Lactonic Compounds of Apricot

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A charcoal adsorption essence of the Blenheim variety of apricot was subjected to repetitive gas chromatographic separations, and the isolated components were characterized by infrared spectroscopy. Compounds not previously reported in apricot include benzyl alcohol, caproic acid, epoxydihydrolinalool IV, γ-caprolactone, δ-octalactone, δ-decalactone, and γ-dodecalactone. The biochemistry of the dihydrolinalools is discussed. Possible biogenetic pathways to the γ- and δ-lactones via hydroxy fatty acids are postulated.

Early work on the nonvolatile constituents of apricot was reviewed in a previous paper which, on the basis of gas chromatographic retentions and infrared data, also identified a number of the volatile constituents (Tang and Jennings, 1967). Rhoades and Millar (1965) also performed gas chromatographic studies of apricot volatiles, but did not identify the components. The present study was devoted to further characterization of this essence, with some attention to the mechanisms by which some of these compounds might be produced.

APPARATUS

Gas chromatographic separations utilized Beckman Thermotrac program units with Hamilton glass-lined injectors and Carle microcell detectors. Both packed and wide-bore capillary columns were used.

Infrared spectra were determined on thin films in a Beckman IR8 fitted with a beam condenser as described by Tang and Jennings (1967).

Epoxydihydrolinalool I, II, III, and IV were prepared from linalool (Klein and Rojahn, 1964) and isolated by gas chromatographic separation. Caproic acid and benzyl alcohol were obtained from Eastman Organic Chemicals and γ-caprolactone from K and K Laboratories. δ-Decalactone and γ-dodecalactone were supplied by Hubert M. Cole, Firmenich, Inc., New York, N.Y., and δ-octalactone by R. E. Wrolstad, Oregon State University. K. L. Stevens, USDA, Albany, Calif., furnished samples of epoxydihydrolinalools I and II.

The essence used in this study was obtained by charcoal adsorption (Tang and Jennings, 1967). Two pounds of activated charcoal (8- to 12-mesh, Matheson Coleman & Bell) were placed in the water aspirator discharge line of a vacuum deaerator operated by a large commercial cannery processing select, ripe apricots to puree. After 48 hours, the charcoal was freeze-dried to remove water and extracted with ethyl ether in a Soxhlet extractor. The major portion of the solvent was removed by fractional distillation, leaving ca. 8 ml. of a light yellow oil. To concentrate further the higher boiling compounds which were the object of this study, a stream of dry nitrogen was then blown across the surface of the open container until the total volume was reduced by approximately 50%.

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METHODS AND PROCEDURES

Initial gas chromatographic separations were performed on a 18-foot × 1/4-inch stainless steel column packed with 10% Triton X-305 on 40- to 60-mesh Gas Pack F, programmed from 70° to 210° C. Collected fractions (restricted to those emerging after linalool) were reinjected on a 10-foot × 1/8-inch Carbowax 20M column (Figure 1) and then further purified on an SF96 (50) column. Final purifications utilized 500-foot × 0.03-inch stainless steel capillary columns coated with Carbowax 20M or SF96 (50).

Fractions were collected from the exit ports of the gas chromatographs by a method first suggested by Kratz (Heinz, 1965). An aluminum foil triangle (about 3 inches on each side) was wrapped around one end of a 1-foot length of thin-walled glass capillary tubing. The tubing was inserted into the exit port of the chromatograph, and the rolled foil used as a sliding tapered seal. This ensured a tight fit and also achieved a gradient cooling effect in the glass tube to help minimize aerosol formation. A cheesecloth bag containing crushed dry ice was placed on the capillary tubing, and for the higher boiling fractions, which are prone to form aerosols, a hot plate at approximately 100° C. was placed under the tubing and the dry ice bag was set on the top side. This arrangement produced a hot wall–cold wall effect and eliminated losses due to fogging (Teranishi *et al.*, 1962).

For those samples requiring repeated collections or storage, two Teflon caps were used to stopper both ends of the glass capillary tubing. Caps were made of Teflon rod (1/4 × 1/8 × 1/8 inch) with a center-drilled 0.059 × 3/16 inch well. This achieved satisfactory sealing and eliminated the danger of pyrolytic changes occasionally induced by flame sealing. A few microliters of carbon tetrachloride were used to recover the purified fraction from the glass capillary with a microsyringe. The recovered fraction was then reinjected on a dissimilar column or transferred to a salt plate where the solvent was allowed to evaporate.

RESULTS AND DISCUSSION

Chromatograms typical of the original apricot essence under these chromatographic conditions have been published previously (Tang and Jennings, 1967). Figure 1 shows a chromatogram of this more concentrated essence on a 10-foot × 1/8-inch column packed with 5% Carbowax 20M on 60- to 80-mesh Chromosorb G. The positions occupied by newly identified apricot volatiles—benzyl alcohol, caproic acid, epoxydihydrolinalool IV, γ-capro-

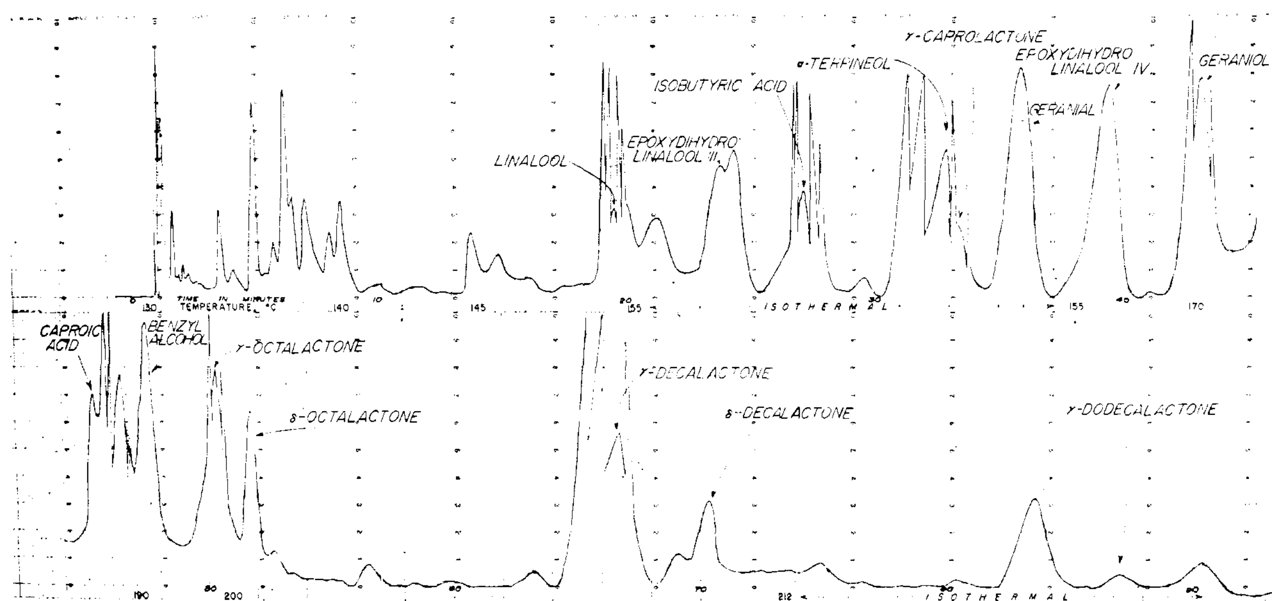


Figure 1. Chromatogram of apricot essence concentrate on 10-foot × 1/8-inch column, 5% Carbowax 20M on 60- to 80-mesh Chromosorb G

lactone, δ-octalactone, δ-decalactone, and γ-dodecalactone—are as indicated. γ-Caprolactone was resolved from α-terpineol by reinjecting collections on the SF96 (50) column, on which γ-caprolactone has a shorter retention time. Each of the above compounds had the same retention as the authentic compound under various temperature programs, and the infrared spectra of the isolated components matched those of the authentic compounds.

Epoxydihydrolinalools I and II were among the compounds identified in the previous paper, IV was isolated in this study, and III is suspected to be present, based on an infrared spectrum obtained with insufficient amount of material.

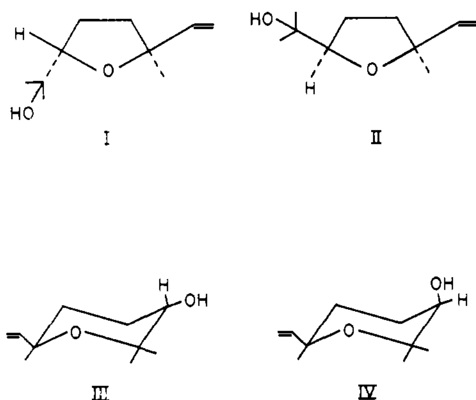


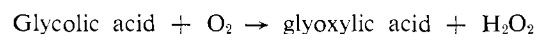
Table I. Even-Numbered Lactones of Apricot,[a] Peach,[b,c] and Pineapple[d]

	Apricot	Peach	Pineapple
γ-Butyrolactone			+
γ-Caprolactone	+	+	+
γ-Octalactone	+	+	+
δ-Octalactone	+		+
γ-Decalactone	+	+	
δ-Decalactone	+	+	
γ-Dodecalactone	+		

[a] Tang and Jennings (1967).
[b] Jennings and Sevenants (1964).
[c] Sevenants and Jennings (1966).
[d] Creveling (1967).

The infrared spectra of I, II, and IV agree with those published by Felix *et al.* (1963). These authors checked the stereochemistry by oxidation of the terminal methylene to a carboxyl group; lactone formation is possible only for those which possess the isopropyl alcohol and carboxyl groups in cis positions.

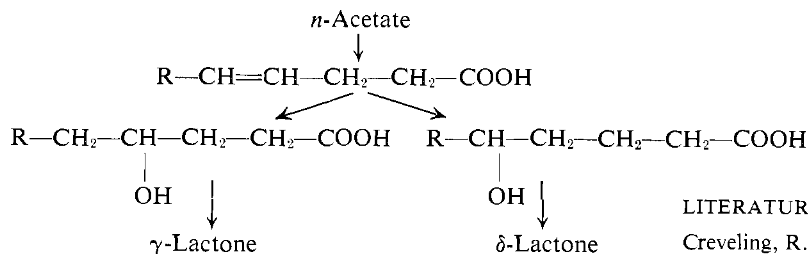
Epoxydihydrolinalools have been identified previously as fruit volatiles, but it has not been established whether they are products of biological systems or artifacts introduced during preparation (Stevens *et al.*, 1966). However, peroxide is known to be produced by enzymatic reactions between reduced flavin nucleotide and O_2 in biological systems, such as the reaction catalyzed by glycolic oxidase which occurs widely in plant tissues and serves as an excellent catalyst for the formation of H_2O_2.



$$\text{Glycolic acid} + O_2 \rightarrow \text{glyoxylic acid} + H_2O_2$$

Wilkoff and Martin (1963) confirmed that in biosynthesis of *trans*-L-epoxysuccinic acid from fumaric acid by *Aspergillus fumigatus* epoxide oxygen was derived solely from molecular O_2.

A number of fruits have been reported to contain lactones (Table I). Their rather general occurrence suggests that they may be produced by some common biogenetic pathway. Muys *et al.* (1962) obtained optically active γ- and δ-lactones by microbiological reduction of the corresponding keto acids. Yamada and Stumpf (1965) studied the enzymatic degradation of long-chain hydroxy fatty acids in plants and reported that one of the breakdown products of 10-hydroxypalmitic acid was 4-hydroxydecanoic acid. The biosynthesis of coumarin has been studied, and it was concluded that coumarin would lactonize spontaneously from coumarinic acid under the acidic conditions normally encountered in the plant (Kosuge and Conn, 1961). While it is reasonable to postulate the formation of lactones via the catabolic pathway, other routes to their production are also possible. One such scheme could involve the following biosynthetic sequence:



A mechanism that would specifically direct the double bond to the γ-δ position is unknown, but this could proceed through β-γ unsaturation similar to the system described in certain eubacteria (Scheuerbrandt *et al.*, 1961) followed by a double bond shift through rehydration and dehydration. β-γ- and δ-ϵ-unsaturated fatty acids, which have been found in plant seeds (Wolff, 1966), could also produce lactones of this type by hydrating to place the hydroxy group on the γ or δ position. If the β- or ϵ-hydroxy fatty acid were formed, lactonization would not occur because of the steric effect. Other possibilities are the introduction of a hydroxyl group at the nonterminal position of a paraffinic carbon chain by an oxidative mechanism (Galliard and Stumpf, 1966).

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