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Sesquiterpene Hydrocarbons in Pineapple Fruit

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The volatile constituents of pineapple fruit have been isolated under enzyme inhibition, enriched by liquid–liquid extraction, and fractionated on silica gel. Analysis of the nonpolar fraction by gas chromatography and coupled GC–MS showed at least 20 sesquiterpene hydrocarbons to be present. α-Copaene, β-ylangene, α-patchoulene, γ-gurjunene, germacrene D, α-muurolene, and δ-cadinene were identified via authentic samples and/or published data. One of the minor compounds of the fraction seems to be responsible for the fragrant odor (reminescent of fresh-cut pineapple).

The first comprehensive studies of pineapple volatiles resulted from collaboration between the Pineapple Research Institute of Hawaii and the Stanford Research Institute and showed the fruit to contain aliphatic, hydroxy, and acetoxy esters, γ-lactones, sulfur compounds, linalool oxide, and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Creveling et al., 1968; Rodin et al., 1965, 1966; Silverstein et al., 1965). Flath and Forrey (1970) reported the presence of a number of additional compounds, mainly carboxylic esters, and also of three monoterpene alcohols. The occurrence of sesquiterpenoid structures ($M^+ = 204$) in pineapple extracts has been mentioned by Näf-Müller and Willhalm (1971), but only one compound, an alcohol with a selinane skeleton, was identified by GLC and mass spectrometry. The interesting sensory properties of the hydrocarbon fraction were the reason to study the chemical composition in more detail.

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EXPERIMENTAL SECTION

Materials. Pineapple fruits (*Ananas comosus* Merr.) from the Ivory Coast were obtained from a local supplier.

Aroma Isolation. Whole ripe pineapples were peeled, cut into methanol, and mixed in a Waring Blendor (final methanol concentration 66%; Drawert et al., 1973). The methanol–juice mixture was separated immediately in a Sorvall-type centrifuge (0 °C, 1500*g*, 10 min) and extracted with a pentane–methylene chloride (2:1) mixture. The solvent was removed by distillation through a Vigreux column (20 cm, 40 °C) (Drawert and Rapp, 1968; Drawert et al., 1969), and the total concentrate was subjected to ascending column chromatography using pentane–ether mixtures as the migrating solvents (Schreier et al., 1974). The hydrocarbon fraction was obtained by elution with

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Table I. Mass Spectral Data of Sesquiterpenes in Pineapple[a]

peak no.	compound	m/e (rel intensity)
1	M^+ = 204	41 (74), *91 (100)*, 105 (78), 119 (40), 133 (58), 147 (41), 148 (44), 161 (40)
2	M^+ = 204	*41 (100)*, 55 (56), 79 (53), 91 (85), 93 (63), 105 (65), 108 (74), 133 (71)
4	α-copaene, FiT 999	
6	M^+ = 204	41 (82), 79 (44), 91 (97), 93 (63), *105 (100)*, 108 (46), 119 (44), 133 (40)
7	β-ylangene, FiT 946[b]	
10	α-patchoulene, FiT 872	
11	M^+ = 204	*41 (100)*, 55 (62), 79 (76), 91 (58), 93 (55), 95 (78), 105 (58), 204 (52)
12	γ-gurjunene, FiT 984	
15	germacrene D[c]	
16	M^+ = 204	*41 (100)*, 55 (61), 91 (76), 105 (86), 119 (64), 161 (86), 189 (64), 204 (77)
17	M^+ = 204	*41 (100)*, 55 (64), 79 (55), 81 (79), 91 (57), 93 (75), 105 (53), 107 (61)
19	α-muurolene, FiT 996[b]	
20	δ-cadinene, FiT 997	

[a] Identification by comparison of mass spectra and retention data of authentic samples if not otherwise indicated. Maximum identity of the sample spectrum and NBS Library spectrum results in a FiT of 1000. [b] Retention time compared with published data. [c] Sample spectrum and retention time compared with published data.

200 mL of pentane/1 kg of fruit concentrate. All solvents (analytical grade) were redistilled before use.

GLC Conditions. A Carlo Erba Fractovap 2350/AC gas chromatograph equipped with a flame ionization detector was used. Columns used were as follows: (1) OV-101 50 × 0.32 mm i.d. glass capillary column (Jaeggi), carrier gas H_2 0.5 bar = 1 mL/min, injector and detector temperatures 250 °C, programmed from 40 to 230 °C at 2 °C/min; (2) Carbowax 20M 40 m × 0.5 mm i.d. SCOT glass capillary column (SGE), carrier gas H_2 0.38 bar = 1 mL/min, injector and detector temperatures 200 °C, programmed from 65 to 170 °C at 2 °C/min (Drawert and Berger, 1982).

Combined GLC–Mass Spectrometry. A mass spectrometer, Finnigan 4021 (quadrupole), was directly coupled with a Finnigan 9610 gas chromatograph. The column used was as follows: Carbowax 20M 25 m × 0.25 mm i.d. glass capillary column (Mega), carrier gas He = 40 cm/s, injector and transfer line temperatures 220 °C, temperature programmed at 65 °C for a 3 min isotherm and then 1.5 °C/min up to 170 °C. Ionization chamber operated at 70 eV. Recording and searching of mass spectra were done by an integrated Nova 4/CDC disc drive system (32 Mbyte, NBS Library with 33.000 reference spectra) (Drawert et al., 1982).

RESULTS AND DISCUSSION

Unsaturated hydrocarbons are known to undergo different changes if conventional separation methods are applied (Tressl et al., 1970). In order to avoid or reduce the formation of secondary aroma compounds, the pineapple volatiles have been isolated under enzyme inhibition by adding methanol.

Figure 1 shows a part of the reconstructed ion chromatogram and the corresponding mass chromatograms of typical sesquiterpene hydrocarbon fragments including the molecular ion. Compounds with molecular ions M^+ = 136, 202, 206, or 208 were not detected. A list of identified compounds and mass spectra of not identified main compounds is given in Table I. Peaks 3, 5, 8, 9, 13, 14, and 18 were too small or not clean enough to give good spectra; their fragmentation patterns fit with that of sesquiterpen hydrocarbons (see also Figure 1).

Comparing the mass spectra published in the literature it has to be stated that not all of them were obtained from authentic samples and many data are not consistent. This may also be attributed to different mass spectrometric conditions. In the present study authentic samples served as references as far as available.

Literature data of retention times and mass spectra were taken from Hunter and Brogden (1964), Hayashi et al. (1967), Andersen and Falcone (1969), Moshonas and Lund (1970), Schreier et al. (1976), Bruns (1978), and Seifert and Buttery (1978).

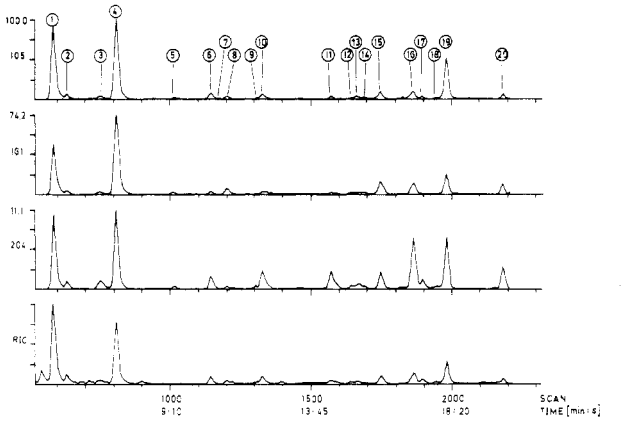

Figure 1. Reconstructed ion and mass chromatograms of the nonpolar fraction of pineapple volatiles.

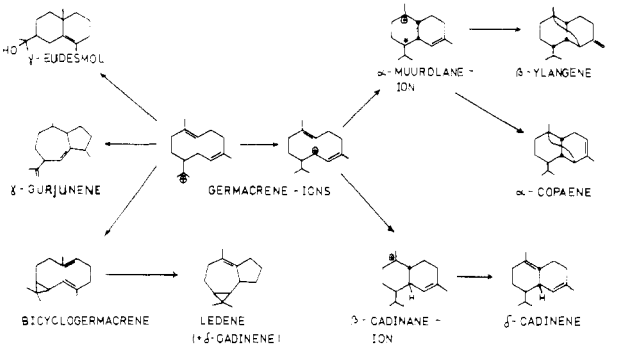

Figure 2. Proposed pathways to sesquiterpene skeletons found in pineapple.

All of the identified sesquiterpenes possess bi- or tricyclic skeletons and can formally be derived from both the *cis*, *trans*- and the *trans,trans*-cyclodecadiene systems (Croteau, 1974). Figure 2 shows the biogenetic connections between the compounds of Table I with germacrene ions as key intermediates.

The extreme ease with which some of the germacrene types can undergo acid-catalyzed cyclization in vitro (Jain and McCloskey, 1969) may also take place in vivo (cytoplasma pH in ripe pineapple fruit cells is 3.2–3.5; Singleton and Gortner, 1965). Eudesmane-type compounds like γ-eudesmol—a constituent of pineapple volatiles (Näf-Müller and Willhalm, 1971)—can be derived in vitro by treatment of germacratrienes with electrophilic reagents

(Brown et al., 1967). Various *Ambrosia* species elaborate germacranolides together with either eudesmanolides or pseudoguaianolides (Yoshioka et al., 1970). These pathways may lead to γ-gurjunene and α-patchoulene in pineapple (the latter being tentatively identified).

The direct correlation between the germacrane and the aromadendrane skeleton was performed by Shinoda et al. (1969), who demonstrated the in vitro conversion of bicyclogermacrene to a mixture of ledene and δ-cadinene in weakly acid media. The formation of δ-cadinene may as well happen via germacrene D and the β-cadinene ion. The latter proposition is supported by the simultaneous occurrence of α-muurolene, α-copaene, and β-ylangene (see Figure 2).

Germacrene D has been observed to photoisomerize to α- and β-bourbonene and to transform readily into γ-muurolene, α-amorphene, δ-cadinene, and γ-cadinene in contact with silica gel (Yoshihara et al., 1969), but prefractionation of fruit extracts is a precondition for obtaining clean mass spectra. When a 100-g fruit sample was extracted with pentane and the total extract submitted to GLC–mass spectrometry, 12 compounds with a molecular ion m/e = 204 were detected.

These results for the small unfractionated extract and the absence of the expected artifacts α- and β-bourbonene, γ-muurolene, α-amorphene, and γ-cadinene in the prefractionated extracts permit the conclusion that the described aroma separation procedure yields samples with a composition similar to that of the intact fruit. Calculated from the internal standard, the concentrations of the main compounds (peaks no. 1 and 4) are in the range of 0.2 ppm, but the stage of ripeness and unequal distribution in the fruit influence the values (results not shown).

Of all the compounds identified α-patchoulene seems to contribute to the strong fruity–spicy odor of the fraction. Sniffing runs have indicated that quantitatively minor components provide considerable aroma character. Enrichment and purification of these trace volatiles are in progress.

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Registry No. α-Copaene, 3856-25-5; α-ylangene, 20479-06-5; α-pathoulene, 560-32-7; γ-gurjunene, 22567-17-5; germacrene D, 23986-74-5; α-muurolene, 10208-80-7; δ-cadinene, 483-76-1.

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