W98 was superior to SE-30, but the best column contained Reoplex 400 in both the 4 and 8 ft lengths. Although excellent analytical scale separations were possible using this phase, it was not suitable for use on a preparative scale.

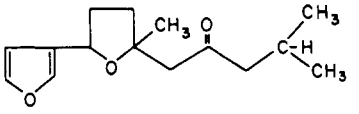

Figure 1. Structure of ipomeamarone

With repeated injections of large samples (about 100 μl), performance of the Reoplex 400 columns declined rapidly. Apparently some component(s) of the partially purified product binds irreversibly with the column material when the column temperature is held within the 180–200° C recommended maximum operating temperature of the column. Prior injection of silylating agent (Silyl 8, Pierce Chemical Co.) did not alleviate this problem. All other columns tested had considerably higher maximum allowable operating temperatures.

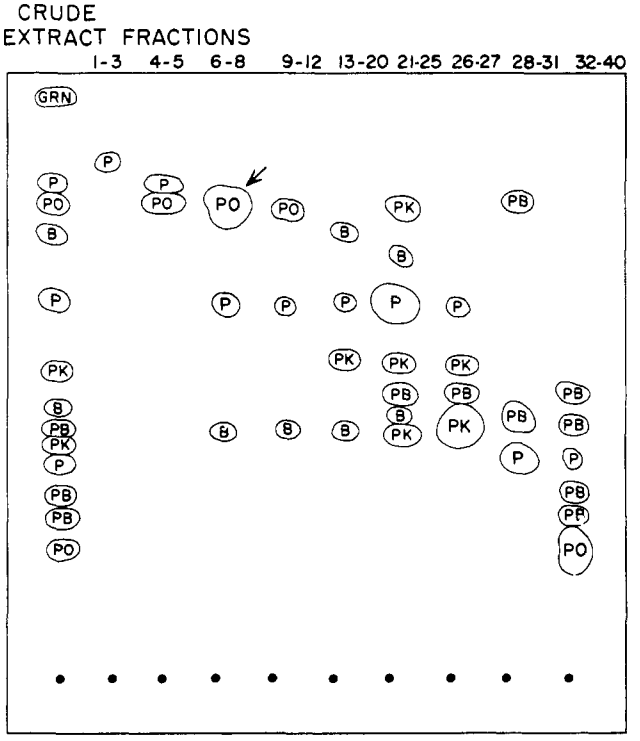

Figure 2. Thin-layer chromatograms of crude extract and silicagel column fractions. Fractions 6–8 contained ipomeamarone (indicated by arrow). Spot colors are coded as follows: PK = pink, PB = brownish purple, P = purple, PO = pinkish orange—(changes to dark gray), B = blue, and GRN = green

Because of the problems encountered with Reoplex 400 as the stationary phase for prep-scale separation of ipomeamarone, UC-W98 (a methyl-vinyl silicone) was substituted with good results. The time required per run with UC-W98 was approximately twice as long as that for a comparable one with a Reoplex column. However, this requirement was well compensated for by the much longer column life and higher maximum operating temperature.

Preparative Gc of Ipomeamarone. An 8 ft × $^3/_8$ in. ($^1/_4$ in. i.d.) glass column containing 18% UC-W98 on 80/100 Chrom Q was constructed. Injector and detector temperatures were 285° and 280° C, respectively, and the carrier (helium) flow rate was 120 ml/min. Thermal conductivity detection was used.

A variable temperature program permitted more rapid elution of the components with retention times greater than ipomeamarone and reduced the total time required per run. The column was maintained isothermally at 170° C for 22 min. Ipomeamarone was eluted during the period of 14.7–20.7 min after injection. The temperature was then increased to 250° C (15°/increase/min), held for 18 min, and returned to 170° C before the cycle was repeated with a new injection.

Injections of 100–200 μl each of partially purified ipomeamarone were made per run. The ipomeamarone peak was received in a small collector bottle immersed in ice water. The product obtained gave only a single peak on gc analysis and a single spot on thin-layer chromatograms. In one series of injections, using a total of 340 mg of partially purified sample, 87 mg of pure ipomeamarone was collected. Since the material injected contained 130 mg of ipomeamarone (see analytical section), the overall efficiency of collection of ipomeamarone was 67%. Similarly, with injection of a total of 50 mg of pure ipomeamarone, 41 mg was collected, giving a collection efficiency of 82% without the presence of the components of the mixture.

Infrared (Figure 3), nmr, and mass spectra were obtained on the collected ipomeamarone and were consistent with previously reported data (Akazawa, 1960; Yang *et al.*, 1971).

To verify the toxicity of ipomeamarone, two mice were given intraperitoneal injections of the gc purified material. The first received 40 mg and died within 3 hr. The second mouse died 12 hr after receiving 35 mg. Gross and histological examinations of the various organs in both animals revealed acute hepatotoxicity (Wilson *et al.*, 1970) and other pathological phenomena.

Quantitative Analytical Gc of Ipomeamarone. A 6 ft × $^1/_4$ in. ($^5/_{32}$ in. i.d.) glass column containing 10% UC-W98 on 100/120 Chrom Q was prepared. Injector and detector temperatures were held at 290° C and a carrier (helium) flow rate of 60 ml/min was maintained. Flame ionization detection was used. Air and hydrogen flow rates were 250 ml/min and 60 ml/min, respectively. The oven temperature was 180° C. Under these conditions, ipomeamarone had a retention time of 7.3 min (Figure 4).

A calibration plot was prepared showing the amount of pure ipomeamarone injected *vs.* detector response. Detector response was measured both in terms of peak area (disc integrator) and peak height. Peak height was the preferred method in this case, since it gave somewhat better reproducibility. The plot was prepared for the range of 50–500 ng. Five determinations were made on each of ten points, and the average values were plotted. The average percent deviation for determinations on each point was 2.6%. The plot showed good linearity. The minimum detection limit was determined to be 0.7 ng.

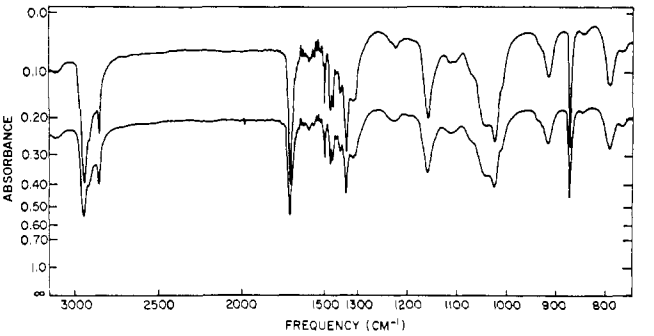

Figure 3. Infrared spectra (liquid film) of authentic ipomeamarone (upper curve) and gc purified ipomeamarone (lower curve). Spectra were recorded using a Perkin-Elmer, Model 337, infrared spectrophotometer

An adaptation of the method used recently by Oguni *et al.* (1969) was employed for the quantitative extraction of ipomeamarone from sweet potatoes. A blemished sweet potato was weighed and shredded. A well-mixed 100-g portion was chopped to small particle size with dry ice in a food blender with a well vented bowl. A 10-g representative sample of the thawed material was homogenized in 100 ml of chloroform-methanol (95:5, v/v) in a glass homogenizer. The homogenate was filtered through a Büchner funnel containing a spun glass filter. The residue was washed three times with 25-ml portions of the solvent mixture, and the filtrates were combined, washed with two 25-ml portions of water and dried over anhydrous sodium sulfate.

The extract was concentrated or diluted with the solvent until a gc analysis (Figure 4) could be obtained from which the ipomeamarone content of the material injected (5 μl of solution) could be determined directly from the calibration plot. After each analysis the column temperature was increased to 270° C for 15 min. With the total volume of the extract solution and the weight of potato tissue known, the average ipomeamarone content per g of sweet potato tissue as well as that of the entire tuber could be calculated.

To establish the reproducibility and accuracy of measurements on crude extract, the plots shown in Figure 5 were prepared. As before, multiple determinations were made on each point for which the average percent deviation was 2.5%. From the points with added quantities of pure ipomeamarone, an average percent recovery was calculated as 98 ± 6%. An additional plot (not shown) was prepared in which quantities of pure ipomeamarone in the range of 1–10 × 10^{-8} g

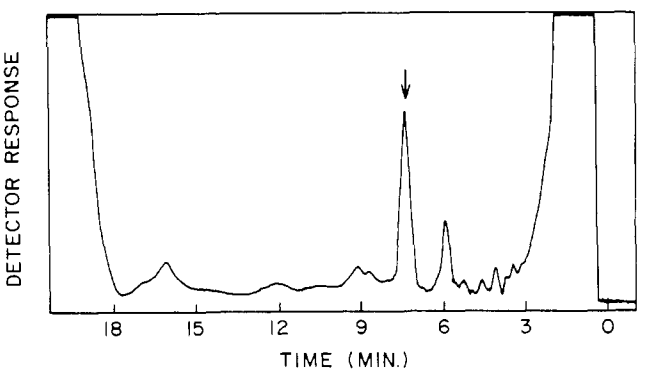

Figure 4. Gas chromatogram at 180° C of crude sweet potato extract (attenuation, 8; range 10^{-11}). Ipomeamarone is indicated by an arrow. At time = 18 min, temperature was increased to 270° C

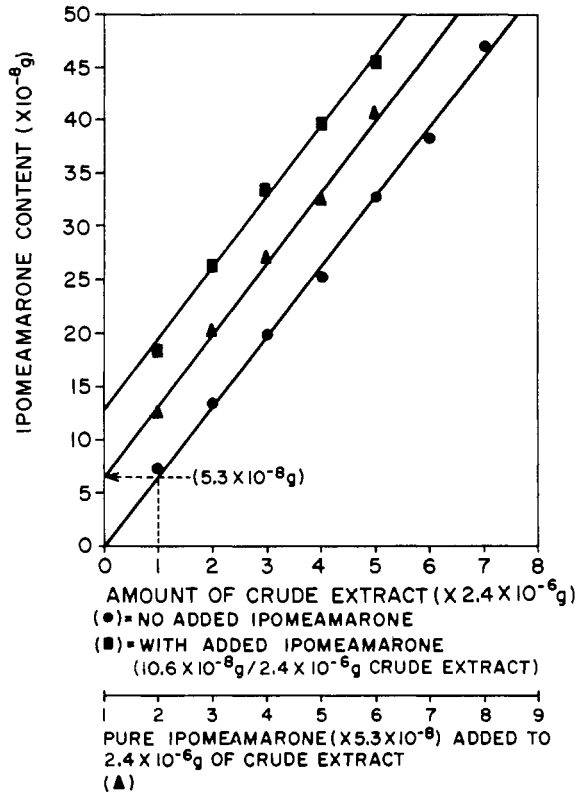

Figure 5. Plots showing recovery of ipomeamarone from crude sweet potato extract (●) including a sample to which additional ipomeamarone was applied initially (■) to crude extract with different amounts injected and uniform individual samples of crude extract to which increments of pure ipomeamarone (▲) were added

were added to 9.6 × 10^{-6} g of crude extract (ipomeamarone content, 21.2 × 10^{-8} g). The linearity of this plot was considered further evidence against the possibility of another component underlying ipomeamarone in the chromatograms.

Recovery was also evaluated by adding known amounts of pure ipomeamarone to the crude potato homogenate before extraction. Quantities added were within the range of 0.3–3.0 mg/g of potato. The average recovery was 99 ± 8%.

Application. The foregoing analytical method was used to measure the ipomeamarone content of sweet potatoes obtained at local food stores.

A typical sweet potato, showing only minor blemishes, was analyzed for its ipomeamarone content. Tlc of the ether extract showed several Ehrlich-positive spots, in addition to ipomeamarone. Glc analysis, by the previous method, showed an ipomeamarone content of 1.1 mg per g of sweet potato tissue.

Similar samplings of sweet potatoes were made at several retail and wholesale markets both in the vicinity of Nashville, Tenn., and Lexington, Ky. Numerous potatoes were found to contain ipomeamarone and other toxic compounds. The ipomeamarone contents of representative samples are summarized in Table II.

DISCUSSION

The discovery that formation of ipomeamarone and other furanoterpenoids is induced in sweet potatoes by various agents has led to the belief that the normal carbohydrate and fatty acid metabolism of the plant must be altered to synthesize these abnormal metabolites (Uritani and Akazawa, 1959). With the structure of ipomeamarone well established, the elucidation of its biosynthesis has become important for unveiling the pathological and biochemical role of the compound. The use of glc may provide a useful tool to those investigators currently active in this endeavor.

Table II. Ipomeamarone Content of Selected Sweet Potato Samples Obtained at Food Stores in Nashville, Tenn., and Lexington, Ky.

Sample No.	Source	Weight of Whole Potato (g)	Average Ipomeamarone Content (mg) per g of Sweet Potato Tissue	Ipomeamarone Content, Whole Potato (mg)
1	Nashville	125	1.1	138
2	Nashville	85	0.24	20.4
3	Lexington	94	0.10	9.4
4	Nashville	117	0.12	14.1
5	Lexington	125	7.6	950

Although the present report is concerned with the isolation and analysis of ipomeamarone, similar methods may be applicable to studies on other toxic (Wilson *et al.*, 1970) and nontoxic furanoterpenoids produced by the sweet potato. Preparative and quantitative analytical procedures for most of these abnormal metabolites have not been previously reported. Investigations are continuing in these areas and will be reported later.

The figures given in Table II for the ipomeamarone content of five sweet potato specimens represent 2 to 125 mouse i.p. LD_{50}'s (Taira and Fukagawa, 1958). Whether the finding of ipomeamarone and other natural toxicants in marketable sweet potatoes (Wilson *et al.*, 1970) has implications for human health can only be a matter for speculation at this time. However, their occurrence appears to be more prominent than has been appreciated heretofore. Furthermore, preliminary studies (Wilson *et al.*, 1970) have shown the toxic furanoterpenoids are not destroyed by normal cooking procedures. It is evident that these observations merit further consideration and study.

ACKNOWLEDGMENT

The authors gratefully acknowledge support from U.S. Public Health Service grant, AI-07000, and a Center in Toxicology grant, ES-00267, to Vanderbilt University. M. R. B. also acknowledges support by the Vivian Allen student fund.

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Received for review November 9, 1970. Accepted January 11, 1971.