Isolation and Characterization of 4-Ipomeanol, a Lung-Toxic Furanoterpenoid Produced by Sweet Potatoes (*Ipomoea batatas*)

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Column chromatography was used to obtain a fraction enriched with lung toxic material from an ether extract of moldy sweet potatoes. Preparative gas chromatography permitted isolation of a partially purified toxic component from this mixture, but the toxic principle was thermally unstable under the conditions required for separation. The silyl ether derivative, however, was stable to heat and silyl toxin was separated in pure form. After acid hydrolysis, pure toxin was recovered which was shown to produce a respiratory disease in mice characterized by severe pulmonary edema, pleural effusion, and death.

Recently the toxicity of mold-damaged sweet potatoes (*Ipomoea batatas*) has been described (Wilson *et al.*, 1970). Toxic furanoterpenoids are produced by the sweet potato in response to certain fungus infections, presumably as a defense mechanism (Uritani and Akazawa, 1959). Such damaged tubers (or their ether extracts) when fed to experimental animals produce a characteristic and often lethal respiratory disease (Wilson *et al.*, 1970).

The presence of a hepatotoxic substance, ipomeamarone (Figure 1), in damaged sweet potatoes has been recognized for many years (Wilson *et al.*, 1970). In addition, a hydroxylated derivative of ipomeamarone called ipomeamaronol (Figure 1) has been isolated from damaged sweet potatoes and is also toxic to the liver (Yang *et al.*, 1971). However, neither of these compounds is responsible for the respiratory pathology seen in examples of poisoning by moldy sweet potatoes (Wilson *et al.*, 1970).

Wilson *et al.* (1970) reported the presence of another component, termed lung edema factor, in sweet potatoes infected with *Fusarium javanicum.* This substance produces a respiratory disease in mice similar to that seen in natural outbreaks of poisoning in cattle caused by mold-damaged sweet potatoes (Wilson *et al.*, 1970). In a recent communication we reported (Wilson *et al.*, 1971) the isolation and structural characterization of a lung-toxic compound, 4-ipomeanol [1-(3-furyl)-4-hydroxypentanone] (Figure 2). In this report we describe in detail procedures used in the isolation of this substance from moldy sweet potatoes.

METHODS AND RESULTS

The procedure used for isolation of 4-ipomeanol was similar to that reported recently for the separation and purification of ipomeamarone from damaged sweet potatoes (Boyd and Wilson, 1971). Column chromatography was used first to obtain a fraction enriched with lung-toxic material. Preparative gas chromatography permitted the isolation of individual components from the toxic mixture in quantities sufficient for toxicity studies and structural characterization.

Crude Extract from Moldy Sweet Potatoes. Bioproduction of the furanoterpenoids was accomplished as previously described (Wilson *et al.*, 1970). Sweet potato slices (30 kg) were inoculated with *Fusarium javanicum* and incubated at 20°C for 6 days. The slices were then shredded and extracted with ether. The extracts were combined, dried over anhydrous sodium sulfate, filtered, and the ether was removed. The viscous dark brown residue weighed approximately 20 g.

Column Chromatography. A 12 × 5 cm column was prepared using 11 cm of 0.05–0.2 mm Silica Gel (Brinkmann) topped by 1 cm of 0.2–0.5 mm adsorbent suspended in *n*-hexane. Six grams of crude sweet potato residue was dissolved in 10 ml of ethyl acetate and applied to the column. Elution at a rate of 40 ml/hr was begun with 200 ml of *n*-hexane, followed by hexane–ethyl acetate mixtures in which the amount of ethyl acetate was increased stepwise at a rate of 5% for every 200 ml of eluent. The collected fractions (50 ml each) were monitored for Ehrlich-positive furanoterpenoids using the tlc technique previously described (Boyd and Wilson, 1971). When the eluent concentration of ethyl acetate reached 100%, collections were terminated and fractions were examined for toxin content.

Toxicity Tests. Solvent was removed from the fractions and the residues were tested individually for toxicity in mice. A 10–20 mg dose of each fraction dissolved in 50–100 μl of propylene glycol was administered either by intraperitoneal injection or gastric intubation. Lung toxicity was found to be associated with fractions 28–35. Tlc analyses of these fractions indicated the presence of two strongly Ehrlich-positive (purple) spots (PB and P, Figure 3) with low R_f values relative to ipomeamarone (Boyd and Wilson, 1971) (arrow). Only fractions 6–8 (not shown in Figure 3) contained ipomeamarone.

Gas Chromatography. In a previous study we found the liquid phase UC-W98 useful for separation of several volatile components of sweet potato extracts (Boyd and Wilson, 1971). This material also was used successfully in the present work to isolate components of the lung toxic fractions.

Equipment. A Varian Model 1520-B instrument was used with an 8 ft × 3/8 in. (1/4 in. i.d.) glass column containing 18% UC-W98 on 80/100 Chrom Q. Injector and detector temperatures were 265 and 260°C, respectively, and the carrier (helium) flow rate was 115 ml/min. Thermal conductivity detection was used.

Gc Analysis of Column Fractions Containing Toxin. Column fractions 28–35 were combined (450 mg), and injection of 5 μl of this material at an oven temperature of 170°C produced the chromatogram shown in Figure 4. Lowering the temperature to 150°C expanded the chromatogram so that the

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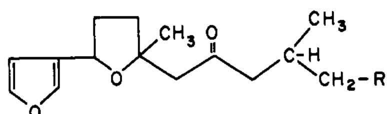

Figure 1. Structure of ipomeamarone (R = H) and ipomeamaronol (R = OH)

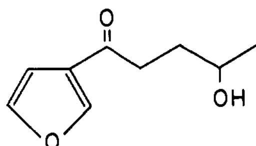

Figure 2. Structure of 4-ipomeanol

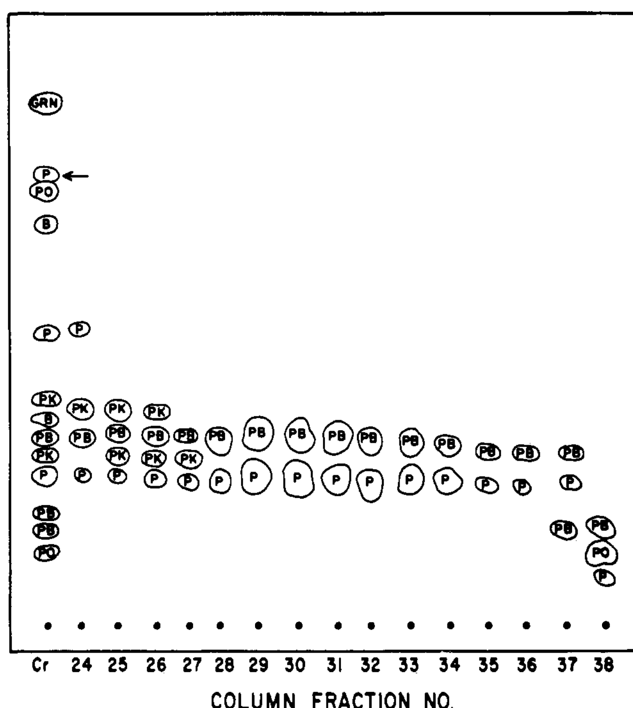

Figure 3. Thin-layer chromatograms of the crude mixture (Cr) and fractions 24–38. Ipomeamarone is indicated by an arrow. (Tlc plate eluted in 10% MeOH-benzene and developed with Ehrlich's reagent as previously described) (Boyd and Wilson, 1971): PK = pink, PB = brownish purple, P = reddish-purple, PO = pink-orange (changes to grey), B = blue, GRN = green

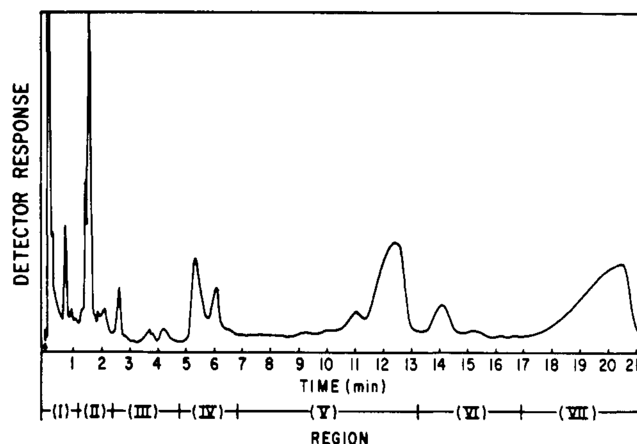

Figure 4. Gas chromatogram at 170°C of the lung toxic fractions 28–35. Regions I–VII were collected individually and tested for toxicity in mice

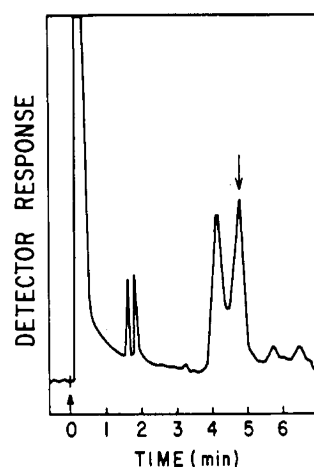

Figure 5. Gas chromatogram at 125°C of region II (collected by preparative gc). The peak at t = 4.8 min (arrow) was collected and examined for toxicity

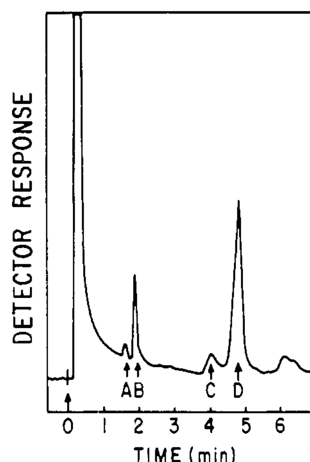

Figure 6. Chromatogram obtained at 125°C on reinjection of the peak collected at t = 4.8 min in Figure 5. Peaks A and B due to components of mol wt 150 and C and D with mol wt 168

last peak eluted 25.5 min after injection. This lower temperature allowed use of larger samples for preparative separations. The chromatogram was arbitrarily divided into the regions I–VII shown in Figure 4.

Preparative gc and Toxicity Tests of Components in the Toxic Mixture. For preparative gc, repeated injections of 35–50 μl each were made. The seven regions were collected in small receptor bottles immersed in ice water. After 10–20 mg of condensate was obtained for each region, toxicity tests were done as before on each sample. Lung toxicity was shown to be associated with region II.

Gc conditions were then determined by which the major peaks in region II could be individually isolated and tested for toxicity. The chromatogram shown in Figure 5 was obtained at an oven temperature of 125°C. The peaks at t = 4.1 min and t = 4.8 min correspond to the pair of peaks at t = 1.6 min in Figure 4. Again, repeated injections (35–50 μg each) of the mixture were made and the larger peak (t = 4.8 min) was collected. Intraperitoneal administration of 5 mg of this material in a mouse produced the characteristic respiratory disease. However, reinjection of 2 μl of this sample (oven temp 125°C) yielded the chromatogram shown in Figure 6. Peak D corresponds to the one originally collected (t = 4.8 min, Figure 5); peak C corre-

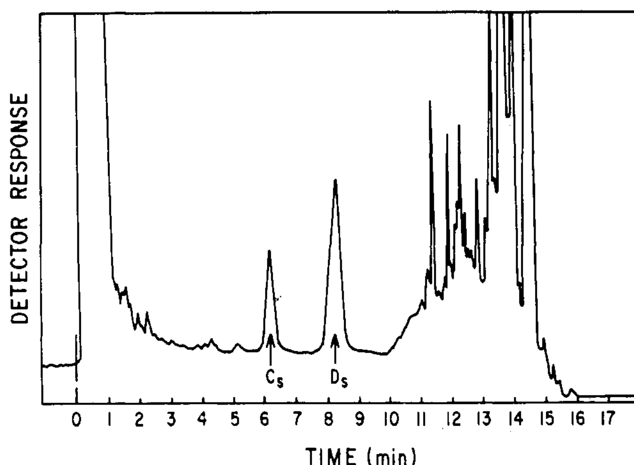

Figure 7. A typical chromatogram obtained for preparative gc of silylated column fractions 28–35. Peaks C_S and D_S correspond to the silyl ether derivatives of the compounds producing peaks C and D (Figure 5). (Temp increased to 250 °C at t = 10 min)

sponds to the peak at t = 4.1 min (Figure 5) and its presence was presumably due to an imperfect preparative separation.

Molecular weights were determined for peaks A–D. An LKB-9000 gc–ms instrument with gc injector temperature of 180°C and column temperature of 80–110°C was used for the determinations. (Under the given conditions the chromatogram obtained was essentially the same as in Figure 6.) Peaks A and B were shown to be due to compounds with similar mass spectra and both having the parent ion at m/e 150. Likewise the mass spectra obtained from peaks C and D were very similar and both had parent ions at m/e 168. Thus it was suspected that the substances giving peaks C and D were closely related structurally and an intense peak at m/e 150 in the spectra of both C and D suggested that they were hydroxylated compounds (m/e 168-H_2O → m/e 150). The substances A and B therefore were presumed to be the products of thermal dehydration of C and D which occurred within the chromatograph. It was not possible to use lower temperatures for preparative gc due to the presence of several considerably less volatile impurities in the toxin-containing column fractions. A similar problem was encountered with the preparative gc of ipomeamarone (Boyd and Wilson, 1971). Thus it was necessary to form heat-stable silyl derivatives of C and D to permit their isolation and purification.

Isolation of Pure Toxin *via* the Silyl Ether Derivative. Silylating reagent (Tri-Sil/BSA, Formula "P"; Pierce Chemical Co., Rockford, Ill.) was added (1.0 ml of reagent/50 mg of sample) to the combined column fractions 28–35 (425 mg). The mixture was allowed to stand at 50°C in a water bath for 30 min. At the end of this period, a gas chromatogram of the material (5 μl sample) under the same conditions as indicated in Figure 5 showed the disappearance of the peaks at t = 4.1 min (C) and t = 4.8 min (D) and the appearance of two new peaks at t = 6.2 min (C_s) and t = 8.3 min (D_s). A typical chromatogram is shown in Figure 7. Molecular weight determinations on each peak indicated a parent ion for both C_s and D_s at m/e 240, which is consistent with the formation of monosilyl derivatives of C and D. The gc oven temperature was decreased to 125°C and a preparative scale separation was begun. Repeated injections of 100–200 μl of the silylated mixture were made and peaks C_s and D_s, respectively, were obtained. Reinjection of small amounts of each showed only single peaks.

The silyl ether derivative, D_s, was hydrolyzed by dissolving it (35 mg) in 0.5 ml of CCl_4 and 0.3 ml of 0.5 N HCl and shaking the mixture at room temperature for 3 hr. At the end of this period, an additional 0.5 ml of CCl_4 was added, and the solution was separated from the water layer with a pipette. A 100-mg portion of anhydrous potassium carbonate was added and shaken with the CCl_4 solution in order both to dry it and to neutralize any residual acid. The solution was filtered and the solvent (and desilylation by-products) carefully removed by evaporation under a stream of dry nitrogen. Under the same gc conditions as shown for Figure 5, a chromatogram of an 0.5-μl sample of the hydrolyzed material showed a major peak corresponding in retention time to peak D (t = 4.8 min). Also, as expected, a peak due to the dehydration product of D appeared at t = 1.9 min. On tlc, a single Ehrlich-positive compound was observed, corresponding in R_f to the reddish-purple spot (lowest R_f) seen in crude fractions 28–35 (Figure 3).

Toxicity of the D compound was determined. Injection of 1-mg doses in mice reproduced the respiratory disease caused by moldy sweet potatoes (Wilson *et al.*, 1970), confirming the role of the compound as a lung edema factor.

Subsequent chemical studies of the substance have indicated its identity as a pure compound, 4-ipomeanol (Wilson *et al.*, 1971) (Figure 2). This structure assignment has also been confirmed by synthesis which will be reported separately. In addition, our studies have shown that C is an isomer, 5-(3-furyl)-5-hydroxy-2-pentanone, of 4-ipomeanol. The toxicity of this compound is presently under investigation.

DISCUSSION

The isolation of 4-ipomeanol has allowed, for the first time, a clear differentiation of individual agents responsible for different manifestations of the experimental disease syndrome produced by crude extracts from moldy sweet potatoes (Wilson *et al.*, 1970). Ipomeamarone and ipomeamaronol are principally hepatotoxic, whereas 4-ipomeanol is toxic primarily to the lungs, producing no significant gross pathological changes in abdominal viscera. Perhaps the greater potency of the lung edema toxin or greater susceptibility of cattle to this toxin may account for failure to note liver damage in many of the natural outbreaks of moldy sweet potato poisoning in this species.

The finding of all these toxic metabolites in marketable sweet potatoes may have implications for human health (Wilson *et al.*, 1970; Boyd and Wilson, 1971). The methods described in this report should be adaptable to studies designed to evaluate this possibility. It should also be possible to develop an accurate and reliable quantitative test for 4-ipomeanol and its isomer, utilizing the gas chromatographic methods and conditions established in this and previous reports (Boyd and Wilson, 1971).

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Received for review August 10, 1971. Accepted September 28, 1971. 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. Michael R. Boyd also acknowledges support by a Vivian Allen M.D.-Ph.D. Fellowship.