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Red Squill Modified by *Lactobacillus acidophilus* for Rodenticide Use

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Red squill (*Urginia maritima*, Liliaceae) bulb and root preparations were treated with three strains of *Lactobacillus acidophilus*, fortifying the cultures with dry milk and oat, wheat, and rice flour. *Lactobacillus* growth with the production of a β-glucosidase converted bitter glucoside scilliroside to its tasteless aglycon scillirosidin. These products were blended into rat diets at 0.03% scillirosidin levels, and 95% of the female rats died. Although male rats usually ate more bait than the females, none ate enough for a lethal dose of scillirosidin. The rats learned to avoid the baits if they did not die after initial ingestion of these fast-acting rodenticides. Technical scillirosidin mixed into rat diets had a toxic effect on female rats similar to the *L. acidophilus* treated red squill products.

Red squill is being investigated as a new economic crop for the southwest United States where it grows well (Gentry et al., 1987). The bulb, roots, and other plant parts contain scilliroside [6β-(acetyloxy)-3β-(β-D-glucopyranosyloxy)-8,14-dihydroxybufa-4,20,22-trienolide], a highly toxic, emetic, and bitter bufadienolide glucoside (Verbiscar et al., 1986a, b). Because of high toxicity and the emetic safety factor, dried bulb powders have been used in rat baits for centuries. However, rats and mice, which are unable to vomit, may not eat a lethal amount of red squill baits when first exposed, resulting in formulation problems.

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Our initial attempts to improve acceptability involved conversion of scilliroside to its aglycon scillirosidin, which is tasteless and equally toxic. The aerobic fungus *Aspergillus niger* was used as a source of β-glucosidase to elicit this cleavage (Verbiscar et al., 1987). *A. niger* was grown in extracts of red squill, producing the enzyme necessary for the hydrolysis of scilliroside to scillirosidin. The resulting aglycon extracts were administered orally to Charles River rats. The scillirosidin aglycons were found to be more toxic to female rats than to males, which is also the case for scilliroside.

In addition to the *A. niger* study we tested 12 strains of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* on hand from a jojoba detoxification project. It seemed reasonable that because Lactobacilli cleave lactose, a galactosylglucose, the active enzyme could also cleave the glucose from scilliroside. The Lactobacilli are nontoxic and microaerobic, which facilitates processing. The Lactobacilli

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also grow well in nonsterile media, lowering the pH and producing carbon dioxide which limits growth of random volunteer microorganisms. We were successful growing Lactobacilli on jojoba seed meal, modifying the cyano group of simmondsin and related glucosides, thereby detoxifying the meal and making it acceptable as a feed additive (Verbiscar et al., 1981). Our goal in the current study was to identify Lactobacilli that would grow on nutrient-fortified red squill preparations, producing a β-glucosidase capable of splitting the glucose from scilliroside and thereby producing scillirosidin for improved acceptability in rodent baits.

EXPERIMENTAL SECTION

Materials. Eight strains of *L. acidophilus* (NRRL 629, 1833, 1868, 1910, 1911, 1912, 2092, 2178) and four strains of *L. bulgaricus* (NRRL 548, 734, 1909, 1918) originally obtained from the Culture Collection, U.S. Department of Agriculture, Peoria, IL, courtesy of C. W. Hesseltine, are maintained at Anver. Red squill plants were obtained from the Gentry Experimental Farm, Murrieta, CA, courtesy of H. S. Gentry. Nutrient supplements included Albers yellow corn meal, old-fashioned Quaker oats, Springfield Enriched long-grain rice, Gold Medal whole wheat flour, and Kingsford corn starch. The cereal grains were comminuted to flours in a Weber hammer mill through an 0.04-in. screen.

Culture Conditions. The Lactobacilli were cultured and maintained in a 3% solution of Carnation nonfat dry milk in distilled water (Verbiscar et al., 1981). Propagation was carried out at 37 °C incubation temperature, and the cultures were stored at 8 °C in a refrigerator. A gyrotory shaker was used at times during the incubation to accelerate bacterial growth and increase β-glucosidase production. Cotton plugs or milk filter disk cloth, sometimes covered with Saran wrap to retain moisture, were used as culture flask closures.

Assay Methods. Assay methods for bufadienolides developed in prior studies (Verbiscar et al., 1986a,b, 1987) were followed with use of lead acetate to precipitate phenolics. Thin-layer chromatography (TLC) was carried out on silica gel G plates using ethyl acetate–2-methoxyethanol (9:1) developer, which gave R_f values for scilliroside of 0.31 and for scillirosidin of 0.77. An acetonitrile–water (95:5) developer gave R_f values for scilliroside of 0.22, for scillirosidin of 0.68, and for desacetylscillirosidin of 0.52. These scilla compounds fluoresce bright green under UV light after acid spraying with 10% sulfuric acid and gentle heating. Affinity high-performance liquid chromatography (HPLC) was difficult to apply quantitatively to several of the fermentation products. The cereal flour supplements needed for good *Lactobacillus* growth and β-glucosidase production, and *Lactobacillus* metabolism products, introduced an assortment of interfering contaminants. In some instances this problem was overcome by making a final assay extraction into chloroform. Most HPLC assays were carried out on a 3.2 × 500 mm Lichrosorb Si 60 5-μm column protected by a 3.2 × 40 mm Porasil A column. With acetonitrile–water (95:5) eluant at a flow rate of 1.0 mL/min, retention of scilliroside was ~9 min. Scillirosidin was assayed effectively on a 4.6 × 250 mm Sepralyte octyl reversed-phase column with water–acetonitrile (2:1) eluant at a flow rate of 1.0 mL/min. It showed retention of scillirosidin at 20.7 min and scilliroside at 5.7 min (Figure 1). The detector was set at 300 nm for both compounds.

Material Preparation. Collections of bulbs were made on three occasions. Two of these collections were used in the preparation of dry powders as described in this section, and the third was used for a bulb + root mash as described in another section. The highest total scilliroside content in bulbs occurs during summer dormancy. The highest percent scilliroside in bulbs occurs during the flowering period in September.

On March 26 during a period of growth, 12 red squill bulbs (clone 868) were harvested with roots, washed, and air-dried (13.57 kg). The bulbs were sliced into small pieces and tray-dried in a forced-air oven at 60–65 °C for 20 h. The resulting material was ground in an Alpine Model 25MZ hammer mill with a rasp-trapeze screen. The resulting meal powder 0–124 weighed 2.47 kg (18.7%) and contained 7.7% moisture and 0.19% scilliroside. On August 4, during summer dormancy 10 bulbs of clone 868 without roots were harvested and cleaned (11.2 kg). After processing as before, meal 0–154 weighed 2.42 kg (21.6%) and contained 0.13% scilliroside. The lower scilliroside content here is due principally to the fact that high-toxicity roots were not processed with the bulbs (Verbiscar et al., 1986b).

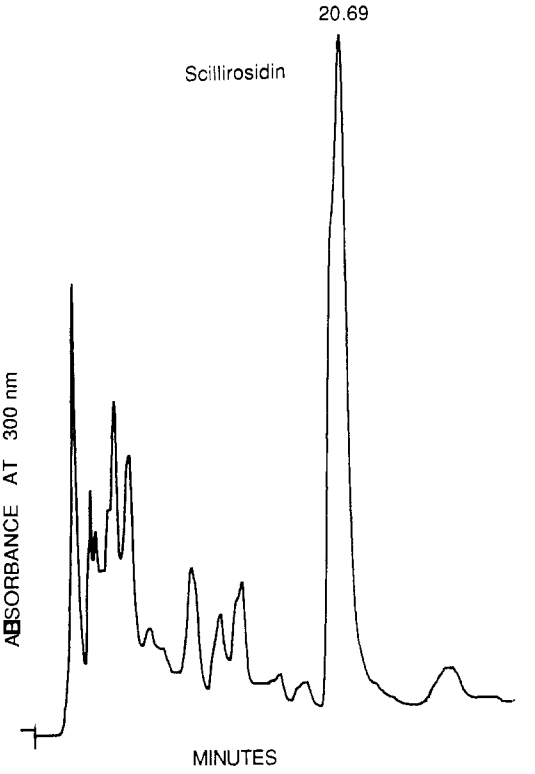

Figure 1. Reversed-phase HPLC of *L. acidophilus* 1910 oat-fortified red squill powder. Conditions: Porasil A precolumn; Sepralyte octyl column, 4.6 × 250 mm; water–acetonitrile (2:1) eluant at 1.0 mL/min; detector at 300 mm.

Fortified Red Squill Extracts. A 225-g quantity of red squill powder 0–124 was extracted with 1100 mL of distilled water in a Waring blender. The mixture was separated in a basket centrifuge, the cake was washed with another 450 mL of water, and the filtrates were combined. Ten 125-mL Erlenmeyer flasks with milk filter disk cloth closures were each charged with 30 mL of the extract and 2 g of one of the five flour nutrients including corn, oats, rice, wheat, and corn starch. A second collection of five 500-mL Erlenmeyer flasks were each charged with 100 mL of the extract and 10 g of the nutrients. The media were sterilized in an autoclave for 15 min at 121 °C. The cooled 125-mL flasks were inoculated with 3 mL of *L. acidophilus* 1910 and 1912 cultures grown in 3% milk solids. The 500-mL flasks were inoculated with 10 mL of strain 2092 culture. The fermentations were carried out in an incubator at 37 °C with occasional gyrotory shaking at 125 rpm. After 11 days, acetone was added to each flask to quench the fermentation, dissolve the scilla toxicants, and render the mass filterable. After filtration the filter cake was washed well with acetone. The filtrates were reduced in volume under vacuum on a rotary evaporator and then extracted several times with chloroform. The chloroform extracts were concentrated to dryness in a rotary evaporator and then transferred with methanol to a 10-mL volumetric flask for assay. HPLC was carried out on the Lichrosorb column using acetonitrile–water (100:0.5) eluant, which resulted in retention times for scillirosidin of 6.5 min and for desacetylscillirosidin of 12.5 min. Results are in Table I.

Fortified Red Squill Mash. Fresh bulbs with roots of clone 871 containing 80% water were macerated in a Waring blender and then fortified with rice, wheat, and oat nutrients. The mashes were autoclaved because earlier tests on nonsterilized mashes produced considerable random microorganism growth. After inoculation with standard cultures of *L. acidophilus* 1910 and 2092, the mash cultures were incubated at 37 °C for 18 days. TLC assays indicated that all of the scilliroside was converted to scillirosidin and desacetylscillirosidin in each culture including

Table I. *L. acidophilus* Treatment of Fortified Red Squill Extracts

	scillirosidin, % theoretical yield					
strain	corn	oats	rice	wheat	starch	av
2092	61	69	75	64	75	69
1910	59	66	76	70	58	66
1912	51	58	55	62	20	49
av	57	64	69	65	51	

the milk solid controls. The 1910 oats and 2092 milk solids cultures gave the best yields of scillirosidin. However, considerable desacetylscillirosidin hydrolysis product occurred in four of the six cultures. Acetoxy group hydrolysis could have occurred during the incubation period or during the long tray drying of the mashes at 60 °C. This hydrolysis of the acetoxy group discouraged further mash experiments.

Fortified Moist Red Squill Powders. Six Fernbach flasks (2800 mL) were charged with 200 g of red squill powder 0–154. Four of these systems were fortified with 40 g each of rice (two flasks), oat, and wheat flours, and two flasks were fortified with 3 g each of dry milk solids. The six systems were then inoculated with 125 mL of *L. acidophilus* 1910 and 2092 grown in 5% solutions of dry milk solids, by spraying onto the meal in a rotating flask. The flasks were closed with milk filter disks and incubated at 37 °C. At day 6 mycelia growth was very evident below the surface and on the bottoms of the fortified meals. Saran wrap was installed over the closure to reduce moisture loss. Monitor samples on day 15 showed 40–60% conversion of scilliroside including two milk solid only controls. On days 22 and 23 TLC monitors indicated that 1910 rice and 2092 rice were essentially complete so they were removed from the incubator and dried in a forced-air oven at 65 °C. The other four systems still contained scilliroside and had partially dehydrated, so 100 mL of water was added to each and the Saran wrap was removed. On day 30, the remaining samples were removed from the incubator, oven-dried, and comminuted in a Weber hammer mill. HPLC assay for scillirosidin was made on a Sepralyte C_8 reversed-phase column. Residual scilliroside in three products was not well separated from nutrient source contaminants and was estimated by semiquantitative TLC comparisons with scillirosidin. Very small amounts of desacetylscillirosidin were also estimated by TLC comparison with the HPLC assayed scillirosidin. Results are in Table II.

Rat Testing. Efficacy tests were carried out at the University of California, Davis, using Sprague–Dawley rats weighing over 200 g. The prepared samples from Table II were mixed into an EPA-type specified rodent diet comprising ground corn (65%), ground oats (25%), corn oil (5%), and powdered sugar (5%). The test diets were blended with the six prepared samples at a 0.03% level of scillirosidin–scilliroside, with several tests run at 0.01% and 0.05% toxicant levels. Technical scillirosidin (67%) in ethanol was added to the EPA diet at three dose levels of 0.01%, 0.03%, and 0.05% and then allowed to evaporate overnight. For each formulation, six rats, sexes equal, were caged individually in suspended wire cages, each with a tray to collect any bait spillage. The rats were deprived of food for about 6 h prior to the start of the test. The measured amount of bait was placed in the cages at about 4:45 p.m. on a no-choice basis, exposing the rats overnight undisturbed. The next day, any remaining bait was weighed and deaths were recorded. The health of surviving animals was monitored for at least 7 days following this 1-day bait exposure

Table II. *L. acidophilus* Treatment of Fortified Moist Red Squill Powders

		toxicant[a]					
		I		II		III	
strain nutrient	meal dry wt yield, g		mg	%	mg	%	mg
1910 oats	105	0.31	320	0	0	0.02	20
1910 wheat	107	0.27	290	0	0	0.05	50
2092 rice	131	0.19	250	0	0	0	0
1910 rice	130	0.17	220	0.04	50	0.04	50
2092 milk solid	127	0.14	180	0.2	250	0	0
1910 milk solid	170	0.08	140	0.1	170	tr	tr

[a] Key: I, scillirosidin; II, scilliroside; III, desacetylscillirosidin.

Table III. Tests of *L. acidophilus* Treated Red Squill Powders in Rat Diets[a]

		av bait consumed, g		av toxicant consumed, mg/kg		mortality[c]	
strain nutrient	toxicant total,[b] %	M	F	M	F	M	F
1910 milk	0.01	2.2	1.2	0.72	0.54	0	3[d]
1910 milk	0.03	1.3	0.8	1.63	1.17	0	3
1910 rice	0.03	0.5	0.6	0.70	0.88	0	3[e]
1910 wheat	0.03	0.9	0.7	1.12	1.05	0	3
1910 oats	0.03	0.7	1.0	0.92	1.43	0	3
2092 milk	0.03	1.7	1.4	2.08	1.94	0	3
2092 milk	0.05	1.2	1.0	2.10	2.24	0	3
2092 rice	0.03	0.5	0.6	0.66	0.83	0	3

[a] Each value is an average of three rats. Average weights: males (M), 253 g; females (F), 216 g. [b] Scillirosidin (I) plus residual scilliroside (II) (Table II). [c] All rats died on day 1 unless noted otherwise. [d] One rat died on day 4. [e] One rat died on day 7.

Table IV. Tests of Technical Scillirosidin in Rat Diets[a]

		av bait consumed, g		av toxicant consumed, mg/kg		mortality[c]	
test group	toxicant total,[b] %	M	F	M	F	M	F
X	0.01	3.1	1.7	1.44	0.84	0	0
Y	0.03	1.5	1.2	2.11	1.85	0	3
S[f]	0.03	1.2	1.3	1.48	1.81	0	3[d]
Z	0.05	1.3	1.1	2.95	2.60	0	2[e]

[a] Each value is an average of three rats. Average weights: males (M), 221 g; females (F), 210 g. [b] Composition: 67% scillirosidin, 8% desacetylscilliroside, and 4% desacetylscillirosidin in ethanol. [c] All rats died on day 1 unless otherwise noted. [d] One rat died on day 2. [e] One rat died on day 4. [f] Sugar increased in diet from 5% to 10% and less corn by 5%.

test. Results of the fortified culture baits and technical scillirosidin baits are in Tables III and IV, respectively.

RESULTS AND DISCUSSION

In a preliminary screening of 12 strains of Lactobacilli for their ability to split glucose from scilliroside, four *L. bulgaricus* strains had no activity, whereas five of eight *L. acidophilus* strains showed varying degrees of activity when tested in a nonfat milk medium. *L. acidophilus* strains 1910, 1912, and 2092 were most active, and these were screened further in aqueous extracts, mashes, and moist powders. These cultures were fortified with cereal grain flours to supplement the nutrients in red squill to support Lactobacilli growth with β-glucosidase production.

The β-glucosidase produced by the Lactobacilli converted toxic and bitter scilliroside to a tasteless and highly toxic scillirosidin (Rothlin and Schalch, 1952; Verbiscar et al., 1986a,b, 1987). In addition, *L. acidophilus* strains 1868, 1910, 1911, and 2092 also showed a glucosyl transferase reaction. For strain 1911, conversion of scilliroside to glucosylscilliroside during the test period, as monitored by TLC, was about 85%. Among 12 Lactobacilli, we have found nitrilase (Verbiscar et al., 1981), β-glucosidase, and glucosyl transferase activity. Glycoside hydrolase and glycosyl transferase activity can be two functions of the same enzyme (Nisizawa and Hashimoto, 1970). The effect of glucosylation of scilliroside in the preparations was not examined, although it would probably decrease and/or delay toxicity of orally ingested rat baits.

Conversion of scilliroside to scillirosidin occurred readily in the *L. acidophilus* cultures where aqueous red squill extracts were fortified with cereal grain flours and incubated at 37 °C for 11 days (Table I). Highest average conversions to scillirosidin were observed with strains 1910 and 2092 at 66% and 69%, respectively. Strain 1912 was less active and produced more of the low-toxicity desacetylscillirosidin. The oats, rice, and wheat nutrients with average scillirosidin yields of 64%, 69%, and 65% were slightly better than corn and corn starch. Accordingly, *L. acidophilus* strains 1910 and 2092 with oats, rice, and wheat were chosen for further study. Furthermore, these *L. acidophilus* strains are an alternative to *A. niger* (Verbiscar et al., 1987) for the preparation of pure scillirosidin from scilliroside.

After red squill mashes were explored with limited success, the preferred physical state for carrying out this conversion effectively was found to be fortified moist red squill powders. The best yields of scillirosidin with a minimum of side products occurred when the cultures were maintained at 37 °C rather than ambient temperature and when the fortified moist red squill powders were run without autoclaving. In one of the experimental runs, sterilization temperatures caused water-soluble protein to denature, thereby reducing nutrient value for the Lactobacilli which grew more slowly with less β-glucosidase production. The preparative runs using nonsterilized fortified, moist red squill powder resulted in high-yield conversion to scillirosidin, with only minor production of the undesirable desacetylscillirosidin. The scilliroside in these cultures of strains 1910 and 2092 was almost completely converted to aglycon when fortified with grain nutrients. Despite the lengthy culture time of 30 days, the four cereal-fortified dry products were of good quality, with a pleasant cereal odor, and only a slight fermentation odor. The six products are assembled in Table II in decreasing order of scillirosidin yield.

Rodenticide tests were carried out on the six *L. acidophilus* treated red squill powder preparations as summarized in Table III. Two of these six samples, 1910 milk and 2092 milk, were prepared without cereal flour fortification and still contained significant amounts of residual scilliroside. Results can be interpreted generally in terms of scillirosidin (I) and scilliroside (II) concentrations. These two compounds are nearly equal in potency (Rothlin and Schalch, 1952), whereas desacetylscillirosidin (II) is far less toxic (Verbiscar et al., 1987). Accordingly, the *L. acidophilus* treated preparations were formulated into a basal EPA challenge diet at scilla toxicant I + II levels of 0.01%, 0.03%, and 0.05%. Eight bait tests were conducted on three male rats and three females for each test.

Only female rats ate lethal doses of the baits. All 24 females fed the eight experimental baits died, 22 of them on day 1. Toxicant average consumption per test group ranged from 0.54 to 2.24 mg/kg for the females for an overall mean of 1.26 mg/kg of mixed toxicants I + II. Eliminating the data for samples 1910 milk and 2092 milk, which were high in scilliroside, the mean lethal dose for female Sprague–Dawley rats was calculated as ~1.0 mg/kg of scillirosidin. None of the male rats ate enough bait to die. It is known that scilliroside and scillirosidin are more toxic to female than to male rats (Rothlin and Schalch, 1952). In prior studies (Verbiscar et al., 1986a,b, 1987) an LD_{50} for orally administered scillirosidin was 2–3 mg/kg for female Charles River rats and 4–5 mg/kg for males. The lethal dose values in each of these studies are in the same general range with somewhat higher toxicity to the female Sprague–Dawley rats. This could be a strain difference, or a difference in the method of administration of the toxicants to the rats, i.e., by gavage or feeding in baits.

Both male and female rats consumed more of the 1910 milk and 2092 milk baits containing residual scilliroside than other formulated baits. This indicates that the bitter taste of scilliroside in red squill preparations is not the primary reason for the limited consumption by rats and the resultant learned bait avoidance. It may be that a rapid toxic action rather than palatability causes rats to stop feeding on baits containing scillirosidin. Ingested scillirosidin apparently acts faster than scilliroside, causing feeding rats to feel toxic symptoms sooner. Also, the higher degree of toxicity to females may have caused them to stop eating sooner than males. The absorption, distribution, and toxicological action are slow enough for female rats to ingest a lethal dose of scillirosidin, but not males.

As an alternative rodenticide, a technical grade of scillirosidin (Verbiscar et al., 1987) was tested for acceptance in the EPA type diet. Baits were formulated at three toxicant concentration levels and fed to Sprague–Dawley rats (Table IV). At the 0.01% scillirosidin level rats ate more of the bait than at higher levels but did not consume a lethal dose. At 0.01% males consumed an average of 1.44 mg/kg and females 0.84 mg/kg of scillirosidin, which is less than the average lethal dose of the red squill powder treatment tests. At 0.03% and 0.05% toxicant concentrations, females consumed more than the lethal dose of 1 mg/kg and eight of nine died. None of the males died at these higher dose levels. Increasing the sugar level in the baits did not increase consumption (test group S), which further suggests that taste is not the inhibiting factor.

In these tests with red squill fermented powders and technical scillirosidin preparations, only females ate enough bait for a lethal dose. Males ate slightly more than females but never enough for a lethal dose of scillirosidin. This suggests that scillirosidin acts relatively fast, causing rats to stop feeding when they feel the toxic effects. By the time the females experience symptoms, they have already

consumed a fatal dose as females are considerably more susceptible to the toxicant than the males. Illness-based aversion learning is known (Robbins, 1980). The characteristic of slow toxic action allowing the animals to consume a full lethal dose of bait has been observed for bromethalin (Dreikorn and O'Doherty, 1985). Alternately, the concentration of a palatable toxicant in the rodenticide bait should be high enough for a lethal dose after initial feeding.

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Registry No. Scilliroside, 507-60-8; scillirosidin, 507-59-5.

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Studies on the Enzymic Hydrolysis of Bound Aroma Components from *Carica papaya* Fruit

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HRGC and HRGC–MS identifications of bound volatiles from papaya fruit (*Carica papaya*, L.) were achieved after isolation of an extract obtained by Amberlite XAD-2 adsorption and methanol elution followed by simultaneous enzyme catalysis extraction (SECE) using glycosidase (emulsin) and acid phosphatase. Aromatic substances, such as, e.g., benzaldehyde, benzyl alcohol, 2-phenylethanol, and benzyl isothiocyanate as well as (*E*)-3,7-dimethylocta-2,6-dienoic acid, were liberated by glycosidase, while the monoterpene alcohols linalool and 2,6-dimethyloct-7-ene-2,3,6-triol were released by phosphatase activity. As precursor of the phosphate-bound 2,6-dimethyloct-7-ene-2,3,6-triol, the phosphorylated 6,7-epoxylinalool is discussed.

In the past, the composition of volatiles from *Carica papaya* fruit has been extensively studied. Linalool and benzyl isothiocyanate have been found to be its major aroma constituents (Katague and Kirch, 1965; Flath and Forrey, 1977; McLeod and Pieris, 1983; Idstein and Schreier, 1985). Recently, investigations of bound forms of volatiles have also been carried out leading to structural elucidation of several aryl β-D-glucosides such as benzyl, 2-phenylethyl, (4-hydroxyphenyl)-2-ethyl, and four isomeric malonated benzyl β-D-glucosides in this fruit (Schwab and Schreier, 1988a). Meanwhile, the availability of more uncommon aglycons such as terpenoids has been provided by introduction of simultaneous enzyme catalysis extraction (SECE) (Schwab and Schreier, 1988b). Thus, it was interesting to study again the composition of bound volatiles in papaya fruit with use of this versatile technique. This paper concerns the results obtained after SECE using two different types of hydrolases, β-glucosidase (emulsin) and acid phosphatase.

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EXPERIMENTAL SECTION

Fruits. Fresh, ripe papaya fruits (*C. papaya* L. var. Solo) were obtained from the local market.

Isolation of an Extract by the XAD Method (Gunata et al., 1985). Fruits (sample weight 3 kg) were cut and the seeds removed. After homogenization with 1 L of 0.2 M phosphate buffer (pH 7.5) containing 0.2 M glucono-δ-lactone and centrifugation (30 min, 15000*g*) the supernatant was subjected to LC chromatography on Amberlite XAD-2 adsorbent (glass column, 25 × 900 mm). After being washed with 1500 mL of H_2O, 500 mL of pentane, and 750 mL of ethyl acetate, the extract was isolated by eluting with 1000 mL of MeOH. The MeOH fraction was concentrated under reduced pressure to dryness and redissolved in 50 mL of 0.2 M phosphate buffer (pH 5.5). Remaining volatiles were separated by diethyl ether extraction.

SECE (Schwab and Schreier, 1988b). After the SECE apparatus was filled with 0.2 M phosphate buffer (pH 5.5), the aqueous layer of the papaya extract was transferred to a dialysis

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