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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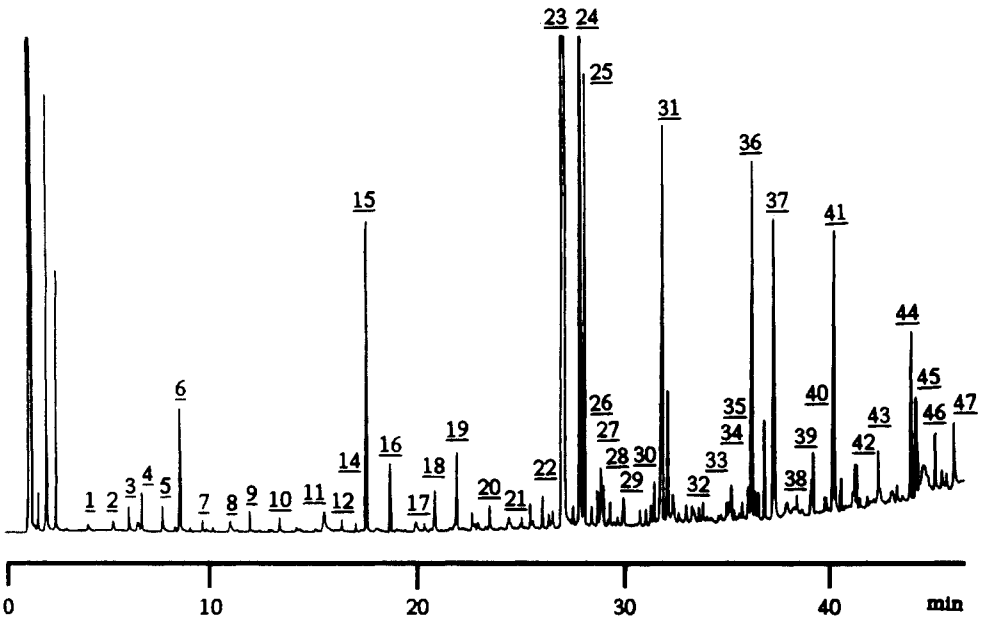

Figure 1. HRGC separation (J&W; 30 m, 0.25-mm i.d., fused silica WCOT CW20M capillary column, df = 0.25 μm) of compounds liberated by SECE of a XAD-separated papaya extract after 12 days of hydrolysis using emulsin.

membrane, 50 mg of emulsin (Boehringer) added, and the tube carefully closed. This tube was placed into the SECE reaction vessel, and the liquid–liquid extractor of SECE was filled with diethyl ether. Finally, the two parts of the system were connected, and micropump and heater were started. The flow rate was 2 mL/h, and the temperature 25 °C. SECE was performed over 14 days, and each day the flask with the organic layer was exchanged and the liberated aglycons were analyzed by means of HRGC and HRGC–MS. SECE with emulsin was repeated under the above-mentioned condition but performed at 37 °C over 7 days. In an analogous experiment, acid phosphatase from sweet potato (Sigma) was used. In this study, carried out at 37 °C over 7 days, the phosphate buffer used contained additionally 0.2 M glucono-δ-lactone and 0.2 M EDTA in order to inhibit the glycosidase activities detected in this enzyme preparation. Quantification of the released volatiles was achieved by addition of 0.5 mg of 1-octanol as standard before HRGC analysis.

Screening of Enzyme Activities. Enzyme activities in emulsin (Boehringer) and acid phosphatase (Sigma) were screened with use of the commercially available API ZYM test kit (Api-Mérieux) (Satyanarayana et al., 1985).

Phosphate Determination. Phosphate content of the papaya extract was measured before and after acid hydrolysis with $HClO_4$ according to Schriever et al. (1957).

Synthesis of Geranyl Phosphate and Pyrophosphate. Preparation of geranyl phosphate and pyrophosphate was performed by a standard phosphorylation method (Cramer and Rittersdorf, 1967) followed by purification described by Gafni and Shechter (1979).

Hydrolysis of Geranyl Phosphate and Pyrophosphate by Acid Phosphatase and Emulsin. Six milligrams of a geranyl phosphate and pyrophosphate mixture was dissolved in 6 mL of 0.2 M phosphate buffer (pH 5.5) and divided exactly into two parts. To the first part was added 1 mg of acid phosphatase from sweet potato (Sigma) and the resultant mixture incubated overnight at room temperature, while the second remained without enzyme addition. The next day 3 mg of 1-octanol as standard was added to each solution, and the volatiles were extracted and analyzed by means of HRGC–MS. TLC was used for identification of the substances in the remaining aqueous layer.

Capillary Gas Chromatography (HRGC). A Carlo Erba Fractovap 4100 gas chromatograph with FID equipped with a J&W fused silica CW20M capillary column (30 m, 0.25-mm i.d., film thickness 0.25 μm) was used. Split injection (1:50) was employed. The temperature program was 3 min isothermal at 40 °C and then 40–240 °C at 4 °C/min. The flow rates for the carrier gas were 2.0 mL/min of He, for the makeup gas 30 mL/min of N_2, and for the detector gases 30 mL/min of H_2 and 300 mL/min of air, respectively. The injector temperature was kept at 200 °C and the detector temperature at 250 °C. Volumes of 1.0 μL were injected.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 1440 gas chromatograph equipped with a Carlo Erba water-cooled on-column injection system was coupled by an open-split interface to a Finnigan MAT 44 mass spectrometer with SS 200 data system. A J&W CW20M fused silica capillary column (30 m, 0.25-mm i.d., film thickness 0.25 μm) connected to a 2-m uncoated piece of fused silica capillary column as the retention gap was used. The conditions were as follows: temperature, from 40 to 240 °C at 5 °C/min; carrier gas flow rate, 2.5 mL/min of He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA; injection volume, 1.0 μL. Results of qualitative analyses were verified by comparison of HRGC retention (R_t) and mass spectral data with those of authentic reference substances. Quantitative HRGC determinations were carried out on a Hewlett-Packard 3388 A laboratory data system.

RESULTS AND DISCUSSION

SECE with Emulsin. In a first series of experiments, SECE of an extract obtained from papaya fruit pulp by XAD adsorption and methanol elution was carried out over 14 days at 25 °C. As a representative example, Figure 1 shows the HRGC separation of the liberated compounds identified by HRGC–MS after 12 days of hydrolysis (Table I). In agreement with our previous studies performed with apple fruit (Schwab and Schreier, 1988b) during the first days of SECE preferably aromatic aglycons were released, while in later phases of the process also monoterpenes were hydrolyzed. The structures of monoterpenes identified after SECE of papaya extract are represented in Figure 2.

Comparison of SECE with Emulsin and Acid Phosphatase. Since in the emulsin preparation phosphatase activity was detected and a large amount of bound phosphate (4.3 mg/kg) was determined in the XAD-separated papaya extract, our interest was focused on bound phosphorylated papaya constituents. First of all, in model reactions the ability of commercial acid phosphatase to cleave synthesized geranyl phosphate and pyrophosphate in phosphate buffer was confirmed. The phosphate esters were also hydrolyzed by emulsin. Furthermore, in the acid phosphatase preparation glycosidase activities were also

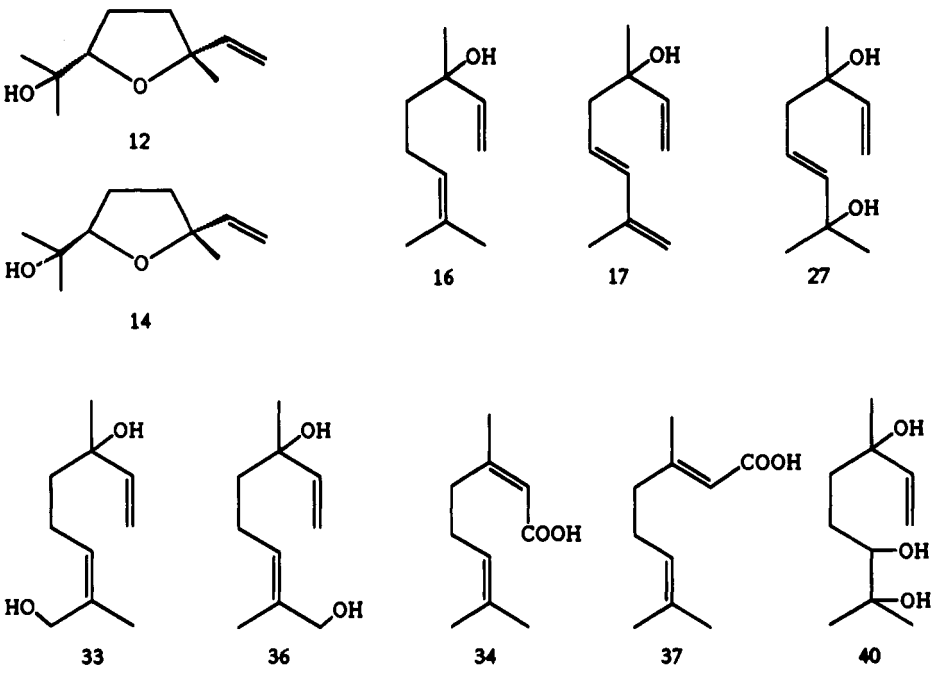

Figure 2. Terpenoids released by SECE during emulsin treatment of a XAD-separated papaya extract. The numbers of compounds correspond to the numbers in Table I.

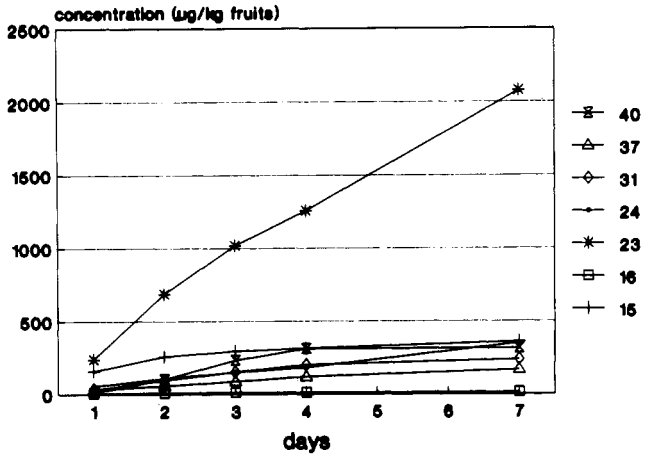

Figure 3. Enzymic release of compounds **15**, **16**, **23**, **24**, **31**, **37**, and **40** during SECE with emulsin of a XAD-separated papaya extract. For details, see the Experimental Section.

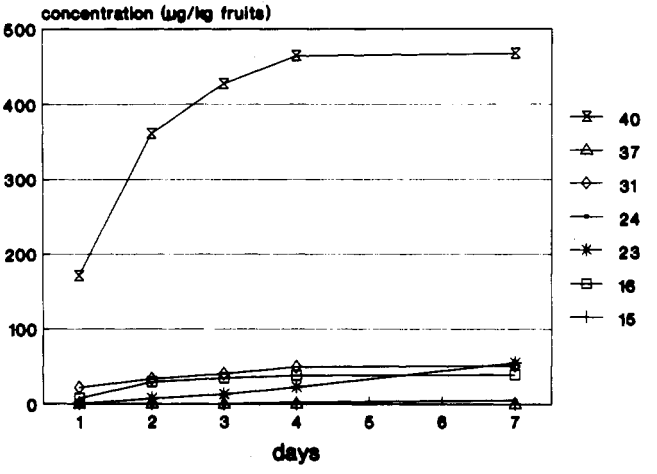

Figure 4. Enzymic release of compounds **15**, **16**, **23**, **24**, **31**, **37**, and **40** during SECE with acid phosphatase of a XAD-separated papaya extract. For details, see the Experimental Section.

detected, which could be inhibited by glucono-δ-lactone and EDTA.

In a second series of experiments, two SECE processes were carried out over 7 days at 37 °C using both emulsin and acid phosphatase. Figures 3 and 4 represent the quantitative results of the release of compounds after SECE of papaya extracts using emulsin (exhibiting acid phosphatase side activity) and acid phosphatase (glycosidase activities inhibited), respectively. In contrast to the aromatic substances **15**, **23**, **24**, and **31**, the monoterpene alcohols **16** and **40** were not liberated by glycosidase, but by phosphatase activity. This can be clearly seen from the results of hydrolysis using both enzymes, in which more than 400 μg/kg of **40** and approximately 30 μg/kg of **16** were released (Figures 3 and 4) by emulsin and acid phosphatase. These data strongly suggest the occurrence of phosphates of **40** and **16**. The monoterpene acid **37**, however, was not liberated by acid phosphatase (Figure 4), but by glycosidase activity (150 μg/kg; Figure 3).

The monoterpene triol **40**, first identified as natural compound in grapes (Williams et al., 1980), is regarded to be formed by opening the epoxide ring of 6,7-epoxylinalool under mild acid conditions. Recently, 6,7-epoxylinalool has been detected for the first time as natural plant constituent in papaya fruit (Winterhalter et al., 1986). Because of the sensitivity of the epoxide hydrolysis, the phosphate-bound 6,7-epoxylinalool can be postulated as precursor of the phosphorylated triol **40**. Similar compounds have been previously described by Banthorpe et al. (1977). With cell-free extracts of different plants incubated with [^{14}C]-labeled substrates such as isopentenyl and geranyl pyrophosphate, as well as isopentenol and geraniol, epoxides and polyols formed from these compounds by "salvage enzymes" have been identified. As incorporation rates for the above-mentioned substrates, 96, 80, 25, and 15%, respectively, have been determined. These high rates evaluated for monoterpene pyrophosphate incorporation together with our observation of the presence of phosphorylated **40** in papaya fruit indicate that the substrates for the salvage enzymes are not the free terpene alcohols, but the pyrophosphates of these compounds. The free terpene alcohols can be obtained after phosphatase hydrolysis of the phosphorylated compounds. An acid phosphatase from papaya fruit has been already

Table I. Compounds Identified in Papaya Fruit by HRGC and HRGC–MS after SECE Treatment (Emulsion) of a Methanolic Eluate Obtained from XAD-Separated Fraction

peak no.[a]	R_t[b]	compound
1	1000	2-butanol
2	1083	2-methyl-1-propanol
3	1110	3-penten-2-one
4	1110	2-pentanol
5	1124	1-butanol
6	1191	2-methyl-1-butanol
7	1236	3-methyl-2-buten-1-ol
8	1250	1-pentanol
9	1311	2-methyl-2-buten-1-ol
10	1345	1-hexanol
11	1403	acetic acid
12	1421	(*Z*)-linalool oxide furanoid
13	1441	1-heptanol
14	1461	(*E*)-linalool oxide furanoid
15	1502	benzaldehyde
16	1529	linalool
17	1585	(*E*)-3,7-dimethylocta-1,5,7-trien-3-ol
18	1591	butanoic acid
19	1643	2-methylbutanoic acid/3-methylbutanoic acid
20	1708	4-methylacetophenone
21	1734	1-phenylethanol
22	1830	2-dodecenal
23	1848	benzyl alcohol
24	1871	2-phenylethanol
25	1877	phenylacetonitrile
26	1920	1-dodecanol
27	1925	2,6-dimethylocta-3,7-diene-2,6-diol
28	1951	2-methylbenzyl alcohol
29	1975	phenol
30	2033	2-methylphenol
31	2051	benzyl isothiocyanate
32	2131	1-tetradecanol
33	2197	(*Z*)-2,6-dimethylocta-2,7-diene-1,6-diol
34	2210	(*Z*)-3,7-dimethylocta-2,6-dienoic acid
35	2254	1-pentadecanol
36	2254	(*E*)-2,6-dimethylocta-2,7-diene-1,6-diol
37	2311	(*E*)-3,7-dimethylocta-2,6-dienoic acid
38	2365	1-hexadecanol
39	2401	benzoic acid
40	2425	2,6-dimethyloct-7-ene-2,3,6-triol
41	2463	phenylacetic acid
42	2558	1-octadecanol
43	2563	3-hydroxy-β-damascone
44	2637	1-nonadecanol
45	2643	3-oxo-α-ionol
46	2656	3-hydroxy-β-ionol
47	2698	vomifoliol

[a] The peak numbers correspond to the numbers in Figure 1. [b] R_t = linear retention index based on a series of *n*-hydrocarbons. For HRGC conditions, see the Experimental Section.

purified by Carreno and Chan (1982), which showed an optimum pH (pH 6.0) similar to that of acid phosphatase from sweet potato.

Regarding the identification of terpene acid **37** after SECE of papaya extract (Figure 3), several publications dealing with terpene carbohydrate esters (Hase et al., 1982; Iwagawa and Hase, 1983; Gross et al., 1987) suggest the presence of such conjugates also in papaya fruits.

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