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6,7-EPOXY-LINALOOL AND RELATED OXYGENATED TERPENOIDS FROM *CARICA PAPAYA* FRUIT

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Key Word Index—*Carica papaya*; Caricaceae; papaya fruit; 6,7-epoxy-linalool; epoxy-linalool oxides; terpene polyols.

Abstract—Oxygenated terpenoids derived from linalool, a major constituent of papaya fruit volatiles, were studied by HRGC and HRGC-MS. Using a sample preparation technique suitable for the separation and enrichment of polar compounds, the two diastereoisomers of 6,7-epoxy-linalool, 2,6-dimethyl-octa-1,7-diene-3,6-diol, 2,6-dimethyl-octa-3,7-diene-2,6-diol, (*E*)- and (*Z*)-2,6-dimethyl-octa-2,7-diene-1,6-diol and 2,6-dimethyl-oct-7-ene-2,3,6-triol were identified. Additionally, each of four diastereoisomeric epoxy-linalool oxides in their furanoid and pyranoid forms were detected for the first time as natural plant constituents. Biogenetic pathways for formation and metabolism of the oxygenated linalool derivatives are discussed.

INTRODUCTION

Most studies on plant volatiles have been undertaken with the aim of identifying the substances responsible for the characteristic aroma and flavour [1]. In recent years, however, there has been an increasing interest in the investigation of biogenetic flavour formation [2]. The reasons for this new trend are obvious; all over the world there is an increased demand for natural flavours and—as the natural sources are no longer sufficient—new ways of production are being sought, e.g. use of plant cell or tissue cultures, microorganisms or enzymes [3, 4]. At present, these efforts are rather limited, especially for plant volatiles where only a few experimentally documented biogenetic pathways are known [2–5]. In this paper, some results of our studies on papaya (*Carica papaya*) fruit volatiles are presented.

RESULTS AND DISCUSSION

Linalool has been found to be one of the main volatile constituents of papaya fruit pulp [6]. At the biological pH of 5.6, drastic chemical degradation reactions of linalool during sample preparation should not be expected. Nevertheless, in model experiments, we examined the possible degradation of linalool at this pH value. Despite the relatively low acidity, linalool was partially decomposed to a series of hydrocarbons, 2,6,6-trimethyl-2-vinyl-tetrahydropyran and α-terpineol [7].

To investigate the influence of pH of the fruit pulp on the composition of volatiles, another sample preparation technique at pH 7.0 was used [8]. With HRGC and HRGC-MS analysis, several linalool derivatives were characterized, which were not previously described as papaya fruit or natural plant constituents. These compounds comprised the two diastereoisomers of 6,7-epoxy-linalool (**1**), 2,6-dimethyl-octa-1,7-diene-3,6-diol (**2**), 2,6-dimethyl-octa-3,7-diene-2,6-diol (**3**), 2,6-dimethyl-oct-7-ene-2,3,6-triol (**4**), (*Z*)- and (*E*)-2,6-dimethyl-octa-2,7-diene-1,6-diol (**9**, **10**) and each of four diastereoisomeric epoxy-linalool oxides in their furanoid and pyranoid forms **11**–**18** (cf. Figs 1–3). The compounds **2**–**4** have already been reported in *Cinnamomun camphora* Sieb. [9], grapes [8, 10, 11] and passion fruit [12], but not in papaya. Among the remaining substances **1** and **11**–**18** were detected for the first time in a natural plant material.

As outlined in Fig. 1, the acid catalysed formation of triol **4** from 6,7-epoxy-linalool (**1**) seems plausible, whereas enzymic reactions leading to **2** and **3** from **1**, as previously discussed [8], are speculative.

Previously, the isomers of 6,7-epoxy-linalool (**1**) had been proposed as possible precursors of hydroxy ethers **5**–**8** (Fig. 2), the so-called linalool oxides, without any experimental evidence for their occurrence in natural plant material [13, 14]. Apart from the lack of enzymic studies, the identification of **1** together with the already detected isomeric linalool oxides **5**–**8** in papaya fruit pulp [6, 15] supports this hypothesis (Fig. 2).

As shown in Fig. 2 the triol **4** had also been discussed as a natural precursor of the furanoid linalool oxides **5** and **6**, since at an acidic pH (< 3.5), and/or upon heat treatment during sample preparation, **4** has been found to be converted to **5** and **6** [16]. In these previous experiments there was no indication of the formation of the corresponding pyranoid linalool oxides **7** and **8**. As we were unable to detect any formation of the linalool oxides from triol **4** in model experiments carried out under natural conditions (i.e. pH 5.6) this pathway might be excluded for papaya fruit.

The isomeric diols **9** and **10** (Fig. 3) were determined in a ratio of *ca* 1:6 (*Z*:*E*) in the fruit pulp. Biogenetic formation via enzymatic ω-hydroxylation seems plausible and has previously been proposed for several bacterial [17, 18] and fungal [19] transformations of linalool.

In higher plants, the (*E*)-isomer **10** has been found in different *Nicotiana* species [20, 21]. The β-D-glucosides of (*E*)-**10** and (Z)-diol **9** have been isolated from *Betula alba*

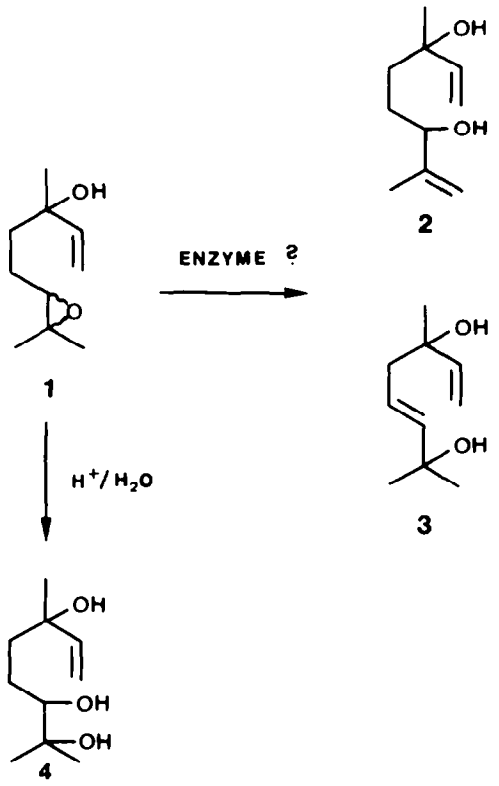

Fig. 1. 6,7-Epoxy-linalool (1) as precursor of terpene diols 2 and 3 and triol 4.

leaves and from the fruits from *Chaenomeles japonica* [22].

The four diastereoisomeric epoxy derivatives of linalool oxides in their furanoid (**11–14**) and pyranoid forms (**15–18**) are represented in Fig. 3. The natural occurrence of eight epoxy-linalool oxides and their quantitative distribution in the fruit pulp (e.g. furanoid forms, $E:Z = 5:1$) seemed to exclude a subsequent epoxidation of the corresponding linalool oxides. Therefore, a hypothetical diepoxy-linalool derivative is postulated as a possible biogenetic precursor of structures **11–18** (Fig. 3). This hypothesis is supported by the findings of Osborne [23], who detected the formation of the diepoxy derivatives of geraniol and nerol after feeding these alcohols to *Pisum sativum* cell cultures.

EXPERIMENTAL

MS were determined at 70 eV by HRGC-MS, scanning from m/z 41 to 250 with total ion current monitoring. HRGC and HRGC-MS were carried out using a fused silica WCOT column (30 m × 0.259 mm, df = 0.25 μm) coated with DB-wax. On-column inj was used (0.4 μl). The column was held at 50° for 3 min and then programmed at 4°/min to 250°. FID temp. 250°; carrier gas He 2 ml/min. Linear HRGC retention indices [24] were compared with those of authentic reference samples. HRGC-FTIR was carried out using a fused silica WCOT DB-5 column (30 m × 0.32 mm i.d., df = 0.25 μm). PTV injection (40–200°) was performed. Temp. prog. 60–250° at 5°/min. FID temp. 300°; carrier gas He 2 ml/min. Light pipe and transfer line were held at 200°; vapour phase spectra were recorded from 700 to 4000 cm^{-1} with 1 scan/sec.

Decomposition of linalool at pH 5.6 during sample preparation.

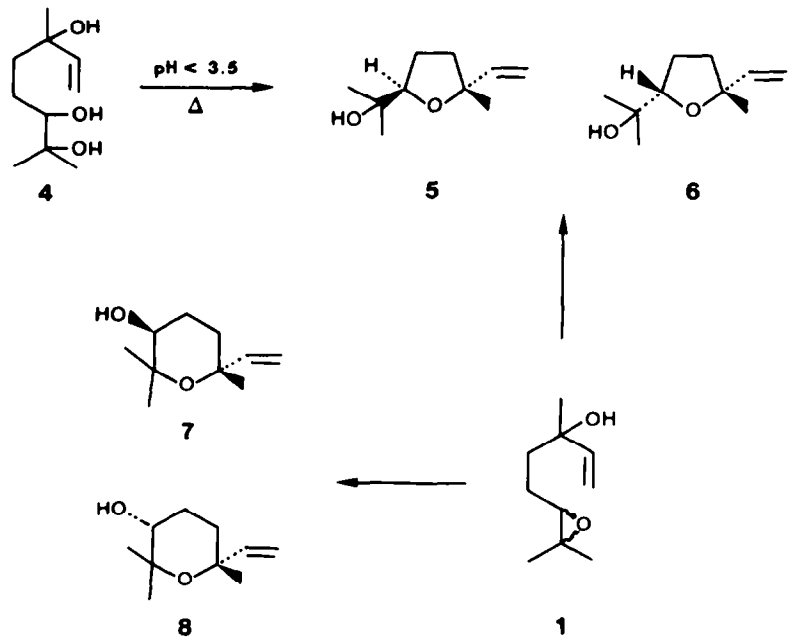

Fig. 2. Chemical formation of furanoid linalool oxides 5 and 6 from triol 4 and biogenetic formation of the four linalool oxides 5–8 from 6,7-epoxy-linalool (1).

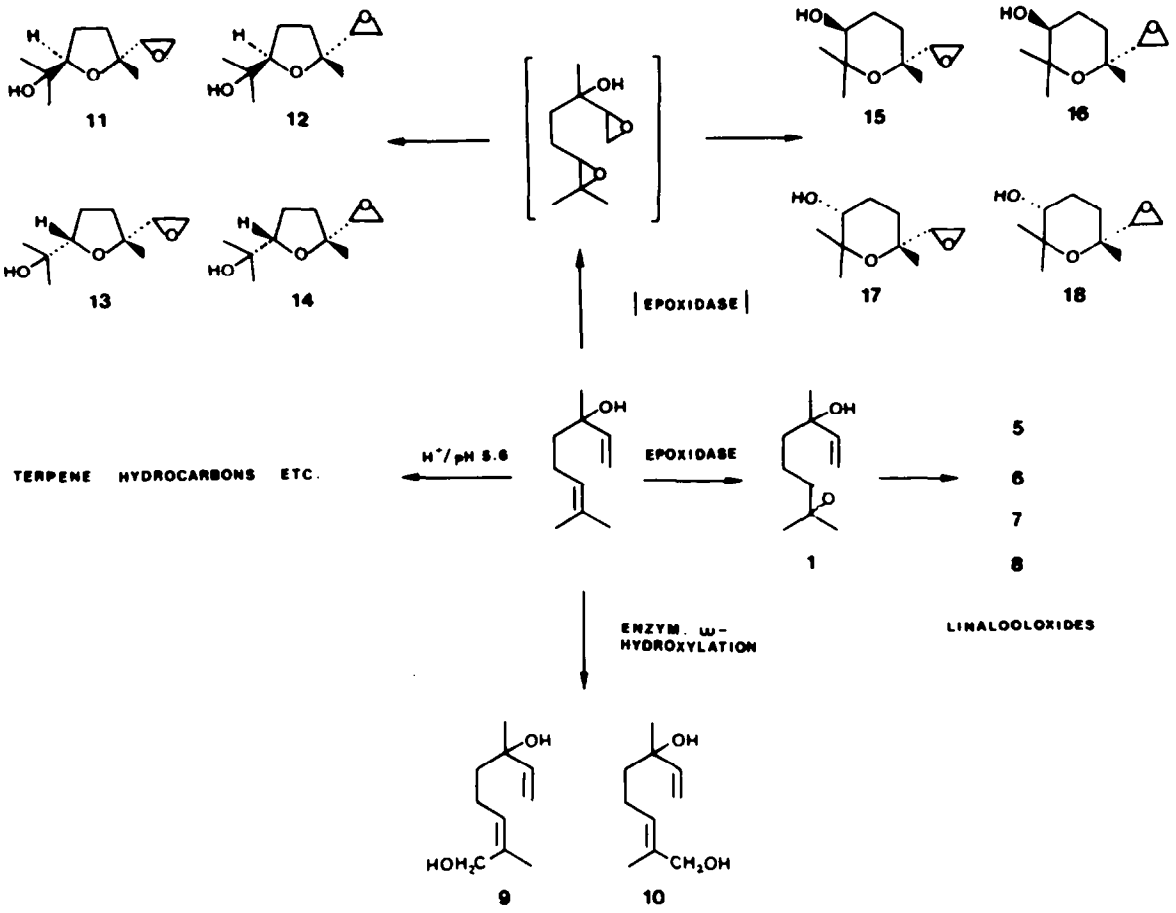

Fig. 3. Chemical and biogenetic formations of linalool derivatives.

Linalool (2 mg) in 900 ml H_2O (acidified with 20 ml of 1 M Pi buffer pH 5.6) was extracted with pentane–CH_2Cl_2 (2:1) as previously described [6]. After drying and concn of the extracts the degradation products were analysed by HRGC and HRGC-MS.

Decomposition of triol **4**. Triol **4** (2.5 mg in 900 ml 0.1 M Pi buffer pH 5.6) was stored in the dark at 25° for 3 days and subsequently liquid extracted as described above.

Extraction of polar compounds (ref. [8], *modified)*. Fresh papayas (Solo variety, shipped by air-freight from Brazil) were held at room temp until full ripeness. After peeling and careful removal of seeds, the sample was homogenized with 0.1 M Pi buffer pH 7.0 (sample wt 700 g). After centrifugation (30 min, 4000 *g*) the supernatant was extracted with $CHCl_3$ and carefully (< 30°) concd to dryness. The residue was taken up in H_2O (2 × 10 ml) and washed with pentane. The aq. soln was extracted with $CHCl_3$ (3 × 50 ml), the organic phase dried (Na_2SO_4), concd to 0.1 ml and subjected to HRGC and HRGC-MS analysis. Identifications were performed by comparison of the HRGC R_t and MS data of separated compounds with those of synthetic specimens.

Preparation of authentic reference samples. Compound **1**: to 40 mmol (6.17 g) (−)-*R*-linalool in 100 ml dry Et_2O 40 mmol (6.9 g) *m*-chloroperbenzoic acid (MCPB) was added in small portions with cooling (−5°). After stirring for 2 hr (0°) the reaction mixture was washed with H_2O, 10% NaOH soln, satd NaCl soln and dried (Na_2SO_4). Purification by LC on silica gel 60 using pentane–Et_2O (7:3) afforded diastereoisomer **1** as a colourless oil. HRGC: R_ts 1781 and 1791, respectively. FTIR (vapour phase) ν cm^{-1}: 3659, 3086, 2983, 1842, 1645, 1459, 1373, 1230, 1073, 989, 915. EIMS *m/z* (rel. int.): 155 $[M - Me]^+$ (0.5), 137 $[M - Me - H_2O]^+$ (0.5), 97 (7), 85 (5), 79 (8), 71 (34), 68 (29), 59 (33), 55 (21), 43 (100). ^{1}H NMR (60 MHz, $CDCl_3$) was in close agreement with published data [14, 25]. Compounds **2** and **3** were prepared by photooxygenation of linalool, followed by reduction with $NaBH_4$ [26, 27]. HRGC: R_ts 1927 and 2106, respectively. For spectral data see refs [9–11, 26, 27]. Triol **4** was prepared according to the method of ref. [8] using diastereoisomeric 6,7-epoxy-linaloyl acetate, and showed similar spectral properties. In our hands, **4** is directly amenable to HRGC: R_t 2427. Derivatization afforded the acetonide derivatives with R_ts 1828 and 1837, respectively (the natural **4** co-chromatographed with the isomer at R_t 1828). Synthesis of **9** and **10** was accomplished by oxidation of linalool with SeO_2 [20], and afforded, after reductive work-up, the (*E*)- and (*Z*)-isomers of 2,6-dimethyl-octa-2,7-diene-1,6-diol in a ratio of *ca* 10:1 (10% yield). After purification by LC on silica gel 60 using pentane–EtOAc (1:1) compound **9** was characterized by FTIR, ^{1}H NMR and MS. Spectral properties corresponded to those published [20, 22]. HRGC: R_t 2294. The (*Z*)-isomer **10** was examined by HRGC (R_t 2254) and HRGC-MS. For spectral differences between **9** and **10** see ref. [28].

Preparation of the furanoid epoxy-linalool oxides **11–14**. Isomeric linalool oxides **5** and **6** (23 mmol, 4 g) were epoxidated

(as described for 1) with 23 mmol (4 g) MCPB for 20 hr. Work-up and purification by LC on silica gel 60 using pentane–Et_2O (7:3 up to 2:8) afforded the diastereoisomeric (*Z*)- and (*E*)-epoxy-linalool oxides (150 and 65 mg, respectively) as colourless oils. ^1H NMR spectra agreed with those published [29]. (*E*)-isomers **11** and **12**, HRGC: R_is 1867 and 1877, respectively. FTIR (vapour phase) $\nu\,cm^{-1}$: 3606, 3049 (epoxy), 2984, 1379, 1180, 1109, 1058, 905. EIMS *m/z* (rel. int.): 171 $[M-Me]^+$ (1), 153 $[M-Me-H_2O]^+$ (1), 143 (6), 127 (4), 97 (5), 84 (31), 81 (27), 71 (20), 59 (55), 43 (100). (*Z*)-Isomers **13** and **14**: HRGC: R_is 1792 and 1797, respectively. FTIR (vapour phase) $\nu\,cm^{-1}$: 3608, 3528 (intramol. H-bonding), 3067 (epoxy), 2983, 1378, 1179, 1130, 1053, 900. EIMS *m/z* (rel. int.): 171 $[M-Me]^+$ (0.5), 143 $[M-expoxy]^+$ (6), 125 $[M-epoxy-H_2O]^+$ (3), 110 (2), 97 (7), 84 (40), 81 (19), 71 (20), 59 (37), 43 (100). The epoxides of the pyranoid linalool oxides **15–18** were prepared analogously to the furanoid forms **11–14** (10 day epoxidation at 25°). (*E*)-Isomers **15** and **16**, HRGC: R_is 2141 and 2166, respectively. FTIR (vapour phase) cm^{-1}: 3660, 3051, 2985, 1457, 1375, 1233, 1071, 1006. EIMS *m/z* (rel. int.): 171 (0.5), 153 (0.2), 143 (14), 125 (5), 107 (3), 84 (21), 71 (20), 59 (69), 55 (25), 43 (100). (*Z*)-Isomers **17** and **18**, HRGC: R_is 2116 and 2119, respectively. FTIR (vapour phase) $\nu\,cm^{-1}$: 3660, 3490 (intramol. H-bonding), 3060, 2984, 2945, 1457, 1377, 1173, 1004. EIMS *m/z* (rel. int.): 186 $[M]^+$ (0.2), 171 $[M-Me]^+$ (0.4), 153 (0.2), 143 (13), 125 (6), 84 (21), 71 (18), 59 (71), 55 (28), 43 (100).

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